TIME-RESOLVED SPECTROSCOPY OF *Arabidopsis thaliana* LEAVES

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Light absorbed by plants is split into three major parts: photosynthesis, fluorescence, and heat. In this work we describe the results of time-resolved fluorescence spectroscopy obtained for Arabidopsis thaliana wild-type and recessive mutant *npq4-1*, deprived in the PsbS protein. Prior to the experiment parts of the plants (approximately halves of rosettes) were exposed to excess light at the wavelength of 620 nm. In the case of the mutant, the fluorescence decay exhibits drastic shortening, and measured transients are independent upon the excitation power. In addition, decays measured for non-illuminated parts of the plants are generally longer than for the illuminated parts. Wild-type plants feature much weaker response to the excess light. This result indicates a key role of PsbS protein in regulation of excess energy dissipation.

ACKNOWLEDGEMENTS

Financial support provided by the Welcome/2008/1 and Welcome/2008/2 projects awarded by the Foundation for Polish Science.

PROTEIN-QUANTUM DOTS CONJUGATES

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Colloidal quantum dots (QD) are crystals of semiconductor materials (e.g. CdSe or CdTe), of diameter from few to several nanometers. QDs are of special interest of (bio)nanotechnology because of its spectral properties - broad absorption spectrum, size-tunable and narrow emission spectrum and significant resistance for photobleaching [1]. Surface of quantum dots may be modified with other molecules. When biological molecules are used, the product is called nanohybrid. Application of proteins for modification of QD surface increase usefulness of hybrid, depending of the protein properties. Here we present covalent conjugates of QD with enzymatic protein, ferredoxin-$NADP^+$ oxidoreductase (FNR) [2], examining the changes in protein and QD properties by several techniques. FNR is photosynthetic protein, involved in electron transfer from photosystem I to NADPH or plastoquinone pool. FNR is also oxidoreductase of potential biotechnological application. We are showing that FNR-QD hybrid sustained its activity, however parameters of enzyme kinetics were impaired.

We also present noncovalent, stable conjugates of QDs and membrane-scaffold proteins (MSP) [3] as novel option for hydrophobic QDs solubilization. Application of MSP as a QD cover was possible due to amphipathic character of these proteins, usually used for formation of lipid nanodiscs. We developed a procedure, resulting in nanohybrids of monomeric QDs, stable for several days in water solutions as well as sustaining its fluorescent properties. Such methods may results in easier preparation of water-soluble QD-protein conjugates.

ACKNOWLEDGEMENT

The research was financed by National Science Centre grant No. N N202 130039. Research was performed in laboratories of NanoFun, POIG.02.02.00-00-025/09.

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ANTICANCER ACTIVITY OF NOVEL FERROCENYL-FLAVONE COMPLEXES

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Bioorganometallic chemistry focuses on the biological function of organometallic compounds. A special efforts have been dedicated toward ferrocene-containing anticancer agents. In this field compounds based on the conjugation of the ferrocenyl moiety with a biologically relevant molecule are of special interests. Recently conjugates of ferrocenes with flavonoids have focused the attention of scientists as a new promising class of biologically active compounds for medical application.

In this study we have evaluated the cytotoxic and cytostatic properties of novel ferrocenyl-flavone compexes (Fc): (*E*)-6-ferrocenylvinyl-chromen-4-one (4), (*E*)-6-ferroce-nylvinyl-2-methyl-chromen-4-one (5), (*E*)-6-ferrocenyl-vinyl-2-phenyl-chromen-4-one (6) and (*E*)-6-ferrocenylvinyl-chromen-4-one-3-propionic acid (7) against a range of human cancer cell lines derived from estrogen-responsive (MCF-7) and estrogen-negative breast adenocarcinoma (MDA-

MB-231), hepatocellular carcinoma (HepG2) and T lymphoblast-like polymorph cells (CCRF-CEM). The cells were exposed for 24-72 h to a range of Fc concentrations (0-120 μM) and the fraction of viable cells were estimated by MTT test.

CCRF-CEM cells were the most sensitive to investigated ferrocenyl-flavones showing the lowest IC_{50} concentration (37.5 ± 0.9 μM) for compound 4. The remaining cell lines expressed little response (IC_{50} > 120 μM). Prolonged exposure to low doses of Fc promoted HepG2 cell proliferation, which was an undesirable effect in the context of the assumed anticancer potential of these compounds. A different degree of ferrocenyl-flavone cytotoxicity and differences in their antiproliferative properties suggest both SAR (*structure activity relationship*) and the specific response of human cancer cells to these bioorganometallic compounds.

COMPARISON OF ANTIBODY BINDING WITH NATIVE *Proteus mirabilis* (S1959) O3 LIPOPOLYSACCHARIDE AND ARTIFICIAL EPITOPE LYS-GALA-PAA

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The focus of the presented studies is lipopolysaccharide isolated from *Proteus mirabilis* (1959) O3 strain, one of human opportunistic pathogens. The peculiar feature of O3 LPS is the presence of 6 N^{alpha}-(D-galacturonoyl)-L-Lysine residues recognized by human and rabbit antibodies (Kononov L.O., et al. Clycoconj J, 1991, Knirel Y.A, et al. Inn.. Immun. 2011). Immune complexes of rabbit antibodies with native O3 LPS and synthetic, artificial epitope Lys-GalA coupled to polyacrylamide (Lys-Gal-PAA) were tested by a label-free optical detection techniques. Two methods - Total Internal Reflection Ellipsometry (TIRE) and atomic force microscopy (AFM) were used. The TIRE experimental was set-up with SE 800 SENTECH spectroscopic ellipsometer operating in the spectral range of 280-850 nm. The lowest amount of LPS *Proteus mirabilis* (S1959) O3 and artificial epitope Lys-GalA-PAA that bind anti-O3 antibodies were 0.5 and 0.00195 mg/ml, respectively. The measurements of adsorbed immunocomplexes were carried out and ellipsometric parameters $\Psi(\lambda)$ and $\Lambda(\lambda)$ for different incident wavelength in a spectral range between 400 and 850 nm were obtained. The wave shift changes between phases, depending on the two antigens (O3LPS and Lys-GalA-PAA) were observed. Atomic force microscopy (AFM) experiments were carried out using Magnetic AC mode. The images were acquired at 20^{o}C in a PBS aqueous solution on the samples previously used for ellipsometric experiments. The RMS surface roughness was determined from AFM topography for the O3 LPS or Lys-GalA/-PAA and anti O3 rabbit serum immobilized on a gold surface. The measured values were 3.02 nm and 3.84 nm respectively and reflected the differences in a molecular structure of the components forming immunocomplexes. In conclusion, TIRE and AFM methods confirmed their efficiency in immuno-complexes studies.

ACKNOWLEDGEMENTS

This work was supported by grant from NRC N 304 275540.

THE REGULATION OF LIPOSOME AGGREGATION PROCESSES IN MOLECULAR CROWDING CONDITIONS FOR LIPOSOMAL TRANSDERMAL DRUG FORMULATIONS

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Liposomes as enhancers of transdermal drug delivery have been firstly described in 1980. Mezei (Mezei M., Gulasekharam V., Life Sciences, 1980, 26 (18), 1473-77) presented experimental data showing that the concentration of steroids in the skin was five times higher when liposomes were used in the formulation. Since then it has been demonstrated in numerous studies that liposomes can serve as a permeability enhancers for anesthetics, antibiotics and many other biologically active compounds.

Despite years of studies the exact molecular mechanisms of liposome permeability enhancement have not been determined. In order to correctly identify the liposome mode of action, the rarely used parameter needs to be considered, namely the molecular crowding. The high density of macromolecules, aggregates or polymers results in altered water activity therefore the state of biological structures can be modified and this in turn will change the activity of the compound of interest, mainly by altering its pharmacokinetic profile.

The dermis is a highly crowded space therefore the crowding effect needs to be accounted for regardless on the exact physicochemical nature of the compound-skin interaction. It has been shown, for example, that liposomes aggregate and fuse when dehydrated using highly hydrophilic polymers.

Having all that in mind, the method to measure the liposome (LUV) aggregation process induced by the macromolecular crowding is proposed. The method is based on the fluorescence resonance energy transfer (FRET) so the membrane fusion can be detected and quantitated. Using this method the liposome composition, which ensures their stability in crowded spaces

therefore making the liposomal formulation stable, has been determined. Controlling the aggregation process opens the door for designing the encapsulated compound release triggering mechanism, which would depend exclusively on the molecular density of the environment.

ACKNOWLEDGEMENTS

Presented works were co-financed by the European Union as part of the European Social Fund.

LUMINESCENCE OF UPCONVERTING Gd_2O_3: (Zn^{2+}, Er^{3+}, Yb^{3+}) NANOPARTICLES

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Gadolinium oxide (Gd_2O_3) due to its chemical stability, thermal stability, high melting point (~2320^oC) and low phonon energy (phonon cutoff 600 cm^{-1}) works very well as a host matrix for upconversion [1]. Low phonon energy decreases the probability of non-radiative relaxation thus increasing the quantum yield of upconversion process. The wide bandgap - 5,4 eV allows on easily doping with rare earths (RE) luminescence ions [2, 3].

Such RE doped material is, due to the high density (ρ=7.6 g/cm^3), suitable for use in X-ray detection and imaging. The properties of Gd_2O_3 make it applicable in contrast-enhanced magnetic resonance imaging (MRI) technique. RE-doped Gd_2O_3 opens up new perspectives for selective treatment of local tissues and for early diagnosis neoplastic diseases.

We synthesized Gd_2O_3 nanoparticles doped by Er^{3+}(1%) Yb^{3+}(18%) ions by the solution combustion method with adding Zn to starting materials. Entering of zinc ions into the Gd_2O_3 matrix introduces oxygen vacancies, leading to an increase of photoluminescence intensity due to reduction of the site symmetry of rare earth ions [4].

Transmission electron microscopy, scanning electron microscope and X-ray diffraction served for characterization of the studied nanoparticles. Using photoluminescence techniques we investigated the relationship between the intensity of emission and the excitation power, providing information on the amount of photons involved in the upconversion process. The quantum yield of photoluminescence was determined for Gd_2O_3: Er^{3+},Yb^{3+} nanoparticles as a function of Zn concentration in the starting materials and of excitation power. The highest quantum yield of the studied nanoparticles is 0.09% upon 980 nm excitation (continuous wave) for nanoparticles about 70 nm diameter.

Because of the good quantum yield this material is adequate for biomedical imaging. The nanoparticles were passivated with PVP and introduced into HeLa tumor cells. We examined their location inside HeLa cells for various incubation times and nanoparticles concentra-tions.

ACKNOWLEDGEMENTS

The research was partially supported by the European Union within European Regional Development Fund, through grant Innovative Economy (POIG.01.01.02-00-008/08) and by the grant from the Polish National Centre for Research and Development NR13004704, the Polish National Science Center NN 303 663 940, and NN 302 663 940 and the Center of Excellence. This work has been done in the NanoFun laboratories co-financed by the European Regional Development Fund within the Innovation Economy Operational Programme, the Project No. POIG.02.02.00-00-025/09/.

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THE EFFECT OF EXTRACT FROM PRIMROSE (*Oenothera paradoxa*) ON ERYPTOSIS INDUCED BY TERT-BUTYL PEROXIDE *in vitro*

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Oenothera paradoxa is a rich source of polyunsaturated fatty acids, particular linoleic acid. Human organism is not able to synthetize those acid, thus they must be supplied with diet. Because polyunsaturated fatty acids plays different functions in human organism

including regulation of various biochemical processes they are commonly used in the prevention of numerous diseases, which are associated with oxidative stress.

The purpose of the study was to assess if extract from primrose has protective role on eryptosis induced by tert-buthyl peroxide in vitro. The erythrocytes (5% hematocrit) where preincubated with extract from primrose at concentrations ranging from 5 to 20 mg/ml for 30 min. Then, the samples were washed and incubated with tert-butyl peroxide in the concentrations of 200, 300 and 400 μM.

Treatment of the erythrocytes with different concentrations of primrose or tert-butyl peroxide at 200 μM and 300 μM did not enhance eryptosis in comparison to control sample, where tert-butyl peroxide at 400 μM caused 10% eryptosis.

Preincubation of the erythrocytes with different concentrations of oil from primrose for 30 min and 1 hour incubation with tert-butyl peroxide at 200 and 300 μM did not cause eryptosis. However, the treatment of the cells with tert-butyl peroxide at 400 μM and different concentration of oil from *Oenothera paradoxa* induced 30% eryptosis. These results show that the oil from *Oenothera paradoxa* seeds synergistically with the highest concentration of tert-butyl peroxide

THE EFFECT OF EXTRACT FROM PRIMROSE (*Oenothera paradoxa*) ON HUMAN ERYTHROCYTES EXPOSED TO OXIDATIVE STRESS INDUCED BY TERT-BUTYL PEROXIDE in vitro

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Oenothera paradoxa is a rich source of polyunsaturated fatty acids. Human organism is not able to synthetize those acid, this they must be supplied with diet. Diet supplementation with oil from primrose has beneficial effects because it plays a essential role in the immune response reactions, with are associated with alterations of pro- and antioxidative balance. Oxidative stress occurs when reactive oxygen species formation exceeds antioxidative capabilities of the cell, with contributes do damage to cellular components.

The purpose of the study was to assess whether extract from primrose may protect cells from oxidative stress induced by tert-buthyl peroxide in vitro. The erythrocytes (5% hematocrit) where preincubated with extract from primrose at concentrations ranging from 5 to 20 mg/ml for 30 min. Then ,the samples were washed and incubated with tert-butyl peroxide in the concentrations of 200, 300 and 400 μM. In the study hemolysis, lipid peroxidation, methemoglobin formation the level of hydroksyl radical and catalase activity were assessed.

The conducted analysis showed that the oil from primrose did not cause any changes in hemolysis, lipid peroxidation, hemoglobin oxidation, catalase activity or hydroxyl radical level. Preincubation of the erythrocytes with extract from primrose and following incubation with tert-butyl peroxide at 300 and 400 μM, caused an increase of all parameters studied.

The obtained results show synergistic effect of the extract from primrose and tert-butyl peroxide in human erythrocytes.

EFFECT OF Ca^{2+} IONS ON THE ACTIVITY OF VACUOLAR ION CHANNELS IN *Physcomitrella patens* MOSS

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A majority of environmental stimuli cause an increase in the cytoplasmic calcium $[Ca^{2+}]_{cyt}$ concentration in plant cells. Changes in the concentration of this ion initiate a series of processes, including regulation of the activity of ion channels in cell organelle membranes. One of the largest organelles with calcium-dependent ion channels is the vacuole.

Previous research on vacuolar channels in higher plants indicates their high dependence on $[Ca^{2+}]_{cyt}$. SV and VK are among channels that open at high $[Ca^{2+}]_{cyt}$. Both types of channels are activated upon binding of Ca^{2+} to the EF motifs located on the cytoplasmic side of the vacuolar membrane (tonoplast). The dependence of SV and VK channels on varies $[Ca^{2+}]_{cyt}$. SV channels open at concentrations higher than 10 μM, whereas VK channels are active at concentrations of up to 5 μM. The activity of SV channels is additionally influenced by vacuolar calcium $[Ca^{2+}]_{vac.}$, whose increased concentration causes a decrease in channel activity.

Until now, there have been no investigations of SV and VK channels in the vacuoles of lower plants. The moss *Physcomitrella patens*, whose genome comprises protein coding sequences similar to the SV and VK channels in higher plants, is a model organism.

The investigations were carried out using the patch-clamp method at different concentrations of $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{vac}$. The measurement conditions applied facilitated simultaneous observation of the activity of the SV and VK channels. SV channels were shown to be more dependent on $[Ca^{2+}]_{cyt}$ than the VK channels, which opened in the absence of Ca^{2+} in the cytoplasm. This trait was not observed in the VK channels investigated previously in higher plants. Experiments performed in the absence of Ca^{2+} in the vacuole revealed increased numbers of active SV channels, which more likely to open in these conditions.

CHANGES IN THE BIOELECTRICAL POTENTIAL GENERATED IN *Arabidopsis thaliana* LEAVES AND STEM BY AN INJURY STIMULUS

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Arabidopsis thaliana is a model organism and a highly valued research material for investigations of excitability and transmission of the bioelectrical potential. Bioelectrical changes propagating over long distances include action potentials (AP) and variation potentials (VP). AP and VP differ from each other considerably, but both have a fundamental signalling role in plants [Fromm, Lautner 2007].

The present investigations were conducted on three strains of *Arabidopsis thaliana* ecotype Columbia: a wild-type strain (WT) and two strains with a knocked out gene encoding the potassium channel, i.e. AKT2-2 and Gork [Michard et al. 2005]. We used an extracellular method. The investigations were carried out in two experimental systems, in which electrodes were inserted into plant stem or leaves. An injury stimulus (burn) was applied, which evoked action and variation potentials recorded in all the analysed strains. The mean velocity of propagation of the bioelectrical changes elicited by leaf blade burning was 2 $mm \cdot s^{-1}$ (AKT2-2), 1 $mm \cdot s^{-1}$ (GORK), and 0,4 $mm \cdot s^{-1}$ (WT). The mean value of the amplitude of bioelectrical changed generated in the leaves was 85 mV (AKT2-2), 47 mV (GORK), and 40 mV (WT). The bioelectrical changes induced by burn injury in the stem of the AKT2-2 strain were primarily variation potentials. The mean amplitude of these changes was 65 mV and its propagation velocity was 2 $mm \cdot s^{-1}$.

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THE INFLUENCE OF FULLERENOL $C_{60}(OH)_{36}$ ON HUMAN PERIPHERAL MONONUCLEAR BLOOD CELLS

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The aim of this work was to assess the influence of fullerenol ($C_{60}(OH)_{36}$) on human peripheral mononulear blood cells (MNBC) under oxidative stress induced by hydrogen peroxide.

MNBC ($1x10^6$cells/ml) were incubated with fullerenol (75 mg/L or 150 mg/L) and/or H_2O_2 (0.5 mM). Cell viability by Trypan Blue assay, LDH release, caspase 3 activity, mitochondrial membrane potential with potentiometric dye $DiOC_{6(3)}$ as well as cytofluorimetric assay of size (FSC) and granularity (SSC) of MNBC were determined. On the basis of changes in the viability it was assessed that fullerenol was toxic to the cells but the decrease in viability was lesser than after H_2O_2 treatment. Fullerenol combined with H_2O_2 did not produce synergistic effect on MNBC. It was observed that $C_{60}(OH)_{36}$ neither influenced the LDH release nor caspase 3 activity. However, when MNBC were treated with fullerenol combined with H_2O_2 the caspase 3 activity decreased. Fullerenol at 75 mg/L did not influence the mitochondrial membrane potential of the cells, whereas fullerenol at 150 mg/L decreased the potential. Fullerenol at both concentrations protected from H_2O_2-induced mitochondrial potential decrease after 3-hour incubation. Fullerenol did not influence the size of MNBC whereas the cells granularity increased proportionally to the fullerenol concentration. Fullerenol combined with H_2O_2 induced the greater increase of the granularity of the cells than H_2O_2 itself.

In conclusion, fullerenol at 150 mg/L decreased the viability and mitochondrial potential of the cells and increased granularity of MNBC. Changes induced by fullerenol, especially at 150 mg/L, were comparable to changes induced by H_2O_2. However, fullerenol at both concentrations protected from H_2O_2-induced mitochondrial potential decrease.

APPLICATION OF INHIBITORS OF SELECTED METABOLIC PROCESSES FOR IDENTIFICATION BIOLOGICAL SOURCE OF BIOSPECKLE PHENOMENON IN APPLE TISSUE

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The biospeckle phenomenon – dynamic interference pattern formed by scattering of coherent light on living objects – is used in experimental, non-destructive methods of evaluation of fruit and vegetables quality. The physics of biospeckle is well-developed, but biological background of biospeckle of plant tissues is not clearly defined. Biospeckle activity (measure of biospeckle dynamics) is the result of movement inside the tissue, therefore physical (diffusion, Brownian motion) and biological processes (cytoplasmic streaming, organelle movement, cell division and growth) as a sources of biospeckle activity are considered.

The goal of this study was to investigate the effect of: cytochalasin B (CB), lantrunculin B (LB), colchicine (CO), cycloheximide (CY) and a mixture of ion channel inhibitors (ICI) as a substances non-

destructively and selectively influencing the processes associated with the movement in the cell, on biospeckle activity. CB inhibits polymerization and LB causes depolymerization of actin filaments, and both cease transport associated with the actin cytoskeleton as well as cytoplasmic streaming. CO prevents microtubule polymerization, stopping their reorganization. CY - an inhibitor of translation - blocks protein synthesis and can potentially reduce the number of emerging and moving scattering centers in the cytoplasm in the form of the protein. Since changes in intracellular ions concentration alter cytoplasmic streaming, an ICI, blocks the transport of H^+, K^+, $Ca^{2+}$and A^-, and can affect movements of cytoplasm and a number of secondary processes.

Results indicate that about 74% of biospeckle activity is caused only by biological processes and reconstruction of actin filaments and functioning of ion channels are a main sources of biospeckle activity in case of apple tissue.

HEMOLYTIC AND OXIDATIVE PROPERTIES OF METABOLITES AND IMPURITIES OF GLYPHOSATE

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The toxicity of herbicides is an issue of worldwide concern. Glyphosate [*N*-(phosphonomethyl) glycine] is used all over the world to protect agricultural and horticultural crops and it's not safe as it had been considered before. Poisonings still pose a challenge and problems for toxicological investigations.

The present study was undertaken to evaluate the toxic potential of a widely used glyphosate, its metabolites: aminomethylphosphonic acid, methylphosphonic acid and impurities: *N*-(phosphonomethyl)iminodiacetic acid, *N*-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. The analysis of noxious effects of metabolites and impurities seem to be very important to evaluate the toxicological risk that is exerted by these substances (EU regulations 1107/200/EC).

The erythrocytes were exposed to different concentrations of these compounds (0.01; 0.05; 0.1; 0.25; 0.5; 5mM) for 1 and 4 h.

We evaluated the effect of these compounds on hemolysis, hemoglobin oxidation and reactive oxygen species formation in human erythrocytes. Moreover, the changes in the size (FSC-A) and the shape (SSC-A) of red blood cells were assessed using flow cytometry.

It was proven that glyphosate at the highest concentration 5 mM during 4 h incubation changed the parameters examined in human erythrocytes, except FSC-A and SSC-A parameter. Glyphosate metabolites and impurities increased hemolysis (about 1 %) and methemoglobin level (about 1.5 %) but did not change the size and shape of the erythrocytes. The changes were observed only for the concentrations ranging from 0.5-5 mM. Most of the investigated compounds induced ROS formation from 0.25 mM (increase ROS level about 20%), except the *N*-methylglyphosate that caused changes from 0.5 mM. The investigated metabolites and impurities caused stronger damage to human erythrocytes than glyphosate itself after 4 h incubation.

BASIC HIPPOCAMPAL CELL RESPONSES TO VIOLOGEN-PHOSPHORUS DENDRIMERS

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Two zero generation (G0), water-soluble viologen-phosphorus dendrimers (VPD) were tested on murine hippocampal cells. VPD belong to the new class of dendrimers characterised by the presence of viologen moieties and phosphorus atoms in their structure. Several biological properties of this group of dendrimers have been already discovered, among others, the antibacterial activity, toxicity to B14, N2a cell lines and erythrocytes, as well as the effect on cholinoesterases involved in neurodegenerative diseases. Nevertheless, due to the lack of data on the influence of these new type of dendritic compounds on cell processes, we analysed chosen cell responses after 24 h treatment with five concentrations of VPD (1 - 20 μM). Performed tests comprised cytotoxicity assay, generation of reactive oxygen species (ROS), oxidative activity of mitochondria, mitochondrial membrane potential ($\Delta\Psi m$) alterations, and changes in catalase activity. The results revealed only small changes in cellular processes, indicating that VPD are only slightly toxic to mouse hippocampal cells. Interestingly, in contrast to other well-known classes of dendrimers, VPD seem to possess weak antioxidative activity, which may be very useful feature in the context of their potential biomedical applications. In general, these compounds can be considered good candidates for further studies.

VIOLOGEN-PHOSPHORUS DENDRIMERS DO NOT INDUCE CELL DEATH IN MOUSE HIPPOCAMPAL CELLS

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Viologen-phosphorus dendrimers (VPD), possessing both phosphorus atoms and viologen groups in their architecture, are new class of dendritic compounds. The biomedical potential of VPD is dependent on their cytotoxicity but so far, only MTT test was performed to examine the impact of these dendrimers on cell viability. For this reason, we performed a number of

tests to check if VPD induce apoptosis or necrosis, which included double staining methods, DNA fragmentation assay, cell cycle analysis, and assessment of cell morphology. We chose embryonic mouse hippocampal cell line (mHippoE-18) to analyse the cell condition after 24 h treatment with two zero generation (G0) VPD (VPD1 and VPD2). These two dendrimers differ in the core structure, the number of viologen units and surface groups. The results show that, at the tested range of concentrations, VPD caused only slight induction of apoptosis and alterations in the cell cycle phases distribution in mouse hippocampal cells, while no changes in the cell morphology were observed. These findings indicate that VPD can be considered as relatively safe compounds, useful for further biomedical investigations.

EVALUATION OF THE OXIDATIVE PROPERTIES OF HYBRID NANOSPHERES IN HUMAN BREAST CANCER CELLS

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Development of novel and effective non-toxic compounds with anticancer activity has been receiving a considerable attention because of the increasing number of deaths caused by cancer. Recently, the casein kinase CK2 has been shown to play crucial role in proliferation and apoptosis of cancer cells. Overexpression of this enzyme has been demonstrated as associated with the progression of cancer. Nanoparticles like polyoxometalates (POM) act as effective inorganic inhibitors of protein kinase CK2 and thus have ability to neutralize its activity. Little is known about other mechanisms of their activity in cancer cells.

This study aimed at evaluating the ability of PA66/POM hybrid nanospheres (POM clusters stabilized within non-toxic polyamide 66) to generate oxidative stress in cancer cells. For this purpose GSH, -SH groups and total antioxidant potential of estrogen-responsive MCF-7 breast cancer cells treated with PA66/POM have been estimated.

The cells were incubated with different amount of PA66/POM for 0, 24, 48 and 72 h and then subjected to analysis. Microplate spectrofluorimetric method with O-phthaldialdehyde (OPA) has been used for estimation of cellular GSH. OPA reacts with GSH which generates fluorescence allowing its specific quantification. Content of –SH groups was determined by the EPR spectroscopy using spin labeling with RSSR [bis(2,2,5,5-tetramethyl-3-imidazoline-1-oxyl-4-il)disulfide], a stable biradical nitroxide containing disulfide bond. The total antioxidant potential of cells was determined using 2,4,6-tripirydyl-s-triazine (TPTZ) method based on reduction of Fe^{3+} to Fe^{2+} in low pH conditions which yields formation of a colored complex with 2,4,6-tripirydyl-s-triazine (TPTZ). The investigation showed that PA66 and PA66/POM hybrid materials caused a time-dependent decrease in the level of all of the investigated parameters which could suggest the generation of oxidative stress in cancer cells by these nanomaterials.

ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY IN THE INVESTIGATION OF OXIDATIVE STRESS IN PLANTS

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Electron paramagnetic spectroscopy (EPR) is a technique used in the study of paramagnetic materials such as organic and inorganic radicals, transition metal ions and rare earth metal ions, appearing in solids and solutions. Due to the high sensitivity, EPR is widely used in biology. Not being a destructive method, it allows measurement of biological material without damage of the integrity and structure of the tissue. The aim of our experiment was the characteristics of EPR spectra of wheat grains, which show different sensitivity to oxidative stress and leaves of the seedlings obtained from these grains, cultured in stress conditions. Oxidative stress was induced in plants by differentiating the water uptake by root system by application in the hydroponic conditions of polyethylene glycol (PEG 6000) or NaCl.

EPR studies and elemental analysis of grains and seedlings showed the presence of Mn(II), Fe(III) and Cu(II) in higher contents in sensitive genotypes. Besides, in investigated tissues some stable organic radicals, localized in protein and carbohydrate structures were found. The content of radicals differentiated sensitive and tolerant wheat genotypes. The number of radicals in grains and seedlings of control plants (not stressed) of sensitive wheat genotypes was higher and increase in the stress conditions. The dependence between content of radicals and osmotic stress intensity was also established.

ACKNOWLEDGMENTS

This work was partly supported by grant of NCN, No: 2011/03/B/NZ9/00074

COUPLING OF GOODWIN'S LOOPS OF REPRESSION AND INDUCTION

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Circadian rhythms are generated in a cell by oscillatory systems in the net of transcription factors. Negative feedback loops constitute necessary components of such systems. We consider two coupled Goodwin's loops. The loop of repression has negative feedback and the loop of induction has a positive feedback. Both loops are coupled by a common promoter. Transcription of the two respective genes takes place simultaneously at high concentration of the inductor and low concentration of the repressor. We analyzed numerical solutions of the mathematical model in two kinds of systems. 1. Both loops have the same number of elements and have identical rate constants. 2. The loop of induction is shorter or longer than the loop of repression. Oscillations have the shortest period when the induction loop is by one or two elements longer than the loop of repression. The period increases and approaches to a constant value at longer induction loops. The oscillations are slower at shorter loops of induction. They are fully damped when the induction loop is by three or four elements shorter than the repression loop.

METAL-ENHANCED FLUORESCENCE OF AMPHOTERICIN B

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In this work we used confocal fluorescence microscopy to investigate the influence of plasmonic excitations in metallic nanoparticles on the fluorescence intensity of Amphotericin B (AmB), a molecule with important pharmaceutical function, in particular for serious systemic mycoses. For AmB molecules placed in the vicinity of silver nanowires synthesized in aqueous solution we observed strong increase of fluorescence emission when using 405 nm excitation wavelength. This excitation wavelength is resonant with plasmon excitation in the silver nanowires. We find that the emission intensity for the Am molecules located at the ends of the nanowires is stronger as compared to the AmB molecules located along the wires. Fluorescence decays measured for the AmB molecules coupled with silver nanowires remain unaffected by the coupling, suggesting increase of excitation rate as a source for the observed increase of fluorescence intensity. The results can be a starting point for further optimization of the design of a hybrid nanostructure composed of AmB and silver nanowires for achieving more efficient fluorescence detection and describing in detail molecular arrangement of AmB in biologically relevant architectures.

ACKNOWLEDGMENTS

Financial support provided by the Welcome/2008/2 project awarded by the Foundation of Polish Science.

EFFECT OF TIME ON LIPOSOME MEMBRANE FLUIDITY DOPED BY LIPOPOLYSACCHARIDES OF *Hafnia alvei* STRAIN PCM 1200: ESR STUDY

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Liposomes prepared from egg yolk lecithin (EYL) by sonication method were doped with lipopolysaccharide (LPS) of *Hafnia alvei* strain PCM 1200 in following concentrations: 16.8, 5, 0.5% in molar ratio to lecithin. To monitor the fluidity at different depths and different regions of the membranes two spin probes were used. Spin probe 2, 2, 6, 6-tetramethylpiperidine-1-oxyl (TEMPO) can freely diffuse in the membrane and provide information about both the water and lipid phases. Thus, the ESR spectrum of TEMPO in a membrane is a superposition of two components coming from TEMPO in water and in lipid phase. The relative extent of partitioning of TEMPO between the hydrophobic and hydrophilic phases can be measured from the ra-tio of signal intensities. In this study the partition coefficient F was measured. It varies as a function of fluidity of surface membranes over time. Spin probe 2-(14-Carboxytetradecyl)-2ethyl-4,4-dimethyl-3-oxazolidinyloxy (16DOXYL) was used to measure fluidity change in the deep hydrophobic region of the lipid bilayer. The data was obtained by calculating the rotational correlation time τ which various over time. Studies conducted for 80 hours gave following results:

1. surface membrane stabilizing effect was observed in all LPS concentrations;
2. the strongest surface membrane stabilizing effect was induced by 5% LPS;
3. 5% LPS induced also a very strong stabilization effect deep in the lipid bilayer.

Liposomes doped with 5% LPS formed very stable structure with low sensibility over time in studied 80 hours period. Due to their properties liposomes modified in such way may be used as drug carriers.

MICRO- AND MACRORHEOLOGY OF NEWTONIAN FLUIDS AND COMPLEX MACROMOLECULAR SYSTEMS – - COMPARISON OF DYNAMIC LIGHT SCATTERING, OPTICAL TWEEZERS AND ROTARY RHEOMETRY

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Rheological properties of substances used in pharmaceutical industry are essential for production process and functionality of the final product. In this paper, a comparison of techniques determining the rheological properties at macro and micro scale is presented. Dynamic light scattering (DLS), optical tweezers and rotary rheometry were used in order to determine viscosity coefficient of medium. DLS method uses tracking of nanoscopic objects of known size to determine viscosity of studied medium while optical tweezers uses thermal fluctuations of probe of known properties. These two techniques allow to determine rheological properties of medium in microscale whereas rotary rheometry enables to obtain information about global viscosity. Measurements were carried out for two substances which differ in viscoelastic properties: propylene glycol and aristoflex AVC. Propylene glycol was measured at concentration in a range 10–50% and aristoflex AVC in concentration range 0.05–0.35%. In DLS and optical tweezers measurements polystyrene nanobeads as probes were used. Diameters of nanospheres were 50, 100, 200, 500, 800, 1000, 1500 and 2000 nm. The viscosity coefficient of propylene glycol obtained with DLS and rotary rheometry were consistent with tabulated values. The viscosity values of artistoflex AVC obtained with rotary rheometry were different from values obtained with DLS and optical tweezers technique. Moreover viscosity coefficient determined with DLS using probes of different diameter were significantly different. The results provided information on both mechano-elastic properties of complex materials as well as their level of structuring at the microscale.

THE EFFECT OF THE DUAL FLUORESCENCE IN SELECTED 1,3,4 THIADAZOLES

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The spectroscopy researches of the new and biologically active compound (4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole (FABT) have been recently done. The compound is derived from the group of 1,3,4-thiadazoles. The compounds are characterized by antibacterial and antifungal features as well as they show a quite high level of the nerves protection and anticancer protection.

The researches which were done by using the fluorescence spectroscopy, allowed to observe the effect of the double fluorescence which was induced by the concentration of the hydrogen ions, the changes of the temperature as well as the aggregations effects in water environment. Regarding the analogical measurements which were done in methanol (as well as other solvents) only the single fluorescence was noticed.

After the process of the methanol acidification till pH 1, two separated, partly covered with each other bands were observed. Regarding the crystallography data as well as the fluorescence researches two types of the conformations FABT molecules were noticed: the conformation ”S” – type (including – OH group from resorcil ring which is placed on the side of the sulphur atom from 1,3,4-thiadazol ring. The “S” – type shows the single fluorescence band. The second type is the “N” – type with the same group as mentioned above, however, on the side of the nitrogen atom showing the double fluorescence.

The calculations which were done by using DFT methods show the differences in the energy between the two above conformations which is 3.2 kcal / mol. The rotary barrier in case of tested molecule, according to calculations is 12.6 kcal /mol. The analysis using oscillatory and spectroscopy method – FTIR as well as Roman method indicates that pseudo-hetero–aromatic system may have been created in the case of the “N” – conformation in FABT molecule.

This system may induce the setting inner molecular transfer of the CT load. Additionally this may led to the changes in chemical as well as biological features in the element. FABT molecules (as well as others from 1,3,4-thiadiazols group can be used as fluorescence probes which sensitive to pH changes and the polarization of the environment.

CHLORINATED PERSISTENT POLLUTANTS INDUCE APOPTOTIC ALTERATIONS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES (*In vitro* STUDY)

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Persistent Organic Pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health. In this study, we have assessed the effect of selected POPs, i.e. 1,2,4-trichlorobenzene (1,2,4-TCB), hexachloro-benzene (HCB), lindane and dieldrin on apoptosis induction in human peripheral blood lymphocytes.

The cells were incubated with xenobiotics for 2 h for ROS analysis and for 4 h for analysis of other parameters. Using fluorescent probe H_2DCFDA we observed an increase in ROS formation in lymphocytes incubated with all of the compounds examined in the concentrations range from 0.05 to 5 μg/mL.

Analysis of changes in transmembrane mitochondrial potential ($\Delta\Psi m$) was conducted using JC-9 fluorescent probe. It was noted that chlorobenzenes, and particularly lindane and dieldrin in the concentrations ranging from 0.2 to 10 μg/mL increased the number of cells, which were characterized by $\Delta\Psi m$ reduction. ROS formation and changes in mitochondrial membrane potential could have affected caspase-3 activation, which was observed in the lymphocytes incubated with all of the compounds studied, particularly with lindane and dieldrin in the concentrations of 5 and 10 μg/mL.

Changes in cell's membrane permeability (test with YO-PRO-1) and translocation of phospha-tidylserine (test with Annexin-V conjugated with fluorescein) were assessed to confirm apoptotic alterations in human lymphocytes. It was found that all compounds studied from 0.2 to 10 μg/mL increased the above parameters in the incubated cells.

The observed apoptotic changes in human lymphocytes provoked by relatively low concentrations of 1,2,4-TCB, HCB, and particularly lindane and dieldrin suggest that these compounds can disturb function of immunological system among people environmentally, and in particular occupationally exposed to these substances.

TOWARD THE STANDARDIZATION OF OBTAINING CRITICAL MICELLE CONCENTRATION, ENTHALPY OF MICELLIZATION AND MICELLE IONIZATION DEGREE FROM ISOTHERMAL TITRATION CALORIMETRY AND CONDUCTOMETRY MEASUREMENTS

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Micellization is one of the most widely studied phenomenons occurring in solutions of amphiphilic molecules. Thermodynamics of micellization is characterized first of all by the critical micellar concentration (CMC). It is a measure of stability of the micellar form of solutes, like surfactants or amphiphilic polymers, with respect to their monomeric form in solution. The basic thermodynamic functions of the micellization process: Gibbs free energy, enthalpy and entropy of micellization, are obtained from measurements directly or indirectly, being calculated from the values of CMC and other parameters like e.g. degree of micelle ionization for ionic amphiphiles. Among a wide variety of experimental methods used for study micellization processes the isothermal titration calorimetry (ITC) and conductometry (for ionics) are of special interest. ITC allow obtaining in one measurement CMC and enthalpy of micellization, and conductometry CMC and degree of micelle ionization. Micellization is not an abrupt transition in the solution properties, but rather some continuous process of formation of molecular aggregates from monomers. Therefore there is not unique definition of CMC, and the obtained values depend also on the experimental method used. Additional problem in determining CMC from measurements arise due to very diverse behavior of different compounds during micellization. In the present work an attempt is presented to standardize the method of determining CMC, enthalpy of micellization and degree of micelle ionization based on data obtained from ITC and conductometry measurements, by fitting some mathematical functions. The proposed approach is compared with methods used in literature.

DETERMINATION OF A GLASS-TRANSITION TEMPERATURE FOR SOME MAMMALIAN ALBUMINS

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The temperature at which the properties of material change from liquid-like to solid-like is called glass-transition temperature T_g. In the present paper T_g for human serum albumin (HSA), equine serum albumin (ESA), ovine serum albumin (OSA), porcine serum albumin (PSA) and rabbit serum albumin (RSA) has been obtained from viscosity measurements of aqueous solutions of the albumins and from the Avramov's model. The viscosity measurements have been performed with an Ubbelohde-type capillary microviscometer at temperatures ranging from 278 K to 318 K and over a wide range of concentrations. For each protein the viscosity-tempera-ture dependence, for a fixed concentration, is analyzed on the basis of equation resulting from the Avramov's model. The model gives three-parameter dependence of liquid viscosity on tem-perature, and one of those parameters is T_g. It appears that the glass-transition temperature of a solution, for each studied albumin, increases with increasing concentration. To establish the glass-transition temperature of a particular albumin, in turn, a modified Gordon-Taylor formula is applied. The formula shows that the glass-transition temperature of a solution depends on both T_g for a dissolved albumin $T_{g,a}$ and for water $T_{g,w}$, and on a parameter describing the strength of the albumin-water interaction k. The glass-transition temperature for pure bulk water is well-known and its the most frequently cited value is $T_{g,w}$ = 136 K. The quantities $T_{g,a}$ and k has been taken as adjustable parameters in a modified Gordon-Taylor formula. Thus obtained numerical values of the parameters are as follows: $T_{g,a}$ = (245.5 ± 3.8) K, k =

0.2616 ± 0.0294 for HSA at pH 4.7; $T_{g,a}$ = (245 ± 6.2) K, k = 1.473 ± 0.154 for HSA at pH 7.0; $T_{g,a}$ = (217.1 ± 4.3) K, k = 1.435 ± 0.159 for ESA; $T_{g,a}$ = (217 ± 6.1) K, k = 0.7702 ± 0.1198 for OSA; $T_{g,a}$ = (215.5 ± 5.8) K, k = 0.5707 ± 0.089 for PSA; $T_{g,a}$ = (215.4 ± 7.0) K, k = 0.6624 ± 0.1102 for RSA.

SOME HYDRODYNAMIC PROPERTIES OF HUMAN SERUM ALBUMIN IN SOLUTIONS AT ISOELECTRIC POINT

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Paper presents a viscometric study of human serum albumin (HSA) aqueous solutions at isoelectric point (pH 4.7). Viscosity measurements were made with an Ubbelohde-type capillary microviscometer for concentrations from 9.5 kg/m^3 up to 328 kg/m^3 and at temperatures ranging from 278 K to 318 K. The viscosity-temperature dependence, for each concentration, was analyzed on the basis of a modified three parameters Arrhenius equation. Each of those parameters increases with increasing of a solution concentration. Analysis of these relations showed that, for HSA in solution at isoelectric point, the activation energy of viscous flow E = 8.77 10^5 kJ/mol, the entropy of the process of viscous flow S = 5.58 10^6 J/mol K and the effective specific volume x=1.78×10^{-3} m^3/kg. Hydrated HSA molecule was approximated by an ellipsoid of revolution with one long semi axis a = 8.2 nm and two shorter semi axes b = 2.1 nm. Dependence of the proteins solutions viscosity on concentration can be quantitatively described by the Mooney equation. One of the parameters in these equation is the self-crowding factor. Its numerical value for HSA at isoelectric point is 1.74 and it lies in the range (1.35 - 1.91) which was originally obtained by Mooney. At low concentrations, the intrinsic viscosity and the Huggins coefficient were obtained. The intrinsic viscosity, which measures a contribution of albumin to the viscosity of the solution, decreases from 6.46×10^{-3} m^3/kg (5^{0}C) up to 6.29×10^{-3} m^3/kg (45^{0}C). The Huggins coefficient, in turn, increases from 0.775 (5^{o}C) up to 0.782 (45^{o}C). The existence of three ranges of concentrations: dilute, semi-dilute and concentrated, was proved. In the semi-dilute regime, the Mark-Houvink-Kuhn-Sakurada (MHKS) exponent was calculated. It slightly increases with increasing temperature from 0.333 (5^{0}C) up to 0.343 (45^{0}C).

VISCOMETRIC STUDY OF BOVINE, OVINE, AND RABBIT SERUM ALBUMIN IN DILUTE, SEMI-DILUTE, AND CONCENTRATED AQUEOUS SOLUTIONS

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The viscosity of bovine serum albumin (BSA), ovine serum albumin (OSA) and rabbit serum albumin (RSA) aqueous solutions has been measured at temperatures ranging from 278 K up to 318 K in the mono-disperse range i.e. in a range of concentrations up to 363 kg/m^3 for BSA, up to 317 kg/m^3 for OSA, and up to 300 kg/m^3 for RSA. The measurements were conducted with an Ubbelohde-type capillary microviscometer. A convenient method of data presentation, in the case of viscosity-concentration relationship, consists of using reduced variable [hc], where [h] is the intrinsic viscosity in m^3/kg and c is the solute concentration in kg/m^3. For each studied albumin and at each measured temperature the log-log plot of the specific viscosity h_{sp} versus [hc] gives a master curve, which shows the existence of three ranges of concentrations: dilute, semi-dilute and concentrated region. In the dilute and concentrated region the above mentioned plot is linear, and in the semi-dilute one is non-linear. In the semi-dilute region the relation between the relative viscosity and concentration can be described by Lefebvre's relation. One of the parameter in this relation is the Mark-Houvink-Kuhn-Sakurada (MHKS) exponent. In general, it is considered as an indicator of protein conformation in solution. The obtained values of the MHKS exponent change slowly from 0.35 ± 0.006 (5^{0}C) up to 0.353 ± 0.006 (45^{0}C) for BSA, from 0.355 ± 0.005 (5^{0}C) up to 0.349 ± 0.006 (45^{0}C) for OSA, and from 0.346 ± 0.006 (5^{0}C) up to 0.35 ± 0.006 (45^{0}C) for RSA. It indicates that for each studied albumin the MHKS exponent is, in the frame of error estimation, constant. The slope of the master curve in the concentrated region is an indicator of protein stiffness. It decreases from 6.64 (5^{0}C) up to 5.61 (45^{0}C) for BSA, from 4.91 (5^{0}C) up to 4.39 (45^{0}C) for OSA, and from 4.78 (5^{0}C) up to 4.26 (45^{0}C) for RSA. The above results shows that stiffness of the studied albumins decreases with increasing temperature.