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PHASE TRANSITIONS IN CHOLESTEROL CRYSTALLIZED FROM VARIOUS SOLVENTS

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ABSTRACT

Cholesterol was crystallized under various conditions from a range of solvents, and the effects of the solvents on the crystal structure were studied. The phase transitions and the latent heat, ΔH_p, were measured and were found to vary from 0.18 kcal mole^{-1} (for CCl_4) to 1.1 kcal mole^{-1} (for acetonitrile). It seems possible that the polymorphic transitions of cholesterol at 37°C may be divided into several subtransitions, each one corresponding to a slight configurational change that can be attributed to a possible flip-over of the aliphatic chain of the cholesterol.

INTRODUCTION

The role of cholesterol in the pathogenesis of atherosclerosis [1] and gallstones [2] is well established. In an attempt to assess the importance of the actual state of the cholesterol in human plasma, it has been shown that mainly plaques (or plates) are deposited [3], and that the form in which cholesterol is made available to the body is of great importance [3].

Besides the observed crystal-habit modifications, two crystal structure modifications (polymorphs) of cholesterol have been detected by X-ray diffraction [4]. It has not yet been established whether the crystal habit is in any way correlated with the crystal structure.

A study of the effect of solvents and conditions of crystallization was carried out in this laboratory [5]. It was shown that the nature of the solvent and the level of supersaturation both affect the crystal habit [5]. In the present study, an attempt was made to assess the effect of the solvents on the crystal structure of cholesterol, hopefully through phase-transition effects.

A phase transition in anhydrous cholesterol at 37°C has been reported by Labowitz using the DTA and DSC techniques [6]. The close proximity of the temperature of this phase transition to normal human body-temperature seems to be very significant. The presence of the undesirable phase of cholesterol can thus be attributed to the body temperature, resulting in atherosclerosis. Furthermore, good correlation was found between naturally occurring atherosclerosis in certain mammals and their blood temperature,

that is, species whose body temperature is below 37°C are not subject to that disease [6].

A latent heat, ΔH_p, of 0.7 kcal mole^{-1} for the reversible endothermic polymorphic crystalline transition has been reported using the DSC technique [7]. Thermograms indicating the endothermal phase change at 37°C were measured using both anhydrous cholesterol and samples ground with water and were compared with data obtained from previous equilibrium spreading-pressure techniques [8].

No attempts have been made to correlate the type of solvent from which cholesterol was crystallized with its crystal structure and no explanations have as yet been given as to the nature of the phase transition. Phase transitions of freshly crystallized cholesterol indicate the distinct possibility of the existence of subtransitions, i.e. partial steps induced by the particular sol-solvents in which the crystals have been grown.

EXPERIMENTAL

Materials

Cholesterol (obtained from the Aldrich Co.) was purified by repeated crystallizations from various solvents to >99.5% purity. The absence of solvates and impurities was established by gas chromatography. The melting point was 149°C and elemental analyses showed C = 83.88% and H = 11.92%.

The solvents for crystallization were of spectroscopic grade, purchased from Malinkrodt or Baker, and were redistilled before use.

Methods

Simultaneous DTA, TG and DTG determinations were carried out on a Mettler Thermoanalyzer under a controlled dry nitrogen flow at a rate of 5 l h^{-1}.

The samples were of the order of 150—200 mg in weight, and the heating rate was 2°C min^{-1}. The DTA sensitivity was 50—200 μV.

The latent heat of melting (ΔH_m) and of polymorphic transition (ΔH_t) were calculated from DTA peak areas. The calibration for the heat of transition (which occurs in the range of 36—40°C) was carried out with lauric acid, which has a melting point at 43—44°C and a latent heat of melting of 43.72 cal g^{-1}. The calibration obtained from the data of indium melting at 154.6°C (with a latent heat of 6.8 cal g^{-1}) was used to measure the heat of melting of cholesterol which occurs at 147—150°C. X-Ray measurements were carried out on carefully ground powdered samples, with a Philips diffractometer using Cu radiation and a Ni filter. Diffraction analysis was repeated several times using samples crystallized from various solvents under various conditions, at room and at elevated temperatures (40°C).

RESULTS

The DTA curves of phase transition and melting of cholesterol crystallized from two different solvents are shown in Fig. 1. A marked difference between the heats of phase transition is clearly evident.

The temperatures, and heats of transition and of melting, are listed in Table 1. The Table is divided into two sets of data: the temperatures and heats of the crystals taken directly from the crystallization process, and the data for the temperature and heat of transition of the solidified melt left in the crucible for 24 h after completion of the first melting.

The heat of transition of cholesterol after melting and resolidification is in the range 0.68—0.81 kcal $mole^{-1}$, irrespective of the solvent used and the mode of crystallization, in excellent agreement with the values cited in the literature [7]. Also, the temperatures of melting are in the narrow range 147—150°C, indicating the purity of the crystals. The latent heats vary between 5.16 and 7.12 kcal $mole^{-1}$, also in reasonably good agreement with the values reported in the literature [7]. The slight variations do not correlate with the polarity of the solvent from which cholesterol was precipitated, with the mode of formation, or with the previous, somewhat peculiar, thermal history of the samples, namely, the excessive variations of the transition heats of the freshly prepared crystals. These latter values vary between 0.14 and 1.04 kcal $mole^{-1}$.

It may be argued that the freshly prepared crystals contain some trapped solvent, and that the evolution of this solvent at the transition point affects the measurements. There are, however, two contra-arguments which refute this suggestion. First, the DTA determinations were carried out simultaneously with the TG measurements. The balance of the thermoanalyzer has a sensitivity of 0.01 mg, and so a weight loss of 0.1 mg, had it occurred, would

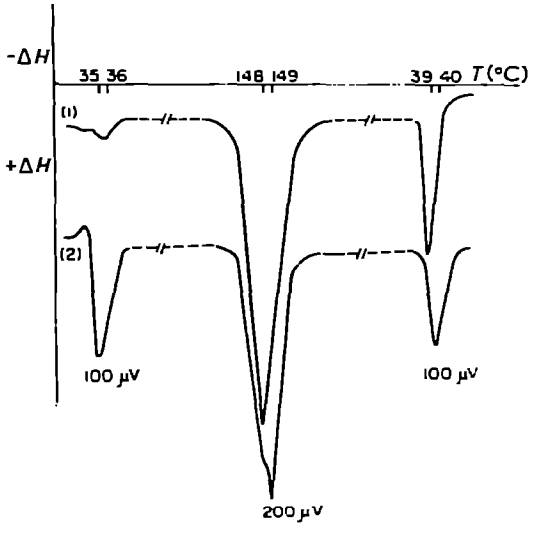

Fig. 1. DTA curves of phase transition and melting of cholesterol crystallized from: (1) carbon tetrachloride — 196.08 mg; and (2) acetonitrile — 137.89 mg.

TABLE 1

Heat of transition and melting of freshly precipitated and resolidified cholesterol

No.	Solvent	Condition of solution	Freshly precipitated crystals				Melted and resolidified crystals	
			T_t (°C)	ΔH_t (kcal mole^{-1})	T_m (°C)	ΔH_m (kcal mole^{-1})	T_t (°C)	ΔH_t (kcal mole^{-1})
1	Carbon tetrachloride	Quiescent	39	0.18	148	6.82		
2	Carbon tetrachloride	Quiescent	40	0.25	147	5.40	40	0.70
3	Ethanol	Quiescent	41	0.20	150	6.24	—	—
4	Ethanol	Shaken	44	0.14	150	6.84	40	0.81
5	Ethanol	Stirred	40	0.15	—	—	—	—
6	Methanol	Quiescent	47	0.20	147	6.33	39	0.68
7	Methanol	Shaken	38	0.40	148	5.16	39	0.75
8	Benzene	Quiescent	40	1.01	148	6.70	39	0.68
9	iso-Propanol	Quiescent	44	0.77	148	7.12	40	0.73
10	Acetonitrile	Quiescent	35	0.89	149	5.40	40	0.70
11	Acetonitrile	Quiescent	36	1.04	148	—	—	—

have given a very pronounced signal. Moreover this significantly large signal would have represented only about 0.05% of the sample weight. No such weight loss was recorded. Second, evolution of solvent is, as a rule, endothermic, and as the latent heat of transition is also endothermic, the assumption of solvent being driven off could only affect the result in one direction, namely to increase the total effect. Most of the changes were the reverse: the measured heats of transition of the freshly prepared crystals were lower than those measured after melting and recrystallization. An additional feature of the transition of the freshly prepared crystals is the wide variation of the transition temperature, ranging from 35 to 47°C.

It is well known that thermal measurements are strongly affected by the purity of the sample [9] and by the experimental conditions [10]. Therefore, several of the crystal samples were finely ground and tightly packed. The same dependence upon the solvent used as indicated in Table 1 was found.

The crystals deposited from ethanol with a heat of transition of 0.2 kcal $mole^{-1}$ were recrystallized from methanol. The transition heat of the new batch of crystals was 0.45 kcal $mole^{-1}$.

It might be supposed that some of the crystals with a very low heat of transition were precipitated at temperatures higher than 40°C, thus acquiring the crystal structure of the high-temperature phase which was partly retained after slow cooling to room temperature. The low heat of transition could then be explained as the energy input needed to transform only a part of the crystal structure to the high-temperature arrangement. To test this assumption, crystals were grown from acetonitrile along with crystals from slowly evaporating solutions of ethanol and carbon tetrachloride during two weeks in a bath thermostated at 47°C. Again the latent heats of the polymorphic transition correlated with the nature of the solvent in the same way as in Table 1, namely they were higher than 0.7 kcal $mole^{-1}$ for crystals grown from acetonitrile and appreciably lower than 0.7 kcal $mole^{-1}$ for those grown from carbon tetrachloride and ethanol.

In the absence of any other acceptable explanation, it may be tentatively inferred that the structure of the crystals grown in carbon tetrachloride, methanol and ethanol may vary from the structure of crystals grown in isopropanol, acetonitrile and benzene. After the crystals have been melted and recrystallized, their origin is no longer relevant.

The powder X-ray diffractograms of cholesterol crystals grown from various solvents differed mainly in the relative intensities of the peaks. Single-crystal studies showed that the difference between cell lengths (along the *c*-axis) of the low temperature and high-temperature phases was 0.2 Å, about 0.5% of the 38.1 Å length of the cell [4]. It seems therefore that the powder diffraction technique is not suitable to establish even differences of this magnitude, and since the differences may be significantly smaller, as evidenced by some of the extremely low heats of transition, it was concluded that the technique was of no further use in this study.

The single cholesterol crystals used in the aforementioned study were grown by slowly evaporating ethyl ether solution [4]. Attempts to grow single crystals from the solvents employed in this study were unsuccessful —

even relatively large and transparent crystals turned out to be polycrystalline.

As the ring component in the cholesterol molecule is rigid it follows that the energetic and structural transition results from the flip-over of the aliphatic chain. In a detailed topographic study it was calculated that the distance from C_3 to C_{27} in a steroid molecule nearly identical with cholesterol was 18.2 Å [11]. Substituting OH for the extreme C—C bonds would make the stretched cholesterol molecule 19 Å long, in excellent agreement with the 38.1 Å length for a two-molecule cell. The aliphatic seven-membered side-chain contributes 8.43 Å to the molecule's overall dimensions, and it can be arranged in various configurations with only a very slight effect on the length of the projection in the *c*-axis direction.

It seems possible that the polymorphic transition of cholesterol at 37°C may be divided into several subtransitions, each one corresponding to a slight configurational change. Some of these subtransitions may be preferentially induced by specific solvent—solute interactions. The existence of solvent—cholesterol interactions will also be demonstrated by NMR studies shortly to be reported.

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