

INTERACTION OF AMYLOID LYSOZYME WITH LIPID MONOLAYERS

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It is becoming increasingly recognized that deposition of well ordered filamentous β -sheet-rich protein aggregates (amyloid fibrils) is a main factor responsible for pathological pathways in a number of human disorders, such as Alzheimer's, Parkinson's diseases, type II diabetes, etc. Numerous studies suggest that toxicity of amyloid species is related to their interactions with cell membrane. Despite extensive research efforts, modifying effects of toxic protein aggregates are still far from being fully understood. In view of this, the present work has been undertaken to shed light on the molecular details of interactions between amyloid aggregates of lysozyme and model lipid membranes. Using Langmuir monolayer technique, we tried to ascertain whether amyloid lysozyme fibrils possess an ability to insert into phospholipid monolayer composed of DMPC and DMPC:DMPG (40 mol. %). The reaction of lysozyme fibrillization was initiated by protein incubation under denaturing conditions (glycine buffer, pH = 2.2, 60 °C) during 8 days. The initial step of amyloid-lipid interaction, leading to membrane damage is the penetration of fibrillar protein into lipid monolayer, which can be traced as an increase of surface pressure. Prior to fibril injection, the monolayer was compressed to a target surface pressure (initial pressure). The addition of amyloid lysozyme into sub-phase resulted in the raise of the surface pressure for both types of the above monolayers. During about 10 minutes the monolayer was saturated with protein as judged from the presence of plateau in penetration kinetics. To determine the maximal initial surface pressure at which fibrillar lysozyme can still insert in the lipid monolayer (exclusion pressure), we performed experiments at four different values of initial surface pressure (π_0) and approximated the $\Delta\pi(\pi_0)$ dependence by linear regression. The obtained values of exclusion pressures were 32 mN/m for DMPC monolayer and 51 mN/m for DMPC:DMPG (40 mol. %). It is known that the packing density of lipids in biological membranes corresponds to surface pressure 30 mN/m. The results obtained indicate that amyloid lysozyme readily inserts into DMPC:DMPG (40 mol. %) monolayer under physiological conditions in contrast to DMPC monolayer. A significantly less exclusion pressure recovered for monolayers composed of zwitterionic lipids in comparison with negatively charged monolayer revealed that electrostatic interactions represent a dominant factor in the insertion of lysozyme fibrils into phospholipids monolayers.

Taken together, the results obtained allowed us to conclude that fibrillar aggregates of lysozyme efficiently insert into phospholipid monolayers. This work was supported by the grants from the President of Ukraine (VT, project number GP/F32/109) and Fundamental Research State Fund (project number F.41.4/014).