X-ray spectral diagnostics of the local environment of zinc in the arachidic acid layers

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Abstract. The article describes the behaviour of the Zn ions in aqueous subphase in presence of arachidic acid films at the air/liquid interface first studied by XANES spectroscopy in the fluorescent mode under total external reflection conditions. Fingerprint analysis of experimental spectra was carried out to determine the local structure of zinc ions in arachidic acid. It showed that zinc is surrounded by oxygen atoms, which may correspond to the interaction of zinc ions with polar groups of arachidic acid.

1. Introduction

Interest in the study of ordered lipid monolayers is due to the fact that in terms of their composition and morphology they represent an adequate model of biological membranes [1]. Vitally important cellular processes depend on the controlled transport of metal ions across lipid bilayers. It is known that the biological lipid bilayer membrane contains a mixture of lipids, some of which alone in aqueous solution do not form bilayers at all [2]. In these cases, the Langmuir method on the air-water interface is the best model for studying lipid-metal interactions.

Investigations by Langmuir method on the air-water interface have shown that arachidic acid monolayers are sensitive to the inorganic ions in the subphase. The presence of inorganic ions in the subphase changes the ordering of surface molecules of arachidic acid monolayers at the air-water interface, leads to ordered or disordered properties of the fatty acid monolayers on the subphases of different inorganic ion solutions, different inorganic ions have different effects on surface-bulk partitioning of stearic and acid monolayers [3].

Arachidic acid is found in many body fluids (blood and urine). Inside the cell, arachidic acid is mainly found in the cytoplasm and membrane. It is known that the carboxyl group of arachidic acid can coordinate with metal ions from aqueous subphases for various types of bonds. The carboxylate anion can coordinate with metal atoms as a monodentate or polynodentate ligand. In a Langmuir monolayer on subphase, the carboxyl groups of arachidic acid can change the type of coordination with a change in the nature of the ions. Due to the high mobility of arachidic acid molecules, when the monolayer contacts metal ions, a structural reorganization of the entire monolayer occurs.

At present, a powerful arsenal of physicochemical methods has been developed for studying biochemical processes in cells. However, the study of the influence of trace elements on the development of diverse cellular processes remains a rather difficult task due to the fact that trace
elements are present in the body in small quantities. Along with such ions as Na, K, Ca the dynamics of zinc and iron across biological membranes is the least studied.

Unlike other methods, the X-ray absorption near-edge spectroscopy (XANES) techniques can be applied to almost any ion and any concentration.

XANES spectroscopy is very sensitive to the local chemical environment of the specific element, such as the nature of the chemical bonding, the number and type of ligands, metal-ligand distances, and the oxidation [4, 5]. The XANES measurement in the fluorescent mode under total external reflection conditions (TER) on the surface of liquid subphase is developing method that provides new possibilities for in situ monitoring of structure formation processes in two-dimensional nanosystems and studying objects with extremely low concentrations of absorbing atoms (for example, monolayers of metalloproteins and lipids) [6].

The presented studies are devoted to the study of the behavior of the Zn ions in present of arachidic acid films, organized in the form of a monolayer on the surface of an aqueous subphase in a Langmuir bath.

2. Methods

Arachidic acid solution was spread on the surface of subphase (aqueous solution of ZnCl$_2$) in a Langmuir trough. Arachidic-acid were purchased from Sigma-Aldrich Co. The concentration of ZnCl$_2$ in the solution was $6*10^{-5}$ M. The layer was compressed to a surface pressure of $\pi=20$ mN/m and kept constant during X-ray measurements.

The XANES measurements on arachidic acid films at the air/liquid interface have been performed at the bending magnet beamline LANGMUIR (Kurchatov Center for Synchrotron Radiation). The Zn K-edge XANES spectra were recorded in the fluorescent mode under TER conditions. The channel-cut monochromator Si(111) with a spectral width of $\sim 2$ eV has been used. The beam was tilted to the surface of the liquid using two flat mirrors. XANES measurements have been carried out at a fixed incidence angle of $0.8$ critical angle $\theta_C$. The depth of penetration of X-rays, which determines the sensitivity of the measurements, was $\sim 85$ Å. To ensure good statistics of XANES spectra, we applied a multipass method. Reproducibility of the energy position of the monochromator in multipass mode was determined to be within 0.18 eV. Total time of measurement was 20 h.

3. Results and discussion

A series of Zn K-XANES spectra in arachidic acid films at the aqueous solution of ZnCl$_2$ in the fluorescent mode under TER conditions were obtained (Fig. 1). As can be seen in the figure, a change in the shape of the XANES spectra after the first measurement was observed. All presented spectra have three main spectral features: the main maximum A, shoulder B and peak C. Over time, relative to the initial spectrum (solid), a shift of the main maximum A was observed from the energy 9665.7 eV by 1.2 eV to the left, while its intensity decreased and an additional shoulder A’ appeared (dot). The maximum B at an energy of 9679.4 eV increased in intensity, and an additional feature B’ appeared at the 9673.6 eV. A shift of the peak C from an energy of 9709.5 eV by 3 eV to the right and emergence of the peak D is observed. This can be explained by a change in the local environment of zinc ions in the presence of arachidic acid monolayer of over time. Subsequent measurement did not lead to significant changes in the spectrum (Fig.1, final spectrum).

As shown in [7] metal ions from the subphase can react with surfactant molecules and under certain conditions leads to the formation of metal nanoparticles under the monolayer. Thus, arachidic acid on the surface of NiCl$_2$ solution promotes appearance of nickel nanoparticles or their clusters [8]. Besides Zn$^{2+}$ ions give rise to considerable condensation effects for the fatty acid monolayers. In accordance with the above, we decided to test two hypotheses that could explain the observed changes in the spectra: the interaction of zinc ions with polar groups of arachidic acid molecules and the formation of nanoparticles or nanoclusters under a monolayer.
To get closer to answering that question the «fingerprint» method for interpretation of spectra was applied. Both experimental spectra were compared with the standard compound’s spectra (Fig. 2): ZnCl₂ solution, Zn(II) hexahydrate ion (Zn(OH)₆) and ZnO nanoparticle spectra. As can be seen from Figure 2, the initial spectrum has a shape similar to the ZnCl₂ solution spectrum. It indicates that zinc ions have not yet interacted with arachidic acid at the beginning of the experiment. Comparison of the spectra of the ZnCl₂ solution and Zn(OH)₆ showed that the Zn ions in the solution are surrounded by water molecules.

Significant differences in the shape of the final spectrum with the spectrum of ZnO nanoparticles (energy range 9650 - 9680 eV) indicate that the formation of ZnO nanoparticles at the interface is not observed, but the interaction of zinc ions with polar groups of arachidic acid may take place.

4. Conclusion
The behavior of the Zn ions in aqueous subphase in presence of arachidic acid films at the air/liquid interface was first studied by XANES spectroscopy in the fluorescent mode under TER conditions. The observed changes in the XANES spectra indicate a change in the local environment of zinc ions for different stages of measurements. Analysis of the Zn K-edges spectra local environment of zinc ions in the layers of arachidic acid was carried out using the «fingerprint analysis». It is shown that the interaction of zinc ions from the aqueous subphase with layers of arachidic acid on its surface is possible, and the formation of ZnO nanoparticles at the interface is not observed. Thus, the interpretation of X-ray absorption spectra makes it possible to obtain information on changes in the local environment of zinc ions and study the interaction mechanisms of the lipid layer with metal ions.

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