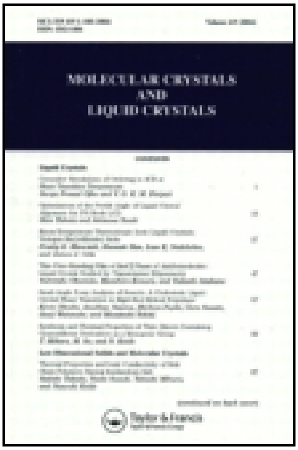

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The Smectic Phase of Lipid-Water Systems: Properties Related to the Nature of the Lipid and to the Presence of Net Electrical Charges

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Abstract—The lamellar phase of the lipid-water systems, when studied as a function of the water content, displays properties that belong to one of the following classes, according to the nature of the lipid. (a) The lamellar phase takes up an almost indefinite amount of water intercalated between the lipid layers (lipids bearing net electrical charges). (b) The maximum amount of intercalated water is rather small, and the water in excess stays as a separate phase (lipids devoid of net charges). (c) The amount of water in the lamellar phase is limited: further addition of water induces a transition to another liquid-crystalline phase (soaps and detergents). It is shown here that if small amounts of ionizable lipids, either anionic or cationic, are incorporated to a non-ionizable lipid, the lamellar phase takes up very large amounts of water, as in the lipids of class (a): this phenomenon is not observed if the added lipids are an equinormal mixture of anionic and cationic species. These experiments stress the rôle of the electrical charges as stabilizing factor in the lipid-water phases. The rôle of this and other factors is discussed.

Introduction

One of the most widespread phases of the lipid-water systems, that we have called Lα,[1] is typically smectic. Its structure consists of planar lipid leaflets, all parallel and equidistant, separated by layers of water; the lipid leaflets are filled by the highly disordered paraffin chains, their surface being covered by the hydrophilic groups of the lipid molecules (Fig. 1). When, for a variety of

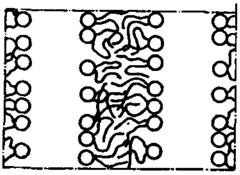

Figure 1. Structure of the Lα (smectic) phase of lipid-water systems: schematic representation of a section perpendicular to the lamellae. The polar group is represented by a circle, the paraffin chains by a wriggle.

lipids, the properties of this phase are analyzed, as a function of the amount of water, three cases can be distinguished:

(1) The thickness of the water layers varies enormously (from 5 Å to more than 250 Å), without altering either the thickness or the regular stacking of the lipid leaflets. Several lipids, that all possess ionizable groups, have been shown to belong to this class: complex lipid extracts of biological origin brain,[2] mitochondria,[3] red cells,[4] chloroplasts polar lipids[5] as well as phosphatidyl inositol, cardiolipins, phosphatidic acid (our unpublished results).

(2) The thickness of the water layer is limited to a rather narrow range, in most cases 5 to 25 Å; the water in excess remains as a separate phase, in equilibrium with the most hydrated smectic phase. The lipids that belong to this class are all devoid of net electrical charge: lecithins,[6,7,8] monoglycerides,[6,9,10] cerebrosides,[6] glycolipids.[5]

(3) The thickness of the water layer is again bounded within narrow limits; the excess water, in this case, induces a phase transition into another lipid-containing phase (cubic, hexagonal). Soaps and detergents belong to this class.[1]

These properties of the smectic phase are related to the problem of the forces responsible for the stability of the lipid-water phases. Our desire to investigate this problem was stimulated by a discussion with Dr. A. Parsegian. We were thus led to undertake some experiments aimed at clarifying the role of the electrical charges. The results are described in this paper.

Experimental

Our work was carried out on lipids devoid of net electrical charge (mainly lecithin), to which we added small amounts of non ionic, cationic and anionic lipids.

The following lipids were used: natural lecithin extracted from hen egg yolk by the usual procedures,[11] Lα-dipalmitoyl lecithin (from Sigma Chem. Comp.), monopalmitin (from Sigma Chem. Comp.), triton (from Sigma Chem. Comp.), CTAB (cethyl trimethyl ammonium bromide), sodium oleate, sodium stearate.

Homogeneous dry lipid mixtures were obtained by evaporating dilute solutions in chloroform or methanol. Water and lipids were mixed at the desired concentrations. The X-ray diffraction experiments were carried out as described elsewhere.[1]

The concentrations are defined by c = (weight of lipids)/(weight of lipids plus water) and γ = (weight of added lipid)/(weight of lecithin plus added lipid).

Results

This paper deals only with the lamellar phase, type Lα[1,3] characterized by X-ray diffraction diagrams containing a few equidistant small angle ($s < (10\ \text{Å})^{-1}$) sharp reflections and the broad band around $s = (4.5\ \text{Å})^{-1}$, characteristic of the " liquid " paraffins.

In each experiment the repeat distance d and the concentration c were measured. A simple relation exists between d, c, the thickness d_l of the lipid layer and the ratio $(\bar{v}_l/\bar{v}_0)$ of the partial specific volumes of lipid $(\bar{v}_l)$ and water $(\bar{v}_0)$ (see ref. 1). It is:

$$d_l = d\left[1 + \frac{v_0}{v_l}\left(\frac{1-c}{c}\right)\right]^{-1} \tag{1}$$

If d_l and $(\bar{v}_0/\bar{v}_l)$ are independent of concentration, there is a linear relation between d and $(1-c)/c$: the slope and intercept determine the values of d_l and of $(\bar{v}_l/\bar{v}_0)$. The relation between the experimental values of d and $(1-c)/c$ is shown in Fig. 2.

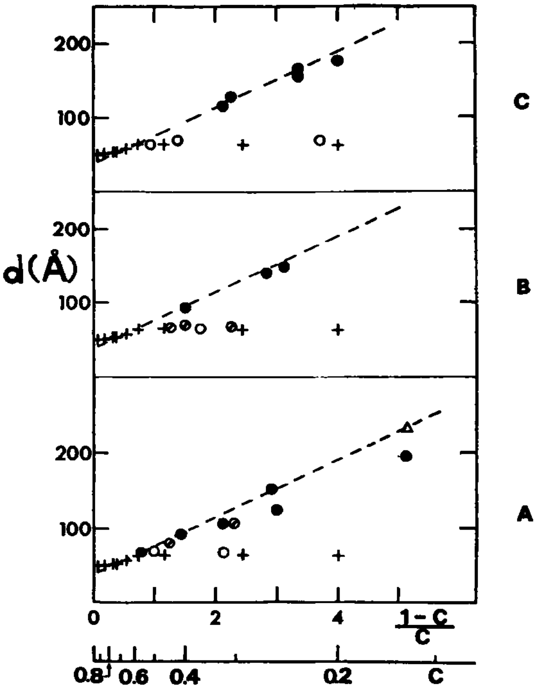

Figure 2. Observed repeat distance d of the smectic phase of the egg lecithin-water system, in the presence of various charged lipids. The total lipid concentration is defined by c = (weight of lipids/weight of lipids plus water). The concentration of added charged lipids is defined by γ = (weight of additive/total weight of lipids) and by σ, the average area available to one charged group of the added lipids on the surface of the lipid leaflets. The dotted straight lines correspond to the ideal case of a smectic phase incorporating all the water of the system, with $d_l = 38$ Å and $(\bar{v}_0/\bar{v}_l) = 1$ (see Eq. 1).

(A) CTAB as added lipid

	γ	$\sigma(\text{Å}^2)$
+	0	–
○	0.006	5200
⊘	0.008	3900
⊙	0.009	3470
●	0.015	2080
△	0.035	890

(B) Na-oleate as added lipid

	γ	$\sigma(\text{Å}^2)$
+	0	–
○	0.005	5150
⊘	0.007	3680
●	0.011	2340

(C) Na-stearate as added lipid

	γ	σ(Å^2)
+	0	–
○	0.005	5220
●	0.011	2380

As mentioned above lecithin belongs to class 2; the water content of the smectic phase increases as the amount of water increases, until a maximum hydration is reached, beyond which the excess water remains in a separate phase, in equilibrium with the most hydrated smectic phase. This is shown in Fig. 2: d increases from $c = 1$ to $c = 0.45$, and remains constant for $c < 0.45$, while the X-ray reflections remain quite sharp.

The addition of non charged lipids (mono- and di-glycerides, tritons), up to $\gamma = 0.05$, has no effect on the X-ray diffraction properties of the smectic phase of lecithin.

If the added lipids bear a net electrical charge the following phenomena are observed. At low water content the swelling of the smectic phase by water is independent of the amount and the nature of the added lipids. In the presence of excess water the first effect becomes appreciable at about $\gamma = 0.002$; the X-ray reflections, become diffuse, showing that the stacking of the lipid lamellae is somewhat disordered. At still higher concentrations of added lipids the maximum value of d (i.e. the maximum hydration) increases, as well as the sharpness of the X-ray reflections. Both effects become more pronounced with increasing γ (Fig. 2). When γ reaches 0.03 the smectic phase takes up any amount of water, at least in the range of our experiments ($c > 0.15$).

It may be noted that in the three cases of Fig. 2, the envelope of the experimental points, at $c < 0.40$ is a straight line, corresponding to a lipid leaflet of thickness $d_l = 38$ Å and $(\bar{v}_0/\bar{v}_l) = 1$ (see Eq. 1). At the highest lipid concentrations the hypothesis of a concentration-independent lipid leaflet appears to break down.[6]

The outstanding rôle of the electrical charges is stressed by two additional observations. One is that the simultaneous presence of anionic and cationic lipids, in equivalent amounts, counteracts the effects of each of the lipids taken separately. The second is that the addition of electrolytes (NaCl or KCl up to 0.5 M) to water damps down the effects of the charged lipids, but remains without effect on lecithin alone.

Finally it should be noted (see Fig. 2) that the effects of the charged lipids are hardly dependent on the sign of the electrical charge and on the chemical nature of the lipid.

Discussion

Among the different factors that are likely to play a rôle in the stability of the lipid-water phases, three have been taken into consideration previously:

(a) electrostatic interactions of mobile and fixed charges[12]

(b) interfacial interactions at the water-paraffin boundary[12]

(c) rubber-like properties of the paraffin chains, entailing conspicuous entropy changes as the dimensions of the lipid-containing structure elements are varied.[1]

Parsegian has presented a theoretical justification of the phase transitions of the soap-water systems,[12] and of the properties of the lamellar phase of the lecithin-water systems,[13,14] based upon the first two factors, namely the electrostatic and the interfacial interactions. We[1] have put forward an explanation of the thermal variations observed in lipid-water systems by taking into account the last two factors, interfacial interactions and rubber like properties. The results presented here clearly indicate that the rôle of the electrostatic interactions is very important indeed, and provide a support to the theoretical approach proposed by Parsegian.[12,14]

Nevertheless other lines of evidence that suggest that factors in addition to the above are likely to play a rôle, should not be neglected. One of the puzzling observations is the fact that the lipids of class 2 are both zwitterionic (lecithin), and non-ionic (monoglycerides, cerebrosides, galactolipids). Electrostatic interactions can hardly provide an adequate explanation for the similar properties of lipids so different from an electrochemical standpoint. Another phenomenon rather difficult to explain within the framework of the previous discussion is the effect on the soap-water systems of fatty acids and of fatty alcohols. These additives stabilize a lamellar phase incorporating large amounts of water, under conditions (temperature, concentration) in which non-lamellar phases would be observed in the absence of them (ref. 15 and our unpublished observations). In this case, the transition from the behaviour of class 3 to that of class 1 is obtained either by " diluting " the electrical charges on the lipid leaflets (fatty alcohol), or by changing the ionic environment (fatty acid).

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