INSERTION OF BENZANTHRONE DERIVATIVES INTO MODEL MEMBRANES: LANGMUIR MONOLAYER STUDY

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Membrane physical properties are known to control a variety of biological processes, such as partitioning of proteins and peptides into lipid bilayer, membrane fusion, modulating the enzyme activity, just to name a few [1]. One powerful physical technique for detecting physical and chemical properties of model and biological membranes is based on the use of fluorescent probes [2-5]. Of special interest in this regard are newly synthesized fluorescent compounds. Along with increasing attempts to monitor the behavior of real biological systems, the models consisting of an insoluble amphiphile spread as a Langmuir monolayer and a second component (amphiphiles, proteins, polymers, dyes or pharmaceutics) dissolved in the subphase are becoming more and more fascinating. .The lipid monolayer, as one of the simplest models of biomembranes, represents a promising system for studying various processes including, in particular, lipid-protein or dye-lipid interactions at the molecular level. Despite a broad use of Langmuir monolayer technique, the exact mechanisms of dye-lipid interaction are poorly investigated. Therefore, in the present study the behavior of dimyristoylphosphatidylcboline (DMPC) monolayer affected by the penetration of novel benzanthrone derivatives, ABM, A8, A4, AM12 and AM4(2-3) has been addressed.

Lipid monolayers were formed at the air/water interface using a Langmuir tensiometer (DeltaPi, Kibron Inc., Espoo, Finland). The through was filled with 1.3 ml buffer solution onto which a lipid solution was spread and equilibrated. After addition of the dyes solution to the subphase the plots of surface pressure versus time were recorded to follow adsorption of benzanthrone dyes to the lipid layer at different initial surface pressures. Insertion of fluorescence dyes into DMPC monolayer at the air/water interface can be monitored as an increase in the surface pressure of the monolayer. The results

obtained indicate that the lower is initial surface pressure, the higher is $\Delta\pi$. After addition of the dye solutions to the subphase a sudden increase of surface pressure was observed during the first few seconds, followed by a smooth increase (for ABM and A4) or decrease (for A8, AM12 and AM4(2-3)) to the equilibrium value. Furthermore, ABM exhibited ambiguous behavior. At initial surface pressures above 20 mN/m, ABM is gradually adsorbed reaching the equilibrium in less than 1 min. In contrast, for initial surface pressures below 20 mN/m, a sudden increase was observed during the first few seconds after probe addition to lipid monolayer, followed by a smooth increase to the equilibrium value. Surface pressure increase/initial surface pressures dependencies are represented in Fig.1.

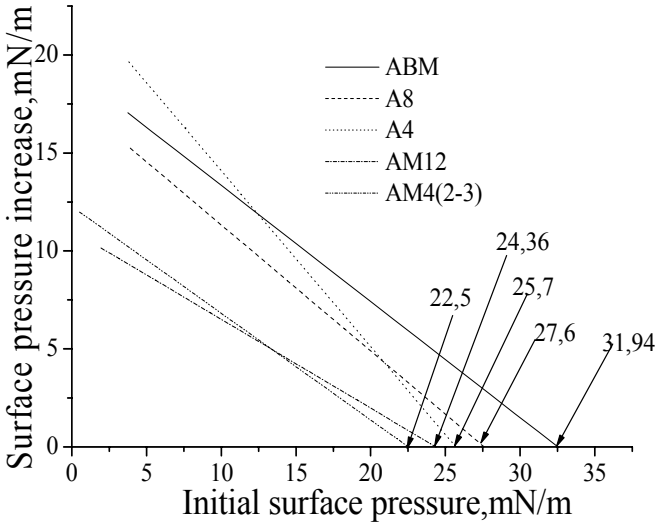

Figure 1 – Surface pressure increase under the influence of benzanthrone probes as a function of initial surface pressure. The maximum initial surface pressures at which the dyes under study can still cause an increase in surface pressure are varied from 22,5 for AM4(2-3) to 31,9 mN/m for ABM

To test the ability of newly synthesized benzanthrones to insert into membranes *in vivo*, we determined the maximum initial surface pressures at which the dyes can still cause the increase in surface pressure, a quantity also known as the "limiting surface pressure". The results obtained show that benzanthrone derivatives insert into DMPC monolayer with linear dependences of $\Delta\pi$ on the initial surface pressure (Fig. 1). The surface pressure of biological membranes is known to be ca. 30 mN/m. It is clear from Fig.1, that ABM possesses the highest ability to insert into DMPC monolayer. Furthermore, taking into account the fact that ABM limiting surface pressure exceeds the value for

biological membranes, we can assume that this probe is capable of inserting into lipid monolayer at physiological packing density, the property favoring the use of this dye in membrane studies. This assumption is fully supported by the results reported elsewhere [6].

After injection of fluorescent probes to the lipid Langmuir monolayer the dyes must migrate from the subphase to the air-water interface. Since aminobenzanthrone probes are uncharged, their adsorption at the air–water interface is most probably driven by van-der-Waals interactions and hydrophobic effect. The air/water interface acts as a hydrophobic surface and adsorption is dominated by the rate of probe reorientation. In addition, the results reported here indicate that the adsorption of benzanthrone derivatives at the air–water interface is unequivocally influenced by the packing conditions at the interface.

To summarize, the effects of benzanthrone aminoderivatives on DMPC monolayer at different initial surface pressures have been examined using Langmuir monolayer technique. It has been shown that the probes under study are capable of inserting into lipid monolayer. The results presented here provide additional evidence for the dye ability to insert into biological membranes *in vivo*.

This work was supported by the grants from European Social Fund (project number 2009/0205/1DP/1.1.1.2.0/09/APIA/VIAA/152), Fundamental Research State Fund of Ukraine (project number F.41.4/014).

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