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Non-Equilibrium Melting of Amylose-V Complexes

C. G. Biliaderis

Department of Food Science, University of Manitoba, Winnipeg, Manitoba,
Canada R3T 2N2

C. M. Page and T. J. Maurice

General Foods Inc., Research Department, Cobourg, Ontario, Canada K9A 4L4

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SUMMARY

Thermal analysis of solution-grown crystalline amylose-V complexes of amylose, amylodextrin and β-cyclodextrin with a series of saturated 1-monoglycerides (C_{10}–C_{18})*, lysolecithin, lauric acid and 1,3-dipalmitin was carried out using differential scanning calorimetry (DSC). At intermediate water contents (60%) and moderate heating rates (10°C* min^{-1})*, some of these complexes exhibited two melting transitions separated by an exothermic effect. A mechanism of partial melting, followed by recrystallization and final melting, is proposed to account for such non-equilibrium melting. These phenomena are strongly influenced by the amount of water present in the system; the higher the moisture content, the more cooperative the melting and the lower the occurrence of secondary crystallization processes. The overall thermal behavior of the complex/*H_2O *mixture can be explained in terms of both thermodynamic melting point depression due to the diluent and structural reorganization upon heating. The latter is related to the crystallite morphology/habit of the complex and appears to be dictated by the nature of the ligand molecule. Good amylose complexing agents induce metastable, less perfected crystalline structures that are inclined to reorganization upon heating in the DSC, presumably via a lamella thickening mechanism. Due to the non-equilibrium character of the melting process of these complexes, theoretical treatment of melting data*

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using expressions such as the Flory–Huggins equation is not applicable.

INTRODUCTION

Differential scanning calorimetry (DSC) data on phase transitions of granular starches often deal with irreversible (non-equilibrium) melting processes (Slade & Levine, 1984; Maurice *et al.*, 1985; Biliaderis *et al.*, 1986). Thus, reorganization, crystallization and recrystallization superimposed on melting lead to complicated DSC thermal profiles, particularly when the amount of water present in the system is insufficient to facilitate a cooperative melting path, and a moderate heating rate is employed. The non-equilibrium nature of these phenomena has often not been recognized and thus made the interpretation of the calorimetric data very difficult (Wirakartakusumah, 1981; Bulpin *et al.*, 1982; Donovan *et al.*, 1983).

In recent calorimetric studies on crystalline amylose–lipid complexes it was helpful to interpret the thermal behavior of these systems using the same principles that govern the crystallization and melting phenomena of semicrystalline synthetic polymers (Biliaderis *et al.*, 1985). At intermediate moisture contents, for the multiple melting transitions of amylose–monopalmitin and amylose–lauric acid complexes, a process of partial melting followed by recrystallization and final melting during the DSC heating experiment was suggested as an explanation of their thermal profiles. Thus, the recorded DSC curve does not reflect the initial crystallite distribution nor their structure. Instead, it is a composite of all thermal events occurring simultaneously during heating. On the other hand, a single endotherm was observed for the amylose–lysolecithin complex under all conditions. Although it was obvious that the melting/recrystallization phenomena were strongly dictated by the nature of the ligand molecule, the overall relationship was not elaborated in great detail. It is the aim of the present report to summarize our DSC studies on these systems by focusing attention on the crystallization habit and melting properties of solution-formed amylose-V complexes as influenced by the type of the host lipid molecule, the molecular size of the polysaccharide and the water content. The main emphasis here has been placed on complexes grown from dilute solutions. However, for comparative purposes the thermal characteristics of a 'melt-crystallized' sample (i.e. melted and then recrystallized) were also investigated.

EXPERIMENTAL

Materials and methods

Lysolecithin from egg yolk (Type I), lauric acid (C_{12}), monocaprin (1-C_{10}), monolaurin (1-C_{12}), monomyristin (1-C_{14}), monopalmitin (1-C_{16}), monostearin (1-C_{18}), 1,3-dipalmitin (1,3-C_{16}) and β-cyclodextrin (cycloheptaamylose) were products of Sigma Chem. Corp. (St Louis, Missouri, USA). The molecular characteristics of the faba bean amylose fraction ($\overline{DP}$: 1400) were previously reported (Biliaderis *et al.*, 1985). Amylodextrin was prepared from faba bean starch by immersing granules (1·0 g (40 ml)$^{-1}$) in 2·2 N HCl at 35°C for 30 days. The insoluble residue was then neutralized and washed with deionized water and ethanol. Its structural characterization was carried out according to Biliaderis *et al.* (1981): it had a $\overline{DP}$ of 20 and was slightly branched (β-amylolysis: 86·1%). The conditions for the preparation of the amylose inclusion complexes (AM–ligand) were essentially those reported by Biliaderis *et al.* (1985). The concentration of the amylose solution was 0·8% (w/v) and the weight ratio of amylose to the ligand was 5/1. Complex formation was allowed to take place at 85°C for 2 h and the solutions were then slowly cooled to room temperature. β-Cyclodextrin–1-C_{16} complex was prepared according to Szejtli & Banky-Elod (1975). The DSC studies were carried out using a DuPont 1090 Thermal Analyzer equipped with a DuPont 910 cell base and a pressure DSC cell (pressure 1400 kPa with N_2). Details for the calibration and operation of this unit have been reported elsewhere (Biliaderis *et al.*, 1985; Maurice *et al.*, 1985). All samples (6–12 mg) of a specified water content were prepared in DuPont coated aluminum pans and hermetically sealed. Data were recorded at 0·4 s time intervals and stored on 8 in floppy disks. Data analyses and file normalization to a constant sample weight were performed using the DuPont software analysis programs.

RESULTS AND DISCUSSION

Metastable melting of amylose-lipid complexes

The most important parameter determining the ability of amylose molecules to crystallize is their chemical-structural regularity (i.e. homopolysaccharide, mainly linear). Furthermore, upon addition of a complexing ligand the induced coil → helix transitions provide additional conformational order that is required for molecular nucleation to proceed. The crystallization process is thus enhanced in the presence of an effective complexing agent.

Polymer crystallization from solution is usually associated with a folded-chain lamellar morphology in the crystalline structure. In fact, flexible linear macromolecules will crystallize first from the random disordered state into a chain-folding macroconformation. The lamellar folded chain crystals are, however, metastable and thus are prone to annealing when sufficient mobility exists in the solid state (Shalaby, 1981; Wunderlich, 1981). Typically, a macromolecular sample may exhibit change in the crystal habit or increase in crystallite dimensions upon annealing mainly through changes in the fold-length. A portion of the sample may also melt and recrystallize into a more perfected form of greater lamella thickness. Accordingly, during a DSC heating experiment a semicrystalline material is exposed to annealing conditions. These could well change the structure of the original crystallites. Partial melting followed by recrystallization seems to be a common feature in the thermal profiles of most synthetic polymers (Runt & Harrison, 1980; Wunderlich, 1980). Recrystallization can be treated as normal crystallization modified by the presence of the remaining unmelted crystallites that act as nuclei. It is therefore controlled kinetically by the molecular nucleation step (Wunderlich, 1981). Thus, the extent of such secondary crystallization will depend on the rate of crystal growth in relation to the heating rate. Evidence for a lamella-like molecular organization of amylose-V complexes was mainly provided by electron- and low-angle X-ray diffraction patterns on single crystals (Manley, 1964; Yamashita, 1965; Zobel *et al.*, 1967; Buleon *et al.*, 1984). On the basis of the calculated long spacings using the Bragg equation, a lamella thickness of 75–100 Å was suggested for these complexes. Furthermore, recent structural studies on amylose-V complexes using α-amylases to selectively hydrolyze the amorphous regions gave resistant amylodextrin fragments of $\overline{DP}$ 75–123 (Jane & Robyt, 1984). These $\overline{DP}$ values correspond to a repeating fold-length of 100 Å. In the light of these findings and taking into account the molecular size of the amylose used in this study ($\overline{DP}$: 1400), it seems reasonable to suggest that the polysaccharide molecules in these complexes assume multiple chain-folding within the same and/or successive lamellae.

Figure 1(a) illustrates the DSC thermal profiles of the solution-grown AM-1-C_{16} complex heated at three different water contents. By

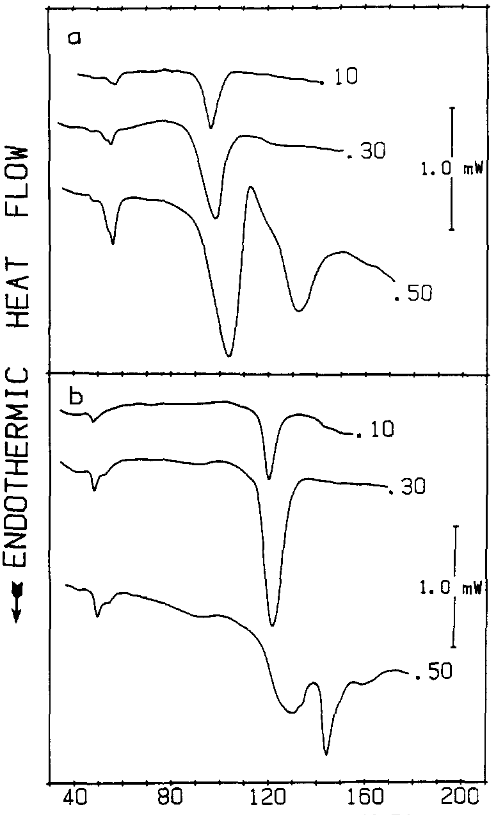

Fig. 1. DSC thermal curves of (a) solution-grown and (b) melt-crystallized AM-1-C_{16} complex: numbers designate the weight fraction of the solid material. Weight of complex from top to bottom (mg): 1·01, 2·44, 5·55, 1·02, 3·25 and 4·76. Heating rate 10°C min^{-1}.

varying the water content, the change from a more cooperative to a metastable type of melting is apparent. Thus, at a 50% water concentration, two melting transitions separated by an exothermic effect can be seen. Such multiple melting characteristics can be interpreted as partial melting, followed by recrystallization and final melting. To investigate the thermal behavior of this complex crystallized at higher temperatures than those employed for its solution-grown counterpart, the complex (50%) was first melted at 170°C (10 min) and subsequently recrystallized at 110°C (4 h). The thermal curves of this sample are shown in Fig. 1(b) while a summary of the DSC data for both the solution-grown and melt-crystallized AM-1-C_{16} complex is given in Table 1. At all concentrations studied, both the onset transition temperature (T_i) and the melting peak temperature (T_p) of the melt-crystallized complex were higher than those of the solution-formed crystalline material. Furthermore, the half-height width of the transition endotherm was less, indicative of a narrower distribution of crystallite dimensions. The higher thermal stability (increased T_p) can be explained on the basis of the higher crystallization temperature. By analogy with other synthetic polymers, one would expect the crystal-

TABLE 1

Effect of Water Content on the Melting Characteristics of Solution-Grown and Melt-Crystallized AM-1-C_{16}

Moisture content (%)	*Solution-grown*				*Melt-crystallized*			
	T_i[a] (°C)	T_p[b] (°C)	*Half-height width* (°C)	ΔH ($J\,g^{-1}$)	T_i (°C)	T_p (°C)	*Half-height width* (°C)	ΔH ($J\,g^{-1}$)
10	83	97	6·5	20·4 ± 0·8[c]	108	121	5·0	21·6 ± 0·7[d]
20	83	97	6·5	20·1	108	121	5·5	21·7 ± 1·1[c]
30	84	99	9·0	20·6	108	122	8·0	22·9
40	84	101, 125	—	21·0	109	127	—	19·9
50	86	104, 132	—	21·4 ± 0·7[d]	111	128, 143	—	24·0 ± 0·0[c]

[a] T_i = initial transition temperature.
[b] T_p = peak transition temperature.
[c] $n = 2$.
[d] $n = 3$.

lite thickness and the melting point to increase with increasing crystallization temperature (Shalaby, 1981; Wunderlich, 1981). It was also interesting to observe that the melt-crystallized complex was prone to further reorganization, although less than the solution-grown complex, under conditions favoring this process (i.e. 50% water content).

Effect of moisture content and heating rate

It is well known that the presence of a diluent can cause depression of both the melting point (T_m) and the glass transition temperature (T_g) of a polymer. The magnitude of this depression depends on the amount of the diluent and its compatibility with the polymer (Jenkins, 1972). Looking first at T_m, it has been established that for a semicrystalline polymer the equilibrium melting point of the polymer, T_m°, will be depressed to a new value, T_m, if the diluent is included in the amorphous phase. The relationship between T_m and volume fraction of the diluent is that described by the Flory–Huggins equation (Flory, 1953) and rests on the assumption of equilibrium melting which is not normally achieved with macromolecular crystals. Nevertheless, reasonably good agreement with this expression has been observed in non-equilibrium experiments for synthetic polymers when zero-entropy production melting conditions (i.e. a melting path under which reorganization is negligible) were established (Mandelkern, 1964; Wunderlich, 1980). The lowering of T_m by incorporation of the diluent is a thermodynamic effect and as such it does depend on the degree of interaction between diluent and polymer: i.e. it is a function of the polymer–diluent interaction parameter, x_1. A diluent also acts as a plasticizer for the amorphous phase and thus lowers its T_g. While different theories exist to account for this depression, it can be considered to arise from a free volume increase effect that results in enhanced mobility of the amorphous phase (Sears & Darby, 1982). The importance of recognizing the depression of both T_m and T_g by the diluent is obvious if one considers that it is within the $T_m - T_g$ temperature region where processes such as crystallization and annealing occur. Thus, by appropriate choice of the solvent and its volume fraction in the polymer–diluent mixture one can change the ($T_m - T_g$) range and thus alter the rate of these processes. Water, for example, has been shown to be a very effective plasticizer for semicrystalline synthetic and natural polymers (Salmen & Back, 1977; Marshall & Petrie, 1980;

Bair, 1981; Maurice *et al.*, 1985). At intermediate water contents it was found that the (T_m-T_g) range of hydrated starch granules increases and thus allows for reorganization to occur during the DSC heating experiment (Biliaderis *et al.*, 1986).

The effect of water content on the melting behavior of AM-1-C_{18} is shown in Fig. 2. Melting was highly cooperative in excess moisture situations (>70%) yielding a single endothermic transition. As the water content is decreased, however, the peak temperature of the first

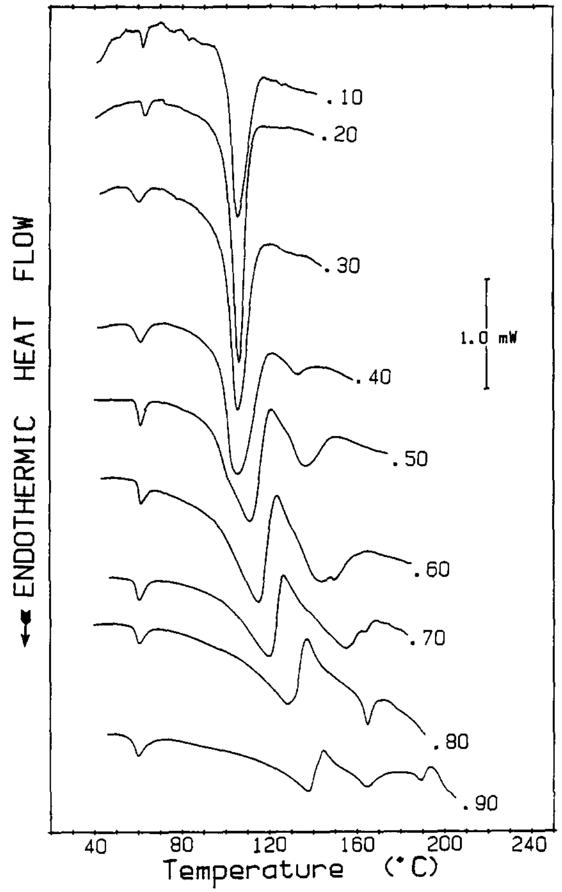

Fig. 2. DSC thermal curves of AM-1-C_{18} at various water contents: numbers designate the weight fraction of the solid material. Weight of complex from top to bottom (mg): 1·22, 1·91, 2·93, 4·41, 4·77, 5·61, 7·01, 7·36 and 8·05. All data files were normalized to a constant sample weight of 5.00 mg. Heating rate 10°C min^{-1}.

transition is elevated and the overall thermal profile becomes indicative of metastable melting. The well-defined exothermic effect between the two endotherms provides strong evidence that such structural rearrangements do occur during heating in the DSC. This behavior was exhibited by most of the amylose–lipid complexes studied (see later section) at intermediate to low water contents. This implies that, after partial melting, recrystallization of the complex to a more perfected structure, presumably of greater lamella thickness, is strongly influenced by the amount of water present in the system. It also indicates that zero-entropy production melting conditions can be approximated by heating these samples in contact with excess amounts of water. The thermal curves of Fig. 2 clearly show that the rate of secondary crystallization processes is decreased with increasing water concentration.

If recrystallization occurs upon heating, its extent will depend on the heating rate: i.e. the slower the heating rate, the longer the time the amylose chains have to recrystallize and thus the more pronounced the secondary crystallization/melting phenomena will be. Figure 3 illustrates the effect of heating rate on the DSC thermal profile of AM-1-C_{14}. At low to intermediate heating rates (3–30°C $\min^{-1}$) two melting endotherms are seen, where the low and high temperature transitions correspond to the melting of the original and recrystallized material, respectively. In fact, the first peak reflects the composite thermal effect of two processes: melting of the initial crystallites and recrystallization. Thus, the measured T_m is no longer characteristic of the starting material. On the other hand, at higher heating rates the sample has very little time to reorganize and therefore gives a single melting endotherm (e.g. 60°C $\min^{-1}$). The slight elevation of the melting peak temperature at such high heating rates can be explained on the basis of superheating; the temperature of the sample rises faster than it can melt. The effect of heating rate on the multiple melting peaks of both AM-1-C_{14} and AM-1-C_{18} is also apparent from Fig. 4 where the enthalpies of both endotherms are plotted as a function of heating rate. With increasing heating rate the enthalpy of the first transition increases while that of the recrystallized material is reduced. The overall effect of water on the thermal behavior of amylose–lipid complexes can be thus explained by considering the thermodynamic melting point depression as well as the structural reorganization during thermal analysis.

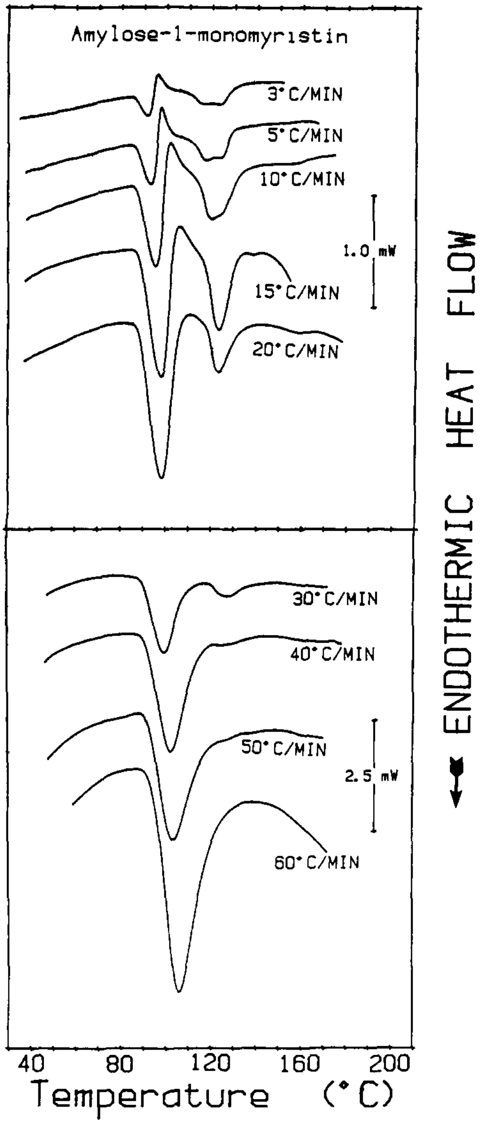

Fig. 3. DSC thermal curves of AM-1-C_{14} (40% w/w in H_2O) at different heating rates. Weight of complex from top to bottom (mg): 3·35, 3·30, 3·53, 3·45, 3·45, 3·31, 3·39, 3·71 and 3·68.

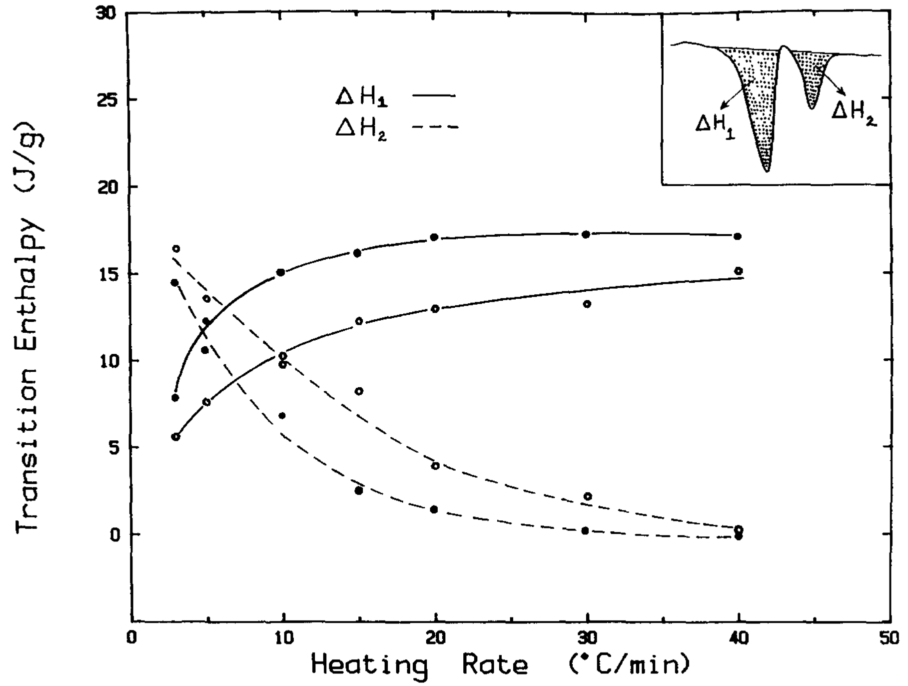

Fig. 4. Effect of heating rate on the melting transition enthalpies (ΔH_1, ΔH_2) of AM-1-C_{14} (○) and AM-1-C_{18} (●). Complex concentration 40% w/w in H_2O.

Effect of ligand and molecular size of amylose

Another factor that can influence the melting behavior of these complexes is the possibility of morphological variations in the crystallites of these materials. Thus, if the isolated complex has reached a high degree of perfection (i.e. large crystallites with a minimum number of defects) during crystallization, it will show less tendency to reorganize under whatever heating rate–moisture content regime is used.

Figures 5 and 6 illustrate the thermal profiles of faba bean amylose complexes with a series of ligand molecules. The thermal curves of Fig. 5 are those of the 10% complex concentration, while those of Fig. 6 are of 40%. Single melting transitions were shown by all materials in excess water (Fig. 5), indicative of melting conditions approaching

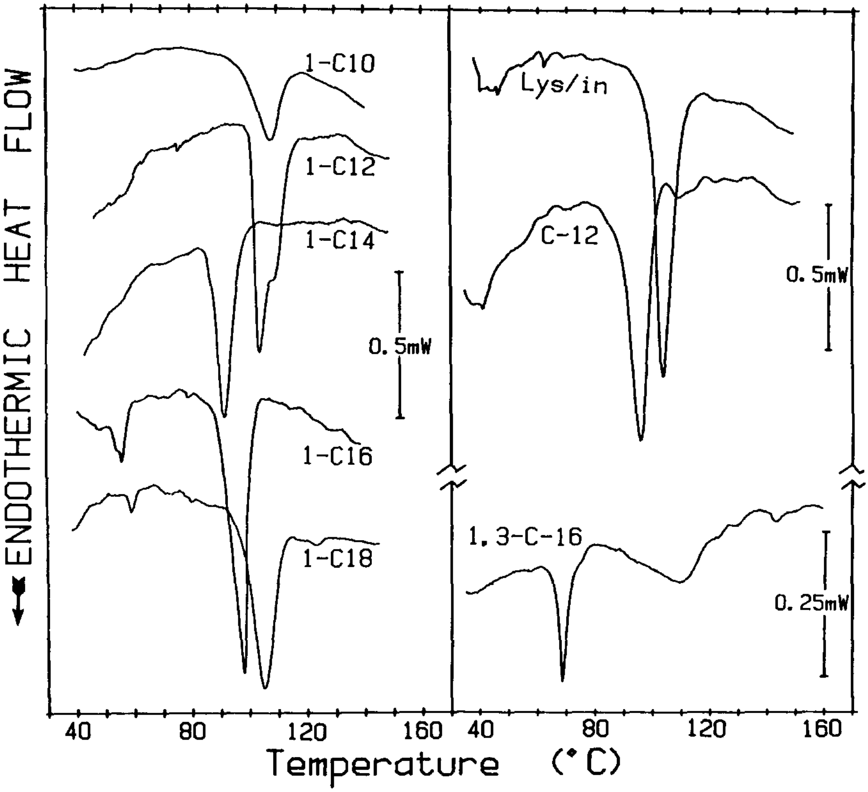

Fig. 5. DSC thermal curves of 10% (w/w solids) amylose-lipid complexes. Weight of solid material (mg): AM-1-C_{10}, 1·30; AM-1-C_{12}, 1·20; AM-1-C_{14}, 1·20; AM-1-C_{16}, 1·01; AM-1-C_{18}, 1·22; AM-lysolecithin, 1·58; AM-C_{12}, 1·07; AM-1,3-C_{16}, 0·98. Heating rate 10°C min^{-1}.

zero-entropy production. The melting peak temperatures, however, varied among these samples. In general, the ligands with the poorer complexing ability exhibited the higher thermal stability. Thus, for the series of saturated monoglycerides studied, the trends in the transition peak temperatures (Fig. 7) resembled that of the complexing index vs. chain length of the fatty acid (Fig. 8, generated from data published by Krog (1971)). This must reflect differences in the crystallization habit of these materials which is controlled by the nucleation step. The latter is affected by the coil → helix conformational changes induced upon effective association of the ligand molecule with the poly-

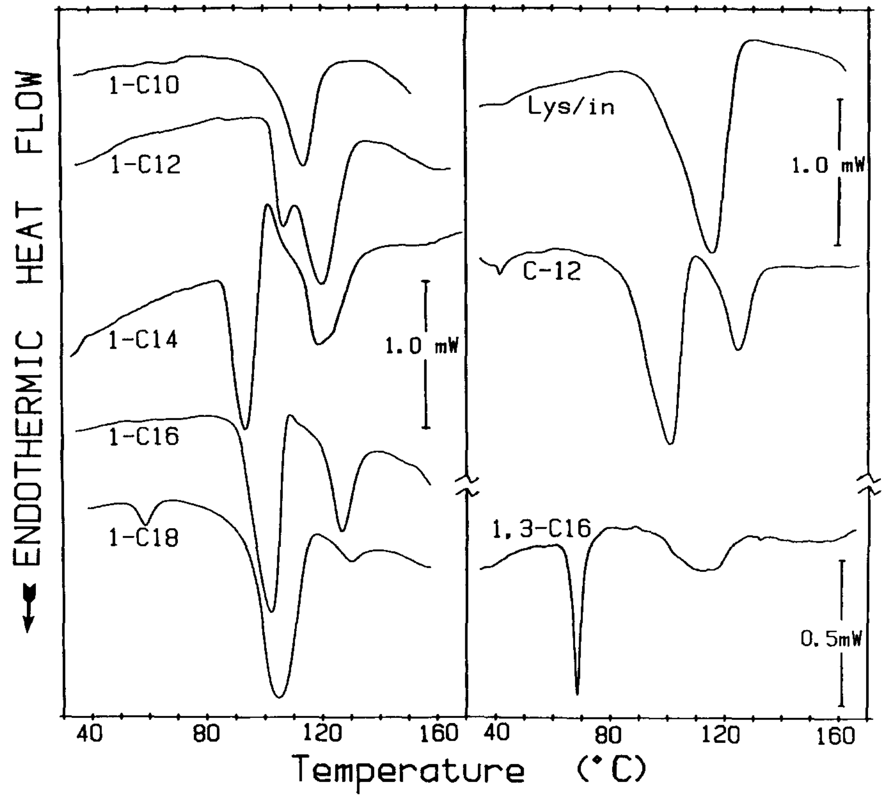

Fig. 6. DSC thermal curves of 40% (w/w solids) amylose–lipid complexes. Weight of solid material (mg): AM-1-C_{10}, 4·22; AM-1-C_{12}, 4·08; AM-1-C_{14}, 3·52; AM-1-C_{16}, 3·84; AM-1-C_{18}, 4·41; AM-lysolecithin, 4·28; AM-C_{12}, 4·72; AM-1,3-C_{16}, 3·92. Heating rate 10°C min^{-1}.

saccharide chain. It appears that in the presence of a good complexing agent (e.g. 1-C_{14}) relatively poorer, less thermostable, complexes are grown under the same effective degree of supercooling. With a good complexing agent, the nucleation density and the rate of crystallization would be increased, thus leading to a polycrystalline structure of smaller and less perfected crystallites. The transition temperatures of the 40% complexes also exhibited a similar trend (Figs 6 and 7). Furthermore, the less perfected complexes of the better complexing ligands were the ones that showed higher tendencies for recrystallization upon heating.

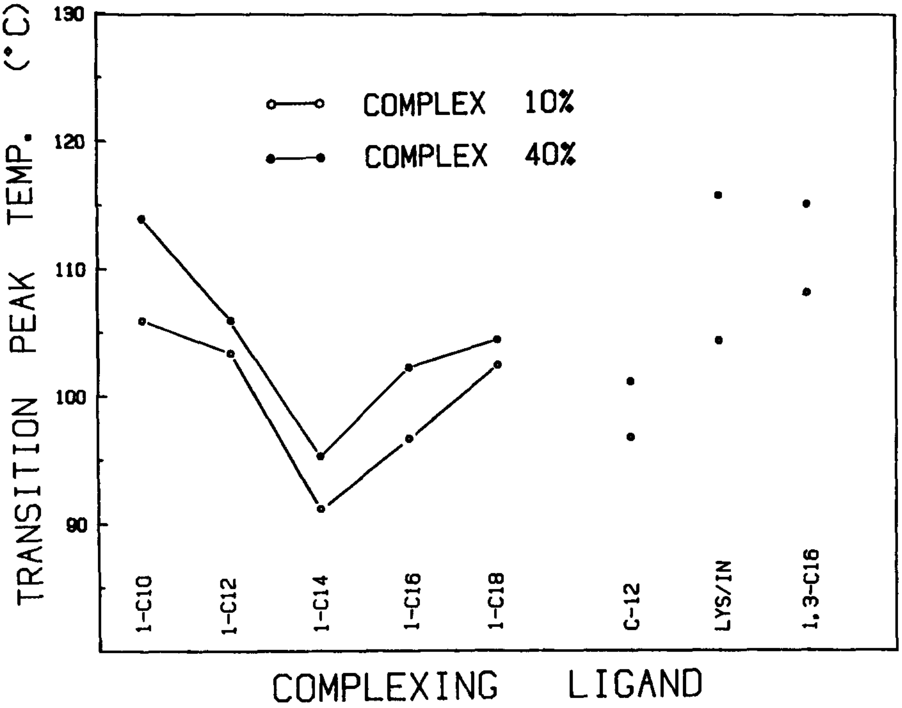

Fig. 7. Dependence of transition peak temperature of the solution-grown crystalline amylose–lipid complexes on the nature of the ligand molecule.

In order for the argument of chain folding to be valid, it was necessary to demonstrate that metastable melting is absent for complexes where, due to the molecular characteristics of the polysaccharide, chain folding is not the morphological feature of the crystallite structure. Therefore, the melting properties of two such complexes, amylodextrin-1-C_{16} and β-cyclodextrin-1-C_{16}, were investigated at various moisture contents. The fixed cyclic structure of β-cyclodextrin (yields doughnut-like complexes (Bender & Komiyama, 1978)) as well as the relatively short chain length of the amylodextrin ($\overline{DP}$: 20) do not allow crystallization to proceed through a chain-folding mechanism. In this case one would expect lamella thickness to approximate to the molecular chain length and, hence, melting to occur without crystal thickening. The thermal profiles shown in Fig. 9 were indeed in

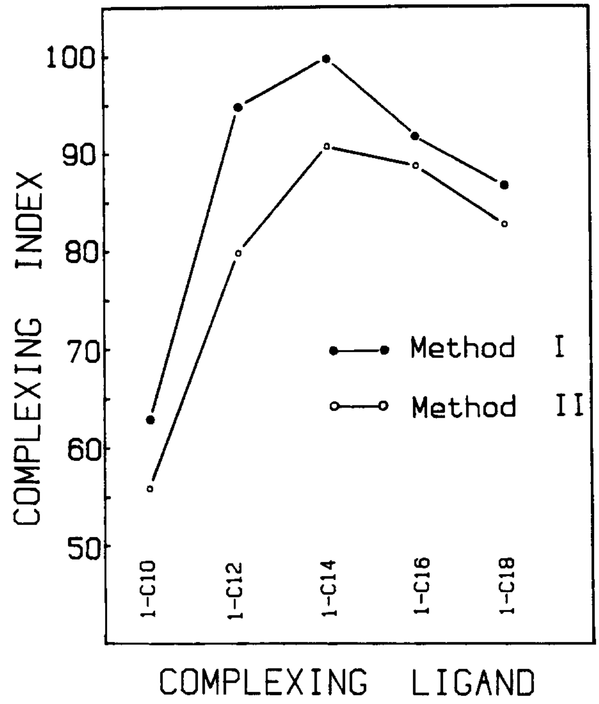

Fig. 8. Amylose complexing ability of saturated 1-monoglycerides with chain length from C_{10} to C_{18}. Method I is based on the determination of remaining amylose in solution after separation of the precipitated complex. Method II is based on the determination of the reduction in iodine affinity of the amylose in the presence of added monoglyceride (adapted from Krog (1971)).

accord with this concept. Both complexes of β-cyclodextrin and amylodextrin showed no evidence for any recrystallization during heating. On the other hand, the corresponding sample of faba bean amylose complex exhibited the typical irreversible metastable melting at intermediate water contents. It was also interesting to observe a reversible high temperature melting peak (160°C, 50%, dashed lines) for the noncomplexed amylose sample. This endothermic transition occurs at 82°C and 111°C in the case of amylodextrin and β-cyclodextrin, respectively. Further studies are required, however, to provide additional insight into these transitions.

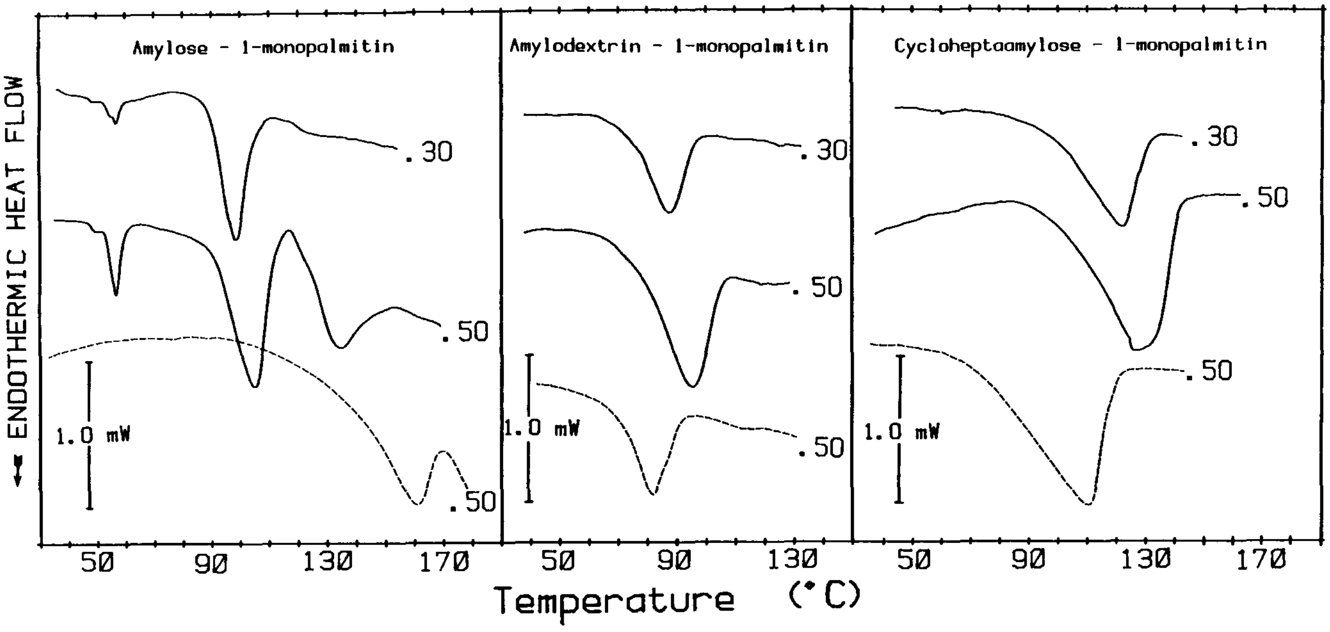

Fig. 9. DSC thermal curves of amylose-1-C_{16}, amylodextrin-1-C_{16}, β-cyclodextrin-1-C_{16} (30% and 50% complex, solid lines), and amylose, amylodextrin, β-cyclodextrin (50%, dashed lines). Normalized weight of solid material (mg): 3·00 and 5·00 for the 30% and 50% samples, respectively. Heating rate 10°C min^{-1}.

CONCLUSIONS

In concluding the above discussion, perhaps it would be beneficial to schematically illustrate the interplay between all the parameters involved in phase transitions of amylose–lipid complexes (Fig. 10). The top part illustrates the processes that take place simultaneously during heating in the calorimeter and the resultant thermal curves one would anticipate experimentally. The bottom part presents the parameters which appear to influence these processes. The overall mechanism can be best rationalized by considering both thermodynamic and non-thermodynamic contributions. For the former one must take into account the depressing effect of diluent (water) on the melting temperature, T_m. Water also acts as a plasticizer, lowering the glass transition temperature (T_g) of the amorphous regions of these complexes. In excess water the amorphous chain segments are fully plasticized and thus crystallite melting becomes more cooperative and occurs at lower temperatures. At decreasing levels of diluent, partial melting of the less perfected material takes place in a temperature region where recrystallization is very rapid.

With regard to the non-thermodynamic effects one factor that appears to influence the melting behavior of these complexes is the degree of crystallite perfection in the semicrystalline structure. Growing amylose–lipid complexes in solution under the same supercooling conditions, crystallite perfection/size seems to be related to the type of the ligand molecule and the molecular size/structure of the polymer chain. Thus, if the solution-grown crystalline complex has already achieved a high degree of perfection (e.g. in the presence of a poor complexing agent), it would exhibit very little or no tendency for recrystallization. Furthermore, inherent with the dynamic nature of the DSC technique, and in relation to the rate of recrystallization, the melting curves of the complexes will be modified by the heating rate. The major consequences of such metastable melting is that as the heating rate increases the higher melting endotherm would decrease in magnitude. This effect was indeed observed experimentally (Fig. 4). Similarly, any other factor that can affect the ability of these semicrystalline materials to anneal upon heating will alter the character of the first melting endotherm and thus the apparent peak temperature (T_m): note that $T_{m_3} > T_{m_2} > T_{m_1}$ (Fig. 10). In summary, then, conditions such as low moisture content, low degree of crystallite perfection and

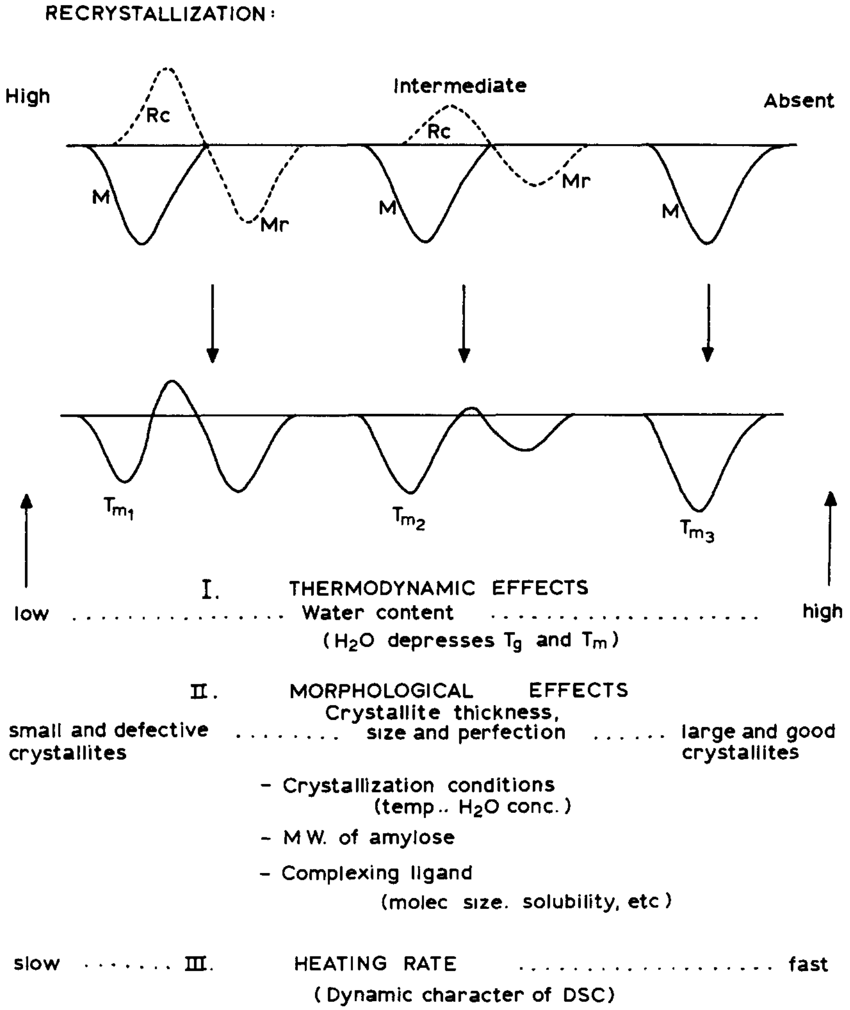

Fig. 10. Schematic representation of the mechanism proposed to account for the melting behavior of solution-grown amylose-V complexes. The processes designated as M, Rc and Mr refer to melting, recrystallization and melting of recrystallized material, respectively.

slow heating rates will tend to foster secondary crystallization processes. In this case, the resultant behavior will be a composite of the peaks due to the melting of the original crystallites, the recrystallization exotherm and the melting of the recrystallized material. On the other hand, excess of water, higher perfection of crystallite microstructure and high heating rates will retard lamella thickening and, hence, yield single endothermic transitions. Due to the non-equilibrium nature of the melting phenomena, it is obvious that any thermodynamic analysis on the melting data, using expressions like the Flory–Huggins equation (describes only equilibrium melting), has no theoretical basis.

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