Influence of Oxygenated Sterol Compounds on Phase Transitions in Model Membranes. A Study by Differential Scanning Calorimetry†

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ABSTRACT: A marked influence of oxygenated sterol compounds (OSC: 7α-, 7β-, and 25-hydroxycholesterol and 7-ketocholestanol) on the reversible gel to liquid-crystalline phase transition behavior of cholesterol-free and cholesterol-containing model membranes is evidenced by high-sensitivity differential scanning calorimetry. Liposomes of dipalmitoylphosphatidylcholine (DPPC) were chosen as model membranes. Each of the investigated OSC exerts an individual influence on the phase transition of DPPC liposomes, which expresses itself in the temperature range, the corresponding enthalpy, and the peak shape of calorimetric curves. The onset temperature of the phase transition is lowered in the following range of effectiveness: 7β-hydroxycholesterol > 7α-hydroxycholesterol > 7-ketocholestanol > cholesterol. The mutual presence of cholesterol and of OSC leads to the following order: 7α-hydroxycholesterol ≈ 7β-hydroxycholesterol > 7-ketocholestanol > cholesterol (without OSC) > 25-hydroxycholesterol. The enthalpy of the phase transition is decreased with increasing content of cholesterol, 7α- or 7β-hydroxycholesterol, or 7-ketocholestanol. At a concentration of about 10 mol % of the latter three OSC, the corresponding enthalpy value of the transition is lowered from 9.1 kcal/mol for pure DPPC to about 7.5 kcal/mol, whereas 10 mol % cholesterol lowers the enthalpy value to 7.0 kcal/mol. Incorporation of both cholesterol and one of the OSC into the DPPC liposomes leads to enthalpy values depending on the total sterol content only, except in the case of 7β-hydroxycholesterol at relatively high concentration (about 10 mol %); here, we found a higher value. Cholesterol-free DPPC liposomes containing 25-hydroxycholesterol display a thermotropic behavior very similar to that of pure DPPC liposomes. This effect is attributed to the sparing solubility of 25-hydroxycholesterol in DPPC bilayers. The mutual presence of cholesterol and 25-hydroxycholesterol in DPPC liposomes, however, leads to a thermotropic behavior resembling that of model membranes containing cholesterol and either 7α- and 7β-hydroxycholesterol or 7-ketocholestanol.

A group of oxygenated sterol compounds (OSC)[1] has been identified to influence a variety of important processes in living cells (Kandutsch et al., 1978). Besides the inhibition of sterol synthesis, OSC also affect membrane-associated functions and membrane morphology in a wide range (Yachnin et al., 1979). OSC exhibit their greatest activity if they possess the 3β-hydroxyl group of cholesterol and the complete eight-carbon side chain at carbon atom 17. Oxygenated derivatives of 5α-cholestanol, which lack the double bond between carbon atoms 5 and 6, also act as potent suppressors of cholesterol synthesis.

Cells cultured in the presence of 25-hydroxycholesterol display markedly abnormal characteristics provided exogenous cholesterol is absent. All of these biochemical abnormalities can be at least partially prevented or reversed by the addition of exogenous cholesterol to the 25-hydroxycholesterol-containing cell cultures. These abnormalities have been attributed to altered membrane properties produced by the lipid imbalance of the cell membrane (Yachnin et al., 1979). OSC, when incubated with human erythrocytes in lipoprotein-depleted medium, are inserted into the cell membrane. In some cases, echinocytic transformation of the red blood cells was observed. Echinocyte-forming as well as non-echinocyte-forming OSC, when incorporated in red cell membranes, result in expansion of the surface area of red cell ghost membranes, in an increase of the hemolytic volume, and, as a consequence, in diminished osmotic fragility of erythrocytes. The most potent inhibitors of osmotic lysis were found to be 7β-hydroxycholesterol, 22-ketocholesterol, and 20α-hydroxycholesterol (Streuli et al., 1981a,b).

Calorimetric studies of the influence of OSC on the phase transition behavior of red cell ghost membranes would provide some important information about their thermodynamic properties. Since biological membranes are composed of a great variety of different lipids and proteins, such systems are too complex for the first orienting study. Hence, investigations of liposomes containing one chemically pure phospholipid provide basic information of the influence of OSC on more complex systems. Calorimetric examination of liposomes consisting of dipalmitoylphosphatidylcholine- (DPPC) containing cholesterol has been the topic of several publications (Ladbrooke et al., 1968; de Kruyff et al., 1973, 1974; Demel & de Kruyff, 1976; Estep et al., 1978; Mabrey et al., 1978). In the present work, differential scanning calorimetry (DSC) studies on cholesterol-free and cholesterol-containing aqueous dispersions of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) were carried out in order to obtain thermodynamic and kinetic information about the influence of OSC on the gel to liquid-crystalline phase transitions in these liposomal systems. The following OSC were employed: 7α-, 7β-, and 25-hydroxycholesterol and 7-ketocholestanol.

Egg lysolecithin and cholesterol, suspended in water, form stoichiometrically bimolecular lamellar structures as found in phosphatidylcholine bilayers. An excess of cholesterol leads to the occurrence of crystalline cholesterol within the lamellar phase, as revealed by X-ray analysis (Rand et al., 1975). Crystalline anhydrous cholesterol exhibits a reversible endothermic phase transition at about 308 K (Spier & van Senden, 1965). Although this transition has not been described in liposomal systems yet, domains of pure crystalline cholesterol may appear in cholesterol-rich liposomes. This also holds for

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[1] Abbreviations: DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DSC, differential scanning calorimetry; DTA, differential thermal analysis; OSC, oxygenated sterol compounds; TG, thermogravimetry.

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systems containing OSC. Therefore, the phase behavior of pure cholesterol, of cholesterol monohydrate, and of the OSC used in this study was investigated prior to the examination of the model membrane systems.

Experimental Procedures

Materials. Cholesterol (biochemical grade; Merck, West Germany), 7α-, 7β-, and 25-hydroxycholesterol and 7-ketocholestanol (Steraloids Inc., Wilton, NH), and 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (purissium; Fluka, Switzerland) were commercially purchased. All substances were checked for contamination by thin-layer chromatography. 7α-Hydroxycholesterol and 7-ketocholestanol contained small amounts of impurities. The compounds were purified by thin-layer chromatography on silica gel plates in 50:50 (v/v) ethyl acetate:*n*-heptane and recrystallized from analytical-grade acetone. Cholesterol monohydrate was prepared by crystallization from aqueous acetone.

Preparation of Model Membranes. All DPPC dispersions were prepared by mixing appropriate aliquots of the corresponding lipids in chloroform. The solvent was removed at room temperature under a stream of nitrogen. After vacuum drying for at least 15 h, a phospholipid concentration of about 0.1 M was obtained by addition of doubly distilled water. The sample was vortexed for 20 min at 320 K under a nitrogen atmosphere. Samples were stored at 270 K. According to Suurkuusk et al. (1976), liposomes with a radius between 500 and 5000 Å are produced by this procedure, whereas ultrasonic treatment leads to vesicles with a radius of about 110 Å. Such vesicles, however, are metastable and therefore not qualified for calorimetric investigation.

Thermal Analysis. Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC-2 calorimeter under a flowing helium or nitrogen atmosphere at a scanning rate of 10 °C/min in the heating and cooling mode. The pure crystalline sterols were investigated in standard aluminum pans. The liposomal suspensions (about 7 μL) were hermetically sealed in special aluminum pans. After completion of the DSC runs, samples of pure sterols as well as samples of liposomes were quantitatively recovered from the pan, dissolved in a 1:1 (v/v) mixture of chloroform and methanol, and checked by thin-layer chromatography on silica gel plates either in ethyl acetate/*n*-heptane (1:1 v/v) (pure sterols) or in chloroform/methanol/water (130:70:8 v/v) (liposomal systems). DPPC was visualized by iodine staining; the sterols were visualized by staining with a 1:1 (v/v) mixture of absolute sulfuric acid and a saturated aqueous solution of ammonium cerium(IV) sulfate. After several heating/cooling cycles, a small amount of an impurity with a spot at $R_f = 0.04$ (R_f for DPPC was 0.11) was found only in samples containing 25-hydroxycholesterol.

Dehydration and phase transitions of cholesterol monohydrate were investigated by simultaneous thermogravimetry (TG) and differential thermal analysis (DTA) using a Stanton-Redcroft STA 781 instrument. In addition, the exact weight losses were measured with a high-sensitivity thermobalance (Perkin-Elmer TGS-2). DTA/light microscopy experiments were performed on a Mettler 2000-B cell modified to allow simultaneous observation in a polarizing microscope.

In thermoanalytical studies, the temperature of the extrapolated onset, T_{eo}, is usually indicated to mark the beginning of a reaction. The intersection of the extrapolated base line with the tangent through the inflection point of a DSC peak was taken to be the onset of a transition. Transition enthalpies were calculated from DSC scans by either electronic or graphic integration of the corresponding peaks areas.

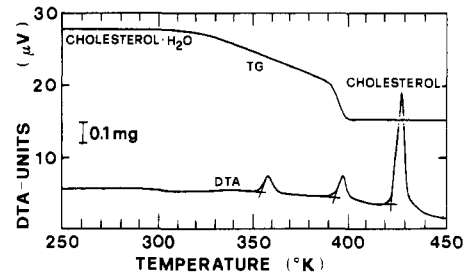

FIGURE 1: Simultaneous thermogravimetry (TG) and differential thermal analysis (DTA) curves of crystalline cholesterol monohydrate (10.50-mg sample, heating rate 5 °C/min, flowing N_2 atmosphere).

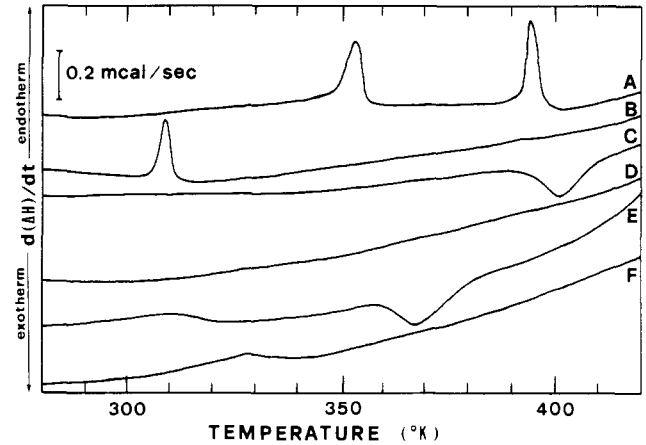

FIGURE 2: DSC scans of pure crystalline sterols (heating rate 10 °C/min, flowing He atmosphere). (A) Cholesterol monohydrate (2.21 mg); (B) dehydrated cholesterol (from scan A); (C) 25-hydroxycholesterol (2.89 mg); (D) 7-ketocholestanol (2.09 mg); (E) 7α-hydroxycholesterol (2.00 mg); (F) 7β-hydroxycholesterol (3.25 mg). Curves C through F are the first heating scans. Curves after melting of the sample may differ markedly.

Results

Pure Sterols. In the anhydrous form of pure cholesterol, we find an endothermic solid/solid phase transition at 308 K with a transition enthalpy of about 0.70 kcal/mol (curve B in Figure 2). The transition is completely reversible with a temperature hysteresis of 15–20 K on cooling. Figure 1 presents our results of simultaneous TG/DTA investigations of crystal plates of cholesterol monohydrate carefully crystallized from aqueous acetone. The purity of the samples was checked by elemental analysis and X-ray diffraction. Two endothermic irreversible transitions at 353 K and at 393 K and also melting of anhydrous cholesterol at 422 K are indicated by the DTA curve. The enthalpy values for the first two transitions determined by DSC analysis fluctuate around mean values of 2.0 and 4.6 kcal/mol, respectively. They depend on crystal size, crystal morphology, and experimental conditions. The loss of the water molecule of cholesterol monohydrate occurs in the temperature range of 310–400 K with a marked increase of the reaction rate at 393 K, where the second transition is evident in the DTA curve. Both transitions at 353 and at 393 K also clearly appear in simultaneous DTA/light microscopy experiments. The crystal morphology remains unchanged during both transitions, whereas a darkening of certain areas within the crystal plates is observed.

None of the investigated OSC (curves C–F in Figure 2) exhibits thermotropic behavior similar to that of cholesterol. The endothermic curvatures of 7α- and 7β-hydroxycholesterol (curves E and F in Figure 2) at $T_{max} = 312$ K and $T_{max} = 328$ K, respectively, represent a slow irreversible process, presumedly due to the loss of moisture. The exothermic peaks

Table I: Thermodynamic Characteristics of Aqueous Dispersions of DPPC/Cholesterol/OSC Mixtures[a]

	composition (mol %)	transition temp (K) heating T_{eo}(±0.3)	heating T_{max}(±0.2)	heating $\Delta T_{1/2}$	cooling T_{max}(±0.2)	enthalpy of transition, ΔH (kcal/mol of DPPC)
DPPC, pure						
pretransition		305.8	308.5	3.3	300.0	1.5 ± 0.2
transition		313.5	315.0	1.0	312.0	9.1 ± 0.2
DPPC and cholesterol	10.4	312.2	313.0	1.4	312.0	7.0 ± 0.3
	14.2	311.8	313.5	2.3	311.5	6.0 ± 0.3
	20.2	311.0	313.5	5.9	311.5	4.4 ± 0.3
	23.5	310.5	314.0	8.5	311.3	3.7 ± 0.3
DPPC and cholesterol/25-hydroxycholesterol						
pretransition	0.0/9.4	302.0	305.2	4.0	?	0.5 ± 0.1
transition	0.0/9.4	312.3	313.3	1.3	312.3	7.6 ± 0.2
pretransition	0.0/21.0	304.8	306.8	2.3	?	0.3 ± 0.1
transition	0.0/21.0	313.0	314.0	1.1	312.0	7.7 ± 0.2
	10.9/3.0	311.8	313.0	1.7	312.0	6.0 ± 0.2
	10.5/5.2	311.8	313.0	1.9	312.0	5.3 ± 0.2
	11.5/5.2	311.5	313.0	1.9	312.0	5.3 ± 0.2
	9.6/10.0	311.8	314.0	3.8	312.0	4.5 ± 0.2
DPPC and cholesterol/7-ketocholestanol	0.0/10.8	311.7	313.0	1.6	312.0	7.5 ± 0.3
	0.0/20.1	309.5	312.5	6.3	311.5	5.8 ± 0.3
	9.7/10.0	310.3	313.0	6.9	312.0	4.8 ± 0.3
DPPC and cholesterol/7α-hydroxycholesterol	0.0/10.9	311.0	312.8	1.5	311.2	7.5 ± 0.2
	0.0/19.3	308.0	313.0	5.6	312.0	5.8 ± 0.2
	10.4/9.3	309.0	313.5	7.1	312.8	4.6 ± 0.2
DPPC and cholesterol/7β-hydroxycholesterol	0.0/7.0	311.5	313.0	2.0	312.0	8.6 ± 0.2
	0.0/12.8	309.0	312.0	3.5	310.5	7.3 ± 0.3
	0.0/14.6	308.0	311.7	3.8	310.4	6.4 ± 0.3
	0.0/18.3	306.0	311.0	5.8	310.3	5.6 ± 0.3
	8.7/5.1	310.5	312.5	3.0	310.7	6.2 ± 0.3
	10.1/9.3	309.5	312.0	3.8	310.8	5.7 ± 0.3
	23.3/4.3	307.0	315.5	11.5	312.5	2.9 ± 0.3

[a] T_{eo}, temperature of the extrapolated onset; T_{max}, peak temperature; $\Delta T_{1/2}$, peak width at half peak height.

of 25-hydroxycholesterol at T_{max} = 402 K, 7α-hydroxycholesterol at T_{max} = 368 K (curves D and E in Figure 2), and various exothermic reactions of the other investigated OSC at temperatures above 420 K (not shown in Figure 2) may be due to rearrangement or decomposition reactions. Thin-layer chromatography of samples scanned several times up to the melting point showed several minor spots; the main spot still was the initial substance, however. Since the study of aqueous dispersions of DPPC/cholesterol/OSC mixtures was confined to an upper temperature limit of 335 K, no further investigations of those high-temperature exothermic reactions of the pure OSC were carried out.

Liposomal Systems. The results of DSC experiments on aqueous dispersions of DPPC containing various amounts of cholesterol and/or OSC are listed in Table I. The data are mean values of at least four heating/cooling cycles. The indicated errors of temperature and enthalpy were estimated from repeated heating/cooling experiments.

Pure and 25-hydroxycholesterol-containing DPPC dispersions exhibit two DSC peaks, denoted as the pretransition and the transition, respectively. The occurrence of the pretransition is suppressed by all other investigated sterols even when present in small amounts (<10 mol %). Increasing cholesterol content leads to asymmetrically shaped DSC peaks. A similar behavior can be observed in liposomes containing 7-ketocholestanol. In contrast to those systems, dispersions of DPPC and either 7α- or 7β-hydroxycholesterol, even up to contents of 20 mol %, yield quite symmetric DSC curves. If about 10 mol % cholesterol and about 10 mol % OSC were admixed, asymmetry in peak shape occurred in all investigated systems. With the exception of 25-hydroxycholesterol, increasing sterol content of the DPPC liposomes decreases the initial transition temperature (T_{eo}) in the following rank order: 7β-hydroxycholesterol > 7α-hydroxycholesterol > 7-ketocholestanol > cholesterol. The peak maximum (T_{max}) is only slightly shifted. Increasing cholesterol content decreases the transition enthalpy, as does the coexistence of cholesterol and OSC to approximately the same extent with the exception of dispersions containing both 7β-hydroxycholesterol and cholesterol. These show a higher enthalpy than liposomes with an equivalent content of cholesterol. Cholesterol-free samples to which about 20 mol % 7α- or 7β-hydroxycholesterol or 7-ketocholestanol was added yield transition enthalpies in the range of 5.6–5.8 kcal/mol compared to 4.4 kcal/mol in samples containing cholesterol only.

With the exception of 25-hydroxycholesterol, increasing total sterol content leads to a broadening of the DSC peaks. Peak broadening is most pronounced for liposomes containing 7β-hydroxycholesterol. According to cooling scans, no distinct hysteresis occurs.

The individual influence of OSC or cholesterol on the phase transition behavior of DPPC liposomes becomes even more evident if the kinetics of the transitions are evaluated. The heat absorbed or emitted during a reaction is proportional to the amount of the system which has reacted. Therefore, the heat absorbed at a certain temperature (ΔH_T) divided by the total heat absorption (ΔH) equals the degree of the reaction: $\alpha = \Delta H_T/\Delta H$. The plots of α vs. temperature for some representative samples are given in Figure 3. Since in DSC experiments the temperature is a function of time (established by the scanning rate), the slope of the tangents of these plots is a measure of the velocity of the transition. From the data shown in Figure 3, it is evident that the main phase transition in pure DPPC liposomes is faster than the pretransition. Increasing sterol content drastically lowers the reaction rate except for DPPC dispersions containing 25-hydroxycholesterol. Cholesterol-rich systems exhibit a retarded reaction rate at temperatures corresponding to α values higher than 0.5.

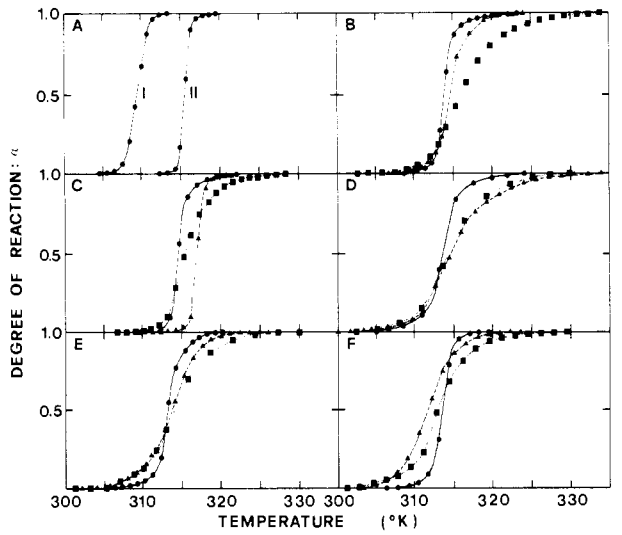

FIGURE 3: Reaction degree (α) of the gel to liquid-crystalline transition vs. the temperature of aqueous dispersions of DPPC/cholesterol/OSC mixtures. Data were calculated from DSC curves registered under the same conditions as indicated in Figure 2. (A) Pure DPPC dispersions: (I) pretransition; (II) transition. (B) DPPC/cholesterol mixtures: (solid line) 10.4 mol % cholesterol; (dashed line) 14.2 mol % cholesterol; (dotted line) 20.2 mol % cholesterol. (C–F) DPPC/cholesterol/OSC mixtures: (solid line) about 10 mol % OSC (no cholesterol); (dashed line) about 20 mol % OSC (no cholesterol); (dotted line) about 10 mol % OSC and about 10 mol % cholesterol. The inserted OSC is 25-hydroxycholesterol in (C), 7-ketocholestanol in (D), 7α-hydroxycholesterol in (E), and 7β-hydroxycholesterol in (F). For the exact sample composition, see Table I.

Samples containing 7α- or 7β-hydroxycholesterol gave symmetric α vs. T plots only.

Whereas the thermal behavior of DPPC dispersions containing only 25-hydroxycholesterol is dependent on the thermal history of the sample investigated, all the other samples of freshly prepared liposomes exhibit identical DSC curves even after repeated heating/cooling cycles. In Figure 4, DSC scans of DPPC liposomes containing only 25-hydroxycholesterol are presented. With increasing cycle number, both the pretransition and the transition peak decrease in height and area; the pretransition peak, however, does so in a more pronounced manner than the transition peak. The width at half-maximum height ($\Delta T_{1/2}$) of the transition peak increases with consecutive scans. After the third cycle, peak shapes, heights, and areas remain constant. If the sample is stored for 3 h at 280 K, the DSC curve registered is almost identical with the first one (panel D in Figure 4). Further cycling decreases the pretransition and the transition peaks again. Cooling scans of the third cycle show a small and extremely broad pretransition peak. This may be due to the fact that the pretransition is much slower than the transition.

Discussion

The solid/solid phase transition found at 308 K for pure crystalline anhydrous cholesterol is in accordance with literature data (Spier & van Senden, 1965; Petropavlov & Kostin, 1976; Loomis et al., 1979; Garti et al., 1981). The reversibility of this transition and the influence of the thermal history of the samples on the DSC curve shape have been earlier described by us (Dubler & Kamber, 1980). In a NMR study, this transition has been attributed to a change in the packing of the terminal CH_3 groups of the aliphatic side chain (van Putte et al., 1968).

Contradictory results are reported on the phase behavior of pure crystalline cholesterol monohydrate (Faiman, 1977; Loomis et al., 1979; Garti et al., 1981). Our DSC/TG in-

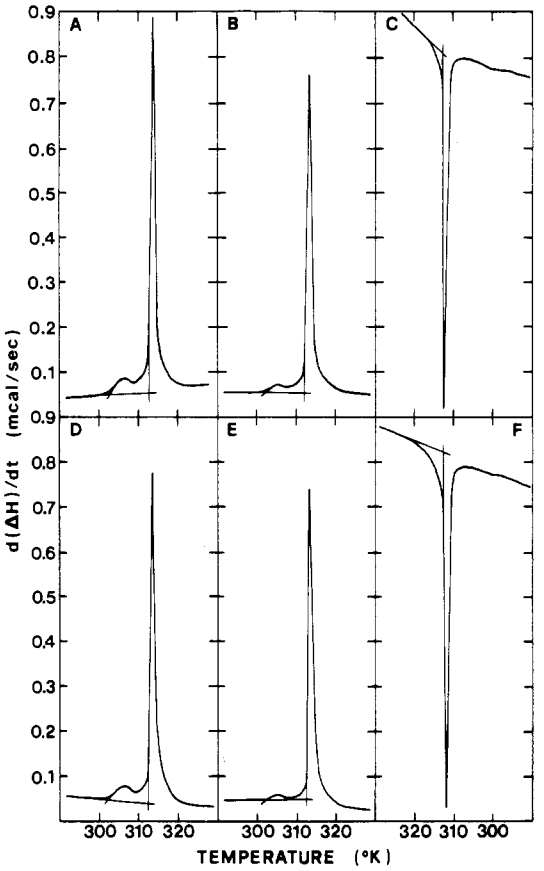

FIGURE 4: DSC scans of an aqueous dispersion of a DPPC/25-hydroxycholesterol (9.4 mol %) mixture at a scanning rate of 10 °C/min. The DPPC content was 673 μg (0.1 M). (A) First heating run of a sample stored for 15 h at 270 K; (B) third heating run; (C) cooling curve after the third heating run; (D) first heating run after the sample (from scan C) had been stored for 3 h at 280 K; (E) third heating run of the sample; (F) cooling curve after the third heating run (scan E).

vestigations of crystal platelets in flowing nitrogen atmosphere substantiate the occurrence of two endothermic phase transitions at 353 and 393 K. In contrast to the DSC experiments of cholesterol monohydrate in hermetically sealed pans with an equal amount of water by Loomis et al. (1979), both transitions are irreversible under our experimental conditions. Whereas the transition at 393 K clearly coincides with the maximum dehydration reaction rate, the nature of the transition at 353 K remains unclear.

None of the pure OSC investigated as well as the pure cholesterol monohydrate show any distinct phase transition in the temperature range from 290 to 335 K. Therefore, the DSC investigation of the gel to liquid-crystalline phase transition in liposomes would not be affected by the occurrence of crystalline domains of OSC or cholesterol monohydrate within the liposomes. Domains of pure anhydrous cholesterol would give rise to overlapping thermal effects in the DSC curve due to the transition of anhydrous cholesterol at 308 K and the gel to liquid-crystalline transition in the liposome appearing in the temperature range between 305 and 315 K. Such overlapping thermal effects, however, may be ruled out since according to Bogren & Larsson (1963) only the monohydrate form of cholesterol crystallizes in biological systems.

The two DSC peaks at T_{eo} = 305.8 and 313.5 K in aqueous dispersions of pure DPPC were explained by a pretransition and a transition from a gel to a liquid-crystalline phase (Demel & de Kruyff, 1976). According to Suurkuusk et al. (1976), this behavior of DPPC dispersions is typical for Bangham-type

multilamellar liposomes (prepared in a similar manner as described in this paper) which are stable with respect to time and thermal treatment.

According to Janiak et al. (1976), the pretransition of aqueous dispersions of pure DPPC is associated with a structural transformation from a one-dimensional (below the pretransition temperature) to a two-dimensional monoclinic lattice. The latter consists of lipid lamellae distorted by a periodic undulation or ripple predominating in the temperature region between the pretransition and the main transition. Whereas DPPC liposomes containing either cholesterol, 7α- or 7β-hydroxycholesterol, or 7-ketocholestanol do not exhibit the pretransition peak in DSC scans, 25-hydroxycholesterol/DPPC dispersions stored below the pretransition temperature show a behavior similar to that of liposomes of pure DPPC. Hence, we conclude that 25-hydroxycholesterol is almost insoluble in double layers of pure DPPC below the phase transition temperature. In consecutive heating/cooling cycles of 25-hydroxycholesterol/DPPC dispersions, enthalpy and peak shape are slightly affected. This effect may be explained in terms of a slight solubility of 25-hydroxycholesterol above the main transition.

From paramagnetic resonance spectra of spin-labels (Recktenwald & McConnell, 1981), fluorescence probe studies, and freeze–fracture electron microscopy (Lentz et al., 1980), it is known that below the main transition temperature an ordered microscopic phase separation occurs in cholesterol-containing double layers of DPPC. In samples containing up to about 20 mol % cholesterol, one of the two phases essentially consists of pure DPPC, and the other phase contains about 20 mol % cholesterol and remains in a quasi-liquid state (Recktenwald & McConnell, 1981; Copeland & McConnell, 1980). The asymmetric DSC peaks of cholesterol-containing liposomes can be separated into two parts. One of them is relatively sharp and is most probably associated with chain melting of the pure DPPC phase. The other peak is broad and is related to the existence of an interfacial region between pure DPPC and cholesterol-rich domains (Estep et al., 1978). If OSC are soluble in DPPC, they might interact with DPPC in a manner similar to cholesterol and therefore lead to an analogous phase separation.

Whereas 25-hydroxycholesterol is almost insoluble in cholesterol-free DPPC liposomes, as concluded from DSC experiments, it has a high solubility in the cholesterol-rich phase due to the similarity of the two sterol molecules. Considering a similar situation in biological cell membranes leads to a possible explanation of the reversibility of membrane abnormalities which result upon insertion of OSC. Cells grown in a cholesterol-free medium, which, however, contains 25-hydroxycholesterol, exhibit markedly abnormal membrane characteristics which can be reversed by adding cholesterol to the culture medium (Yachnin et al., 1979).

7β-Hydroxycholesterol was found to be the most potent of the sterol compounds examined with regard to its ability to decrease the extrapolated onset temperature of the gel to liquid-crystalline phase transition in DPPC liposomes. This result may be connected with the marked effect of 7β-hydroxycholesterol to inhibit osmotic lysis of erythrocyte membranes (Streuli et al., 1981a). Consequently, DSC studies on red cell ghost membranes would contribute to the elucidation of such membrane aspects.

Acknowledgments

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Registry No. 7β-Hydroxycholesterol, 566-27-8; 7α-hydroxycholesterol, 566-26-7; 7-ketocholestanol, 7591-17-5; cholesterol, 57-88-5; 25-hydroxycholesterol, 2140-46-7; dipalmitoylphosphatidylcholine, 2644-64-6; cholesterol monohydrate, 5808-12-8.

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