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# Differences between conformations of lecithin and phosphatidylethanolamine polar groups and their effects on interactions of phospholipid bilayer membranes

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SUMMARY

The preferred orientation of the polar group in lecithin has the zwitterion extended normal to the bilayer so that the end-group contribution to the X-ray long spacing is about 11 Å. This conformation contributes a repulsive dipolar energy to the interactions of lecithin molecules within and across bilayers, helping to produce a rapid uptake of water to shield the mutually repelling dipoles. In contrast, there is a net neutralisation of charge between the polar groups of phosphatidylethanolamine, which are arranged so that the zwitterions are either approximately tangential to the plane of the bilayer, or interdigitated with opposing groups in a multilamellar system (net contribution 8 Å to the X-ray long spacing). The resulting cohesion makes phosphatidylethanolamines difficult to hydrate. The differences in zwitterion conformation, and hence hydration and motional freedom, result in the different stabilities of lecithin and phosphatidylethanolamine dispersions.

The extensive studies of phospholipid systems in the last decade have resulted in a good understanding of the physical states assumed by the hydrocarbon chains (for a review, see ref. 1). In contrast, the conformations, motions and interactions of phospholipid polar groups are poorly understood. This is clearly a major omission, because with lecithins and phosphatidylethanolamines it must be the rather small structural differences in the polar group regions which determine the quite different phase behaviour in water of these two classes of phospholipids[2].

Although the conformations of the zwitterionic polar groups of lecithin and

phosphatidylethanolamine have been studied by several workers, these studies have resulted in much disagreement, and the situation is confused. Thus, there are those[3–5] who consider that the zwitterion is orientated with its axis normal to that of the chains, whereas others[6] consider that the two axes are parallel, and still others[7] consider that the zwitterion is in some intermediate position. The motions and hydration of the zwitterions have not been directly investigated, apart from a determination[8,9] of the amount of water which is unfreezeable at 0 °C and an NMR investigation[10] of the motions of this "bound" water in lecithin/water systems. In this paper, we show how the X-ray long spacings of saturated lecithin and phosphatidylethanolamine homologues may be used to obtain the polar group orientations. We also show how these orientations affect the polar group motions and hydration, as determined using NMR spectroscopy, and discuss the differences in interactions which result from the differences in these properties.

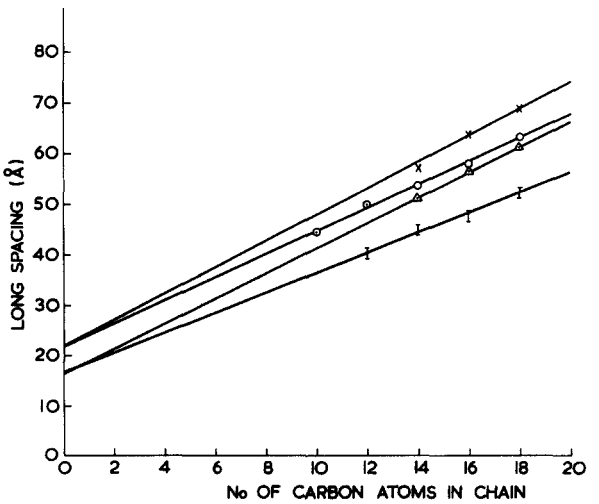



Fig. 1. Variation of X-ray long spacings ($d_{100}$) with number of carbon atoms per hydrocarbon chain ($n$) for saturated 1,2-diacyllecithin and phosphatidylethanolamine bilayer systems. x——x, lecithins at maximum hydration at 25 °C (data from ref. 8); ⊙——⊙, lecithin $\alpha_1$ monohydrates at 23 °C (a least-squares fit to data from ref. 8); △——△, anhydrous $\beta$ phosphatidylethanolamines at room temperature (data from ref. 19); I, least-squares line through spacings measured 3 °C above the liquid crystal transition temperature ($T_C$) of phosphatidylethanolamines at maximum hydration. These dimensions are based on only the first order diffraction of the lamellar repeat, so the accuracy is probably no better than ± 1 Å. Details of X-ray experimental methods can be obtained from refs 8 and 19. Values of $T_C$ can be obtained from ref. 9.

Fig. 1 shows X-ray long spacings (determined in the present study and taken from the literature) for lecithins and phosphatidylethanolamines as a function of the number of carbon atoms ($n$) in the fatty acyl chains. Extrapolation of the X-ray long spacings of the $\beta$ crystals of the phosphatidylethanolamine homologues to $n = 0$ indicates that each chain length-independent end group contributes about 8 Å (*cf.* refs 11, 12) to the bilayer

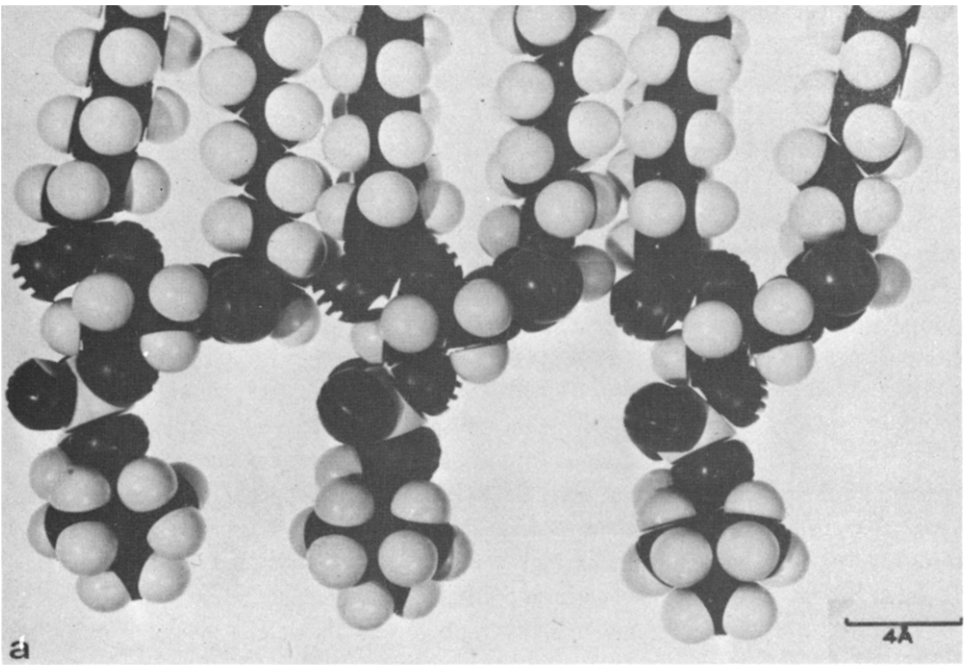
thickness*. This length of 8 Å is occupied by the glycerylphosphorylethanolamine moiety. Examination of molecular models indicates that the zwitterions must be either folded back to give charge neutralization within each monolayer of zwitterions (Fig. 2b), or interdigitated in a trans-lamellar fashion.

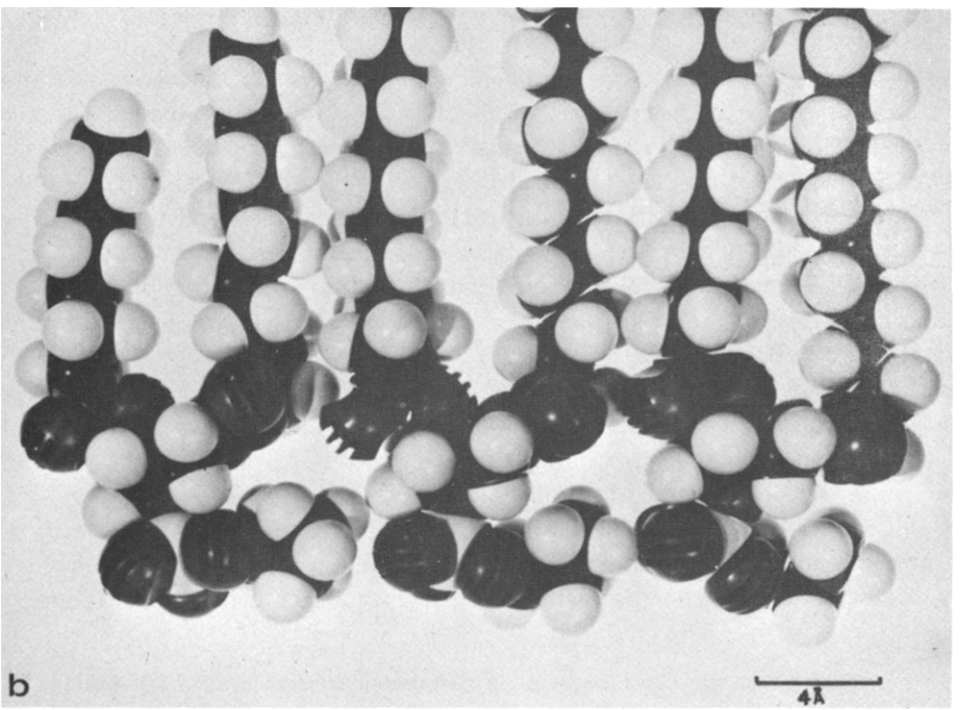
Lecithins are different from phosphatidylethanolamines in that they usually crystallize as monohydrates and are difficult to obtain in an anhydrous state. For the $\alpha$ monohydrates the end-group contribution per lecithin molecule is 11 Å (Fig. 1) and this can only arise from the zwitterions being extended parallel to the hydrocarbon chains (Fig. 2a) and abutting the polar groups of the neighbouring bilayer. The difference from phosphatidylethanolamine may be due to the increased size of the lecithin zwitterion (leading to steric repulsion and reduced attractive electrostatic energy for the bent-up conformation) and to the inability of the $N(CH_3)_3$ group to participate in hydrogen bonds. In the extended conformation (Fig. 2a) the phosphate groups of adjacent lecithin molecules are not shielded from each other and obviously the energy of the system can be greatly reduced by the presence of water molecules hydrogen bonded between anionic oxygen atoms. We propose that this is the reason for the great stability of lecithin monohydrates; it should be noted that an infinite phosphate–water hydrogen-bonded ribbon has been found in crystals of the complex glyceryl phosphorylcholine $CdCl_2 \cdot 3H_2O$ [13].

In the $\alpha$-gel phase at maximum hydration, Fig. 1 shows that the contribution of each lecithin end group to the bilayer long spacing is still 11 Å, *i.e.* is unaltered by the addition of about 9 molecules of water. This means that either the water molecules pack into the polar group region in such a way as not to contribute to the long spacing, or the contribution of the water is cancelled by a tilting of the zwitterion. A slight tilting or curling may occur in any case, since measurement of molecular models indicates that with the zwitterion in the *gauche* conformation about the OC–CN bond[14,15] and fully extended parallel to the chains (as in Fig. 2a), the end-group contribution to each lecithin molecule is 12 Å. However, the data in Fig. 1 cannot be extrapolated with sufficient confidence to warrant further discussion of this point. It can be seen that the data for dimyristoyllecithin ($n$ = 14) in the fully hydrated gel phase give a long spacing 1.5 Å less than would be predicted from a linear extrapolation (Fig. 1); this is because the polar groups in this molecule are rotating[9] at the temperature studied, whereas those of the other two lecithins are not. This motion therefore causes a reduction in the long spacing of 0.8 Å per head group. Since this motion is that characteristic of the liquid crystal phase (which for this molecule occurs at a temperature only a few degrees higher than the temperature studied), it can be seen that there is no gross change in the time-averaged polar group conformation during the gel-to-liquid crystal transition.

Application of this extrapolation method to lecithins above the gel-to-liquid crystal transition temperature ($T_c$) yields a spacing which contains contributions from both the lipid polar group and the interlamellar water layer; separation of these two is not possible

*Such an extrapolation assumes that the end-group conformation is independent of chain length for $n$ = 10–18.


a
4Å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at present. This problem does not arise for phosphatidylethanolamines, and the end-group contributions (Fig. 1) show that there is no change on going through $T_c$, each molecule contributing 8 Å. Furthermore, the presence of bound water does not affect this spacing, indicating that this water is packed near the glycerol backbone. Deuteron NMR results on lecithin–$^2H_2O$ and phosphatidylethanolamine–$^2H_2O$ systems (E.G. Finer and A. Darke, unpublished results), which give information on the bound water and the polar group motions above $T_c$, are entirely consistent with the difference in conformation between the lecithin and phosphatidylethanolamine end groups being maintained above $T_c$. The lecithin polar group, not being bonded to its neighbour, undergoes motions which are about twice as fast as those of phosphatidylethanolamine. Both molecules bind about 4 water molecules round the phosphate group, and a further 8 in the rest of the polar region; however, lecithin can trap another 11 molecules of water between bilayers because of the extended conformation and fast motions[16] of the polar group (M.C. Phillips, E.G. Finer and H. Hauser, unpublished results), whereas the attractive dipolar potential resulting from the bent-up conformation of phosphatidylethanolamine (Fig. 2b) keeps opposing bilayers abutting each other and does not permit further water to separate the bilayers.

The different conformations of the polar groups of the two phospholipid classes lead to differences in behaviour on the macroscopic level. Because the zwitterion conformation shown in Fig. 2a leads to dipolar repulsion between adjacent bilayers, the activation energy for water uptake by lecithin is less than that of phosphatidylethanolamine and therefore lecithins above the liquid crystal transition temperature swell much more readily in water to hydrate the polar groups. The attractive potential in phosphatidylethanolamine keeps the molecules more closely packed than for lecithin, and thus $T_c$ is higher than in the equivalent lecithin–water system. The stabilities of the phospholipid dispersions parallel the abilities to accept trapped water between bilayers in a multilamellar system. The interaction of the zwitterions with other species is also affected by their orientation. The arrangement of the phosphatidylethanolamine zwitterion coplanar to the lipid–water interface enables the polar groups to bind small amounts of $Ca^{2+}$, whereas under the same conditions no binding to lecithin is detected[17].

Finally, it is interesting to note that it has been suggested[18] that the phospholipid distribution in erythrocyte membranes is asymmetrical: the $NH_2$-containing phosphatidylethanolamine and phosphatidylserine molecules are on the interior surface whereas the $N(CH_3)_3$-containing lecithin and sphingomyelin are on the exterior surface. Because of their orientation, the zwitterionic polar groups in lecithin molecules cause a net repulsion (M.C. Phillips, E.G. Finer and H. Hauser, unpublished results) between pure lecithin

Fig. 2. Photographs of space-filling models of: (a) Lecithin molecules arranged so that the O–C–C–N group is extended below the chains in a *gauche* conformation. This gives an end-group contribution to the bilayer long spacing of 2 x 11 A. (b) Phosphatidylethanolamine molecules arranged with the zwitterions bent at right angles to the hydrocarbon chains. The end-group contribution to the bilayer long spacing is 2 x 8 Å. In both (a) and (b) the conformations of the phospholipid molecules, apart from the zwitterions, are arbitrary.

bilayers. It follows that the effects of the extended zwitterions on the exterior cytoplasmic surface may be supplementing the negative membrane zeta potential in preventing agglutination of erythrocytes. Such a repulsion would not arise from the bent-up zwitterions on the interior membrane surface and this asymmetry may contribute to the structural and functional differences between the intra- and extracellular parts of biological membranes.

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