AFM investigation of alteration in lipid-protein model membrane caused by heavy ions

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In this study we report the visualisation by AF microscopy of damages caused by lead ions in phosphlipid bilayer and Ca^{2+}ATPase/phospholipid films. Calcium ATPase is an integral membrane protein that pumps Ca^{2+} ions across the membrane. The molecule fits in a box of 100Åx80Åx140Å. Investigation of the mechanism of alteration in model lipid/protein membranes by the heavy ions is of considerable interest because incorporation of heavy metal ions in the protein molecules (due to intoxication) might perturb the conformation of the proteins and, in some cases, inhibit their functions.

Phosphlipid bilayers. Bilayers of phosphotidylethanolamine PE (purchased from Sigma) were deposited onto the hydrophobic silicon substrate by vertical Langmuir-Blodgett technique. The first sample (control film) was transferred onto solid substrate from pure water subphase at a surface pressure of 40 mN/m. For the second sample PE monolayer was formed on water subphase containing $1x10^{-4}$ M acetate lead.

Protein/lipid films. Calcium ATPase was provided by Prof. A.M.Rubzov (Moscow State University, Russia). Monolayer of PE was formed on pure water subphase and compressed to a surface pressure of 40 mN/m. The hydrophobic silicon substrate was withdrawn through the interface with a spread monolayer. Ca^{2+}ATPase solution was deposited onto substrate (control film). After incubating the substrate with the Ca^{2+}ATPase solution for a 30 hours the substrate was washed with pure water and withdrawn again through a freshly spread monolayer of PE. For deposition of the second film Ca^{2+}ATPase was incubated in $1x10^{-3}$ mg/ml solution of acetate lead.

AFM investigations were carried out in air in tapping mode with the microscope P47-SPM-MDT (Russia, NT-MDT) and Si cantilevers NSC11 (Estonia, Mikromasch). Image analysis of PE bilayer transferred onto silicon substrate from subphase containing acetate lead (fig.1a) revealed that this film is not uniform (in contrast to control PE film), clearly visible are holes with size ranging from some 10 nm up to some 100 nm. A comparison of Ca^{2+}ATPase/phospholipid films showed that film morphology of the control film differs significantly from that of the film composed of Ca^{2+}ATPase molecules incubated in acetate lead solution (fig.1b). In the latter case the density of protein molecules bound to the phospholipid monolayer is much lower.

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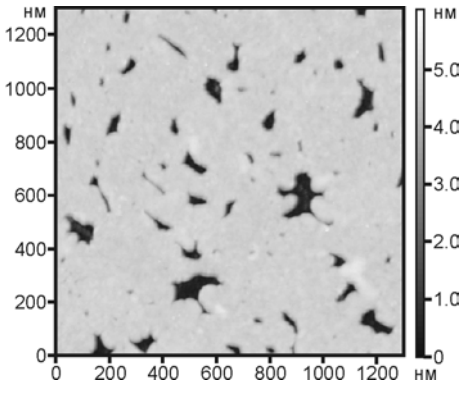
Fig



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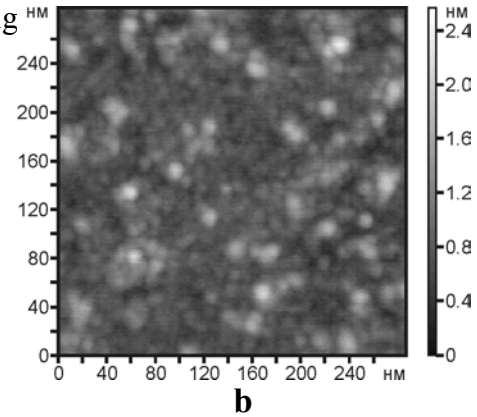

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