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Lipid headgroups mediate organization and dynamics in bilayers

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ABSTRACT

We report on the fluorescence lifetime and anisotropy decay dynamics of the tethered chromophore NBD in unilamellar vesicles comprised of phosphoglycerol and phosphocholine lipids with C_{12} and C_{18} saturated acyl chains, with or without cholesterol and/or sphingomyelin. For the phosphocholine vesicles, we use the chromophore 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (NBD-PC), and for the phosphoglycerol vesicles, we use the chromophore 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (NBD-PG). The addition of cholesterol and/or sphingomyelin to the PC vesicles restricts the chromophore environment, in agreement with the known rigidizing effect of cholesterol on PC membranes. The PG systems do not exhibit an analogous effect with the addition of cholesterol and/or sphingomyelin. The motional freedom of the NBD chromophore is, in general, more restricted in the PC bilayers than it is in the PG bilayers, and we understand this behavior in the context of the role of the lipid headgroups in mediating bilayer organization.

1. Introduction

Plasma membranes are central to the function of mammalian cells. Despite the fact that a single mammalian plasma membrane may contain as many as 500 different lipid and sterol compounds, it has emerged that the distribution of these constituents within the membrane is not homogeneous [1–7]. Lipid bilayer membranes are heterogeneous, complex structures, exhibiting phase separation even in comparatively simple model systems. The existence of such phase separation has given rise to the notion of "lipid rafts" [2–5,8–20], and their presence and characterization in both model bilayers and plasma membranes are an active area of research. Although there is ample evidence of phase separation in two- and three-component systems, the existence of lipid raft structures in plasma membranes remains to be proven. We are interested in these heterogeneous bilayer systems to determine whether or not phase segregated regions in simple model systems can be used to support transmembrane proteins in their active forms. To address this issue, we have investigated organization and dynamics within one, two and three component model bilayer structures and have examined how organization and dynamics varies with the chemical functionalities present, in both the polar and nonpolar regions of the bilayers [21]. In this manuscript, we consider the role of the lipid head group identity in mediating the dynamics of chromophores placed in close spatial proximity to the bilayer head groups.

In the vicinity of the lipid bilayer head group region, the environment is dominated by the presence of water, and optical spectroscopic probes indicate that this environment is slightly more viscous than bulk water [22]. Despite the distinct structural differences between the polar and nonpolar regions of lipid bilayers, it is clear that each has a structural influence on the other, both in terms of the local motional freedom available to individual molecules, and in terms of longer range translational freedom. A key issue in understanding the organization of lipid bilayer structures is knowledge of how the polar phospholipid head groups interact with the aqueous environment in which they are in contact. The most widely used phospholipid family for such studies is the phosphocholines (PCs). The headgroups of these lipids are terminated by a trimethylamino moiety that carries a formal charge which is strongly solvated by water, but does not possess lone pairs which could hydrogen-bond extensively with surrounding water. The phosphoester functionality, however, does possess the lone pairs with which to hydrogen-bond with water. Phosphoglycerols (PGs), in addition to having the charged phosphoester functionality, also contain two hydroxyl moieties, enabling more extensive hydrogen bonding with water. The issue we are concerned with in this paper is whether or not the structural differences between the PC and PG lipid head groups give rise to significant differences in the dynamics of bilayers made from these lipids, and we interrogate the bilayer dynamics by means of a tethered chromophore.

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We use the chromophore 7-nitrobenz-2-oxa-1,3-diazole, NBD, a molecule that has been used extensively for interrogating biological and biomimetic systems [23–31]. This chromophore is characterized by a fluorescence spectral profile and lifetime that both depend sensitively on the polarity of its immediate environment [23–31]. In addition, the chromophore NBD is available with a variety of side-groups, or attached to lipid head groups or acyl chains, making it a particularly attractive choice for the study of lipid bilayers. We report here on the dynamics of NBD tethered to the acyl chains of PG (NBD-PG) and PC (NBD-PC) lipids. Our data show that in PC vesicles, the NBD chromophore exists in an environment that depends sensitively on the composition of the bilayer. Our infinite time anisotropy data, interpreted in the context of the hindered rotor model [32,33], indicate that the composition of the PC-based bilayer mediates the mesoscopic rigidity of the system, with the cone angle of the chromophore varying from ca. 60° for the pure lipid bilayer to ca. 10° for bilayers comprised of lipid, cholesterol and sphingomyelin. This effect has been seen before [34] and our data point collectively to the creation of molecular scale disorder and a mesoscopic increase in bilayer rigidity. The only structural motif consistent with these findings is that of a heterogeneous, phase separated bilayer structure. In contrast, when the analogous measurements are made for the PG vesicle systems, we find that the recovered cone angles and local freedom do not depend significantly on the presence of cholesterol and/or sphingomyelin. We also find that, in general, the tethered NBD chromophore exhibits more motional freedom (lower local viscosity) in the PG vesicles than in the PC vesicles. The most apparent differences between the PG and PC systems are the ability of the PG headgroups to hydrogen-bond extensively both among lipid headgroups and with surrounding water, and the charged nature of PG in contrast to the zwitterionic PC. The data we present here show that the identity of the lipid headgroup plays a significant role in determining the local environment of the tethered chromophore, and these differences are likely accounted for by differences in the nature of the interactions the lipid headgroup moieties are capable of experiencing.

2. Experimental

2.1. Materials

The fluorescent probes 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (NBD-PG) and 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (NBD-PC) were obtained from Avanti Polar Lipids, Inc. and were used without further purification. For the construction of unilamellar vesicles, the lipids 1,2-dilauroyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DLPG, mp = −3 °C), 1,2-distearoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DSPG, mp = 55 °C), 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC, mp = −1 °C), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC, mp = 55 °C), egg sphingomyelin (SPM) and powdered cholesterol were obtained from Avanti Polar Lipids, Inc. The vesicle constituents were prepared in a 1:1:1 mole ratio of phospholipid:cholesterol:sphingomyelin or a 2:1 mole ratio of phospholipid:cholesterol for the multicomponent systems. The tagged phospholipids in these systems were present at ca. 0.1 mol%. A total of twelve vesicle solutions were produced: (1) NBD-PG/DLPG, (2) NBD-PG/DLPG/cholesterol, (3) NBD-PG/DLPG/cholesterol/SPM, (4) NBD-PG/DSPG, (5) NBD-PG/DSPG/cholesterol, (6) NBD-PG/DSPG/cholesterol/SPM, (7) NBD-PC/DLPC, (8) NBD-PC/DLPC/cholesterol, (9) NBD-PC/DLPC/cholesterol/SPM, (10) NBD-PC/DSPC, (11) NBD-PC/DSPC/cholesterol, and (12) NBD-PC/DSPC/cholesterol/SPM. Chloroform was evaporated from the lipid solution, and the remaining solid was dissolved in a 4:1 benzene:methanol solvent system. The benzene:methanol solvent was then evaporated, and the resulting lipid mixture was dissolved in 10 mM Tris® buffer (Aldrich) adjusted to pH 8 in MilliQ water. For further mixing, the solution was put through five freeze-thaw-vortex cycles, where for each cycle the sample is placed in liquid nitrogen for 5 min, then in a hot water bath for 5 min, then vortexed for 2 min. A syringe-based mini-extruder from Avanti Lipids was used to extrude the lipid suspension through a polycarbonate filter 11 times to produce unilamellar vesicles of ∼100 nm diameter [11,22].

2.2. Steady state measurements

Absorption spectra were recorded on a Cary model 300 double beam UV–visible absorption spectrophotometer, with 1 nm spectral resolution. Emission spectra were recorded on a Spex Fluorolog 3 spectrometer at a spectral resolution of 3 nm for both excitation and collection monochromators.

2.3. Time correlated single photon counting measurements

All lifetime and anisotropy data were acquired using a time correlated single photon counting (TCSPC) system. This system has been described in detail elsewhere [22,35,36] and we recap only its salient features here. The source laser is a CW mode-locked Nd:YAG laser (Coherent Antares 76-S) that produces 30 W average power at 1064 nm with 100 ps pulses at 76 MHz repetition rate. The third harmonic (355 nm, 1 W average power) of the output of this laser excites a cavity dumped dye laser (Coherent 702-2), operating at 460 nm using Stilbene 420 dye (Exciton). The dye laser output is typically 5 ps pulses at a repetition rate of 3.8 MHz. Fluorescence signals are spectrally filtered using a subtractive double monochromator (American Holographic) and are detected using a Hamamatsu R3809U-51 microchannel plate photomultiplier tube (MCP-PMT) detector. The signals are processed using a Tennelec 454 quad constant fraction discriminator and Tennelec 864 time-to-amplitude converter and biased amplifier. For this system the instrument response time is ca. 35 ps fwhm. Fluorescence was collected at 0°, 54.7° and 90° with respect to the vertically polarized excitation pulse. Collection wavelength, polarization and data acquisition were controlled using National Instruments LabVIEW® 7.1 software.

2.4. Pressure-area isotherms

Pressure-area isotherms were generated using a Langmuir–Blodgett trough (Nima Technology Ltd. Model 612D). The lipid solutions were prepared at a concentration of 1 mg/mL in chloroform, with a 2:1 molar ratio of phospholipid:cholesterol and 1:1:1 phospholipid, cholesterol, SPM.

3. Results and discussion

We are interested in understanding the motional dynamics of the chromophores NBD-PG and NBD-PC in unilamellar vesicles comprised of phosphoglycerol and phosphocholine lipids, respectively (Fig. 1), with and without cholesterol and/or sphingomyelin. We are concerned not only with the dynamics of the chromophore, but also the specific region within the vesicles where the tethered chromophore localizes. For our experimental conditions, we have predominantly unilamellar vesicles, although it is possible that there is some contribution from multilamellar vesicles. We have shown previously that the molecular scale dynamics of imbedded