X-ray standing wave studies of molecular organization in lipid/protein films on the water surface

Introduction

Investigations of protein–metal complexes in biological systems have attracted great interest, because metals play an exceptionally important role in processes occurring in the human body. It has long been commonly believed that the most important biochemical functions in the organism are performed by sodium, potassium, magnesium, and calcium, which account for 99% of all metal atoms in the human body. However, in recent years, increasing attention has been given to so called microelements (iron, molybdenum, cobalt, copper, and zinc), which are present in the body in trace amounts.

In our studies the X-ray standing wave technique was used to analyze the elemental composition and molecular organization of ordered protein films based of hemoglobin treated by different toxic reagents (urea, chelating compounds). It was found that conformational rearrangements caused by toxic compounds are accompanied by a considerable increase in the ability of protein molecules to bind metal ions.

Formation of Protein Films of Hemoglobin on the Liquid Surface

Protein films were formed by the adsorption of the protein on a surfactant (octadecylamine) Langmuir monolayer deposited on the liquid surface. The following substances were used: the protein hemoglobin as a lyophilized powder (Sigma), urea (Sigma), and octadecylamine (Sigma).An aqueous solution of hemoglobin (9 mg/L) was placed in a Langmuir trough. Then a solution of octadecylamine in chloroform (0.5 mg/mL) was deposited onto the surface of this subphase. The octadecylamine monolayer was compressed to a surface pressure of 20 mN/m. The X-ray measurements were started within 40 min. The surface pressure of the layer was maintained constant during X-ray measurements.

X-ray fluorescence measurements of protein films formed on the surface of an aqueous subphase were performed at the beamline ID10. The incident and reflected beams were in the vertical plane. The characteristic fluorescence emission spectra were recorded for angles of incidence smaller than the critical angle for TERs of water. The VORTEX energy dispersive X-ray fluorescence detector was placed above the surface of the aqueous subphase at an angle of 90°. The energy of the incident beam was 13.4 keV.

X-ray fluorescence measurements

Changes in the elemental composition of the protein films of hemoglobin after the treatment with urea were studied in a series of X-ray fluorescence measurements under X-rat total external reflection (TER) conditions. It is well known that urea has a toxic effect on the human body. Thus, an increased urea level in the blood is characteristic of metabolic disorders in patients with chronic kidney failure. Hemoglobin was treated with urea according to the following scheme. Urea was added to a protein solution (the concentration of urea in the working solution was 0.09 and 0.17 M), and the incubation of the mixture was performed at room temperature for 12 h. It should be noted that the concentration of urea in the solution was fairly low (a urea solution with a concentration of ~8 M is commonly used to denaturize protein molecules).

Results

Figure 1 shows the characteristic fluorescence spectra of mixed protein films of hemoglobin after treatment with urea and of the reference thin film of hemoglobin without preliminary treatment. The spectra were recorded near the critical angle for TER of water, where the fluorescence yield intensity of the protein film reached a maximum. It is clearly seen that treatment with urea led to a considerable change in the elemental composition of the films. The spectrum of the reference thin film shows a peak of iron ions bound to the active sites of hemoglobin (Fig. 1a) and a weak peak of copper. In the spectra of thin films based on the protein treated with urea, the intensity of the peak of copper is substantially higher and, in addition, an intense peak of zinc appears (Figs. 1b, 1c). It should be noted that the intensity of the peak of Zn ions substantially increased when the protein was treated with a urea solution at a higher concentration.

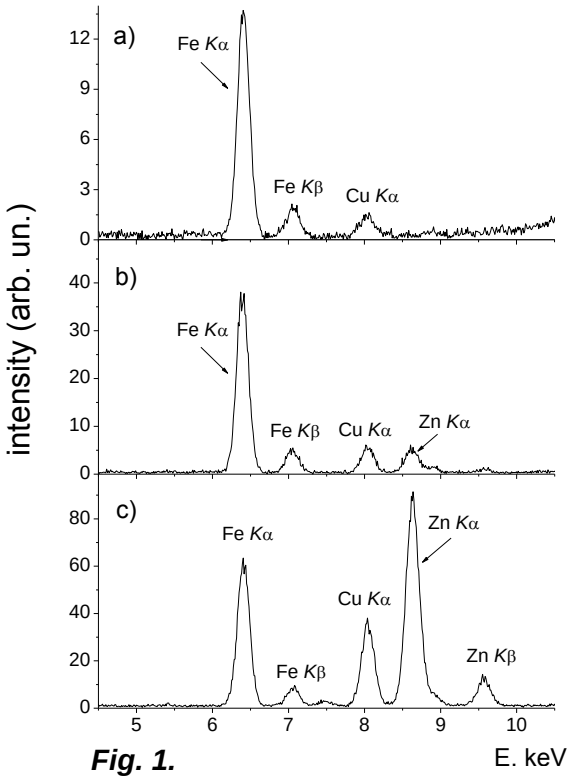

Fig. 1.

It should be emphasized that the protein solutions used in all measurements were prepared with high purity water (the resistance was higher than 18 MΩ/cm) produced by a Millipore Corp. purification system. The data on the changes in the elemental

composition of protein films can be explained by an increase in the accessibility of adsorption sites on the hemoglobin molecule due to conformational changes of the protein macromolecules after treatment with urea. Hemoglobin is the main oxygen transport protein in mammals. The protein consists of four globules of two types (two α subunits and two β subunit). Each subunit has a heme prosthetic group containing one iron ion. An increase in the adsorption capacity of hemoglobin after treatment with a urea solution is, apparently, attributed to the fact that treatment with urea leads to the loosening of the near-surface structure of the protein due to the breaking of structure forming hydrogen bonds, resulting in the extensive penetration of water molecules. This gives rise to a new conformational state of protein: the so called molten globule. The surface of the molten globule of hemoglobin contains a large number of aminoacid residues capable of binding metal ions, which are present in solution.

Data on the changes in the elemental composition of the protein films reported in the present study provide evidence of an increase in the ligand binding properties of proteins altered by endotoxins, which are accumulated in the body of patients with metabolic disorder diseases. Metabolic disorders in the body are responsible for a number of severe chronic diseases, primarily, atherosclerosis. In patients with metabolic disorders, pathological products of metabolism (homocysteine and intermediate molecules) and physiological products of metabolism in high doses (urea, uric acid, hormones, neuromediators, and peroxidation products), as well as various xenobiotics (including drugs), act as toxicants. These endogenous and exogenous factors can cause changes in the conformation of protein macromolecules, resulting in new adsorption sites becoming accessible, which, in turn, lead to a substantial increase in the ability of the protein to bind metal ions. These changes lead to the impairment of functional properties of protein molecules, and the latter become foreign to the body. In response to these foreign proteins (antigens) antibodies are produced in the body. These antigen/antibody complexes can deposit in tissues with the development of an immune based inflammatory process (vascular inflammation in atherosclerosis). In addition, protein molecules, which have an altered conformation and encapsulate microelements, can be excluded from physiological processes and be removed from the body through various excretory systems, in particular, with urine.

The substantial increase in the ligand binding properties of the protein molecules observed in the model experiments with the use of protein films indicates that it is necessary to revise the knowledge on the reasons for the violation of the microelement composition in particular organs and tissues of patients with metabolic diseases.