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Gel to liquid-crystalline transitions of aqueous dispersions of positional isomers of a heteroacid unsaturated phosphatidylcholine mixed with epicholesterol and cholesterol

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The gel to liquid-crystalline phase transitions of dispersions of two positional isomers of a phosphatidylcholine, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) and 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (OSPC) mixed with 13 and 17 mol% cholesterol or epicholesterol were observed by differential scanning calorimetry. Cholesterol caused a greater reduction in the enthalpy change of the transitions than did an equal amount of epicholesterol. Both sterols had a more pronounced influence on OSPC than on SOPC. Endotherms of PC plus sterol were resolvable into two components whose properties were similar for both sterols. The results suggest that the sterols interact differently with positional isomers of PC, and that the interaction is not strongly influenced by the orientation of the hydroxyl group of the sterol.

When equal amounts of cholesterol were mixed with the positional isomers of unsaturated heteroacid phosphatidylcholines (PC) in bilayers the steroid had a more pronounced influence on the gel to liquid-crystalline transition of one isomer in comparison to the other [1,2]. At cholesterol concentrations of less than 17 mol%, there was a difference in the shapes of calorimetrically observed endotherms for the gel to liquid-crystalline phase transitions between bilayers made of 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) plus cholesterol and bilayers made of cholesterol plus the positional isomer 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (OSPC) [1]. Similar differences were seen with the PC isomers which contained eicosanoate (archidate) and oleate in the 1 and 2 positions [2]. Huang [2,4] suggested that there may be preferential interaction between the saturated and unsaturated chains of PC and the two faces of cholesterol, these being determined by a hydrogen bond between the 3β-OH group of cholesterol and the carbonyl oxygen at the 1-position of the PC.

Studies in which the physicochemical properties of bilayers composed of lipids plus cholesterol or epicholesterol (the epimer of cholesterol with a 3α-hydroxyl group) have shown that epicholesterol has a less profound influence on the properties of membranes than does cholesterol (see, for example, Refs. 5 and 6). Whether or not a specific hydrogen bond is formed between the 3β-OH of cholesterol and some group in the headgroup or backbone region of the phospholipid is still not demonstrated unequivocally (see, for example, Refs. 7 and 8 and references therein), although many possible sites for such an interaction have been considered (see, for example, Ref. 9 and references therein).

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In this report we describe the effect of epicholesterol and cholesterol on the gel to liquid-crystalline phase transitions of SOPC and OSPC to see whether the change of orientation of the hydroxyl group of cholesterol alters the observed calorimetric behaviour in these systems. If the differences in the shapes of the endotherms of OSPC-cholesterol and SOPC-cholesterol were determined exclusively by an orientation of the cholesterol which was dependent on the formation of a specific hydrogen bond with the PC headgroup, one might except that the influence of epicholesterol on the endotherms of the transitions of the two PC isomers would be opposite in 'sense' to that seen with cholesterol.

SOPC and OSPC from two sources (synthesized in our laboratory [1] or purchased from Sigma Chemical Co, St. Louis, MO) were mixed with 13 or 17 mol% of cholesterol (Sigma) or epicholesterol (Steraloids Inc., Wilton, NH). The sterols each gave a single spot on thin-layer chromatography. The PC were pure by thin-layer chromatographic, fatty acid and ultraviolet light analyses. They were found to contain between zero and five percent of their respective positional isomers when analyzed as described before [10].

Lipid-sterol mixtures were made in chloroform. After removal of the chloroform solvent and drying under vacuum overnight in the presence of P_2O_5, the lipid mixtures were dispersed in deionized, doubly-distilled water (approx. 33% by weight) by vortexing for 1 min at room temperature. Samples were analyzed by differential scanning calorimetry using a Perkin Elmer DSC-2 operated at scan rates of 5 Cdeg/min and full scale sensitivities of 0.2 to 2 mcal/s [1,2]. After calorimetric analysis a very small amount of lysoPC was observed in one each of the SOPC and OSPC samples. Analysis of the endotherms from gel to liquid-crystalline transitions and their deconvolution into components was carried out as described before [1].

Endotherms where the excess specific heat has been normalized per mole of phospholipid are shown in Fig. 1. Dispersions with cholesterol or epicholesterol produced endotherms which had different shapes depending upon with which PC isomer they were mixed. As seen before, the endotherms from SOPC-cholesterol were resolvable into two components [1], a narrow component with a T_{max} slightly below that of the PC and a broad component with a T_{max} near that of the PC. Endotherms from SOPC-epicholesterol had similar shapes to those of SOPC-cholesterol, and were resolvable into similar narrow and broad components. The endotherms from dispersions of OSPC-cholesterol were also resolvable into two components but in this case the narrow component had the smaller proportion of the total enthalpy change and the broad component the greater proportion. The endotherms from OSPC-epicholesterol were similar in shape to those of OSPC-cholesterol, and different from those of SOPC-sterol dispersions. One series of thermograms was obtained for each PC together with 17 mol% epicholesterol. The shapes were similar to those of the corresponding PC with 13 mol% cholesterol.

Quantitative data from the curve analyses are given in Table I. Epicholesterol caused a smaller reduction in the total enthalpy change of the transition of each PC isomer than did cholesterol. This is consistent with previous observations that epicholesterol is less effective on a molar basis than cholesterol at reducing the enthalpy change of the transition of OSPC [5], condensing monolayers of OSPC [12], and reducing the permeability of egg phosphatidylcholine liposomes [13]. A recent study employing ^{2}H-NMR spectroscopy has shown that the effects of epicholesterol on the 'fluidity' of dimyristoyl PC below and above its phase transition are similar in kind to those of cholesterol, but they are of a reduced magnitude in comparison to those of cholesterol [6].

Both sterols influenced the transitional endotherm of any one positional isomer in the same 'sense'. For endotherms from samples containing 13 mol% sterol the following points apply. (i) The shapes of the endotherms from SOPC-cholesterol and SOPC-epicholesterol are similar to one another; the shapes of the endotherms from OSPC-sterols are also similar to each other, but they are different from those of SOPC-sterols. (ii) Of the total ΔH for a given PC, the two steroid isomers caused the same proportion to be distributed to the narrow and broad components. For example, the ΔH of the narrow component of SOPC-sterol was between 44 and 64% of the total

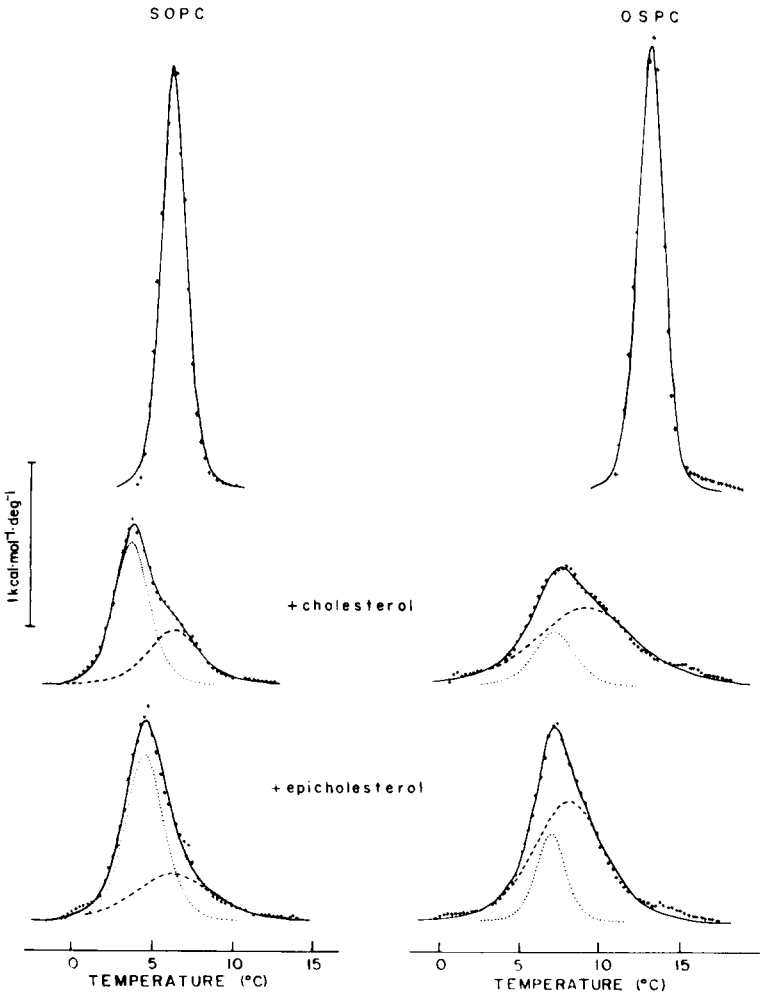

Fig. 1. Normalized endotherms for the calorimetric scans of SOPC, OSPC, SOPC-cholesterol (87 : 13, mol/mol), SOPC-epicholesterol (87 : 13, mol/mol), OSPC-cholesterol (87 : 13, mol/mol) and OSPC-epicholesterol (87 : 13, mol/mol). The solid lines show the total normalized excess specific heat curves. The dotted lines are for the narrow components and the dashed lines for the broad components. +, Normalized data points taken every 0.25 Cdeg along the original DSC trace. The program used for deconvolution assumes that two independent two-state transitions, each obeying the van't Hoff equation, contribute to the total excess heat capacity [1,14].

ΔH for dispersions with cholesterol and epicholesterol. For OSPC-steroid the narrow components for mixtures with both cholesterol and epicholesterol were between 15 and 23% of the total ΔH. The endotherm of samples containing 17 mol% cholesterol indicated that this sterol had much less influence in the transition than did cholesterol (see Ref. 5). The trend in the effects of the two sterols were similar, with cholesterol and epicholesterol removing more of the total ΔH from OSPC than from SOPC at any given concentration of sterol. Also the narrow component was removed to a greater extent in OSPC than in SOPC. These differences and similarities are not due to limited solubility of the steroids because both are below their solubility limits [13].

The results indicate that for both cholesterol isomers there may be a preferential orientation between one face of the sterol and the chain located on a given position on the glycerol backbone as suggested previously for the interaction of cholesterol and PC [1–4]. Both isomers would appear to have the same preference, although their influence differs in magnitude. It would appear that the differential interaction is not strongly dependent on the configuration of the cholesterol hydroxyl, but is more likely influenced by the other parts of the steroid known to be important in cholesterol-phospholipid interactions (see, for example, Ref. 13). Perhaps the energy gain from the appropriate matching of sterol faces with saturated and unsaturated chains [3,4] is sufficient

TABLE I

DISTRIBUTION OF ENTHALPY CHANGE FOR MIXTURES OF PC-STEROL

T_{max}, temperature at which the maximum excess specific heat occurred. Data for samples No. 1 containing cholesterol were estimated from Ref. 1.

PC	Sterol	mol% sterol	Sample No.	Total		Narrow component			Broad component		
				T_{max} (°C)	ΔH (kcal·mol^{-1})	T_{max} (°C)	ΔH (kcal·mol^{-1})	% of total ΔH	T_{max} (°C)	ΔH (kcal·mol^{-1})	% of total ΔH
SOPC	None	0	1	6.4	6.6						
		0	2	7.4	5.3						
	Cholesterol	13	1	6.6	5.5	6.6	2.4	44	9.0	3.1	56
		13	2	3.8	3.9	3.8	2.5	64	6.4	1.4	36
		17	1	6.2	4.0	1.5	0.3	8	6.2	3.7	92
	Epicholesterol	13	1	5.5	7.5	5.2	3.8	51	6.6	8.7	49
		13	2	4.6	5.0	4.6	3.2	64	6.3	1.8	36
		17	1	5.5	4.4	5.3	3.3	75	8.2	1.1	25
OSPC	None	0	1	9.0	6.4						
		0	2	13.0	5.8						
	Cholesterol	13	1	8.1	3.8	6.6	0.7	18	11.1	2.9	82
		13	2	7.4	5.1	7.0	1.1	22	9.0	4.0	78
		17	1	7.9	3.5	–[a]	–[a]	–[a]	7.9	3.5	100
	Epicholesterol	13	1	10.1	5.2	9.2	0.8	15	11.2	4.4	85
		13	2	7.1	5.7	7.0	1.3	23	8.1	4.4	77
		17	1	6.0	3.8	5.7	1.7	45	6.9	2.1	55

[a] No narrow component.

to overcome any disadvantage associated with a loss of hydrogen bonding potential in the presence of epicholesterol.

It is not possible to say if the association of these molecules in the gel state, implied by the shapes of the endotherms, exists in the liquid crystal where rotational and translational diffusion rates are much higher than in the gel.

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