Interactions between a permeabilizing biopolymer and a model membrane: insights from fluorescence lifetime correlation.

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Abstract

The cell membrane is a highly dynamic and pliable entity, whose structure and functionality depends on its interaction with numerous biomolecules. These interactions – which are crucial for the normal operation of the cell and also for the optimization of drug delivery strategies – can be studied using simplified systems composed of model membranes (i.e. supported lipid bilayers, SLB) and synthetic polymers.[1] Such simplified systems permit the fine tuning of key parameters such as the lipid composition of the SLB and the hydrophobicity of the synthetic polymers. Furthermore, the interactions can be modelled using simulation methods like, for example, the bond fluctuation method.[2] Here we present a study of the interactions between a SLB and PP-50, a synthetic biopolymer which facilitates the internalization of trehalose into human erythrocytes.[3]

The SLB was formed by disruption of large unilamellar vesicles onto ozone-treated glass coverslips. The SLB was composed of DOPC, as well as of two fluorescently labelled lipids: DOPE labelled with ATTO 655 and DOPE labelled with 2,6-Diethyl-1,3,5,7-tetramethyl-8-[(2-fluorophenyl)-6-methoxy-1,5-naphthyridine-3-N-hydroxysuccinimydyl carboxylate]-4,4'-difluoroboradiazaindacene (NapthBodipy). It has been reported that ATTO 655 is primarily located between the polar heads of the lipids and the buffer solution, whereas NapthBodipy is primarily located between the hydrophobic tails of the lipids.[4] The lipophilicity of the dyes determines their preferential location in the lipid bilayer. The ratio of labelled to unlabelled lipids was approximately 1×10^{-5}. The photophysical and diffusional properties of the system were studied by fluorescence lifetime correlation spectroscopy and by fluorescence lifetime imaging microscopy.

Upon addition of the polymer, the lateral diffusion coefficient (D_L) of DOPE attached to ATTO 655 (DOPE-ATTO) remained unaltered: before and after the addition of PP-50 the D_L values for DOPE-ATTO are 6.76 ± 0.05 and 6.61 ± 0.16 $\mu m^2 s^{-1}$, respectively. Contrariwise, the presence of the polymer significantly increased the D_L value of DOPE labelled with NapthBodipy (DOPE-Bodipy), from 5.66 ± 0.16 to 7.86 ± 0.36 $\mu m^2 s^{-1}$. Additionally, the presence of the polymer quenched the fluorescence of both dyes, with the highest quenching efficiency ($E_Q \approx 0.7$) being observed for DOPE-Bodipy. The aforementioned results suggest that PP-50 is preferentially located in the bilayer core, among the hydrophobic tails of the phospholipids. The presence of the polymer produces a small decrease in the fluorescence lifetime of the dyes, which is not sufficient to explain the observed quenching efficiencies. Hence, the value of E_Q must be primarily attributed to a static quenching mechanism, such as dye aggregation. Accordingly, the reduction in the average number of fluorescent molecules in the confocal volume correlates strongly with the value of E_Q.

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