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Solute effects on the thermal stability of glycerol monostearate–amylose complex superstructures.

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ABSTRACT

Thermally induced order–disorder transitions of two distinct structural forms of the glycerol monostearate–amylose complex (form I, Tm 99° and form II, Tm 117°) have been investigated by differential scanning calorimetry in various solvent environments. For neutral salts, the effectiveness of anions and cations in stabilizing or destabilizing the ordered chain domains of the complex followed, in general, the classical Hofmeister series. Thus, with Na^+ as the sole counterion, the Tm of the transition (at <1.0M) increased in the order of $SCN^- < I^- < NO_3^- < F^- < Cl^- < CH_3COO^- < SO_4^{2-}$. With Cl^- as the common anion, the ranking of the cations was $NH_4^+ < K^+ < Na^+ < Li^+ \leqslant Ca^{2+} \leqslant Mg^{2+}$. Interestingly, ranking of certain ions (*e.g.*, CH_3COO^-) differed between the two forms of the complex, particularly at high electrolyte concentrations. This implies that some solutes can act differently at various levels of supermolecular structure. Glucose and malto-oligosaccharides were effective stabilizers and resulted in non-equilibrium phase transition behaviour for the metastable superstructures of the complex (melting with reorganization during heating). These effects were proportional to the molecular weight and concentration of the small carbohydrate solute.

INTRODUCTION

The stability of macromolecular structures is a sensitive function of their solvent environment[1]. Following the pioneering work of Hofmeister, it is well established that neutral salts regulate order–disorder transitions and association–dissociation equilibria of biopolymers in solution (proteins, nucleic acids, etc.). In this context, there is a characteristic ranking of ionic effectiveness in promoting stability of macromolecular conformation known as the lyotropic or Hofmeister series. It is also known that these phenomena extend beyond the conformational behaviour of ordered macromolecules to salting out of non-electrolytes (small molecules), stability of lyotropic sols, as well as surface tension and kinetics of chemical reactions in solution. In view of this generality, it would appear that such effects are a consequence of solvent structure modification by various ions. However, specific interactions between macromolecules and ions can occur which, in turn, modify solvent–polymer interactions and thereby affect the stability of the ordered structure. For example, conformational ordering and aggregation of charged polysaccharides (*e.g.*, alginates and carrageenans) are sensitive to cation

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type[2-4]. Furthermore, in considering aggregated structures, salt effects may be more complicated than those affecting helix–coil transitions of macromolecules in solution. Intermolecular assosiations could influence the stability of ordered chains in the solid state and thereby modulate the sensitivity of a polymer matrix to solutions of electrolytes or other small molecular weight solutes.

The linear starch component, amylose, is precipitated from aqueous solutions of aliphatic compounds or iodine in the form of single-stranded helical complexes in which the guest molecule occupies the central cavity of the helix[5,6]. X-ray powder diffraction of amylose complexes gives rise to a characteristic pattern, designated as V, which has different d-spacings from the other two polymorphs of amylose, the A and B forms. The latter double-helical structures consist of more extended helices than the V-form: pitch height 21.04 and 20.80 Å for A- and B-forms, respectively, in comparison with 7.92–8.17 Å for the V-forms[7,8]. On the basis of electron and X-ray diffraction data[9-12], a lamellar morphology has been proposed for the organization of helices in V-amylose; *i.e.*, polysaccharide chains are folded and lie with their axes perpendicular to the crystal surfaces. From the Bragg equation, the calculated long spacings were 75–100 Å[9,10], while the enzyme structural analysis data of Jane and Robyt[13] suggested a lamellar thickness of $\sim$100 Å.

Recent calorimetric studies on phase-transition behaviour of amylose–lipid complexes[14-16] have indicated that V-amylose helices exist in various states of aggregation. Using saturated monoglycerides as complexing ligands, two thermally distinct forms (form I and II) were identified and characterized by X-ray diffraction, by calorimetry, and by enzymic structural analysis methods. A morphological model for these metastable forms has also been suggested[14]. Form I (low Tm), obtained under conditions favouring rapid nucleation, was described as an aggregated state where ordered polymer chains are distributed randomly (amorphous X-ray pattern). In contrast, form II (high Tm) appeared as a polycrystalline aggregate with well developed long-range order, giving the typical reflections of V-diffraction pattern. Our observations were recently confirmed by Whittam *et al.*[17] for complexes of amylose with linear alcohols (4–8 carbon atoms). Crystallization and annealing studies for amylose–monoglyceride complexes, carried out under controlled temperature–time storage protocols[14,16], further suggested that structural forms I and II belong to two distinct free-energy domains that are separated from each other by high-energy barriers; *i.e.*, conversion of the kinetically preferred form I of the complex to the thermodynamically favoured structure II (state of low free energy) occurs only after partial melting of the former. Understanding the supermolecular structure, stability, and transformations between the various forms is of considerable inportance since it is known that the functional properties of starch-containing foods are strongly influenced by the complexation of amylose with lipids during thermal processing. Our recent observations that sucrose stabilized both forms of the glycerol monostearate–amylose complex, while CsCl at high concentrations causes chain dissociation in form II without affecting the conformation of individual helices[16], prompted us to examine the effect of various solutes on the thermal stability of the complex. In this communication, we report on the interac-

tions between water and V-amylose superstructure as affected by the addition of neutral salts of the lyotropic series and the homologous glucose-based oligosaccharides (G_1–G_7).

EXPERIMENTAL

Materials. — Amylose, from potato starch with a d.p. of 1150 ([η] in N KOH 156 $mL.g^{-1}$), was obtained from Aldrich Chemical Co., while glycerol monostearate was a product of Sigma Chemical Co. Salts of ACS grade (CH_3COONa, NaCl, NaF, NaI, $NaNO_3$, Na_2SO_4, NaSCN, NH_4Cl, $CaCl_2$, $LiCl_2$, KCl and $MgCl_2$) were supplied by Fisher Scientific Co., while D-glucose and malto-oligosaccharides (G_2–G_7) were products of Boehringer Mannheim, Canada Ltd. (Dorval, Quebec).

Preparation of glycerol monostearate–amylose complexes. — The conditions for preparation of complexes were according to Biliaderis and Galloway[14]: amylose concentration 0.25% (w/v), amylose-to-ligand (glycerol monostearate) ratio 5:1, isothermal crystallization at 60° (Form I) and 90° (Form II). Complexes were washed repeatedly with $CHCl_3$ to remove the free ligand, as assessed by differential scanning calorimetry (d.s.c.). Samples used for X-ray analysis were kept in the hydrated state, while those intended for d.s.c. studies were freeze dried.

X-ray diffraction analysis. — Wet amylose-V complexes were deposited as 1-mm thick films on aluminium holders and analyzed with a Philips PW 1710 powder diffractometer equipped with a graphite monochromator: Cu K_α radiation, voltage 40 kV, sampling interval 0.45 s, scan speed 0.1 × $2\theta°s^{-1}$.

Differential scanning calorimetry (d.s.c.). — The d.s.c. studies were carried out using a 9900 Thermal Analyzer equipped with a DuPont 910 cell base and a pressure d.s.c. cell. The system was calibrated with In[18] and was operated under pressure by purging N_2 into the cell (1400 kPa). All measurements were carried out at 20% (w/v) solids in aqueous solutions of electrolytes and at a heating rate of $10° min^{-1}$. Under these conditions melting proceeds without reorganization of the metastable structures during heating[18] (*i.e.*, zero-entropy production melting). For the homologous glucose-based oligosaccharide series, d.s.c. was carried out at two different weight ratios of complex: sugar:water (2.0:1.6:6.4 and 1.0:2.0:2.0). Data were collected at 0.4-s intervals and analyzed by the DuPont software analysis programs; reported transition enthalpies ($J.g^{-1}$) and peak melting temperatures are means of triplicate analyses. Statistical differences between various solvent environments were determined, by analysis of variance, in conjunction with a Duncan's Multiple Range Test, and a paired T-Test.

RESULTS

The d.s.c. thermal curves (Fig. 1) of glycerol monostearate–amylose complexes grown isothermally at 60 and 90°, indicated that forms I (Tm 99.4 ± 0.3°) and II (Tm 116.6 ± 0.2°) were homogeneous preparations. The respective X-ray diffraction patterns of the two superstructures indicated that only form II has the three major

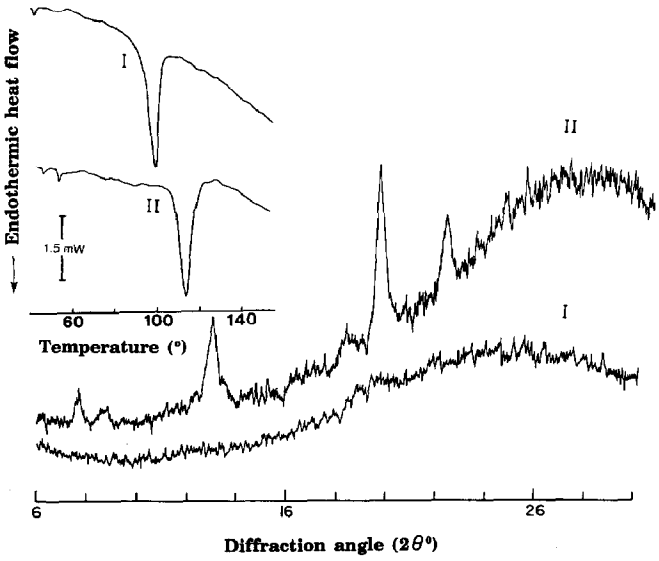

Fig. 1. D.s.c. thermal curves (20% w/w complex in water) and X-ray diffraction patterns of wet glycerol monostearate–amylose complex superstructures (forms I and II).

reflection peaks of V crystals at 7.36, 13.1, and 20.1 $2\theta°$. In contrast, an amorphous pattern was shown for form I. These results are consistent with the view that only form II has sufficiently developed long-range order in its structure, typical of a partially crystalline polymer[14].

The effect of neutral salts, with Na^+ as the sole counterion present, on the melting temperature of the complex superstructures is shown in Fig. 2 as a function of solute concentration. The relative ranking of the anions (at molar concentration of salt < 1.0) in stabilizing both structural forms was: $SCN^- < I^- < NO_3^- < F^- < Cl^- < CH_3COO^- < SO_4^{2-}$.

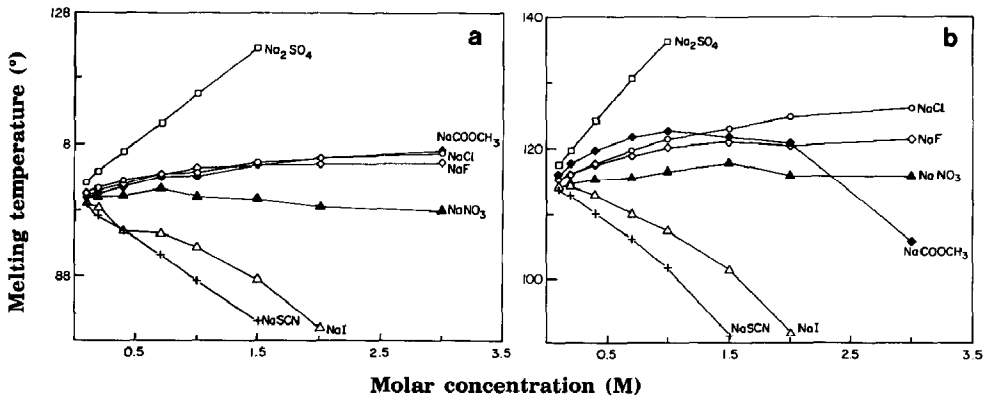

Fig. 2. Changes in the melting temperature (Tm) of glycerol monostearate–amylose complexes [form I(a) and II(b)] as a function of molar concentration (M) of various sodium salts.

This order followed closely the classical Hofmeister series that is operative for stabilizing/destabilizing macromolecules as diverse in structure and conformation as DNA, proteins, and synthetic polymers in aqueous solutions[1]. Thus, SO_4^{2-} is clearly an effective stabilizer, while SCN^- and I^- destabilize the structure of forms I and II, decreasing the Tm considerably; the elevation or depression of Tm was a linear function of salt concentration. Other salts (NaCl, $NaNO_3$, NaF) had minimal effect on Tm over the entire range of salt concentrations tested. For I^- and SCN^-, in addition to effects on Tm, these ions markedly decreased the magnitude of the transition enthalpy (Table I).

TABLE I

Transition enthalpies (ΔH, J. g^{-1}) of glycerol monostearate–amylose complexes (forms I and II) as a function of molar concentration (M) of various sodium salts[a]

Concentration (M)	*CH_3COONa*	*NaCl*	*NaF*	*NaI*	*$NaNO_3$*	*Na_2SO_4*	*NaSCN*
Form I							
0.1	21.6 ±0.3a	20.3 ±0.1a	20.3 ±0.2a	20.7 ±0.2a	20.7 ±0.7a	23.7 ±0.7a	19.8 ±0.4a
0.2	22.4 ±0.1a	21.6 ±0.4a	20.3 ±0.4a	19.9 ±0.1ab	21.4 ±0.9a	21.7 ±0.7a	17.4 ±0.5b
0.4	20.1 ±0.2ab	21.1 ±0.4a	22.9 ±0.1e	18.3 ±0.1c	20.2 ±0.2a	21.6 ±0.1a	17.0 ±0.3b
0.7	20.6 ±0.1b	20.7 ±0.1a	20.4 ±0.2e	18.5 ±0.3c	23.8 ±3.4bc	21.6 ±0.2a	16.5 ±0.2c
1.0	20.7 ±0.2b	19.5 ±0.7a	21.6 ±0.1de	16.8 ±0.8d	19.7 ±0.1a	NM	14.8 ±0.4d
1.5	21.3 ±0.8ab	19.8 ±0.3a	21.7 ±0.2e	14.6 ±0.2e	20.1 ±1.2a	NM	9.9 ±0.9e
2.0	23.1 ±1.3b	21.4 ±0.5a	20.6 ±0.1ab	8.8 ±0.9f	18.8 ±0.9a	NM	4.6 ±1.1f
3.0	26.7 ±0.4c	19.8 ±0.3a	21.4 ±0.1bc	—	18.9 ±0.2a	NM	—
Form II							
0.1	21.5 ±1.3a	22.4 ±0.1a	21.4 ±2.1a	21.3 ±0.2a	24.3 ±0.4a	22.1 ±0.1a	20.4 ±0.4a
0.2	22.9 ±0.2a	22.4 ±0.3a	22.9 ±0.1a	20.2 ±1.1ab	19.6 ±0.1a	21.9 ±3.5a	19.7 ±0.1ab
0.4	23.9 ±0.9a	21.4 ±0.2a	22.3 ±0.1a	19.3 ±1.2b	18.4 ±0.1a	21.9 ±1.1a	19.0 ±0.7b
0.7	21.3 ±0.7a	23.5 ±1.3a	24.2 ±0.4a	17.0 ±0.1b	18.4 ±1.6a	22.8 ±0.4a	13.6 ±0.3c
1.0	23.5 ±0.1a	23.6 ±0.6a	23.0 ±0.9a	15.5 ±0.1c	21.2 ±2.0a	NM	10.9 ±0.3d
1.5	22.1 ±1.1a	22.9 ±0.1a	22.4 ±0.4a	10.1 ±0.5d	18.1 ±0.6a	NM	4.2 ±0.6e
2.0	20.8 ±0.5a	21.6 ±1.3a	22.6 ±0.1a	4.7 ±0.3e	19.2 ±1.7a	NM	3.5 ±0.5f
3.0	23.8 ±0.6a	21.7 ±1.6a	22.5 ±0.9a	—	17.7 ±0.8a	NM	—

[a] Column values followed by the same letter are not significantly different ($P \leq 0.01$) as determined by the Duncan's Multiple Range Test; NM values not reported due to non-equilibrium melting.

At concentrations greater than 2.0M, NaSCN, and NaI completely disrupted the ordered domains of both forms of the complex as evidenced by the lack of an endothermic transition.

To verify that the conformational responses and therefore the d.s.c. transition data (ΔH, Tm), as described above, were not substantially influenced by the time of exposure of the complex superstructure to a particular ionic environment, the time dependence of the thermal properties of forms I and II were examined using the most potent destabilizers. Table II summarizes the values of the transition parameters for complexes equilibrated with varying NaSCN concentration solutions, for periods of 5

TABLE II

Time-dependent effects on the thermal properties of glyceryl monostearate–amylose complexes (forms I and II) in the presence of NaSCN[a]

Concentration (M)	*Form I*				*Form II*			
	Tm (°)		*ΔH (J.g^{-1})*		*Tm (°)*		*ΔH (J. g^{-1})*	
	5 (min)	*24 (h)*	*5 (min)*	*24 (h)*	*5 (min)*	*24 (h)*	*5 (min)*	*24 (h)*
0.4	95.3a ±0.1	95.4a ±0.3	19.5a ±0.3	19.6a ±0.3	110.1a ±0.1	109.9a ±0.1	19.5a ±0.1	18.3a ±0.1
1.0	87.2a ±0.1	87.9a ±0.4	14.3a ±0.1	13.4a ±0.1	101.6a ±0.5	101.1a ±0.4	10.9a ±0.2	10.7a ±0.4
1.5	82.8a ±0.1	84.3b ±0.1	9.0a ±0.2	4.8b ±0.5	91.2a ±0.5	93.8a ±0.4	3.9a ±0.5	2.2a ±0.2
2.0	72.9a ±0.1	71.2b ±0.	3.6a ±0.5	2.0a ±0.2	—	—	—	—

[a] Data followed by the same letter for each pair of values (5 min *vs.* 24 h) are not significantly different ($P \leqslant 0.001$), as determined by a paired T-Test.

min and 24 h, prior to d.s.c. analysis. In general, concentrations up to 1.5M did not result in significant differences in ΔH and Tm between the two storage regimes. Similar observations were made for complexes exposed to NaI, where no differences were observed up to 2.0M concentrations (data not shown). These results imply that conformational disordering of V-amylose is essentially independent of exposure time to the solvent at 25° and, instead, occurs mainly upon dynamic heating during d.s.c. analysis.

The thermal responses of forms I and II to the series of anions tested exhibited similar trends at low salt concentrations (Fig. 2). However, at high salt concentrations, anions which fell into the middle of the lyotropic series (NaCl, NaF, and $NaCO_2CH_3$) exerted different effects (relative magnitude and direction) on the complex superstructures. Thus, while the Tm–concentration relationships were clustered in the case of form I, the respective plots of form II were quite spread. It is also interesting to contrast the responses in Tm with molar concentration of CH_3CO_2Na between the two forms. Acetate was shown to stabilize form I over the entire concentration range, while it depressed Tm of form II at concentrations above 2.0M. It would appear from these data that certain anions act differently at various levels of structural organization of amylose–lipid complexes.

For aggregated states, which consist of ordered polymer chains (*e.g.*, amylose–lipid complexes) neutral salts, and other perturbants, are expected to affect structural order at two levels: one, the interchain assosiation–dissociation processes (supermolecular level), and the other, helix–coil transitions (molecular level). Figure 3 presents the thermal responses of both forms of the glycerol monostearate–amylose complex to varying molar concentration of NaI. With increasing concentration of this salt, there was a progressive decrease in Tm and enthalpy, as well as a broadening of the endotherm. There was no indication that a multistep pathway is involved in the order →

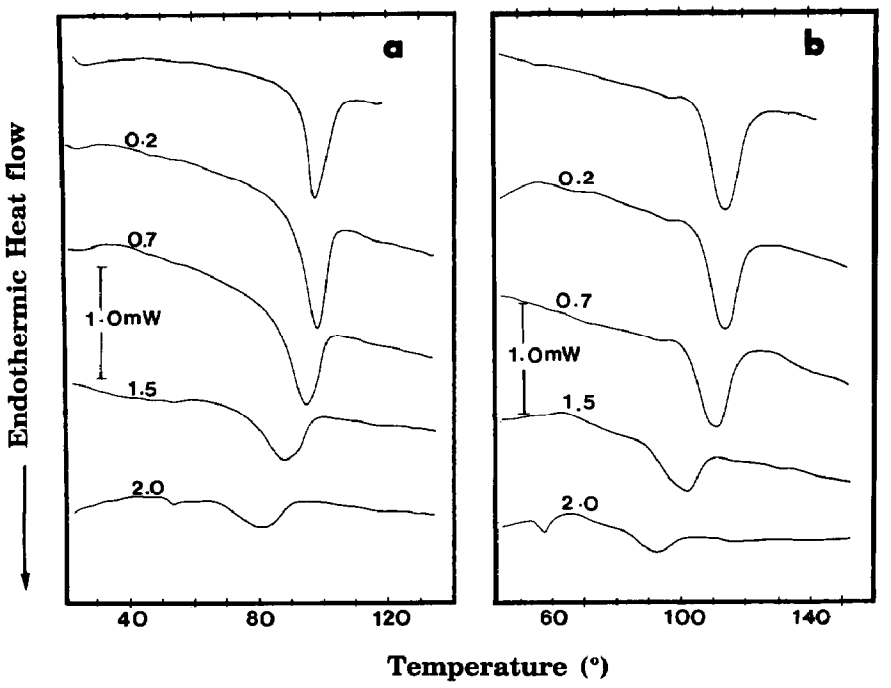

Fig. 3. D.s.c. thermal curves of structural forms I (a) and II (b), (20% w/w) in NaI solutions of various molar concentrations (0.2–2.0M). Mass of complex from top to bottom (mg): (a) 2.05 (control), 2.14, 2.13, 2.05, and 2.30; (b) 2.09 (control), 2.07, 1.99, 1.92, and 2.03. Heating rate 10°. min^{-1}.

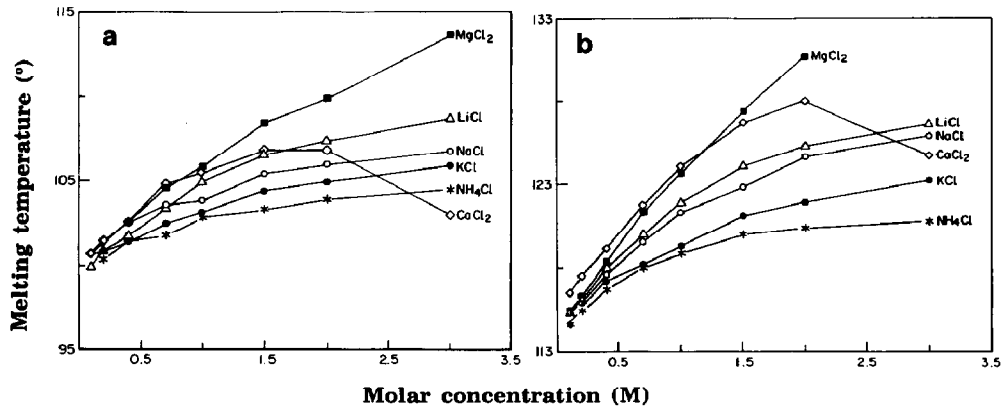

Fig. 4. Changes in the melting temperature (Tm) of glycerol monostearate–amylose complexes, form I (a) and II (b), as a function of molar concentration (M) of various salts with chloride as common counterion.

disorder transition of the structural domains of forms I and II. Under the experimental conditions employed in these studies (heating rate, solids:solvent ratio, electrolyte concentration), dissociation of aggregated helices and their conformational disordering seem to pake place as a one step thermal event. At 2.0M NaI, the low temperature (53–57°) endotherm corresponds to unbound monoglyceride liberated upon disruption of helices.

Cations also exerted stabilizing effects on both complexes, as evidenced by the positive relationships between Tm and salt concentrations (Fig. 4), using Cl^- as the common counterion. The ranking of the cations in terms of their ability to stabilize the complex at low concentrations (< 1.0M) was $NH_4^+ < K^+ < Na^+ < Li^+ \leqslant Ca^{2+} \leqslant Mg^{2+}$, a sequence that follows the Hofmeister series for cations. Values of Tm for the range of salts examined suggest that cations, across the Hofmeister series, differ less than anions in their molar effectiveness of stabilizing the complex superstructures. For $CaCl_2$, the Tm decreased markedly with increasing concentration of this salt at concentrations above 2.0M. Furthermore, form I exhibited higher sensitivity (dissociation) in the presence of 2.0M $CaCl_2$ than did form II.

The ΔH values of thermal dissociation of forms I and II remained relatively unchanged with the concentration of all neutral salts (Tables I and III), except for NaI and NaSCN. Although minor differences in ΔH were observed (at $P \leqslant 0.01$) among certain concentrations for some of the electrolytes, the enthalpy values did not display any specific trends. It should be also noted that enthalpy estimates for transitions at > 1.0M $NaSO_4$ were hampered due to non-equilibrium melting (Table I)[16]; *i.e.*, after partial melting, the complexes underwent reorganization during thermal analysis.

Glucose and the homologous series of malto-oligosaccharides stabilized the complexes at both concentrations (*e.g.*, Form I, Fig. 5); the effects were greater for the high ratio of sugar to water (Fig. 5b). These results agree with the findings of earlier reports[19–22], which showed that addition of sugar to starches increases their pasting and gelatinization temperatures. One consequence of this behaviour is inhibition of starch gelatinization and possibly restrictive effects on the conformational disordering of starch molecules during heating of high sugar content food formulations. At the high oligosaccharide-to-water ratio (1:1), the d.s.c. thermal profiles provided strong evidence of metastable melting (Fig. 5b). Thus, following melting of the original structure, a second high-temperature endotherm was observed when sugars were included. The magnitude of this transition increased with increasing molecular weight of the oligosaccharide. The well-defined exothermic effect, between the two endotherms for G_5, G_6, and G_7, is indicative of structural rearrangement of form I during heating in the calorimeter. This behaviour is typical of non-equilibrium macromolecular crystals and has been previously reported for metastable V-amylose structures heated in the presence of limited amounts of water[18,23]. Non-equilibrium melting was also apparent, although to a lesser extent, for Form II at 1:2:2 mixtures of complex:sugar:water (data not shown). Furthermore, there was a slight reduction in the apparent transition enthalpy with increasing molecular weight of malto-oligosaccharide which suggests that the sugars restricted the extent of conformational disordering of helices upon heating; *e.g.*,

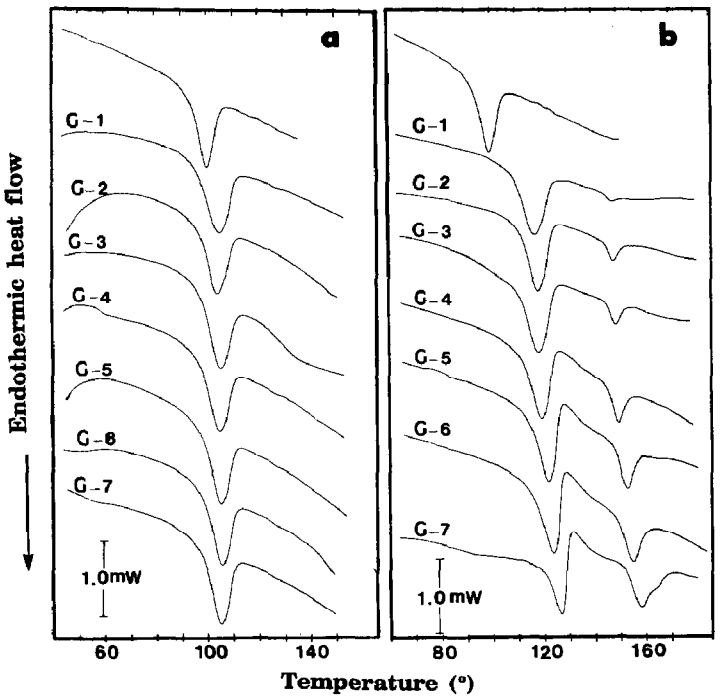

Fig. 5. D.s.c. thermal curves of glycerol monostearate–amylose complex (form I) at complex to sugar to water weight ratios of 2.0:1.6:6.4 (a) and 1.0:2.0:2.0 (b). G_1–G_7 denote the D-glucose residues in the oligosaccharide. Mass of complex from top to bottom (mg): (a) 2.05 (control), 2.30, 2.32, 2.20, 2.09, 2.11, 2.13, and 2.12; (b) 2.05 (control), 1.94, 1.97, 1.88, 1.91, 2.05, 1.86, and 1.96. Heating rate 10°. min^{-1}.

in the presence of G_1 and G_7, the respective values were 22.1 and 20.3 $J.g^{-1}$ for form I, and 23.8 and 20.2 $J.g^{-1}$ for form II.

DISCUSSION

The molecular and structural features of V-amylose with respect to chain conformation and unit-cell dimensions have been revealed by a large number of X-ray studies[5,6,11,12,24,25]. The main structural motifs of this polymorph are helical chain segments stabilized by Van der Waals forces, hydrophobic interactions, and hydrogen bonding (between adjacent anhydroglucose residues O-2–O-3′, and interturn H-bonds O-2–O-6 and O-3–O-6)[8]. However, despite the similarity in chain conformation, a detailed morphological description of V-amylose in the solid state, particularly as it forms under dynamic conditions of hydrothermal and mechanical processing of food materials, is difficult to produce since organization of helices with varying degree of randomness can yield different supermolecular structures. Chain packing (positional and orientational) thus becomes an important determinant of local structural order and is expected to have an imprint on various macroscopic properties of this polymer. From the d.s.c. and X-ray diffraction data of the present report and previous studies[14–16], we

infer that there is a relationship between thermal stability and structure of the two identified superstructures of amylose–monoglyceride complexes, forms I and II. While the thermodynamic and kinetic arguments for the existence of these forms have been presented in detail elsewhere[14], most of the structural data point to differences in the degree of localized order. Form II has sufficiently developed long-range order, typical of a partially crystalline structure and detectable by both d.s.c. and X-ray diffraction techniques. On the other hand, form I seems to consist of structural domains of short-range order which, although not detectable by X-ray due to their size and/or imperfections, do respond thermally in the calorimeter. The latter structures represent a molecular organization between those of a crystalline state and a "truly" amorphous (glassy) state. Additional evidence supporting the suggestion of localized order in form I was obtained by freeze drying the wet complexes, where the reflection lines at $2\theta^\circ$ 13.1, and 20.1 began to be visible[16]; displacement and alignment of chains upon freezing improves the three-dimensional order of the aggregated helices without affecting their d.s.c. thermal responses.

Changes in solvent quality by addition of neutral salts affected the conformational stability of both complex superstructures. In general, the effects of salts on the properties of glycerol monostearate–amylose complexes were a strong function of the ionic species present (type and concentration) and followed the lyotropic series. With Na^+ as common counterion, "chaotropic" co-anions of high Hofmeister number, such as I^- and SCN^-, destabilized the structure (Tm was markedly shifted toward lower values and transition enthalpy was reduced with increasing salt concentration), whereas anions of low Hofmeister number, such as SO_4^{2-}, enhanced the stability of V-complex superstructures. Although the phenomenology of lyotropic series on biopolymer conformational stability is known, the origin of these effects still remains obscure[26–28]. There are indications, however, that these ubiquitous effects are a manifestation of changes in water structure[1,26] and that preferential binding of ions on macromolecules, as frequently reported in protein literature[29–31], may modulate the nature and balance of intra- and inter-molecular forces responsible for the stability of ordered states as well as the preferential hydration of the macromolecule. According to the work of Timasheff and co-workers[30,31] on proteins, stabilizing or salting-out effects on macromolecules are characterized by a large preferential hydration of the polymer, whereas extensive binding of the solute to macromolecule is frequently observed for those agents having structural perturbing effects.

In general, the order of effectiveness of various anions and cations (<0.1M) in displacing the Tm of the order–disorder transition of forms I and II resembles those for the effect of ions on pasting, gelatinization, solubilization, and retrogradation of starch[32,33]. The destabilizing anions SCN^- and I^- progressively decreased the Tm and increased the breadth of the transition of both forms without any evidence for interconversion between the two aggregated states, as was found in the presence of CsCl (4.0–5.0M)[16]. Although most structural perturbants exerted similar effects on both supermolecular structures, sodium acetate at high molar concentration (>2.0M) exhibited destabilizing action on form II, while it stabilized form I. These findings suggested

that certain ions may affect differently the forces which influence the association–disassociation of helices than those involved in helix stabilization and thereby alter the thermal stability of the complex, depending on its chain organization in the aggregated state. In contrast to the stabilizing action of most cations (Fig. 4) throughout the range of salt concentrations examined, $CaCl_2$ beyond the dilute salt level (> 2.0M) lowered the transition temperature of forms I and II. This salt is also known to cause disruption of starch granules at room temperature (2.5–3.0M)[34,35] and has proven a potent destabilizer of native protein conformations[27,30,31]. At much higher concentrations (> 5.0M), $CaCl_2$ solutions increased again the transition temperature of the complexes (data not shown), in agreement with the findings of Evans and Haisman[35] on wheat starch.

With the exception of NaSCN and NaI, the results of transition enthalpy of the complex in various solvent environments (Table I, III) indicated that there were no wide differences between the two superstructures and over a wide range of electrolyte concentrations examined (0.1–2.0M). The enthalpy values of forms I and II in water were 21.2 $\pm$ 0.6 and 22.2 $\pm$ 0.8 $J.g^{-1}$, respectively. The slightly higher transition enthalpy of form II was maintained for almost all ionic environments. The process of complex dissociation involved the disruption of various stabilizing forces operating at both molecular and supermolecular level by heating in an aqueous medium. Energy is, therefore, required to dissociate the aggregated chains (*i.e.*, to overcome intermolecular H-bonding between adjacent helices) as well as to disrupt the ordered conformation of chains. From the enthalpy data presented in Tables I and II, and in the light of the postulated structural morphologies of forms I and II (ref. 14), it would appear that melting enthalpies of amylose–monoglyceride complexes represent mainly the energy for helix $\rightarrow$ coil transitions and that contributions from chain dissociation are minimal. Recent calorimetric data of Whittam *et al.*[17] indicate higher enthalpy values for crystalline than "amorphous" complexes (1.58 *vs.* 0.90 $J.g^{-1}$). The differences were attributed to a substantial contribution from intermolecular interactions in the case of crystalline complexes. However, the reported values were at least one order of magnitude lower than those of our studies as well as of other workers[36,37]. Variations in estimated enthalpy values could arise from the different thermal analysis systems used and their calibration, the crystallization conditions (temperature, ligand type), and purity of the amylose. Such magnitude of difference in ΔH needs to be resolved first before any conclusions can be made on the relative contributions of intra- and interchain interactions to the overall transition enthalpy.

Glucose and malto-oligosaccharides (G_2–G_7) raised the transition temperatures of both V-complex superstructures (Fig. 5). This is in accord with earlier calorimetric studies which indicated that sugars and polyhydroxy organic compounds elevate the gelatinization temperature of starch[19,21,22,35,38]. Although the stabilizing effects of sugars on starch gelatinization are of considerable importance in processing of high-sugar-content bakery items, the exact mechanism by which these solutes influence the transition behaviour of starch is unclear. A number of different explanations have been advanced, including competition for water, lowering of water activity, and interactions of sugars with the amorphous parts of the granule[38,39]. However, it has been difficult to

TABLE III

Transition enthalpies (ΔH, J. g^{-1}) of glycerol monostearate–amylose complexes (forms I and II) as a function of molar concentration (M) of various salts with chloride as common counterion[a]

Concentration (M)	*NH_4Cl*	*$CaCl_2$*	*LiCl*	*$MgCl_2$*	*KCl*
Form I					
0.1	21.3 ±0.2a	21.7 ±0.1ab	20.8 ±0.1a	20.0 ±0.3a	20.4 ±1.2a
0.2	21.2 ±0.1a	21.6 ±0.3ab	19.7 ±0.4a	20.3 ±0.1ab	20.2 ±1.0a
0.4	20.6 ±0.1b	18.4 ±1.2bc	20.9 ±0.8a	20.6 ±0.4ab	21.7 ±0.2a
0.7	20.5 ±0.2b	20.1 ±0.1ac	20.4 ±0.1a	20.4 ±0.1abcd	21.4 ±0.2a
1.0	20.6 ±0.3a	20.1 ±0.4a	18.2 ±0.7a	21.5 ±0.1bc	21.2 ±0.3a
1.5	20.5 ±0.1ab	20.5 ±0.2a	19.1 ±0.3a	21.3 ±0.1bc	21.2 ±0.6a
2.0	20.5 ±0.3b	20.0 ±0.9ab	19.7 ±0.8a	21.9 ±0.1c	20.5 ±0.1a
3.0	17.2 ±0.2c	21.6 ±0.1a	21.5 ±0.2a	23.1 ±1.4d	18.9 ±1.9a
Form II					
0.1	22.8 ±1.6a	21.8 ±0.1ab	21.9 ±0.3a	22.1 ±0.3a	22.3 ±0.1a
0.2	22.7 ±0.6a	21.8 ±0.2ab	21.9 ±1.3a	23.7 ±0.6bc	21.7 ±1.2a
0.4	21.9 ±0.6a	22.5 ±2.9b	22.1 ±0.3a	23.3 ±0.3c	21.7 ±0.5a
0.7	21.8 ±0.6a	21.9 ±0.1ab	21.3 ±1.6a	23.4 ±0.3bc	21.0 ±0.4a
1.0	22.1 ±0.1a	21.9 ±0.9ab	21.9 ±1.2a	23.5 ±0.3bc	22.4 ±0.1a
1.5	22.3 ±0.1a	22.9 ±0.6b	24.4 ±0.1a	24.3 ±0.3c	21.1 ±0.4a
2.0	22.4 ±0.4a	23.2 ±0.6b	23.2 ±1.0a	24.0 ±0.1dc	21.8 ±0.8a
3.0	22.1 ±0.1a	17.5 ±0.4a	22.5 ±0.2a	23.6 ±0.1dc	21.1 ±0.3a

[a] Column values followed by the same letter are not significantly different ($P \leq 0.01$) as determined by the Duncan's Multiple Range Test.

rationalize all the results on the basis of a particular theory. In view of the partially crystalline character of granular starch, Lelievre[40] had applied a thermodynamic treatment of the melting data of starch–solute–water ternary mixtures using an extended version of the Flory[41] equation which relates the Tm of a polymer to the diluent concentration. Aside from the simplifying assumptions made with regard to the magnitude of interaction coefficients between the three components (polymer–solute–solvent), this theoretical framework is based on the assumption of equilibrium states and processes, which usually are not valid in the case of heated aqueous starch systems (see discussion below). A similar relationship between Tm, water activity and volume fraction of water in the granules derived by Evans and Haisman[35] from the Flory theory also suffers the same drawback.

By analogy with partially crystalline synthetic polymers, van der Berg[42] has first suggested that a glass–rubber transition must precede the melting of starch crystallites and provided a theoretical analysis for the dependence of this transition on the moisture content of starch. This view was later supported by calorimetric data[43–46]. The d.s.c. thermal curves thus indicated that melting is controlled by the mobility of the amorphous material surrounding the crystallites; *i.e.*, melting can proceed only after exceeding the characteristic glass transition temperature (Tg) of the glassy regions of the granule. It has been also shown[46,47] that Tg is highly sensitive to water/heat plasticiza-

tion; *i.e.*, Tg of dry starch is greatly depressed by small amounts of water (plasticizer). In this context, starch gelatinization was described as a non-equilibrium process since melting of crystallites is kinetically constrained by the immobile glass at temperatures below Tg[43–46]. Furthermore, within the Tm–Tg range, where molecular mobility of the amorphous chain segments is enhanced, composite thermal effects due to partial melting of metastable crystallites (endothermic), reorganization (exothermic), and final melting of more stable crystallites have been reported for heated aqueous starch systems[18,23,46]. The thermal curves in Fig. 5b clearly indicate that such events also occur for V-amylose complexes in the presence of low molecular weight carbohydrates. The reason why V-amylose superstructures undergo reorganization during heating lies in the metastable (non-equilibrium) nature of their ordered domains. Following partial melting of the less stable helices, reorganization is favoured since the remaining helical chain segments can act as nuclei, and the temperature is still below the melting point of the equilibrium crystals. Reorganization and formation of regularly packed arrays of helices yield a state of much lower free energy (*i.e.* thermodynamically more stable).

An alternative approach has been taken by Slade and Levine[45] to explain the elevation of starch gelatinization by sugars. According to these workers, a water–sugar mixture is a plasticizing cosolvent which is less effective in depressing the Tg of starch relative to water alone (*i.e.* a sugar–water mixture exerts an antiplasticizing action). In view of the molecular weight dependence of Tg for polymeric materials[48], they further suggested that antiplasticization is enhanced by increasing the molecular weight and concentration of the solute. Their experimental data for the effects of homologous series of sugars, present as cosolvents, confirmed this prediction. As the mol. wt. of the cosolvent increases, the cosolvent becomes less efficient in suppressing the Tg of

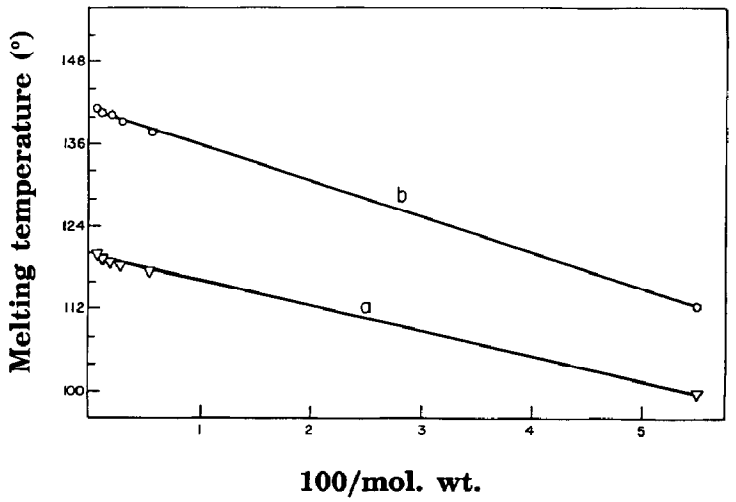

Fig. 6. Transition temperature (Tm) as a function of 100/mol.wt. of cosolvent (water + D-glucose oligomer) for three-component mixtures of glycerol monostearate–amylose:sugar:water (1.0:2.0:2.0 parts by weight): (a), form I; (b), form II.

granular starch and the Tm of crystallites is raised. Using these insights, we have tested their hypotheis for the glycerol monostearate–amylose superstructures. As shown in Fig. 6, the mol. wt. of the water–malto-oligosaccharide cosolvents was indeed inversely related to the melting temperature of the complex. These results add further support to the argument that polyhydroxy compounds influence the melting of starch structures by elevating the Tg of the respective amorphous phase, which in turn causes the melting events to commence at a higher temperature.

CONCLUSIONS

The d.s.c. data presented herein indicate that small solutes have a pronounced impact on the stability and conformational responses of V-amylose. In considering the phase-transition dynamics of this material, as affected by temperature and solvent environment, one has to take into account the various levels of structural order in the solid state, such as secondary and supermolecular structure. Using salt or oligosaccharides, it may be possible to develop processing protocols (temperature–time, water content–solute concentration) that would foster the formation of a particular superstructure of this polymer and thereby control its properties in processed food systems. The physical state and behaviour of amylose in starch-containing foods is of great technological importance because of their role in imparting functionality of starch, *e.g.*, staling of baked items, stickiness of pasta, solubility of extruded cereal products, thermomechanical stability of processed grains (*e.g.*, parboiled rice).

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