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Revisiting the bilayer structures of fluid phase phosphatidylglycerol lipids: Accounting for exchangeable hydrogens

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ABSTRACT

We recently published two papers detailing the structures of fluid phase phosphatidylglycerol (PG) lipid bilayers (Kučerka et al., 2012 J. Phys. Chem. B 116: 232–239; Pan et al., 2012 Biochim. Biophys. Acta Biomembr. 1818: 2135–2148), which were determined using the scattering density profile model. This hybrid experimental/computational technique utilizes molecular dynamics simulations to parse a lipid bilayer into components whose volume probabilities follow simple analytical functional forms. Given the appropriate scattering densities, these volume probabilities are then translated into neutron scattering length density (NSLD) and electron density (ED) profiles, which are used to jointly refine experimentally obtained small angle neutron and X-ray scattering data. However, accurate NSLD and ED profiles can only be obtained if the bilayer's chemical composition is known. Specifically, in the case of neutron scattering, the lipid's exchangeable hydrogens with aqueous D_2O must be accounted for, as they can have a measureable effect on the resultant lipid bilayer structures. This was not done in our above-mentioned papers. Here we report on the molecular structures of PG lipid bilayers by appropriately taking into account the exchangeable hydrogens. Analysis indicates that the temperature-averaged PG lipid areas decrease by 1.5 to 3.8 $Å^2$, depending on the lipid's acyl chain length and unsaturation, compared to PG areas when hydrogen exchange was not taken into account.

1. Introduction

Lipid membranes play important roles in biological systems [1–4]. Because of this, solving the structures of fully hydrated lipid bilayers has been a longstanding goal of membrane biophysics [5–13]. In the case of biologically relevant membranes, thermal fluctuations preclude the determination of individual atomic positions [14,15]. Lipid bilayer structures are thus best described by statistical averages of atomic groups which exhibit similar characteristics, for example scattering density. Indeed, X-ray and neutron scattering have been extensively used to elucidate the average structures of flexible lipid bilayers [16–19]. However, each technique is only capable of resolving specific features of the bilayer. For example, X-ray scattering is sensitive to the headgroup's electron dense phosphate moiety [20,21], whereas neutron scattering is better suited to revealing the position of the glycerol/carbonyl backbone — due to its lack of hydrogen atoms. To exploit the strengths of each technique, the matter density-based scattering density profile (SDP) model was developed [22], which allows for the joint refinement of the different contrast neutron and X-ray data, as well as the inclusion of independently obtained volumetric data. However, SDP analysis requires that the bilayer's chemical composition is known. Specifically, in the case of neutron scattering, neutrons "see" deuterium (D) atoms (the heavy, stable isotope of hydrogen) very differently than they do hydrogen (H) atoms, as the coherent neutron scattering lengths for H and D atoms differ substantially in both phase and magnitude.

The terminal glycerol of phosphatidylglycerol (PG) lipids contains two OH groups which are capable of fast hydrogen exchange with the surrounding solvent, as demonstrated by nuclear magnetic resonance

Abbreviations: SDP, scattering density profile; SANS, small angle neutron scattering; SAXS, small angle X-ray scattering; MD, molecular dynamics; NSLD, neutron scattering length density; ED, electron density; vP, volume probability; PG, phosphatidylglycerol; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylglycerol; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol; SOPG, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol; DLPG, 1,2-dilauroyl-*sn*-glycero-3-phosphatidylglycerol; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylglycerol; DSPG, 1,2-distearoyl-*sn*-glycero-3-phosphatidylglycerol

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(NMR) studies [23]. The exchange between PG hydrogens and water has a profound effect on the neutron scattering length density (NSLD) of PG bilayers when in contact with water other than pure H_2O (*i.e.*, varying ratios of H_2O/D_2O). It is then obvious that accurate NSLD and electron density (ED) profiles can only be obtained if the bilayer's chemical composition is known, *i.e.*, exchangeable hydrogens are fully accounted for. This was not done in our previously published papers detailing the structures of PG bilayers [24,25]. Here, we report on the molecular structures of PG lipid bilayers, including the effects of exchangeable OH hydrogens. We find that when accounting for exchangeable hydrogens, temperature-averaged PG lipid areas decrease by 1.5 to 3.8 Å^2, compared to our previous analyses.

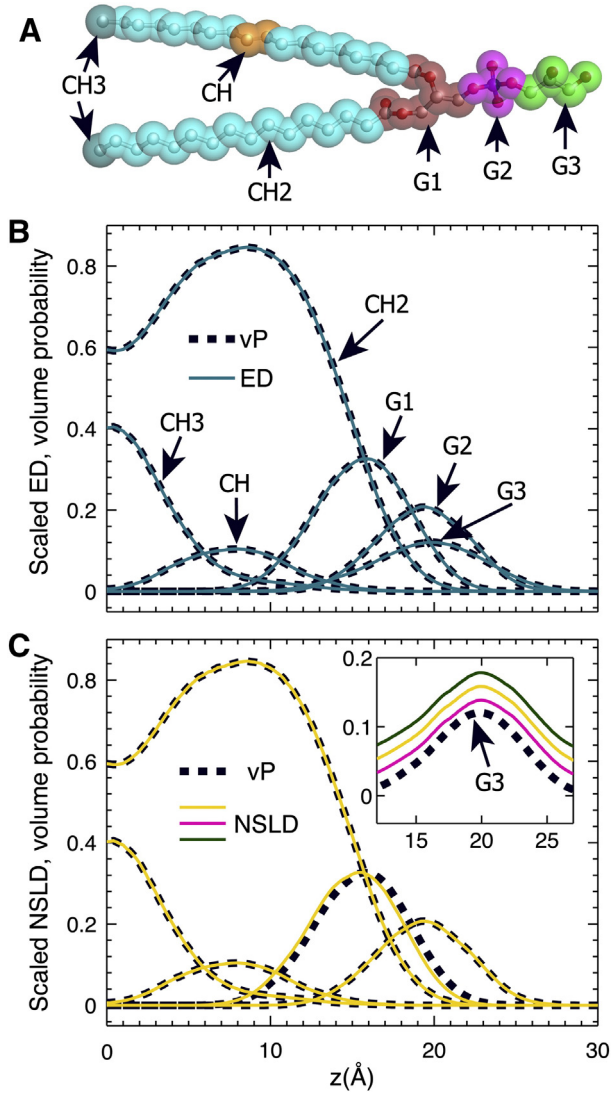

Fig. 1. Comparing component ED and NSLD profiles to their volume probabilities. Three sets of profiles were calculated from *NPT* simulations of a POPG bilayer with an average lipid area of 62.9 Å^2. (A) Parsing of POPG into six components, each is represented by semi-transparent spheres of different colors. (B) Component EDs (solid cyan lines) were scaled by the ratio of the component volume and electron number. Good overlap was obtained between EDs and the corresponding volume probabilities (vPs, dark dashed lines) for all components. (C) Similar to EDs, component NSLDs (yellow solid lines) were scaled to match their corresponding vPs (dark dashed lines), except in the case of the terminal glycerol (G3). The three G3 NSLDs (inset) were calculated assuming 100% (magenta), 70% (yellow) and 50% (green) deuteration of the two hydroxyls. G3 NSLDs for 50% and 70% OH deuteration are shifted vertically for better visualization when comparing to their vP (dark dashed line). It is clear that each component's NSLD and vP have similar centers and shapes. The only discrepancy is the position of G1, most likely the result of a biased distribution of hydrogens between the interfacial glycerol and backbone carbonyl moieties.

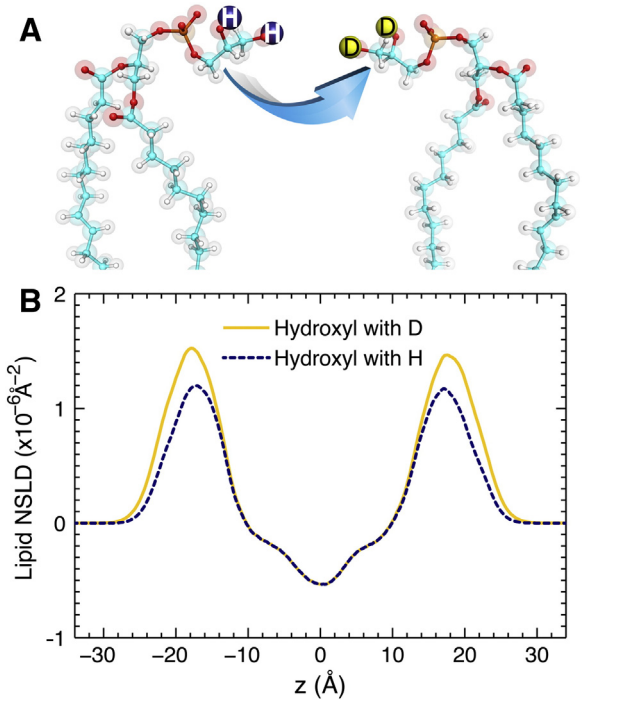

Fig. 2. The effect of hydrogen exchange on the NSLD of POPG. (A) A schematic of hydrogen exchange for the two hydroxyls on the PG headgroup. At 0% D_2O, two hydrogens (blue spheres) are associated with the hydroxyl oxygens. When 100% H_2O is exchanged for 100% D_2O, the two hydroxyl hydrogens are replaced by two deuteriums (yellow spheres). (B) NSLD of a POPG bilayer (water not included). The yellow solid line corresponds to a bilayer with deuterated hydroxyls and the blue dashed line corresponds to a bilayer with protiated hydroxyls. The two profiles were obtained from atom number density distributions (*NPT* simulations with a POPG lipid area of 62.9 Å^2) after being multiplied by their corresponding neutron scattering power.

2. Methods

To simultaneously refine small angle X-ray scattering (SAXS) and neutron scattering (SANS) data, an SDP model was developed for PG bilayers [24]. In this model, bilayer hydrocarbon chains were parsed into components made up of terminal methyl (CH3), methylene (CH2) and methine (CH) groups, and the headgroup was parsed into components encompassing the glycerol–carbonyl backbone (G1), phosphate (G2) and terminal glycerol (G3) groups (Fig. 1A). Such a parsing scheme enables ED and NSLD component profiles to be commonly described by component volume probability (vP) distributions. An example is shown in Fig. 1B. The dashed lines represent component volume probabilities calculated from constant number, pressure and temperature (*NPT*) simulations of a POPG bilayer with an average lipid area of 62.9 Å^2. Each component's ED (solid cyan lines) was scaled by the ratio of the component volume and electron number. Good overlap

Table 1
Structural parameters of seven PG lipid bilayers obtained from SDP analysis, where hydrogen exchange with solvent deuterium was accounted for. The units for lipid area *A*, overall bilayer thickness D_B, and hydrocarbon thickness $2D_C$ are in Å^2, Å and Å, respectively. The estimated uncertainty for each parameter is~2%.

	20 °C			30 °C			50 °C			60 °C		
	A	D_B	$2D_C$	*A*	D_B	$2D_C$	*A*	D_B	$2D_C$	*A*	D_B	$2D_C$
DLPG	60.2	31.4	21.8	62.1	30.7	21.3	65.3	29.5	20.6	67.1	28.9	20.3
DMPG	NA			62.5	33.8	24.5	66.0	32.6	23.7	67.5	32.0	23.4
DPPG	NA			NA			64.7	36.7	27.8	66.8	35.9	27.2
DSPG	NA			NA			NA			66.8	39.1	30.4
POPG	62.5	38.5	29.2	64.3	37.6	28.6	68.4	36.1	27.6	69.6	35.7	27.4
SOPG	62.9	40.2	31.0	64.3	39.6	30.5	67.6	38.1	29.5	69.0	37.6	29.2
DOPG	67.9	37.1	28.5	69.1	36.6	28.2	71.1	36.0	27.9	71.7	35.9	27.8