

## СПИСЪК НА ПРЕДСТАВЕНИ РЕЗУЛТАТИ НА КОНФЕРЕНЦИИ

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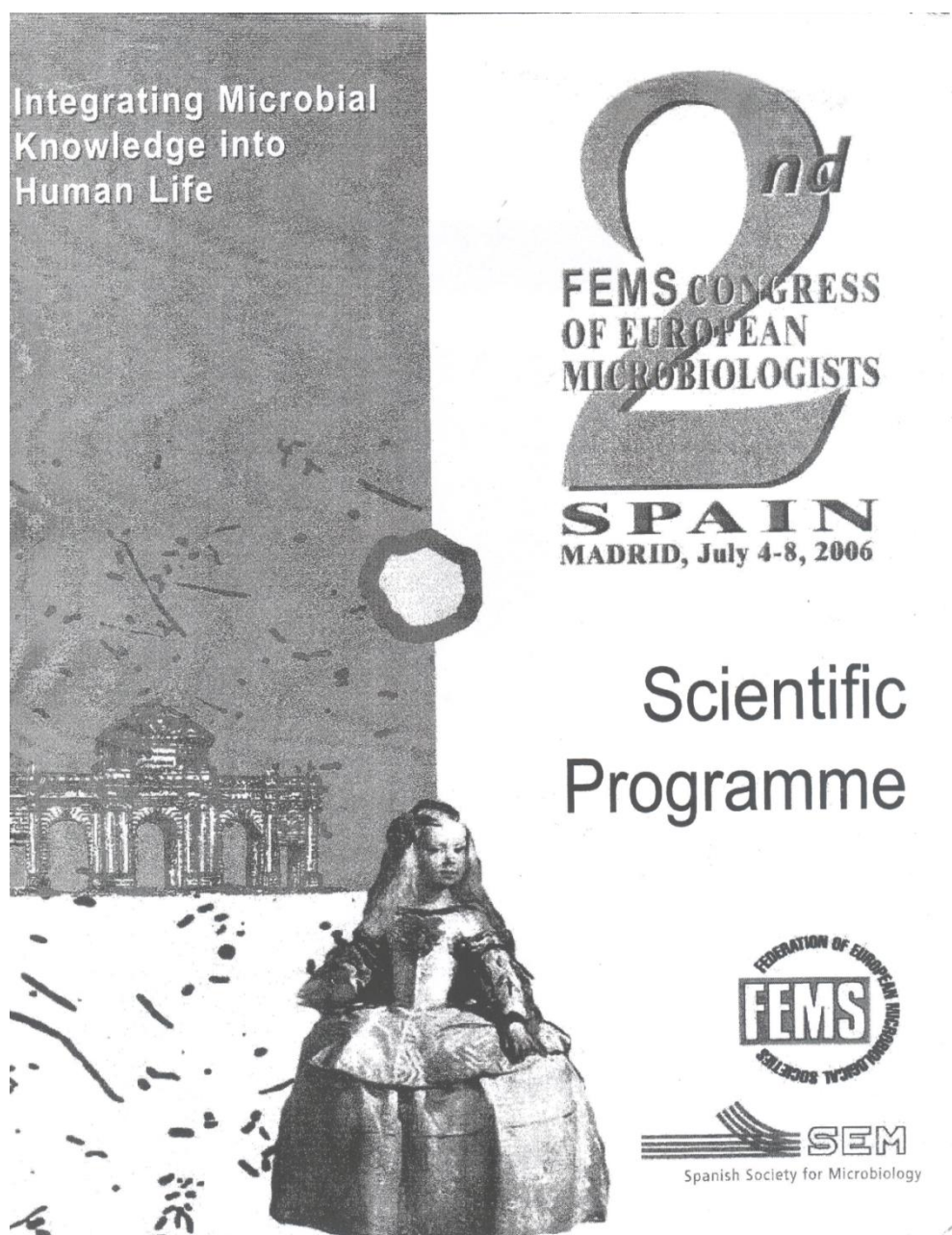
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51. L. Velkova, **A. Dolashki**, R. Velikova, P. Dolashka. Identification of peptides and glycopeptides with antimicrobial activity from garden snail *C. aspersum* using mass spectrometry. (Lecture), 4-th Glycobiology World Congress, 17-19 September 2018, Rome, Italy.
52. R. Abrashev, E. Krumova, **A. Dolashki**, N. Kostadinova, J. Miteva-Staleva, L. Velkova, B. Spasova, M. Angelova, P. Dolashka. *Cornu aspersum* - derived peptides with biological activity. (Poster), 4-th Glycobiology World Congress, 17-19 September 2018, Rome, Italy.
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**СПИСЪК**  
**на участия на гл. ас. Александър Долашки в конференции**  
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## MICROBIAL PHYSIOLOGY AND METABOLISM

### P.PHY.1

Correlation between heat shock and oxidative stress responses of *Aspergillus niger*

Abrashev, R. Dolashki, A. Stevanovic, S. Dolashka, P. Voelter, W. Stefanova, L. Pashova, S. Hristova, R. Angelova, M.

### P.PHY.2

An extremely thermostable trehalose-hydrolyzing  $\beta$ -glucosidase from *Thermus thermophilus* HB27

Alarico, S. Empadinhas, N. Da Costa, M.S.

### P.PHY.3

Essential involvement of lipid-II-tripeptide in cell division in *Escherichia coli*

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### P.PHY.4

A protein interaction network for phospholipid synthesis in the inner membrane of *Escherichia coli*

Bouveret, E. Gully, D. Battesti, A. Leduc, D.

### P.PHY.5

The thermophilic bacterium *Persephonella marina* uses a two-step pathway for the synthesis of glucosylglycerate

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### P.PHY.6

Response of *Cupriavidus metallidurans* CH34 to low-shear modeled microgravity (LSMMG) as determined by microanalyses

De Boever, P. Benotmane, M.A. Crabbé, A. Leys, N. Mergeay, M.

### P.PHY.7

Compatible solutes in the radiation-resistant bacterium *Rubrobacter xylanophilus*

Empadinhas, N. Mendes, V. Simoes, C. Mingote, A. Lamosa, P. Santos, H. Da Costa, M.S.

### P.PHY.8

The PQQ-Alcohol Dehydrogenase of *Gluconacetobacter diazotrophicus* PAL5

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### P.PHY.9

The inositol-1-phosphate synthase from *Rubrobacter xylanophilus*

Fernandes, C. Empadinhas, N. Da Costa, M.S.

### P.PHY.10

Molecular crowding of DnaA on the membrane surface – a switch for nucleotide exchange

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### P.PHY.11

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### P.PHY.12

Reductive precipitation of technetium by a thermophilic gram-type positive dissimilatory Fe(III)-reducer

*Thermoterrabacterium ferrireducens*

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### P.PHY.13

D-arabinitol metabolism in the human fungal pathogen *Candida albicans*: functions of the dehydrogenase encoded by

Gola, S. Plá, J.

### P.PHY.14

Dihydrolipoamide dehydrogenase involved in bacterial stress response when grown on a non-ionic surfactant as sole carbon source

Huang, S.L. Sun, Y.L. Hung, G.C. Chen, C.H. Guo, G.L.

### P.PHY.15

All three J-domain proteins of the *Escherichia coli* DnaK chaperone machinery are DNA binding proteins

Katz, C. Gur, E. Ron, E.Z.

### P.PHY.16

Giant colonies of yeasts

Klisova, T. Patakova, P. Herman, J.

### P.PHY.17

Changes in Growth Capability and Metabolism of *Trichoderma viride* Vegetative Mycelia Associated with Ageing

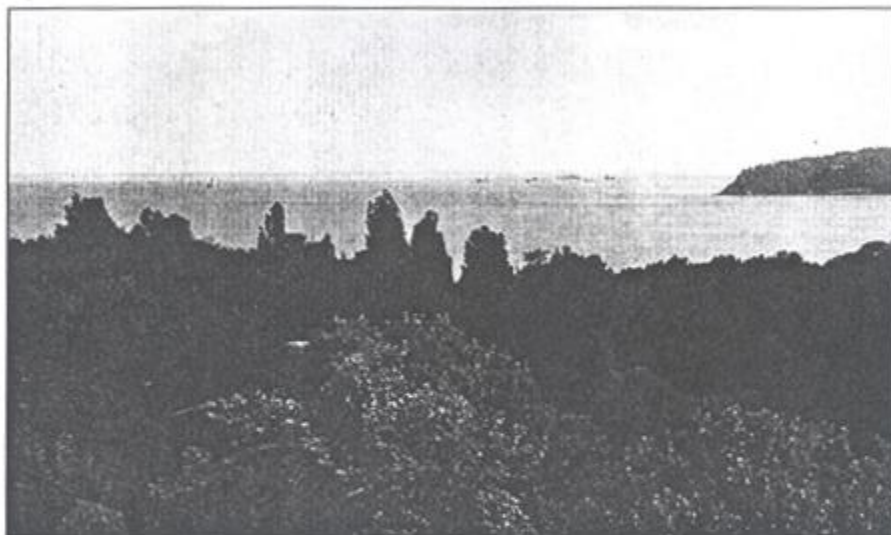
Lakatos, B. Simkovic, M. Ditte, P. Chovanec, P. Varecka, L.

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**ELEVENTH CONGRESS  
OF THE BULGARIAN MICROBIOLOGISTS**  
with International Participation

**PROGRAM AND ABSTRACTS**



*St. Constantine, Varna, October 5-7, 2006*



GAM47

## ADAPTIVE STRESS RESPONSE OF *HUMICOLA LUTEA* 103 TO COPPER EXPOSURE

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M. Angelova<sup>1</sup>

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Copper is an essential element for fungal metabolism, but can be toxic to microorganisms at high concentrations. It is widely considered that copper ions exert its effect at the cellular level through induction of oxidative stress. Adaptive response to heavy metal treatment refers to the ability of cells to better resist the damaging effects of the toxic agent when first preexposed to a lower dose. It is a widespread phenomenon that has been observed in prokaryotes and eukaryotes. In this study, the effect of pretreatment of the fungal cells of *Humicola lutea* 103 with copper salts on growth and antioxidant defense was investigated.

The changes of biomass production, protein carbonyl content, synthesis of reserve carbohydrates and antioxidant enzyme activities in the experiments with pretreatment of *H. lutea* cells in comparison to the stress conditions were analyzed. Pretreatment with 70  $\mu\text{g/ml}$   $\text{Cu}^{2+}$  resulted in enhanced resistance of the conidiospores and mycelia taken from exponential growth phase to higher doses of the heavy metal. Adaptive cell response

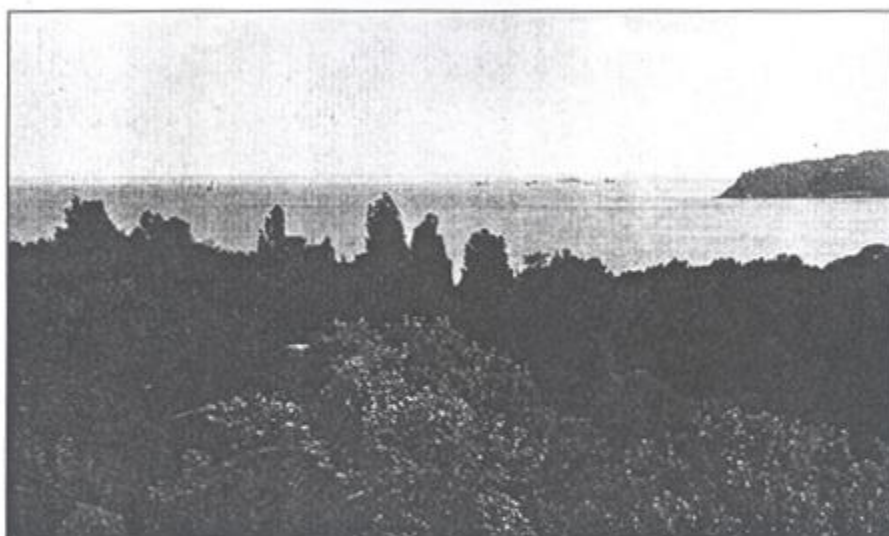
3. R. Abrashev, A. Dolashki, S. Stevanovic, P. Dolashka, W. Voelter, L. Stefanova, S. Pashova, R. Hristova, M. Angelova, Cellular response and antioxidants enzymes in *Aspergillus niger* strain against temperature stress. GAM, Eleventh Congress of the Bulgarian Microbiologists with International Participation International House of Scientists Frederic Joliot-Curie St. Constantine, 5-7 October 2006, Varna, Bulgaria.



## **ELEVENTH CONGRESS OF THE BULGARIAN MICROBIOLOGISTS**

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### **PROGRAM AND ABSTRACTS**



**St. Constantine, Varna, October 5-7, 2006**

with SOCS from other eukaryotic sources.

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4. **R. Toshkova, E. Ivanova, M.-D. Nastke, L. Velkova, S. Stevanovic, R. Hristova, A. Dolashki, M. Gardeva, I. Dimitrov, W. Voelter, P. Dolashka-Angelova. Hemocyanins as immunostimulators. III16, Eleventh Congress of the Bulgarian Microbiologists with International Participation International House of Scientists Frederic Joliot-Curie St. Constantine, 5-7 October 2006, Varna, Bulgaria.**

III16

## HEMOCYANINS AS IMMUNOSTIMULATORS

Reneta Toshkova<sup>1</sup>, Emilia Ivanova<sup>2</sup>, Maria-Dorothea Nastke<sup>3</sup>, Lyudmila Velkova<sup>4</sup>, Stefan Stevanovic<sup>3</sup>, Romyana Hristova<sup>4</sup>, Alexandar Dolashki<sup>4</sup>, Maria Gardeva<sup>4</sup>, Ivan Dimitrov<sup>4</sup>, Wolfgang Voelter<sup>5</sup>, **Pavlina Dolashka-Angelova<sup>4</sup>**

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Hemocyanin from the giant keyhole limpet *Megathura crenulata* has been a subject of biomedical interest because of its remarkable immunostimulatory properties in experimental animals and man. Molluscan *Helix vulgaris* (HvH) and *Rapana venosa* (RvH), and arthropodan *Carcinus aestuarii* (CaH) hemocyanins have been studied in order to evaluate their potential biochemical and medical applications.

It was established that the serum IL-2 production was better expressed in animals immunized by HvH and CaH than with the native molecule of KLH. Increased IL-2 production in supernatants of *in vitro* cultivated lymphocytes was observed in animals immunized with native CaH and KLH. Spleen cells from the mice immunized with other hemocyanins showed negligible stimulation. It was found that CaH causes increased

5. **P. Dolashka-Angelova, L. Velkova, K. Sandra, A. Beck, A. Dolashki, B. Devreese, S. Stevanovic, J. Van Beeumen.** Complete oligosaccharide structure of *Rapana venosa* hemocyanin. P21, Sofia School of Protein Science - 2007, For Students and Young Researchers, Institut Curie & Institute of Organic Chemistry with Center of Phytochemistry-BAS, 3-5 October 2007, Sofia, Bulgaria.



BULGARIAN ACADEMY OF SCIENCES  
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## SOFIA SCHOOL OF PROTEIN SCIENCE – 2007

FOR STUDENTS AND YOUNG RESEARCHERS

Sofia, 3-5 October 2007

**Topics:** Isolation, crystallization and crystallographic study of proteins, protein thermodynamics and kinetics, physico-chemical methods to study proteins and protein-ligand complexes, proteomics, protein bioinformatics and simulation

**Invited speakers:** J.Luis Arrondo (Spain), Jozef Van Beeumen (Belgium), Jacqueline Cherfils (France), Constantin Craescu (France), Plamen Demirev (USA), András Dér (Hungary), Christine Ebel (France), Ilian Jelesarov (Switzerland), Andrey Karshikoff (Sweden), Rudolf Ladenstein (Sweden), Wei Liu (Sweden), Maria Miteva (France), Liliane Mouawad (France), Stefan Stevanovic (Germany), Stefan Szedlacsek (Rumania), Nikolay Todorov (England)

**Organizers:** Petya Christova (Bulgaria), Andrey Karshikoff (Sweden), Constantin Craescu (France)

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## Complete oligosaccharide structure of *Rapana venosa* hemocyanin

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The complete oligosaccharide structures of *Rapana venosa* hemocyanin (RvH) were studied by sequencing of glycopeptides using LC/ESI-MS, Nanoflow-ESI mass spectrometry, CE or by MALDI-TOF-MS after enzymatically liberation of the N-glycans from both structural subunits RvH1 and RvH2. The structural information of these glycans was obtained by sequencing of them by Q-Trap mass spectrometer. Mass spectrometry revealed a highly heterogeneous mixture of different glycans from RvH2 comprising at least 37 different compositions of Hex<sub>0.9</sub> HexNAc<sub>2-4</sub> Hex<sub>0.3</sub> Pent<sub>0.3</sub> Fuc<sub>0.3</sub> and the deoxyhexose and pentose residues. Oligosaccharides with approximately the same structures were identified in structural subunit RvH1. Also novel types of N-glycans with a internal Fuc connecting one GalNAc(β1-2) and one hexuronic acid was confirmed in both structural subunits RvH1 and RvH2.

## THERMODYNAMIC ANALYSIS AND MOLECULAR MODELING OF RAPANA VENOSA HEMOCYANIN – FUNCTIONAL UNIT RVH2-E

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**Keyword:** *Rapana venosa Hemocyanin functional unit RvH2-e, Circular dichroism spectra, Reversible denaturation, Thermodynamic characteristics, 3D- structure*

pH-T diagram is typical "phase portrait" for stability of functional unit RvH2-e. Using different techniques the T-transition curves at different pH for RvH2-e were analyzed and the parameters of the thermodynamic functions were obtained. Increasing temperature and within the T range 25-55°C the reversibility increases and "opens a reversibility window" within the range of pH 5.5-9.0, for which were calculate at standard temperature the thermodynamic functions  $\Delta H_0$  and  $\Delta G_{0exp}$ .

Molecular modeling of correct 3D structure of functional unit RvH2-e was done which allows us to fix most probably position of missing 9 residues now presented in existed x-ray model at very poor resolution of 3.30Å.



6. **A. Dolashki, A. Gushterova, B. Tchorbanov, W. Voelter, P. Dolashka-Angelova. O-Diphenoloxidase activity of copper-containing proteins. P-75. VIII Научна конференция по химия, 18-19 юни 2010г., гр. Копривщица, България.**

8<sup>th</sup> Chemistry Conference      Koprivshtitsa 18-19 June 2010

P-75

### O-DIPHENOLOXIDASE ACTIVITY OF COPPER-CONTAINING PROTEINS

A. Dolashki<sup>1</sup>, A. Gushterova<sup>2</sup>, B. Tchorbanov<sup>1</sup>, W. Voelter<sup>3</sup> and P. Dolashka<sup>1</sup>

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Hemocyanins and tyrosinases are Type 3 copper-containing proteins with similar active sites, but they differ largely with respect to size, structure and their physiological functions. While hemocyanins transport dioxygen, tyrosinases uses one oxygen of the dioxygen molecule for chemical transformations.

Hemocyanins are large multisubunit copper proteins composed of different subunit types and found freely-dissolved in the hemolymph of arthropods and molluscs. Tyrosinases are nearly ubiquitously distributed in nature and are essential for pigmentation, important factors in wound healing and primary immune response.

The enzyme tyrosinase was purified from bacterium *Laceyella sacchari* using Servacell DEAE 52 resin and SEC Sephacryl S-100 column. The molecular mass of the purified enzyme was determined by MALDI mass spectrometry to be 30 025 Da which corresponds to the obtained results from SDS-PAGE.

*o*-Diphenoloxidase activities (*o*-diPO) of functional unit RvH1-a of molluscan hemocyanin *Rapana venosa* and tyrosinase from bacterium *Laceyella sacchari*, were studied using L-Dopa and Dopamine as substrates. With L-DOPA as substrate the native RvH1-a did not show any *o*-diPO activity. The native FU RvH1-a was converted to enzymatic active form, after treatment with SDS, trypsin, urea and different values of pH and its *o*-diPO activity was studied. The highest artificial induction of *o*-diPO activity was observed after incubation with 2.9 mM SDS, and RvH1-a shows both, L-DOPA ( $K_m=6.53$  mM,  $K_{cat}=8.48$ ) and Dopamine ( $K_m= 2.0$  mM,  $K_{cat}= 4.2$ ) activity due to a more open active sites of the enzyme and better access of the substrates. The  $K_m$  value of RvH1-a is very close to those of *Helix pomatia* and *Sepia officinalis* Hcs, but several times higher compared to those of Octopus Hcs. Also RvH1-a has a very high enzyme activity compared with other molluscan Hcs.

Using the diphenol L-DOPA and Dopamine as substrates, the kinetic parameters of tyrosinase for both substrates,  $K_m = 7.8$  mM and 0.5 mM and  $k_{cat}/K_m = 157$  mM<sup>-1</sup> s<sup>-1</sup> and 23 mM<sup>-1</sup> s<sup>-1</sup>, respectively, were determined.

**Acknowledgement:** The work is financially supported by research grants of the Bulgarian National Science Fund TK01-496/2009 and POSTDOC-06.

7. L. Velkova, A. Dolashki, S. Shishkov, P. Dolashka-Angelova. Complete oligosaccharide structure of *Rapana venosa* hemocyanin. P-77. VIII Научна конференция по химия, 18-19 юни 2010 г., гр. Копривщица, България.

8<sup>th</sup> Chemistry Conference Koprivshitsa 18-19 June 2010

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## COMPLETE OLIGOSACCHARIDE STRUCTURE OF *RAPANA VENOSA* HEMOCYANIN

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Hemocyanins act as oxygen-transporting glycoproteins in many arthropod and mollusc species. There are large differences in their molecular masses, structure and monosaccharide composition.

Molluscan hemocyanins (Hcs) have recently received particular interest due to their significant immunostimulatory properties. This is mainly related to their high carbohydrate content and highly specific monosaccharide composition. Now we analysed the oligosaccharides and the carbohydrate linkage sites of the *Rapana venosa* hemocyanin (RvH).

The oligosaccharide structures of the structural subunits RvH1 and RvH2 of *R. venosa* hemocyanin (RvH) were studied by sequence analysis of glycans using MALDI-TOF-MS and tandem mass spectrometry on a Q-Trap mass spectrometer after enzymatical liberation of the N-glycans from the polypeptides. Our study revealed a highly heterogeneous mixture of glycans of the compositions Hex<sub>0-9</sub> HexNAc<sub>2-4</sub> Hex<sub>0-3</sub> Pent<sub>0-3</sub> Fuc<sub>0-3</sub>. A novel type of N-glycan, with an internal fucose residue connecting one GalNAc(β1-2) and one hexuronic acid, was detected, as also occurs in subunit RvH1. A glycan with the same structure but with two deoxyhexose residues was observed as a double charged ion. Several site-occupancies were subsequently elucidated by precursor ion scanning of the intact glycopeptides using the Q-trap-LC/MS and Nanoflow-ESI mass spectrometry.

Following reports on antiviral properties from arthropod hemocyanin, we conducted a study of antiviral effects of the native molecules of RvH and of *Helix lukorum* hemocyanin (HlH), of their structural subunits, and of the glycosylated functional unit (Fu) RvH2-e and the non-glycosylated unit RvH2-c on HSV virus type 1. Only glycosylated Fu RvH2-e exhibits this antiviral activity. The carbohydrate chains of the Fu are likely to interact with specific regions of glycoproteins of HSV, through van der Waals interactions in general or with certain amino acid residues in particular. Several clusters of these residues can be identified on the surface of RvH2-e.

**Acknowledgement:** This work was supported by a research grant by the Bulgarian National Science Fund TK01-496/2009 and UV-L-301, DAAD -17/2007 and DFG-01/2008 (Germany) and the Bulgaria Academy of Sciences (BAS).



8. V. Moshtanska, V. Borisova, A. Dolashki, T. Dimanov, W. Voelter, P. Dolashka-Angelova. Purification, characterization and molecular identification of antimicrobial peptides from the hemolymph of marine snail *Rapana venosa*. P-78. VIII Научна конференция по химия, 18-19 юни 2010 г., гр. Копривщица, България.

8<sup>th</sup> Chemistry Conference      Koprivshtitsa 18-19 June 2010

P-78

**PURIFICATION, CHARACTERISATION AND MOLECULAR IDENTIFICATION OF ANTIMICROBIAL PEPTIDES FROM THE HEMOLYMPH OF MARINE SNAIL *RAPANA VENOSA***

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In the last two decades, antimicrobial peptides (AMPs) have been gaining attention as antimicrobial alternatives to chemical food preservatives and commonly used antibiotics. Small antimicrobial peptides act by disrupting the structure or function of microbial cell membranes. In this study, for the first time we have explored the isolation, identification and characterisation of a novel antimicrobial peptide (AMP) produced by the hemolymph of mollusks. Hemolymph of *Rapana venosa* snails is a complex mixture of biochemically and pharmacologically active components such as peptides and proteins. Several peptides were identified from the hemolymph by ultrafiltration and reverse-phase high-performance liquid chromatography (RP-HPLC). Mass spectrometry showed the precise molecular weight of the peptides between 3000 and 9500 Da. Their isoelectric points were acidic. Edman degradation identified the sequence of about 29 amino acids of their N-terminal which matched no peptide in the MASCOT search database, indicating novel molluscan peptides.

UV spectra revealed that these substances possessed the characteristics of protein peptides. Four of them showed strong antimicrobial activities against tested microorganisms including Gram-positive/negative bacteria.

**Acknowledgement:** This work was supported by a research grant by the Bulgarian National Science Fund TK01-496/2009 and UV-L-301, DAAD -17/2007 and DFG-01/2008 (Germany) and the Bulgaria Academy of Sciences (BAS).

9. P. Dolashka, L. Velkova, A. Dolashki, F. Zal, L. Francois, S. Stevanovic, W. Voelter, L. Molin, P. Traldi, B. Salvato. Oligosaccharide structures of hemocyanins determined by mass spectrometry. Lecture, W2. 29th Informal Meeting on Mass Spectrometry, 15-19 May 2011, Fiera di Primiero, Italy.

29<sup>th</sup> IMMS

## W2 OLIGOSACCHARIDE STRUCTURES OF HEMOCYANINS DETERMINED BY MASS SPECTROMETRY

*Pavlina Dolashka<sup>(1)</sup>, Lyudmila Velkova<sup>(1)</sup>, Aleksander Dolashki<sup>(1)</sup>, Franck Zal<sup>(2)</sup>, Lallier François<sup>(2)</sup>, Stefan Stevanovic<sup>(3)</sup>, Wolfgang Voelter<sup>(3)</sup>, Laura Molin<sup>(4)</sup>, Pietro Traldi<sup>(5)</sup>, Benedetto Salvato<sup>(5)</sup>*

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Molluscan hemocyanins (Hcs) are glycosylated proteins with high molecular mass and carbohydrate content about 7-9%. Molecular masses of the native and dissociated molecules of molluscan *Octopus vulgaris* (OvH), *Sepia officinalis* (SoH), *Rapana venosa* (RvH) and *Helix lucorum* (HlH), as well as arthropodan *Curcuma aestuarii* (CaH) hemocyanins were determined to be in the region of 4000 to 9000 kDa by Electrospray ionisation mass spectrometry (ESI-MS), Matrix assistant laser light spectrometry (MALLS) and Matrix assistant laser desorption mass spectrometry (MALDI-MS) analyses. The differences of the masses measured by mass spectrometry and calculated by their gene sequences represent the carbohydrate content of Hcs.

Two approaches were applied and the oligosaccharide structures of several Hcs were proposed based on both, sequence analysis of the glycopeptides and the glycans by MALDI-TOF-MS, CE-MS  $\mu$ LC/ESI-MS/MS and LC-MS-Q-Trap.

The oligosaccharide structures of the glycans were studied by preliminary sequence analysis by MALDI-TOF-MS before and after treatment with the specific exoglycosidases  $\beta$ 1-3,4,6-galactosidase and  $\alpha$ 1-6(>2,3,4) fucosidase, followed by sequence analysis of Q-trap/MS/MS spectra. These analyses revealed specific differences in the oligosaccharide moieties of molluscan RvH (hexuronic acid and internal fucose), HlH (methyl-galactose), *Haliothis tuberculata* (methyl-hexosa and internal fucose), KLH (1,3 galactose linked to 1,4 GlcNAc) as well as high mannose, fucose and xylose residues. In fact, the presence of hexuronic acid residue, with a neighboring internal fucose to which also an N-acetylhexosamine is linked, stand for a novel N-glycan motif.

## 29<sup>th</sup> INFORMAL MEETING ON MASS SPECTROMETRY

organized by:

Consiglio Nazionale delle Ricerche  
Istituto di Scienze e Tecnologie Molecolari  
Sezione di Padova

and

Mass Spectrometry Department of Chemical  
Research Center, Hungarian Academy of Sciences

Under the auspices of:

Divisione di Spettrometria di Massa - Società Chimica Italiana

15<sup>th</sup> - 19<sup>th</sup> May 2011  
Auditorium Intercomunale di Primiero  
Fiera di Primiero (TN) - ITALY

SC EDITORIALE  
VIA PADOVA, 12



10. L. Velkova, L. Nikolaeva-Glomb, L. Mukova, A. Dolashki, P. Dolashka, A. Galabov. Antiviral effect of molluscan haemocyanines. *Antiviral Research*, 90 (2), p.A47, 24<sup>th</sup> ICAR, Sofia, Bulgaria, 8 -11 May 2011.

### Antiviral Effect of Molluscan Haemocyanines

Lyudmila Velkova<sup>1,\*</sup>, Lubomira Nikolaeva-Glomb<sup>2</sup>, Lucia Mukova<sup>2</sup>, Aleksander Dolashki<sup>1</sup>, Pavlina Dolashka<sup>1</sup>, Angel S. Galabov<sup>2</sup>

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Hemocyanins (Hcs) are oxygen-binding glycoproteins, freely dissolved in the hemolymph, of many arthropods and mollusks. The structure and oligosaccharide moieties of the molluscan Hcs *Rapana venosa* and *Helix lucorum* have been determined and recently received particular interest due to their immunostimulatory properties. Hemocyanins also have been found to show antiviral activity. In the present study the antiviral effect is tested against the *in vitro* replication of human respiratory syncytial virus (hRSV) and influenza virus A/Aichi/2/68/H3N2 by the CPE-inhibition assay. The complete molecules of Hcs do not show antiviral effect. But a marked antiviral activity of the structural subunits and the functional units is found against the replication of hRSV. Their effect

- 11 A. Dolashki, W. Voelter, B. Tchorbanov, P. Dolashka-Angelova. Conversion of hemocyanin from *Rapana* into enzyme. P13. XVI International conference on oxygen binding and sensing proteins, 22-26 August, 2010, Antwerp, Belgium.



XVI<sup>th</sup> International conference on oxygen  
binding and sensing proteins  
22-26 August 2010, Anwerp

P13: Conversion of hemocyanin from *Rapana* into the enzyme

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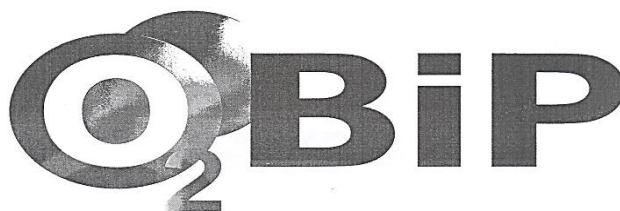
<sup>2</sup> Interfacultary Institute of Biochemistry, University of Tübingen, Tübingen, Germany

**Abstract**

*o*-Diphenoloxidase activities (*o*-diPO) of functional unit RvH1-a of molluscan hemocyanin *Rapana venosa* and tyrosinase from bacterium *Lacceyela sacchari*, were studied using L-Dopa and Dopamine as substrates. With L-DOPA as substrate the native RvH1-a did not show any *o*-diPO activity. Therefore the native FU RvH1-a was converted to enzymatic active form, after treatment with SDS, trypsin, urea and different values of pH and its *o*-diPO activity was studied. The highest artificial induction of *o*-diPO activity was observed after incubation of RvH1-a with 3.0 mM SDS, and FU shows both, L-DOPA ( $K_m=2.0$  mM,  $=102.1$ ) and Dopamine ( $K_m= 6.53$  mM,  $k_{cat}/K_m = 53.3$ ) activity due to a more open active sites of the enzyme and better access of the substrates. The  $k_{cat}/K_m$  value of activated RvH1-a is higher compared to the native FU and those of *Helix vulgaris* and *Octopus vulgaris* Hcs, but several times lower compared to those of tyrosinase from bacterium *Lacceyela sacchari* ( $k_{cat}/K_m = 157$  mM<sup>-1</sup> s<sup>-1</sup>).



12. V. Moshtanska, P. Dolashka, V. Borisova, A. Dolashki, S. Stevanovic, T. Dimanov, W. Voelter. Mass spectrometric analyses of antimicrobial peptides isolated from hemolymph of molluscs. 29th Informal Meeting on Mass Spectrometry, 15-19 May 2011, Fiera di Primiero, Italy.



XVI<sup>th</sup> International conference on oxygen  
binding and sensing proteins  
22-26 August 2010, Anwerp

**P12: Antimicrobial peptides from the hemolymph of molluscs**

Vesela Moshtanska<sup>1</sup>, Velika Borisova<sup>2</sup>, Aleksander Dolashki<sup>1</sup>, Stefan Stevanovic<sup>3</sup>, Zvetan Dimanov<sup>2</sup>, Wolfgang Voelter<sup>4</sup>, Pavlina Dolashka-Angelova<sup>1&</sup>

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**Abstract**

In the last two decades, antimicrobial peptides (AMPs) have been gaining attention as antimicrobial alternatives to chemical food preservatives and commonly used antibiotics. For the first time we have explored the isolation, identification and characterisation of a novel antimicrobial peptides (AMPs) produced by the hemolymph of mollusc *Rapana venosa* and *Helix aspersa* snails. Several peptides were identified from the hemolymph of these snails by ultrafiltration and reverse-phase high-performance liquid chromatography (RP-HPLC). Their molecular masses were determined by MALDI-TOF to be between 3000 and 9500 Da. Edman degradation identified 2 or 3 prolin residues in their N-terminal sequences which matched no peptide in the MASCOT search database, indicating novel molluscan peptides.

Seven from eleven peptides isolated from the hemolymph of *Rapana venosa* exhibited antimicrobial activity against two bacterial strains, one Gram-positive (*Staphylococcus aureus*) and one Gram-negative (*Klebsiella pneumoniae*). Antimicrobial activity against the same bacterial strains showed only two peptides from the hemolymph of *Helix aspersa*.

13. V. Moshtanska, P. Dolashka, V. Borisova, A. Dolashki, S. Stevanovic, T. Dimanov, W. Voelter.  
Mass spectrometric analyses of antimicrobial peptides isolated from haemolymph of molluscs.  
P26. 29-th Informal Meeting on Mass Spectrometry, 15-19 May 2011, Fiera di Primiero, Italy

P62

MASS SPECTROMETRIC ANALYSIS OF ANTIMICROBIAL  
PEPTIDES ISOLATED FROM HEMOLYMPH OF MOLLUSCS

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Hemolymph of molluscan snails is a complex mixture of biochemically and pharmacologically-active components such as peptides and proteins. Antimicrobial peptides are gaining attention as antimicrobial alternatives to chemical food preservatives and commonly used antibiotics. Therefore, for the first time we have explored the isolation, identification and characterisation a novel antimicrobial peptides produced by the hemolymph of *Rapana venosa* snail from Black sea and the garden snail *Helix lucorum*. Several peptides with acidic isoelectric points were isolated from the hemolymph by ultrafiltration and reverse-phase high-performance liquid chromatography (RP-HPLC) and their N-terminal sequences identified by Edman degradation matched no peptides in the MASCOT search database. Mass spectrometric analysis indicated novel proline-rich peptides with molecular weight between 3000 and 9500 Da. Their UV spectra and orcinol/sulfuric test revealed that these substances possessed the characteristics of peptides and glycopeptides. Several of the Pro-rich peptides also showed strong antimicrobial activities against tested microorganisms including Gram-positive and Gram-negative bacteria.

14. P. Dolashka, F. Zal, A. Dolashki, L. Molin, P. Traldi, B. Salvato. ESI-MS and MALLS analysis of quaternary structure of molluscan and arthropodan hemocyanins. XVII International meeting "Oxygen binding and sensing proteins". 29th August - 1st September 2012, Parma, Italy.



## ESI-MS and MALLS analysis of quaternary structure of molluscan and arthropodan hemocyanins

Pavlina Dolashka<sup>1</sup>, Franck Zal<sup>2</sup>, Alexandar Dolashki<sup>1</sup>, Laura Molin<sup>3</sup>, Pietro Traldi<sup>3</sup> and Benedetto Salvato<sup>4</sup>

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Understanding the function of macromolecular complexes is related to a precise knowledge of their structure. Recently, the development of suitable mass spectrometric techniques (ESI and MALDI) and multi-angle laser light scattering (MALLS) has enabled absolute mass determination of the native complex and of their subunits. Therefore, using these techniques the structure and association/dissociation behavior of huge molecules of molluscan *O. vulgaris*, *S. officinalis* and *R. venosa*, as well as arthropodan *C. aestuarii* Hcs were characterized. Molecular masses of the native and dissociated molecule of cephalopodan Hcs *O. vulgaris* (3 545 and 359.3 kDa, respectively) and *S. officinalis* (4 134 and 443.8, respectively) revealed that only one type subunit organize their molecules, while two isoforms with different masses (422.8 and 400.0 kDa) were measured for gastropodan *R. venosa* Hc, aggregated into didecamers. The difference of their structural subunits also was established after limited proteolysis with TPCK-trypsin. Eight FUs with masses of ~50 kDa were isolated from both subunits of RvH and isoform of SoH, while seven FUs were purified from OvH. Further characterization of proteins by ESI-MS and MALDI-MS gave insight into post-translational modification such as glycosylation. Glycosylation of *O. vulgaris* and *S. officinalis* was suggested based on the differences (11.6 and 40.0, respectively) between the masses measured by ESI-MS and those calculated by their gene sequences.

### Acknowledgments

This work was supported by a research grant by the Bulgarian National Science Fund № BG051PO001-3.3-05/0001



15. L. Velkova, P. Dolashka, A. Dolashki, S. Stevanovic, W. Voelter, J. Van Beeumen, B. Devreese. Analysis of glycoprotein from hemolymph of *Helix lucorum* garden snail. 33rd European Peptide Symposium, 31.08-05.09.2014, Sofia, Bulgaria.

## Posters P162-170: Peptide Biochemistry & Ribosomal Peptide Synthesis

**P162.**

### EXPRESSED PROTEIN LIGATION AS A MODIFICATION TOOL FOR A NOVEL NON-ANIMAL COLLAGEN-LIKE PROTEIN

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Collagen is the most abundant structural protein as substantial part of the extracellular matrix. The protein is built of three polypeptides which super-coil to form a triple-helix. These triple-helices form bundles and fibres to form larger tissue structures.[1]

Collagen is used in a variety of medical products, but concerns about possible transfer of diseases due to extraction from animal sources call for cost-effective production of recombinant collagen.[2]An alternative approach makes use of stable collagen-like triple-helical sequences, derived from various bacteria which are disease-free and their production by fermentation provides high yields. The bacterial collagen VCL (V: globular domain, CL: collagen-like domain) examined in this work has been fermented giving yields up to 19 g/L and being biologically inert which makes it a versatile template for further modifications.[3,4]

In the current project, expressed protein ligation (EPL) is used to modify VCL to introduce new and improved functions that will enhance the properties of this collagen-like protein. EPL allows the ligation via a native amide bond which is not limited to peptide sequences but also provides the opportunity to incorporate unnatural amino acids such as  $\beta$ - or D-amino acids, a single fluorescent label or a functional side chain which are not accessible by classic recombinant protein expression. Structural activity of collagen as material can be studied more extensively and information can be

collected about how the material properties change when modified.

**Key words:** expressed protein ligation, collagen, cell-biomaterial interaction, tissue engineering

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**P163.**

### ANALYSIS OF GLYCOPROTEIN FROM HEMOLYMPH OF *HELI*X *LUCORUM* GARDEN SNAIL

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Hemolymph of garden snail *Helix lucorum* is a complex mixture of biochemically and pharmacologically active components such as proteins, peptides and glycopeptides. Hemocyanin is a copper-containing, a respiratory glycoprotein. To analyse its oligosaccharide structures, peptides and glycopeptides are isolated by reverse-phase HPLC after treatment of the hemocyanin with trypsin. The isolated fractions are analyzed by the orcinol/H<sub>2</sub>SO<sub>4</sub> test. Using the nano-LC/MS/MS-Q-Trap system allowed to determine the glycosylation sites and the carbohydrate structure. The glycopeptides,

16. M. Ivanov, E. Todorovska, P. Dolashka, A. Dolashki, M. Radkova, O. Georgiev. Cloning, characterization and phylogenetic analysis of actin coding cDNA from *Rapana venosa* 33rd European Peptide Symposium, 31.08-05.09.2014, Sofia, Bulgaria.

## Posters P162-170: Peptide Biochemistry & Ribosomal Peptide Synthesis

selectively are detected in a proteolytic mixture by oxonium marker ions at  $m/z$  163[Hex]<sup>+</sup> and 204[HexNAc]<sup>+</sup>. Some glycans, after digestion with PNGase F, are identified by tandem mass spectrometry using Q-Trap mass spectrometer. Our study revealed a high-mannose and the complex type structures.

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**P164.**

### CLONING, CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF ACTIN CODING CDNA FROM *RAPANA VENOSA*

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Actin coding cDNA (AccNo KF410817.1) named here *RvAct1* from tissue of veined rapa whelk (*Rapana venosa*) was isolated using RT-PCR, 3' and 5'RACE synthesis reactions.

After sequencing, a phylogenetic analysis of *RvAct1* was performed. Sequences from representatives of all *Mollusc* classes (*Gastropoda*, *Bivalvia*, *Cephalopoda*) and invertebrates such as *Caenorhabditis elegans* (class *Chromadorea*), *Agelena silvatica* (class *Arachnida*), *Marsupenaeus japonicus* (class *Malacostraca*) showing amino acid sequence identity of 97-99% with *R. venosa* actin were selected for phylogenetic analysis. Amongst vertebrates, six *Homo sapiens* actin isoforms with sequence identity of 93-99% with *RvAct1*, six chicken actins and one fish actin from

*Acipenser transmontanus* (class *Actinopterygii*) were used.

A common N-terminal sequence pattern in all invertebrate actins was found:

1 Met, 2 Cys, followed by a stretch of three acidic amino acids of either Glu or Asp specific for vertebrate non-muscle actins, were confirmed in (*Act1*). Compared with muscular actins, the vertebrate cytoplasmic actins are featured by 20 AK residues. The comparison between the *R. venosa* actin amino acid sequence (*Act1*) and the available molluscs and human actin AA sequences showed a total of 15 matches: 6 Ala, 10 Val, 16 Met, 17 Cys, 76 Val, 103 Val, 162 Thr, 176 Leu, 201 Thr, 225 Gln, 260 Ala, 267 Leu, 287 Val, 297 Thr, 365 Ser with the amino acid residuals diagnostic for vertebrate cytoplasmic actins.

The sequence comparison indicated that *Rapana venosa* cDNA is related to both beta actin isotype and gamma cytoplasmic isotype. Phylogenetic analysis also revealed high similarity with cDNA from different taxonomy groups and a clearly defined distinction between *Rapana venosa* (*Act1*) from other actin genes in its own taxon *Mollusca*.

**Acknowledgments:** This work was supported by №BG051PO001-3.3.06-0025 financed by the European Social Fund and Operational Programme Human Resources Development (2007 – 2013) and co-financed by Bulgarian Ministry of Education, Youth and Science, DMU 03/26, Bulgaria; DFG-STE 1819/5-1/2012, Germany and FWO - VS.025.12N, Belgium.

**P165.**

### CLONING, ISOLATION AND CHARACTERIZATION OF HEMOCYANIN CODING CDNA FROM *CANCER PAGURUS*

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17. A. Dolashki, P. Dolashka, S. Stevanovic, L. Molin, P. Traldi, L. Velkova, J. Van Beeumen, B. Devreese, W. Voelter. Antimicrobial activity of peptides isolated from the hemolymph of *Helix lucorum* snails 33rd European Peptide Symposium, 31.08-05.09.2014, Sofia.

## Posters P232-274: Antimicrobial Peptides, Glycopeptides and Lipopeptides

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### P253.

#### SYNTHETIC PEPTIDES DERIVED FROM BOVINE LACTOFERRICIN: EFFECT OF L-, D-AMINO ACIDS EXCHANGE

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Bovine Lactoferricin (LfcinB) is a peptide with demonstrated antimicrobial activity. This activity is directly related with its structure. Structure-activity studies have established two main requirements for its antimicrobial activity: a cationic charge and an amphipathic conformation [1,2].

In this study we have evaluated the effect of the replacement of L by D-amino acids, on the secondary structure peptide and its antimicrobial activity. As model, a sequence derived from LfcinB was selected (<sup>1</sup>RRWQWRMKKLG<sup>14</sup>). Designed peptides were synthesized, purified, and characterized using RP-HPLC, MALDI-TOF MS and Circular Dichroism (DC). For each peptide, the antibacterial activity against *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 was evaluated. RP-HPLC analysis showed that D-amino acids in sequence peptide, induce a variation in retention times with respect to L-amino acids analogue, indicating differences in the physicochemical behavior of the peptides. Furthermore, it was possible to identify, by DC, changes in secondary structural elements, which depends on type and position of D-amino acid. Finally, inclusion of D-amino acid residues in the peptide sequence had minor influence on the

antimicrobial activity against both studied strains (MIC~130 µM).

The results underlined the importance of secondary structural elements and suggest that an appropriate position of D-amino acid residues might be affecting the antimicrobial activity.

**Keywords:** Peptides, Bovine Lactoferricin, D-Amino Acids, Secondary Structure.

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[2] D. Chapple. *Infection and Immunity*, **66**(6), 2434 (1998)

### P254.

#### ANTIMICROBIAL ACTIVITY OF PEPTIDES ISOLATED FROM THE HEMOLYMPH OF *HELIX LUCORUM* SNAILS

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The recent appearance of a growing number of bacteria resistant to conventional antibiotics, has become a serious medical problem. To overcome this resistance, the development of new compounds is encouraged. Hemolymph of *Helix lucorum* garden snail is a complex mixture of biochemically and pharmacologically active

18. P. Dolashka, A. Dolashki, L. Velkova, S. Stevanovic, L. Molin, P. Traldi R. Velikova, W. Voelter. Bioactive compounds isolated from garden snails. Втора Национална младежка конференция „Биологически науки за по-добро бъдеще“ 30-31.10.2015. Пловдив.

„БИОЛОГИЧЕСКИ НАУКИ ЗА ПО-ДОБРО БЪДЕЩЕ“, 2015 г.



Секция: Биотехнологии, Бионанотехнологии и Приложна биология



### *Bioactive Compounds Isolated from Garden Snails*

Dolashka P.<sup>1\*</sup>, Dolashki A.<sup>1</sup>, Velkova L.<sup>1</sup>, Stevanovic S.<sup>2</sup>, Molin L.<sup>3</sup>, Traldi P.<sup>3</sup>, Beeumen J.<sup>4</sup>, Devreese B.<sup>4</sup>, Voelter W.<sup>5</sup>

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**Abstract.** The recent appearance of a growing number of resistant to conventional antibiotics, has become a serious medical problem. To overcome this resistance, the development of new compounds is encouraged. Hemolymph and mucus of *Helix lucorum* and *Helix aspersa* garden snails are a complex mixture of biochemically and pharmacologically active components.

Glycoprotein 'hemocyanin' and antimicrobial peptides from the hemolymph and mucus are important components of the innate immunity. Some isoforms and peptides serve as effector molecules of the defense system, providing an efficient initial effect against infectious pathogens.

The in vitro antitumor activity of *Helix hemocyanin* and its isoforms with different oligosaccharide structures was established on the bladder carcinoma permanent cell lines Cal-29, T-24. This is probably due to the specific oligosaccharide structures of hemocyanins which are exposed on the surface of the molecule.



19. P. Dolashka, A. Dolashki, L. Velkova, S. Stevanovic, L. Molin, P. Traldi R. Velikova, W. Voelter. Poster P80. Bioactive compounds isolated from garden snails. Втора Национална младежка конференция „Биологически науки за по-добро бъдеще“ 30-31.10.2015, Пловдив.

„БИОЛОГИЧЕСКИ НАУКИ ЗА ПО-ДОБРО БЪДЕЩЕ“, 2015 г.

## P80

### *Bioactive Compounds Isolated from Garden Snails*

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The in vitro antitumor activity of *Helix* hemocyanin and its isoforms with different oligosaccharide structures was established on the bladder carcinoma permanent cell lines Cal-29, T-24. This is probably due to the specific oligosaccharide structures of hemocyanins which are exposed on the surface of the molecule.



20. L. Velkova, P. Dolashka-Angelova, A. Dolashki, B. Devreese, J. Van Beeumen. Carbohydrate structures of molluscan hemocyanins from snails *Helix lucorum* and *Rapana venosa*, determined by mass spectrometry. Втора Национална младежка конференция „Биологически науки за по-добро бъдеще“ 30-31.10.2015, Пловдив.

„БИОЛОГИЧЕСКИ НАУКИ ЗА ПО-ДОБРО БЪДЕЩЕ“, 2015 г.



**Carbohydrate Structures of Molluscan Hemocyanins from Snails *Helix lucorum* and *Rapana venosa*, Determined by Mass Spectrometry**

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**Abstract.** Hemocyanins (Hcs) are copper-containing respiratory ycoproteins with quaternary structure, freely dissolved in the hemolymph of several arthropods and molluscs. The carbohydrate structure of molluscan Hcs has recently received particular interest due to their immunostimulatory properties, antitumor and antiviral activity. This is mainly related to their high carbohydrate content and specific monosaccharide composition. We have determined and analysed oligosaccharide structures of molluscan hemocyanins, isolated from the hemolymph of Bulgarian garden snails *Helix lucorum* and of marine snail *Rapana venosa*. The oligosaccharide structures of the structural subunits of *Rapana venosa* hemocyanin (RvH) and the isoform  $\beta$ c-*Helix lucorum* hemocyanin ( $\beta$ c-HIH) were studied by sequence analysis of N-glycans using MALDI-TOF-MS and tandem mass spectrometry on a Q-Trap mass system, after enzymatical liberation of the N-glycans from the polypeptides. Our study revealed a highly heterogeneous mixture of N-glycans with compositions Hex3-7HexNAc2-5MeHex0-4Pent0-1Fuc0-1 and Hex0-9HexNAc2-4MeHex0-1Pent0-2Fuc0-3, in the isoform  $\beta$ -HIH and subunits of RvH, respectively. Identified glycans of  $\beta$ -HIH have predominantly monoantennary and diantennary structure with complex type and hybrid type. As in most molluscan hemocyanins, the glycans of  $\beta$ -HIH contain mainly a terminal MeHex residue, in some cases even two, three and four. Several carbohydrate chains in the HIH we analyzed are core-fucosylated, and also possess a high degree of methylation. Identified glycans in RvH have mainly

21. L. Velkova, P. Dolashka-Angelova, A. Dolashki, B. Devreese, J. Van Beeumen. Carbohydrate structures of the  $\beta$ c-HIH structural subunit of *Helix lucorum* hemocyanin. P100. Втора Национална младежка конференция „Биологически науки за по-добро бъдеще“ 30-31.10.2015, Пловдив.



„БИОЛОГИЧЕСКИ НАУКИ ЗА ПО-ДОБРО БЪДЕЩЕ“, 2015 г.

P100

**Carbohydrate structure of the  $\beta$ c-HIH structural subunit of *Helix lucorum* hemocyanin**

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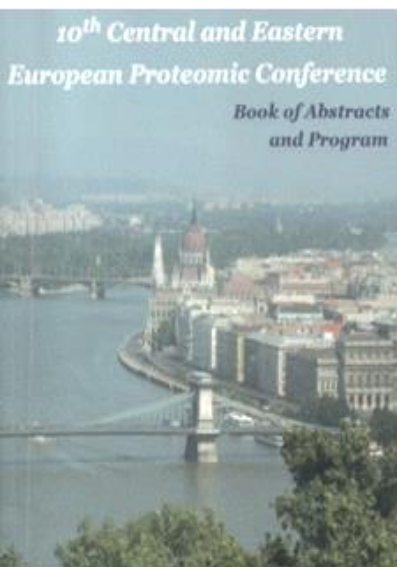
**Abstract.** Hemocyanins (Hcs) are copper-containing respiratory coproteins with quaternary structure localized in the hemolymph of several arthropods and molluscs. Molluscan Hcs usually have are powerful immunogens, probably due to their high carbohydrate content and specific monosaccharide composition. We analyzed the oligosaccharides and the carbohydrate linkage sites of the structural subunit  $\beta$ c-*Helix lucorum* hemocyanin ( $\beta$ c-HIH) isolated form hemolymph of garden snail *Helix lucorum* using tandem mass spectrometry.

Monosaccharide sequencing and determination of the configurations of N-glycans released from  $\beta$ c-HIH after PNGase F treatment was performed by Q-Trap mass spectrometry. The carbohydrate structures of  $\beta$ c-HIH contained various larger N-glycans with a number of methylated sugars, which could be identified as 3-O-methyl-mannose and 3-O-methylgalactose, in addition Fuc and/or Xyl residues were found in polysaccharides. In total 26 glycans, were identified as a highly heterogeneous mixture with compositions Hex3-7HexNAc2-5 MeHex0-4 Pent0-1Fuc 0-1. The primary structures of N-glycans from high mannose, complex- and hybrid type have been determined.

**Acknowledgements.** This work was supported by Bulgarian Ministry of Education, projects DHRC/01/6 and Youth and Science, DMU 03/26, Bulgaria and co-financed by FWO – VS.025.12N, Belgium and DFG-STE 1819/5-1/2012 – Germany



22. P. Dolashka, A. Dolashki, O. Antonowa, L. Velkova, R. Velikova, J. Van Beeumen, S. Stevanovic, W. Voelter, H.-G. Rammensee, B. Devreese. Proteomic analysis of antitumor activity of molluscan hemocyanin. 10th Central and Eastern European Proteomic Conference (11-15.10.2016) Budapest, Hungary.



Or02

10<sup>th</sup> CEEPC 2016

## PROTEOMIC ANALYSES OF ANTITUMOR ACTIVITY OF MOLLUSCAN HEMOCYANINS

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Bladder cancers are wide-spread in the world with a high morbidity rate and considerable social and economic implications. Therefore, the development of novel agents with mechanisms of action is a persisting issue. Hemocyanins, the huge oxygen-transporting glycoproteins of some mollusks, are used as immunomodulatory proteins with proven anti-cancer properties.

In our previous studies 'hemocyanins' glycoproteins, isolated from the hemolymph of molluscan brown garden snail *Helix lucorum* (HHH) and marine snail *Rapana venosa* (RvH) were analysed in comparison to the hemocyanin from keyhole limpet hemocyanin *Megatara crenulata* (KLH). The antitumoral effect of the mentioned hemocyanins and its isoforms were studied *in vitro* on the bladder cancer permanent cell HT-1197, CAL-29, T-24, and the normal urothelial cell line HL 10/29 (1). We demonstrated that the total molecule of HHH has a superior growth inhibiting effect than KLH and RvH on CAL-29 and T-24 bladder cancer cells and the mechanism of antitumor activity of the tested hemocyanins includes the induction apoptosis. Gene expression data showed upregulation of genes involved in the apoptosis as well as of the immune system activation and downregulation of genes associated with response to angiogenesis (2).

Inhibition of Cal-29 tumor cell growth was observed after incubation with native hemocyanin of *H. lucorum* hemocyanin. The effect and mechanism of action of active hemocyanins were studied at the level of their molecular organization, amino acid sequence, and carbohydrate structure using several new methods and techniques. In this study, the antitumor activities of HHH were investigated before and after treatment with hemocyanin by changes in protein expression of T-24 and CAL-29 bladder cancer cell lines. Since KLH is used increasingly in biomedicine as a carrier for vaccines and immunotherapeutic agent for bladder transitional cell carcinoma was used as a control.

Proteomic profiling of bladder carcinoma permanent cell lines T-24 and CAL-29, treated with HHH, show different protein expression by two-dimensional (2D) gel electrophoresis. 2D profiles of protein expression before treatment were compared to those after treatment with HHH and the different spots were analysed by MALDI-TOF-TOF. Mass spectrometry analysis

23. P. Dolashka, A. Dolashki, L. Velkova, S. Stevanovic, L. Molin, P. Traldi R. Velikova, W. Voelter. BO3. Bioactive compounds isolated from garden snails. XIth работна среща с международно електронно участие “Биологична активност на метали, синтетични съединения и природни продукти” 14-16.12.2016 г.

На **14-16 декември 2016** г. в Института по експериментална морфология, патология и антропология с музей при Българска академия на науките ще се проведе:

ЕДИНАДЕСЕТА РАБОТНА СРЕЩА  
с международно електронно участие

**“БИОЛОГИЧНА АКТИВНОСТ НА МЕТАЛИ,  
СИНТЕТИЧНИ СЪЕДИНЕНИЯ И  
ПРИРОДНИ ПРОДУКТИ”**



Главен организатор: Институт по експериментална морфология, патология и антропология с музей

### Bioactive compounds isolated from garden snails

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#### ABSTRACT

The recent appearance of a growing number of resistant to conventional antibiotics, has become a serious medical problem. To overcome this resistance, the development of new compounds is encouraged. Hemolymph and mucus of *Helix lucorum* and *Helix aspersa* garden snails and *Rapana venosa* marine snail are a complex mixture of biochemically and pharmacologically active components.

Glycoprotein 'hemocyanin' and antimicrobial peptides from the hemolymph and mucus are important components of the innate immunity. Some isoforms and peptides serve as effector molecules of the defense system, providing an efficient initial effect against infectious pathogens.

The *in vitro* antitumor activity of *Helix* and *Rapana* hemocyanins and their isoforms with different oligosaccharide structures was established on the bladder carcinoma permanent cell lines T-24. This is probably due to the specific oligosaccharide structures of hemocyanins which are exposed on the surface of the molecule.

**Key words:** Antibacterial activity, Antitumore activity, Hemocyanins, *Helix lucorum*, *Helix aspersa*, peptides.



24. L. Velkova, A. Dolashki, P. Dolashka. BO4. N- Linked carbohydrate structures of molluscan hemocyanins from snails. XIth работна среща с международно участие “Биологична активност на метали, синтетични съединения и природни продукти” 14-16.12.2016 г.

На **14-16 декември 2016 г.** в Института по експериментална морфология, патология и антропология с музей при Българска академия на науките ще се проведе:

ЕДИНАДЕСЕТА РАБОТНА СРЕЩА  
с международно електронно участие

**“БИОЛОГИЧНА АКТИВНОСТ НА МЕТАЛИ,  
СИНТЕТИЧНИ СЪЕДИНЕНИЯ И  
ПРИРОДНИ ПРОДУКТИ”**



Главен организатор: Институт по експериментална морфология, патология и антропология с музей

**N- Linked carbohydrate structures of molluscan hemocyanins from snails**

Lyudmila Velkova<sup>\*1</sup>, Aleksandar Dolashki<sup>1</sup>, Pavlina Dolashka<sup>1</sup>

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**Abstract**

Molluscan hemocyanins (Hcs) have recently particular interest due to their significant immunostimulatory properties. This is mainly related to their high carbohydrate content and specific monosaccharide composition. We present comparative studies in oligosaccharide structures of structural subunits from *Rapana venosa* (RvH), *Haliotis tuberculata* hemocyanin and *Helix lucorum* hemocyanin by mass spectrometry.

Two approaches were applied to analyse the isolated glycans. The first approach included sequencing of the glycans by specific glycosidases and analysis of the fragments via MALDI-TOF-MS before and after treatment with the enzymes giving only preliminary results about the structures of the glycans. Therefore, the second approach, tandem mass spectrometry was applied, and the glycan structure being derived from their MS/MS spectra, obtained by tandem mass spectrometry, on a hybrid quadrupole-linear ion trap mass spectrometer - ESI-Q-Trap system.

The characterization of the N-linked glycans found in HlH, RvH and HtH, in this study, is revealed in part novel structural motifs which might contribute to the pronounced immunogenicity of this gastropod glycoprotein. It is obvious that gastropods have a wide capacity to modify the basic biantennary N-glycan structure with many species-specific peculiar structures. The oligosaccharide moieties found in HlH, RvH and HtH are a potential source of novel N-glycans that are important for the stimulation of the immune response and/or for the production of antibodies used in diagnosis and therapy.

**Keywords:** molluscan hemocyanins (Hcs), *Rapana venosa* (RvH), *Haliotis tuberculata* hemocyanin (HtH), *Helix lucorum* hemocyanin (HlH), structural subunit; mass spectrometry; ESI-Q-Trap; N-glycans.

25. A. Dolashki, P. Dolashka, A. Stenzl, S. Stevanovic, W.K. Aicher, L. Velkova, R. Velikova, W. Voelter. Poster Proteomics analysis of antitumor activity of Helix and Rapana hemocyanins. First Balkan Conference on Personalized Medicine 26-27.10.2017, Sofia, Bulgaria.



## Proteomics analysis of antitumoractivity of Helix and Rapana hemocyanins

Dolashki A.<sup>1</sup>, Dolashka P.<sup>1</sup>, Stenzl A.<sup>2</sup>, Stevanovic S.<sup>3</sup>, Aicher W.K.<sup>2</sup>, Velkova L.<sup>1</sup>, Velikova R.<sup>1</sup> and Voelter W.<sup>5</sup>

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Hemocyanins (Hcs) are copper-containing glycoproteins that act as oxygen transporting proteins in many arthropods and mollusk species. Hemocyanins from the molluscs *Helix aspersa* (HaH), *Helix lucorum* (HIH) and *Rapana venosa* (RvH) exhibiting different oligosaccharide structures have been investigated for potential use in therapy of bladder cancer permanent cells. In vitro studies on the antitumor activities of these proteins were performed in T-24 cells and compared to doxorubicin and mitomycin-C. Control experiments were performed using normal urothelial HL 10/29 cells. The obtained results show that the human tumor T24 cell lines are sensitive to the action of the tested hemocyanins and their isoforms. The inhibition of the tumor cell growth was dose and time dependent and was observed after incubation with native HaH and HIH and FUs  $\beta$ c-HIH-h and RvH-c. Cells treated with both FUs,  $\beta$ c-HIH-h and RvH-e, showed apoptotic and necrotic cells and this inhibition was stronger than the effect measured for doxorubicin treated cells. No growth inhibition of the normal urothelial cell line HL 10/29 was observed after treatment with HIH, HaH, RvH and their isoforms. The impact of hemocyanins on tumor cells was investigated by 2D-gel PAGE and several proteins showed indeed altered abundancies. The most effective inhibition of tumor cells is probably caused by a specific novel and unusual N-glycan oligosaccharide structure on HIH with methylated hexoses, an internal fucose residue connecting one GalNAc( $\beta$ 1-2) and one hexuronic acid.



26. R. Velikova, L. Velkova, A. Dolashki, P. Dolashka. Structure and conformational stability of hemocyanin from garden snail *Cornu aspersum*. XV Национална конференция с международно участие „Природни науки 2017“ (NCNS2017) 29.09.2017 - 01.10.2017, Варна, България.



XV НАЦИОНАЛНА КОНФЕРЕНЦИЯ С МЕЖДУНАРОДНО УЧАСТИЕ

“ПРИРОДНИ НАУКИ '2017”

СЕКЦИЯ

БИОРАЗНООБРАЗИЯ, БИОМЕДИЦИНА, БИОРЕСУРСИ И ЕКОЛОГИЯ

Section

Biodiversity, Biomedicine, Bioresources and Ecology

### Structure and conformational stability of hemocyanin from garden snail *Cornu aspersum*

R. Velikova, L. Velkova, A. Dolashki, P. Dolashka

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#### **Abstract**

The hemolymph of molluscan snails is a complex mixture of biochemically and pharmacologically-active components such as peptides and proteins. The various aspects of biomedical applications of molluscan hemocyanins, associated with their immunogenic properties and antitumor activity, give major importance to structural studies of these glycoproteins. Hemocyanins (Hcs) are copper-containing respiratory glycoproteins with quaternary structure localized in the hemolymph of several arthropods and molluscs. Molluscan hemocyanins are large glycoproteins, usually have a higher carbohydrate content with different structures and quantities of the oligosaccharide moieties.

The native molecule hemocyanin CaH isolated from hemolymph of garden snail *C. aspersum* is constructed from three different subunits -  $\beta$ c-CaH,  $\alpha$ N-CaH and  $\alpha$ D-CaH (MW~ 400 kDa), each of them containing eight functional units (MW~ 50kDa).

The structural and conformational stability of the CaH,  $\beta$ c-CaH subunit and the  $\beta$ c-CaH-g functional unit were analyzed by CD in wide pH-T range ( $T^{\circ}\text{C}$  20÷85°C and pH 2.5÷12). The obtained results lead to the conclusion that:

- The mechanism of thermal denaturation of CaH is of a complicated character and the process of thermal unfolding is irreversible. The relatively small changes of initial especially  $[\theta]_{222}$  at high temperatures indicate that many secondary structure elements are preserved, especially at neutral pH and even at extreme high temperatures.
- The native CaH molecule is more stable compared to the  $\beta$ c-CaH subunit and  $\beta$ c-CaH-g functional unit, it was explained by the formation of quaternary structures, which introduces additional factors, namely non-ionic forces (intra-subunit, hydrophobic and hydrogen-bonded networks of carbohydrate moiety interactions). The difference in the stability of the native CaH and  $\beta$ c-CaH SU indicates the influence of the quaternary structure and the difference in the stability of  $\beta$ c-CaH and  $\beta$ c-CaH-g Fu shows the influence of the tertiary structure.
- The carbohydrate structure influences the stability of the native molecule CaH and  $\beta$ c-CaH subunit, but does not affect the stability of one isolated functional unit.

**Keywords:** hemocyanin,  $\beta$ c-CaH subunit (SU),  $\beta$ c-CaH-g functional unit, circular dichroism (CD), stability, temperature and pH-dependent denaturation.

**Acknowledgments:** This work was supported by a research grant for young scientists №72-00-40-111/09.05.2017, by the Bulgarian Academy of Sciences.

27. P. Dolashka, D. Kowalewski, A. Dolashki, S. Stevanovic, P. Hristova and W. Voelter. Bioactive peptides isolated from the hemolymph of arthropodan crab *Eriphia verrucosa*. P32.7-th Bulgarian Peptide Symposium 10 – 12.06. 2016, Blagoevgrad, Bulgaria.



7<sup>th</sup> Bulgarian Peptide Symposium – 2016 with international participation is organized by the Bulgarian Peptide Society and South-West University "Neofit Rilski", Blagoevgrad, Bulgaria

The Symposium is organized under the auspices of European Peptide Society and is dedicated of the

*40<sup>th</sup> Anniversary of South-West University "Neofit Rilski"*

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**Bioactive peptides isolated from the hemolymph of  
arthropodan crab *Eriphia verrucosa***

Pavlina Dolashka, Daniel Kowalewski, Aleksander Dolashki, Stefan  
Stevanovic, Petya Hristova and Wolfgang Voelter

Hemolymph of arthropods and mollusks is a complex mixture of biochemically and pharmacologically active components. Moreover, the antimicrobial peptides from the hemolymph are important components of the innate immunity. Some isoforms and peptides serve as effector molecules of the defense system, providing an efficient initial effect against infectious pathogens. Recently, the interest to these compounds increases because the resistant to conventional antibiotics has become a serious medical problem.

Several peptides were identified in the hemolymph of arthropod crab *Eriphia verrucosa*. Their molecular weights between 600 and 2000 Da and amino acid sequences were determined by electrosprey ionisashin massspectrometry (ESI-MS). Quite a series of proline-rich peptides were determined. They show high homology with hemocyanin, arginine kinase, cytochrome c oxidase, actin, antimicrobial peptide etc.,

We tested the antimicrobial activities of peptides isolated from the hemolymph of the crab *E. verrucosa* against Gram+ and Gram- bacteria. Peptides in the hemolimph exhibited inhibition effects against *Bacillus subtilis*, *Salmonella enteritis*, *Staphylococcus epidermidis* 1093 and *Escherichia coli* 3397 and were considered to be promising candidates for the treatment of microbial infections and suppression of microbial resistance.



28. A. Dolashki, W. Voelter, P. Dolashka and B. Lieb. Intact and chemically modified functional unit-e of molluscan *Helix* hemocyanin. P 33. 7-th Bulgarian Peptide Symposium 10 – 12.06.2016, Blagoevgrad, Bulgaria.

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7<sup>th</sup> Bulgarian Peptide Symposium – 2016 with international participation is organized by the Bulgarian Peptide Society and South-West University "Neofit Rilski", Blagoevgrad, Bulgaria

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**P 33**

**Intact and chemically modified functional unit-e of molluscan *Helix* hemocyanin**

Aleksandar Dolashki<sup>a</sup>, Wolfgang Voelter, Pavlina Dolashka and Bernhard Lieb

Molluscan hemocyanins (Hcs) are a family of extracellular oxygen transport proteins with high molecular mass. We have analysed and compared the structure of functional unit "e" of hemocyanins isolated from garden snail *Helix aspersa* (HaH) and *Helix lucorum* (HlH). A partial cDNA sequence of *H. aspersa* hemocyanin encoding a protein of 415 AK was isolated and compared with a gene sequence of *H. lucorum* hemocyanin which is known. The deduced amino acid sequence shows a high degree of similarity with hemocyanin unit  $\beta$ -HlH-e of *H. lucorum* (72,5%), suggesting for a common structure. Both functional units,  $\beta$ -HaH-e and  $\beta$ -HlH-e, were isolated using ion-exchange chromatography and their *o*-Diphenol oxidase activity (*o*-diPO) was analysed using L-Dopa and dopamine as substrates. With L-Dopa as substrate the native FUs did not show any *o*-diPO activity. Therefore the native FUs were converted to enzymatic active form, after treatment with trypsin. It is known that the access to the active site is covered by the C-terminal domain in a functional unit of molluscan hemocyanin, which could explain a very low catalytic activity of molluscan *Helix* hemocyanins where the entrance to the active site is probably blocked by Leu or Phe residues, respectively. After trypsinolysis and removal of the C-terminal peptide of the FU-e of *H. aspersa* (KLIPEPSVLFVAAHEDVHVE, Mw 2229,5 Da) and *H. lucorum* (IQIIPDGAIDLHSHLLHEPTVSFVPAHDLHLK Mw = 3654,2 Da) hemocyanins a channel in Hcs is opened and the binuclear copper active site becomes accessible for phenolic substances. Two peptides were identified and analysed by MALDI-TOF\_TOF after incubation of *H. aspersa* (KLIPEPSVLFVAAHEDVHVE, Mw 2229,5 Da) and

29. R. Velikova, P. Dolashka, A. Dolashki, Tch. Vasilev, A. Pashov. Mass spectrometry analysis of immunoglobulin at different conditions. P 54. 7-th Bulgarian Peptide Symposium 10-12.06.2016, Blagoevgrad, Bulgaria.

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7<sup>th</sup> Bulgarian Peptide Symposium - 2016 with international participation is organized by the Bulgarian Peptide Society and South-West University "Neofit Rilski", Blagoevgrad, Bulgaria

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*40<sup>th</sup> Anniversary of South-West University "Neofit Rilski"*

**P 54**

### **Mass spectrometry analysis of immunoglobulin at different conditions**

Velikova R, Dolashka P, Dolashki A, Vasilev Tch, Pashov A

The immune system is capable of producing a large repertoire of antibodies that can recognize a wide variety of different molecular structures and conformations. Polyspecific antibodies represent a first line of defense against infection and regulate inflammation, properties hypothesized to rely on their ability to interact with multiple antigens.

The specific binding portion of the antibody (paratope) binds very specific to a particular part of the antigen (epitope) that led to the formation of the antibody. Through this connection immunoglobulin has its effect. The polyspecificity of a fraction of IgG antibodies, present in all healthy individuals, can be increased in vitro by transient exposure to protein destabilizing agents:

- ❖ low or high pH
- ❖ high salt concentration
- ❖ chaotropic agents

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7<sup>th</sup> Bulgarian Peptide Symposium - 2016 with international participation is organized by the Bulgarian Peptide Society and South-West University "Neofit Rilski", Blagoevgrad, Bulgaria

30. A. Dolashki, L. Velkova, P. Dolashka. Mass spectrometric analyses of bio-antioxidants: peptides and glycoproteins. "First International Conference on Bio-antioxidants" 25-29.07.2017, Sofia, Bulgaria.



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ICBA 2017

# **First International Conference on Bio-antioxidants ICBA 2017**

"Natural bio-antioxidants – as a base for new  
synthetic drugs and food additives/supplements"

## **BOOK OF ABSTRACTS**

### **Young Scientists School on Bio-antioxidants YSSBA 2017**

**25-29 June 2017**  
**Bulgarian Academy of Sciences - Presidium**  
**Sofia, BULGARIA**

<http://icba2017.orgchm.bas.bg>



## YSSBA 2017 Keynote Lectures

### Mass spectrometric analyses of bio-antioxidants: peptides and glycoproteins

Aleksander Dolashki<sup>1</sup>, Lyudmila Velkova<sup>1</sup>, Paylina Dolashka

<sup>1</sup> Institute of Organic Chemistry with Centre of Phytochemistry – BAS, Acad. G. Bontchev str. Bl 9, 1113 Sofia, BULGARIA

Several peptides and glycoproteins with antioxidante activity have excellent potential as pharmaceutical products. Inhibitory effect of the fraction, containing, on the reduction of NBT by photochemically generated superoxide anion radicals.

Tandem mass spectrometry, combines MALDI-MS/MS and Q-Trap MS/MS, was applied to analyse the structure of ten biologically active peptides isolated from *Cornu aspersum* with antioxidant activity. The molecular masses of peptides were measured by their MS spectra and the primary structures were determined by their MS/MS spectra.

Tandem mass spectrometry as a very useful method was also apply to analyse the primary structure of glycoproteins, superoxide dismutase from *K. marxianus* NBIMCC 1984 yeast (Cu/Zn-KmSOD) and from fungal strain *Hemicola lutea* 103 (Cu/Zn-HISOD). The obtained fractions after treatment of the enzyme with trypsin were separated by HPLC and their amino acid sequences were determined by MALDI-TOF-TOF.

Both SODs are glycoproteins and their carbohydrate structures were also identified by MALDI-MS/MS and Q-Trap MS/MS. After treatment of the Cu/Zn-KmSOD with PNGase F a single peak at 1257.3 [M+Na]<sup>+</sup> was detected which suggests a uniform oligosaccharide chain. The structure of this carbohydrate chain was determined by Q-Trap MS/MS.

One putative linkage site was observed in the sequence of both glycoproteins. This linkage site (-Asn-Leu/Ile-Thr-) at position 33-35 is the glycosylated and is conserved in several SODs.

**Keywords:** antimicrobial peptides, glycopeptides, glycoproteins, *Cornu aspersum*, mass spectrometry

**Acknowledgement:** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.

31. A. Nissimova, A. Dolashki, L. Velkova, P. Dolashka. Structure and antibacterial activity of glycopeptide, isolated from snail *Cornu aspersum*. "First International Conference on Bio-antioxidants" 25-29.06.2017, Sofia, Bulgaria.





## YSSBA 2017 Oral Presentations

### Structure and antibacterial activity of glycopeptide, isolated from snail *Cornu aspersum*

Alexandra Nissimova<sup>1</sup>, Aleksander Dolashki<sup>1</sup>, Lyudmila Velkova<sup>1</sup>, Pavlina Dolashka<sup>1</sup>

<sup>1</sup> Institute of Organic Chemistry with Centre of Phytochemistry – BAS, Acad. G. Bontchev str. Bl. 9, 1113 Sofia, BULGARIA, [anissimova@yahoo.com](mailto:anissimova@yahoo.com)

The world provides a rich source of peptides with antimicrobial, antiviral and antitumor activity. Glycopeptides are an expanding group of structurally complex anti Gram positive antibacterial agents, representatives of which have been used in human and veterinary medicine. Quite a series of proline-rich peptides, isolated from arthropods and molluscs, were considered to be promising candidates for the treatment of microbial infections and suppression of microbial resistance.

We now report on the primary structure and mass of a peptide isolated from the mucus of the molluscan garden snail *Cornu aspersum*, and compare its antimicrobial activities with those of other similar peptides isolated from the hemolymph of the marine snail *R. venosa* and garden snail *H. lucorum*.

The structure of a glycopeptide isolated by high performance reverse phase chromatography (HPLC) from the mucus of the garden snail *Cornu aspersum* was determined by mass spectrometric analyses. Its molecular mass of 4113 Da was measured by MALDI-TOF and the amino acid sequence of the glycopeptide KARYCGAEVTAN was determined by its MS/MS analysis.

In the test against Gram+ (*C. perfringens*, *P. acnes* и *S. aureus*) and Gram- (*E. coli*) bacteria the peptide shows an inhibition effects against *S. aureus* and *E. coli*.

**Keywords:** antimicrobial peptides, glycopeptides, *S. aureus*, *E. coli*, *Helix lucorum*, *Rapana venosa*, *Cornu aspersum*, mass spectrometry

**Acknowledgement:** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.



32. R. Velikova, L. Velkova, A. Dolashki, P. Dolashka. Structure and stability of peptides and hemocyanin from garden snail *Cornu aspersum* „First International Conference on Bio-antioxidants (ICBA2017) 25-29.07.2017, Sofia, Bulgaria.



**Structure and stability of peptides and hemocyanin from garden snail *Cornu aspersum***Radostina Velikova<sup>1</sup>, Lyudmila Velkova<sup>1</sup>, Aleksandar Dolashki<sup>1</sup> and Pavlina Dolashka<sup>1</sup><sup>1</sup>Institute for Organic Chemistry, Center for Phytochemistry, BAS, Acad. Georgi Bonchev ", bl.9, Sofia 1113, Bulgaria; [radostina.1988@abv.bg](mailto:radostina.1988@abv.bg)

The hemolymph of molluscan snails is a complex mixture of biochemically and pharmacologically-active components such as peptides and proteins with antioxidante activity.

The peptides with antioxidant activity were isolated from the extract of garden snail *C. aspersa* and the temperature and pH-dependent denaturations of one isolated peptide were determined by circular dichroism (CD). Loss of  $\alpha$ -helical structure occurred in the range of 50-85°C depending on pH. The peptide was not stable towards thermally-induced unfolding.

A copper-containing respiratory glycoprotein (hemocyanin) was also isolated from the hemolymph of garden snail *C. aspersa* (CaH). The structural and conformational stability of CaH,  $\beta$ c-CaH subunit and functional unit  $\beta$ c-CaH-g were analyzed by CD in wide pH-T range (T°C 20-85°C and pH 2-12).

The obtained results show that the native CaH is more stable compared to the  $\beta$ c-CaH subunit and  $\beta$ c-CaH-g functional unit, which was explained by the formation of quaternary structures, and non-ionic forces (intra-subunit, hydrophobic and hydrogen-bonded networks of carbohydrate moiety interactions). The difference in the stability of the native CaH and  $\beta$ c-CaH indicates the influence of the quaternary structure and the difference in the stability of  $\beta$ c-CaH and  $\beta$ c-CaH-g shows the influence of the tertiary structure.

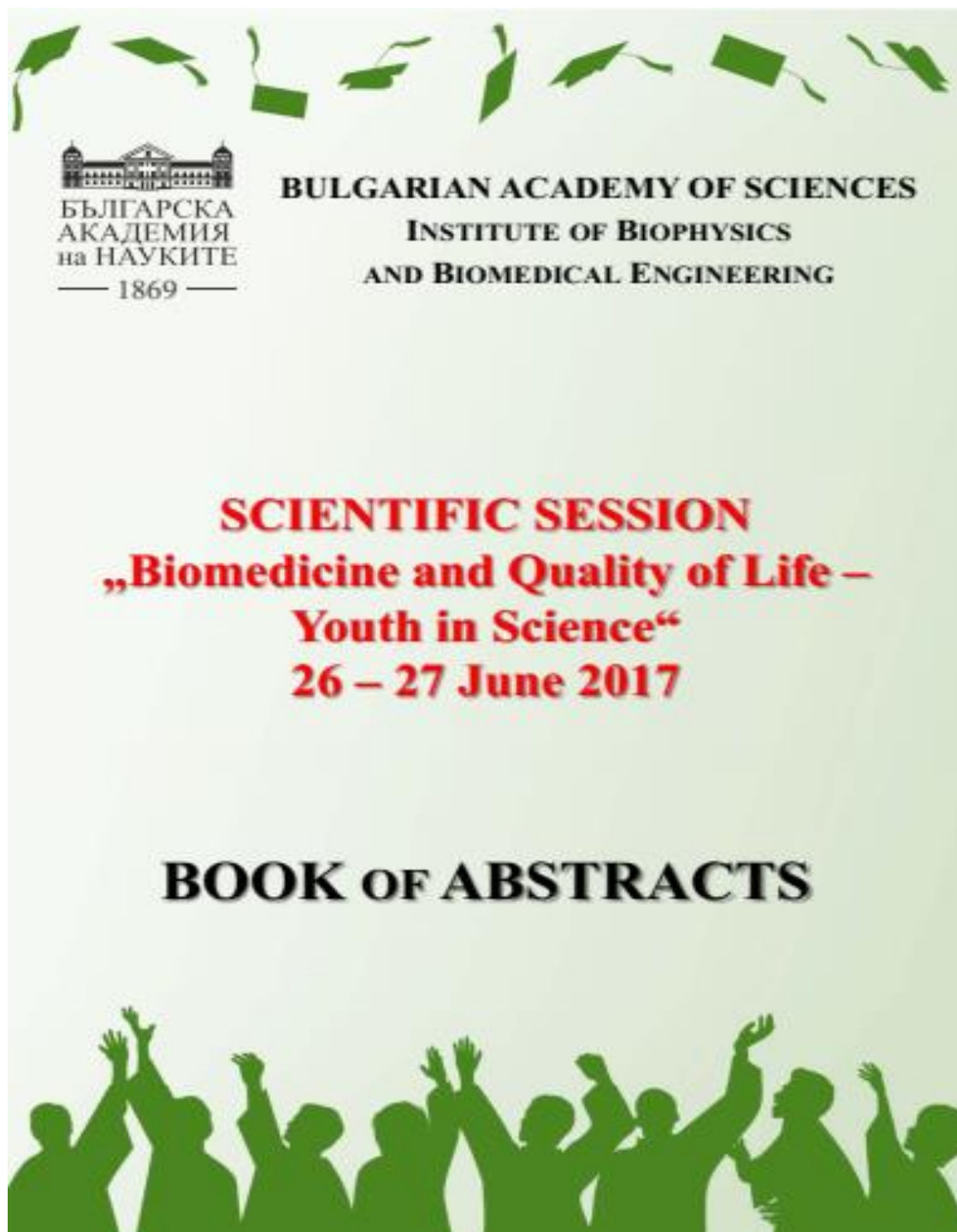
The carbohydrate structure influences the stability of the native molecule CaH and  $\beta$ c-CaH subunit, but does not affect the stability of one isolated functional unit.

**Keywords:**  $\beta$ c-CaH subunit (SU),  $\beta$ c-CaH-g functional unit (Fu), circular dichroism (CD), stability, temperature and pH-dependent denaturation.

**Acknowledgments:** This work was supported by a research grant № ДН 01/14 from 19.12.2016, to the National Science Fund of Republic Bulgaria.



33. Y. Voynikov, R. Abrashev, N. Kostadinova, E. Krumova, A. Dolashki, L. Velkova, P. Dolashka. Antioxidant Screening of Peptides Isolated from the Hemolymph of Garden Snail *Cornu aspersum*. "Biomedicine and Quality of Life" Conference 26-26.07.2017.





## Antioxidant Screening of Peptides Isolated from the Hemolymph of Garden Snail *Cornu aspersum*

**Yulian Voynikov<sup>1,2</sup>, Radoslav Abrashev<sup>3</sup>, Nedelina Kostadinova<sup>3</sup>, Ekaterina Krumova<sup>3</sup>, Aleksandar Dolashki<sup>1</sup>, Lyudmila Velkova<sup>1</sup>, Pavlina Dolashka<sup>1</sup>**

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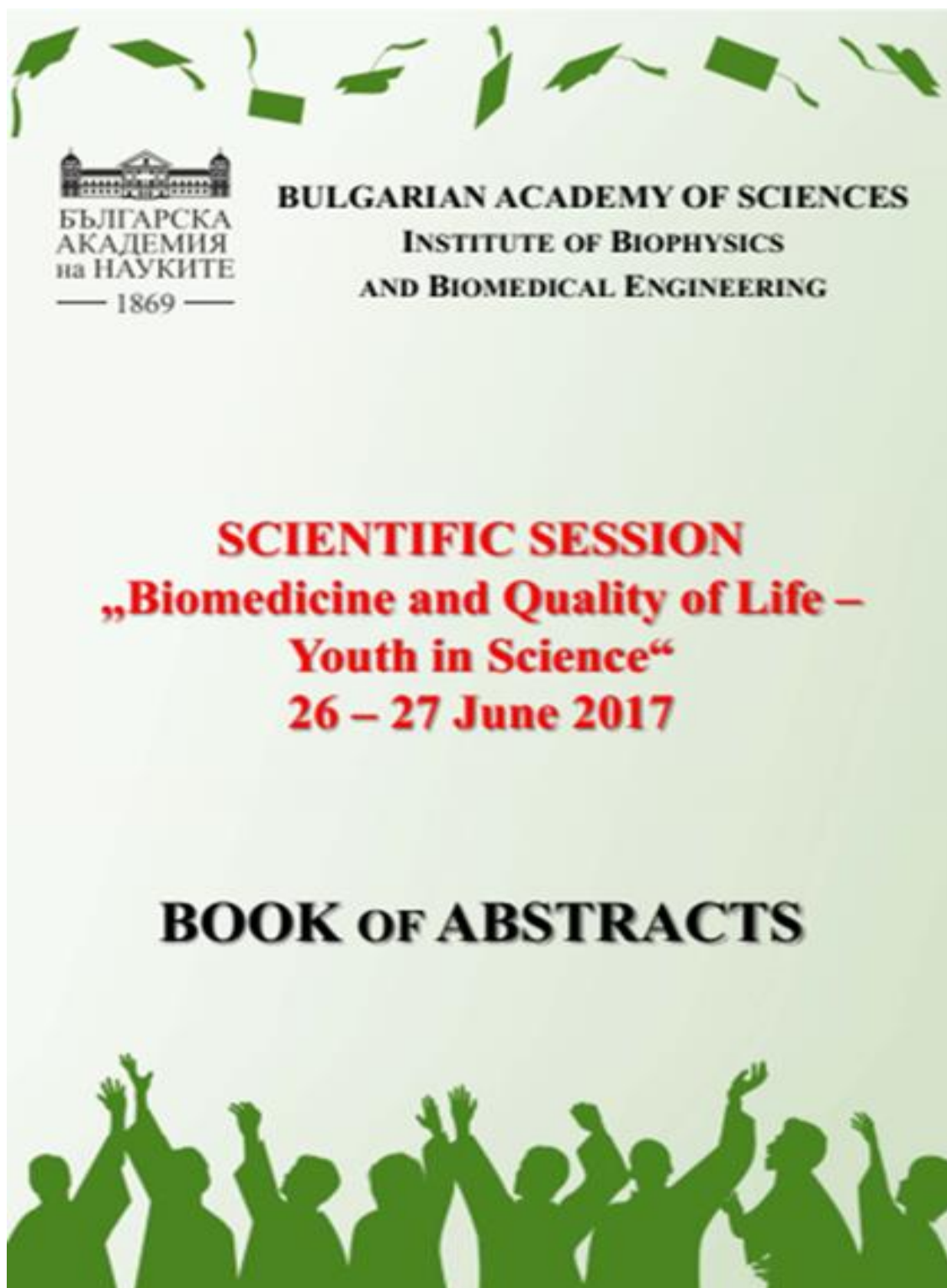
**Abstract:** Antioxidative peptides have been utilized by various species to combat pathogenic microorganisms and stress. In the present study, the antioxidant potential of protein fractions obtained from the hemolymph of the garden snail *Cornu aspersum* was evaluated. Proteins were separated from the isolated moluscan hemolymph into three fractions with masses of < 10 kDa, 10-30 kDa and > 30 kDa. Two complementary test methods were employed for the preliminary antioxidative screening, involving measurement of the radical scavenging activity on 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH), and the inhibition of *nitro blue tetrazolium* (NBT) reduction by photochemically generated O<sub>2</sub><sup>-</sup>. Caffeic acid was used as a positive control. On the DPPH assay all fractions were able to reduce the stable free radical DPPH to the yellow-colored 1,1-Diphenyl-2-picrylhydrazyl, with the peptide fraction below 10 kDa exhibiting better free radical scavenging activity than those with higher molecular weight. All tested fraction revealed comparable inhibition on the reduction of NBT by photochemically generated superoxide anion radicals, but the effect was less pronounced than that of caffeic acid.

**Keywords:** *Cornu aspersum*, Peptide, Protein, DPPH, NBT.

### Acknowledgement

*This work was supported by a research grant № DN 01/14 from 19.12.2016 of the National Science Fund of Bulgaria.*

34. A. Nissimova, A. Dolashki, L. Velkova, P. Dolashka. Antibacterial Activity of Peptides from Snail Cornu aspersum. “Biomedicine and Quality of Life” Conference 26-26.07.2017.



## Antibacterial Activity of Peptides from Snail *Cornu aspersum*

**Alexandra Nissimova, Aleksander Dolashki, Lyudmila Velkova, Pavlina Dolashka**

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**Abstract:** The emergence of multi-resistant bacteria requires the development of antibiotics with new mechanisms of action. Antimicrobial peptides are a unique and diverse group of molecules that have a great potential for use in new antimicrobial drugs, as many of them have a pronounced cytotoxicity to a number of multi-drug resistant bacteria. It is known that *Cornu aspersum* snail mucus contains a number of biologically active components. Ten biologically active peptides from *Cornu aspersum* snails contained in a fraction below 10 kDa were isolated by high pressure reverse phase chromatography (HPLC) on a Nucleosil column C18.

The molecular masses of these peptides between 1 and 5 kDa were determined by mass spectrometry analysis (MALDI-TOF). The amino acid sequences of the peptides were determined by their MS/MS spectra which indicate that they are tryptophan, tyrosyl and proline rich peptides. We also analyzed a glycopeptide with a mass of 4113 Da, whose amino acid sequence was determined by MS/MS analysis – KARYCGAEVTAN.

Some of these peptides show antibacterial activity against bacterial strains *E. coli*, *C. perfringens*, *P. acnes* and *S. aureus*.

**Keywords:** Antimicrobial peptides, *Cornu aspersum*, Mass spectrometry, *E. coli*, *C. perfringens*, *P. acnes*, *S. aureus*.

### Acknowledgements

*This work was supported by a research grant № DN 01/14 from 19.12.2016 of the National Science Fund of Bulgaria.*



35. R. Velikova, L. Velkova, A. Dolashki, P. Dolashka.. Structure and Stability of Hemocyanin and Peptides from Garden Snail *Cornu aspersum*. “Biomedicine and Quality of Life” Conference 26-26.07.2017



## Structure and Stability of Hemocyanin and Peptides from Garden Snail *Cornu aspersum*

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**Abstract:** The hemolymph of molluscan snails is a complex mixture of biochemically and pharmacologically-active components such as peptides and proteins. The structures stability, temperature and pH-dependent denaturation of one of isolated peptide was determined by CD. Loss of  $\alpha$ -helical structure occurred in the range 50-85 °C depending on pH. The peptide was not stable towards thermally-induced unfolding.

The native molecule of CaH, isolated from hemolymph of garden snail *C. aspersa*, is constructed from three different subunits –  $\beta$ c-CaH,  $\alpha$ s-CaH and  $\alpha$ d-CaH (Mw ~ 400 kDa), and each of them containing eight functional units (Mw ~ 50kDa).

The structural and conformational stability of the CaH,  $\beta$ c-CaH subunit and the  $\beta$ c-CaH-g functional unit (Fu) were analyzed by CD in wide pH-T range (T °C 20÷85 °C and pH 2÷12). To provide details on the stability of the protein, Fu  $\beta$ c-CaH-g was compared with the native molecule of CaH and the subunit  $\beta$ c-CaH via pH-T diagrams, typical phase portraits for stability and denaturation reversibility. Increasing the temperature from 25 °C to 60 °C, the reversible denaturation of the molecule of protein also increases, opening a reversibility window within the range of pH 5.5-8.0 for subunit  $\beta$ c-CaH and the range of pH 5.0-9.0 for functional unit  $\beta$ c-CaH-g and as a result of which the real thermodynamic parameters ( $\Delta C_p$ ,  $\Delta H^\circ_{ap}$ , and  $\Delta G^\circ_{ap}$ ) were determined.

The obtained results lead to the conclusion that the carbohydrate structure influences the stability of the native molecule CaH and  $\beta$ c-CaH subunit, but does not affect the stability of one isolated functional unit.

**Keywords:** Photosystem  $\beta$ c-CaH subunit (SU),  $\beta$ c-CaH-g functional unit (Fu), Circular dichroism (CD), Stability, Temperature and pH-dependent denaturation.

### Acknowledgements

*This work was supported by a research grant № DN 01/14 from 19.12.2016 of the National Science Fund of Bulgaria.*

36. P. Dolashka, A. Dolashki. Successful collaboration for more than 25 years. Bio-active peptides and glycoproteins. Bioactive Peptides and Proteins, Tübingen, Germany, 14-16.07. 2017

#### Bioactive Peptides and Proteins

Interfakultäres Institut für Biochemie der Universität Tübingen (IFIB) Hoppe-Seyler-Str. 4, im Horsaal

Tübingen, July 14, 2017

A meeting with contributions from former students of Prof. Wolfgang Voelter

8.30-9.45 Opening Remarks

Welcome in the Lecture Hall of the Interfaculty Institute of Biochemistry, University of Tübingen Prof. Dr. Ralf-Peter Jansen, Director, Interfaculty Institute for Biochemistry, University of Tübingen

Welcome Address from the University of Tübingen Prof. Dr. Karin Amos, Pro-Rector of the Univ. of Tübingen

A Tribute to a Living Legend: Wolfgang Voelter Prof. Dr. Dr. h. c. mult. Atta-ur-Rahman, former Director of International Center for Chemical and Biological Sciences,

Karachi University, Minister of Science and Technology, Islamabad, Unesco Science Laureate

From  $^{19}\text{F}$  NMR of O-trifluoroacetyl derivatives to the De-coding of Molecular Structures by  $^{13}\text{C}$  NMR

Prof. em. Dr. Eberhard Breitmaier, University of Bonn

A walking Tour from Chemistry to Biochemistry Prof. em. Dr. Dr. hc. mult. Wolfgang Voelter, Univ. of Tübingen

16.00-16.30 Prof. Dr. Pavlina Dolashka, Prof. Dr. Aleksandar Dolashki Academy of Sciences, Sofia

Successful collaboration for more than 25 years on bioactive peptides and glycoproteins

16.30-16.45 Concluding Remarks Prof. Dr. Iqbal Choudhary, Director International Center for Chemical and Biological Sciences, University of Karachi

19.00 Banquet

Hotel IBIS, Tübingen, Friedrichstr. 20, spe



37. Y. Voynikov, A. Dolashki, P. Hristova, S. Stevanovic, E. Krumova, J. Miteva-Staleva, M. Angelova, P. Dolashka. Antimicrobial activities of fractions from the mucus of garden snail *Cornu aspersum*. 10th Balkan Congress of Microbiology Microbiologia Balkanica '2017 Sofia Park Hotel Moskva, November 16th – 18th, 2017.



BALKAN SOCIETY FOR MICROBIOLOGY



# 10<sup>th</sup> BALKAN CONGRESS OF MICROBIOLOGY

MICROBIOLOGIA BALKANICA '2017



Sofia, Bulgaria  
November 16<sup>th</sup> – 18<sup>th</sup>, 2017

# ANTIMICROBIAL ACTIVITIES OF FRACTIONS FROM THE MUCUS OF GARDEN SNAIL *CORNU ASPERSUM*

Y. Voynikov<sup>1</sup>, A. Dolashki<sup>1</sup>, P. Hristova<sup>2</sup>, S. Stevanovic<sup>3</sup>, E. Krumova<sup>2</sup>, J. Miteva-Staleva<sup>2</sup>, M. Angelova<sup>2</sup>, P. Dolashka<sup>1</sup>

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**Aim.** Terrestrial slugs and snails excrete biological fluid (mucus) that has been recognized as a rich source of bioactive natural compounds and also protects them against microbial invasion. The present study aimed at evaluating the antibacterial (*Propionibacterium acnes*) and antifungal (*Aspergillus fumigatus*, *Aspergillus niger* and *Penicillium hrysogenum*) activities of extracts derived from the mucus of the garden snail *Cornu aspersum*.

**Methods.** The isolated molluscan mucus was separated into several fractions on Millipore filters (fraction with Mw <10 kDa, <20 kDa, > 30 kDa, <100 kDa and >100 kDa). They were tested for their antimicrobial and antifungal activity by the well diffusion method. The amino acid sequences of several peptides were determined by MALDI-TOF/TOF.

**Results.** The fraction having < 20 kDa displayed the highest antibacterial activity against *P. acnes* followed by that of >100 kDa. Amino acid sequencing by MALDI-TOF/TOF showed that Pro, Gly, Tyr and Trp – rich peptides are present in the fraction <20 kDa. All fractions possess fungistatic activity on the mycelium growth of *A. niger* strain with the most significant effect exerted by the fraction below 10 kDa. However, a slight activity was demonstrated by the fractions between 10-30 kDa and above 30 kDa against *P. hrysogenum* strain and *E. coli*.

**Conclusion.** Among the tested fractions, the one having < 20kDa exerted the highest antimicrobial activity. Several peptides with less than 10 amino acids, rich in Pro, Gly, Tyr and Trp, were sequenced by mass spectrometry and identified as potential cause for the exerted antimicrobial effect.

**Acknowledgement.** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.

**Keywords.** snail mucus, *Cornu aspersum*, antibacterial, antifungal

**Acknowledgments.** This work was supported by a research grant № ДН 01/14 from 19.12.2016, to the National Science Fund of Republic Bulgaria.

**Keywords.** antimicrobial activity, extract, snail *C. aspersum*,  $\beta$ c-CaH, peptides

38. L. Velkova, A. Dolashki, L. Molin, P. Traldi, Y. Voinikov, R. Velikova, W. Voelter, P. Dolashka. Antimicrobial activity of proteins and peptides isolated from extract of the garden snail *Cornu aspersum*. 10th Balkan Congress of Microbiology Microbiologia Balkanica '2017 Sofia Park Hotel Moskva, November 16th – 18th, 2017.



BALKAN SOCIETY FOR MICROBIOLOGY



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MICROBIOLOGIA BALKANICA '2017



Sofia, Bulgaria  
November 16<sup>th</sup> – 18<sup>th</sup>, 2017



## ANTIMICROBIAL ACTIVITY OF PROTEINS AND PEPTIDES ISOLATED FROM EXTRACT OF THE GARDEN SNAIL *CORNU ASPERSUM*

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<sup>3</sup>Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen

The rapid increase in drug-resistant infections has presented a serious challenge to antimicrobial therapies, because it emphasizes the urgent need to develop novel antimicrobials drugs agents. One area of interest is peptides and proteins produced by invertebrates as part of their defence system.

Extract collected from the garden snails *C. aspersum* is a complex mixture of biochemically and pharmacologically active compounds as peptides and proteins.

The structural subunits ( $\beta$ c-CaH,  $\alpha$ <sub>N</sub>-CaH and  $\alpha$ <sub>D</sub>-CaH) with molecular mass of about 420 kDa of oxygen transporter glycoprotein *C. aspersum* hemocyanin (CaH) were isolated. Surprisingly, the structural subunit  $\beta$ c-CaH not only shows strong antimicrobial activities against *S. aureus* and the likewise Gram-positive *Streptococcus epidermidis*, but also against the Gram-negative bacterium *Escherichia coli*. Furthermore, the subunit  $\beta$ c-CaH, carries immunomodulatory properties, which makes it especially interesting compound for the development of novel therapeutics.

The isolated 14 fractions from the extract (mucus and hemolymph) of *C. aspersum* after applying ultrafiltration and reverse-phase high-performance liquid chromatography (RP-HPLC), containing different peptides, have molecular weights between 600-7000 Da, determined by MALDI-TOF-MS.

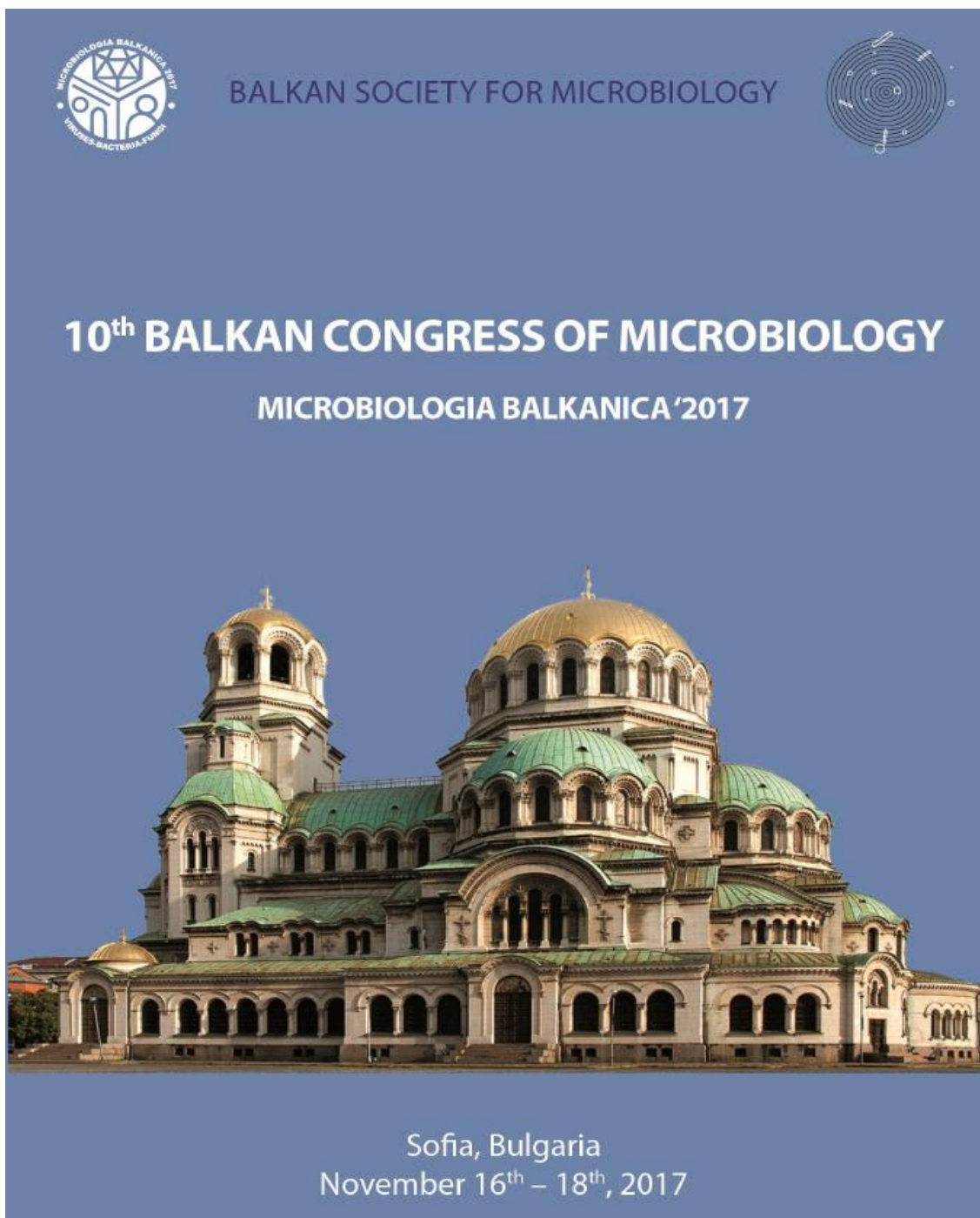
Our *in vitro* study has shown that the three fractions from the extract of *C. aspersum*, have a demonstrable antimicrobial activity against two different strains of *Propionibacterium acnes* (PA266 and KPA) and *E. coli* (3548 and 3397). Two of the fractions show strong antimicrobial activities against *S. epidermidis*, as a  $\beta$ c-CaH subunit.

We suggest that these compounds therefore have the potential to become a substitute for the commonly used antibiotics against which bacterial resistance has gradually been developed.

**Acknowledgments.** This work was supported by a research grant № ДН 01/14 from 19.12.2016, to the National Science Fund of Republic Bulgaria.

**Keywords.** antimicrobial activity, extract, snail *C. aspersum*,  $\beta$ c-CaH, peptides

39. R. Velikova, L. Velkova, A. Dolashki, P. Dolashka. AntM-39 Antimicrobial properties of molluscan hemocyanins. 10th Balkan Congress of Microbiology Microbiologia Balkanica '2017 Sofia Park Hotel Moskva November 16th – 18th, 2017, Sofia, Bulgaria.





## ANTIMICROBIAL PROPERTIES OF MOLLUSCAN HEMOCYANINS

R. Velikova, L. Velkova, A. Dolashki, P. Dolashka

Institute for Organic Chemistry, Center for Phytochemistry-Bulgarian Academy of Sciences, Sofia

Hemocyanins (Hcs) are oxygen-binding glycoproteins, freely dissolved in the hemolymph, of many arthropods and mollusks. They have been shown to exhibit antitumor, antiviral and antimicrobial activity and are therefore considered to be "natural antibiotics". Hemocyanins are a promising alternative to conventional preparations.

The native molecule and three structural subunits were isolated from the hemolymph of the garden snail *Cornu aspersum* from the farms. Physicochemical analyzes and conformational stability of hemocyanin by fluorescence spectroscopy and circular dichroism confirmed that the protein is very stable. The antibacterial activity of hemocyanin from snail *Cornu aspersum* and its isoforms were also determined *in vitro* against bacterial strains of *E. coli* and *S. aureus*, identified as pathogenic with a high risk of resistance.

Hemocyanin from *C. aspersum* and its isoforms are new biologically active natural compounds, alternatives to chemical antibiotics, potential for application and treatment of therapeutic problems. Information will be gathered to create new concepts to overcome multi-drug resistance and create new types of antimicrobials.

**Acknowledgments.** This work was supported by a research grant of Bulgarian Academy of Science № 72-00-40-111/ 09.05.2017.

**Keywords.** antibacterial activity, circular dichroism (CD,) snail *C. aspersum*



40. P. Dolashka, Y. Voynov, L. Velkova, A. Dolashki. Proteomics analysis of Alzheimer's and antitumor activity of glycoproteins against bladder carcinoma permanent cell lines. 9th International Conference and Expo on Molecular Medicine Proteomics & Bioinformatics November 13 - 15, 2017 Paris, France.

**conferenceseries.com**

Pavlina Dolashka et al., J Proteomics Bioinform 2017, 10:11(Suppl)  
DOI: 10.4172/0974-276X-C1-106

JOINT EVENT

9<sup>th</sup> International Conference and Expo on

**Proteomics and Molecular Medicine**

9<sup>th</sup> International Conference on &  
**Bioinformatics**

November 13-15, 2017 Paris, France

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### **Proteomics analysis of Alzheimer's and antitumor activity of glycoproteins against bladder carcinoma permanent cell lines**

**Pavlina Dolashka, Voynikov Y, Velkova L and Dolashki A**  
Bulgarian Academy of Sciences, Bulgaria

Alzheimer's disease (AD) is the most common form of dementia. It is the sixth leading cause of death and affects nearly 30 million people worldwide. Scopolamine and streptozotocin are widely utilized in chemically-induced dementia animal models to mimic specific pathophysiological pathways thought to underlie AD. To the best of our knowledge, there is no report describing proteome analysis on scopolamine or streptozotocin AD animal models. Therefore, we conducted a comparative proteome analysis on CSF isolated from rats with chemically-induced dementia with the purpose of identifying protein biomarkers. Rodents were divided into three groups: rats with scopolamine-induced dementia, rats with streptozotocin-induced dementia and healthy controls. Proteins and peptides were separated from the isolated CSF into four fractions. Two low molecular peptide fractions, with mass below 3 kDa and another with mass ranging from 3 to 10 kDa were analyzed by mass spectrometry, while two other protein fractions, with mass between 10 and 50 kDa and with mass higher than 50 kDa, were characterized by 2D-PAGE and the results were compared. The impact of hemocyanin on tumor cells was investigated by 2D-gel PAGE and several proteins showed indeed altered abundancies. The most effective inhibition of tumor cells is probably caused by a specific novel and unusual N-glycan oligosaccharide structure on H1H with methylated hexoses, an internal fucose residue connecting one GalNAc( $\beta$ 1-2) and one hexuronic acid.

#### **Biography**

Pavlina Dolashka and her group has wide experience in the isolation, purification and characterization of biologically active compounds. She has more than 130 publications on these topics, 3 book chapters and 6 patents. She is Editor-in-board of 3 journals and representative IUPAC. She is coordinating several international research projects, sponsored by NATO (Brussels), the European Commission, Germany (DFG and BMBF), CNR (Italy), FWO (Belgium), China and Ukraine.

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41. R. Velikova, A. Dolashki, A. Stenzl, S. Stevanovic, W.K. Aicher, J. Van Beeumen, L. Velkova, W. Voelter, P. Dolashka. Proteomics analysis of antitumor activity of Helix and Rapana hemocyanins. 9th International Conference and Expo on Molecular Medicine Proteomics & Bioinformatics November 13 - 15, 2017 Paris, France.

**conferenceseries.com**

Radostina Velikova et al., J Proteomics Bioinform 2017, 10:11(Suppl)  
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JOINT EVENT

9<sup>th</sup> International Conference and Expo on

**Proteomics and Molecular Medicine**

9<sup>th</sup> International Conference on &

**Bioinformatics**

November 13-15, 2017 Paris, France

### **Proteomics analysis of antitumor activity of Helix and Rapana hemocyanins**

Radostina Velikova<sup>1</sup>, Dolashki A<sup>1</sup>, Stenzl A<sup>2</sup>, Stevanovic S<sup>3</sup>, Aicher WK<sup>2</sup>, Jozef Van Beeumen<sup>2</sup>, Velkova L<sup>1</sup>, Voelter W<sup>2</sup>, Bart Devreese<sup>2</sup> and Dolashka P<sup>1\*</sup>

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<sup>2</sup>University of Tübingen, Germany

<sup>3</sup>Ghent University, Belgium

Hemocyanins (Hcs) are copper-containing glycoproteins that act as oxygen transporting proteins in many arthropods and mollusk species. Hemocyanins from the molluscs *Helix aspersa* (HaH), *Helix lucorum* (HIH) and *Rapana venosa* (RvH) exhibiting different oligosaccharide structures have been investigated for potential use in therapy of bladder cancer permanent cells. *In vitro* studies on the antitumor activities of these proteins were performed in T-24 cells and compared to doxorubicin and mitomycin-C. Control experiments were performed using normal urothelial HL 10/29 cells.

The obtained results show that the human tumor T24 cell lines are sensitive to the action of the tested hemocyanins and their isoforms. The inhibition of the tumor cell growth was dose and time dependent and was observed after incubation with native HaH and HIH and FUs  $\beta$ c-HIH-h and RvH-c. Cells treated with both FUs,  $\beta$ c-HIH-h and RvH-e, showed apoptotic and necrotic cells and this inhibition was stronger than the effect measured for doxorubicin treated cells. No growth inhibition of the normal urothelial cell line HL 10/29 was observed after treatment with HIH, HaH, RvH and their isoforms. The impact of hemocyanins on tumor cells was investigated by 2D-gel PAGE and several proteins showed indeed altered abundancies. The most effective inhibition of tumor cells is probably caused by a specific novel and unusual N-glycan oligosaccharide structure on HIH with methylated hexoses, an internal fucose residue connecting one GalNAc( $\beta$ 1-2) and one hexuronic acid.

### **Biography**

Radostina Velikova is currently working in the Institute for Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences.

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42. L. Velkova, A. Dolashki, Y. Voinikov, R. Velikova, E. Daskalova, Y. Topalova, P. Hristova, E. Krumova, J. Miteva-Staleva, M. Angelova, P. Dolashka. Antibacterial and antifungal activity of mucus extracts from garden snail *Cornu aspersum*. (Lecture) "Ecology and health" XVII-the National Scientific conference with international participation, 7 June 2018, Plovdiv, Bulgaria.

СДРУЖЕНИЕ С НЕСТОПАНСКА ЦЕЛ „ТЕРИТОРИАЛНА ОРГАНИЗАЦИЯ НА НАУЧНО-ТЕХНИЧЕСКИ СЪЮЗИ С ДОМ НА НАУКАТА И ТЕХНИКАТА-ПЛОВДИВ”  
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НАЦИОНАЛНО ДРУЖЕСТВО „ЕКОЛОГИЧНО ИНЖЕНЕРСТВО И ОПАЗВАНЕ НА ОКОЛНАТА СРЕДА”  
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**ЕКОЛОГИЯ И ЗДРАВЕ, 2018**  
**ECOLOGY AND HEALTH, 2018**

**ПРОГРАМА**  
**PROGRAMME**

XII-та НАЦИОНАЛНА НАУЧНО-ТЕХНИЧЕСКА  
КОНФЕРЕНЦИЯ С МЕЖДУНАРОДНО УЧАСТИЕ

*XII-th NATIONAL SCIENTIFIC  
CONFERENCE WITH INTERNATIONAL  
PARTICIPATION*

07 юни 2018 година  
Дом на науката и техниката – Пловдив, ул. „Гладстон” № 1  
7 June 2018  
*House of Science and Technique – Plovdiv, 1 Gladston Str.*



## Antibacterial and antifungal activity of mucus extracts from garden snail *Cornu aspersum* (Lecture)

Lyudmila Velkova<sup>1</sup>, Aleksandar Dolashki<sup>1</sup>, Yulian Voynikov<sup>1</sup>, Radostina Velikova<sup>1</sup>, E. Daskalova<sup>2</sup>, Yana Topalova<sup>2</sup>, Petya Hristova<sup>2</sup>, Ekaterina Krumova<sup>3</sup>, Jeni Miteva-Staleva<sup>3</sup>, Maria Angelova<sup>3</sup>, Pavlina Dolashka<sup>1</sup>

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<sup>2</sup>*Sofia University "St.Kliment Ohridski", Faculty of Biology, Bulgaria*

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### Abstract

Natural compounds have a great potential for use in new antimicrobial drugs, as some of them have a pronounced cytotoxicity to a number of multi-drug resistant bacteria. Snails produce biological fluid (mucus) which protects them against microbial invasion. The mucus of garden snail *C. aspersum* is a complex mixture of bioactive compounds with potential pharmacological application.

We have performed in vitro studies on the antimicrobial activities of different extracts, obtained from mucus of *C. aspersum*, against Gram<sup>+</sup> and Gram<sup>-</sup> bacteria and a fungal strain *Aspergillus niger*. Our preliminary results have shown that the mucus fraction below 10kDa possess strong antibacterial activity against the pathogen *E. coli*. The fraction having below 20kDa displayed the highest antibacterial activity against *Propionibacterium acnes* 266 AI, followed from fraction above 100kDa. The fraction between 10-50 kDa inhibits the strongest mycelial growth of *A. niger* than fractions below 10 kDa, between 50-100 kDa and over 100 kDa.

Using tandem mass spectrometry we identified the primary structures of many novel antimicrobial peptides in active fraction below 10kDa. Most of them contain high level of glycine and leucine residues into the amino acid sequences and belong of a class of Gly/Leu-rich AMPs, but others, contain proline, tryptophan and valine residues which are typical for peptides with antimicrobial activity. We compared of protein expression profiles of bacterial cells of strain *P. acnes* 266 AI before and after treatment with the fraction below 20kDa and found a significant change in the expression of seven proteins in bacterial cells. After analyzed by MALDI-MS, Mascot and searches across the different databases, these peptides were identified.

Our results may be considered as basic information for further investigations on bioactive compounds from *C. aspersum* and their potential biomedical applications.

**Keywords:** mucus extracts, garden snail *Cornu aspersum*, antibacterial and antifungal activity, antimicrobial peptides (AMPs), mass spectrometry.

**Acknowledgement:** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.

43. P. Dolashka, Y. Voynikov, L. Velkova, A. Dolashki, L. Tancheva, L. Alova, W. Voelter. Exothermic processes in rat brain homogenate investigated by proteomic analyses. (Lecture) "Ecology and health" XVII-the National Scientific conference with international participation, 7 June 2018, Plovdiv, Bulgaria.

СДРУЖЕНИЕ С НЕСТОПАНСКА ЦЕЛ „ТЕРИТОРИАЛНА ОРГАНИЗАЦИЯ НА НАУЧНО-ТЕХНИЧЕСКИ СЪЮЗИ С ДОМ НА НАУКАТА И ТЕХНИКАТА-ПЛОВДИВ”  
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## ЕКОЛОГИЯ И ЗДРАВЕ, 2018 ECOLOGY AND HEALTH, 2018

### ПРОГРАМА PROGRAMME

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7 June 2018  
House of Science and Technique – Plovdiv, 1 Gladston Str.

# Exothermic processes in rat brain homogenate investigated by proteomic analyses

(Lecture)

**P. Dolashka<sup>1</sup>, Y. Voynikov<sup>1</sup>, L. Velkova<sup>1</sup>, A. Dolashki<sup>1</sup>, L. Tancheva<sup>2</sup>, L. Alova<sup>2</sup>, W. Voelter<sup>3</sup>**

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## Abstract

Alzheimer's disease (AD) is now considered the most prevalent progressive neurodegenerative disorder, responsible for 75% of all dementia cases. It affects approximately 35.6 million people worldwide, mainly the elderly ones. At present time only symptomatic treatments are available. From recent data it is suggested that for the development of AD, mainly  $\beta$ -amyloid peptides and neurofibrillary assemblies consisting of phosphorylated tau proteins are responsible for plaque formation. The structure, dynamic behavior and spatial organization of microtubules in neurons in the brain are regulated by their associated tau proteins. Although tau's structure function has been intensively studied for many years, little is known about the molecular mechanisms of interaction between tau proteins and microtubules and promotion of the assembly of microtubules.

Herein, we present results of changes occurred at 45-50°C in the brain proteome of an experimental animal model (rat) with scopolamine-induced neurodegenerative disorder, type AD, using two-dimensional gel electrophoresis (2D-PAGE) coupled with MALDI-TOF-TOF. After homogenization of brain tissue from hippocampus, striatum and cortex, a comparative 1D- SDS-PAGE analysis was performed on samples with and without heating to 45°C.

The main observation from our investigations is the different behavior of the hippocampal proteome from the healthy rats before and after heating to 45°C. Due to the demonstrated change in protein level of tau and tubulin in the rat hippocampus after heating to 45°C, it was suggested that the observed exothermic process at 35-45°C in mice may be due to the partial unfolding of tau protein which leads to the release of tubulin. Both proteins together are involved in protein fibrillation and aggregation. The reported results from this study can help clarify the molecular mechanisms of scopolamine-induced dementia and neurodegenerative processes in general.

**Keywords:** Scopolamine-induced neurodegenerative disorder; Brain homogenates; Hippocampus; Tau protein; Proteomic analyses; Mass spectrometry.

**Acknowledgement:** *This research was carried out with the support of a project under contract No. 03/13/2016, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.*



44. P. Dolashka, L. Velkova, A. Dolashki, Y. Voynikov, R. Velikova, E. Daskalova, Y. Topalova, P. Hristova. Proteomic assay of *Propionibacterium acnes* treatment with mucus extract from garden snail *Cornu aspersum*. (Poster) "Ecology and health" XVII-th National Scientific conference with international participation, 7 June 2018, Plovdiv, Bulgaria.

СДРУЖЕНИЕ С НЕСТОПАНСКА ЦЕЛ „ТЕРИТОРИАЛНА ОРГАНИЗАЦИЯ НА НАУЧНО-ТЕХНИЧЕСКИ СЪЮЗИ С ДОМ НА НАУКАТА И ТЕХНИКАТА-ПЛОВДИВ"  
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INSTITUTE OF MICROBIOLOGY „STEFAN ANGELOV" - BAS

НАЦИОНАЛНО ДРУЖЕСТВО „ЕКОЛОГИЧНО ИНЖЕНЕРСТВО И ОПАЗВАНЕ НА ОКОЛНАТА СРЕДА"  
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## ЕКОЛОГИЯ И ЗДРАВЕ, 2018 ECOLOGY AND HEALTH, 2018

### ПРОГРАМА PROGRAMME

XII-та НАЦИОНАЛНА НАУЧНО-ТЕХНИЧЕСКА  
КОНФЕРЕНЦИЯ С МЕЖДУНАРОДНО УЧАСТИЕ

XII-th NATIONAL SCIENTIFIC  
CONFERENCE WITH INTERNATIONAL  
PARTICIPATION

07 юни 2018 година  
Дом на науката и техниката – Пловдив, ул. „Гладстон" № 1  
7 June 2018  
House of Science and Technique – Plovdiv, 1 Gladston Str.

**Proteomic assay of *Propionibacterium acnes* treatment with mucus extract from  
garden snail *Cornu aspersum*  
(Poster)**

Pavlina Dolashka<sup>1</sup>, Lyudmila Velkova<sup>1</sup>, Aleksandar Dolashki<sup>1</sup>, Yulian Voynikov<sup>1</sup>, Radostina Velikova<sup>1</sup>, E. Daskalova<sup>2</sup>, Yana Topalova<sup>2</sup>, Petya Hristova<sup>2</sup>.

<sup>1</sup> Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences

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**Abstract**

Acne vulgaris is one of the most common an inflammatory skin disease. which is observed in about 80% of young adults. The pathophysiological factors that cause the development of acne are excessive sebum production, abnormal keratinization and desquamation of pilosebaceous follicular epithelium (comedogenesis), follicular proliferation of *Propionibacterium acnes* and inflammation. *P. acnes* appears to play a central role in the induction of inflammation. Recent microbiological and dermatological studies have reproducibly pointed to the strong associations between *P. acnes* and acne vulgaris, *S. aureus* and atopic dermatitis, and *Malassezia* species with dandruff. Resistance development in *P. acnes* is another serious problem in using conventional antibiotics such as erythromycin. In these contexts, we investigated several mucus extracts from garden snail *Cornu aspersum* for antibacterial activity.

The isolated mucus extract was separated into several fractions by ultrafiltration on Millipore membrane filters from 10kDa, 20kDa, 30kDa and 100 kDa. The obtained fractions were tested for antimicrobial activity by the well diffusion method against *Propionibacterium acnes* 266 (AI).

The fraction having below 20kDa displayed the highest antibacterial activity against *P. acnes* 266 (AI), followed from fraction above 100kDa. Using tandem mass spectrometry we identified the primary structures of many novel antimicrobial peptides in active fraction below 20kDa. Most of them contain high level of glycine, proline, tryptophan and valine residues which are typical for peptides with antimicrobial activity.

We have applied a combination of two-dimensional electrophoresis (2-DE) and mass spectrometry (MALDI-MS) to identify changes in expression of proteins secreted by *P. acnes* 266 (AI), before and after treatment with active fraction below 20kDa. These proteins are: glycerol-ester hydrolase A PPA2105 (GehA; MW 35972Da, pI 6.59); PROAC0001\_1849 (MW 31987Da, pI 5.00) putative lysozyme M1 [*P. acnes* SK137]; PPA0687 (MW 28612 Da, pI 9.61) CAMP factor 2; hypothetical protein, specific to *P. acnes* PPA1715 (MW 49335Da); hypothetical protein specific to *P. acnes*; PPA1939 (MW 16862Da, pI 6.17); PPA2106 (MW 54063Da, pI 6.34) putative endoglycoceramidase; PPA0721 (MW 40716Da, pI 9.44) putative invasion-associated protein, NlpC/P60 family.

**Keywords:** mucus extracts, garden snail *Cornu aspersum*, proteomic assay, two-dimensional electrophoresis (2-DE), mass spectrometry MALDI-MS.

**Acknowledgement:** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.

45. L. Velkova, Y. Voynikov, A. Dolashki, L. Tancheva, L. Alova, W. Voelter, P. Dolashka. Proteomic analyses of brain homogenate and cerebrospinal fluid of an experimental animal model (rat). (Poster) "Ecology and health" XVII-the National Scientific conference with international participation, 7 June 2018, Plovdiv, Bulgaria.

СДРУЖЕНИЕ С НЕСТОПАНСКА ЦЕЛ „ТЕРИТОРИАЛНА ОРГАНИЗАЦИЯ НА НАУЧНО-ТЕХНИЧЕСКИ СЪЮЗИ С ДОМ НА НАУКАТА И ТЕХНИКАТА-ПЛОВДИВ"  
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## ЕКОЛОГИЯ И ЗДРАВЕ, 2018 ECOLOGY AND HEALTH, 2018

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7 June 2018  
House of Science and Technique – Plovdiv, 1 Gladston Str.



**Proteomic analyses of brain homogenate and cerebrospinal fluid of an experimental animal model (rat)  
(Poster)**

L. Velkova<sup>1</sup>, Y. Voynikov<sup>1</sup>, A. Dolashki<sup>1</sup>, L. Tancheva<sup>2</sup>, L. Alova<sup>2</sup>, W. Voelter<sup>3</sup>, P. Dolashka<sup>1</sup>

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**Abstract**

The most common neurodegenerative disorder and the most important cause of dementia in elderly people appear to be Alzheimer's disease (AD). The AD-associated problems decrease life expectancy, reduce quality of life, cause physical disability, and eventually lead to serious problems in daily life activities such as social and occupational functions. To reduce the burden of the disease on patients and their families and the social and economic costs, many scientific teams make the remarkable efforts to find diagnostic markers which predict the disease earlier. Recently, it is suggested that for the development of AD, mainly  $\beta$ -amyloid peptides and neurofibrillary assemblies consisting of phosphorylated tau proteins are responsible for plaque formation.

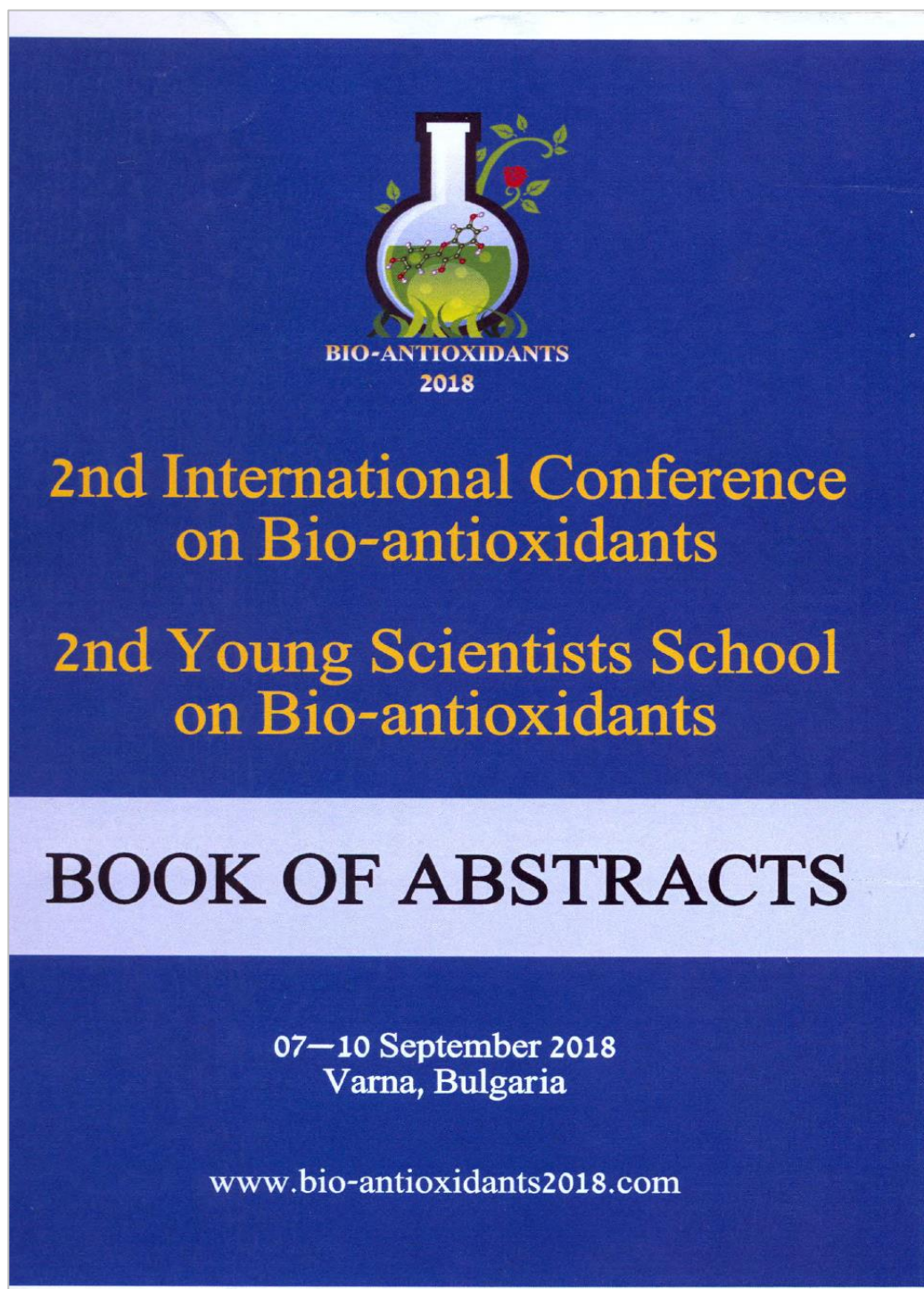
In this study, brain homogenate (BH) and cerebrospinal fluid (CSF) of an experimental animal model (rat) in normal and scopolamine-induced neurodegenerative disorder (type AD), were analyzed by using proteomic techniques, mass spectrometry (MALDI-TOF-TOF) and bioinformatics' analysis.

Comparisons between controls and scopolamine-induced neurodegenerative disorder (type AD) of BH and CSF have showed several common proteins (as microtubule associated proteins, tau proteins, tubulins, and beta amyloid precursor) which are expressed significantly differences. Recent studies have shown, the amyloid-beta ( $A\beta$ ) and amyloid  $\beta$ 1-42 play a key role in the pathogenesis of AD. The intact  $A\beta$  from soluble aggregates comperes are especially relevant biochemical marker because they are believed to be the most toxic form of  $A\beta$ . Therefore, we investigated different fractions <10kDa from BH and CSF, using mass spectrometry (MALDI-TOF-TOF). We found a diversity of  $A\beta$  peptides between 3.0-6.5kDa, and identified some of them. We observed high heterogeneity of proteoforms, which associates with post-translational modifications. This heterogeneity of  $A\beta$  proteoforms deepens our understanding of AD and offers many new avenues for investigation into pathological mechanisms of the disease, with implications for therapeutic development.

**Keywords:** Scopolamine-induced neurodegenerative disorder (type AD); Brain homogenate (BH); Cerebrospinal fluid (CSF); Amyloid-beta ( $A\beta$ ) peptides ; Proteomic analyses; Mass spectrometry.

**Acknowledgement:** *This research was carried out with the support of a project under contract No. 03/13/2016, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.*

46. P. Dolashka, A. Dolashki, L. Velkova. From science to business. Opening Lecture, 2nd International Conference on Bio-antioxidants, 07 – 10 September 2018, Varna, Bulgaria.





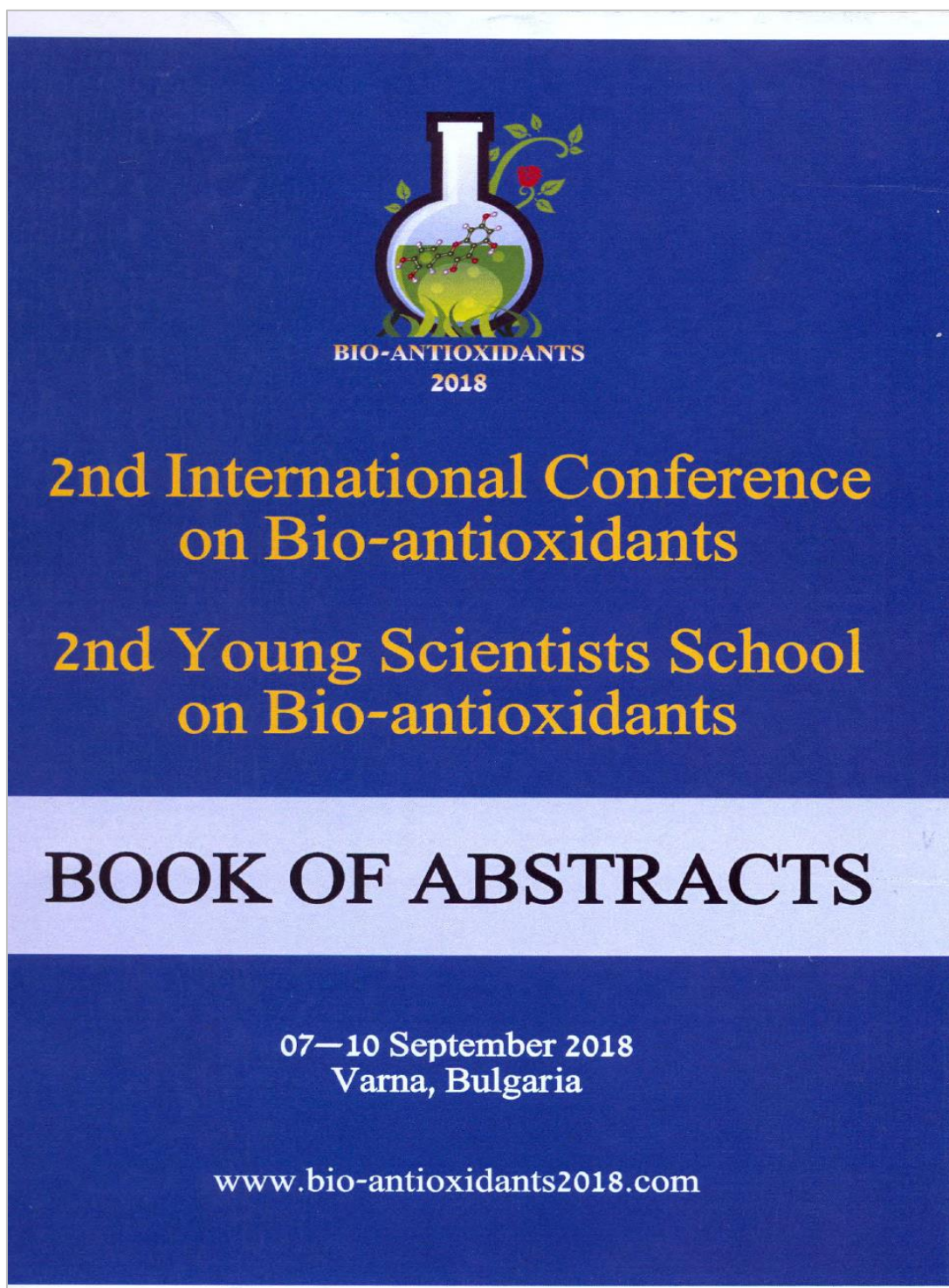


## Final Scientific Program

Friday, 7 <sup>th</sup> September 2018		
<b>Registration and information Company for International Meetings</b>	<i>Romance Splendid Hotel, St. Constantine and Helena Resort</i>	<b>12.00-18.00</b>
<b>Welcome and Opening Ceremony</b>		<b>17.00-18.30</b>
Prof. Vessela Kancheva Chair of organizing committee, IOCCP – BAS, Bulgaria	Welcome address	17.00-17.10
Dr. Stefan Dochev Coordinator Young Scientists School, Austria	Welcome address	17.10-17.20
Prof. Pavleta Shestakova IOCCP-BAS, Bulgaria	Welcome address	17.20-17.30
Prof. Pavlina Dolashka, IOCCP-BAS, Bulgaria	Opening Lecture <i>From science to business</i>	17.30-18.00
Mrs. Anelia Vasileva, Orange Holiday, Varna, Bulgaria	Welcome to Varna	18.00-18.10
Students from Varna, Bulgaria	Welcome to Bulgaria	18.10-18.30
<b>Poster session I</b>	<b>Topics A, B and C</b>	<b>18.30-20.00</b>
<b>Welcome Reception</b>		<b>20.00-21.30</b>



47. P. Dolashka, A. Dolashki, L. Velkova, B., Devreese Y. Topalova, P. Hristova, J. Van Beeumen, W. Voelter. Food supplements with novel bioactive extracts from garden snails. (Lecture), 2-nd International Conference on Bio-antioxidants, 07 – 10 September 2018, Varna, Bulgaria



## E-KL7. FOOD SUPPLEMENTS WITH NIVEL BIOACTIVE EXTRACTS FROM GARDEN SNAILS

Pavlina DOLASHKA,<sup>a</sup> Aleksander DOLASHKI,<sup>a</sup> Lyudmila VELKOVA,<sup>a</sup> Bart DEVREESE,<sup>b</sup> Yana TOPALOVA,<sup>c</sup> Petya HRISTOVA,<sup>c</sup> Jozef VAN BEEUMEN,<sup>b</sup> Wolfgang VOELTER<sup>d</sup>

<sup>a</sup> Institute of Organic Chemistry, Bulgarian Academy of Sciences, G. Bonchev 9, Sofia 1113, BULGARIA

<sup>b</sup> Laboratory of Protein Biochemistry and Biomolecular Engineering, Ghent University, KL Ledeganckstraat 35, 9000 Ghent, BELGIUM

<sup>c</sup> Sofia University "St. Kliment Ohridski", Faculty of Biology, BULGARIA

<sup>d</sup> Interfaculty Institute of Biochemistry, University of Tübingen, Hoppe-Seyler-Strasse 4, D-72076 Tübingen, GERMANY

Hemolymph and mucus of *Cornu aspersum* garden snails provide a rich source of peptides and proteins with antioxidant, antimicrobial, antiviral and antitumor activity. Antimicrobial peptides and glycoproteins from the mucus and hemolymph are important components of the innate immunity. Some isoforms and peptides serve as effector molecules of the defense system, providing an efficient initial effect against infectious pathogens. Therefore quite a series of proline-rich peptides isolated from the mucus of *C. aspersum* garden snails were analysed and were considered to be promising candidates for the treatment of microbial infections and suppression of microbial resistance.

Using tandem mass spectrometry the primary and oligosaccharide structures of many novel peptides with molecular masses between 1 and 20 kDa displayed the highest antibacterial and antifungal activities were identified. Most of them contain high level of glycine, proline, tryptophan and valine residues which are typical for peptides with antimicrobial activity.

Applying a combination of two-dimensional electrophoresis (2-DE) and mass spectrometry (MALDI-MS) the proteins secreted in the mucus also were identified. The carbohydrate structures of both glycoproteins, superoxide dismutases (SODs) and hemocyanins, having also antioxidant activity were determined by MALDI-MS/MS and Q-Trap MS/MS.

Based on obtained results the active compounds were applied to develop several novel food supplements to overcome the recent appearance of a growing number of resistant to conventional antibiotics, which has become a serious medical problem.

**Keywords:** antimicrobial peptides, **bio-antioxidants**, glycopeptides, glycoproteins, *Cornu aspersum*, mass spectrometry

**Acknowledgement:** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.



48. P. Dolashka, A. Dolashki, L. Velkova, V. Petrova, A. Kujumdzieva. Specific glycosidases in quiescent *S. cerevisiae* culture and their functions. (Lecture), 4-th Glycobiology World Congress, 17-19 September 2018, Rome, Italy.



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# 4<sup>th</sup> Glycobiology World Congress

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# 4<sup>th</sup> Glycobiology World Congress

September 17-19, 2018 | Rome, Italy

## Specific glycosidases in quiescent *S. cerevisiae* culture and their functions

Dolashka P<sup>1</sup>, Dolashki A<sup>1</sup>, Velkova L<sup>1</sup>, Petrova V<sup>2</sup> and Kujumdzieva A<sup>2</sup><sup>1</sup>Institute of Organic Chemistry with Centre of Phytochemistry-Bulgarian Academy of Sciences, Bulgaria<sup>2</sup>Sofia University "St. Kliment Ohridski", Bulgaria

Several glycosidases such as alpha-glucosidase I and alpha-mannosidase are located in the endoplasmic reticulum of *Saccharomyces cerevisiae* and they cleave the terminal alpha1, 2-linked glucose alpha1, 2-mannose. Therefore, the effect of *S. cerevisiae* in quiescent state G0 and active cells on the glycosylated hemocyanin *Rapana venosa* (RvH) and *Haliotis tuberculata* (HtH) is analyzed in comparison to the enzyme zymolysis. The carbohydrate structure of both the hemocyanins is known to have mainly mannose-type structures. The structural units of RvH1 and HtH1 are composed of various functional units with 45–60 kDa molecular masses measured by MALDI-TOF/TOF-MS ligand-bound by protease-sensitive peptides. However, we propose the model that the individual polypeptides of the various FUs are linked together in a network generated by oligosaccharides. Our hypothesis is confirmed by the generated fragments, with molecular masses of about 50 kDa, after incubation of HCS in *S. cerevisiae* growth medium. It is based on the assumption that yeasts produce glycosidases that break the oligosaccharides connecting the different functional units. After four days incubation of RvH and HtH in medium with *S. cerevisiae* in quiescent state G0 and active cells, several different fractions were isolated which showed a negative orcinol/H<sub>2</sub>SO<sub>4</sub> test. The results show a higher effect after treatment of both hemocyanins in *S. cerevisiae* active cells medium than in quiescent state G<sub>0</sub>. However, the observed effect is lower than that after incubation of both HCS with zymolyase.

### Biography

Dolashka P has wide experience in the isolation, purification, and characterization of biologically active compounds. She has more than 130 publications on these topics, three book chapters and six patents. She is the Editor-in-board of three journals and Representative of IUPAC. She is coordinating several international research projects, sponsored by NATO (Brussels), the European Commission, Germany (DFG and BMBF), CNR (Italy), FWO (Belgium), China, and Ukraine.

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### Notes:

49. L. Velkova, A. Dolashki, R. Velikova, P. Dolashka. Identification of peptides and glycopeptides with antimicrobial activity from garden snail *C. aspersum* using mass spectrometry. (Lecture), 4-th Glycobiology World Congress, 17-19 September 2018, Rome, Italy.



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# 4<sup>th</sup> Glycobiology World Congress

September 17-19, 2018 | Rome, Italy

## Identification of peptides and glycopeptides with antimicrobial activity from garden snail *C. aspersum* using mass spectrometry

Lyudmila Velkova<sup>1</sup>, Aleksandar Dolashki<sup>1</sup>, Radostina Velikova<sup>1</sup> and Pavlina Dolashka<sup>1</sup><sup>1</sup>Institute of Organic Chemistry with Centre of Phytochemistry-Bulgarian Academy of Sciences, Bulgaria

The recent appearance of a growing number of bacteria resistant to conventional antibiotics has become a serious medical problem. Therefore the development of antibiotics, with novel mechanisms of action is a current issue. The mucus from the garden snail *C. aspersum* is a complex, multi-component mixture including different biochemical active substances. The mucus extract was separated into several fractions using Millipore filters with different size (10, 20 and 100 kDa). We have performed *in vitro* studies on the antimicrobial activities of different extracts, obtained from mucus of garden snail *C. aspersum*, against Gram+, Gram- bacteria and fungal strains *A. niger* and *A. fumigatus*. Our results have shown that fractions < 10 kDa, <20 kDa and between 1-10 kDa possess strong antimicrobial activity against the tested pathogens. To explain the observed effects against various microbial organisms, the peptides and glycopeptides in active fractions were purified by reversed phase high-performance liquid chromatography (RP-HPLC). All fractions were tested of the orcinol/H<sub>2</sub>SO<sub>4</sub> test. The positive fractions (glycopeptides) were lyophilized and further studied by Q-trap-LC/MS/MS. We identified the carbohydrate chains and amino acid sequences of several glycopeptides. Using tandem mass spectrometry MALDI-TOF-MS/MS, we identified the primary structures of 15 novel antimicrobial peptides in active fraction below 10kDa. Most of them contain high levels of glycine and leucine residues, but others contain proline, tryptophan and valine residues, typically for peptides with antimicrobial activity. Our results may be considered as basic information for further investigations on bioactive compounds from mucus of garden snail *C. aspersum*.

**Keywords:** mucus extracts, garden snail *Cornu aspersum*, antimicrobial peptides (AMPs), glycopeptides, mass spectrometry

**Acknowledgement:** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.

### Biography

Lyudmila Velkova works at laboratory "Chemistry and Biophysics of proteins and enzymes" at the Bulgarian Academy of Sciences (BAS). Her research interests include isolation, characterization and application of bioactive substances from natural sources, primarily proteins, glycoproteins, antimicrobial peptides, investigation of carbohydrate structures of glycoproteins by mass spectrometry, as and proteomics analysis. She defended PhD on the topic "Structure and function of carbohydrate chains of hemocyanin, isolated from the marine snail *Rapana venosa*" in Institute of Organic Chemistry with Centre of Phytochemistry - BAS, 2013. She has published more than 25 papers in reputed international journals.

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### Notes:



50. R. Abrashev, E. Krumova, A. Dolashki, N. Kostadinova, J. Miteva-Staleva, L. Velkova, B. Spasova, M. Angelova, P. Dolashka. Cornu aspersum - derived peptides with biological activity. (Poster), 4-th Glycobiology World Congress, 17-19 September 2018, Rome, Italy.



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# 4<sup>th</sup> Glycobiology World Congress

September 17-19, 2018 | Rome, Italy

## *Cornu aspersum*-derived peptides with biological activity

R Abrashev<sup>1</sup>, E Krumova<sup>1</sup>, A Dolashki<sup>2</sup>, N Kostadinova<sup>1</sup>, J Miteva-Staleva<sup>1</sup>, L Velkova<sup>2</sup>, B Spasova<sup>1</sup>, M Angelova<sup>1</sup> and P Dolashka<sup>2</sup><sup>1</sup>The Stephan Angeloff Institute of Microbiology-Bulgarian Academy of Sciences, Bulgaria<sup>2</sup>Institute of Organic Chemistry with Centrum of Phytochemistry-Bulgarian Academy of Sciences, Bulgaria

Recently, bioactive peptides have received close scientific attention for their broad scope of bioactivities, mainly including antioxidant and antimicrobial properties. Such peptides are especially prominent for their notable contributions to human health improvement through the scavenging of dangerous free radicals. Moreover, they have shown to be good candidates for the development of alternative antimicrobial agents. The aim of the present study was to derive different fractions from the mucus of the garden snail *Cornu aspersum* and to evaluate their antioxidation and antifungal activity. The snails, *Cornu aspersum* were collected in Bulgaria and the mucus was purified. After that, the fractions with different molecular mass were obtained by ultrafiltration on Millipore filters. Mass spectrometry analyses on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a nanoelectron spray ion source was performed for the peptide characterization. The total antioxidant potential of the tested fractions was assessed by the DPPH and ABTS radical scavenging activity methods and the nitroblue tetrazolium (NBT) reduction assay; superoxide dismutase (SOD) activity was evaluated as well. The results showed that the natural peptides derived from *C. aspersum*, specifically the low molecular mass fractions possess potential antioxidant activity confirmed by hydroxyl and superoxide radical scavenging activity and radical cation decolorization assay. These peptides exhibited fungicidal and fungistatic activity against *Candida membranifaciens*, *Aspergillus fumigatus* and *Aspergillus niger*.

**Acknowledgments:** This work was supported by the National Scientific Fund of the Ministry of Education and Science, Bulgaria (DN 01-14/20168), which is greatly acknowledged.

### Biography

Radoslav Abrashev is an Associate Professor in the Department of Mycology at The Stephan Angeloff Institute of Microbiology (IMiB), Bulgarian Academy of Sciences. He has completed his PhD from IMiB and Post-doctorate from the University of Strathclyde, Glasgow, UK. He has published more than 30 papers in reputed journals. His research interest is focused on production of biological active compounds from natural sources, their purification, chemical and biological characterization in terms of antioxidant and antifungal properties.

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### Notes:

51. N. Kostadinova, P. Dolashka, E. Krumova, L. Velkova, A. Dolashki, R. Abrashev, J. Miteva-Staleva, B. Spasova, M. Angelova. Mucus from *Cornu aspersum* – a perspective resource of antioxidant substances. 14th Congress of Microbiologists in Bulgaria with International Participation, October 10th – 13th, 2018, Hisarya, Bulgaria.



**14<sup>th</sup> Congress of Microbiologists  
in Bulgaria  
with International Participation**

**PROGRAM AND ABSTRACTS**



**Hisarya, October 10<sup>th</sup> – 13<sup>th</sup>, 2018**



## MUCUS FROM *CORNU ASPERSUM* – A PERSPECTIVE RESOURCE OF ANTIOXIDANT SUBSTANCES

Nedelina Kostadinova<sup>1</sup>, Pavlina Dolashka<sup>2</sup>, Ekaterina Krumova<sup>1</sup>, Lyudmila Velkova<sup>2</sup>, Alexander Dolashki<sup>2</sup>, Radoslav Abrashev<sup>1</sup>, Jeni Miteva-Staleva<sup>1</sup>, Boryana Spasova<sup>1</sup>, Maria Angelova<sup>1</sup>

<sup>1</sup>The Stephan Angeloff Institute for Microbiology, Bulgarian Academy of Sciences, Sofia;

<sup>2</sup>Institute of Organic Chemistry with Centrum of Phytochemistry, Bulgarian Academy of Sciences, Sofia

**Introduction.** Snail mucus is a rich source of bioactive natural compounds with a variety of functions, including facilitating of the treatment of wounds and skin conditions. More importantly, the mucus is well known for its anti-aging properties and frequently investigated for antibacterial potency. Generally, both of these properties facilitate the ability of the organism to cope with the generation of free radicals.

**Aim.** The aim of the present study was to evaluate the extent of antioxidant potential of fractions from mucus of garden snail *Cornu aspersum*.

**Methods.** The model snail species were collected in Bulgaria and the mucus was purified and subjected to ultrafiltration. Three different samples were investigated – lyophilized fractions (molecular mass < 10 kDa and > 20 kDa) and liquid fraction (> 20 kDa). Inhibitory effect of the fractions was determined by calculating of the inhibition of NBT reduction by photochemically generated superoxide. Superoxide dismutase (SOD) activity and dose-dependence of the superoxide anion scavenging effect were studied as well.

**Results.** The fraction with molecular mass <10 kDa showed the highest SOD activity. It is possible that the detected SOD value represents the so called “SOD-like” activity that could be displayed by some peptides with low molecular mass capable of superoxide scavenging.

**Conclusions.** The results from the antioxidant screenings of *C. aspersum* mucus and its fractions show that this naturally derived product (specifically the low molecular weight fractions), has a good potential to counteract the formation of reactive oxygen radicals.

**Keywords:** *Cornu aspersum*, superoxide radicals, SOD, antioxidant activity

**Acknowledgment:** This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.