Correlation between molecular shape and hexagonal H_{II} phase promoting ability of sterols

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1. INTRODUCTION

Sterols are main constituents of biological membranes. Their exact function is unknown, but many studies indicated that sterols greatly affect the physical properties of the lipid part of membranes. In particular, the observations that cholesterol by its condensing effect increases the chain order in the liquid crystalline state and by its liquifying effect decreases the chain order in the gel state, has led to the concept that cholesterol-containing membranes are in an 'intermediate state of fluidity' [1].

Both model membrane experiments and studies on biological membranes have revealed that the sterol structure is of critical importance in the sterol–lipid interaction. In general, a planar ring system, a 3β-OH group and a long flexible chain at C_{17} are prerequisite for both condensing and liquifying effects [2–3]. The same structural requirements are found for sterols which promote growth of the sterol-requiring mycoplasmas [4]. A relationship between these structural characteristics and membrane permeabilities is observed in pure lecithin liposomes [5] and also in biological membrane systems [6–8].

Considerable interest has arisen in the possible occurrence of non-lamellar lipid structures in membranes [9]. These possibilities are reinforced by the observation that every biological membrane tested so far contains substantial amounts of lipids which upon isolation, when dispersed in aqueous buffers, organize themselves in inverted structures such as the hexagonal H_{II} phase or intrabilayer inverted micelles. For instance, natural phosphatidylethanolamines (PEs) undergo temperature-dependent bilayer → hexagonal H_{II} transitions such that at physiological temperatures the hexagonal H_{II} phase is preferred [10,11]. Phosphatidylcholines stabilize bilayer structure of PEs whereas cholesterol promotes hexagonal H_{II} phase formation in mixed phosphatidylcholine–PE systems [12,13].

Nothing is known about the effect of sterol structure on the phase preference of lipids. Therefore, we have investigated by ^{31}P NMR the effect of different sterols on the bilayer → hexagonal H_{II} transition [9] of a synthetic PE with two questions in mind:

(i) Is the chemical structure of the sterol molecule of importance for the phase behaviour of the system?

(ii) Can phase changes induced by the sterol be correlated with the molecular shape of the sterol?

2. EXPERIMENTAL

Dielaidoyl phosphatidylethanolamine (18:1$_t$/18:1$_t$ phosphatidylethanolamine, DEPE) was synthesized as in [14]. Sterols were purchased from the following commercial sources: cholest-5-en-3β-ol (cholesterol), cholest-(5,7)-dien-3β-ol (7-dehydrocholesterol), cholest-(5, 7, 22)-trien-24-methyl-3β-ol (ergosterol), cholest-4-en-3-one (Fluka AG, Buchs); (cholest-(4,6)-dien-3-one (BDH, Poole); 5α-choles-tan-3-one (Koch-Light,

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Colnbrook); cholest-5-en-3α-ol (epicholesterol); (Schwarz/Mann, New York); 5α-androstan-3β-ol (Ikapharm, Ramat-Gan).

2.1. *Preparation of samples*

Aqueous dispersions of phospholipids with or without added sterols were carried out from a chloroform solution containing appropriate amounts of lipids (~40 mg) dried under nitrogen. Residual traces of chloroform were removed by submitting the samples to high vacuum over 3–4 h. The lipid samples were then hydrated by addition of 0.8 ml Tris–HCl 0.01 M (pH 7.4), NaCl 0.1 M, EDTA 2 mM and 0.2 ml of the same buffer made in ^2H_2O. Dispersions were accomplished upon exhaustive vortex mixing.

2.2. *^{31}P NMR*

Proton-decoupled 36.4 MHz ^{31}P NMR spectra were obtained as in [10]. The % of lipid organized in extended bilayers or hexagonal H_{II} phases were determined by computer subtraction methods, using the characteristic pure 'bilayer' and 'hexagonal' types of ^{31}P NMR line shape [9] as references. In no case were isotropic ^{31}P NMR signals observed.

3. RESULTS AND DISCUSSION

The effects of the different sterols used here on the bilayer→hexagonal H_{II} phase transition temperature are presented in fig.1. Cholesterol, 7-dehydrocholesterol and ergosterol affect only to a small extent the transition temperature. Upon changing the 3β-OH into a 3α-OH group (epicholesterol) the transition temperature is shifted to lower values by almost 10°C. Replacement of the 3β-OH group by a keto-group (5α-cholestan-3-one) leads to almost the same change as for the 3α-OH derivative. Addition of one or two conjugated double bonds (cholest-4-en-3-one and cholest-(4,6)-dien-3-one) produces an additional temperature shift of ~10°C. Removal of the isocaproic side-chain (5α-androstan-3β-ol) has the same effect as the conjugated 3-ketosteroids and the H_{II} phase transition temperature becomes 34°C.

Gel → liquid crystalline phase transitions can also be monitored by ^{31}P NMR, as below the transition temperature a large increase in linewidth occurs [15]. For pure DEPE we found the transition temperature to be ~35°C as reported [10]. Incorporation of equimolar amounts of epicholesterol and the 3-ketosteroids did not affect the gel → liquid crystalline phase transition of the DEPE in agreement with previous calorimetric studies on phosphatidylcholine systems [6]. Ergosterol had no effect. In contrast, incorporation of 50 mol% of cholesterol and 7-dehydrocholesterol eliminated this phase transition confirming the known liquifying effect of these sterols [1].

Thus, the structural requirements of the sterol molecule to decrease the bilayer → hexagonal H_{II} phase transition temperature are very similar to those for the liquifying and condensing effects that these sterols exert in bilayer systems.

Those sterols which most strongly destabilize bilayer structure (e.g., decrease the bilayer → hexagonal H_{II} phase transition temperature) inhibit the growth of sterol-requiring mycoplasmas [4]. Also incorporation of the 3-ketosteroids into erythrocytes greatly increases the permeability and fragility of the membrane [8] which might be related to their strong bilayer-destabilizing effect.

In [9] a correlation was proposed between the molecular shape and the phase preference of lipid molecules. Lipids with a small polar head group

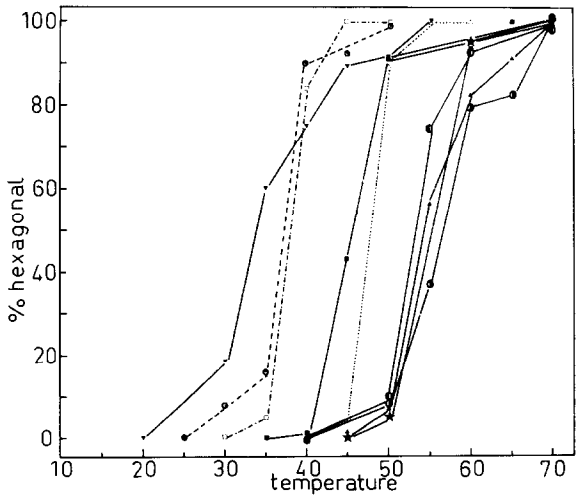

Fig.1. Representation of the bilayer → hexagonal H_{II} phase transition as a function of temperature if DEPE samples containing: (★) no sterol; (▲) cholesterol; (◐) ergosterol; (◑) 7-dehydrocholesterol; (■) epicholesterol; (▼) 5α-androstan-3β-ol; (△) 5α-cholestan-3-one; (○) Cholest-4-en-3-one; and (□) cholest-(4,6)-dien-3-one.

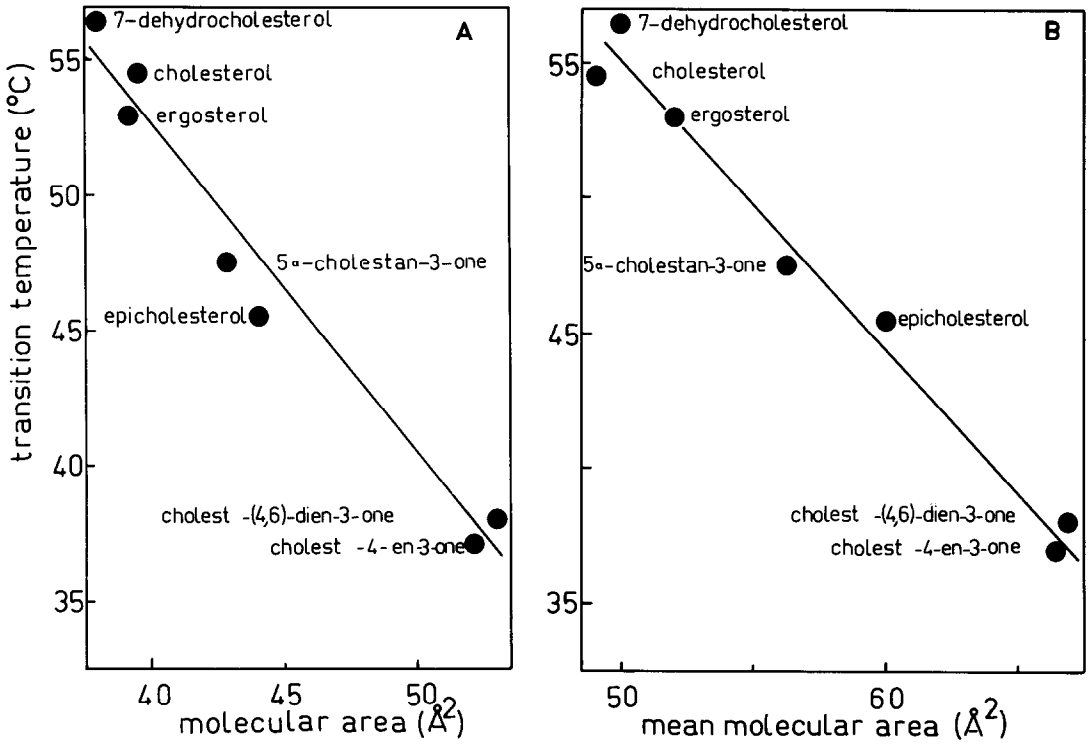

Fig.2. Representation of the bilayer → hexagonal H_{II} phase transition temperature as a function of:
(A) the molecular area of pure sterols in monolayer films;
(B) the mean molecular area of mixed sterol–lecithin monolayer films (surface pressure: 12 dynes/cm) [2].

and a relatively large hydrocarbon area are 'cone-shape' and thus prefer to organize themselves into inverted structures such as the hexagonal H_{II} phase or inverted micelles.

The exact dynamic shape of phospholipid and sterol molecules in mixed films is unknown. However, some insight into the shape can be obtained from monolayer data. As the polar part of the sterol molecule in general is much smaller than the hydrocarbon region, sterols are 'cone-shaped'. The magnitude of the dynamic cone shape can be derived from the mean molecular area of sterols in monolayers at the air–water interface. For instance, the 3-ketosteroids occupy a much larger molecular area in monolayers as compared to cholesterol [2]. This could indicate that the ring system of the 3-ketosteroid is not perpendicularly oriented to the air–water interface and that due to fast axial rotation the angle of the dynamic cone is larger than that of the cholesterol molecule.

The bilayer → hexagonal H_{II} phase transition temperature is plotted in fig.2 as a function of the molecular area of sterols (surface pressure = 12 dynes/cm) as obtained from published monolayer studies using pure sterols (fig.2A) or mixed phosphatidylcholine–sterol (fig.2B) films. Straight lines are obtained with correlation coefficients of 0.985 (fig.2A) and 0.990 (fig.2B), respectively. This clearly demonstrated the good correlation between the molecular shape and the phase preference of the lipid. The only exception is for 5α-androstan-3β-ol which induced the largest bilayer destabilization but which has a limiting area of 39 Å, comparable to that of cholesterol (39.5 Å) [2]. Apparently, the lack of the *iso*-caproid acid side chain at C_{17} induces disorder in the end of the acyl chains in the DEPE thus inducing a more cone-like shape of the DEPE molecules, thereby decreasing the bilayer → hexagonal H_{II} phase transition temperature.

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