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# On the Supermolecular Structure and Metastability of Glycerol Monostearate–Amylose Complex

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*ABSTRACT*

*Calorimetry of glycerol monostearate–amylose complexes reveals the presence of at least two thermally distinct metastable forms, which thus implies a definite connection between thermal properties and the supermolecular organization of these materials. On the basis of X-ray-diffraction, calorimetric, and structural-analysis data, it is postulated that form I ($T_m$ 99·4°C), obtained at low crystallization temperatures, has a uniformly distributed partial order where no separate crystallites can be identified. Such a structure would have an internal energy and entropy between those of an amorphous melt and a structure of discrete crystallites, such as form II ($T_m$ 116·6°C). Conversion of form I to form II is readily carried out by isothermal annealing at temperatures above its melting point. The conformational responses of the complex (forms I and II) in various stabilizing ($Na_2SO_4$, sucrose, CsCl) and destabilizing (urea, guanidine hydrochloride) environments further suggest that interconversion between the various forms can be explained by considering that structural orders at two levels are affected: association–dissociation of aggregated helices and helix-coil transitions; at high concentrations, CsCl caused disruption of the crystallites without altering the conformation of individual helices. The transition enthalpy of the complex shows very little change with increases in the long-range order of the supermolecular structure (X-ray-diffraction data) and is interpreted to reflect mainly contributions from conformational disordering of helices.*

## INTRODUCTION

Experimental data on the melting of macromolecular crystals are often indicative of irreversible (non-equilibrium) processes. As a result,



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crystallite reorganization or recrystallization can occur before final melting. This behavior is a direct manifestation of the metastability of partially crystalline states, and, for synthetic linear polymers, it has been attributed to small crystal size, chain-folding, and defects in the crystal structure (Wunderlich, 1973, 1980). Such non-equilibrium states exhibit much lower melting temperatures than equilibrium crystals, and their metastability can be assessed by calorimetry (Shalaby, 1981; Wunderlich, 1980, 1981).

Differential scanning calorimetry (DSC) measurements of phase transitions of starch (Slade & Levine, 1984; Maurice *et al.*, 1985; Biliaderis *et al.*, 1986*a*) and amylose–lipid complexes (Stute & Konieczny-Janda, 1983; Biliaderis *et al.*, 1985, 1986*b*) have also shown that the thermal curves do not reflect the initial crystallite distribution or the morphology of the crystallites. Instead, when the amount of water present in the system is insufficient to facilitate a co-operative melting path or a moderate heating rate is employed to allow annealing during heating, or both, melting and reorganization can occur simultaneously, which thus yields composite thermal profiles. Approximation of zero-entropy production melting (i.e. melting without change in the metastability of the system) is therefore essential if one is to deduce the degree of metastability and information regarding the structure of original crystallites from their melting behavior. For aqueous starch systems, crystallite melting approaches zero-entropy production conditions in excess moisture situations and under relatively fast heating rates (Biliaderis *et al.*, 1986*b*). One limit of the method of fast heating, however, is the possibility of excessive superheating (the rate of heating exceeds the rate of response of the system), particularly with crystals of more extended chain character and a large number of tie (strained) chain segments (Wunderlich, 1980). The DSC data of Shiotsubo and Takahashi (1984) on potato-starch gelatinization indicate that, at heating rates above 0·5°C/min, crystallite melting is subject to kinetic limitations (e.g. water diffusion, thermal lags) since $T_m$ increases with the heating rate. Nevertheless, DSC measurements at 5–10°C/min in conjunction with excess moisture (80%) do provide useful qualitative information about the structure and metastability of the initial sample, particularly for amylose–lipid complexes (Biliaderis *et al.*, 1985, 1986*a*, *b*).

In a recent study of the effect of crystallization temperature on the supermolecular organization of amylose–monoglyceride complexes (Biliaderis & Galloway, 1989), crystallized from dilute solution, two thermally distinct forms were identified: form I (low $T_m$), which shows an amorphous X-ray diffraction pattern and readily undergoes reorganization upon heating, and form II (high $T_m$), which gives the typical V-type

crystallographic pattern. A working structural model for these forms was also suggested. Form I was assumed to be obtained when rapid nucleation occurred and was morphologically described by a random distribution of helices having little crystallographic register in the aggregated state. In contrast, form II appeared to have the classical structure of well-defined crystallites embedded in disordered regions. The present paper is concerned with differences in the annealing behavior, thermal stability, and interconversions between these metastable structural forms of glycerol monostearate–amylose complex in both stabilizing (sodium sulphate and sucrose) and destabilizing (urea, guanidine hydrochloride) aqueous environments. Furthermore, the effects of CsCl on the structure of the complex, as probed by X-ray and DSC, provided additional supporting evidence to the postulate that forms I and II are two distinct thermodynamic states of different internal energy and entropy. The subject is of practical importance since both forms may be found in processed starch-based food products (bread, parboiled rice, extruded cereals, etc.), depending on their thermomechanical history.

## EXPERIMENTAL

### Materials and methods

Glycerol monostearate was a product of Sigma Chemical Company (St Louis, MO), and amylose, from potato starch, was obtained from Aldrich Chemical Company (Milwaukee, WI). Guanidine hydrochloride (Gdn. HCl), sodium sulphate ($Na_2SO_4$), and urea (ACS Chem. reagent grade) were products of Sigma Chemical Company. Cesium chloride (CsCl) was obtained from Pharmacia Ltd (Montreal, Canada). The molecular properties of the amylose fraction were: $[\eta]$ in 1 N KOH 156 ml/g, corresponding to a $\overline{DP}$ of 1150, iodine affinity of 18·9 g $I_2$/100 g, $\beta$-amylolysis 83·8 ± 1·1% and $\lambda_{max}$ of its iodine complex 620 nm. Gel-filtration chromatography of this material on a Sephacryl S-1000 superfine column (2·6 × 90 cm, flow rate 0·4 ml/min) eluted with 40% v/v dimethyl sulfoxide ($Me_2SO$) in distilled water gave a broad symmetrical chain distribution.

The conditions for the preparation of amylose–monostearin complexes were essentially those reported by Biliaderis *et al.* (1985). Complex formation was carried out for 24 h in aqueous solutions (0·25% w/v amylose; weight ratio of amylose to added ligand 5:1) maintained at a constant temperature (60°C for form I and 90°C for form II). The insoluble complexes were recovered by centrifugation (8000 *g*) and washed repeatedly with chloroform to remove the free ligand (as

assessed by DSC analysis). Samples used for X-ray diffraction and acid-etching experiments were kept in the hydrated state, whereas those intended for DSC analysis were freeze-dried.

Acid hydrolysis of the complexes in a heterogeneous reaction was carried out at 40°C by suspending 1·5 g of solids in 300 ml 1·2 N HCl under continuous gentle agitation. At specified time intervals, the reaction mixtures were neutralized and centrifuged, and the supernatants were assayed for total carbohydrates by using the orcinol–sulphuric acid method (0·1% w/v orcinol in 70% v/v $H_2SO_4$; Miller *et al.*, 1960). The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial complex. Iodine staining of the resistant amylodextrin residues was done according to Bailey and Whelan (1961). After solubilization with $Me_2SO$, gel permeation of the amylodextrin residues was carried out on a Sephacryl S-200 superfine column (0·9 × 131 cm, flow rate 19·0 ml/h) eluted with degassed distilled water (35°C). Carbohydrates in the eluent (orcinol–sulphuric acid; absorbance at 450 nm) were continuously monitored with an auto-analyzer.

X-ray diffraction analysis was performed on hydrated or lyophilized residues deposited on aluminum holders by using a Philips PW 1710 powder diffractometer equipped with a graphite-crystal monochromator. The operating conditions were: copper $K_a$ radiation, voltage 40 kV, recorder time constant 0·5 s, sampling interval time 0·4 s, recorder speed 10 mm/2$\theta$°, and scan speed 0·1 2 $\theta$°/s.

The DSC studies were carried out by using a Du Pont 9900 Thermal Analyzer equipped with a Du Pont 910 cell base and a pressure DSC cell. The system was calibrated with indium (Biliaderis *et al.*, 1985). A pressure of 1500 kPa with $N_2$ was used to eliminate pan failure at temperatures above 120°C. All samples were prepared in Du Pont hermetic pans by adding the required amount of solution to a pre-weighed freeze-dried sample of the complex. In this respect, it is of interest to note that freeze-drying of the hydrated complex did not alter the thermal properties ($T_m$, $\Delta H$) of both forms I and II. All DSC measurements were carried out at 20% solids and 10°C/min to approximate zero-entropy production melting. The lyophilized samples were kept for at least 2 h to equilibrate with the solvent, before DSC analysis, in order to minimize time-dependent changes in conformation of the complex at room temperature, particularly for solutions of high concentration of destabilizing agents. For isothermal annealing studies, the samples were heated in the calorimeter for a specified time–temperature regime, cooled, and weighed to ensure no losses of solvent during heating.

## RESULTS AND DISCUSSION

### Annealing effects and structural considerations

Crystallization of the complex at 60°C and 90°C gave pure preparations of structural forms I ($T_m$ 99·4 ± 0·3°C) and II ($T_m$ 116·6 ± 0·2°C), respectively, as shown in Fig. 1. Although, in a number of calorimetric studies of amylose–lipid complexes, multiple melting transitions have been reported, the underlying causes of this behavior remained unknown (Wirakartakusumah, 1981; Bulpin *et al.*, 1982; Donovan *et al.*, 1983; Stute & Konieczny-Janda, 1983; Kowblansky, 1985; Eliasson, 1988; Raphaelides & Karkalas, 1988). As a result, no attempt was made to provide a possible morphological model for the description of solid state

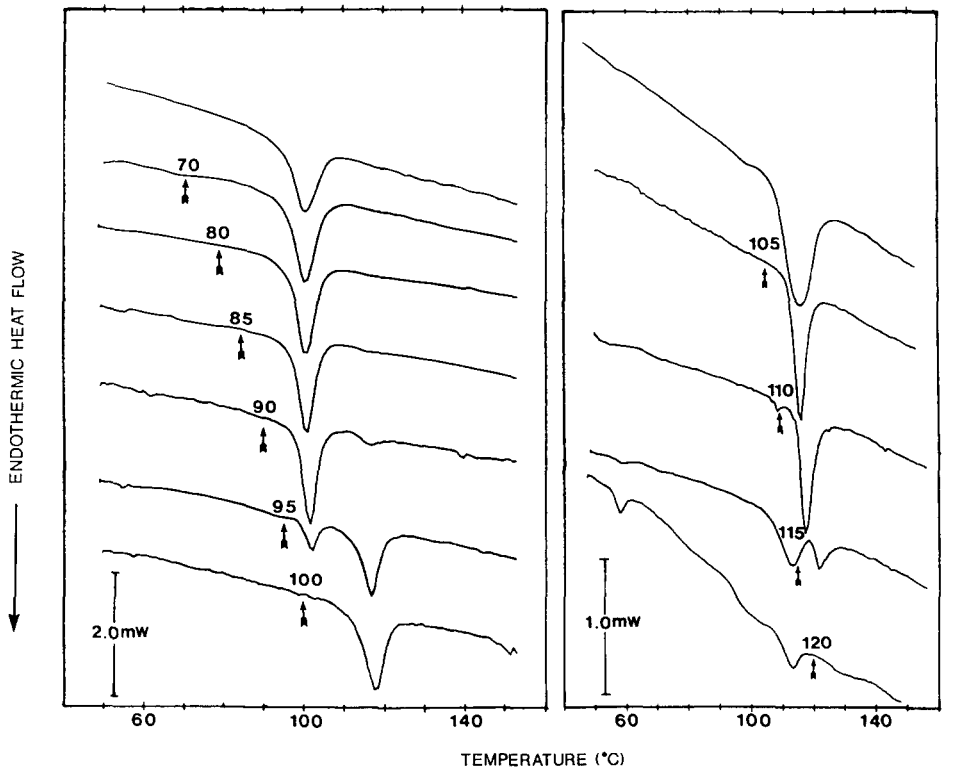


**Fig. 1.** Effect of annealing treatments (2 h at the specified temperature) on the structure of forms I (left) and II (right). Top curves are of control (non-treated) samples. Heating rate of DSC experiments 10°C/min, solids content 20% w/w. Mass of complex from top to bottom (mg): (left) 2·05 (control), 2·07, 2·01, 1·92, 1·97, 1·93, and 1·97; (right) 2·09 (control), 1·32, 1·42, 1·28, and 1·46.

organization of these materials that could account for such thermal responses. On the basis of a number of DSC, X-ray, and structural analysis data, it was proposed that multiple melting transitions of complexes represent metastable states of varying degree of organization of the helical chain motifs in the ordered domains. The thermodynamic and kinetic arguments for their existence were given in detail elsewhere (Biliaderis & Galloway, 1989) and remain valid for the discussion of this paper. A striking feature of form I was the constancy of its melting temperature, which suggested that it is a well-defined state.

In the hydrated state, form I lacks the characteristic diffraction pattern of V-complex (Fig. 2(a)), as compared with form II, which shows the three major reflection peaks of V-crystals at 7·36, 13·1, and 20·1 $2\theta°$ (Fig. 2(c)). The X-ray diffraction of the dry powder of form I gave some of the characteristic spacings of V-form (Fig. 2(b)), although diffusely and not as distinctly as those of form II (Fig. 2(d)). It therefore appears that there is a modification in the structure of form I during lyophilization in

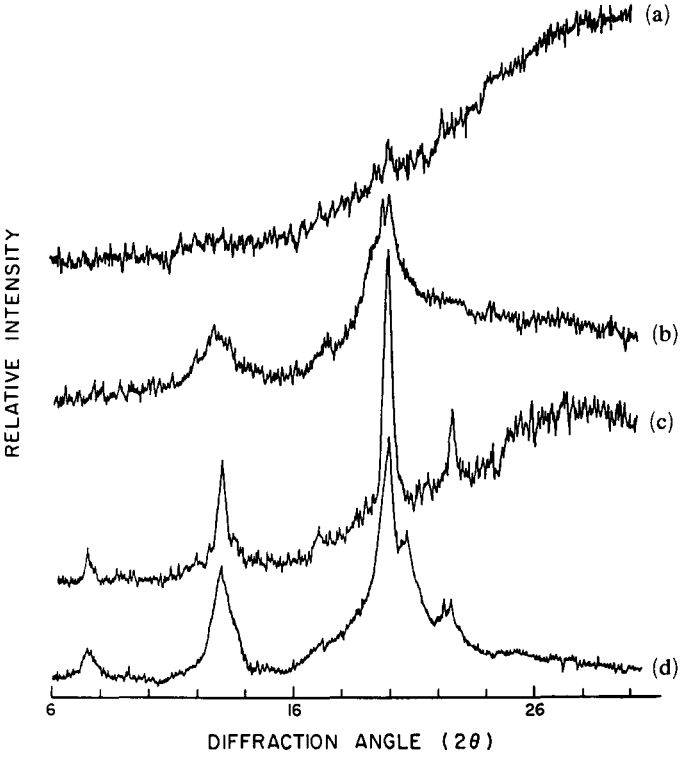


**Fig. 2.** X-ray diffraction diagrams: (a) form I wet; (b) form I dry; (c) form II wet; (d) form II dry.

that chain aggregates of much higher order are formed, which gives rise to a two-line V-pattern. These results are consistent with the view that form I has helices of very little crystallographic register, whereas the structural domains of form II consist of well-developed crystallites. The $T_m$ of form I remains constant regardless of the crystallization conditions (temperature, polymer concentration, etc.). However, it is dependent on the chain length of the ligand (Kowblansky, 1985; Biliaderis *et al.*, 1985; Raphaelides & Karkalas, 1988) and the degree of unsaturation of the aliphatic chain (Stute & Konieczny-Janda, 1983; Eliasson & Krog, 1985). It is also worth commenting here that, despite the differences in X-ray diffraction patterns between the hydrated and lyophilized–rehydrated complex of form I (Fig. 2(a), (b)), the DSC thermal parameters were not altered as a result of freeze-drying, i.e. identical $T_m$ and $\Delta H$ values were obtained for the hydrated and dried–rehydrated samples of form I.

The results of the annealing experiments on structural modification of the complex are shown in Fig. 1. The effect of annealing temperature on form I is negligible up to about 90°C, which is the onset temperature for the melting transition of the initial sample. Annealing at higher temperatures resulted in the development of the second endotherm, whose $T_m$ coincided with that of form II. The fact that form II appears only when the metastable form I is annealed at elevated temperatures implies that high energy barriers exist between the two forms. Hence, unless the structure of form I is partially melted, it would remain practically unchanged at low temperatures. It is likely that the inter-helical chain segments of form I are under considerable strain and thus exhibit an elevated $T_g$ (Biliaderis *et al.*, 1986; Slade & Levine, 1987), very close to the $T_m$ of the helices. This situation leads to a kinetically stable system. However, once some helical chains of form I melt, the structure relaxes, and crystallization can proceed rapidly around the remaining helices that can act as nuclei. Since conversion of form I → II upon annealing does not yield another intermediate state between the two forms, form I must represent a distinct thermodynamic state.

An increase in the melting temperature of form II along with a decrease in the half-height width of the melting transition was observed after isothermal annealing (Fig. 1, right). This suggests the development of larger and more perfect crystallites of narrower size distribution, typical of annealing effects on macromolecular non-equilibrium crystals (Wunderlich, 1980). Assuming a chain-folded (lamellar) macroconformation, as suggested by electron- and low-angle X-ray diffraction work on single crystals and polycrystalline aggregates of V-amylose (Manley, 1964; Yamashita, 1965; Zobel *et al.*, 1967; Buleon *et al.*, 1984)

as well as structural studies by using α-amylases and gel permeation chromatography (Jane & Robyt, 1984; Biliaderis & Galloway, 1989), crystallite perfection or thickening or both would proceed via increased motion of chain defects and ingestion of tie-chain segments (Buckley & Kovacs, 1984). Annealing at temperatures above the $T_m$ of the initial II-crystals yielded multiple melting peaks. Under these conditions, the rate of nucleation decreases rapidly, and thus crystallization in the metastable melt is restricted; the sample appears to crystallize during subsequent cooling.

The possibility that differences in the observed $T_m$ between forms I and II might arise from differences in the size of the crystallites, as predicted by the Thomson–Gibbs equation for chain-folded crystals (Wunderlich, 1980), was further examined. The chain distribution of the ordered domains in the two forms was determined by gel filtration following acid-etching of the complex. Assuming that acid degrades preferentially the amorphous regions (chain folds, loops, and tie molecules), the resistant amylodextrin fragments would represent the chain segments participating in the crystallites. The thermal and chemical properties of the residues at various hydrolysis-time intervals are given in Table 1, and the chromatographic profiles of the six-day treated samples are shown in Fig. 3. The chain distributions of the resistant fragments of form I (Fig. 3(a)) and II (Fig. 3(b)) were similar despite the different solubilization levels reached at this digestion period. These results are in agreement with the authors' previous findings on the characterization of glycerol monomyristate and glycerol monopalmitate–amylose complexes by α-amylase etching/gel chromatography (Biliaderis & Galloway, 1989). Furthermore, they support the hypothesis that structural

**TABLE 1**

Thermal and Chemical Properties of Amylodextrin Residues after Acid-Etching of Glycerol Monostearate–Amylose Complex (forms I and II) in a Heterogeneous Reaction Mixture (1·5 g solids/300 ml 1·2 N HCl; 40°C).

| *Reaction time (days)* | *Solubilization (%)* | | $\lambda_{max}$ *(iodine complex)* | | $\Delta H$ *(J/g)* | |
|---|---|---|---|---|---|---|
| | *I* | *II* | *I* | *II* | *I* | *II* |
| 1 | 13·2 | 15·1 | 602 | 606 | 25·3 ± 1·6[a] | 26·8 ± 1·9 |
| 2 | 29·3 | 27·6 | 594 | 599 | 25·8 ± 0·6 | 27·5 ± 2·1 |
| 6 | 68·1 | 42·4 | 585 | 588 | 19·8 ± 1·1 | 17·9 ± 1·1 |
| 12 | 75·7 | 54·6 | 579 | 580 | 14·3 ± 3·1 | 13·2 ± 2·7 |

[a] $n$: 3.

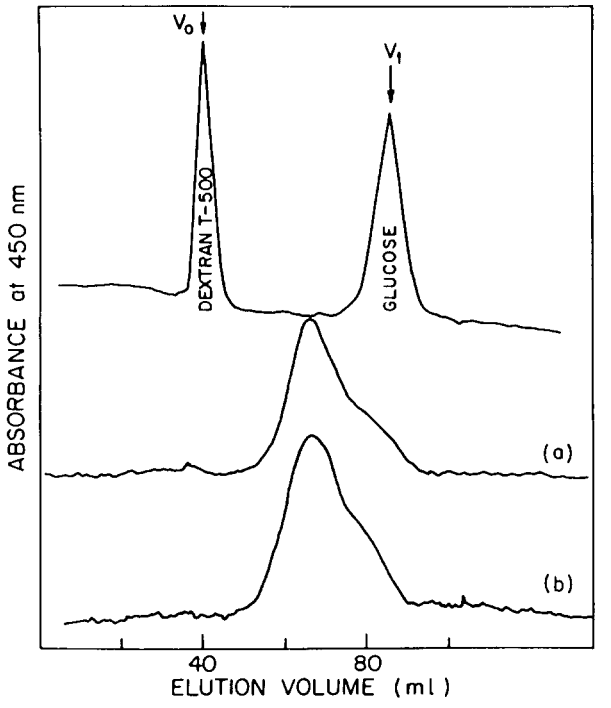


**Fig. 3.** Chromatographic profiles of resistant amylodextrin fragments after acid hydrolysis (6 days) of the complex (1·2 N HCl) on a Sephacryl S-200 column (131 × 0·9 cm) eluted with water (flow rate 19·0 ml/h, 35°C): (a) form I; (b) form II.

differences between forms I and II mainly lie in the degree of organization of the helical chain segments and not in their length (Biliaderis & Galloway, 1989).

## Solvent effects on conformational stability of the complex

The role played by the solvent and small solute–solvent interactions in aqueous solutions of macromolecules is in some aspects far from clear. In general, solutes can affect macromolecular stability and conformational interconversion equilibria by direct interactions with the macromolecule and/or by indirect action through effects on the structure of water (von Hippel & Schleich, 1969). Since the pioneering work of Hofmeister, it has been recognized that neutral salts, for example, drastically alter the solubility and conformation stability of macromolecules; their relative effectiveness follows the lyotropic or Hofmeister ion series (von Hippel & Schleich, 1969). Selective interactions between polymer and ion may, however, distort the normal lyotropic order (Haud

& Smidsrød, 1970; Rinaudo *et al.*, 1979). Furthermore, for polymeric materials having a higher structural order owing to aggregation of chains, salt effects must be interpreted by considering both dissociation of the supermolecular structure and conformational disordering of individual chains. As a result, the ranking may not necessarily parallel the Hofmeister series if the ions act differently at the various levels of ultrastructure, as was shown for reconstituted collagen in the solid state (Chang & Chien, 1973; Chien, 1975). Incorporation of polyhydroxy compounds (e.g. sugars) is also known to stabilize macromolecules in solution (Gerlsma, 1968; Lee & Timasheff, 1981; Suggett, 1974) or in the solid state (e.g. starch; Lund, 1984) against the disruptive effect of temperature and pH. In contrast, urea and guanidinium salts are universal denaturants of proteins and other 'native' structures of macromolecules, including starch (Tanford, 1968, 1970; Erlander & Tobin, 1967; von Hippel & Schleich, 1969; Suggett, 1974).

The thermal responses of forms I and II with increasing concentration of sucrose and $Na_2SO_4$, used as stabilizing compounds, are shown in Figs 4 and 5, respectively. Evidence for structure stabilization of the

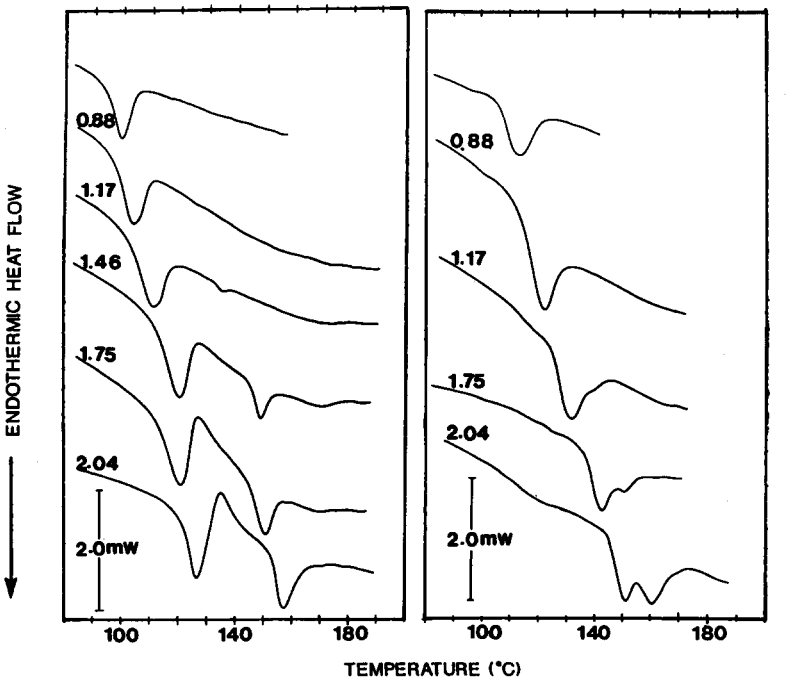


**Fig. 4.** DSC thermal curves of structural forms I (left) and II (right) of the complex (20·0% w/w) in sucrose solutions of varying molar concentrations. Mass of complex from top to bottom (mg): (left) 2·46 (control), 2·05, 2·29, 2·02, 2·33, and 2·35; (right) 2·09 (control), 1·72, 1·84, 2·30, and 1·87. Heating rate 10°C/min.

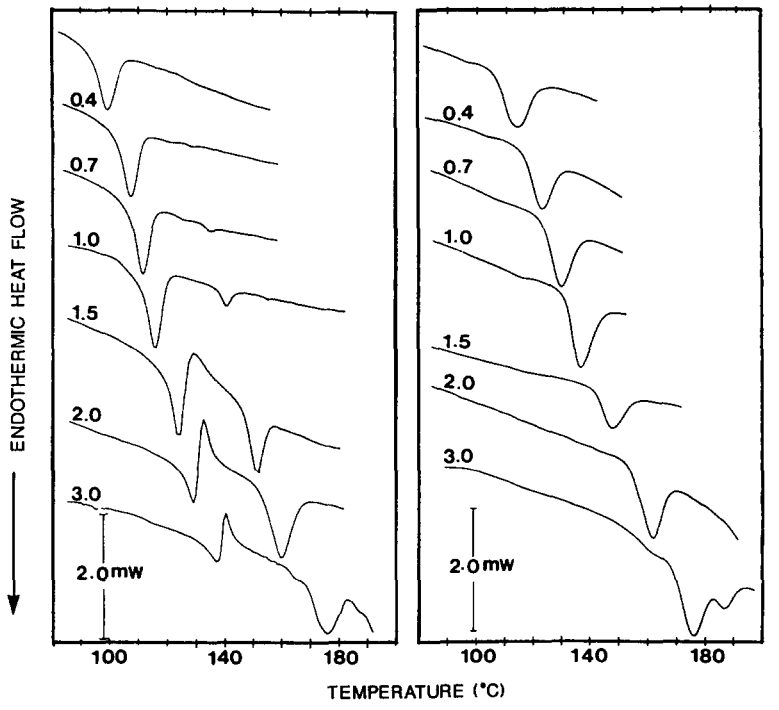


**Fig. 5.** DSC thermal curves of structural forms I (left) and II (right) of the complex (20% w/w) in $Na_2SO_4$ solutions of varying molar concentrations. Mass of complex from top to bottom (mg): (left) 2·05 (control), 2·31, 2·10, 2·39, 2·04, 2·01, and 1·91; (right) 2·09 (control), 1·93, 1·96, 1·97, 1·98, 1·55, and 2·00. Heating rate 10°C/min.

complex was reflected by increases in $T_m$ for both forms. Furthermore, at high solute concentrations (> 1·17 M sucrose, Fig. 4; > 0·7 M $Na_2SO_4$, Fig. 5), the metastable form I undergoes reorganization to form II during thermal analysis. The exothermic effects between the two endotherms are indicative of recrystallization of the complex into a state of lower free energy (a thermodynamically more stable form). Thus the thermal curves represent composite effects of secondary processes superimposed on the primary phenomena. It was, in fact, shown that by increasing the heating rate (from 3 to 50°C/min), the enthalpy of the first transition increased (2·0 M sucrose, 2·0 M $Na_2SO_4$), whereas that of the recrystallized material was reduced (data not shown).

The observed stabilizing role of $Na_2SO_4$ (Fig. 5) on both structural forms of the complex is in accord with its ranking in the lyotropic series. Sulphate salts [$(NH_4)_2SO_4$, $Na_2SO_4$] are known to stabilize the native conformation of proteins and nucleic acids (von Hippel & Schleich, 1969). Other relevant observations of the influence of anions on swelling and gelatinization properties of starch (Mangels & Bailey, 1933; Medcalf

& Gilles, 1966; Evans & Haisman, 1982) and starch retrogradation (Morsi & Sterling, 1963) were also consistent with the lyotropic anion series, $SO_4^{2-} < F^- < Cl^- < Br^- < I^- < SCN^-$; sulphate salts exercise the strongest effect in increasing the gelatinization temperature, retarding swelling, and promoting retrogradation.

The structure-stabilizing action of sucrose (Fig. 4) agrees with earlier findings of the effect of sugars on starch gelatinization. Sugars, as well as other polyhydroxy compounds, increase the gelatinization temperature of starch (Jacobsberg & Daniels, 1974; Bean & Yamazaki, 1978*a*, *b*; Wootton & Bamunuarachchi, 1980; Spies & Hoseney, 1982; Evans & Haisman, 1982). There have been several interpretations of this behavior, including competition between starch and sugars for water, inhibition of granule hydration, and specific sugar–starch interactions (Evans & Haisman, 1982; Spies & Hoseney, 1982; Lund, 1984). Attempts have even been made to explain and predict the thermal responses of starch in sugar solutions on purely thermodynamic grounds by using an extension of the Flory equation to a three-component system (Lelievre, 1976; Blanshard, 1979; Evans & Haisman, 1982). Although this approach provides a convenient framework to analyze melting data, its development is based on equilibrium melting processes and making simplifying assumptions regarding the interaction parameters, $X_{ij}$, between solvent, solute, and polymer. However, evidence suggesting that melting of starch crystallites is a non-equilibrium process (Slade & Levine 1984, 1987, 1988; Maurice *et al.*, 1985; Biliaderis *et al.*, 1986*a*; Blanshard, 1987) has raised doubts on the use of such a thermodynamic treatment. The observed multiple-melting transitions for glycerol monostearate–amylose complex (Figs 4 & 5) at high $Na_2SO_4$ or sucrose concentrations also imply that melting of this material is far from equilibrium or even zero-entropy production melting conditions. Another approach to explain the elevation of the gelatinization temperature by sugars was recently suggested by Slade and Levine (1987). They have considered sugar/water solutions as plasticizing cosolvents that exert less plasticizing effect on the amorphous regions of starch than water alone. As such, they are less effective in depressing the glass-transition temperature of starch ($T_g$) than water. This, in turn, leads to an elevated $T_m$ for the crystallites; crystallite melting can commence only after exceeding the characteristic $T_g$ of the surrounding glassy matrix (Slade & Levine, 1984, 1987, 1988; Maurice *et al.*, 1985; Biliaderis *et al.*, 1986*a*).

In contrast to the results obtained with $Na_2SO_4$ and sucrose, the DSC thermal curves of forms I and II in the presence of urea (Fig. 6) and Gdn.HCl (Fig. 7) exhibited destabilization of the complex structure. The

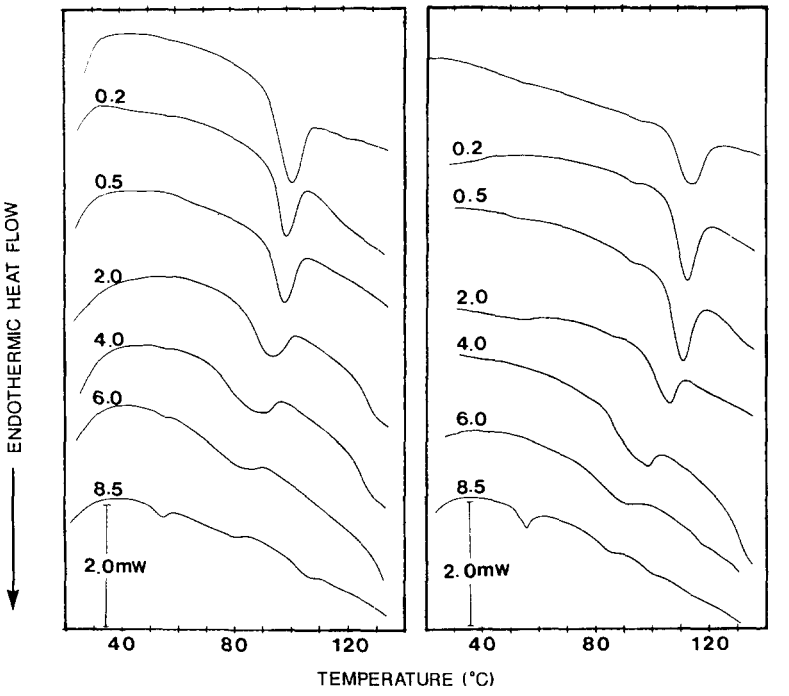


**Fig. 6.** DSC thermal curves of structural forms I (left) and II (right) of the complex (20% w/w) in urea solutions of varying molar concentrations. Mass of complex from top to bottom (mg): (left) 2·05 (control), 2·16, 2·04, 2·45, 2·42, 2·18, and 2·59; (right) 2·09 (control), 2·24, 2·11, 1·93, 2·06, 2·32, and 2·29. Heating rate 10°C/min.

$T_m$ progressively decreased, and the transitions became broader with increasing concentration of the denaturant. These effects were more pronounced at levels above 0·5 M for both agents. At concentrations above 6·0 M, a small endothermic transition at 55–58°C evolved, which corresponds to the melting of liberated monoglyceride, presumably owing to complete disruption of the helices. Urea is also known to reduce the gelatinization temperature of starch and to stabilize amylose in solution (Suggett, 1974); this behavior has been suggested to arise from effects on the structure of water. With respect to the order→disorder transition of the structural domains of forms I and II, there was no clear indication that a multi-step pathway is involved under the experimental conditions (heating rate and denaturant concentration) employed, although the melting endotherms did become less co-operative at high concentrations of urea or Gdn.HCl. It is likely that both dissociation of the supermolecular structure and helix-coil transitions occur simultaneously. In addition to $T_m$, there was also a progressive

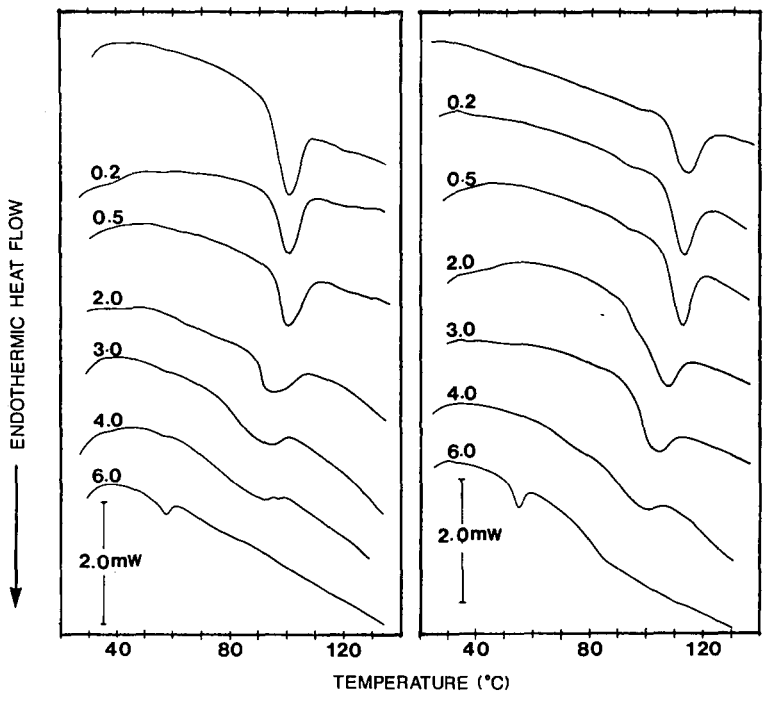


**Fig. 7.** DSC thermal curves of structural forms I (left) and II (right) of the complex (20% w/w) in guanidine hydrochlorite solutions of varying molar concentrations. Mass of complex from top to bottom (mg): (left) 2·05 (control), 2·31, 2·27, 1·99, 2·21, 2·31, and 2·21; (right) 2·09 (control), 2·05, 1·96, 1·93, 2·10, 1·96, and 2·45. Heating rate 10°C/min.

decrease in the melting enthalpy of the complex with increasing concentration of denaturant (Fig. 8). Changes in $\Delta H$ appeared more rapid in the case of Gdn.HCl than urea, particularly for form I. Interestingly, at low concentrations of Gdn.HCl ( $< 3{\cdot}0$ M), the destabilizing potential of guanidinium ion is different for form I from that for form II; i.e. the transition-enthalpy values of form II remained constant, whereas $\Delta H_I$ decreased continuously with increasing molar concentration. Thus Gdn.HCl is relatively ineffective toward the structure of form II. These findings reinforce the idea that there are differences in the supermolecular organization between the various forms of the complex. Urea effects, on the other hand, did not seem to distinguish between forms I and II.

To provide further insight into the structure of the complex, we have examined its thermal behavior in aqueous solutions of neutral salts by DSC. In these studies, in addition to forms I and II, we used an annealed sample of form II prepared by isothermal annealing (30% w/w complex in water, 120°C/2 h); to distinguish between these samples, the solution-

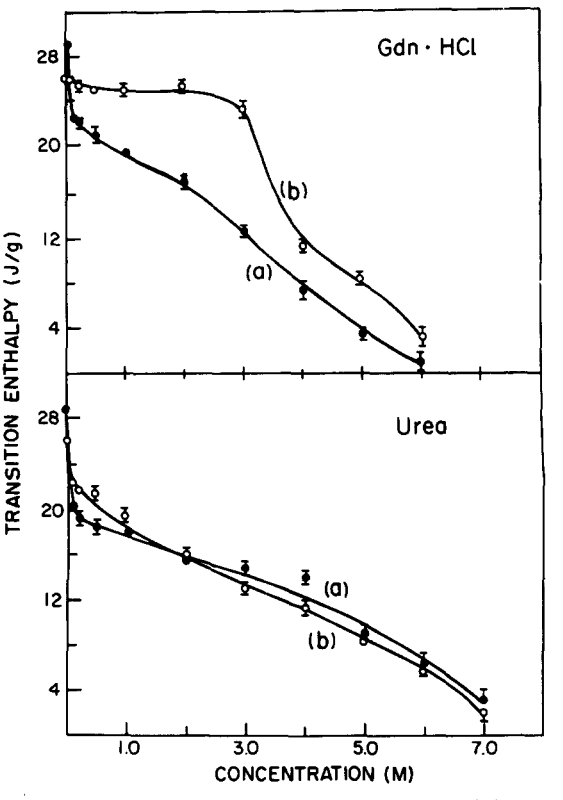


**Fig. 8.** Transition enthalpies of structural forms I(a) and II(b) of the complex with molar concentration of guanidine hydrochlorite (top) or urea (bottom).

crystallized form II is here designated as IIa and the annealed sample as IIb. The X-ray diffraction diagrams of forms I, IIa, and IIb along with the corresponding DSC thermal curves are shown in Fig. 9. The well-defined long-spacings for forms IIa and IIb suggest that only these materials have a partially crystalline structure. One can also notice that the intensity of the lines of V-amylose are enhanced significantly with annealing (compare Fig. 9(b) and (c)). The relative intensities of the diffraction lines at 20·1 $2\theta°$, taken as an index of long-range order, were: 1·0 (I), 8·6 (IIa), 15·2 (IIb). Despite the marked differences in their supermolecular structure, as evidenced by the DSC and X-ray data (Fig. 9), the transition enthalpies of all three forms were of a similar order of magnitude (26·4–29·2 J/g). These results indicate that the major contributor to $\Delta H$ is the enthalpy of helix → coil conformational transitions and thus differences in $T_m$ for the various forms are of entropic origin; $T_m = \Delta H/\Delta S$.

Among the various salts examined, only cesium chloride exhibited mild dissociating effects with respect to the complex superstructure. This

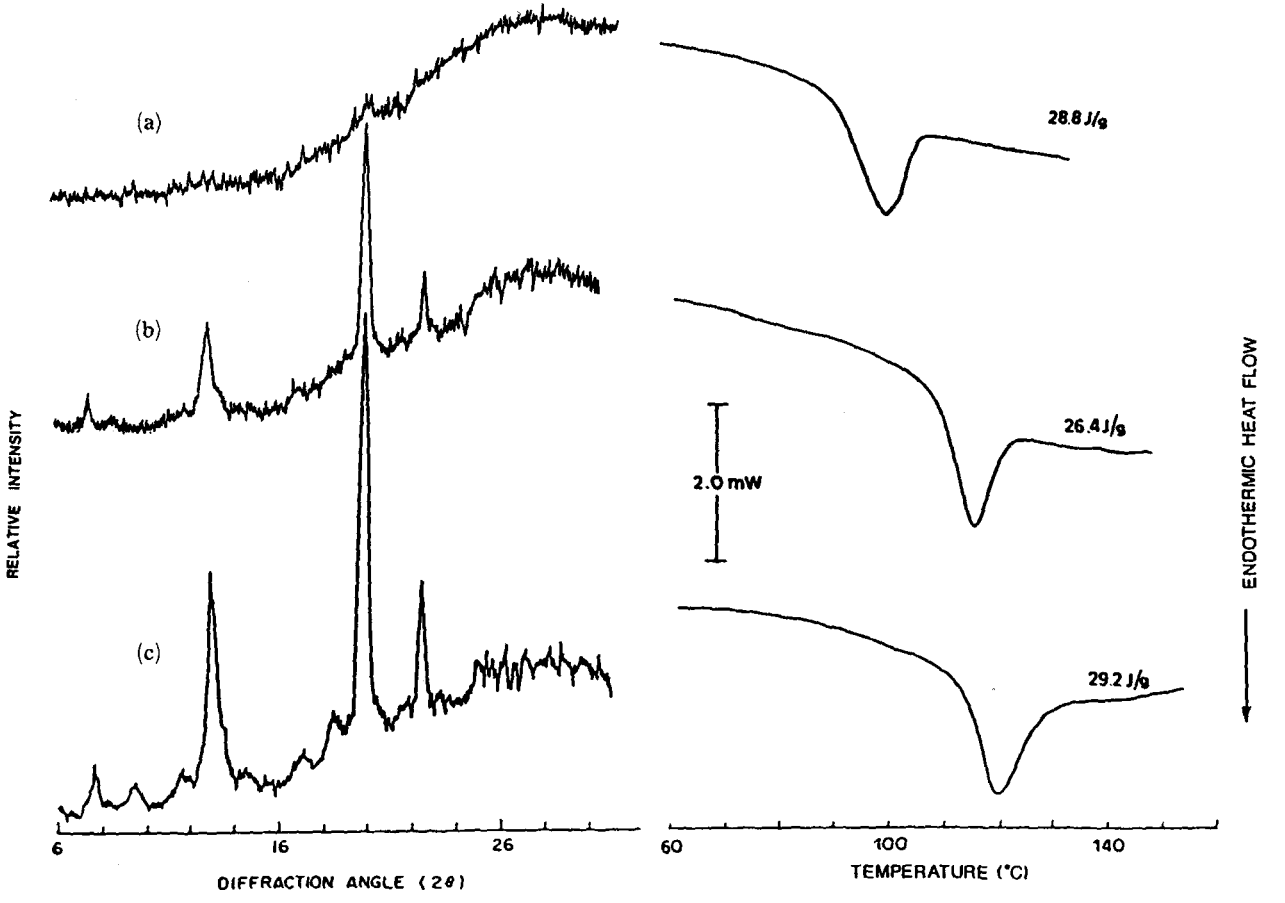


**Fig. 9.** X-ray diffraction patterns (left) of dehydrated freeze-dried samples and DSC thermal curves (20% w/w complex in water): (a) form I; (b) form IIa; (c) form IIb.

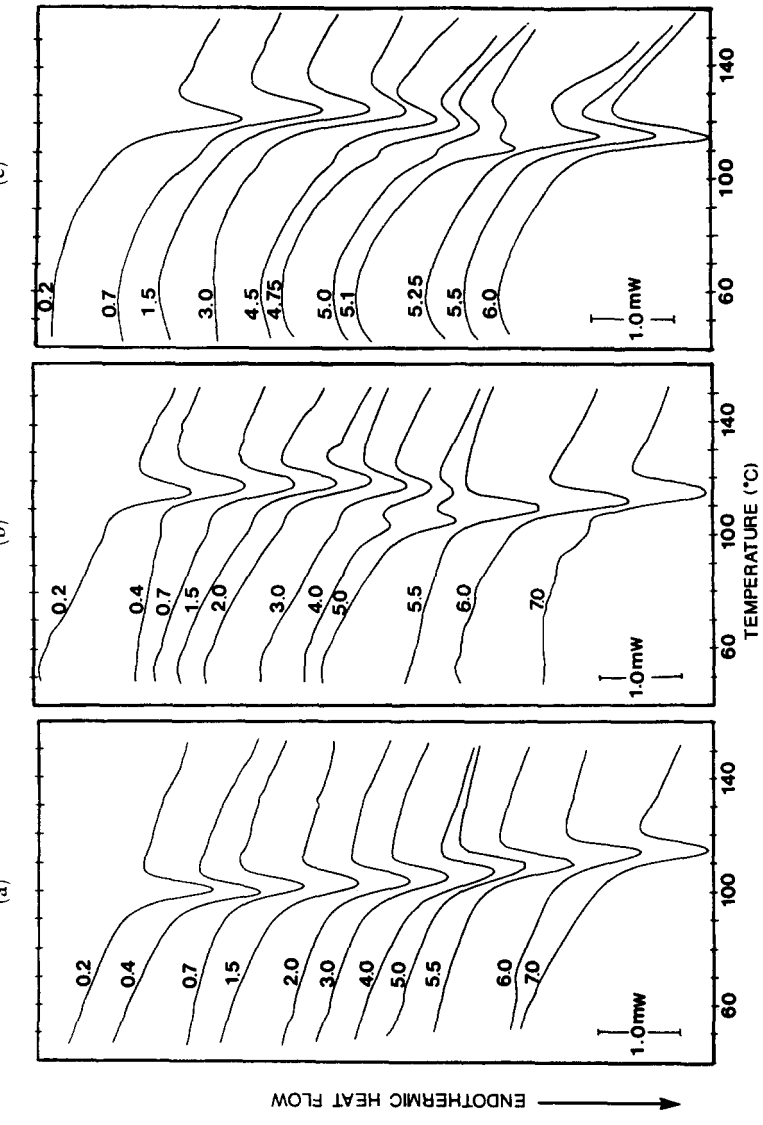


**Fig. 10.** DSC thermal curves of structural forms I (a), IIa (b), and IIb (c) of the complex (20% w/w) in cesium chloride solutions of varying molar concentrations. Mass of complex from top to bottom (mg): (left) 2·16, 2·08, 2·16, 2·14, 2·13, 2·09, 2·05, 2·33, 2·24, 2·06, and 1·99; (middle) 1·90, 2·07, 2·02, 1·91, 2·07, 2·03, 2·48, 1·92, 2·07, 2·14, and 2·13; (right) 2·27, 2·24, 2·34, 2·58, 2·08, 2·13, 2·08, 2·46, 2·15; 2·25, and 2·24. Heating rate 10°C/min.

salt, however, stabilized the structure of form I (Fig. 10(a)); $T_m$ increased with increasing molar concentration of CsCl (0·2–7·0 M CsCl). On the other hand, CsCl seemed to cause dissociation of the supermolecular structure of forms IIa and IIb at molar concentrations above 4·0 M and 5·0 M, respectively, as evidenced by the corresponding thermal profiles (Fig. 10(b) and (c)). In fact, at intermediate salt concentrations (4·0–5·1 M), double endotherms were observed that reflect the disordering process of forms I and IIa (or IIb). At much higher molar concentrations, single transitions were seen that correspond to the melting of form I at equivalent salt concentration. These results therefore suggest that CsCl promotes disaggregation of amylose–lipid helices whereas it maintains their conformational order. This notion was subsequently corroborated by the X-ray diffraction data of Fig. 11. With increasing concentration of

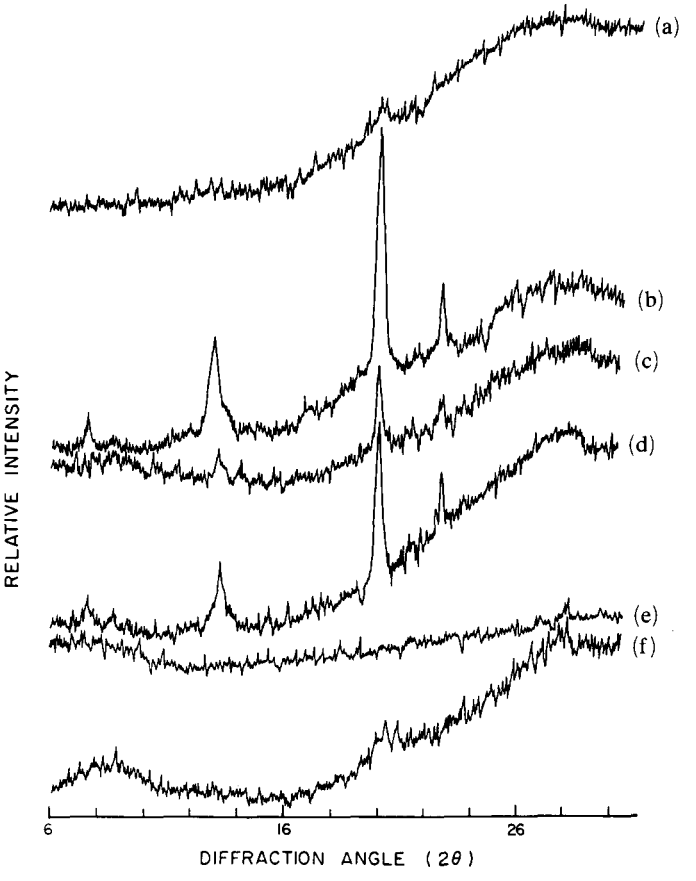


**Fig. 11.** X-ray-diffraction diagrams of wet glycerol monostearate–amylose complexes: (a) form I; (b) form II; (c) form II in 0·5 M CsCl; (d) sample c, washed; (e) form II in 6·0 M CsCl; (f) sample e, washed.

CsCl, form II shows diminishing intensities in the characteristic V-diffraction lines (Fig. 11(b), (c) and (e)). For the 0·5 M-treated sample, after washing out the salt, there was a recovery of the three-dimensional order in the structure (form II), as seen in the pattern of Fig. 11(d); the washed sample was also found to have DSC transition characteristics ($\Delta H$, $T_m$) indistinguishable from form II (data not shown). In contrast, exposing form II in a much stronger CsCl solution (6·0 M) yielded a structure that had the characteristics of form I (X-ray; Fig. 11(b), (e) and (f) and DSC data) before and even after thorough washing of the salt. Consequently, CsCl at high concentrations causes irreversible disruption of the partially crystalline supermolecular structure of the complex without altering, however, the conformation of individual helices. The transition enthalpies for all forms of the complex remained relatively constant (Fig. 12) over the entire concentration range (0·2–7·0 M CsCl), which provides additional evidence that the salt does not perturb the helical structure of the chains. In this context, it is also known that CsCl at high concentrations does not affect the H-bonding of DNA duplex (Erikson & Szybalski, 1964).

## CONCLUSIONS

As with synthetic polymers, whose mechanical behavior is determined by a number of structural parameters (molecular weight, branching, cross-linking, crystallinity and crystallite morphology, molecular orientation, and plasticization; Mandelkern, 1984), the thermomechanical properties of amylose–lipid complexes in bulk or in a composite starch-

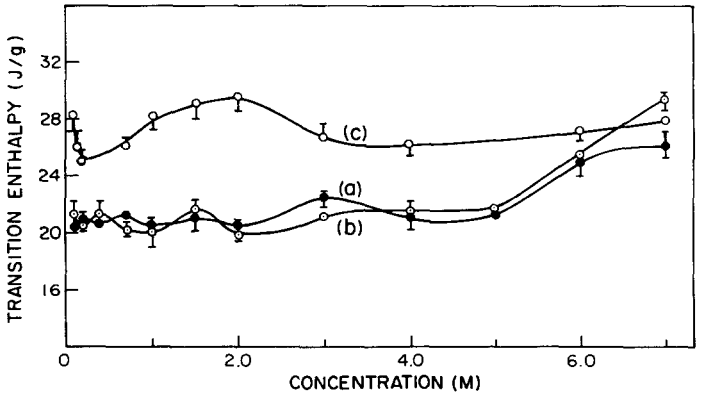


**Fig. 12.** Transition enthalpies of structural forms I (a), IIa (b), and IIb (c) of glycerol monostearate–amylose complexes with molar concentration of CsCl.

containing product would depend on their supermolecular organization, i.e. the way chains intermingle to give ordered and disordered domains in the bulk solid state. Such higher order of chain organization is extremely complex and rather difficult to describe morphologically, since the polymer chains can exist in various aggregated states, depending on the crystallization conditions, molecular constitution, and polymer polydispersity. Different structural forms of amylose–lipid complexes are now being recognized and need to be studied in a systematic manner if one is to establish relationships between properties and morphological features of such non-equilibrium states. The DSC, X-ray, and structural analysis data presented in this paper further support the proposal of earlier work that amylose–lipid complexes, in the solid state, exist mainly as two morphologically distinct supermolecular, metastable structures (Biliaderis & Galloway, 1989). Form I, a kinetically preferred polymorph since its $T_m$ does not vary, seems to consist of helical-chain segments of very little crystallographic register with one another. A diffused two-line V-diffraction pattern obtained for this form upon lyophilization (Fig. 2(b)) suggested the formation of a more ordered tertiary structure via chain aggregation during freezing. However, such limited inter-chain associations in the solid state were insufficient to cause changes in the thermal properties of form I. Instead, form I seems to be a separate thermodynamic state (between those of liquid/melt and classical crystals) that converts into form II only after partial melting of its structure; i.e. large energy barriers exist between forms I and II, which confer kinetic stability into the system. Form II, on the other hand, exhibits a well-defined three-line V-diffraction pattern, typical of this particular crystalline structure. Crystallites of this form can be perfected/enlarged via isothermal annealing. In dealing with transition dynamics among the various metastable forms of the complexes, as effected by temperature, ligand, and dissociating or stabilizing agents, one has to consider the different levels of structural order of these materials in the solid state (i.e. intramolecular and supermolecular structure). In this regard, the stability

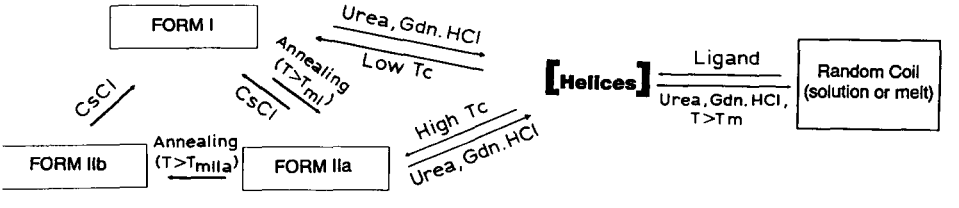


**Fig. 13.** Supermolecular-structure changes in the glycerol monostearate–amylose complex, as governed by temperature, ligand, and various stabilizing or destabilizing agents.

and interconversions in the aggregated state would depend on how the various molecular and environmental factors affect aggregation/dissociation of helices as well as helix-coil transitions, as schematically illustrated in Fig. 13.

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