Phase Behavior of Triolein and Tripalmitin Detected by Differential Scanning Calorimetry

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ABSTRACT

The thermotropic behavior of triolein, tripalmitin and their mixtues was determined by differential scanning calorimetry. Polymorphic behavior was noted for the triglycerides but the triglycerides were converted to a single form after 4 to 5 successive heating scans. Melting points for each triglyceride were determined for the pure samples and mixtures, and plotted as a phase diagram. The phase diagrams indicate that a phase separation of triglycerides occurred at all concentrations of triolein and tripalmitin. However, the melting peak onset temperature of tripalmitin was shifted by triolein from 56 C at 100 mol % tripalmitin to 37 C at 15 mol % tripalmitin. Similarly, the peak onset temperature of triolein was shifted by tripalmitin from -2.5 C at 100% triolein to -4 C at 95% triolein. Enthalpies were also determined for pure samples and mixtures. These data indicated that when either triolein or tripalmitin were present as the minor component of the mixture, the enthalpy of the minor component was reduced whereas that of the major component was not greatly altered.

INTRODUCTION

Differential scanning calorimetry (DSC) is a technique which offers the advantages of measuring heats of melting and fusion as well as melting and freezing points, while requiring only mg quantities of material. The thermal behavior of VLDL has recently been determined by differential scanning calorimetry in human plasma VLDL (1) and VLDL isolated from the perfused rat liver (2). These particles are rich in triglycerides, containing 70 to 80% of their mass as triglyceride. It is believed that the structure of the VLDL triglyceride core and its chemical composition are important in determining the surface characteristics of the particle. However, the complex thermotropic transitions that were noted did not correlate well with the fluorimetrically determined transitions (2-4). The triglycerides in these investigations were highly enriched in oleic acid or palmitic acid. Although phase diagrams of other triglyceride mixtures have been reported (5-7), some of these diagrams were incomplete and contain only melting point data (5). Therefore, we have undertaken an investigation of a phase diagram of triolein, tripalmitin and several mixtures of the two. In this study, we determined the following: (1) the polymorphic behavior of triglycerides, (2) differences in melting and freezing points of pure triglycerides and their mixtures, and (3) differences in ΔH_{cal} for pure triglycerides and their mixtures. These data may help to explain the effect of different triglyceride species on each others' thermal behavior in complex mixtures as may occur in the secreted VLDL.

Abbreviations used are as follows: VLDL, very low density lipoprotein; DSC, differential scanning calorimetry.

MATERIALS AND METHODS

Differential Scanning Calorimetry

Differential scanning calorimetry was performed with a DSC-2 (Perkin-Elmer Corp., Norwalk, CT). The triglycerides (0.5-2.0 mg) were solubilized in 50 µl of chloroform and transferred to 10 µl aluminum sample pans, and warmed to about 50 C to promote solvent evaporation. The pans containing the samples were then lyophilized for 12 hr to remove any residual solvent. The pans are sealed at 24 C, cooled to -50 C at a rate of 1.25 C/min, and equilibrated for 5 min at the lower temperature limit before a scan was initiated. The samples were then reheated to 65 C at a rate of 1.25 C/min. The samples were then cooled to -50 C at 10 C/min. This cycle was repeated 4 times. The recorded traces in the figures were taken during the fourth cycle on heating unless otherwise specified. This ensured that the thermal history of the samples was the same. Sensitivities were 1.0 to 5.0 mcal/sec for large amounts of triglycerides and 0.2 for triglyceride mixtures in which the minor fraction was below 10 mol %. The areas under the peaks of the phase transition were found by weighing the paper; the weights were compared to the weight of a standard area of known enthalpy (Indium) and sample enthalpies were calculated (8,9). ΔH_{cal} is the calorimetrically measured enthalpy change (10,11). Triolein and tripalmitin were obtained from Supelco Inc., Bellefonte, PA.

RESULTS

Polymorphic Behavior of the Pure Triglycerides

Pure triolein or tripalmitin possesses multiple transitions upon first heating (Fig. 1, a and c). These are the β' and β forms typical of the polymorphism displayed by triglycerides (12, 13). α Forms were not detected since they were located at the lower temperature limit of our instrument and since samples were not specially treated to allow detection of α forms (14). As shown in Figure 1, b and d, the β' forms can be converted into β forms after multiple heating and cooling regimens (13). It should also be noted that, when multiple polymorphic forms are present, exotherms separate melting peaks of different forms. Cooling exotherms for each of the samples in Figure 1 were also determined (Fig. 2). A single transition was noted in each case at a temperature near that of the most stable β form. Multiple transitions were not noted even during the first cooling regimen (Fig. 2, a and c). Thus, the triolein and tripalmitin multiple transitions could be converted into a single, reproducible form that appeared on both heating and cooling scans. The reproducibility of the melting temperatures after the fourth run was ± 1 C.

Mixtures of Triolein and Tripalmitin

The effect of mixing tripalmitin and triolein on the onset temperatures of the transition of the respective glycerides is shown in Figure 3. The mixture did not show a single peak on either heating or cooling regimens, indicative of mixing of the 2 components. Instead, 2 transitions similar to those noted for triolein and tripalmitin, respectively, were noted on endothermic scans (fourth heating and cooling regimens). Multiple peaks due to polymorphic forms were not apparent, although they occurred during the first heating regimen. All peaks observed were reversible, as cooling peaks were observed for all samples. The onset temperature of the endothermic transition of the tripalmitin was always higher than triolein. However, increasing molar content of triolein lowered the onset temperature of the tripalmitin from 56 to 40 C on heating scans. Conversely, tripalmitin also affected the onset temperatures of the triolein transition, increasing it from -15 to -3 C on heating scans. Thus, the effect of triolein on the phase transition onset temperature of tripalmitin was greater than the

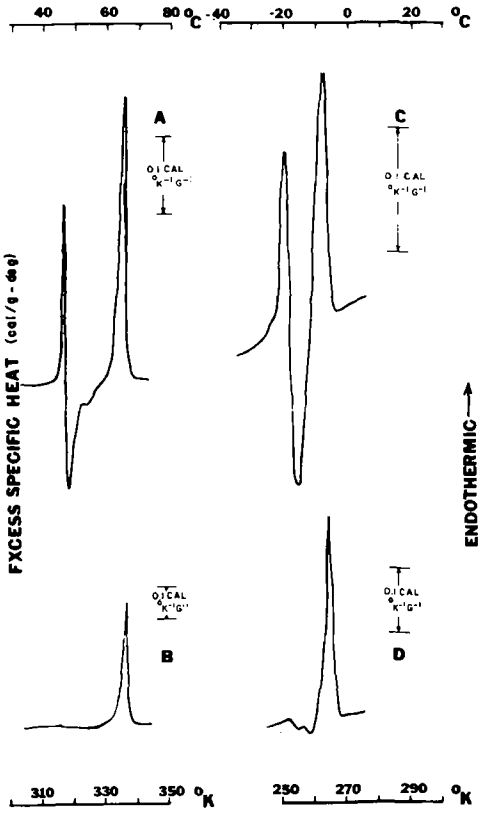

FIG. 1. Conversion of polymorphic triolein and tripalmitin forms into single phases. Triolein (0.66 mg) and tripalmitin (0.80 mg) were heated from 290 to 345 C as described in Methods. (a) Tripalmitin, first heating scan, sensitivity 2 mcal/sec; (b) tripalmitin, fourth heating scan, sensitivity 5 mcal/sec; (c) triolein, first heating scan, sensitivity 1 mcal/sec; and (d) triolein, fourth heating scan, sensitivity 2 mcal/sec.

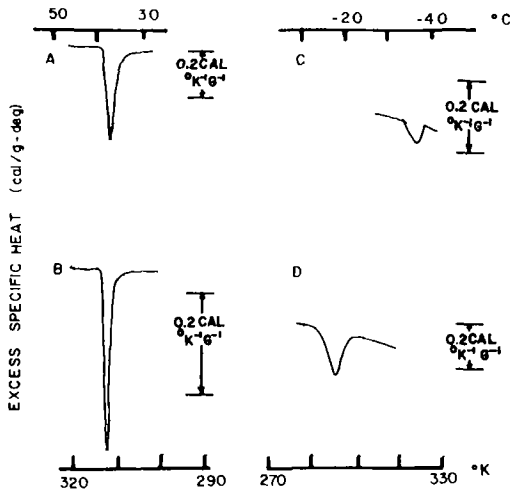

FIG. 2. Cooling scans of triolein and tripalmitin. All conditions were as described in the legend to Fig. 1.

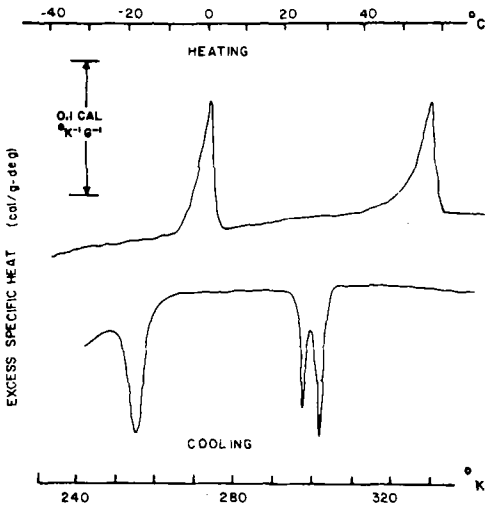

FIG. 3. DSC scans of a tripalmitin/triolein mixture. Heating and cooling scans of a 0.62 mg 60 mol % triolein/40 mol % tripalmitin mixture were determined after 4 heating and cooling cycles. The sensitivity was 1 mcal/sec on heating and cooling from 290 to 345 C.

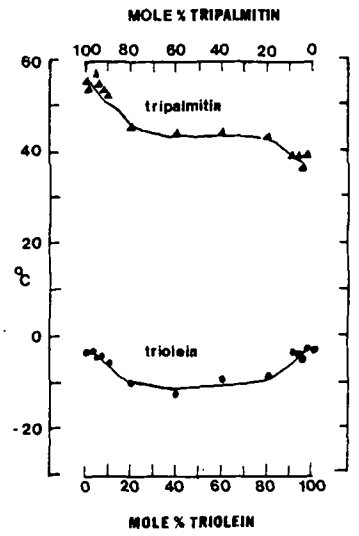

FIG. 4. Melting temperatures of triolein-tripalmitin mixtures. All methods were as described in legend to Fig. 1 except that data were taken only from the final scan (i.e., polymorphic forms were removed).

effect of tripalmitin on the onset temperature of triolein. On heating scans, the onset temperature of the phase transition of the glyceride species was relatively constant only when it constituted between 20 and 80 mol % (Fig. 4). At less than 20 mol %, the onset temperature of the glyceride was shifted toward the onset temperature of the major glyceride species. Conversely, above 80 mol %, the onset temperature of the major glyceride increased to that of the pure melting species. The cooling peak for pure triolein was at -14.0 C whereas, for equimolar mixtures with tripalmitin, it occurred at -11 C. Pure tripalmitin had a cooling peak at 37 C which occurred at a lower temperature in equimolar mixtures with triolein. It is notable that tripalmitin cooling peaks were doublets when tripalmitin was present between 20 and 80 mol % of the mixture, but pure as well as high or low mol % tripalmitin samples had a single cooling peak.

The effect of triolein on enthalpy changes of tripalmitin are shown in Figure 5a. Below 40 mol % tripalmitin, the enthalpy change of the tripalmitin peak decreased from near 35 cal/g to less than 10 cal/g. The enthalpy change of triolein (Fig. 5b) was lower than that of tripalmitin and was also decreased drastically below 50 mol % triolein (from 22 to less than 9 cal/g). Thus, when either glyceride represented about 60 mol % or greater of the mixture, its enthalpy change was not greatly altered, indicating that "impurity" of the other glyceride up to 40 mol % did not greatly alter the enthalpy change of the major species.

The thermal data for pure triolein, tripalmitin, and some mixtures thereof is presented in Table 1. At 100 mol %, the enthalpy change of 25.3 cal/g for triolein compared well with a previously reported value of 25.8 cal/g for β triolein (13). At 100 mol %, the enthalpy change of 37.9 cal/g for tripalmitin was less than the 59.0 cal/g for β tripalmitin reported elsewhere (13). However, at 91 mol %, the enthalpy change was 49.6. Thus, it seems possible that, for some of the tripalmitin

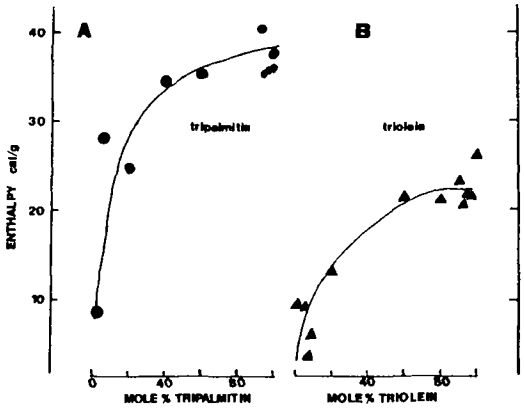

FIG. 5. Dependence of enthalpy on mol % of tripalmitin and triolein. All conditions were as described in legend to Fig. 4.

TABLE 1

Thermal Properties of Triolein-Tripalmitin Mixtures

Mol %		Temperatures (C) Peak onset		ΔH cal/g		ΔT (C)	
Triolein	Tripalmitin	Triolein	Tripalmitin	Triolein	Tripalmitin	Triolein	Tripalmitin
100	0	-2.5	–	25.3	–	8.0	–
95	4	-4	37	21.9	28.0	9.0	13.0
60	40	-8	45	21.4	33.5	11.0	15.5
5	95	-4	55	9.1	35.5	5.0	9.0
0	100	–	56	–	37.9	–	10.0

samples, not all of the sample may have been present in the β form. The effect of one triglyceride on another enthalpy change of melting is presented in Figure 5, a and b. The enthalpy change of each component increased as its mol % in the mixture increased. The width of the transition, ΔT, of triolein was decreased when this triglyceride was the minor glyceride in the mixture (5 mol %). In contrast, the width of the transition of tripalmitin was increased when it was present at 5 mol % compared to 100 mol %. The cooperativity of each of these transitions was not determined due to the complex polymorphic forms and nonideal behavior of these mixtures.

DISCUSSION

As evidenced by our data, even a single acid triglyceride will exhibit complex thermal behavior on heating. Multiple peaks, some of which disappear on successive heating and cooling regimens, do not necessarily imply that the structure of the lipid is polymorphic. In complex systems, alternate explanations such as phase separations or protein denaturation must be considered. Since no protein is present in our samples and because only a single molecular species of triglyceride is present, we interpret our results as being consistent with the presence of polymorphic forms. Such polymorphic forms have been described in detail for other single component triglycerides (13-16). Large exotherms, as noted in the present work, were also noted by others (13-16) and are also indicative of the presence of polymorphic forms (17). Their disappearance after 4 heating and cooling scans is consistent with the presence of a single triglyceride form. From Figure 1, a and c, it can be seen that more than one crystalline form of triolein and tripalmitin exist below their melting points. After a heating scan is initiated, a metastable triglyceride will convert to a stable crystalline form by a reordering of acyl chains (13-16). More unstable forms may disappear upon successive heating scans. Given the problems of multiple peaks, possible differences in sample history and reversibility, it is difficult to determine quantitative parameters on these transitions. Therefore, all samples were subjected to the same treatment regimens ensuring the same sample history. All of the polymorphic forms were converted to a single reproducible β form which was the most stable. It is known that α forms of triglyceride are not reversible and convert to β' and β forms (13-16). The β forms are the most stable and they are clearly reversible, as indicated in Figure 2. Reversibility is consistent with the interpretation that the transition noted is not metastable or due to denaturation. This procedure allowed accurate determination of enthalpies and transition temperatures.

The effect of one triglyceride component on another was determined using mixtures of triolein and tripalmitin. If a eutectic mixture is formed, then only one melting point would be expected to appear. However, the results indicated that at all mol % these triglycerides maintained at least a partial phase separation (Fig. 3). However, in mixtures of triolein and tripalmitin, the melting point of the minor triglyceride was altered in the direction of the melting point of the major triglyceride. This may be due to the partial formation of a eutectic mixture (5) or to the solubilization of the minor glyceride into the major glyceride. In contrast, the minor triglyceride had the effect of depressing the major triglyceride's melting point. Between 20 and 80 mol %, the melting points of both tripalmitin and triolein were relatively constant, but slightly below the melting points of the pure triglyceride species. This indicates that, at more equimolar ratios, there may still be small amounts of one triglyceride present in each major component. However, the data in Figure 3 clearly show phase separation between the triolein and tripalmitin transitions. Heats of melting, ΔH, were determined for each com-

ponent of the mixture by first calculating the areas of both endothermic peaks due to tripalmitin and triolein, respectively. Each area was divided by the weight of tripalmitin and triolein, respectively. This ratio was compared to that of an Indium standard of known enthalpy. Thus, the enthalpy of tripalmitin and triolein in the mixture could be estimated. Figure 5, a and b, indicated that the heat of melting of either triolein or tripalmitin was reduced by the presence of the other glyceride species when the melting glyceride represented less than 60 mol %. Thus, when these triglycerides are present as a minor component of a mixture, their enthalpies were altered. This may be caused by partial eutectic mixture formation, solubilization in the other glyceride species, or the disruption of the chain packing of the minor glyceride. Thus, although phase separation of tripalmitin and triolein clearly occurred, these phases were not pure at all molar mixtures. They appeared to approach purity when the component was present near 60-80 mol % in the mixture.

The physiological importance of these data may be relevant to the contribution of mixed triglycerides in the VLDL. It has been shown that, upon cooling freshly isolated human VLDL from 45 to 10 C, there are no thermal transitions (18). However, thermal transitions in human VLDL were noted below 0 C. Thus, the physiological significance of thermotropic transitions appears questionable. However, it should be noted that these plasma VLDL represent a heterogeneous mixture and that the triglyceride lipid composition is dependent on diet. Out laboratory recently used a perfused rat liver system to produce much more homogeneous VLDL enriched in either palmitate or oleate (2). DSC thermograms of these VLDL indicated that VLDL enriched in oleic acid had no transitions above 0 C but the transitions noted below 0 C resembled those of the human VLDL (1). In contrast, when the VLDL were enriched in palmitate, phase transitions appeared near physiological temperature at 37 C. Thus, factors that can affect the fatty acid composition of plasma VLDL (e.g., diet, drugs, endocrine status, or pathology) may also shift the location of the phase alterations.

The importance of our findings may, therefore, be extended to our previous investigations with the triglyceride-rich VLDL (2-4). The VLDL displayed multiple peaks by DSC. These transitions were due to the triglycerides and did not disappear even after 4 to 5 heating and cooling cycles. The data presented herein indicated that mixtures of triglycerides may alter the characteristic physical properties of an individual triglyceride. Some phase separation of different acid triglycerides is maintained even when a triglyceride is present at only 1 mol %. Indeed, it has been noted that saturated and unsaturated acyl chains have a tendency to partition into separate layers in mixed-acid triglycerides (12). Thus, complex thermal behavior in biological systems such as VLDL triglycerides may be caused not only by polymorphism, but also by phase separations.

ACKNOWLEDGMENTS

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