Modulated Temperature Differential Scanning Calorimetry for Examination of Tristearin Polymorphism: 2. Isothermal Crystallization of Metastable Forms

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ABSTRACT: The transformations of tristearin were examined by modulated temperature differential scanning calorimetry (MTDSC) in order to examine the utility of this technique. Tristearin has been used as a model polymorphic system, showing metastable phases and complicated transformation routes occuring at relatively slow rates. The β′-forms generated by thermal treatment under modulation do not differ significantly from those generated by the corresponding treatment without modulation. While the total heat flow thermograms are similar, the deconvoluted reversing component shows that annealing, especially at 63°C, has a significant effect on the crystal size and perfection of the solid phases formed. MTDSC also enables the melting of β′ to be separated from the simultaneous crystallization of the β form as evidenced in the c_p component. Quantitative interpretations about such systems cannot be drawn from MTDSC at this point in time.

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KEY WORDS: Crystallization, differential scanning calorimetry, metastable forms, MTDSC, modulated temperature, oscillating, phase behavior, polymorphism, triglycerides, tristearin

The physical properties of triglycerides are mostly determined by their complex polymorphism. Generally, triglycerides exhibit three different polymorphic forms, characterized by particular chain packing and thermal stability: alpha (α), beta-prime (β′), and beta (β). The transformations are monotropic and theoretically take place from α to β′ to β (1–4). The melt crystallizes primarily into the α form on cooling, although special thermal treatment or very slow cooling can lead to the formation of the β′ or β form, respectively (5,6). The existence of two (or even more) forms of the β′-form is debated as to whether there are two distinct phases (5) or whether the β'_2 consists simply of imperfect β′ crystals (6).

Differential scanning calorimetry (DSC) is a powerful tool in the study of the polymorphic transformations in these substances (3,6). The recently developed technique of modulated temperature DSC (MTDSC; also known as modulated DSC or oscillating DSC) has been cited as having several advantages in this area (7). These include the ability to improve separation of reversible and irreversible thermal events (over the time scale of the experimental parameters), and improved resolution of closely occuring/overlapping events. In the first part of this work (8), we studied the effect of operational parameters on the information obtained about polymorphism of a pure monoacid triglyceride (tristearin) by MTDSC. Certain guidelines for the examination of complicated polymorphic systems were identified. These guidelines have been used to examine the generation of metastable β′ forms by isothermal annealing in this work.

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MATERIALS

Tristearin ($C_{18:0}$) (1,2,3-trioctadecanoylglycerol) was obtained from Sigma Chemical Co., St. Louis, MO) (T5016), 99% pure, and was used without further purification.

METHODS

Apparatus. MTDSC analyses were performed on a Seiko DSC 220C (Seiko Instruments Inc., Japan) instrument with a SSC 5300 analysis system. Other aspects of instrumental operation are summarized in the previous work (8).

Isothermal crystallization of β′ forms. These experiments were based on the work of Kellens and Reynaers (6). Isothermal heat treatment was used to generate the β′ forms as follows. All scans were started by heating the sample to 90°C for 3 min. The following cycles were used.

A: $90°C \xrightarrow{b2} 63°C \xrightarrow{\text{Rapid}} 22°C \xrightarrow{c} 90°C$
24 / 48 / 72 s hold

B: $90°C \xrightarrow{b1} 56°C \xrightarrow{\text{Rapid}} 22°C \xrightarrow{c} 90°C$
15 / 30 / 45 min hold

C: $90°C \xrightarrow{b1} 56°C \xrightarrow{b2} 63°C \xrightarrow{\text{Rapid}} 22°C \xrightarrow{c} 90°C$
15 / 30 / 45 min hold
24 / 48 / 72 s hold

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Scans *b*1 and *b*2 were made at 2.5°C/min; the scan labeled "Rapid" was made in the instrument at a setting of 90°C/min. "Isothermal" holding times at "56°C" (melting onset α form) were 15/30/45 min, while those at "63°C" (melting onset β'_2-form) was 24/48/72 s, as indicated above. (Note that due to the temperature modulation, true isothermal conditions do not exist during the holding period at "56" or "63°C". These temperatures are therefore placed in quotation marks in the text to emphasize this point.)

The results presented here are from the reheating scan *c* made at 2.5°C/min to analyze the forms generated from the preceding heat treatments. Modulation parameters were 2°C amplitude and 0.02 Hz frequency. These parameters were found to give the best results in the previous work, representing a degree of oscillation of 0.96 (9).

RESULTS AND DISCUSSION

The β′ forms for tristearin can be generated only through special thermal conditioning treatments, because the α- to β-transformation is rapid. Thermal conditioning schemes have been presented by Kellens and Reynaers (6) and involve holding the sample above the melting temperature of the α form. For reference, we repeated the conditioning schemes for the β'_1 and β'_2 forms as given in Kellens and Reynaers (6) using normal DSC.

Normal DSC thermograms. In order to set a basis for the discussion of the MTDSC results, a look at the normal DSC results is warranted. Thermogram #1 in Figure 1A is the reheating scan *c* after heat treatment cycle B. Unlike in Kellens and Reynaers (6), conversion to the β′ form is not completed during the conditioning period in our system, probably as a result of differences in thermal conductivities in apparatus and size of samples. The reheating thermogram shows an endothermic peak for melting of residual α followed by a broad overlapping exotherm, indicating conversion of this α melt to β. The crystallization exotherm is overlapped by melting of the (previously formed) β′ crystals around 64.8°C; the melt again rapidly converts to β (exotherm centered around 66.9°C); the β form then melts normally with a peak at 73.4°C.

Thermogram #2 in Figure 1A was obtained from scan *c* on a sample subject to the conditioning in cycle C; the sample was held at 56°C for 30 min followed by 24 s at 63°C and subsequent rapid cooling to 22°C. Again, pure β'_1 form is not obtained during the conditioning. The thermogram shows an endothermic peak for melting of residual α followed by a broad exotherm indicating conversion of this α melt to β. The β'_1 form melts at 65.9°C and immediately converts to β (exotherm centered around 68.6°C); the β-form then melts with a peak at 73°C. The β′ melting is much more pronounced in #2 than in #1, with no indication of the (β'_2) shoulder.

MTDSC total heat flow thermograms. Turning on the temperature modulation does not seem to have any major effect on the total heat flow thermograms of scan *c* (degree of oscillation 0.96; Ref. 9). Some of these are plotted in Figure 1B

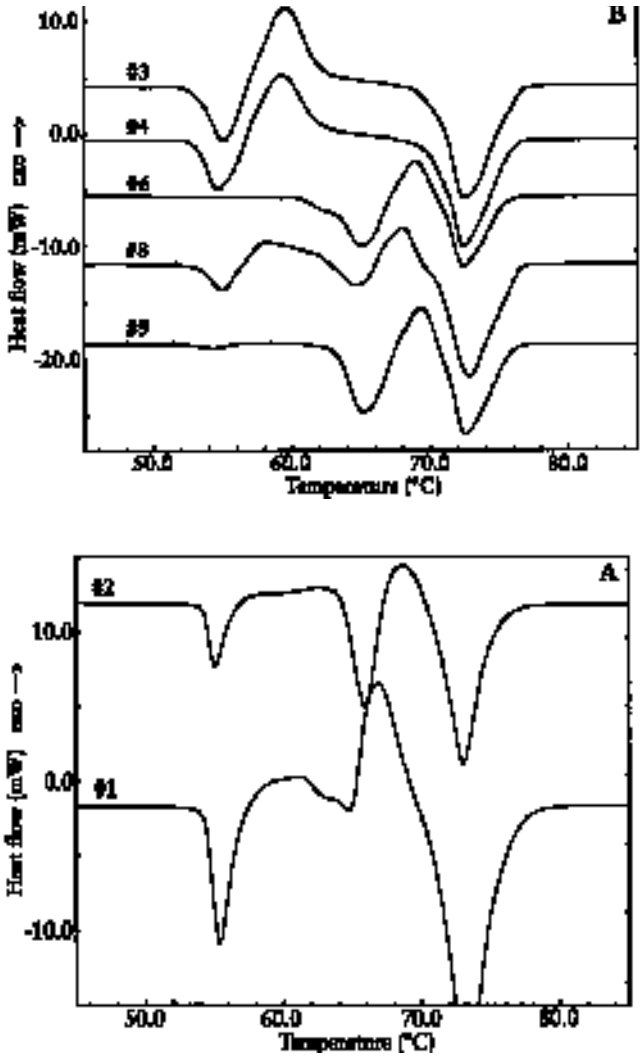

FIG. 1. Comparision of results obtained from a normal differential scanning calorimetry run as opposed to an modulated temperature differential scanning calorimetry (MTDSC) run, showing effect of conditioning cycles. (A) Normal DSC thermograms on reheating tristearin samples after conditioning for #1: 30 min at 56°C (Cycle B); #2: 30 min at 56°C and 24 s at 63°C (Cycle C). (B) Total heat flow MTDSC thermograms from reheating scan *c* after conditioning for #3: 72 s at "63°C" (Cycle A); #4: 15 min at "56°C" (Cycle B); #6: 45 min at "56°C" (Cycle B); #8: 30 min at "56°C" and 72 s at "63°C" (Cycle C); #9: 45 min at "56°C" and 72 s at "63°C" (Cycle C). All scans are made at a heating rate of 2.5°C/min, frequency 0.02 Hz, and amplitude 2°C.

for all the cycles A, B, C. Cycle A, with conditioning at "63°C" for a short period of time (#3), shows no effect of the conditioning. The same can be said for the effect of holding at "56°C" for 15 min (#4). Increasing the holding time to 30 min at "56°C" leads to a partial conversion of the melt to β′ form (not shown); the unconverted portion forms the α phase, which on reheating transforms to β. Holding for 45 min at "56°C", however, leads to complete conversion to the β′ form; a small shoulder on the β′ melting endotherm indicates the likely presence of β'_2 (#6). When the conditioning is performed according to cycle C with holds at both "56°C" and "63°C", the result is similar to that from cycle B. The melting

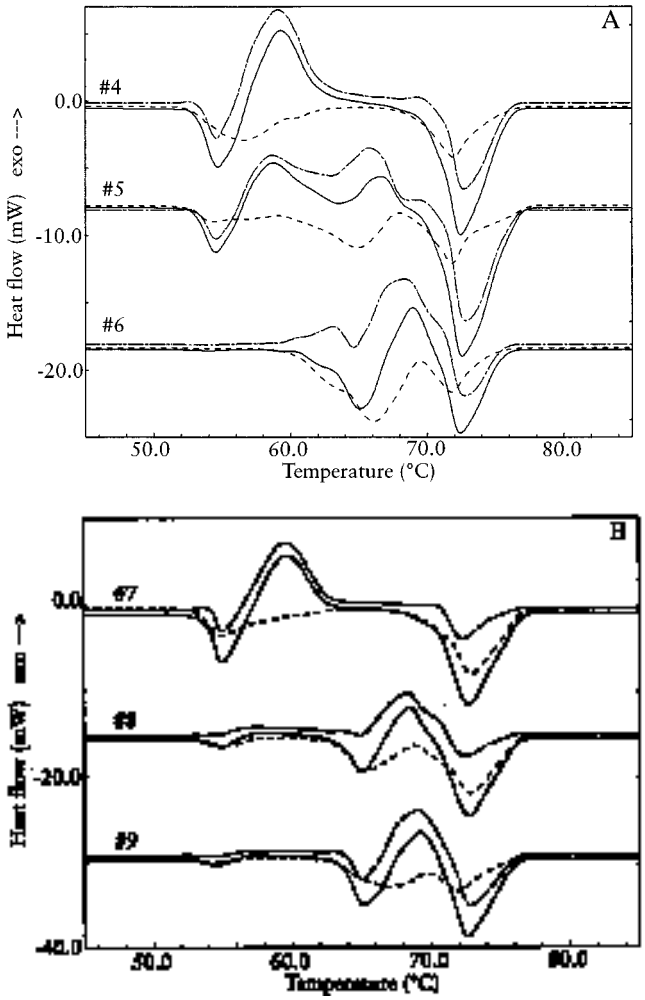

FIG. 2. Effect of conditioning cycles on reheating scan *c*. (A) Conditioning cycle B, #4: 15 min at "56°C"; #5: 30 min at "56°C"; #6: 45 min at "56°C". (B) Conditioning cycle C, #7: 15 min at "56°C" and 72 s at "63°C"; #8: 30 min at "56°C" and 72 s at "63°C"; #9: 45 min at "56°C" and 72 s at "63°C". The deconvoluted total-heat-flow thermogram (——), c_p component (·····), and kinetic component (-—-) are shown. All scans are made at a heating rate of 2.5°C/min, frequency 0.02 Hz, and amplitude 2°C.

endotherm for the β′ form is more pronounced (#8, #9) implying that the annealing at "63°C" leads to a stabilization or improvement of the crystal structure. The β'_2 shoulder is eliminated by holding for an additional 72 s at "63°C" after 45 min at "56°C" (#9 vs. #6); instead, a small α melt is seen in #9. This α form is generated when a portion of the β'_2 crystals melt on annealing at "63°C" and are unable to recrystallize into the β′ form before being cooled down to 22°C. As far as the total heat flow thermogram is concerned, there is no significant effect of the duration of stay at "63°C" in cycle A or C for up to 72 s. However, this is not the whole picture, as will be seen in the discussion of the deconvoluted signals below.

Considering that the above "isothermal" conditioning cycles included a temperature modulation of 2°C, the results are surprisingly similar to those one would expect from true isothermal conditioning. This gives us some confidence in interpreting the corresponding reversing (c_p) and nonreversing (kinetic) heat flow thermograms (Fig. 2).

MTDSC deconvoluted thermograms. Combining the above two conditioning cycles (A and B), as in cycle C, leads to reversing and nonreversing thermograms that correlate to those obtained previously (Fig. 2). Shown in Figure 2B are the results of reheating scan *c* after conditioning at 15/30/45 min at "56°C" and 72 s at "63°C". The overall interpretation of the thermograms is similar to that previously presented. Although the total heat flow thermogram after conditioning at both "56 and 63°C" is not sensitive to the time at "63°C", the same is not true for the reversing (and therefore also the nonreversing) signals. A hold of 15 or 30 min at "56°C" combined with increasing times at "63°C" results in larger c_p endotherms for the β melt (not shown). However, conditioning at "56°C" for 45 min and 72 s at "63°C" leads to a smaller amount of β'_2 when compared to the "56°C" treatment alone. This is because conditioning at "63°C" improves the crystal perfection and size, that is, it promotes the formation of β'_1 form at the cost of the β'_2 form. The resulting melting range of β'_1 form is narrower and at a higher temperature than when the β'_2 form is also present. This is apparent when comparing corresponding scans #6 and #9 in Figure 2. Furthermore, the β'_1 to β transformation is slow (6). Thus, when a larger part of the β′ exists as β'_1 instead of β'_2 (i.e., when conditioning time at "63°C" is increased), the amount of β phase material formed during the reheating by conversion from melted β′ is decreased.

The reversing component of the thermograms also shows that the melting endotherm assigned to β′ shifts to higher temperatures with increasing time at "56°C". Going from a 30-min to a 45-min hold at this temperature thus results in both a larger amount of β′ phase and also perhaps larger or more ordered crystals of this phase (Fig. 2A).

To summarize, the effect of temperature modulation in this experiment is small as far as disturbance in the conditioning and anealing processes is concerned; the same phases are obtained with and without modulation. However, this does not preclude disturbance of crystal size and (im)perfection during the crystallization process itself. The utility of modulation in separating overlapping phenomena is illustrated again. Even though the total heat flow thermograms do not show much difference caused by the holding time at "63°C", the reversing signal is able to bring out the significant effect this annealing step has on the state of the solid phases of the system. Thus, qualitative interpretation of the thermograms is enhanced by the temperature modulation.

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