Thermal Behavior of Potato Amylose and Enzyme-Resistant Starch from Maize[1]

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ABSTRACT

The thermal properties of potato amylose (degree of polymerization 5,500) and of enzyme-resistant starch (RS) (degree of polymerization 65) from autoclaved maize starch in a concentrated aqueous system (22%, w/w) were studied by differential scanning calorimetry (DSC). Heating of amylose and RS in the DSC instrument to 180°C (heating rate 5°C/min) gave melting transitions at 153.6 and 139.8°C, respectively, indicating disordering of fairly thermostable regions. This heating to high temperatures was accompanied by a partial thermal degradation of the linear amylose and RS chains. Reappearance of endotherms during repeated heating and the appearance of exotherms during each cooling phase suggested (re)association of chains from the polymer melts of both amylose and RS. Association of amylose and RS chains appeared to start between 80–75°C and 60–55°C, respectively. Whereas interactions between the long amylose chains were reduced at cooling rates of >10°C/min, interchain contacts between the shorter RS chains seemed not to be affected by the rate of cooling. In the presence of the complexing agent L-α-lysophosphatidylcholine (LPC), the process of association of linear chains was competitively affected by the formation of inclusion complexes of the linear chains with LPC. At an LPC concentration of 10% (w/w), complex formation dominated, and no association of amylose or RS chains was observed.

Formation of enzyme-resistant starch (RS) has been related to association of amylose chains during cooling of gelatinized starch. These RS structures comprise short linear chain fragments with a degree of polymerization (DP) of ~60 (Russell et al 1989, Siljeström et al 1989). They were found to give melting transitions at about 150°C when heated in excess water in the differential scanning calorimetry (DSC) instrument (Sievert and Pomeranz 1989, 1990). Amylose was also reported to give a melting transition in this higher temperature range (at ~155–160°C) (Eberstein et al 1980, Biliaderis et al 1985, Ring et al 1987). In view of the similar thermal properties of amylose and RS, this study further investigated the thermal behavior of these two linear α-glucans. The results could be of interest for high-temperature food-processing operations, such as extrusion- or jet-cooking.

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MATERIALS AND METHODS

Potato amylose (95% amylose, A-9262), maize amylose (practical grade, A-7043), and L-α-lysophosphatidylcholine (LPC) from egg yolk (L-4129) were purchased from Sigma Chemical Co., St. Louis, MO.

RS was prepared as follows. Normal maize starch (15 g) was suspended in distilled water (150 ml), gelatinized at 70°C, allowed to cool to room temperature, autoclaved at 121°C for 30 min, and then stored overnight at 4°C. The retrograded starch was mixed with 10 ml of phosphate buffer (0.05*M*, pH 6.0) and 1 ml of the thermostable bacterial α-amylase, Termamyl L-120 (Novo, Denmark). The mixture was kept at 70°C for 2 hr with continuous stirring. After cooling to room temperature, the mixture was washed with aqueous ethanol at different concentrations: one wash each with 50, 60, and 70% ethanol, then three washes with 80% ethanol. Each washing step was followed by centrifugation. After filtration and final washing with 94% ethanol, the RS residue was dried overnight at 40°C under vacuum and weighed. Expressed as a percentage of the amount of maize starch used as starting material, 5.1% (dm) RS was obtained.

DSC measurements were performed with a Mettler DSC 30 instrument (Mettler, Naenikon-Uster, Switzerland) equipped with a Mettler TC 11 analysis data station. Samples (~20 mg [dm]) were weighed accurately into aluminum pans (Mettler ME 29990,

120 μl) and distilled water (70 μl) was added. The samples were heated from 20 to 180°C (heating rate 5°C/min), cooled to 4°C (cooling rate 10°C/min, unless stated otherwise), and reheated to 180°C (heating rate 5°C/min). An LPC solution (70 μl) was added to the sample in the DSC pan to obtain LPC concentrations of 0.4, 0.9, 1.8, 2.5, 4.0, 7.0, and 10.0% (w/w, dmb), respectively. In all experiments, a pan with water constituted the reference sample. Initial transition temperature (T_i) and completion transition temperature (T_c) of a peak in the DSC thermograms were determined as the points where the scan deviated from linearity before and after the peak. Transition enthalpies (ΔH), computed as joules per gram, were calculated from the area under the curve described by the recording trace and the baseline joining T_i and T_c. Peak transition temperature (T_p) was defined as the temperature at peak maximum. DSC measurements were carried out in triplicate.

For the determination of λ_{max} values, after each DSC heating-cooling step, ~5 mg each of potato amylose and RS was dissolved "as is" in 2 ml of dimethyl sulfoxide. An aliquot (10 μl) of the solution was dissolved with I_2-KI solution (0.2% Lugol's solution, Merck, Germany) in a 10-ml volumetric flask. The absorption of the amylose and RS samples were scanned at 5-nm intervals from 580 to 680 nm and from 500 to 600 nm, respectively, using a Philips PU 8620 spectrophotometer.

Gel-filtration chromatography was used to determine the average DP of potato amylose and RS. The RS residue (10 mg) was dissolved in 1 ml of 4*N* KOH. An aliquot (20 μl) of the solution was chromatographed on a Pharmacia Superose 12 HR 10/30 column (Pharmacia, Uppsala, Sweden) (column length 30 cm, column diameter 1 cm, eluant 0.1*N* KOH, flow rate 20 ml/hr). The effluent was continuously monitored with a high-performance liquid chromatography refractometer (RI-Detector 79877A, Hewlett Packard) maintained at 30°C. The column was calibrated using linear glucan chains of DP 18 and DP 55 obtained by pullulanase-debranching of waxy maize starch as described by Würsch and Hood (1981).

Amylose (1 mg) was dissolved in 1 ml of 0.3*N* NaOH. An aliquot (200 μl) of the solution was loaded on a Sephacryl 500 10/30 column (Pharmacia) (eluant 0.01*N* NaOH, flow rate 30 ml/min). The effluent was continuously monitored with a high-performance liquid chromatography refractometer and fractions of 2 ml were subsequently analyzed for total carbohydrate by the phenol-sulfuric acid method (Dubois et al 1956). The column was calibrated with the dextran standards T 2000, T 500, T 70, and T 40 (Pharmacia) with molecular weights of 2×10^6, 5×10^5, 7×10^4, and 4×10^4, respectively. The molecular weight of amylose was estimated from the molecular weight calibration curve. The approximate DP of amylose was then calculated according to the molecular weight-DP relationship established by Hizukuri and Takagi (1984) for potato, sweet potato, tapioca, and lily amyloses.

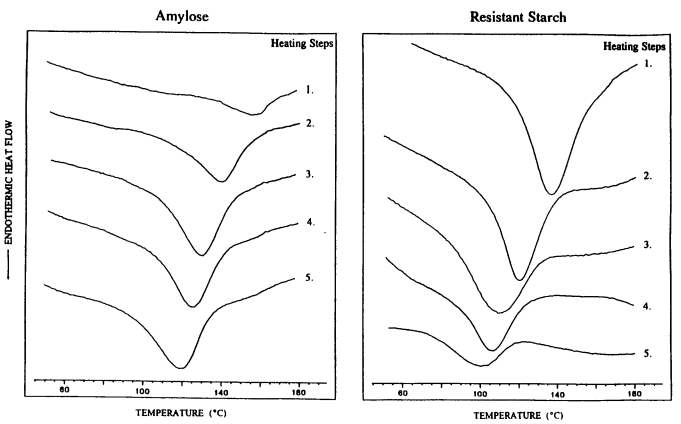

Fig. 1. Differential scanning calorimetry heating curves (1–5) of potato amylose and enzyme-resistant starch from maize heated to 180°C (heating rate 5°C/min).

RESULTS AND DISCUSSION

DSC thermograms of potato amylose (DP 5,500) and RS from maize starch (DP 65) repeatedly heated to 180°C in the DSC instrument are shown in Figure 1. During the first heating, potato amylose gave an endotherm at 153.6°C (T_p) with a melting enthalpy of 5.7 J/g indicating thermal disordering. Amylose from maize (DP 2900) also showed an endothermic transition in the higher temperature range (150.1°C T_p, not shown). Repeated heating of potato amylose was accompanied by a shift of the endotherm to lower temperatures (Figs. 1 and 2 top). The melting enthalpy of the endotherm increased during the first three heating steps, reached a plateau region during the fourth and fifth heating (Fig. 2 bottom), and decreased during the sixth heating (not shown).

Heating of RS yielded an endotherm at 138.8°C (T_p) with a melting enthalpy of 26.9 J/g (Fig. 1). Compared to amylose, the lower melting temperature of RS might indicate smaller cooperative-interacting length between the RS chains. The relatively high melting enthalpy of RS could be due to the RS isolation procedure that effectively isolated enzyme-resistant ordered chain segments in the autoclaved maize starch sample. As observed with amylose, repeated heating of RS caused a shift of the peak to lower temperatures (Figs. 1 and 2 top). However, in contrast to amylose, the melting enthalpy decreased (Fig. 2 bottom), suggesting a continuous degradation of RS structures during repeated heating to 180°C in the DSC.

The λ_{max} values of the amylose and RS samples were determined before the DSC measurements and after each cooling step; they were related to the respective T_p of the amylose and RS melting endotherm recorded during the subsequent heating step. The decrease in T_p of the endotherms of both amylose and RS (Fig. 2 top) was accompanied by a decrease in λ_{max} values (Fig. 3).

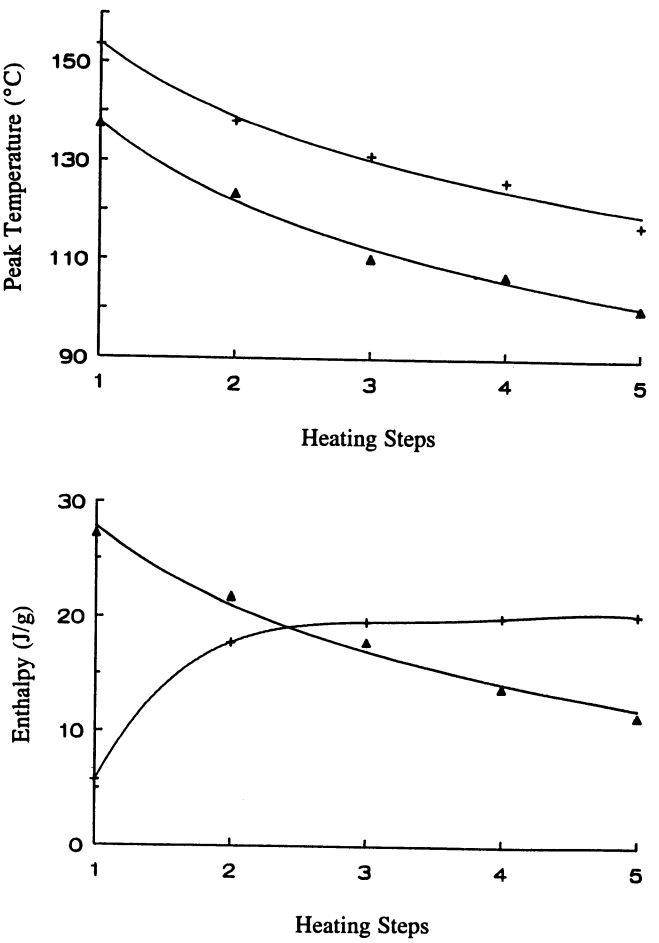

Fig. 2. Effect of 1–5 heating steps to 180°C on peak transition temperatures (top) and melting enthalpies (bottom) of the endotherms of potato amylose (+) and enzyme-resistant starch (▲). SD < 10% mean value, $n = 3$.

This suggests that thermal degradation of the samples occured during repeated heating to high temperatures, resulting in shorter chains that interacted during cooling over increasingly smaller chain sequences. This, in turn, gave rise to lower melting temperatures during the reheating step. The effect of repeated heating on RS structures was also observed using gel-filtration chromatography. The initial DP 65 decreased after the first, second, third, fourth, and fifth heating to DP 55, 40, 35, 33, and 30, respectively. A decrease in the average DP of RS from maize starch during autoclaving at 190°C was also observed by Würsch and Koellreutter (1992).

As mentioned earlier, reappearance of endotherms upon consecutive heating of amylose and RS indicated that a reassociation of the linear chains took place after thermal disordering. Especially, development of the endotherm of amylose during the second heating suggested extended ordering of the chains (Figs. 1 and 2 bottom). In fact, the cooling curves of amylose and RS recorded after each heating step revealed exothermic transitions that could reflect chain association (Fig. 4). Upon cooling, amylose gave an exotherm at T_p 49.8°C with an enthalpy value of 17.3 J/g, suggesting extended ordering of linear chains. RS yielded an exotherm at 39.6°C with an enthalpy value of 22.1 J/g. When amylose and RS were heated to temperatures below their initial melting peak temperatures (<124.1°C and <105.7°C, respectively), no exotherms appeared during the subsequent cooling phase, and no peak development of the amylose melting peak or decrease in enthalpy of the RS melting peak occured during a second heating. This strongly suggests that the exothermic transitions reflect association of linear chains from the melt. The peak transition temperatures of the exotherms of amylose and RS decreased continuously during repeated heating and cooling (Fig. 4). Without a clear T_c, it was impossible to determine the enthalpies

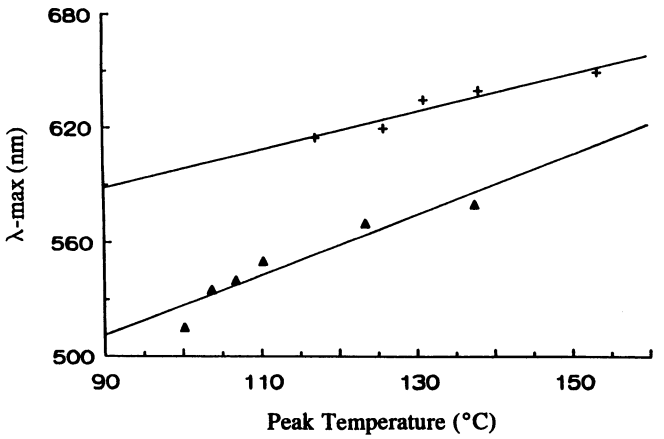

Fig. 3. Relationship between the peak temperatures of the melting endotherms of potato amylose (+) and enzyme-resistant starch (▲) with corresponding λ_{max} values of the samples.

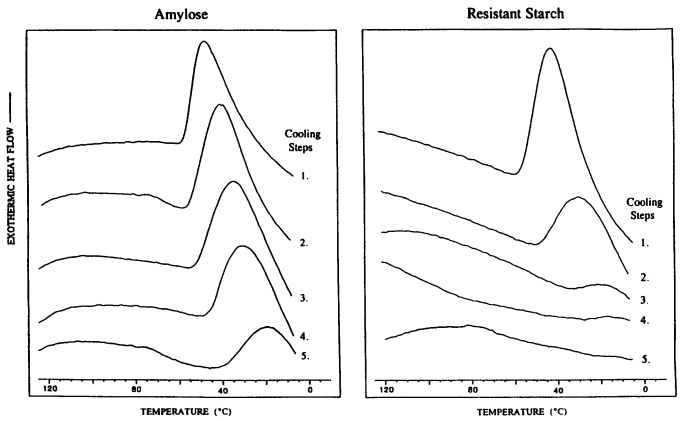

Fig. 4. Differential scanning calorimetry cooling curves (1–5) of potato amylose and enzyme-resistant starch cooled from 180°C (cooling rate 10°C/min).

of the exotherms after repeated heating and cooling. Even when the cooling phase was extended to −20°C, the descending part of curve after the peak maximum decreased continuously without a deviation (not shown). As visually judged from the thermograms shown in Fig. 4, the enthalpies of the exotherms of amylose appeared to change only slightly after the first heating-cooling step, whereas the enthalpies of the exotherms of RS seemed to decrease. This would be consistent with the findings shown in Fig. 1 and 2 (bottom). During repeated heating and cooling, melting enthalpies of amylose remained constant after the third heating and decreased during a sixth heating (not shown), whereas melting enthalpies of RS decreased steadily.

Effect of Final Cooling Temperature

To study the reorganization behavior of amylose and RS, the two samples were heated to 180°C, cooled to various final cooling temperatures, and then reheated to 180°C. The enthalpy of the endotherm that appeared during the reheating step was plotted against the final cooling temperatures after the first heating step (Fig. 5). The curves that reflect association of amylose and RS chains as evaluated by the enthalpy of the melting transitions upon reheating, showed biphasic profiles. Association of amylose chains from the melt started between 80 and 75°C, then proceeded rapidly to about 60°C, and appeared to level off at <52°C. Association of RS chains started between 60 and 55°C, then also proceeded rapidly, and reached a plateau at <40°C. From these profiles, it became evident that the beginning of the leveling-off regions that might indicate a limit of amylose and RS chain association, coinciding with the T_p of the exotherms of amylose (49.8°C) and RS (39.6°C), respectively, during the first cooling (Fig. 4). Because cooling of amylose and RS above the T_i of their exotherms (>67.9°C and >52.8°C, respectively) led to small melting transitions during the reheating step, it would seem that organization of amylose and RS chains upon cooling had already started at temperatures above the appearance of the exothermic transitions. Thus, the DSC method applied in this study does not seem to be sensitive enough to respond to the initial phase of chain association during cooling. This hypothesis, however, is based on the assumption that no organization of linear chains took place during the reheating phase.

A reheating of a heated amylose or RS sample after seven days of storage at 4°C gave no differences in melting enthalpy or T_p compared to counterparts immediately heated after cooling. From these findings, it might be concluded that formation of ordered regions within the heated and cooled amylose and RS sample did not proceed during a storage period of seven days at 4°C.

Effect of Cooling Rate

The phase changes of amylose and RS on cooling the melt (prepared at 180°C), as reported earlier, were based on a cooling

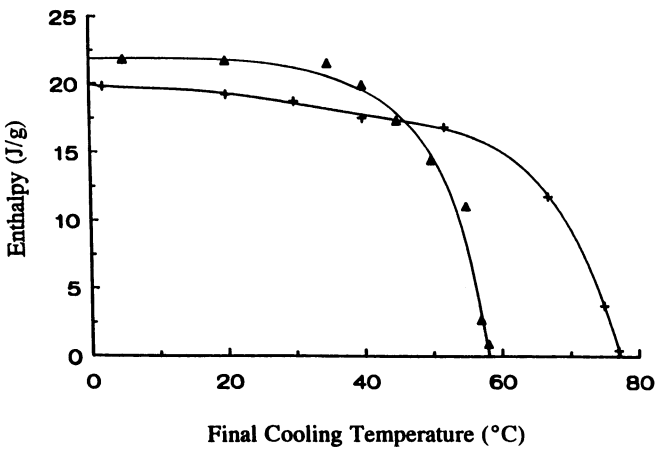

Fig. 5. Effect of the final cooling temperature of the first heating on the melting enthalpy of potato amylose (+) and of enzyme-resistant starch (▲) during reheating. SD < 10% mean value, $n = 3$.

rate of 10°C/min. We also investigated the effects of changes in the cooling rate on the reassociation behavior of amylose and RS chains. Amylose and RS were heated to 180°C (5°C/min), subsequently cooled to 4°C at different cooling rates, and then reheated to 180°C (5°C/min). Figure 6 shows the enthalpy of the high-temperature transition recorded during the second heating as a function of cooling rate after the first heating. Amylose cooled at rates <10°C/min showed a similar melting enthalpy of the endotherm during reheating than amylose cooled at 10°C/min. Cooling rates > 10°C/min resulted in a continuous decrease of the melting enthalpy followed by a plateau at cooling rates >50°C/min. This behavior of amylose might be explained on the basis of chain orientation and alignment during cooling. Slower cooling rates could lead to a greater annealing of the structure and, thus, favor chain alignment and cooperative interactions between amylose chains. Quenching conditions appear to limit the long-term alignment process of the long amylose chains, thereby reducing chain interactions and formation of ordered structures. It should be noted that the exotherms of amylose and RS chain association were no longer detectable at faster cooling rates (>20°C/min). This suggests that the DSC instrument was not able to probe the exothermic process of chain interaction during rapid cooling.

The melting enthalpy of RS during the second heating was not affected by cooling rate after the first heating (Fig. 6). This could be due to the higher mobility of the short RS chains which might allow interchain contacts rapidly to occur independent of the cooling regime.

Interaction with LPC

Amylose heated in the presence of LPC (10% w/w, dmb) showed a T_p at 108.4°C (11.9 J/g) attributable to the melting of amylose-LPC complexes and a T_p at 154.2°C (2.3 J/g) reflecting melting (dissociation) of amylose (Fig. 7). Thus, amylose heated with LPC yielded a significantly lower amylose melting enthalpy (2.3 J/g) than did amylose heated without LPC (5.7 J/g). This, together with appearance of the amylose-LPC melting peak, suggests that amylose partly solubilized during heating in the DSC to form complexes with LPC. Evidence has already been provided that formation and melting of amylose-lipid complexes can occur simultaneously during heating in the DSC (Biliaderis et al 1986). RS heated in the presence of LPC showed no melting of an LPC complex and only gave the peak in the higher temperature range, indicating dissociation of ordered linear chains (Fig. 7). This showed that no complexation with LPC occured during heating.

Amylose and RS, cooled from 180°C in the presence of LPC, both showed an exotherm at about 80°C, demonstrating the formation of inclusion complexes with LPC (Fig. 7). The exotherms in the lower temperature range disappeared, suggesting that no chain association took place. This was confirmed by the reheating curves that showed melting of the LPC complexes but no melting transitions in the higher temperature range (Fig. 7). When amylose and RS were heated in the presence of LPC (10%, w/w) to 121 and 100°C, respectively, (below initial melting peak temperatures), cooled, and reheated to 180°C, amylose gave an amylose-LPC complex (18.4 J/g), whereas RS showed no melting of a complex. The observation that potato amylose already forms a complex with LPC at temperatures below the melting temperature of ordered amylose might indicate the presence of "free" nonordered amylose chains in the sample. In contrast, melting of ordered RS structures is required to induce complexation.

To follow the processes of amylose chain association and amylose-LPC formation in one sample, amylose was heated in the DSC with increasing concentrations of LPC to 180°C (5°C/min), cooled to 4°C (10°C/min), and then reheated to 180°C (5°C/min) (Fig. 8). The cooling curves showed that, with increasing concentrations of LPC, the exotherms at 80°C indicating amylose-LPC complex formation developed, whereas the exotherms in the lower temperature range decreased in enthalpy (Figs. 8 and 9 top). This reveals the competitive character of the processes. At the 5% LPC level, equal enthalpies of the two exotherms were calculated (Fig. 9 top) and recorded (not shown). At the 10% LPC level, amylose-LPC complexation was favored over amylose chain association. The melting enthalpy (27.7 J/g) of the amylose-LPC complex at the 10% LPC level agreed well with the melting enthalpy of 26.5 J/g reported for a solution-grown amylose-LPC complex (Biliaderis et al 1985). Concomitant with increasing amounts of LPC, we observed a shift of the exotherms of amylose-LPC complexation to higher temperatures and a shift of the exotherms of amylose association to lower temperatures (Fig. 8). Cooling rates slower (5°C/min) or faster (50°C/

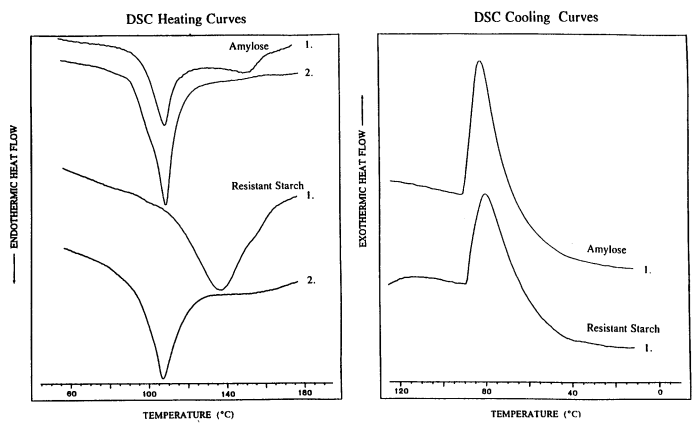

Fig. 7. Differential scanning calorimetry heating and cooling curves of potato amylose and enzyme-resistant starch in the presence of 10% (w/w) L-α-lysophosphatidylcholine. The numbers on the curves refer to the heating and cooling steps.

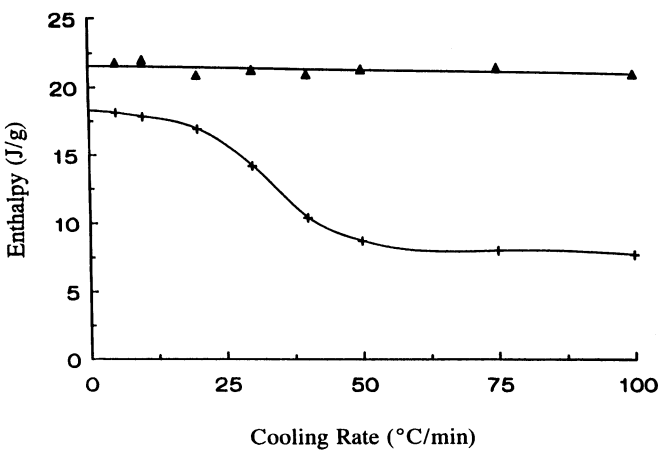

Fig. 6. Effect of the cooling rate after the the first heating on the melting enthalpy of potato amylose (+) and of enzyme-resistant starch (▲) during reheating. SD < 5% mean value, $n = 3$.

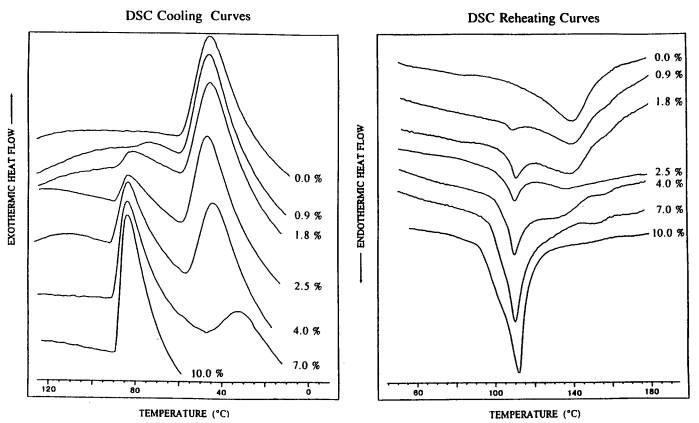

Fig. 8. Effect of different L-α-lysophosphatidylcholine concentrations on differential scanning calorimetry thermograms of potato amylose during cooling from 180°C to 4°C (cooling rate 10°C/min) and during the subsequent reheating to 180°C (heating rate 5°C/min).

min and 100°C/min) than 10°C/min caused no changes in enthalpy of the amylose-LPC complexes upon reheating. These findings, together with the data on amylose association kinetics shown in Figure 6, would seem to indicate that amylose-LPC complexation occurs more rapidly than amylose chain association does.

The reheating curves showed changes that paralleled the events during cooling (Fig. 8). Increasing concentrations of LPC were accompanied by an increase in melting enthalpy of the amylose-LPC complex peak (~110°C T_p) and a decrease of the amylose dissociation peak (between ~140°C T_p and ~155°C T_p) (Figs. 8 and 9 bottom). The cooling curves showed a fairly clear separation between the two competitive mechanisms of complexation and chain association (Fig. 8). The former was best fitted by a second-order polynominal equation, the latter was best described by a linear equation (Fig. 9 top) (equations not given). The corresponding reheating curves showed a less distinct separation (Fig. 8). The increase in melting enthalpy of the amylose-LPC complex with increasing LPC concentrations was best fitted by a third-order polynominal equation (Fig. 9 bottom) (equation not given). The amylose dissociation peak behavior followed fourth-order polynominal equation characteristics (Fig. 9 bottom) (equation not given). This different behavior during cooling and reheating is likely due to the different nature of the two amylose-based structures in the sample. The complexed form is highly crystalline, giving strong V-type X-ray diffraction patterns (Takeo et al 1973, Czuchajowska et al 1991) and well-developed melting peaks (Biliaderis et al 1985). On the other hand, retrograded amylose and RS are poorly crystalline structures (B-type diffraction pattern) composed of amorphous regions interconnected with small or imperfect crystallites that give broader melting transitions

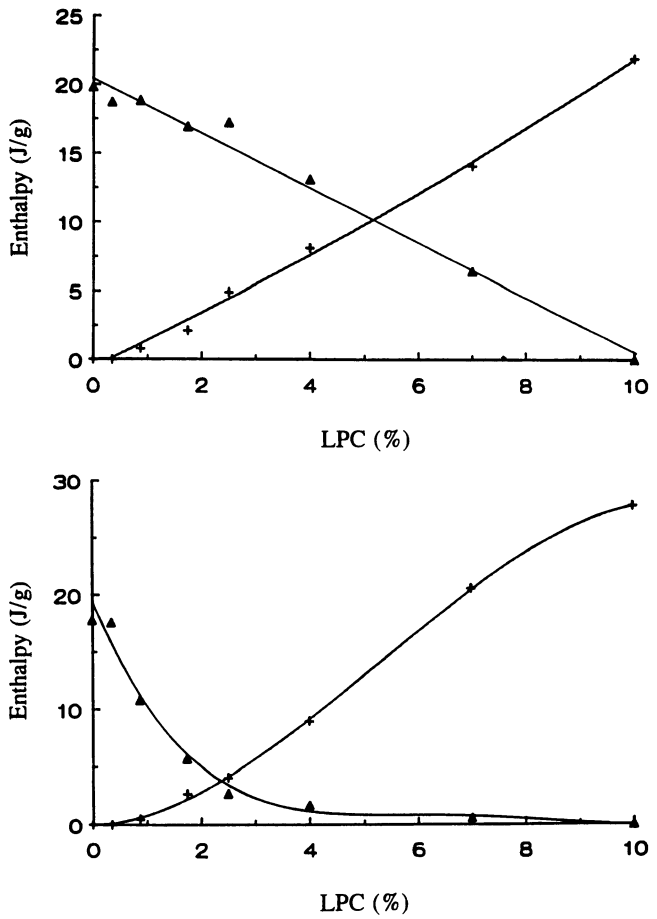

Fig. 9. Effect of different L-α-lysophosphatidylcholine (LPC) concentrations (%, w/w, dmb) on enthalpies of the exothermic transitions of amylose-LPC complexation (+) and amylose chain association (▲) during cooling (top); and on enthalpies of the endothermic transitions of amylose-LPC complex melting (+) and amylose dissociation (▲) during reheating (bottom). SD < 5% mean value, $n = 3$.

at higher melting temperatures (Cairns et al 1990, Sievert et al 1991). Thus, if the V- and B-type structures were heated together, the melting behavior of the highly crystalline V-type structure with a lower melting temperature would tend to predominate and competition for water might occur between the two structures. Furthermore, in a certain temperature range (~120°C), melting of the two structures could take place simultaneously, resulting in overlapping melting transitions.

CONCLUSIONS

The thermal properties of potato amylose (DP 5,500) and RS (DP 65) from autoclaved maize starch in a concentrated aqueous system (22%, w/w) were studied. Both amylose and RS showed melting transitions in the higher temperature range (153.6 and 138.8°C), indicating disordering of fairly thermostable regions. Heating to high temperatures (180°C) was accompanied by partial thermal degradation of amylose and RS. Exothermic transitions during cooling and the reappearance of endotherms during reheating indicated that reassociation of the partially degraded amylose and RS chains occured during the cooling phase. Factors affecting chain association included the chain length, the final cooling temperature, the cooling rate, and the presence of complexing agents. The term "chain association" used in this study might be related to previously investigated mechanisms of amylose precipitation or aggregation (at polymer concentrations <1.0–1.5%, w/w) and gelation (at polymer concentrations >1.0–1.5%, w/w) (Morris 1990). However, the polymer concentration regimes usually used in studies on amylose precipitation and gelation are <10% (w/w) and, hence, well below the 22% (w/w) solids level studied here. Gidley (1989) and Gidley and Bulpin (1989) studied (by viscosity, X-ray, ^{13}C nuclear magnetic resonance, and optical rotation measurements) the association behavior of enzymatically synthesized and commercially available amyloses in aqueous systems. They proposed a mechanism of amylose aggregation and gelation that is dominated by the formation and subsequent lateral aggregation of B-type double helices in crystalline arrays. The model of amylose gelation recently proposed by Leloup et al (1992) involves, in agreement with results of Doublier and Choplin (1989) and Miles et al (1985), an initial process of separation into polymer-rich and polymer-deficient phases. This phase separation allows short-range chain interactions and the establishment of gel junction zones. The junction zones could adopt double helical structures that aggregate. The formation of RS has been related to the reorganization of amylose chains, and it was suggested that RS comprises, apart from amorphous material, small and less-perfect crystalline B-type structures (Sievert et al 1991). These small, poorly developed crystallites might correspond to the small crystalline junction zones formed during amylose gelation. Based on these proposed models of reorganized amylose and RS, the exothermic transitions observed in our study at 49.8°C (T_p) during cooling of the polymeric melt of amylose and at 39.6°C (T_p) during cooling of the polymeric melt of RS, can reasonably be assumed to reflect at least part of the processes of chain interaction, double helix formation, and aggregation.

In light of the multistage process of amylose gelation and precipitation, the question then arises as to what events are represented by the melting peaks of amylose and RS. Gidley and Bulpin (1989) found that heating amyloses in water to 160–170°C is necessary to obtain clear solutions. This would be consistent with the melting transition of amylose observed here (153.6°C, T_p; 124.1°C, T_i; 167.3°C, T_c). The state of amylose in aqueous solutions is not firmly established. There is, however, strong evidence to suggest that amylose adopts coil conformation when solubilized in neutral aqueous solutions (Banks and Greenwood 1975, Kitamura and Kuge 1989, Roger and Colonna 1992). Hence, it might be concluded that the melting of amylose and RS from the solid into the fluid phase involves dissociation of ordered regions composed of double helical segments and double helix-to-single coil transitions. Because the interaction of linear glucan chains with a complexing agent, such as LPC, is believed to

proceed in the initial phase through conformational ordering of the chains (i.e., single coil-to-single helix transition) (Biliaderis 1991), the presence of single coils in the amylose and RS melt would also be corroborated by the complexation we observed while cooling the melts with LPC.

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