Chemistry and Physics of Lipids 160 (2009) 33–44

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Effects of ether vs. ester linkage on lipid bilayer structure and water permeability

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ARTICLE INFO

Article history:
Received 29 January 2009
Received in revised form 29 March 2009
Accepted 26 April 2009
Available online 3 May 2009

Keywords:
Ether lipid
X-ray
Structure
Water permeability
Lipid bilayers

ABSTRACT

The structure and water permeability of bilayers composed of the ether-linked lipid, dihexadecylphosphatidylcholine (DHPC), were studied and compared with the ester-linked lipid, dipalmitoylphosphatidylcholine (DPPC). Wide angle X-ray scattering on oriented bilayers in the fluid phase indicate that the area per lipid A is slightly larger for DHPC than for DPPC. Low angle X-ray scattering yields $A = 65.1$ Å^2 for DHPC at 48 °C. LAXS data provide the bending modulus, $K_C = 4.2 \times 10^{-13}$ erg, and the Hamaker parameter $H = 7.2 \times 10^{-14}$ erg for the van der Waals attractive interaction between neighboring bilayers. For the low temperature phases with ordered hydrocarbon chains, we confirm the transition from a tilted $L_{\beta'}$ gel phase to an untilted, interdigitated $L_\beta I$ phase as the sample hydrates at 20 °C. Our measurement of water permeability, $P_f = 0.022$ cm/s at 48 °C for fluid phase DHPC is slightly smaller than that of DPPC ($P_f = 0.027$ cm/s) at 50 °C, consistent with our triple slab theory of permeability.

1. Introduction

Ether lipids occur in biological systems primarily as plasmalogens, where the first carbon chain is attached to the glycerol backbone via an ether linkage, instead of the more common ester linkage as for the second carbon chain. Plasmalogens have been found in all mammalian tissues examined: nerve myelin can contain up to 52% plasmalogens of total phospholipids (O'Brien and Sampson, 1965). In ox heart, 45% of total phospholipids have ether linkages, primarily occurring in the mitochondria (Warner and Lands, 1961). About 15% of the phospholipids from human red blood cells are ether-linked lipids and this percentage is higher in white blood cells (Williams et al., 1966). Interestingly, ether lipids become elevated in tumor tissue by ~10% of the total lipid content (Snyder, 1972), which may signal macrophages to destroy them (Yamamoto and Ngwenya, 1987). In recent investigations of cancer treatment, synthetic alkyl phospholipids insert into the plasma membrane and kill tumor cells directly through apoptotic and non-apoptotic cell death and indirectly by causing interference in pathways that are critical for phospholipid survival (Mollinedo et al., 1997; Vink et al., 2007).

The effect of the ether linkage, compared to the ester linkage, on the structure and properties of lipid bilayers is of interest because it is a well localized chemical perturbation that affects physical properties. In order to focus solely on this specific linkage, many studies have compared dipalmitoylphosphatidylcholine (DPPC) which has two 16-carbon saturated chains attached to the glycerol backbone via ester linkages, with the model lipid, dihexadecylphosphatidylcholine (DHPC) which has ether linkages on both chains. One dramatic difference is that, whereas DPPC forms a conventional gel ($L_{\beta'}$) phase at room temperature, as DHPC hydrates it converts from a normal gel phase to a chain interdigitated ($L_\beta I$) phase (see Fig. 1) (Ruocco et al., 1985; Kim et al., 1987a; Laggner et al., 1987) and several hypotheses have been advanced for this phase transition (Laggner et al., 1987; Siminovitch et al., 1987; Hatanaka et al., 1997; Batenjany et al., 1997). The roles of headgroup hydration (Haas et al., 1990), headgroup orientation (Hauser, 1981), and membrane dipole potential and hydration force (Gawrisch et al., 1992) of DHPC have been investigated. Probes of DHPC have included changing pH (Furuike et al., 1999), changing hydrostatic pressure (Siminovitch et al., 1987) and binding to trehalose (Takahashi et al., 1997).

One of our main motivations for studying ether vs. ester linkages is to test a recent theory that proposes that the headgroup interfacial region is the major determinant of water permeability through membranes (Nagle et al., 2008). This theory was motivated by comparing structural and permeability data for five ester-linked phosphatidylcholines (Mathai et al., 2008). We now extend this database for this theory by changing the headgroup (our conven-

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doi:10.1016/j.chemphyslip.2009.04.003

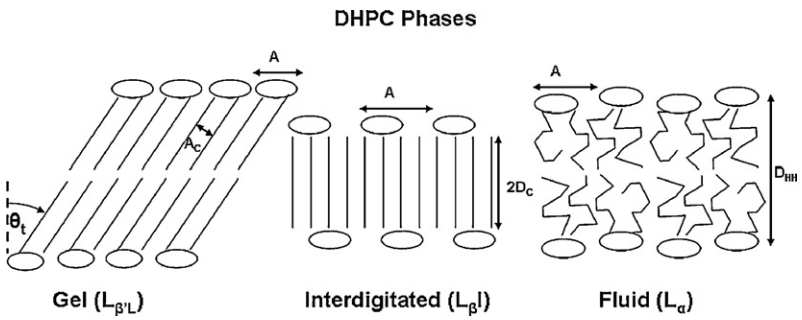

Fig. 1. DHPC phases: gel (partially dehydrated), interdigitated (fully hydrated) and fluid (fully hydrated, $T > 44\,^\circ$C). Important structural quantities such as chain tilt (θ_t), area/chain (A_C), area/lipid (A), hydrocarbon region thickness ($2D_C$) and head-to-head spacing (D_{HH}) are shown.

tion includes the phosphatidylcholine and the glycerol backbone as well as the ester vs. ether linkages in the headgroup) while keeping the chains (starting from the 2nd carbon) the same. Our structural work begins with conventional X-ray methods using both low angle lamellar scattering (LAXS) and wide angle scattering (WAXS) that confirm the room temperature $L_\beta I$ interdigitated structure and a partially dehydrated, $L_{\beta'}$ gel structure of DHPC in multilamellar samples. Enhanced analysis methods are used to refine these chain ordered structures. The fluid (L_α) phase structure of DHPC at 48 °C is obtained using modern analysis methods applied to LAXS diffuse X-ray scattering (Liu and Nagle, 2004) and to WAXS X-ray scattering (Mills et al., 2008). New results for the interactions between DHPC bilayers are also obtained. Both chain ordered and fluid phase structures are compared to our published structures of gel phase DPPC (Tristram-Nagle et al., 1993; Sun et al., 1996; Wiener et al., 1989) and fluid phase DPPC (Kučerka et al., 2006). The structural results are then compared to new measurements of water permeability through fluid phase DHPC bilayers.

2. Experimental

2.1. Materials and sample preparation

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, Lot 160PC-267) and 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine (DHPC, Lot 160DEPC-16 and 160DEPC-18) were purchased from Avanti Polar Lipids (Alabaster, AL) in the lyophilized form and used without further purification. Thin layer chromatography (TLC) using chloroform:methanol:7N NH_4OH (46:18:3, v/v/v) revealed < 0.1% lysolipid when stained with molybdic acid stain for DPPC and both lots of DHPC. HPLC grade organic solvents were purchased from Aldrich. Electrospray ionization mass spectroscopy verified that both lots of DHPC had the same molecular weight of (706 Da) and a purity > 99%. Optical rotatory dispersion (ORD) carried out by Dr. Steve Burgess at Avanti Polar Lipids detected ~20–30% of the D-isomer and ~70–80% L-isomer of DHPC in Lot 16, and ~100% L-isomer in Lot 18.

Four mg DHPC or DPPC was dissolved in 200 μl organic solvent (chloroform/methanol (3:1)) and then oriented onto 30 mm × 15 mm × 1 mm silicon wafers using the rock and roll technique (Tristram-Nagle, 2007), with the modification that for DHPC this technique was performed in an oven at 50 °C, since high temperature helped to orient this lipid. Hydration from water vapor was then carried out in a thick-walled hydration chamber (Kučerka et al., 2005) until the lamellar *D*-spacing was within 1–2 Å of its fully hydrated value.

Fully hydrated *D*-spacings were obtained from unoriented multilamellar vesicle (MLV) samples in excess water prepared by weighing 1–2 mg of dry lipid with 40 μl Milli-Q water and thoroughly mixed in small nalgene vials. These were vortexed and thermally cycled three times between 50 °C and −20 °C and loaded into 1 mm diameter glass capillaries. Large unilamellar vesicles (ULV) of ~60 nm diameter for structural studies were prepared in pure water by extrusion as described by Kučerka et al. (2005).

2.2. X-ray scattering experiments

X-ray data of oriented fluid phase DHPC at 48 °C were obtained at the Cornell High Energy Synchrotron Source (CHESS) using the G1 station on three separate trips on which the wavelength was set with a WB_4/C multilayer monochromater to 1.1797, 1.1808 and 1.2742 Å with a full width at half maximum of ±0.012 Å and the total beam intensity was $\sim 10^{11}$ photons/s. Beam widths were 0.2 mm; our preferred tall beam (0.6–1.0 mm vertical height) was unavailable on one trip when the beam had to be short (0.2 mm). The sample was ~10 μm thick along the normal to the ~2000 bilayers. Its dimension along the direction of the beam for LAXS was either narrow (5 mm) for use with the tall beam or wide (13 mm) for use with the short beam, and for WAXS the sample was narrow (4 mm). The flat samples were rotated from −3° to 7° in θ relative to the beam during the 30–60 s LAXS exposures and were X-rayed at fixed θ for the 10–20 s WAXS exposures. LAXS data from unoriented unilamellar vesicles (ULV) were also obtained as described by Kučerka et al. (2005) and exposure times were 60 s. For WAXS, $\theta = 0.2^\circ$ was used to first collect lipid scattering and then $\theta = -0.2^\circ$ was used to collect background chamber scattering that was subtracted from the lipid data (Mills et al., 2008). Data were collected by a Flicam CCD (Finger Lakes Instrumentation, Lima, NY) with a 1024 × 1024 pixel array and pixel size 69.78 μm/pixel. The sample-to-CCD distance was 400 mm for LAXS and 155 mm for WAXS. Temperature was controlled with a Neslab Controller (Portsmouth, NH) and monitored using a Cole-Parmer Thermistor Thermometer (Vernon Hills, IL).

MLV samples were X-rayed at 20 °C and 48–50 °C using a Rigaku RUH3R microfocus rotating anode (Woodlands, TX) equipped with a Xenocs FOX2D focusing collimation optic. Beam size was 1 mm × 1 mm and 20 min scans were collected using a Rigaku Mercury CCD detector with a pixel size of 68.0 μm/pixel; silver behenate ($D = 58.367$ Å) was used for calibration. A background capillary containing only Milli-Q water was subtracted from the lipid samples. Oriented gel phase data at 20 °C for both DPPC and DHPC (160DEPC-18) and WAXS data for DPPC at 50 °C were also collected using this X-ray setup. Temperature was controlled with a Julabo Controller (Allentown, PA) and monitored with a Cole-Parmer thermistor thermometer (Vernon Hills, IL).

2.3. Analysis of LAXS data from chain ordered phases

Intensities $I(h)$ of lamellar orders h were obtained from background subtracted discrete Bragg peaks. For oriented samples, an absorption correction was applied (Tristram-Nagle et al., 2002) and the usual Lorentz correction of q for oriented samples or q^2 for capillary samples was applied. Our detailed modeling analysis used a