XII International Conference

Biomolecules: Identification and Functions

21-22.10.2016

Book of Abstracts and Program

Krakow, Poland

XII International Conference

Biomolecules: Identification and Functions

21-22.10.2016, Krakow, Poland

organized by:

Department of Biochemistry and Neurobiology
Faculty of Materials Science and Ceramics
AGH University of Science and Ceramics

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Venue:
Faculty of Materials Science and Ceramics
building B8 – Lectures
building B4 - Workshop

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PROGRAM

Day 1 Friday 21st October

Lectures – building B8

9:00 – 10:00 prof. Dominic Desiderio, Department of Neurology, The University of Tennessee, USA

„The use of mass spectrometry to study the human pituitary: The future of neuroendocrinology"

10:00-10:50 prof. Giuseppe Grasso, Dipartimento di Scienze Chimiche, Universita degli Studi di Catania, Italy

„Monitoring the biomolecular interactions and the activity of Zn-containing enzymes involved in conformational diseases: experimental methods for therapeutic purposes"

10:50-11:40 prof. Marcin Majka, Clinical Immunology and Transplantology Department, CMUJ, Poland

„Two faces of transcription factor SNAIL"

11:40-12:00 Dariusz Pianka, Bio Rad, Poland

„Total Protein Normalization in western blotting"

12:00-13:00 COFFEE BREAK

13:00-13:15 Michał Konieczny, Merck, Poland

„Innovative approach for cell analysis and IR protein detection"

13:15-14:05 prof. John Howl, Faculty of Science and Engineering, University of Wolverhampton, UK

„Cell penetrating peptides and Bioportides"

14:05-14:55 prof. Michał Daszykowski, Institute of Chemistry, The University of Silesia, Poland
„Identification of potential markers based on chemical fingerprints”

14:55-15:25 Marcin Gawryś, Shimpol, Poland
„Tissue imaging using MALDI”

15:30-17:00 Poster Session

Day 2 Saturday 22nd October

Laboratories – building B4 (rooms: 131, 233, 234)

10:00-10:40 / 10:45-11:30

Electrochemical cell based system in metabolism simulation.

dr Przemyslaw Mielczarek (KBiN, WIMiC, AGH)

10:00-10:40 / 10:45-11:30 / 12:00-12:40 / 12:45-13:30

V3 Western Workflow with Chemi Doc Touch.

Dariusz Pianka, Bio-Rad

10:00-10:40 / 10:45-11:30 / 12:00-12:40 / 12:45-13:30

Miniaturized Flow Cytometer and IR protein analysis.

Michał Konieczny, Merck

11:30-12:00 COFFEE BREAK

12:00-12:40 / 12:45-13:30

Advances of the new ion source: FAPA in forensic analyses.

dr hab. Marek Smoluch (KBiN, WIMiC, AGH)

12:00-12:40 / 12:45-13:30

Desorption Electrospray Imaging: applications in biomedical sciences.

dr Anna Bodzon-Kulakowska (KBiN, WIMiC, AGH)

POSTER ABSTRACTS

The use of electrospray ionization mass spectrometry on the structural characterization of new ruthenium(II) arene complexes with chromone derivatives

B.M. Pasternak[1], A. Pastuszko[2], E. Budzisz [2]

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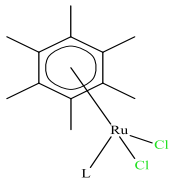
Abstract

Ruthenium(II) arene complexes with the general formula [(η6-arene)Ru(L)Cl2] (where arene = hexamethylbenzene and L = aminoflavone or aminochromone derivatives) have been examined by electrospray ionization mass spectrometry. In our previous paper we have presented the synthesis, lipophilicity and cytotoxic effect of ruthenium(II) complexes with various ligands.[1] The most active complexes against melanoma cells contain 7-aminoflavone and 6-aminoflavone as a ligand. We have observed that the cytotoxicity against the melanoma cell lines of all synthesized complexes seemed in general to be more dependent on structure and less on lipophilicity.



1d - 4d

Where: L = 1) 6-amino-2-phenyl-4*H*-benzopyran-4-one; 2) 7-amino-2-phenyl-4*H*-benzopyran-4-one; 3) 7-amino-2-methyl-4*H*-benzopyran-4-one; 4) 5-amino-8-methyl-4*H*-1-benzopyran-4-one.

Key Words: Ruthenium (II) complexes, chromone, esi-ms, cytotoxic effect
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[1] A. Pastuszko, K. Majchrzak, M. Czyz, B. Kupcewicz, E. Budzisz - J. Inorg. Biochem. 2015 : Vol. 159, s. 133-141.

From isolation and purification of diatom-specific xanthophylls: diadinoxanthin and diatoxanthin to their potential application.

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Abstract

Purpose: Studies were aimed at development of a method of isolation and purification of diatom-specific xanthophylls such as diadinoxanthin (Diadino) and diatoxanthin (Diato). They are engaged in the diadinoxanthin cycle, which protects cells against photoinhibition caused by excessive light energy. Moreover, considering beneficial role of carotenoids for human health, the effect of Diadino and Diato on biological membranes was investigated in model systems in relation to zeaxanthin (Zea) - one of the most common carotenoid found in nature.

Experimental: Illuminated cells of diatom *Phaeodactylum tricornutum* were used as a material for Diadino and Diato isolation and purification through the four steps procedure comprising extraction of pigments, saponification, separation by partition and open column chromatography. Zea was isolated from *Escherichia coli* strain BL21(DE3) containing pAC-ZEAXipi plasmid. Purity of xanthophylls was analysed by HPLC. Diato and Zea were incorporated into DPPC liposomes and used in differential scanning calorimetry and fluorescence anisotropy studies.

Results: It has been developed a high-performance procedure of all-*trans* Diadino and all-*trans* Diato purification from *P. tricornutum*, dedicated to both analytical and preparative scale. Conducted studies have shown a significant effect of examined xanthophylls on physic-chemical properties of phospholipid bilayers as well as their thermotropic phase behaviour.

Conclusions: Hitherto, neither Diadino nor Diato were commercially available in amounts greater than those used as HPLC standards. The developed procedure of isolation and identification opens up possibilities for their use in biochemical studies, in search of pharmaceutical and commercial applications.

Key Words: diatoms, diatoxanthin, liposomes, zeaxanthin

Acknowledgements: This work was supported by the Grant 2013/09/N/NZ1/01031 from the National Science Center Poland. The Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education. A method of diadinoxanthin purification has been reported as patent applications P.412178, P.412177 and PCT/PL2016/000047 (Kuczynska & Jemiola-Rzeminska).

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Biomolecules cooperation in photosynthetic membranes stabilization

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Abstract

Purpose: Photosynthetic organisms possess a multitude of mechanisms to adapt to variable environmental factors providing optimal yield in sun light conversion to biomass production. Although diatoms inhabit thermally unstable water environments they contribute up to about 25% of the yearly biomass production on earth. We present the changes in composition, interactions and ratios of the key biomolecules as the most important strategy to optimize photosynthetic membrane properties at changing temperatures.

Experimental: Experiment was carried out on thylakoid membranes isolated from diatoms adapted at low and moderate temperature (Bojko et al. 2013). The comparative analyses of: fatty acids (GC), proteins (Lowry) and chlorophyll (spectrophotometric method) concentration were done. Role of diadinoxanthin cycle pigments (estimated by HPLC) in temperature-regulated thylakoid membranes structure was additionally estimated by EPR.

Results: Changes in the level of polyunsaturated long-chain fatty acids with a parallel decrease in the content of shorter saturated or mono unsaturated fatty acids is important molecular mechanism of diatom thylakoid membranes in adaptation to low temperatures. Simultaneously decrease of the level of xanthophylls, especially diadinoxanthin, as well as proteins was observed at lower temperature. Furthermore, the increase of diatoxanthin, which is formed from diadinoxanthin, decreases the fluidity of thylakoid membranes of diatoms adapted to moderate temperature more effectively than adapted to low temperature. Effect of temperature on fatty acids, proteins and carotenoids concentration in diatom thylakoid membranes is presented below:

	12°C	**20°C**
Fatty acids/Chl a [µg/mg Chl a]	3642.864 (±378.390)	3796.523 (±193.365)
Unsaturated/saturated fatty acids	2.9129 (±0.125)	2.763 (±0.020)
Proteins [µg/mg Chl a]	1837.116 (±115.001)	2993.862 (±102.093)
Carotenoids [µmol/mmol Chl a]	4551.45 (±718.480)	6496.13 (±131.700)

Conclusions: It was found that regulation of content and interaction of biomolecules such as the fatty acids, carotenoids and proteins is the most important molecular strategy in optimization of diatom photosynthetic membranes under changing environmental temperatures.

Key Words: membrane fluidity, fatty acid composition, carotenoids, didinoxanthin cycle, diatoms
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Aspartic protease 3 (Yps3) on the cell wall of *Candida glabrata* is involved in the interaction of this pathogenic yeast with human epithelial cells

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Abstract

Purpose: One of essential steps in the microbial colonization of human organism is the adherence of pathogen to host epithelial cells. The aim of the present study was to identify some proteins that are exposed on the surface of *Candida glabrata* – the second major cause of human candidiasis after *C. albicans* – including those capable of interacting with human epithelial cells of A431 line, with a particular focus on the role of aspartic proteases in this interaction.

Experimental: A "cell surface shaving" method was used to identify surface-exposed proteins of *C. glabrata* cells, cultured in the YAPD medium known to stimulate the production of extracellular proteases. A mixture of the cell-wall associated proteins was released with beta-1,6-glucanase and allowed to contact with A431 cells. Using the chemical cross-linking and liquid chromatography-coupled tandem mass spectrometry methods we identified several proteins exposed on the *C. glabrata* surface and involved in the interaction with human epithelial cells.

Results: Several fungal proteins, cross-linked to the human cells, were identified. Among them, aspartic protease 3 (Yps3) was found to be exposed on the fungal cell wall under various conditions of reduced nitrogen availability. Other identified proteins such as glyceraldehyde-3-phosphate dehydrogenase 3 (Tdh3), alcohol dehydrogenase 1 (Adh1) and fructose-bisphosphate aldolase (Fba1) are originally cytoplasmic proteins, exported outside the cell and loosely bound with cell wall.

Conclusions: In the present study we identified new proteinaceous partners for epithelial cells on the *C. glabrata* surface. Among them, Yps3 can be an important pathogenic factor, essential for the development of infection.

This work was financially supported by the WBBiB funds for young scientists (KNOW BMN grant no. 15/2016 awarded to D.Z.).

Key Words: *Candida glabrata*, aspartic protease 3
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Solvet vs solventless procedure for determination of average molecular mass of electropolymerised PEDOTs as precursor for nerve guidance channel by means of MALDI TOF MS

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Abstract

Purpose: The main aim of the study was to determine the average molecular weight of the electropolymerised EDOT

Experimental: Mass spectra were obtained on a Shimadzu AXIMA performance MALDI-TOF mass spectrometer operated in linear mode. The laser power was optimized to obtain a good signal-to-noise ratio after averaging 250 single-shot spectra. 1,8,9-trihydroxyanthracene (dith, Mdith = 226.23 g mol^{-1}) was used as a matrix substance. The polymer samples were deposited during electrochemical process in ACN (acetonitrile), in TBABF4 (tetrabuthyl ammonium tetrafluoroborate). 1.5 μL of the matrix solution was applied onto a stainless-steel target plate and then air dried. Afterwards, 1.5 μL of the sample solution was spotted on a dry matrix surface and air dried for several minutes at room temperature. Data were acquired in linear mode with 250 profiles collected by laser firing and 5 shots accumulated into one profile. Data were obtained and analyzed using the Shimadzu Biotech Launchpad program.

Results: Our results indicate that the proposed procedure enable recording of MALDI TOF MS spectra for obtained polymers. During the electropolymerisation homo- and copolymer chains were formed as indicated by peak to peak distance.

Conclusions: Upon the obtained results one can draw the conclusion that both procedure (solvent and solventless one) lead to successful recording of MS spectra. There are several factors that are crucial during sample preparation like grinding or sample homogenization.

Key Words: (3,4,ethylenedioxythiopnehe), copolymerization, MALDI TOF MS, solventless procedure
Acknowledgements: -
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Anti- and pro-oxidative activity measured directly by the extent of 8-oxoguanine production

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Abstract

Purpose: To develop an electrochemical method for the assessment of antioxidative activity of various substances regarded as agents that would protect DNA from oxidative damage potentially leading to mutation. It has been known that guanine has the lowest oxidation potential of all DNA bases. What seems to be even of greater concern, is that it is guanine in telomeres that undergoes oxidation first, and this may contribute to causing cancer. The product of guanine oxidation is 8-oxoguanine, which could be detected electrochemically. The degree to which the production of 8-oxoguanine is reduced in the presence of antioxidants may be a direct measure of the protective capacity of antioxidants.

Experimental: Measurements were done in a typical 3-electrode cell with a glassy carbon as the working electrode, Pt wire as the auxiliary and Ag/AgCl (3 M NaCl) as the reference. A programme of alternating potential step and hold, and ramp stages was used.

Results: Adding many antioxidants to a solution of guanine oxidised by superoxide generated *in situ* results in a steady decrease of 8-oxoguanine produced, as shown in Fig. 1, but some substances regarded as antioxidants demonstrated just the opposite property, under certain conditions, they promoted the oxidation of guanine, as can be seen in Fig. 2.

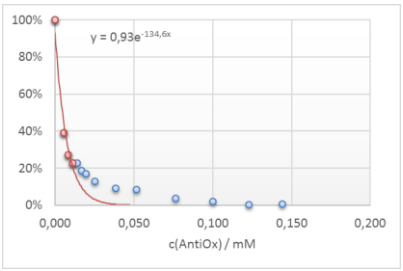

Fig. 1. Relative height of 8-oxoguanine oxidation wave as a function of gallic acid concentration at pH7

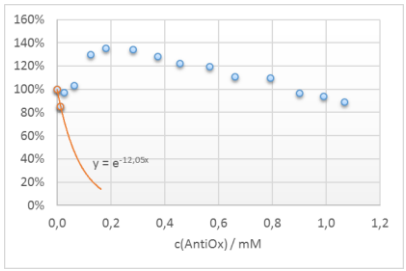

Fig. 2. Relative height of 8-oxoguanine oxidation wave upon addition of oxidised pyrogallol at pH9

Conclusions: The method developed enabled evaluation of antioxidative capacity, but also unexpectedly to show the opposite effect of some known antioxidants under certain conditions.

Key Words: antioxidants, guanine oxidation, 8-oxoguanine
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Insertional mutagenesis for selection of the yeast *Scheffersomyces stipitis* with enhanced ethanol production during alcohol fermentation of xylose and glucose

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Abstract

Purpose: The aim of this work was to develop a method of positive selection of ethanol overproducers from xylose and glucose, based on the usage of 3-bromopyruvate as selective agent. 3-bromopyruvate specifically inhibits key enzymes of glycolysis: hexokinase, pyruvate kinase and pyruvate decarboxylase, so the yeast cells resistant to 3-bromopyruvate should have intensified glycolysis and it may stimulate synthesis of ethanol during the alcoholic fermentation. The method of positive selection was combined with insertional mutagenesis.

Experimental: The yeast strains *PJH53 Scheffersomyces stipitis* was transformed with insertion cassette pUC19_HIS3 and transformants were selected on the medium without histidine and with 25 mM 3-bromopyruvate. One of the obtained insertion mutants with high ethanol production level was used for further work. Strain *Comp_TMI1* was obtained by complementation of *TMI1* gene disruption in strain #4.6 with vector containing native form of this gene.

Results: Among selected transformants, one (strain #4.6) revealed reproducible 2 or 1.5 fold increase of ethanol accumulation during xylose or glucose fermentation, respectively. In this strain, the insertion cassette integrated in the open reading frame of gene that is homologous to *YDL119C* gene of *Saccharomyces cerevisiae* that encodes mitochondrial transporter. We have proposed to call this gene *TMI1* (Transport in Mitochondria). The complement mutant *Comp_TMI1* has produced the same amount of ethanol during the alcohol fermentation of xylose and glucose as a wild type strain, and less than insertion mutant #4.6.

Conclusions: Obtained results revealed that Tmi1 is involved in the regulation of alcohol fermentation of glucose and xylose.

Key Words: Alcoholic fermentation, ethanol, insertional mutagenesis, *Scheffersomyces stipitis*
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Fucoxanthin and diadinoxanthin – anti-cancer biomolecules

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Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland:

1. *Department of Plant Physiology and Biochemistry,*
2. *Department of Cell Biochemistry,*
3. *Department of Physical Biochemistry,*
4. *Department of General Biochemistry,*

Abstract

Purpose: Xanthophylls are a group of ubiquitous pigments, which belong to carotenoids. Fucoxanthin (Fx) is one of the most important antenna xanthophylls in light-harvesting complexes of brown algae and most other heterokonts such as diatoms. In mammals it shows anti-cancer properties. Diadinoxanthin (Ddx) is another example of xanthophylls occurring mainly in diatoms where it participates in photoprotective diadinoxanthin cycle. No anti-cancer properties of Ddx have been tested until now. The aim of the presented work was to compare the influence of Fx and Ddx on the viability of colorectal cancer cells and normal human skin fibroblasts.

Experimental: Fx and Ddx were obtained from *Phaeodactylum tricornutum* using column and thin layer chromatographies. Both pigments were dissolved in ethanol and added to the cell cultures medium. MTT assay was used to determine cell viability.

Results: Both Ddx and Fx decrease the viability of colorectal cancer cells, however the effect of administering Ddx was more visible. Viability of human skin fibroblasts was not affected by the presence of pigments at the concentrations used in experiments.

Conclusions: Described pigments show anti-cancer properties, however this effect was greater when Ddx was used. Neither Fx nor Ddx are active against healthy human cells. Presented results bring a hope for developing new therapies against malignant tumors. Further studies are necessary to determine the molecular mechanism of anti-cancer effect of Ddx.

Key Words: anti-cancer; colorectal cancer; fibroblasts; MTT; nutraceutical; xanthophylls,
Acknowledgements: Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education.

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Quantification of CD25 (IL-2R) expression as a receptor-based tool to evaluate NK cell's cytotoxic activity

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Purpose: Cytotoxic activity is one of the major functions of Natural Killer (NK) cells and critical effector mechanism of innate immune responses against bacteria and virus infected or cancer cells. We can measure NK activity on the basis of their direct lytic effect on target cells, quantification of soluble cytotoxicity-related markers and cytokines, or indirectly be the estimation of viable target cells prior cytotoxic assay. Still the utility of CD25 marker as an indicator of cytotoxic activity has not been yet established. Since, NK cells express IL-2R we aimed to evaluate, whether the variations in CD25 (IL-2R) expression on human NK cells is correlated with their functional cytotoxic activity measured by classic methods and if this surface molecule might serve as a new tool to quantify NK cell activity.

Experimental: Peripheral blood mononuclear cells from 40 donors were isolated and cultured overnight with or without the standard E.coli O:55 lipopolysaccharide (LPS) (25ng/ml). Non-adherent lymphocyte fractions were used as effector cells towards HeLa targets in 4-hour cytotoxic assay measured by MTT reduction assay. Simultaneously lymphocytes were stained (30 min.,4°C) with murine anti-CD3-Cy-5, anti-CD25-PE and anti-CD56-FITC monoclonal antibodies (eBioscience) and analyzed by flow cytometry (LSR2, BD) to quantify CD25-positive cells within total NK cell population ($CD3\text{-}CD56^{+}$). Granzyme B, FasL (GenProbe), IFN-γ and IL-2 (R&D Systems) were measured in supernatants by ELISA.

Results: Our results revealed positive correlation between LPS-driven enhancement of NKs cytotoxic activity measured by MTT assay and the expansion of CD25-positive NK cells ($p<0.05$, $r<0.5$). Positive correlation was also observed between CD25+ NKs and the upregulation of granzyme B ($p=0.0003$), IFN-γ ($p=000.4$) and IL-2 ($p=0.0009$) ($p<0.05$, $r<0.5$) but not in regard to the FasL production ($p>0.05$, $r>0.5$).

Conclusions: This phenomenon is a result of LPS-induced NK cell activation, and according to our study, may serve as a surface marker of bacteria-activated NK cells.

Key words: Natural Killer cells, cytotoxic activity, CD25, MTT, cytokines.
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Preliminary identification of putative plasminogen-binding proteins isolated from *Candida parapsilosis* and *Candida tropicalis* cell walls

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Abstract

Purpose: The current study aimed at identifying the surface-exposed proteins of fungal opportunistic pathogens, *Candida parapsilosis* and *Candida tropicalis*, that interact with human plasminogen (HPG). As HPG is the key component of the fibrinolytic system, involved also in the regulation of complement cascade, its recruitment to the cell surface of microorganisms might highly contribute to their pathogenicity.

Experimental: *C. parapsilosis* and *C. tropicalis* surface-exposed proteins were extracted from the cell walls of pseudohyphae with β-1,3- or β-1,6-glucanase. The mixtures of released proteins were applied onto a HPG-coupled agarose gel and particular putative HPG-binding fungal proteins were eluted under reducing and denaturing conditions and then identified with LC-MS/MS.

Results: With the use of affinity chromatography several putative HPG-binding proteins have been identified for both investigated species. In the case of *C. parapsilosis*, the interactions with HPG occurred via two typical adhesins from agglutinin-like sequence proteins (Als) family and a few "moonlighting proteins", including malate synthase, amidase and 6-phosphogluconate dehydrogenase. And for *C. tropicalis* primarily transaldolase, fructose-1,6-bisphosphatase and 6-phosphogluconate dehydrogenase were indicated as the proteins that are presumably involved in binding to agarose-coupled HPG.

Conclusions: Because of the important role played by HPG in the maintenance of hemostatic balance in human organism, HPG-binding fungal proteins exposed at the pathogens' cell surface, might be considered as potentially useful targets for new therapeutic approaches applied during treatment of candidiasis.

Key Words: fibrinolysis, pathogenic fungi, candidiasis, adhesins, moonlighting proteins, affinity chromatography
Acknowledgements: This work was supported by the Jagiellonian University in Krakow (Statutory Funds of the Faculty of Biochemistry, Biophysics and Biotechnology No.BMN 29/2016 awarded to JKK).
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justyna.karkowska@uj.edu.pl

Modification of peptides for improved detection and quantification: hydrogen-deuterium exchange in betaine motif.

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Abstract

Purpose: The quaternary ammonium (QA) modification of peptides, combined with hydrogen-deuterium exchange (HDX) of α-C hydrogens in *N*,*N*,*N*-trialkylglycine residues provides interesting method of improved detection of peptides by mass spectrometry. The application for quantitative analysis of peptides using liquid chromatography coupled to mass spectrometry (LC-MS) would be possible only if the HD effect is negligible and the isotopologues co-elute.

Experimental: A series of five model peptides were synthesized and modified by formation of QA motives after reaction of N-terminal iodoacetate and N-methylmorpholine (NMM) or 1,4-diazabicyclo[2.2.2]octane (DABCO). The products were subjected to HDX, which occurs in 1% aqueous triethylamine and is not reversible under acidic conditions. The isotopologue mixtures of QA-peptides were analyzed using on-line reversed phase (RP) and hydrophilic interaction (HILIC) LC-MS. Mobile phase consisted of acetonitrile and aqueous ammonium formate (RP, HILIC), 0.1% formic acid or ammonium bicarbonate (RP).

Results: In all chromatographic experiments (RP HPLC, HILIC) we observed identical retention times for native and deuterated QA-peptides as well as preservation of isotopic distribution through chromatographic peak. LC-MS/MS experiments presented simplified fragmentation patterns due to initial localization of charge in ammonium groups.

Conclusions: The results suggest that HDX in methylene group of betaine analogues could be used for stable isotope labeling and generation of efficient isotopic markers for quantitative proteomics.

Key Words: quaternary ammonium salts, hydrogen-deuterium exchange, mass spectrometry, LC-MS, quantitative proteomics, isotopologues

Acknowledgements: This work was supported by grant UMO-2013/09/B/ST4/00277 from the National Science Centre, Poland.

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Biodegradable chitosan hydrogels with bioactive properties for wound dressings

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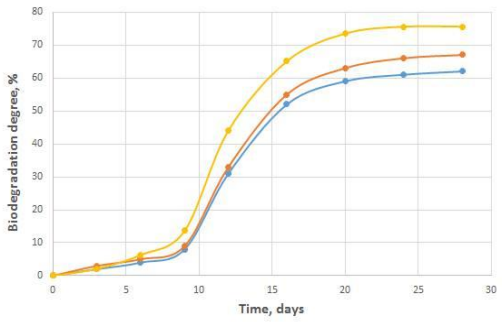
Abstract

Purpose: The aim of the research was to develop chitosan-based bioactive wound dressings with antibacterial properties according to Green Chemistry principles.

Experimental: For the hydrogels obtainment, 1 g of chitosan was dissolved in saturated aquatic solution of aspartic acid. Next step was to add 20 ml propylene glycol and the vessel was placed in microwave reactor Prolabo Synthewave 402 for 10, 15, 20 minutes respectively at 140 °C. Hydrogels were washed out from residuals and dried. Then sorption capacity was determined. Saturated solution of hyaluronic acid was prepared and adsorbed by chitosan gel. Test of antibacterial properties and bioactivity in Ringer's solution and SBF were performed. The curve of release of the active substance was prepared and biodegradation study were carried out by the Sturm Test method.

Results:



Conclusions:

Chitosan hydrogels were successfully obtained with sorption capacity of 5000%. Particles of hyaluronic acid were placed in polymeric matrix and released in a controlled manner outside the structure. Hydrogels were of antibacterial properties and exhibited bioactivity under laboratory conditions simulating human body. All samples underwent biodegradation process in at least 60%, the biodegradability degree was highly correlated with their crosslinking.

Key Words: chitosan, hydrogel, biopolymer, microwave radiation, Green Chemistry
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In vitro biocompatibility of electrodeposited chitosan and chitosan-carbon nanotube tubular implants – possible nerve regeneration materials

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Abstract

Purpose: Designing 3D tubular material chitosan biocomposites is still a challenging task. Especially considering a limited availability of such forms in nerve tissue engineering. Aiming at this problem, we use an electrodeposition phenomenon in order to obtain chitosan (CH) and chitosan–carbon nanotube hydrogel (CH MWCN) tubular implants tested for their *in vitro* biocompatibility.

Experimental: The *in vitro* cytotoxicity was assessed using a mouse hippocampal cell line (mHippoE-18) by standard MTT reduction assay supported by cell imaging by confocal microscopy. In the cytotoxicity assay tested samples fragments were incubated with 200 µL of hippocampal cell suspension (2.5×10^5/mL). Following incubation (24 h, 37 ^oC, 5% CO^2) MTT reduction assay was performed according to standard procedure. Optical densities were measured at a reference wavelength of 570 nm using a Multiskan EX microplate photometer. The experiment (including positive and negative controls) was performed in triplicates and four technical repeats. To determine the influence of biomaterial on cell morphology the samples were placed in the wells of 96-well glass bottom plate and hippocampal cells were gently seeded on materials (5×10^4 cells/well) and incubated for 24 h (37^{o}C, 5% CO_2). Next, each culture was carefully washed (5x) with PBS, fixed (10 min.) with methanol and stained (5 min.) with DiOC6(3) stain. Fixed monolayers were washed (5x) with PBS and the microscopic evaluation was performed with a Leica TCS SP8.

Results: The MTT reduction assay showed that there is no significant loss of cell viability after incubation with CH (84±27%) and CH MWCN (82±10%) implants. The obtained results are comparable to both negative controls of cytotoxicity. In contrast, cell layers treated with cytotoxic agent (1% phenol) exhibit significant loss of cell viability in comparison to control cultures (p=0.02). The confocal microscopy photos allowed measuring the length of axons, length and width of the cells as well as calculating the total number of their fibers. Cells cultured

in the presence of both implants have significantly longer axons (31±2 μm for CH and 82±37 μm for CH MWCN) than control cultures (27±1 μm), $p<0.01$). Similar observations were made for length and width of cells. The cells cultured in the presence of CH were characterized by 36±3 μm cell length and 16±1 μm cell width, whereas the environment of CH MWCN lead to 43±4 μm cell length and 16±1 μm cell width. These values were significantly higher than negative controls (length: 25±1 μm; width: 12±1 μm); $p<0.001$). The number of cell fibers for CH, CH MWCN, and control cultures remained equal.

Conclusions: As both implants did not induce significant cytotoxicity, and promoted axon elongation they were subjected to *in vitro* degradation studies and subsequent *in vivo* animal model experiments.

Key words: chitosan nanotubes, cytotoxicity assay, neuroregeneration, biocompatibility
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Fucoxanthin – algal biomolecule exerting beneficial health effects

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Abstract

Purpose: Fucoxanthin (Fx) is a carotenoid pigment, occurring in many marine photosynthetic organisms, including brown algae and heterokonts. It is found in the chloroplasts, where it acts as an accessory pigment, able to absorb visible light. This pigment was shown to possess many properties which qualify it as a potential pharmaceutical and diet supplement. These include anti-cancer, anti-inflammatory and anti-diabetic activities. This work comprehensively summarizes the recent advancements in elucidating the molecular and cellular functions of Fx, and presents new experimental data concerning the anti-cancer effect of Fx administered in liposomes on human colorectal adenocarcinoma cell line.

Experimental: Fx was isolated from *Phaeodactylum tricornutum* diatoms. The liposomes made of phosphatidylcholine containing 1 mol% Fx (four pigment concentrations) were administered into the cultures of colorectal adenocarcinoma cell line. The control cells received liposomes without Fx. Cell viability was assessed by the means of the MTT assay.

Results: Fx-containing liposomes lowered the viability of tested cancer cell line. This effect was the strongest at the highest tested concentration, where cell viability was significantly lower in the case of Fx-containing liposomes, than in the case of liposomes alone. At lower Fx and lipid concentrations, the differences between the effects of Fx-containing liposomes and liposomes alone were less pronounced. Liposomes without Fx also lowered cell viability to some extent.

Conclusions: In this experimental setup, Fx-containing liposomes exert anti-cancer effect on colorectal adenocarcinoma cell line. Dose-dependency can be observed. However, some of this effect may be attributed to the liposomes themselves.

Key Words: anti-cancer; carotenoid; colorectal cancer; fucoxanthin; MTT

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Identification of selected bovine plasma proteins at parturition

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Abstract

Purpose: The aim of present study was to define protein profile of bovine plasma 2 weeks before and after parturition and to compare it with parturition in order to find any markers of delivery.

Experimental: Plasma samples were obtained from healthy, pregnant cows (n=6) of Holstein breed two weeks before, at parturition and two weeks after parturition. Albumin was removed from plasma samples (Aurum Serum Protein Mini Kit, Bio-Rad), proteins were purified with the ReadyPrep 2-D Cleanup Kit (Bio-Rad). Isoelectric focusing was performed by use of IPG strips (3-10, 17 cm, Bio-Rad) in Protean i12 IEF System (Bio-Rad). The second dimension was made on 12.5% gels (1mm, 17x20 cm) according to Laemmli (1970) in Protean II XL (Bio-Rad). Gel staining was performed according to MS compatible silver staining protocol (Shevchenko et al. 1996). Statistical analysis of gels and spots was made using Delta 2D (Decodon). Statistically significant spots were cut, discolored and prepared for MS analysis (tryptic in gel digestion, purification with C18 ZipTip, Merck). MS and MS/MS was performed on Ultraflextreme (Bruker). Peptide identification was based on PMF with MASCOT software (Swiss-Prot 2016_08).

Results: Statistical analysis which compared the distributions of protein spots as well as the intensity of staining between 2 weeks before, 2 weeks after and the date of delivery revealed 998 spots including 98 with statistical significance (ANOVA $p<0.05$). 18 spots were already identified.

Conclusions: Preliminary study showed few proteins which differed significantly between examined periods of time. Peroxiredoxin -1, L-lactate dehydrogenase B chain and TIMP-2 could be proteins of interest. Whether they can be considered as true delivery markers requires further studies.

Key Words: cows, plasma protein profile, parturition

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