J Fluoresc (2007) 17:65–72
DOI 10.1007/s10895-006-0142-4

ORIGINAL PAPER

Tracing Lysozyme-Lipid Interactions with Long-Wavelength Squaraine Dyes

Valeriya M. Ioffe · Galyna P. Gorbenko · P. K. J. Kinnunen · Anatoliy L. Tatarets · Olga S. Kolosova · Leonid D. Patsenker · Ewald A. Terpetschnig

Received: 21 April 2006 / Accepted: 13 October 2006 / Published online: 17 November 2006

Abstract The applicability of the two newly commercial available squaraine labels Square-670-NHS and Seta-635-NHS to exploring protein-lipid interactions has been evaluated. The labels were conjugated to lysozyme (Lz) (squaraine-lysozyme conjugates below referred to as Square-670-Lz and Seta-635-Lz), a structurally well-characterized small globular protein displaying the ability to interact both, electrostatically and hydrophobically with lipids. The lipid component of the model systems was represented by lipid vesicles composed of zwitterionic lipids egg yolk phosphatidylcholine (PC) and 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC), and their mixtures with anionic lipids either beef heart cardiolipin (CL) or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), respectively. Fluorescence intensity of Square-670-Lz was found to decrease upon association with lipid bilayer, while the fluorescence intensity of Seta-635-Lz displayed more complex behavior depending on lipid-to-protein molar ratio. Covalent coupling of squaraine labels to lysozyme exerts different influence on the properties of dye-protein conjugate. It was suggested that Square-670-NHS covalent attachment to Lz molecule enhances protein propensity for self-association, while squaraine label Seta-635-NHS is sensitive to different modes of lysozyme-lipid interactions—within the L:P range 6–11, when hydrophobic protein-lipid interactions are predominant, an aggregation of membrane-bound protein molecules takes place, thereby decreasing the fluorescence intensity of Seta-635-Lz. At higher L:P values (from 22 to 148) when electrostatic interactions are enhanced fluorescence intensity of Seta-635-Lz increases with increasing lipid concentrations.

Keywords Squaraine label · Lysozyme · Liposomes · Protein-lipid interactions