Thermal Stability of Biological Lipids I: Differential Scanning Calorimetry of Mixtures of Cholesteryl Myristate and Cholesteryl Palmitate

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Abstract □ Differential scanning calorimetry was used to study phase transitions in binary mixtures of mesomorphic (liquid crystalline) saturated cholesteryl esters. The solid to mesomorphic phase transition temperatures for binary mixtures of cholesteryl myristate and cholesteryl palmitate are lower than the phase transition temperature for either pure compound. An apparent eutectic phase formation was found for all compositions of the two components. The mesomorphic phase consists initially of a smectic phase, which converts to a cholesteric phase as the temperature is increased. The cholesteric phase exists over a narrow temperature range, above which the system becomes an isotropic liquid. The phase transition temperatures of the smectic to cholesteric and the cholesteric to isotropic phase transitions are virtually linear functions of the composition of the mixtures. The ΔH values for each phase transition were determined. The major portion of the overall ΔH for the solid to isotropic phase transitions for these mixtures occurs at the solid to mesomorphic phase transition. The behavior of these mixtures is of interest since mesomorphic mixtures of these cholesteryl esters along with other cholesteryl esters are components of the lipid deposits of atheromatous lesions.

Keyphrases □ Cholesteryl myristate and palmitate—differential scanning calorimetry of binary mixtures, phase diagrams □ Phase transitions—binary mixtures of cholesteryl myristate and palmitate studied by differential scanning calorimetry □ Differential scanning calorimetry—used to study phase transitions of binary mixtures of cholesteryl esters, phase diagrams □ Lipids, biological—differential scanning calorimetry of binary mixtures of cholesteryl esters

The significant biological importance of cholesterol and its esters is due to their wide distribution in the body and their role in normal biological chemistry as well as in certain pathological conditions such as atherosclerosis (1–3). Atherosclerosis is the major cause of death in the United States and in many other countries. This disease is characterized by atheromas or atheromatous plaques, which are soft fatty deposits that cause a thickening of the inner layer of large- and medium-sized arteries. The major components of the atheromatous plaque are cholesteryl oleate and cholesteryl linoleate with lesser amounts of cholesterol, cholesteryl palmitate, cholesteryl myristate, and numerous other saturated and unsaturated cholesteryl esters in addition to triglycerides, phospholipids, lipoproteins, and other substances (4).

Cholesteryl esters belong to a class of compounds that do not melt directly from a crystalline solid to an isotropic liquid; instead, as the temperature is increased, they pass through an intermediate phase termed the mesomorphic or liquid crystalline phase. The liquid crystalline phase has the mobility of a liquid, while the molecules remain in domains arranged in an anisotropic ordered array (1). Depending on the chain length of the ester, this phase may consist of a smectic or cholesteric structural arrangement. In certain compounds, the smectic phase may be converted to the cholesteric phase by heating to a particular temperature.

The phase behavior of the cholesteryl ester mixtures is of particular interest because the lipid deposits of atheromatous lesions occur as droplets within cells and in the extracellular spaces of the arterial intima and as streaks on the walls of the arterial lumen (4, 5). These streaks are mesomorphic at body temperature (2), and analysis of the deposits has indicated a large number of cholesteryl esters, many of which have their mesomorphic phase well above body temperature.

The thermal behavior of individual cholesteryl esters has received much attention (6–8). Although the cholesteryl ester deposits in atheromatous lesions are known to exist as mixtures, determination of the physical-chemical properties of such mixtures has only recently begun (6, 9, 10). Visual thermal microscopy of the phase transitions in binary mixtures of saturated cholesteryl esters (cholesteryl myristate and cholesteryl palmitate) has indicated that the transitions from the solid phase to the mesomorphic phase occurred at temperatures below the phase transition temperature for either pure compound (9). However, the mesomorphic to isotropic phase transition occurred at temperatures that were virtually linear functions of the composition of the mixtures, consistent with the liquid nature of the components and, therefore, their expected low degree of intermolecular interaction.

Differential scanning calorimetry (DSC) was used in the present work to study both the qualitative and quantitative thermal properties of binary mixtures of cholesteryl myristate and cholesteryl palmitate.

EXPERIMENTAL

Materials—Samples of cholesteryl myristate and cholesteryl palmitate and mixtures of these two esters were taken from the materials used in the previous visual thermal microscopy studies of their phase transition behavior (9).

Instrumentation—All samples were analyzed on a thermal analyzer[1]. The calorimeter was calibrated with gallium, indium, and tin. Sample weights ranged from 5.25 to 5.80 mg for the various cholesteryl ester mixtures. The samples were contained in aluminum pans with unsealed covers and were maintained under a positive flow of nitrogen at atmospheric pressure. All DSC traces were recorded at a heating rate of 10°/min with a temperature axis scale of 20°/in. as a function of ΔT measured at 0.5°/in. Due to the variation in sample weights, ΔH calculations were normalized to compare the data on an equivalent basis. All samples were

[1] DuPont 900 equipped with a DuPont pressure DSC cell, model 900605.

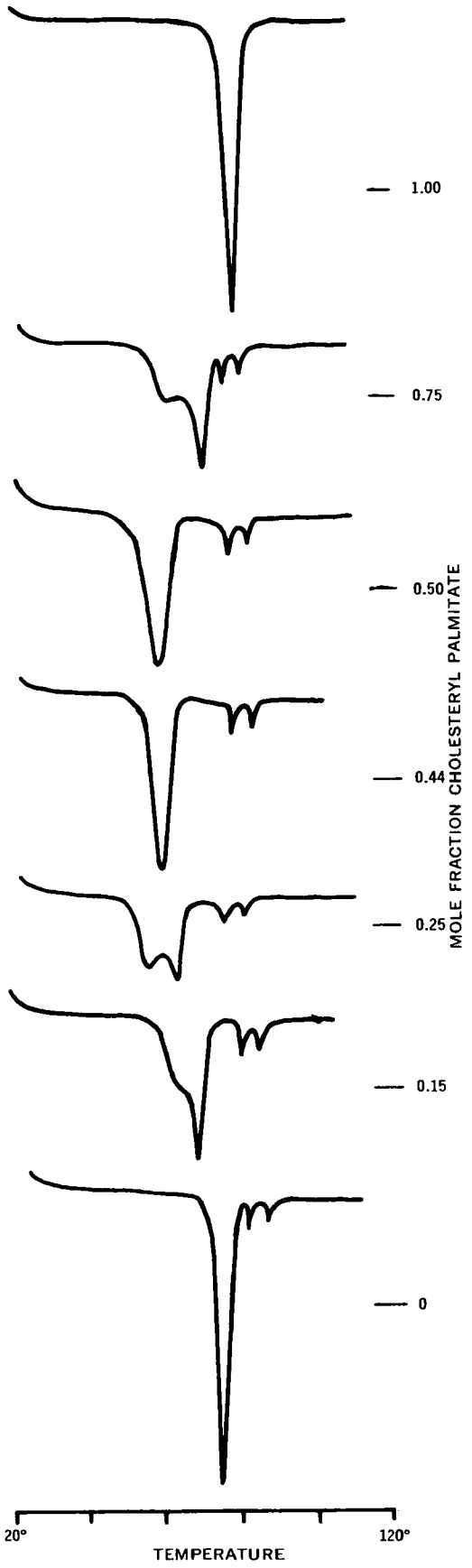

Figure 1—*DSC curves of cholesteryl myristate and cholesteryl palmitate, separately and in mixtures.*

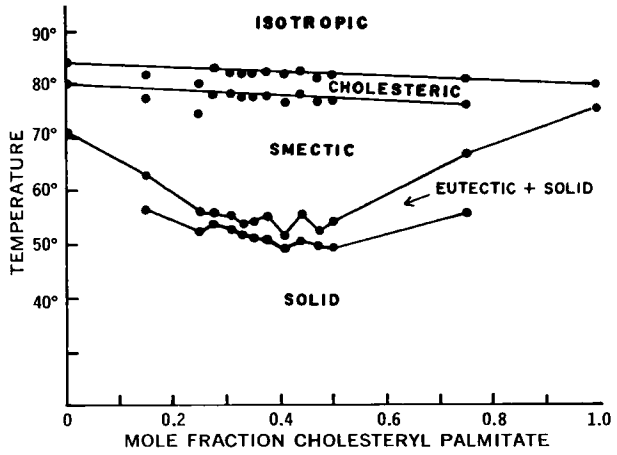

Figure 2—*Phase diagram for mixtures of cholesteryl myristate and cholesteryl palmitate as determined by DSC.*

run in duplicate. Multiple peaks corresponding to the eutectic mixture and to the melting of the excess pure compound were observed in certain cases. The areas of the respective endotherms were arbitrarily assigned by dropping a line perpendicular to the baseline to the maximum point separating the overlapping endotherms. A planimeter[2] was used to measure the areas of the endotherms.

RESULTS AND DISCUSSION

DSC was performed on cholesteryl myristate and cholesteryl palmitate and on mixtures of these materials (Fig. 1). As can be seen in Fig. 1, from the endotherm for cholesteryl myristate (bottom trace), cholesteryl myristate undergoes three endothermic transitions representing from left to right: *(a)* crystalline solid to smectic phase, *(b)* smectic phase to cholesteric phase, and *(c)* cholesteric phase to isotropic liquid. The two mesomorphic phase transitions, smectic to cholesteric and cholesteric to isotropic liquid, are enantiotropic or completely reversible upon heating and cooling (6). Cholesteryl palmitate has, however, only one enantiotropic mesomorphic transition, the transition from the cholesteric phase to the isotropic liquid phase. In this case the smectic phase appears only upon cooling the cholesteric phase and, therefore, would not appear on the endotherm in Fig. 1. The crystalline solid to cholesteric phase and the cholesteric to isotropic liquid phase transitions occur over too narrow a range for the DSC to detect individually, resulting in a single large endotherm.

The major endotherm for cholesteryl myristate (the crystalline solid to mesomorphic phase transition) represents approximately 91% of the total heat of transition from the crystalline solid to the isotropic liquid, and the second and third endotherms account for approximately 6 and 3% of the total transition heat, respectively. These results are in good agreement with those reported by others (8). The transition temperatures are also in general agreement with previously reported values (6, 8).

The DSC traces for the cholesteryl myristate–cholesteryl palmitate mixtures are shifted to lower temperatures from that of either pure compound and have certain distinctly different shape characteristics (Fig. 1). At 0.75 mole fraction of cholesteryl palmitate, four peaks are seen, corresponding, from left to right, to: *(a)* an apparent eutectic mixture appearing as a shoulder on the peak due to excess cholesteryl palmitate, *(b)* excess cholesteryl palmitate, *(c)* the smectic to cholesteric phase transition, and *(d)* the cholesteric to isotropic liquid phase transition. The latter endotherm most likely incorporates the cholesteric to isotropic liquid phase transition of cholesteryl myristate. The shift of the endotherm of the excess cholesteryl palmitate and of the two mesomorphic phase transitions to lower temperatures would be expected in the presence of the eutectic mixture.

At 0.5 mole fraction of each cholesteryl ester, a single large endotherm appears followed by two smaller ones. The large endo-

[2] Gelman Instrument Co.

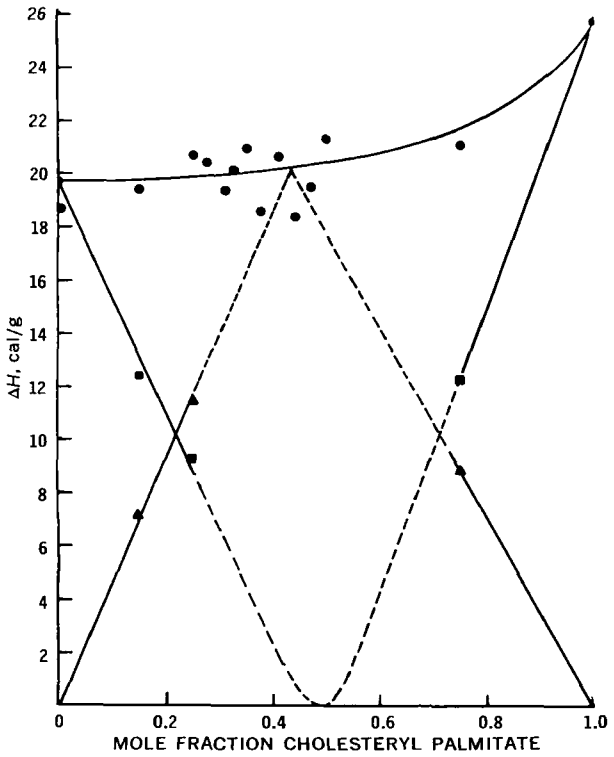

Figure 3—*Crystalline solid to mesomorphic phase transition heats for cholesteryl myristate–cholesteryl palmitate mixtures. Key: ▲, eutectic heat; ■, heat of melting of the excess pure component; and ●, total enthalpy for the phase transition.*

therm corresponds to the eutectic mixture, and the two small endotherms correspond to the smectic to cholesteric and cholesteric to isotropic liquid phase transitions. This pattern of endotherms is also seen at 0.44 mole fraction of cholesteryl palmitate. From the lack of a discernible shoulder and the near symmetry of the large endotherm, it appears that the eutectic mixture occurs close to equimolar quantities of each component. The behavior of the 0.25 mole fraction cholesteryl palmitate mixture is similar to that of the 0.75 mole fraction cholesteryl palmitate mixture. A large double-peaked endotherm appears, corresponding to the eutectic mixture on the left and the excess cholesteryl myristate on the right. At 0.15 mole fraction there is a shoulder, corresponding to the eutectic mixture, on the large endotherm of the excess cholesteryl myristate. As can be expected, it is very difficult to differentiate quantitatively the relative magnitudes of the shoulder on a peak and the peak itself.

The data from the DSC traces in Fig. 1 and from DSC measurements on other mixtures are summarized as a phase diagram in Fig. 2, in which the phase transition temperatures of the cholesteryl myristate–cholesteryl palmitate mixtures are presented as a function of the composition of the mixtures. The data in Fig. 2 correlate favorably with those obtained for these same systems by visual thermal microscopy (9). The appearance of the eutectic mixture and of the smectic to cholesteric phase transition was not determined in the microscopy study. The linearity of the smectic to cholesteric and the cholesteric to isotropic liquid phase transitions indicates that these mesomorphic phases are behaving as ideal liquid mixtures. This would be expected due to the small difference in the ester chain length and the small heat of transition.

In Fig. 3, the eutectic heats, the heat of melting of the excess pure component, and the total heat of the crystalline solid to mesomorphic phase transitions are plotted as a function of the mole fraction of cholesteryl palmitate (10). Extrapolation of the eutectic heats indicates the formation of a eutectic mixture at approximately 0.43 mole fraction of cholesteryl palmitate, whereas the extrapolation of the heats of melting of the pure component indicates that the eutectic mixture composition is about 0.49 mole fraction cholesteryl palmitate. The average of these two values gives an approximate eutectic composition of 0.46 mole fraction of cholesteryl palmitate and 0.54 mole fraction of cholesteryl myristate, or very nearly equimolar quantities of each component.

It may be concluded that binary mixtures of these saturated cholesteryl esters form a eutectic mixture of virtually equimolar composition. Although these substances are not the major components of the lipid deposits of atheromatous lesions, their physical behavior suggests that eutexia may play an important role in the formation and stabilization of the complex mixtures characteristic of these deposits.

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