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CALORIMETRIC INVESTIGATIONS OF PHASE TRANSITIONS IN LIPOSOMES CONTAINING OXYGENATED STEROLS

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ABSTRACT

Liposomes of 1,2-dipalmitoyl-3-sn-phosphatidylcholine (DPPC) containing 7α-, 7β- and 25-hydroxycholesterol and 7-ketocholestanol (oxygenated sterol compounds, OSC) were studied by Differential Scanning Calorimetry. Incorporation of OSC markedly changes the phase transition behaviour of cholesterol-free as well as of cholesterol-containing liposomes. 7β-hydroxycholesterol, being very effective in changing the properties of bio-membranes, is the most potent of the OSC investigated in influencing the phase transition characteristics of liposomes.

INTRODUCTION

Dispersions of phospholipids in water (model membranes) show a sharp, reversible phase transition from a "crystalline" phase to a "liquid-crystalline" phase at about 310 K. Introduction of cholesterol into such systems drastically decreases the enthalpy and the sharpness of this transition; cholesterol exhibits a liquefying effect on lipids in the crystalline state and stabilizes a so-called intermediate gel-state (ref.1). Calorimetric techniques such as Differential Scanning Calorimetry (DSC) are well suited for characterization of phase transitions from this gel state to the liquid-crystalline state. Recently is has been found (ref.2), that in vivo a group of OSC incorporated in cell membranes - replacing cholesterol or in addition to the cholesterol already present in the membranes - affect the physical properties and biological functions of these membranes. As an example, human erythrocytes treated with OSC may show echinocytic transformation (ref.3) as a consequence of the altered membrane properties. OSC possibly also play an active role in the genesis of atherosclerosis in man (ref.4).

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We have studied the influence of four different OSC (see fig.1) on the phase transitions of cholesterol-free as well as of cholesterol-containing model membranes by DSC. Since it cannot be excluded, that using high concentrations of cholesterol or OSC causes a partial formation of crystalline domains of the pure compounds within the liposomes, we also checked the phase transition behaviour of the pure compounds used in this study. Of all compounds investigated, only anhydrous cholesterol - a phase probably not occurring in biological media - exhibits a phase transition in the temperature range of interest (ref.5,6).

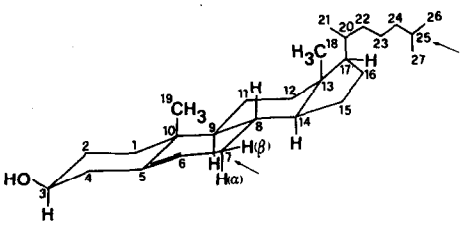

Fig. 1. Numbering scheme of 5-cholesten-3β-ol (cholesterol). Arrows indicate sites of oxidation of the derivatives used.

RESULTS AND DISCUSSION

The results can be summarized as follows:

7α-,7β-Hydroxycholesterol and 7-ketocholestanol as well as cholesterol suppress the occurrence of the small pre-transition at about 306 K when incorporated in amounts of 10 mole% or more.

In contrast to cholesterol and 7-ketocholestanol, 7α- and 7β-hydroxycholesterol give rise to symmetrically shaped DSC peaks corresponding to the main transition when incorporated in amounts of about 20 mole%.

The OSC cause a decrease of the transition temperature as demonstrated in fig. 3(A). This effect decreases in the following rank order: 7β-hydroxycholesterol > 7α-hydroxycholesterol > 7-ketocholestanol > cholesterol.

7α-,7β-Hydroxycholesterol and 7-ketocholestanol diminish the enthalpy of the transition when incorporated in the liposomes (see fig. 3(B)).

The effects of 25-hydroxycholesterol markedly differ from those of the other OSC. A possible interpretation of our DSC data is, that 25-hydroxycholesterol - in contrast to the other OSC investigated - is almost insoluble in cholesterol-free DPPC liposomes below the actual phase transition temperature. A considerable solubility in the cholesterol-rich phase however is indicated by our measurements.

7β-Hydroxycholesterol was found to be the most potent of the oxygenated sterol compounds examined as regards their ability to decrease the extrapolated onset temperature of the gel to liquid-crystalline phase transition in DPPC liposomes.

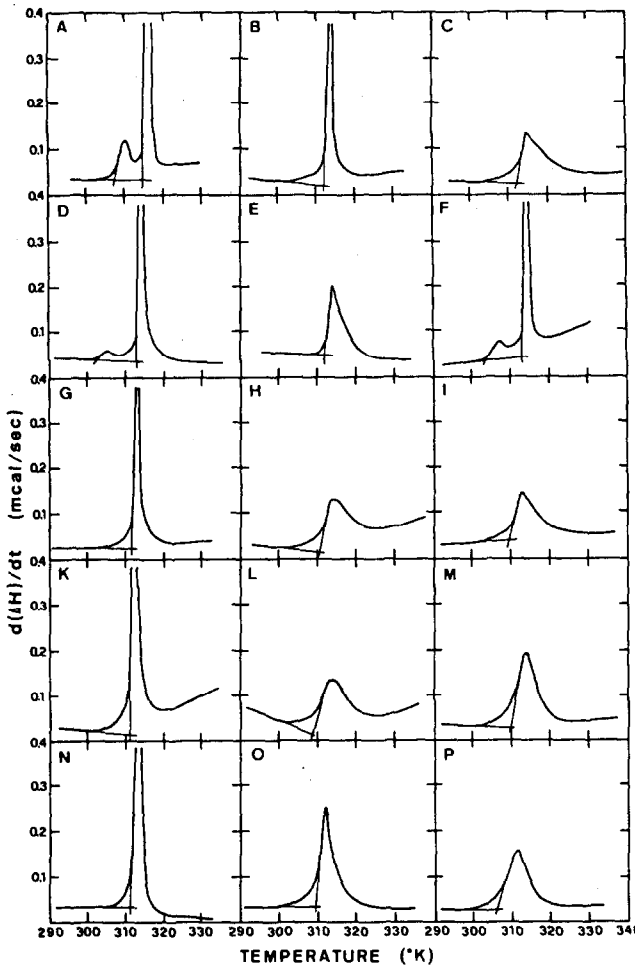

Fig. 2. DSC scans of aqueous dispersions of DPPC/cholesterol/OSC-mixtures (DPPC ~ 0.1 M, Perkin-Elmer DSC-2, scanning rates 10°/min., flowing nitrogen atm.). Sample compositions are given in the table below.

	DPPC (g)	Cholesterol (mol %)	OSC (mol %)	Total Sterol (mol %)	Curve
	739	0.0	0.0	0.0	A
	570	10.4	0.0	10.4	B
	739	20.2	0.0	20.2	C
25-Hydroxy-cholesterol	673	0.0	9.4	9.4	D
	702	9.6	10.0	19.6	E
	573	0.0	21.0	21.0	F
7-Keto-cholestanol	611	0.0	10.8	10.8	G
	768	9.7	10.0	19.7	H
	568	0.0	20.1	20.1	I
7α-Hydroxy-cholesterol	776	0.0	10.9	10.9	K
	753	10.4	9.3	19.7	L
	799	0.0	19.3	19.3	M
7β-Hydroxy-cholesterol	706	0.0	7.0	7.0	N
	790	10.1	9.3	19.4	O
	650	0.0	18.3	18.3	P

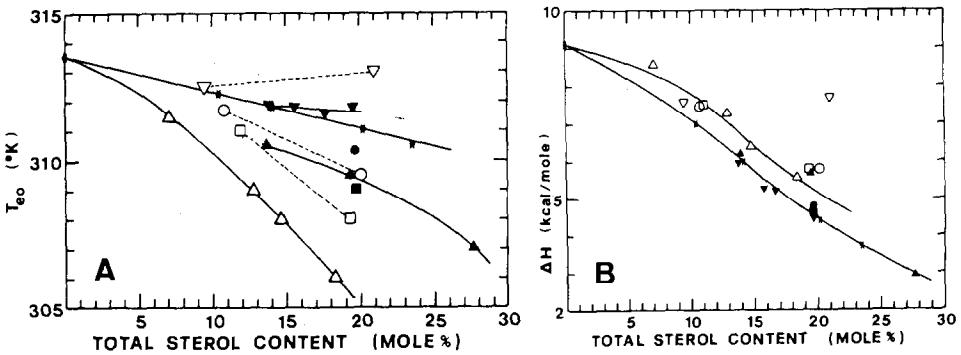

Fig. 3. Temperature of the extrapolated onset T_{eo} (A) and enthalpy ΔH (B) of the gel to liquid-crystalline transition of aqueous dispersions of DPPC/cholesterol/OSC mixtures versus total sterol content. Data were calculated from DSC scans registered under the same conditions as indicated in fig. 2. Crossed points refer to samples containing DPPC and cholesterol, empty dots to samples containing DPPC and OSC and black dots to samples containing DPPC, cholesterol and OSC.

▽ : 25-hydroxycholesterol ○ : 7-ketocholestanol

□ : 7α-hydroxycholesterol △ : 7β-hydroxycholesterol

A kinetic analysis of the DSC data presented here and a discussion of possible phase transition mechanisms is given elsewhere (ref.6).

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