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Influence of the curvature on the water structure in the headgroup region of phospholipid bilayer studied by the solvent relaxation technique

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Abstract

Solvent relaxation (SR) in 1,2-dioleoyl-palmitoyl-*sn*-glycero-3-phosphocholine (DOPC) unilamellar vesicles of different size was probed by 6-hexadecanoyl-2-(((2-(trimethylammonium)ethyl)methyl)amino)naphthalene chloride (Patman), 6-propionyl-2-dimethylaminonaphthalene (Prodan) and 4-[(*n*-dodecylthio)methyl]-7-(*N*,*N*-dimethylamino)-coumarin (DTMAC). Patman probes the amount and mobility of the bound water molecules located at the carbonyl region of the bilayer. Membrane curvature significantly accelerates the solvent relaxation process, but does not influence the total Stokes shift, showing that membrane curvature increases the mobility, without affecting the amount of water molecules present in the headgroup region. This pattern was also verified for other phosphatidylcholines. Prodan is located in the phosphate region of the bilayer and probes a more polar, mobile and heterogeneous environment than Patman. The influence of membrane curvature on SR probed by Prodan is similar, however, less pronounced compared to Patman. DTMAC (first time used in SR) shows a broad distribution of locations along the *z*-axis. A substantial amount of the coumarin chromophores face bulk water. No effect of curvature on SR probed by DTMAC is detectable.

Keywords: Patman; Prodan; Coumarin; Bound water; Time-resolved emission spectra (TRES); *t*(0)-Estimation

1. Introduction

It has been reported in recent work that a change in membrane curvature accompanies membrane fu-

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sion (Tamm et al., 2003), which many biological processes are based on. For instance, the formation of tubules and vesicles inside cells (Zimmerberg and McLaughlin, 2004), the formation of new organelles and enveloping of viruses (Huttner and Zimmerberg, 2001) as well as protein binding (Chao et al., 2002) might be realized via intermediates featuring "membrane bent structures". Efforts are taken to find possible mechanisms of these processes and the characterization of the physical properties of the deformed bilayer state appears to be a key task. Thus, we decided to exploit the advantages of the solvent relaxation (SR) technique and to monitor the changes of the water structure in the interface and headgroup region of phospholipid bilayers of different curvature. The SR approach in general gives direct information on the dynamics and polarity in supramolecular assemblies, in our case in particular regions of unilamellar vesicles. It inspects time-resolved emission (fluorescence) spectra (TRES) of probes, which are well defined located in these particular regions of the bilayer. Electronic excitation leads to an abrupt change in charge distribution of these dyes and is initiating the dynamic process of reorganization of the dyes microenvironment. This so-called solvent relaxation process leads to a dynamic Stokes shift of the TRES. The time course of this SR process is monitored through the observation of the position of the emission maximum frequency, $\nu(t)$, of the recorded TRES and contains information on the organization of the dyes microenvironment.

The important issue of this kind of study is to guarantee that the used probes are well defined located in the particular region of the bilayer. In our work, three dyes that fulfill the location criteria under the applied experimental conditions were chosen for probing the solvation dynamics in the headgroup and interface region. Firstly, the chromophore of Patman is located in the carbonyl region (Hutterer et al., 1996) where all the water molecules are bound to the functional groups (Sýkora et al., 2002a). In a series of applications of the SR method in bilayer characterization, the localization of Patman (Fig. 1) proved to be invariant to parameters like lipid composition (Hutterer et al., 1997a,b; Sheynis et al., 2003), temperature (Hutterer et al., 1996, 1997a), addition of ethanol (Hutterer and Hof, 2002) or protein binding (Hutterer et al., 1997b; Sheynis et al., 2003). Secondly, Prodan is located closer to the interface in the phosphate region (Fig. 1) where the solvation dynamics is faster than in the region probed by Patman (Hutterer et al., 1996; Sýkora et al., 2002a). In addition, the phosphate group is more hydrated, which results in high micropolarity of Prodan microenvironment. It has been recently shown that factors like hydrostatic pressure (Chong et al., 1989) or addition of ethanol (Hutterer and Hof, 2002) partially relocalizes Prodan to a more hydrated and less structured localization. However, in the herein investigated DOPC vesicles, the inspection of the TRES indicates that the Prodan molecules are located at or close to a single main location. Finally, we are using DTMAC, a coumarin-like dye. As already estimated from steady-state data (Epand et al., 1996), the SR data presented herein indicates that the chromophore of DTMAC is found to be located similar to Prodan at the interface region (Fig. 1). Since DTMAC is used in SR studies for the first time, a detailed description of the estimation of the Franck–Condon spectrum ("time-zero spectrum") of DTMAC in vesicles is given. The deformation of the bilayer was simulated by different dimensions of used vesicles, namely 20 nm large sonicated small unilamellar vesicles (SUVs) and 200 nm large extruded large unilamellar vesicles (LUVs). In case of LUVs, the difference between the ratio of the inner and outer surface is approximately 1, whereas in case of SUVs it falls down to 0.5, which naturally influences the packing of the bilayer. In order to reveal these effects, we decided to label either both membrane bilayer leaflets or the outer leaflet alone assuming that the selective labeling will affect the solvation dynamics in case of SUVs more dramatically than in case of LUVs.

2. Materials and methods

Probes 6-hexadecanoyl-2-(((2-(trimethylammonium)ethyl)methyl)amino)naphthalene chloride (Patman) and 6-propionyl-2-dimethylaminonaphthalene (Prodan) were purchased from Molecular Probes and were used without any further purification. 4-[(*n*-Dodecylthio)methyl]-7-(*N*,*N*-dimethylamino)-coumarin (DTMAC) was synthesized as described in (Sterk et al., 1997) and purified by HPLC. Lipids: 1,2-dioleoyl-palmitoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-oleoyl-palmitoyl-*sn*-glycero-3-phosphocholine (OPPC) and 1,2-palmitoyl-