GENERAL FEATURES OF PHOSPHOLIPID CONFORMATION IN MEMBRANES

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1. Introduction

In contrast to the well-established conformational properties of proteins and nucleic acids no equivalent structural elements are associated with the phospholipid molecules in fluid bilayer membranes. Instead the hydrocarbon chains are usually represented by a confusion of entangled lines symbolizing the disordered nature of the bilayer interior. This is certainly an oversimplification. We have shown previously that distinct conformational constraints are imposed on the fatty acyl chains of bilayers composed of 3-*sn*-phosphatidyl cholines [1]. Still it could be argued that these results are unique for the particular class of phospholipids studied. Here we demonstrate that very similar constraints are obtained for zwitterionic 3-*sn*-phosphatidylethanolamine and the negatively charged 3-*sn*-phosphatidylserine. Furthermore, by using an appropriate normalization procedure the results obtained for the different lipids, including those in biological membranes, were found to agree even quantitatively.

Deuterium magnetic resonance (^{2}H-NMR) of selectively deuterated lipids has proved to be a sensitive technique to study the hydrocarbon chain ordering in lipid bilayers [1,2]. The method has been applied mainly to bilayers containing the phosphocholine head group, i.e. 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) [3,4], 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) [5,6], DPPC plus cholesterol [7], 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) [8] and egg-yolk lecithin [9]. In addition, a deuterium order profile has been published for *Acholeplasma laidlawii* membranes biosynthetically enriched with deuterated palmitate [10]. In this report we extend our ^{2}H-NMR studies to membranes with different headgroups, composed of either 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) or 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS). As a second new feature we normalize the deuterium order profiles by referring them to a reduced temperature $\Theta = (T-T_c)/T_c$ ($T \triangleq$ measuring temperature; $T_c \triangleq$ gel-to-liquid crystal transition temperature); T, $T_c \triangleq$ °K). The rationale being to eliminate all effects caused by differences in the gel-to-liquid crystal transition temperatures, which range from −5°C for POPC to 63°C for DPPE (DPPC 41°C, DPPS 51°C). At $\Theta = 0$ each bilayer is at its respective phase transition temperature. It is assumed that membranes which are measured at the same Θ value are approximately in the same physical state. The concept of the reduced temperature is widely used in the theory of phase transitions [11].

2. Materials and methods

DPPC was deuterated at the C-2′ position of both palmitic acyl chains as described earlier [3]. The corresponding DPPE was prepared from DPPC by phospholipase D reaction. DPPS was labeled at carbon atoms 2′, 3′, 6′, 10′ and 14′ again in both hydrocarbon chains. The synthesis of DPPS will be described in more detail elsewhere (J. L. Browning, in preparation). The lipids were generally dispersed in water to yield a final lipid-to-water (buffer) ratio of 50/50 wt/wt and ^{2}H-NMR measurements were made at 13.8 and 55.2 MHz as described earlier [3,6]. The deuterium quadrupole splittings, $\Delta\nu_Q$, were used to calculate the deuterium order parameter, S_{cD}, and the segmental order parameter, S_{mol} [3,5].

3. Results and discussion

The ^{2}H-NMR spectra of C-2′ deuterated DPPE and DPPS are shown in fig.1. Each spectrum consists of three quadrupole splittings with separations of about 14, 20 and 28 kHz. This spectral pattern is unique for the C-2′ segments of the hydrocarbon chains. However, it is a general property of the C-2′ segments of all phospholipids investigated so far, including the lipids of the *Acholeplasma laidlawii* membrane. The qualitative and quantitative similarity of the different lipid systems becomes obvious in fig.2 where the C-2′ deuterium order parameters of four synthetic lipid membranes are plotted as a function of the reduced temperature Θ. Due to the different phase transition temperatures of the various phospholipids, the actual temperature range in fig.2 extends from −5°C to 90°C. Nevertheless, all the data fell in rather narrow bands supporting the hypothesis of a similar physical state at a given Θ temperature. The assignments given in fig.2 are based on results obtained for DPPC and POPC labeled in one chain only [4–6]. For both lipids the *sn*-1 chain gives rise to the largest quadrupole splitting in the spectrum while the *sn*-2 chain produces the two smaller splittings. Figure 3 summarizes the variation of the molecular order parameter, S_{mol}, with the position of the label in the *sn*-1 fatty acyl chain. Again the order

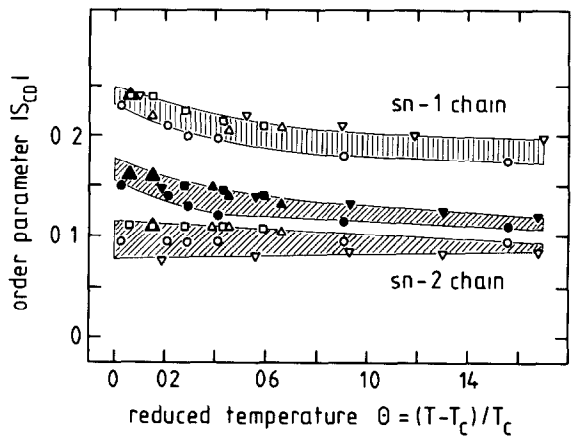

Fig.2. Variation of the deuterium order parameters $|S_{cD}|$ of the C-2′ segments with the reduced temperature. (▽), POPC; (○), DPPC; (□), DPPS; (△), DPPE. All phospholipids were dispersed in water 50% lipid/50% H_2O (wt/wt) except DPPS which was prepared in buffer: 0.5 M Na_2HPO_4–Cl pH 7.2, 0.5 mM Na_2EDTA at a phospholipid/H_2O ratio of 50/150 (wt/wt).

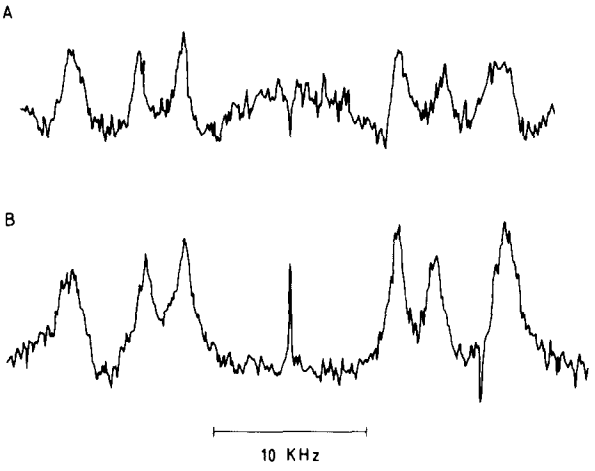

Fig.1. (A) 55.2 MHz ^{2}H-NMR spectrum of bilayers composed of DPPE deuterated at carbon atoms 2′ of both fatty acyl chains (50 wt% lipid, 50 wt% H_2O). Measuring temperature 68°C (5°C above T_c) ca. 20 000 free induction decays. (B) 55.5 MHz ^{2}H-NMR spectrum of bilayers composed of DPPS deuterated at carbon atoms 2′ of both fatty acyl chains. Lipid-to-buffer ratio (50/50 wt/wt). Buffer: 0.5 M Na_2HPO_4–Cl, pH 7.2, 0.5 mM Na_2EDTA. Measuring temperature 60°C. 6400 free inducation decays.

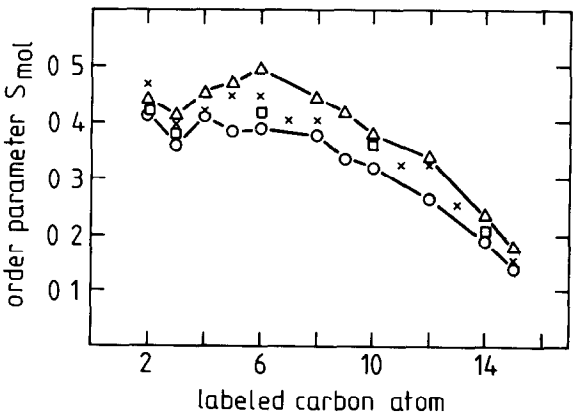

Fig.3. Variation of the molecular order parameter, S_{mol}, with the segment position. (○), DPPC [3]; (△), POPC [5]; (□), DPPS; (×), Acholeplasma laidlawii [10]. The synthetic lipid membranes are measured at the same reduced temperature $\Theta = (T-T_c)/T_c = 0.0605$.

profiles of the three synthetic lipids (POPC, DPPC, DPPS) are plotted at the same reduced temperature $\Theta \approx 0.061$, corresponding to actual measuring temperatures of 11°C, 60°C and 71°C. Also included in fig.3 is the order profile of the *Acholeplasma laidlawii* membrane measured at 42°C [10]. This natural membrane has no well-defined transition temperature, instead it shows a rather broad transition around 25°C [10]. Referred to this approximate phase transition temperature ($T_c \triangleq 298$°K), the measuring temperature of 42°C corresponds to $\Theta = 0.057$ which is tolerable for a comparison with the synthetic lipids measured at $\Theta = 0.061$. Figure 3 then shows that all the order profiles are relatively similar, in contrast to a comparison at the same temperature, e.g. 70°C. It is interesting to note that the extremes are defined by DPPC and POPC while DPPS and the *Acholeplasma laidlawii* membrane fall in between these boundaries. Thus the incorporation of a *cis* double bond is seen to promote larger changes in the order profile than, for example, the introduction of a net negative charge in the polar head group (DPPS) or the incorporation of proteins into the membrane. The relatively small effect of proteins on the deuterium order has also been observed in recent reconstitution experiments with cytochrome oxidase containing bilayers [12].

Concerning the molecular interpretation of figs.2 and 3, we shall neglect individual differences and confine ourselves to the features common to all membranes. By analogy with the analysis presented for DPPC and POPC [4,6], it follows from fig.2 that the beginning of the *sn*-1 and *sn*-2 chains of all lipids studied have different orientations with respect to the bilayer surface. The *sn*-1 chain is extended perpendicular to the membrane surface at all segments, while the *sn*-2 chain begins parallel to the membrane surface and is bent perpendicular to it after the C-2′ segment. With minor modifications this conformation is similar to the single crystal structure of *rac*-1,2-dilauroyl-glycero-3-phosphoethanolamine, which to date is the only known X-ray structure of a phospholipid molecule [13]. The principal features of this structure are supported by recent neutron diffraction studies on DPPC/H_2O membranes [14]. From these ^{2}H-NMR and neutron diffraction data it seems safe to conclude that the single crystal structure is indeed carried over into the liquid crystalline state and is a predominant conformation of phospholipids in fluid membranes. The unique orientation of the beginning of the *sn*-1 and *sn*-2 chains must be regarded as a general property of phospholipid organisation within membranes. The close similarity of the order profiles at a given Θ-temperature (fig.3) further corroborates the idea of a characteristic and ubiquitous phospholipid structure in bilayer membranes. This is reflected not only in the relatively constant order parameters for carbon atoms 2–9 and the subsequent decrease of S_{mol} towards the methyl terminal, but also in such details as the zig-zag in all the order profiles at carbon atoms 2–4. The divergence of the POPC order profile between carbon atoms 5–9 can be explained by a specific stiffening effect of the cis-double bond [5]. In contrast to the C-2′ positions no specific conformation can be singled out to describe the observed order profiles. Owing to rapid isomerisations around carbon-carbon bonds, each chain can assume a manifold of different configurations and a statistical-mechanical treatment is required. The first successful steps towards such a quantitative understanding of bilayer ordering profiles have already been made [15–17]. However, even without these sophisticated calculations inspection of fig.3 reveals that the packing of the hydrocarbon chains in a bilayer must be different from that in a paraffinic liquid, since the very existence of an order profile argues against a completely random bilayer interior.

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