Choline and acetylcholine induce interdigitation of hydrocarbon chains in dipalmitoylphosphatidylglycerol lamellar phase with stiff chains

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1. INTRODUCTION

In some soap gels [1] and in L-DPPG–water system [2] lamellar phases have been observed in which the hydrocarbon chains, stiff and parallel to each other, interdigitate; in other words, the CH_3 ends of the chains of one layer are near to the polar groups of the opposite layer (see fig.1C). From the operational standpoint these phases are characterized by:

(i) The thickness of the lipid bilayer, which is almost one-half of the value expected for the non-interdigitating phase Lβ;
(ii) The area per polar group at the lipid–water interface, which is 4-times the cross-section of one stiff chain;
(iii) The intensities of the reflections.

Moreover, 3 types of organization of the hydrocarbon chains have been observed; these are characterized by a family of sharp reflections in the 0.240–0.780 $Å^{-1}$ range, and correspond to 3 types of two-dimensional lattices: p6, cmm and pgg. A detailed description of these phases can be found in [2].

In our first study [2] the sample, in the form of ammonium salt (L-DPPG–NH_4), was purchased from Serdary International Laboratories Inc. (Ontario) and used without further purification. Later, when a highly pure preparation of rac-DPPG–NH_4 became available to us [5,6] we searched for the interdigitated phase but failed to observe it. This negative result prompted us to study other batches of L-DPPG–NH_4, also from Serdary; again we failed to observe the interdigitated phase. Similarly, Watts et al. [3,4] who studied a L-DPPG–water system as a function of pH, did not report the interdigitated phase. This discrepancy between our early observations and those of all subsequent studies was puzzling. An explanation could be sought in the presence of some impurity in the sample used in our early work: since the preparation of that sample involved a DPPG–glycerol transesterification step, a likely candidate is choline. We demonstrate here that indeed choline, as well as acetylcholine promote interdigitation; we show in [13] that the antibiotic polymyxin B has the same effect.

Abbreviations: rac-DPPG, racemic dipalmitoyl phosphatidylglycerol; L-DPPG, dipalmitoyl L-α-phosphatidylglycerol; Ch, choline; Ach, acetylcholine

2. MATERIALS AND METHODS

rac-DPPG–NH_4–choline and –acetylcholine salts were of synthetic origin and were prepared as follows: The acidic form of the lipid was obtained with an excellent purity after hydrogenolysis of the fully benzylated derivative [5]. Part of this lipid was first neutralized with ammonia and another part neutralized with pyridine and then the pyridine was replaced by choline or acetylcholine. This step was performed as follows: choline chloride (Merck) and acetylcholine iodide (Fluka), converted into acetate form by filtration through an anion-exchange resin (AG I-X2, Bio-Rad) preconditioned

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in the acetate form, were mixed in equimolecular amounts with the rac-DPPG–pyridine using chloroform/methanol (1/1, v/v). Pyridine acetate and organic solvants were removed under reduced pressure. The residual rac-DPPG–Ch and rac-DPPG–Ach salts were lyophilized from benzene solution. All these compounds were pure as indicated by thin-layer chromatography.

The X-ray diffraction experiments were performed and analyzed as in [2,7]. The reflections observed at small angles ($s < (8\ \text{Å})^{-1}$; $s = 2 \sin \theta/\lambda$) characterize the long-range organization. All the phases described here are lamellar. In this case when the concentration c (lipid/sample), the repeat distance d and the ratio of the partial specific volumes of lipid and water $\bar{v}/\bar{v}_w$ are known, it is possible to determine partial thickness of the lipid layer d_l and the average area S available to one lipid molecule at the lipid–water interface [8]:

$$d_l = d\left(1 + \frac{\bar{v}_w}{\bar{v}} \cdot \frac{1-c}{c}\right)^{-1} \quad (1)$$

$$S = 2M\bar{v}/d_l N\ 10^{-24} \quad (2)$$

when M_r is the relative molecular mass of the lipid and N is Avogadro's number. The characterization of 3 types of chain packings (p6 and cmm) is detailed in [2].

3. RESULTS

3.1. *rac-DPPG–NH_4*

The spacings observed with one sample at $c = 0.93$ are plotted in fig.2. At high temperature the phase Lα is observed (fig.1). Below 78°C another lamellar phase Lβ is found; the presence of sharp reflections at 4.12, 2.40 and 2.09 Å^{-1} and the area/polar group 41.8 Å^2 show that the chains are oriented at right angle to the plane of the lamellae [8]. It may be noted that the temperature of the $\alpha \rightarrow \beta$ transition (78°C) is 28°C higher in DPPG than in DPPC [9,10] at the same concentration.

3.2. *rac-DPPG–Ch*

In the system rac-DPPG–Ch–water a phase Lα is observed at $\geq$ 42°C (fig.2). Below that temperature the phase observed is lamellar with stiff interdigitated chains (fig.1c). Over ~ 42–0°C the packing of the chains is of type p6; from − 5°C to − 25°C, the packing of the chains is of type cmm.

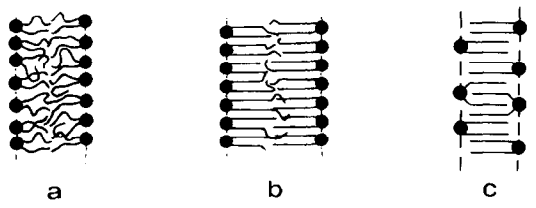

Fig.1. Schematic representation of the structure of the lamellar phases discussed in this work: (•) polar groups; (~) hydrocarbon chains in the liquid-like conformation; (—) chains in stiff conformation. (a) Lα phase: the conformation of the chains is liquid-like; for rac-DPPG–NH_4 sample at $c = 0.93$ and at 78°C (see fig.2) the partial thickness of the lipid layer and the surface area S are 41.1 Å and 57 Å^2, respectively. (b) Lβ phase: the hydrocarbon chains are stiff and parallel, oriented at right angle to the plane of the lamellae; for rac-DPPG–NH_4 sample at $c = 0.93$ and at 20°C, $d_l = 52.6$ Å, $S = 41.8$ Å^2. (c) Interdigitated phase: the chains are stiff and parallel oriented at right angle to the plane of the lamellae and the CH_3 ends of one layer are near to the polar group of the opposite layer; in this phase d_l is smaller (35.5 Å) and S larger (72.5 Å^2) than in the phase Lβ without interdigitation (52.6 Å and 41.8 Å^2, respectively).

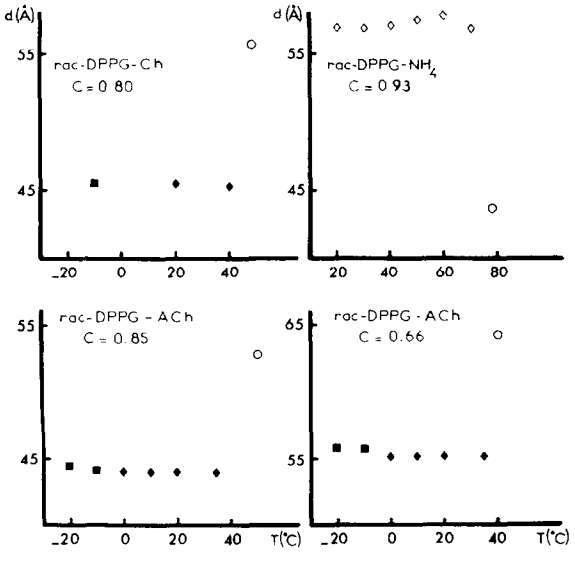

Fig.2. Lamellar repeat distances, d, as a function of temperature. The symbols refer to the organization of the chains; liquid-like (○); two-dimensional lattice p6 (◆); and two-dimensional lattice cmm (■); for the ordered conformations full symbols indicate interdigitating chains, open symbols the non-interdigitating chains.

3.3. *rac-DPPG–Ach*

We studied this lipid at 3 concentrations: c = 1.00, 0.85 and 0.66. In the absence of water, and below 48°C, we observed a lamellar phase (spacing d = 37.4 Å) with stiff interdigitated chains. The organization of the chains is of type p6 over 48–0°C, cmm below – 5°C (down to – 25°C).

The spacings of the lamellar phases observed in the presence of water are shown in fig.2. The $\alpha \rightarrow \beta$ transition temperature is approximately the same (41°C) at the two concentrations and it is lower than in the dry sample (48°C). Below 42°C the lamellar phase contains again stiff interdigitated chains: the organization of the chains is of type p6 over 42–0°C, of the type cmm below – 5°C (and down to – 20°C).

4. DISCUSSION

In agreement with [3,4], the lamellar phases observed in the system rac-DPPG–NH_4/water are of the types Lα and Lβ, the latter without interdigitation. By contrast, the presence of either choline or acetylcholine (in these stoichiometric ratios) has the effect of inducting interdigitation of the hydrocarbon chains.

Therefore, the unforeseen presence of choline seems to provide an explanation for our observations in [2]. Besides, the nature of the optical form (L in our early work, racemic here) does not seem to play a role in the phenomenon of chain interdigitation.

The swelling property is not altered by the presence of choline and acetylcholine; it is worth noting that in the interdigitated phase of rac-DPPG–Ach the thickness of the lipid lamellae is independent of the water content (d_l = 36.6 Å for c = 1.00, 0.85 and 0.66) thus suggesting that only one phase is present over that concentration range.

This work confirms our statement [2] that the non-hexagonal two-dimensional organization of the chains (type cmm and pgg) are only observed in the lamellar phases with interdigitated chains.

Studies on the effect of organic cations to phospholipid bilayers are scarce [11,12]. However, the available data suggest that organic cations such as tetraethylammonium and acetylcholine have quite different effects, as compared to sodium and potassium, on the dispersion properties of phosphatidylserine [11] and of phosphatidylcholine [8]. In particuliar, it turns out from electrophoretic mobility (ξ-potential) and surface potential (ΔV) measurements, that with the former type of cations, the screening of the surface charge of the lipids is somewhat less effective. This might be due [11] to the bulkiness of the organic cations, preventing close approach, and ion-pairing with the phosphate group of the type observed with inorganic cations. Another possibility [11,12] could be that the hydration properties of the whole system (cation/phospholipid) are different. Because of their surface activity, organic cations such as choline and acetylcholine might reduce the hydrophobic contribution to the free energy of stabilization of bilayers [11,12]. In this present case preliminary monolayer experiments clearly show large film expansion for dipalmitoylphosphatidylglycerol spread in the presence of choline and acetylcholine in the subphase, indicative of strong interactions between the lipid and the two organic cations (unpublished). Whether interdigitation is to be accounted for by hydrophobic effect or electrostatic effect or both factors deserves further investigation.

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