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Some factors determining the thermal properties of amylose inclusion complexes with fatty acids

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Abstract

The thermal properties of water-insoluble amylose–stearic acid (18:0) complexes prepared under various conditions were studied by differential scanning calorimetry (DSC). Complexes were studied normally at a concentration of 5% in water at pH ~ 7. Type I complexes formed at ≤ 60°C had dissociation temperatures (T_m) in the range 96–104°C. Type IIa polymorphs formed at ≥ 90°C had T_m = 114–121°C. Various ratios of types I and IIa were formed at 80°C depending on the duration of heating, but no intermediate form was detected. Annealing of the type IIa complex at 105°C and at 115°C gave rise to increasing proportions of type IIb polymorphs with T_m = 121–125°C and dissociation enthalpies of 32–34 J/g of amylose, depending on the temperature and time of annealing. Conversion into the higher polymorphs was retarded at a higher concentration (10%) of the complex under identical conditions, and was delayed at pH ~ 4.7. The dissociation temperatures of amylose complexes with the cis-unsaturated fatty acids oleic (18:1), linoleic (18:2), and linolenic (18:3) also depended on the temperature of formation, and three distinct types were obtained (I, IIa, and IIb). Significant decreases in the T_m of the three polymorphs were observed for each double bond in the fatty acid guest molecule. When type I and type II complexes were made using various proportions of 18:0 and 18:2, mixed acid complexes were obtained with T_m values intermediate between those of the monoacid complexes. The origin of the endothermic transitions on heating the three types of complexes is discussed.

Keywords: Amylose; Fatty acids; Inclusion complexes; Thermal properties

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1. Introduction

It is widely accepted that amylose–lipid complexes have a helical structure, with the linear chain of the guest lipid situated along the central axis of the helical cavity. In complexes with monoacyl lipids each helical turn consists of six glucosyl residues (V_6 helix) [1–8], but seven (V_7) [5,9–12] and eight (V_8) [13] glucosyl residues are possible with bulky guest molecules.

In food systems, complexes with monoglycerides, free fatty acids, lysophospholipids and surfactants are of interest because they can affect the functional properties of the products [14–17]. Numerous studies of amylose–glyceryl monostearate complexes by DSC have revealed the existence of polymorphs which have characteristic dissociation temperatures [18–22]. These complexes are readily formed as insoluble precipitates in neutral aqueous media. In contrast, insoluble complexes with fatty acids are formed only at pH below 7 and in the presence of electrolytes [23]. In cereal starches there are small quantities of naturally occurring lipids which may be exclusively lysophospholipids, or lysophospholipids with similar amounts of free fatty acids [24]. It has been shown recently that these monoacyl lipids occur as inclusion complexes with a fraction of the amylose in the native starch granule [25,26]. Free fatty acids are also much more common components of food lipids than monoglycerides and, therefore, are more likely to form complexes with amylose if conditions are suitable. Comparatively little is known about the properties of these complexes in processed foods, although there is some evidence for the existence of polymorphic forms [19,27,28].

The present work was initiated to elucidate the conditions which govern the formation of polymorphs of amylose complexed with fatty acids, both saturated and unsaturated, in view of the reported diversity of their thermal properties [29–33] which has been tentatively attributed to the ionic nature of the ligand. Further work on interactions between ligand molecules and the amylose helix, using a range of physicochemical methods, is currently in progress.

2. Experimental

Materials.—Potato amylose and fatty acids were obtained from Sigma. The amylose had a beta-amylolysis limit of 94% and was found, by gel permeation chromatography, to be essentially free of amylopectin. Amyloglucosidase (AMG) was obtained from Boehringer-Mannheim (no. 208469, activity 6 units/mg, from *Aspergillus niger*). All other reagents were of analytical grade. Methods for preparing solutions of amylose from the amylose–butanol complex, and solutions of fatty acids for complexing, are given in [23].

Formation of the initial neutral complexes with amylose.—Amylose solution (40 mL, 15 mg/mL of 0.01 M KOH) and a solution of potassium stearate (60 mL, 1 mg/mL of 0.01 M KOH) pre-heated above the Krafft point to 80°C (i.e., until clear again) were mixed, and neutralized with 10 mL of 0.1 M HCl. The complex was then precipitated by adding a few drops of 2 M HCl to pH $\sim$ 4.7. The mixture was then held at 60°C or at 90°C for 24 h. Complexes with unsaturated fatty acids were prepared similarly at 80°C

Table 1
Thermal properties of amylose–stearic acid complexes prepared at a concentration of 5% in water, pH ~ 7

Complexing		ΔH_m	T_m of polymorphs (°C)		
Temperature (°C)	Time	(J/g)	I	IIa	IIb
60	10 min	17.8 [a]	99.2		
	30 min	23.5 [a]	99.1		
	1 h	20.5 [a]	99.3		
	2 h	18.3 [a]	98.3		
	5 h	18.5 [a]	99.8		
	24 h	27.6	99.3		
80	10 min	7.0 + 6.8 [a]	99.6	118.4	
	1 h	6.7 + 18.0 [a]	101.6	118.5	
	2 h	5.5 + 17.8 [a]	101.8	118.9	
	5 h	7.8 + 17.1 [a]	102.6	118.1	
	24 h	28.2		118.0	
90	30 min	15.6 [a]		118.6	
	1 h	17.7 [a]		118.3	
	2 h	18.8 [a]		118.3	
	5 h	21.4 [a]		118.9	
	24 h	26.2 [a]		119.4	
105	50 h	33.1			123.4
115	10 min [b]	24.0 [a]		117.7	
	30 min [b]	29.9 [a]		118.5	
	1 h [b]	14.3 [a]			121.5
	24 h [b]	34.2			125.5

[a] Free fatty acid melting endotherm indicates that enthalpies must be low (see text).
[b] Complex formed at 90°C (24 h) annealed at 115°C for given times.

and held at ambient temperature for 24 h after neutralization. All samples were then centrifuged at 2000*g* for 15 min. The supernatant solution was discarded and the precipitate was resuspended in water and centrifuged as before. This was repeated twice more to obtain a salt-free neutral complex. The complexes were then freeze-dried and passed through a fine sieve.

Dissociating of neutral complexes, followed by complexing and annealing.—Dried amylose–stearic acid complexes were suspended in water (pH ~ 7) to give concentrations of 5 or 10% in tubes provided with screw-caps. In one experiment (Table 1) the water was adjusted to pH ~ 4.7 with 0.1 M HCl. They were then dissociated at 150°C for 30 min, and subsequently held at 60, 80, and 90°C, respectively, for the desired period of time to allow complexing. Samples heated for 24 h at 90°C were further annealed, in separate portions, at 105 and 115°C for different periods of time. After cooling and centrifuging (2000*g* for 15 min) the wet gel-like residues were used directly for DSC. Complexes (concentration 5% in water) with unsaturated fatty acids were dissociated at 135°C for 30 min and protected from oxidation under N_2. They were then recomplexed at 37, 60, 70, 80, or 90°C for 24 h. The gel-like residues were used for DSC.

Complexing in the presence of electrolytes.—Dried neutral complexes were suspended in 0.1 M citrate buffer (pH 6.8) or 0.2 M phosphate buffer (pH 7.05) at a

concentration of 5% in conical flasks provided with screw-caps. The samples were then dissociated at 150°C for 30 min and subsequently held at 90°C for 24 h. After cooling and centrifuging (2000 *g* for 25 min), the wet residue was used for DSC.

Differential scanning calorimetry (DSC).—A DuPont DSC 9900 system was used with a 910 cell base. Dry samples (2–3 mg) were weighed accurately into coated DuPont pans and water equal to 6 times the weight of the sample was added with a syringe. The contents were mixed with a needle before sealing the pans. With wet samples, aliquots of 10–12 mg of gel were weighed into the pan. The concentration of the complex in the pan was in the range of 10–20%. A pan containing water or glycerol was used as a reference. Samples were heated at 10°C/min. The mean enthalpy of dissociation (ΔH_m) of at least three determinations was expressed as J/g of amylose.

Determination of amylose in the DSC pans.—On completion of the DSC scan, amylose was determined by placing the pans, previously opened with pliers, in screw-cap tubes containing Me_2SO (1 mL). The tightly closed tubes were immersed in boiling water for 1 h with occasional vortex mixing, they were then cooled to room temperature, and water (3 mL) was added. Aliquots (1 mL) were then mixed with 1 mL of AMG solution (1 mg/mL citrate buffer, 0.2 M, pH 4.7) and incubated at 60°C for 30 min. Water (8 mL) was added to each tube and 1-mL aliquots were taken for the determination of glucose as described elsewhere [34] (amylose = glucose × 0.9).

Determination of fatty acids.—Fatty acids were converted into methyl esters, in the presence of a suitable internal standard, for quantification by gas chromatography [35]. The methanolysis reagent was MeOH containing 10% of H_2SO_4 (v/v) instead of 14% of BF_3 (w/v), as originally described [35], and samples (10 mg) were heated under N_2 in a boiling water bath for 30 min to obtain quantitative yields.

3. Results

Formation of the initial amylose–stearic acid complex polymorphs at pH ~ 4.7.—Preparing complexes of amylose with fatty acids in warm alkaline solution has distinct advantages because the soluble monomeric potassium salt interacts immediately with the amylose, and there is no risk of forming double-helical retrograded amylose (which does not form inclusion complexes). The complex is recovered by precipitating at pH ≤ 7. Aggregation and precipitation are more efficient at pH ~ 4.7, especially in the presence of a buffer such as 0.1 M citrate [23] (which is also required for assays using AMG) [34]. In the present study the pH was adjusted with 2 M HCl, and electrolytes were later removed by washing. When the quantity of fatty acid used under these conditions does not exceed the stoichiometric amount, there is negligible uncomplexed acid. The neutral complexes gave consistent starting material which could be dissociated and recomplexed under a wide range of conditions that would be difficult to reproduce in any other way.

At pH 4.7, a form dissociating over a low range of temperature (type I, T_m ~ 98°C) was obtained at 60°C. At 90°C, two endotherms were observed with a considerably higher range of dissociation temperatures (type IIa, T_m ~ 106°C; type IIb, T_m ~ 120°C) characterized by Biliaderis et al. [18,20,21] as features of the corresponding type II monoglyceride complexes. Annealing at 115°C gave two overlapping endotherms (T_m

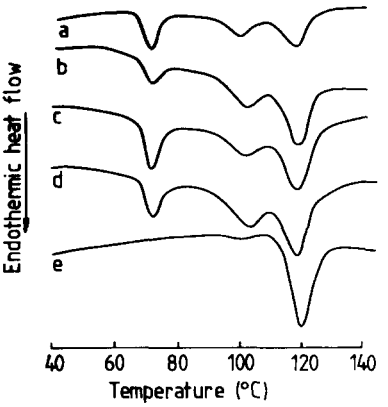

Fig. 1. DSC thermograms of amylose–stearic acid complexes (pH ~ 7) formed at 80°C (a) for 10 min; (b) 1 h; (c) 2 h; (d) 5 h; and (e) 24 h.

~ 118 and 123°C) and a considerable amount of free stearic acid (mp 69°C). Thus, the rearrangement from the low-melting to the high-melting forms was very slow at pH ~ 4.7, and the large melting endotherm of free stearic indicated that there had been some dissociation of the complex at 115°C.

Formation of amylose–stearic acid complexes and annealing at pH ~ 7.—In contrast, samples formed (from dissociated neutral complexes) in water at pH ~ 7 for 24 h gave well-defined endotherms. The type I complex was formed at 60°C (T_m ~ 94–98°C) and the type IIa variant (T_m ~ 118°C) was obtained at 80 and 90°C, all with negligible amounts of free fatty acid. On annealing at 115°C a form with an even higher T_m (~ 123°C) was obtained, similar to that described by Biliaderis and Seneviratne [21] as type IIb for the glyceryl monostearate complex. Higher polymorphs (T_m ~ 122–124°C) of the complex were favoured in the presence of electrolytes at pH ~ 7.

Intermediate stages of complex formation were studied using samples incubated for shorter periods of time. Complexes made at 60°C gave single type I (T_m ~ 99°C) endotherms which became narrower with longer complexing times, suggesting improved homogeneity of the aggregates, and there were small melting endotherms for free stearic acid. At 80°C there was simultaneous formation of type I (T_m ~ 102°C) and type II (T_m ~ 118°C) complexes within 10 min, and there was a gradual increase in the proportion of type II with time (Fig. 1), until type II predominated after 24 h (Fig. 1e). There was a significant melting endotherm for free stearic acid up to 5 h, but very little in the 24-h sample. Complexes formed at 90°C were exclusively type IIa only (with traces of free stearic acid), even at the early stages of formation. When the sample complexed for 24 h was annealed at 115°C no change was observed after 30 min, then there was slow conversion from type IIa into type IIb (T_m ~ 121–125°C) over the next 24 h.

The results reported so far refer to amylose–stearic acid complexes formed at a concentration of 5%. When the concentration of the complex was increased to 10% polymorphic transitions were slightly different. Holding at 60°C for 1, 5, or 24 h to allow complexing gave a single relatively broad endotherm of type I complex (T_m ~ 98°C), and a considerable melting endotherm for free stearic acid (Fig. 2, a–c).

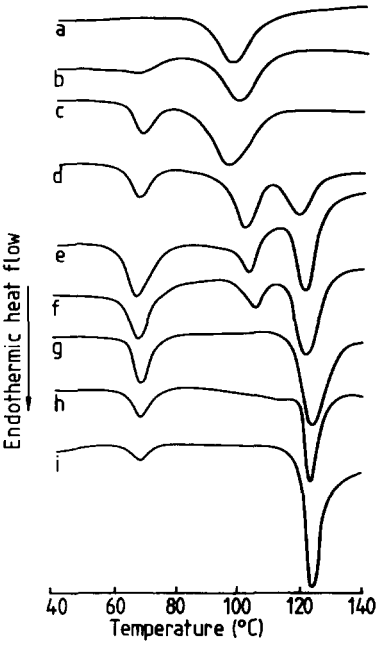

Fig. 2. DSC thermograms of amylose–stearic acid complexes (pH ~ 7) at 10% concentration formed after (a) 1 h at 60°C; (b) 5 h at 60°C; (c) 24 h at 60°C; (d) 24 h at 80°C; (e) 5 h at 90°C; (f) 24 h at 90°C; also complex (f) annealed for (g) 50 h at 105°C; (h) 5 h at 115°C; and (i) 24 h at 115°C.

Holding at 80°C for 24 h and at 90°C for 5 or 24 h resulted in a more homogeneous type I endotherm with a very high T_m (~ 102°C); there were also increasing proportions of the type IIa endotherm (T_m ~ 119–121°C) and the free stearic acid endotherm (Fig. 2, d–f). This implies that complexing and crystallizing as the type IIa polymorph was very slow under these conditions. Further annealing (105°C, 80 h; 115°C, 5 or 24 h) of the sample formed at 90°C gave rise to type IIb complexes with narrow endotherms (T_m 123–124°C), and progressively less free stearic acid (Fig. 2, g–i). Annealing of type IIa to type IIb was clearly less efficient at 10% than at 5% concentration of complex.

The thermal properties of amylose–stearic acid complexes at 5% and at 10% concentration are summarized in Tables 1 and 2. Enthalpy values (ΔH_m) of the type I complex increased with annealing time at 60°C to a maximum value of ~ 28 J/g amylose. Where types I and IIa coexisted, their combined enthalpies showed a similar trend (Table 1, annealed at 80°C), as did the pure type IIa complex (Table 1, annealed at 90°C). This has been observed before with fatty acid [33] and monoglyceride complexes [18]. In experiments where a stearic acid melting endotherm was found, it is implicit that there must have been some free amylose (probably amorphous) as well as complexed amylose. Since amorphous amylose gives no endotherm on DSC, and retrograded amylose (which may form under special circumstances [29]) dissociates at 140–165°C, enthalpies calculated on the basis of total amylose content (or dry weight, as commonly reported by others) must be low [33]. Nevertheless, the data clearly indicate a maximum

Table 2
Thermal properties of amylose–stearic acid complexes prepared at a concentration of 10% in water, pH ~ 7

Complexing		ΔH_m	T_m of polymorphs (°C)		
Temperature (°C)	Time (h)	(J/g)	I	IIa	IIb
60	1	22.4	98.0		
	5	23.9 [a]	98.8		
	24	28.5 [a]	96.0		
80	24	15.4 + 11.7 [a]	101.9 [b]	119.5	
90	5	8.0 + 24.4 [a]	104.1 [b]	——————121.8——————	
	24	3.9 + 24.5 [a]		——————122.1——————	
105	50	32.8 [a]			123.5
115	5	1.5 + 22.0 [a]	————113.9 [c]		123.5
	24	32.8 [a]			124.5

[a] Free fatty acid melting endotherm indicates that enthalpies must be low (see text).
[b] Considered to be an abnormal type I value (see text).
[c] A minor intermediate type, possibly transforming to IIa.

ΔH_m for the type IIb complex of at least 32 J/g, significantly higher than for the IIa complex annealed for comparable times.

Effect of fatty acid unsaturation.—The three common 18-carbon unsaturated fatty acids — oleic (18:1), linoleic (18:2), and linolenic (18:3) — were used to study the effect of cis-unsaturation in the acyl chain on the properties of the complexes. Docosahexaenoic acid (22:6) was included as the most highly unsaturated fatty acid available. In every case, type I, IIa, and IIb complexes were obtained by preparing the complexes at temperatures approximately 20–30°C below T_m (Table 3).

For the type I complex, each additional double bond lowered T_m (relative to the stearic acid complex) by 9.8, 4.7, and 3.2°C, respectively. The corresponding values for the type IIa complex were 10.9, 7.5, and 7.3°C, and for the type IIb complex they were 12.7, 9.5, and 8.2°C. These results are in agreement with previous results for similar complexes prepared under mild conditions [33] (which would be type I) and at higher temperatures [29,31] (which appear to have been type II).

In the case of the docosahexaenoic acid complex, there would be an increase in T_m values due to the longer acyl chain, offset by decreases for the double bonds. T_m of the saturated (22:0) complex was ~ 105°C for the type I complex and 119°C for the type IIa complex; hence the effect of six double bonds was to lower T_m by ~ 30 and ~ 23°C, respectively. Since the three double bonds in linolenate lowered T_m by 18, 25, and 30°C for the three polymorphs, it seems that there is very little effect from more than three double bonds. This can also be seen in the T_m values for complexes made with the series of 20:0 to 20:4 fatty acids [31].

The dissociation enthalpies of the unsaturated fatty acid complexes must be accepted with caution because the presence of free fatty acid (implying free amylose) could not be detected by DSC since the free fatty acids were liquid at ambient temperature. The data in Table 3 certainly show that many ΔH_m values comparable with those of representative stearic acid complexes (Tables 1 and 2) are possible, and a maximum value of 32 J/g for oleic acid type IIa complex seems likely. Although the stoichiometry

Table 3
Thermal properties of amylose complexes with stearic acid and with unsaturated fatty acids [a] recomplexed at various temperatures for 24 h, pH ~ 7

Complexing temperature (°C)	ΔH_m (J/g)	T_m of polymorphs (°C)		
		I	IIa	IIb
Stearic acid				
60	28.5	96.0		
80	28.2		118.0	
90	26.2		119.4	
115	34.2			125.5
Oleic acid				
37		86.2		
70	31.9		106.0	
80	22.7		108.7	
90	25.6			112.8
Linoleic acid				
37	22.5	81.5		
60	22.6		99.0	
70	23.0		100.8	
80	24.1			103.3
Linolenic acid				
37	18.2	78.3		
60	27.0		92.6	
70	28.0			95.1
Docosahexaenoic acid				
37	27.5	70.2		
60	27.9		92.5	
70	21.1			96.3

[a] All cis-unsaturated.

of the complexes was not determined, these results show that the unsaturated acids can complex as well as the corresponding saturated acids under suitable conditions.

In cereal starches some of the amylose is complexed with free fatty acids and lysophospholipids in which there are ca. 40% saturated acids and 60% cis-unsaturated acids (principally 18:2). It was therefore of interest to ascertain whether amylose would form monoacid complexes or mixed-acid complexes when presented with stearate and linoleate together in various proportions. Monoacid complexes would give two distinct endotherms (T_m ~ 98 and 82°C for type I, ~ 118 and 100°C for type IIa), while mixed-acid complexes would be expected to give only intermediate values.

When mixed-acid complexes were made at ambient temperature and pH ~ 4.7 with the theoretical amounts of total fatty acids, excellent yields were obtained and the proportions of the two acids in the complexes were close to the initial values, indicating no selective complexation of either acid. On DSC the complexes gave broad type I endotherms (Table 4) with T_m values intermediate between 18:0 and 18:2 type I complexes (Table 3). There was no trace of separate peaks or shoulders due to monoacid (i.e., pure 18:0 or 18:2) complexes, although this was the pattern when monoacid complexes were mixed in various proportions prior to DSC. The ΔH_m values of the

Table 4
Properties and composition of complexes made with mixtures of stearic (18:0) and linoleic (18:2) acids at ambient temperature and at 80°C for 24 h, pH ~ 4.7

18:0/18:2 ratio		Type I		Type IIa	
Initial	Recovered	T_m (°C)	ΔH_m (J/g) [b]	T_m (°C)	ΔH_m (J/g) [b]
At ambient temperature					
0/100	0/100	87.0	29.0		
25/75	27/73	87.3	29.7		
50/50	47/53	92.0	30.5		
75/25	70/30	96.0	27.6		
100/0	100/0	98.6	33.8		
At 80°C					
0/100	0/100			111.9	22.8
25/75	57/43	97.6	15.7	115.6	13.0
50/50	85/15	100.3	17.7	116.9	5.9
75/25	96/4	99.0	21.4	116.7	3.6
100/0	100/0	100.3	29.6		

[a] Ratio determined by GLC. Ambient complexes contained ca. 8.3 g fatty acids/100 g amylose (corrected for small amounts of uncomplexed fatty acids), while 80°C complexes contained 4.9–6.9 g fatty acids/100 g amylose.
[b] Enthalpies corrected for uncomplexed amylose in complexes made at 80°C.

complexes were similar to the highest values in Tables 1 and 2, showing efficient complex formation at all proportions of the two fatty acids.

When complexes were made at pH ~ 4.7 and held at 80°C for 24 h, close to T_m for the type I 18:2 complex, recoveries of the complexes were only ca. 70% (estimated from uncorrected ΔH_m values and from total fatty acid contents) which suggests that there had been significant dissociation. Since the proportion of 18:0 in the complexes was much higher than in the original mixtures of free acids, it is possible that type I helical segments containing 18:2 dissociated preferentially with negligible recomplexing, or that there was non-selective dissociation of mixed-acid complexes followed by preferential recomplexing with free stearic acid.

On DSC, the mixed-acid complexes made at 80°C gave well-resolved double endotherms. The T_m values were slightly higher than those of complexes made at ambient temperature (allowing for the various proportions of fatty acids) as would be expected from the results in Tables 1 and 2. Similarly, the T_m values were well above T_m for the pure type I 18:0 complex. In practice, the complex containing 47% 18:0 and 53% 18:2 had a T_m expected for a mixed-acid complex, while the other mixed-acid complexes (containing 85 and 96% 18:0) were essentially type IIa stearate complexes. The total ΔH_m values of the complexes were generally low due to inefficient complex formation that was not fully corrected by the method of calculation.

4. Discussion

Polymorphic transitions.—The results presented in this paper show that amylose inclusion complexes with C-18 saturated and cis-unsaturated fatty acids can exist in

polymorphic forms that closely resemble those described for the amylose complex with glyceryl monostearate [18] and other monoglycerides [18–22]. We have therefore used the same nomenclature as for the polymorphs of amylose complexed with saturated monoglycerides.

When complexes are made with monoglycerides, a neutral pH is used to avoid hydrolysis of glyceryl ester bonds, and the complex precipitates spontaneously, indicating a strong tendency to form interhelical hydrogen bonds. If the ligand is a long-chain fatty acid it is convenient to make the complex at pH $\sim$ 12 so that it is soluble, but the complex then stays in solution. When the pH is lowered to $\sim$ 7, the solution becomes turbid but precipitation is slow unless the pH is lowered further to $\sim$ 4.7. Precipitation can also be promoted by using such buffers as citrate. These observations suggest that ionization of the carboxyl group must be suppressed to allow flocculation of microaggregates and promotion of interhelical hydrogen bonding. Significant heterogeneity, implying variable degrees of aggregation and limited ordering of helices in type I polymorphs, is indicated by the variable breadths of DSC endotherms, and by the ranges of values reported for T_m and ΔH_m of fatty acid complexes (Tables 1–3 and [29–33]).

Conversion of type I into type IIa polymorphs probably requires partial 'melting' or disaggregation to obtain sufficient chain mobility for crystallite formation [18]. However, partial dissociation and re-formation of complexes should also be considered, even although amylose–fatty acid complexes have very low dissociation constants at ambient temperature [36].

Conversion of type IIa into IIb seems to be a classical annealing process which requires partial melting of crystallites followed by recrystallization [18], and it was evidently favoured (this study) by using more dilute suspensions of complex. Under the conditions used here some dissociation at intermediate annealing times was indicated by the presence of free stearic acid (Figs. 1a–e, 2d–f) and depressed enthalpy values.

Biliaderis and Galloway [18] proposed a simple generalized mechanism for monoglyceride complex formation from dilute solutions. At lower temperatures, formation of the metastable type I polymorph is the favoured process, determined mainly by the kinetics of complex formation, nucleation, and 'freezing' of helical-chain segments in fairly random positions. At higher temperatures, nucleation is slower and it progresses as a conventional crystallization process. In the present study at pH $\sim$ 7 the lower temperature system prevailed at $\leq$ 60°C, and the higher temperature system at $\geq$ 90°C; while both systems operated at 80°C.

Complexes of amylose with cis-unsaturated fatty acids.—Several early reports [37–41] claim that cis-unsaturated fatty acids complex poorly with amylose, giving low yields and enthalpies of dissociation. This has been attributed to inefficient complexing by the fatty acid which is depicted as non-linear or kinked due to the cis-double bond. However, reasonably efficient complexing was obtained in the present study (Table 3), and others have obtained similar results with unsaturated free fatty acids and monoglycerides [23,31,33,37,39]. This suggests that free rotation about C–C bonds adjacent to C=C bonds allows the unsaturated fatty acids to adopt a quasi-linear conformation around the double bond, which would give the chain a greater diameter than a saturated chain. If this is correct, the amylose helix might need to be expanded from six glucosyl

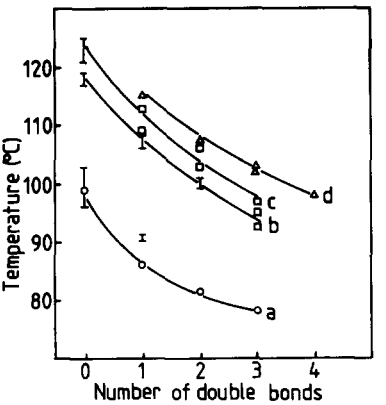

Fig. 3. Dependence of the dissociation temperature of amylose complexes on the number of cis-double bonds in the guest fatty acid: (a) type I complexes of C-18 acids; (b) type IIa complexes of C-18 acids; (c) type IIb complexes of C-18 acids; (d) type II complexes of C-20 acids. Data from present study (a,b), [33] (a), and [31] (c,d).

residues per turn to seven in order to accommodate the unsaturated portion of the acyl chain, as happens with other bulky ligand molecules [1,5,9–12,42,43].

There are two consequences of cis-unsaturation in the fatty acid guest molecule, both consistent with the above hypothesis. The first is that the stoichiometry of the complex is not greatly affected [23] by the postulated proportions of V_6 and V_7 helices. Hence, a similar number of hydrogen bonds and van der Waals interactions will be involved in stabilizing the helix, and the maximum dissociation enthalpies of complexes containing saturated and unsaturated fatty acids should be similar (Table 3 and [31,33]). The second consequence is a systematic lowering of T_m for all three types of complex with increasing number of double bonds (Table 3). Explanations for this effect are discussed in the following section.

The effects of cis-unsaturation on T_m reported in the literature can now be summarized better, knowing that the fatty acid complexes described by Raphaelides and Karkalas [33] were made at 25°C and were thus type I, while those reported by Morrison [31] were incubated for several hours at > 90°C and were therefore type IIa or IIb. Their results and values from Table 3 are combined in Fig. 3. This shows that the well-known effect of chain length (to increase T_m) is retained in fatty acids with the same number of double bonds, and is ca. 6°C per two carbon atoms. The curves also show that the decrease in T_m for each additional double bond is less, so that the plots are not linear. This can also be seen in the T_m values for complexes with 18:0–18:3 monoglycerides [27,37]. With the 20:3 fatty acid complexes [31], the position of the polyunsaturated system in the chain (3 carbons or 6 carbons from the methyl terminus) also affects T_m.

Origin of the endothermic transition.—The primary endothermic event measured by DSC when complexes are heated is generally attributed to the disordering of the complexes (V-helix → coil transition) rather than melting of crystallites [21,22,33], although doubt has been expressed that there is actual dissociation of the complex [44,45]. Results obtained in this study, and by others [29,33], show that dissociation into free ligand and free amylose must occur.

To clarify the probable nature of the transitions, it is necessary to consider the structure of amylose–lipid complexes. The basic unit of both type I and type II complexes is a single-chain helical segment of variable length (enclosing the ligand), normally with six glucosyl residues per turn (the V_6 helix). The V-helix is stabilized by intramolecular hydrogen bonds between glucosyl O-2 $\cdots$ O-3(2) and O-2 $\cdots$ O-6(7) [7,46]. In the unit cell of the crystalline hydrate [7], there are several molecules of water in the interstices between helices hydrogen-bonded to glucosyl O-2,3,5, and 6; there are also numerous intra- and inter-molecular van der Waals contacts. Computer modelling of a fatty acid docked inside the helix suggests that there is only one possible van der Waals contact, between each glucosyl H-5 and a CH_2 group in the fatty acid [8].

The length of a helical segment is probably determined by the length of the lipid molecule within the helix, and by the number of molecules that lie end-to-end. In practice, the stoichiometry of the helical segments and of the complexes (weight basis) is almost independent of the size of the enclosed lipid guest molecule [33]. It is thought that V-helical complex segments are interrupted by short sections of uncomplexed amylose that permit random orientation of the helical segments in type I complexes, and folding into parallel and antiparallel arrays in the microcrystalline type II complexes [18,23,47]. When a type I complex is heated, the endothermic events could be described as disaggregation of helices and chains, and dissociation of helices into free amylose and lipid; melting is only important in type II complexes where there are substantial crystallites. Nevertheless, it is convenient to use the symbols T_m and ΔH_m to characterize all endotherms, as in the recent literature.

It has been suggested that T_m is determined by the conformational entropy of the complex [18,27], which in turn will depend on the state of the polysaccharide chains and the ligand. Thus, the rapid nature of the initial complexation and aggregation of amylose chains at lower temperatures gives a relatively constant T_m for diverse lipid ligands, and transformation to more stable polymorphs lowers the entropy of the amylose chains further with corresponding increases in T_m. However, the contribution of the ligand cannot be ignored. Since there are limited van der Waals contacts between a fatty acid and the helix, the major stabilizing force must be the lower entropy of the fatty acid in the complex compared with the free state, with small differences being inversely related to fatty acid chain length. Introduction of cis double bonds into a fatty acid contained within a helix might cause some strain in the glycosidic bond, which is reflected in the lower T_m values described in this paper.

Enthalpic changes, which can be attributed to the energy required to disorder and dissociate helical complexes, and to melt crystallites in type II complexes, should be amenable to quantitative study. However, precise values are often not available because, as noted above, some samples may contain a proportion of free amylose and their enthalpies will be artificially low [33], or because polymorphs are in an intermediate polymorphic state. Also, two studies of the amylose–LPC complex [48,49] show that reheating and dissociating of the stable polymorph at higher temperatures, followed by cooling to allow complex formation, can give significantly higher ΔH_m values with little change in T_m. Thus, ΔH_m of amylose–lipid complexes can never be regarded as a precise measurement.

If a complex containing a suitable lipid ligand is dissociated in a DSC heating cycle

and is then cooled rapidly, a complex will be re-formed at a temperature ca. 10–20°C below T_m. Complexes are not formed efficiently under these conditions when the ligand is unsaturated, or when it is saturated and crystallizes rapidly, but ligands that are soluble or have a high critical micelle concentration will form complexes efficiently as shown by an exothermic heat of formation equal to ΔH_m.

It is suggested that ΔH_m of type I complexes is determined by the energy required to rupture intrahelical hydrogen bonds which stabilize consecutive helices along the axis of the complex. Only a very small amount of energy would be required to overcome the van der Waals contacts which stabilize the ligand within the essentially hydrophobic inner surface of the spirally arranged glucosyl residues. The energy of dissociation of the two intramolecular hydrogen bonds per glucosyl residue (cf. [7]) will be ~ 230 J/g amylose, while the van der Waals energy is estimated to be 4–5 J/g amylose. Since ΔH_m for type I complexes is ~ 27 J/g amylose, it would appear that only a small proportion of the hydrogen bonds are broken (as in the melting of ice where breaking of $\sim 15\%$ of the bonds induces the phase change), or that exothermic formation of new hydrogen bonds between amylose in solution and water largely offsets the endothermic changes.

It has been claimed that ΔH_m values are of the same order for all three types of complex (with glyceryl monostearate) [20,21]. In the present study ΔH_m of type IIb stearic acid complexes was 32.24 ± 0.66 J/g ($n = 4$), significantly greater than for the lower polymorphs (27.54 ± 0.95 J/g, $n = 6$). The difference (~ 4.7 J/g) is most probably attributable to the net dissociation energy of the numerous van der Waals contacts between helices in crystallites and limited interhelical hydrogen bonding as described by Rappenecker and Zugenmaier [7]. As noted above, variations in ΔH_m with the size of the guest molecule should be small for lipid-saturated complexes, since they have nearly identical stoichiometry (amylose: fatty acid weight ratio) [23,33] and, therefore, nearly the same number of intrahelical H-bonds and van der Waals contacts per glucosyl residue [7].

5. Conclusions

Inclusion complexes of amylose with saturated and cis-unsaturated fatty acids can be made, comparable with the complexes of amylose with saturated monoglycerides. The two principal types of polymorph can exhibit various degrees of heterogeneity (shown by a range of T_m values), and more than one form can occur in a sample, all depending on conditions for their preparation. Heterogeneity of polymorphs is a property of all polymers, but in this study the use of potato amylose, which is polydisperse and has a limited degree of branching, rather than a linear amylose, may have prevented complete transformation of complexes into their most stable polymorphic forms. On the other hand, an ionizable carboxyl group in the ligand made the initial aggregation of complexes more sensitive to pH and salt concentration, but did not affect the polymorphs that were eventually produced. T_m is believed to be of entropic origin, with identifiable contributions from both ligand and V-helices. Enthalpic changes on disordering type I complexes probably involve mostly the breaking of intrahelical hydrogen-bonds

and a few van der Waals contacts, with a smaller contribution from disaggregation of metastable chain structures. Disordering of type II complexes probably involves, in addition, true melting of crystallites with breaking of limited interhelical hydrogen bonds and significantly more van der Waals contacts.

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