Binding of Lysozyme to Phospholipid Bilayers: Evidence for Protein Aggregation upon Membrane Association

Galyna P. Gorbenko,* Valeriya M. Ioffe,* and Paavo K. J. Kinnunen†

*Department of Biological and Medical Physics, V. N. Karazin Kharkiv National University, Kharkiv, Ukraine; and †Helsinki Biophysics and Biomembrane Group, Institute of Biomedicine, University of Helsinki, Haartmaninkatu, Finland

ABSTRACT Biological functions of lysozyme, including its antimicrobial, antitumor, and immune-modulatory activities have been suggested to be largely determined by the lipid binding properties of this protein. To gain further insight into these interactions on a molecular level the association of lysozyme to liposomes composed of either 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine or its mixtures with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-phosphatidylserine, or bovine heart cardiolipin was studied by a combination of fluorescence techniques. The characteristics of the adsorption of lysozyme to lipid bilayers were investigated using fluorescein 5′-isothiocyanate labeled protein, responding to membrane association by a decrease in fluorescence. Upon increasing the content of anionic phospholipids in lipid vesicles, the binding isotherms changed from Langmuir-like to sigmoidal. Using adsorption models based on scaled particle and double-layer theories, this finding was rationalized in terms of self-association of the membrane-bound protein. The extent of quenching of lysozyme tryptophan fluorescence by acrylamide decreased upon membrane binding, revealing a conformational transition for the protein upon its surface association, resulting in a diminished access of the fluorophore to the aqueous phase. Steady-state fluorescence anisotropy of bilayer-incorporated probe 1,6-diphenyl-1,3,5-hexatriene was measured at varying lipid-to-protein molar ratios. Lysozyme was found to increase acyl-chain order for liposomes with the content of acidic phospholipid exceeding 10 mol %. Both electrostatic and hydrophobic protein-lipid interactions can be concluded to modulate the aggregation behavior of lysozyme when bound to lipid bilayers. Modulation of lysozyme aggregation propensity by membrane binding may have important implications for protein fibrillogenesis in vivo. Disruption of membrane integrity by the aggregated protein species is likely to be the mechanism responsible for the cytotoxicity of lysozyme.