SURFACE BEHAVIOUR OF BIOLOGICALLY ACTIVE MOLECULES

PhD dissertation

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Introduction

The living organisms are very complex structures. The compounds form complex dynamically changing network at different levels of organisation. The study of these multicomponent and interrelated system is very difficult, mainly if one is interested in a special process. For this reason, it is worth to establish simplified model systems, which are able to mimic the certain process involving fewer components.

The main part of biological processes takes place at interfaces which are the barrier between two phases. Material and energy transfer can proceed through interfaces. The interfaces may appear at molecular level (eg. surface of an enzyme), inside of the cell (eg. mitochondrium, lysosomes and other cell organelles in cytoplasma), between the cell and surroundings, or the higher level such the absorption through endothelium etc. The nature/character of the interface can influence that what kind of material can interact, adsorb, penetrate or transfer through the interface.

Aim

The aim of the present work was to experimentally model and investigate the interfacial behaviour of such systems where a compound with biological relevance is present at the fluid interface.

Surface activity of water soluble wheat protein extracts containing bioactive compounds were determined in the first part of the work. The adsorption of wheat proteins at air/water and air/dodecane interfaces, and also the rheological properties of adsorbed layer were compared to the corresponding data obtained for well defined proteins used as reference materials. The question was how it is possible to estimate surface activity and characterize rheological behaviour of wheat protein extracts which knowledge might help to reveal the possible correlation between the surface and biological activities.

Monomolecular lipid layer formed in Langmuir balance was used to investigate the interaction of antituberculotic drugs with cell membrane in the second part of the work. On the one hand, the membrane activity of a therapeutic agent isoniazid acid (INH), and its peptide conjugate (INH-redSer) was determined, to asses whether the conjugation may enhance the interaction of drug with lipid monolayer. Furthermore, polarity and lipofility of three new antituberculotic drug candidates (TB2, TB5 and TB8) were determined and compared to the results obtained in Langmuir balance experiments on the interaction with

lipid layer. On the basis of this evaluation the aim was to form a membrane affinity order and apply as a selection tool in the case of high number of drug candidates.

Surface of drug delivery polymeric system was modelled using a biodegradable polymer (PLA) layer modified with a block copolymer (Pluronic) in the last part of the work. The block copolymers proved to be effective in decreasing the hydrophobicity of PLA hence improving its biocompatibility. The aim was to compare the protein (bovine serum albumin) adsorption behaviour of hydrophobic PLA film and that of the hydrophilised, modified PLA films.

Experimental methods

The applied methods were used for characterization of fluid interfaces.
The surface activity at the air/water and dodecane/water interfaces and dilatational rheological behaviour of the adsorbed layers were determined on a pedent drop by axisymmetric drop shape analysis using the OCA15+ instrument (Dataphysics, Germany). The investigation of lipid+antituberculotic drug interaction, and also the study of polymer biocompatibility at air/solution interfaces were performed by forming monolayers using the Langmuir balance technique. Lipid monolayers at the water/air interface for *in situ* SFG studies were also formed in a Langmuir trough. The morphology of the one layer LB films of phospholipon alone or penetrated with INH-redSer or TB5 deposited onto solid mica substrate was visualized with atomic force microscope.

New scientific results

After the given thesis, a number indicates the publication in which the results are presented in details.

1. All of the wheat albumin samples studied show a substantial surface activity decreasing the surface/interfacial tension due to the adsorption of proteins at the air/solution or dodecane/solution interface. Among the six reference proteins the most powerful surface-active substance is BSA, and comparing to wheat protein samples, any of them proved to be even more effective in reduction of surface tension, that means they contain amphiphilic components with a high tendency to adsorption. At dodecane/solution interface comparing to

air/solution surface a higher interfacial activity was observed for all of the wheat fractions owing to molecular expansion unfolding in contact with at hydrocarbon phase.

(1)

2. Characterising the 20 wheat samples there are significant differences among some fractions. Fractions 1-5 proved to be more effective in reduction of surface tension than others which means those have the most hydrophobic character with the highest tendency to adsorb at the air/solution surface. For fraction 2 - with the highest surface and interfacial activity – was appointed that it contains highly hydrophobic, biological active compounds [Szanics, 2007].

(1)

3. The rheological behaviour of wheat protein fractions presents highly compressible films at both air/water and oil/water interfaces, resulting in very low dilatational modulus, ε. This behaviour of wheat proteins is quite unique and highly distinctive from the reference proteins studied here which produce a cohesive film with high ε, which develops fast owing to free expansion even for globular proteins. Wheat fractions might contain small, flexible molecules with weak molecular interaction.

(1)

4. Comparing the affinity of antituberculotic drug isoniazide (INH) and its peptide conjugate (INH-redSer) to lipid membrane monolayer model, I can conclude that conjugation of INH with the peptide chain substantially extended its penetration ability while the INH shows no affinity to lipid monolayer. The possible explanation for this result is that the hydrophobic part of peptide chain of INH-redSer molecules can incorporate into the lipid layer which enhances the possibility of permeation trough cell membrane. The Langmuir balance measurement is a promising method to asses the cell penetration ability of new drug candidates.

(4,5,6)

5. The polarity of three new antituberculotic drug candidates (TB2, TB5 and TB8) were characterized by octanol/water partiton as well as static and dynamic surface activities. It was found that the amphiphilic character grows in the range of TB8<TB5<TB2, which is in accordance with the range of lipofility and lipid membrane affinity obtained by Langmuir film

experiments. It is concluded that the results of lipofility and lipid+drug interaction measurements might be a promising selection method for the future assessment whether the drugs possess ability to interact with the biomembrane.

(2)

6. The results of surface analytical measurement were found to be in a good accordance with the tensiometric results. The sum-frequency *in situ* measurements provided evidence for that INH-redSer can interact and penetrate into lipid monolayer at the molecular level. I can infer that after the penetration of INH-redSer, the sum frequency signal is significantly increased compared to simple lipid monolayer. According to the AFM images of transferred LB films, there are significant difference in structure of pure lipid film and after penetration of TB5 or INH-redSer. The film of lipid is smooth but after the penetration of drugs the roughness increased considerably.

(5,6)

7. Interaction of bovine serum albumin (BSA) with poly(lactic acid) (PLA) layers mixed with poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) triblock copolymers (Pluronic) at air/solution interfaces was studied by the Langmuir balance technique. The protein adsorption to PLA layer is significant owing to the hydrophobic character of the polymer. A significant reduction of protein adsorption was observed by applying the Pluronic 6800 and 12700 with long hydrophilic PEO blocks. The relative block ratio of Pluronics allowing the partial solubilization and hence the hiding of the hydrophobic PPO block by PEO chains might play a role in its interfacial behavior.

(3)

The articles compose the basis of dissertation

1. K. Hill, E. Horváth-Szanics, Gy. Hajós, É. Kiss: Surface and interfacial properties of water- soluble wheat proteins
   Colloids and Surfaces A. 319, 180-187 (2008)

2. K. Hill, Cs. B. Pénzes, B. G. Vértessy, Z. Szabadka, V. Grolmusz, É. Kiss: Amphiphilic nature of new antitubercular drug candidates and their interaction with lipid monolayer
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3. É. Kiss, K. Dravetzky; K. Hill; E. Kutnyánszky; A. Varga: Protein interaction with Pluronic modified poly (lactic acid) Langmuir monolayer
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10. K. Hill, Cs. Pénzes, Sz. Bősze, K. Horváti, F. Hudecz and É. Kiss: Characterization of new antitubercular drug candidates-phospholipid interaction by Langmuir balance technique
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The posters compose the basis of dissertation

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2. K.Hill, Sz. Bősze, K. Horváti, F. Hudecz, É.Kiss:
Molecular interaction between lung surfactant and antibacterial peptide conjugates related to enhanced drug transport
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Molecular interaction between membane lipid and antibacterial peptide conjugates related to enhanced drug transport,
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