Study of Cryostructuration of Polymer Systems. XXI. Cryotropic Gel Formation of the Water–Maltodextrin Systems

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ABSTRACT: The freeze–thaw behavior of water solutions containing dissolved maltodextrin (MD; enzymatically converted potato starch derivative with MW of 8000 Da) over a wide range of MD concentration (0.1–15 g/dL) and freezing temperatures from −24 to −6°C was studied. Cryogenic treatment of these systems resulted in the formation of precipitates or gels, whose yield and thermal characteristics (fusion temperature and enthalpy) depended on the initial polymer concentration and conditions of freezing, frozen storage, and thawing. There appeared to be at least two stages to this process: (i) a rapid stage, when partial insolubilization occurred while the system was freezing, and (ii) a slower stage, the rate of which was dependent mainly on the thawing regimes used or the duration of storage at subzero temperatures. In this respect, the cryostructuration of MD was very similar to the freeze–thaw behavior of amylopectin/amylose and locust bean gum water solutions studied earlier. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 1658–1667, 2002

Key words: maltodexrin–water solutions; freeze–thaw-induced structuration; association; hydrogels; polysaccharides

INTRODUCTION

Maltodextrins (MDs) are the products of a partial controlled chemical or enzymatic degradation of starches isolated from diverse plant sources.[1–3] Currently, these glucose oligomers [MW $\leq$ 1–2 $\times 10^4$ Da as opposed to MW $> 10^5$–10^6 Da for the native starch polysaccharides amylose (AL) and amylopectin (AP)] are widely applied in food technologies, especially for the production of low-fat, low-calorie products.[4,5] In the course of processing or storage of such MD-containing systems, they can be subjected to cryogenic treatment: freezing–storage in the frozen state–thawing, which may affect significantly the properties of the final materials. Such freeze–texturing is a commonplace phenomenon for water solutions and dispersions of starch polysaccharides, which results in the formation of gel-like materials of a macroporous or sometimes spongy nature.[6–10] Similar cryogenically structured gels are the representatives of the wider type of polymeric gels, the so-called cryogels (cryostructurates and cryotexturates are the frequently used synonyms), obtained as a result of the freeze–thaw treatment given to ini-

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tially nonstructured solvent–polymer (or appropriate monomer) systems that are potentially capable of gellling.[11] Starch-based cryogels and those formed from the individual starch polysaccharides, AP and AL, as well as from their artificial mixtures,[12,13] are related to thermoreversible noncovalent cryogels. They are fused upon heating above certain critical temperatures and form again when the resultant biopolymer solutions are refrozen and thawed. In spite of the large amount of information published on starch cryotropic gelation, we were unable to find any data on the consequences of cryogenic processing for MD-containing systems. Therefore, the aim of this work was, first, to recognize the general effects caused by the freezing–frozen storage–thawing process on MD–water solutions of various MD concentrations and to study some properties of MD cryostructurates and, second, to compare the results obtained with the effects observed earlier for high molecular weight starch polysaccharides. Consequently, we used the same experimental approaches and the methodology in these studies as were implemented in our previous research of AP and AL systems.[12,13]

EXPERIMENTAL

Materials

The following substances were used in the work without additional purification: MD was purchased from Avebe (Veendam, The Netherlands) under the trademark of Paselli SA2. This product is enzymatically converted potato starch comprising >99 wt % of oligosaccharides, a dextrose equivalent of 2.8, and molecular weight (MW) of 8000 Da (the manufacturer's data). The MW value was checked by gel chromatography on a Sephadex G-50 (fine) resin (Pharmacia Fine Chemicals, Uppsala, Sweden) column; the eluent was 0.005*N* NaOH. The presence of, along with the major fraction (MW ~8 kDa), additional fractions possessing an MW of about 12–16 kDa was also recorded.

Crystalline iodine, potassium iodide, and sodium acetate, all employed in the preparation of the reagent solution for the determination of the polysaccharide concentration, were purchased from Reakhim (Moscow, Russia) and were of a "chemically pure" grade. All the solutions were prepared with deionized water.

Methods

MD Solutions

Aqueous solutions of MD were prepared as follows: A weighed amount of dry polysaccharide of a known water content was dispersed in the required volume of water, then heated on a boiling water bath for 20 min with stirring. Evaporative losses of water were determined gravimetrically and compensated by addition of the requisite amounts of the solvent. The solutions thus obtained were passed through a sintered glass filter to remove possible undissolved matter and then used in the experiments.

Quantitative Determination of MD Concentrations

Quantitative determination of MD concentrations in the solutions under study was carried out by spectrophotometric analysis of the corresponding iodine–polysaccharide complexes, essentially in accordance with a standard procedure.[6] Calibration plots were obtained first. In practice, 1 mL of the MD solution to be examined was mixed with 1 mL of 0.2*M* Na–acetate buffer (pH 5.3), and then 2 mL of 0.002*M* of the I_2/KI solution was introduced. The absorbance of the solutions thus prepared was recorded at 560 nm using a Model-557 UV-vis spectrophotometer (Hitachi, Tokyo, Japan). The amount of MD remaining in the liquid phase of the heterogeneous dispersions formed by cryogenic conditioning of the initially homogeneous MD–water solutions or their unfrozen equivalents were determined as follows: MD dispersions were separated by centrifugation at 4500 rpm for 30 min at 15–18°C (K 23 refrigerating centrifuge, MLW, former GDR) and 1-mL aliquots were taken from the respective supernatants for analysis of the MD concentration.

Freezing and Frozen Storage of the Samples

Freezing and frozen storage of the samples was performed in the chamber of an F34 MH (Julabo, Seelbach, Germany) programmable precision cryostat (the accuracy of temperature maintenance was ±0.01°C). Thawing of the frozen samples was carried out with controlled heating at a preassigned rate (thawing rate, v_{th}) using the microprocessor-controlled facilities of the cryostat.

Water-absorbing Capacity

The water-absorbing capacity (degree of swelling, $S_{w/w}$) of the MD cryoprecipitates was determined

Table I Data on Cryoprecipitation Yield for the Low-Concentration (0.1–5 g/dL) MD–Water Solutions

Initial MD Concentration (g/dL)	Yield[a] of the Structuration Processes (Y, %) for MD Solutions After Storing at Set Temperatures (°C)			
	+18	−6	−12	−24
0.10	[b]	92.8 ± 2.0	85.6 ± 0.8	88.9 ± 3.5
0.25	[b]	93.5 ± 0.8	91.1 ± 0.7	92.7 ± 1.8
0.50	[b]	93.8 ± 1.2	92.7 ± 0.8	94.3 ± 1.4
1.00	[b]	93.9 ± 0.2	93.0 ± 0.4	94.7 ± 1.0
2.50	[b]	93.6 ± 0.4	93.4 ± 0.9	94.9 ± 0.7
5.00	[b]	93.9 ± 1.1	96.2 ± 0.4	95.7 ± 0.6

Frozen storage duration: 18 h; thawing rate: 0.03°C/min.
[a] Yield values were computed as follows: $Y = (1 - C_i : C_m) \times 100\%$, where C_i is the initial MD concentration, and C_m, the measured MD concentration in a supernatant after centrifugation of the thawed system (see Methods).
[b] No precipitation at all.

by placing samples between several layers of filter paper and pressing-out "free water" under a load of 0.1 MPa. The weight of the wet samples was recorded and compared with their weight after drying at 105°C to a constant weight:

$$S_{w/w} = (W_{wet} - W_{dry})/W_{dry}$$

(g H_2O/g of dry polymer) (W = weight)

where W is the weight of the water.

Differential Scanning Calorimetry

Calorimetric studies of the thermal characteristics of the MD cryostructurates were accomplished using a DASM-4 high-sensitive differential scanning microcalorimeter (Biopribor, Puschino-on-Oka, Russia) over the temperature range of 10–100°C, using a scan rate of 2°C/min. Weighed samples taken from the sediments after centrifuging (see above) were resuspended in enough pure water to give an MD concentration of about 1 g/dL (the exact [MD] was determined with the iodine reaction after melting any solids). The suspension thus prepared was injected into the head probe of the calorimeter.

RESULTS AND DISCUSSION

The phenomena observed as a result of the unfrozen storage of the MD–water solutions showed that similar systems could be grouped qualitatively into three concentration ranges. (i) At low concentration, the sample appearance was not changed after a 1-day incubation at room temperature. (ii) At moderate concentration, under the same conditions, the initial solutions became opalescent or opaque, but remained fluid. (iii) At high MD concentration, the gels had already formed at positive temperatures (cryostructuring of the latter systems was not considered in this work). The consequences of cryogenic treatment of water–MD systems also depended on the initial concentration of MD in the solution to be frozen–thawed. In the case of low-concentration (0.1–5 g/dL) solutions, opaque dispersions were obtained after thawing the system. These could easily be separated by a mild centrifugation into a transparent supernatant and a small amount of compact white sediment (cryoprecipitate). In the case of more concentrated initial solutions (7.5–15 g/dL), freeze–thaw treatment resulted in the formation of pastelike cryostructurates occupying the total volume of the vessel. These two MD-containing systems [of (i) low and (ii) moderate polymer concentration] are now considered separately below.

Cryogenic Influence on Low-Concentration (0.1–5 g/dL) Water Solutions of MD

Low-concentration solutions of MD exhibited a rather interesting behavior when given a cryogenic treatment. Compared to standing at room temperature for 18 h, when no precipitation occurred, the freeze–thaw treatment gave rise, as already noted above, to the formation of cryoprecipitates. The data on the yield (Y) values of this process are summarized in Table I.

One may see that the MD concentration in the solution to be cryogenically treated had virtually

Table II Morphological Features of the 7.5–15-g/dL MD–Water Solutions Subjected or Not to a Cryogenic Treatment

Initial MD Concentration (g/dL)	Morphology[a] of the Systems After Storing at Set Temperatures (°C)			
	+18	−6	−12	−24
7.5	Slightly opalescent liquid	Loose curdlike precipitate. After centrifugation: compacted white cryoprecipitate and a transparent supernatant	Curdlike cryoprecipitate, comprising small needle-shaped particles and a transparent upper liquid layer	A very weak cryogel in the whole sample bulk; squeezes a free liquid under light pressure
10.0	The same	The same	The same	The same
12.5	Opalescent liquid	Loose cryoprecipitate occupying the whole bulk of the sample. After centrifugation: more compacted sediment and a small amount of a transparent supernatant	The same, but the relative amount of cryoprecipitate to liquid layer is increased with the growth of the polymer concentration	Weak cryogel in the whole sample; squeezes a free liquid under light pressure
15.0	Opaque liquid	The same	The same	The same

[a] The morphology of the samples after their incubation for 18 h at a preassigned temperature is given, and then the morphology of the same systems after their centrifugation at 4500 rpm for 30 min is also described.

no effect (except for the very low concentration samples with [MD] = 0.1 g/dL, where some decrease in Y was detected) on the cryoprecipitation yield values at all negative temperatures used. A decrease in the freezing temperature from −6 to −24°C did not result in a significant variation of the cryoprecipitation efficacy either. In all the cases, the freeze–thaw influence was a very effective way to precipitate MD from its dilute water solutions and, typically, more than 90% of the initially soluble MD was transformed into insoluble matter. These results demonstrated, first, a low cryoresistance for such solutions and, second, the powerful influence that cryogenic treatments exert on the association of oligosaccharide molecules, even of a low degree of polymerization.

Evidently, the major reasons for the similar result were the effects of the solute concentrating in those parts of the apparently solid system that remained unfrozen at the negative temperatures indicated in Table I. These liquidlike inclusions have been termed the unfrozen liquid microphase.[14,15] The role this phase plays in the cryostructuration phenomena was reviewed in detail elsewhere[11,16] and has the following essential characteristics: When any solution (even a rather dilute one) freezes at moderate negative temperature, the crystals of a pure solvent are initially formed. This causes an increase in the solute (MD in the case under consideration) concentration in the still unfrozen liquid regions of the system, thus resulting in a strengthening of polymer–polymer interactions, that, in turn, leads to the formation of condensed MD matter. Thus, the freeze concentration has the effect of apparently shifting the critical concentration of MD insolubilization toward lower values, compared to the same process at positive temperatures. Therefore, cryogenic treatment of MD dilute solutions could be considered as an influence strongly facilitating retrogradation phenomena (partial crystallization) in oligosaccharide molecules.

Cryogenic Effects on the 7.5–15-g/dL Water Solutions of MD

The same phenomena were clearly observed for the more concentrated MD–water solutions, as well, when processed cryogenically under analogous freeze–thaw conditions. These results are summarized in Tables II–IV (these record descriptive data

Table III Yields of the Structuration Processes in the 7.5–15-g/dL MD–Water Solutions Subjected or Not to a Cryogenic Treatment

Initial MD Concentration (g/dL)	Yield[a] of the Structuration Processes (Y, %) for MD Solutions After Storing at Set Temperatures (°C)			
	+18	−6	−12	−24
7.5	[b]	92.5 ± 0.8	96.0 ± 0.5	96.6 ± 0.2
10.0	[c]	78.0 ± 0.6	95.1 ± 0.6	95.4 ± 0.4
12.5	[c]	77.9 ± 0.9	94.9 ± 0.5	95.7 ± 1.2
15.0	[c]	73.7 ± 3.2	94.6 ± 0.3	95.6 ± 0.3

[a] See footnotes to Table I for the formula of Y determination and for the freezing–thawing regimes.
[b] No precipitation.
[c] Centrifugation in the regimes used did not separate the system into upper and lower layers.

about the precipitation trends observed together with values of the yields of insoluble matter formation and water-absorbing capacities for the cryoprecipitates and cryostructurates obtained).

When studying these MD-containing solutions, we used only those samples ([MD] ≤ 15 g/dL), which did not gel after 1-day storage at room temperature. Table II shows that at +18°C only gradual growth of turbidity of such solutions was observed. Increasing the MD concentration in the solution up to 20 g/dL and higher resulted in gelation of the initially liquid systems after storing at positive temperature. This gelation behavior is to be expected from concentrated MD–water solutions.[3,17–19] Therefore, solutions containing MD of more than 15 g/dL were not, as mentioned above, considered here, since self-association of MD into gels while still at positive temperatures could interfere with subsequent cryotropic gelation.

In contrast to storage at room temperature, cryogenic treatment of the 7.5–15 g/dL MD–water solutions gave rise to pronounced structuration of the oligosaccharide: The yield values exceeded 70% at all the freezing temperatures studied (Table III). The cryostructurates obtained (Table II) typically possessed a pastelike consistency, where every particle of these thick dispersions had the form of small hydrogel "grains" of various sizes and shapes. Hence, the formation of MD cryoprecipitates could be considered as a specific kind of cryotropic gelation taking place within the volume of each freeze–thaw-precipitated particle. The effect of an apparent shift in the critical concentration of gelation (CCG) was thus inherent to the MD–water solutions of "moderate" concentration, compared to the gelation of the oligosaccharide solutions at positive temperatures. Such a shift of CCG is well documented for numerous other polymers capable of producing noncovalent thermoreversible cryogels, for instance, agar–agar, gelatine, locust bean gum, various starches and their individual polysaccharides (i.e., AP and AL), and poly(vinyl alcohol),[6,11–13,16,20–22] that is,

Table IV Water-Absorbing Capacities of the Samples of Insoluble Matter Formed from the 7.5–15-g/dL MD–Water Solutions Subjected or Not to a Cryogenic Treatment

Initial MD Concentration (g/dL)	$S_{w/w}$[a] Values (g H_2O/g dry polymer) for the Insoluble Specimens Formed at Set Temperatures (°C)			
	+18	−6	−12	−24
7.5	[b]	2.42 ± 0.15	1.90 ± 0.09	1.93 ± 0.08
10.0	[c]	3.58 ± 0.10	1.82 ± 0.08	1.66 ± 0.12
12.5	[c]	3.56 ± 0.11	1.74 ± 0.16	1.65 ± 0.11
15.0	[c]	3.39 ± 0.07	1.57 ± 0.08	1.54 ± 0.08

[a] See Methods for the formula of $S_{w/w}$ determination.
[b,c] The same as in Table III.

this effect is common for the processes of the formation of similar cryogels. Its "driving force" is the increase in the solute concentration in the unfrozen liquid microphase of macrofrozen solutions of gelling polymers, as discussed above, when gelation occurs in the freeze-concentrated medium (i.e., in the considerably more concentrated medium than in the initial solution). However, MD cryostructurates were formed at significantly higher polymer concentrations compared with the above-listed high-molecular gelling agents. This, certainly, was due to the reduced molecular weight of the carbohydrate chains present in such starch derivatives, inasmuch as cryogels of, for instance, AP (MW $\sim 10^6$–10^7 Da) could be obtained under identical freeze–thaw conditions even from the 0.5-g/dL initial polysaccharide solutions.[12,13]

The curdlike MD cryoprecipitates or rather weak cryogels obtained as a result of the freeze–thaw treatment (Table II) had the appearance of structured, milk-white materials. These were separated by centrifugation into a compacted sediment and virtually transparent supernatant, where the residual concentration of MD was measured with an iodine-staining reaction. A comparison of the *Y* values determined for the 0.1–5- and 7.5–15-g/dL MD-containing systems (Tables I and III, respectively) revealed a diminishment in the yield for the case of the latter systems at a freezing temperature of −6°C. For the cryostructuration processes conducted at −12 or −24°C, only an insignificant influence of [MD] and the freezing temperature on *Y* was inherent in both MD concentration ranges, whereas for the 7.5–15-g/dL systems, a certain decrease in the yield values with increase of the MD concentration was found at −6°C. We observed a lowering of the *Y* values from ~93% for the 7.5-g/dL system to ~74% for the 15-g/dL system (Table III). It is thought that these latter results could be explained as follows:

The phase state of any frozen (i.e., crystallized, but not vitrified) solution under equilibrium conditions is known to depend on the cryoscopic properties of the solvent, freezing temperature, and initial solute concentration, which, in turn, determines the volume of the unfrozen microphase and concentration of the solutes therein.[15] In the absence of a real "liquid–solid" phase diagram for such a system (as is the case for the MD–water–ice phase diagram), a semiempirical rule can be used. This says that for a given negative temperature the higher the initial solute concentration, the higher is the volume of unfrozen inclusions, provided that all the solutes are concentrated in the still liquid moiety of the macrofrozen sample, that is, the system is above the "solidus" line of the phase diagram. Apparently, −6°C is a sufficiently "high" negative temperature for MD systems with an initial concentration ≥10 g/dL to affect the cryostructuration yield markedly. In this case, the volume of the unfrozen liquid microphase turned out too large for a significant freeze concentration of MD to drive efficient intermolecular interactions of the oligosaccharide molecules and, so, subsequent cryostructuration was low. Thus, for solutions frozen at −6°C, this combination of factors could cause a decrease in the *Y* values with the increase of the initial MD concentration. However, at the initial MD concentrations lower than 7.5 g/dL, the extent of the freeze concentration needed for the efficient intermolecular interactions was, nonetheless, achieved because a greater amount of water was frozen out and, therefore, high-yield cryoprecipitation took place (Table I). On the other hand, efficient cryostructuring of the 10–15-g/dL MD systems was made possible by a decrease in the freezing temperature (Table III), which also led to a freezing-out of an additional amount of water and resulted in an increase in solute concentration in the unfrozen liquid microphase.

The above explanations of decreases in the *Y* values with increase in the MD concentration for the cryogenic process at −6°C was, from our viewpoint, also supported by the measurements of the swelling characteristics of the MD cryostructurates qualitatively described in Table II. The values of 1.5–2 g/g for the water-absorbing capacities of the freeze-structured samples (Table IV) testified to a rather compact morphology for the gel phase of the specimens prepared at −12 and −24°C, when the extent of cryoconcentrating was high. In contrast, loose cryoprecipitates formed at −6°C (i.e., in the less concentrated medium) consisted of more swollen gel particles, as their $S_{w/w}$ values were significantly (up to two times) higher and the solid matter content in them was lower.

It should be also pointed out that the water-absorbing capacities of the MD cryostructurates formed at −6°C are around 3.5 g H_20 per gram of the polymer, which turned out to be very close to the $S_{w/w}$ values found earlier for the AL cryoprecipitates.[13] The latter species were prepared from the 0.5–2-g/dL solutions of this high-crystalline polysaccharide, that is, AL, which was preliminary dissolved in a highly alkaline medium, neu-

Table V Influence of the Thawing Rate on the Yield of Matter Cryogenically Precipitated from MD–Water Solutions

Initial MD Concentration (g/dL)	Conditions of the Cryogenic Treatment			Yield of the Cryoprecipitated Matter (%)
	Freezing Temperature (°C)	Frozen-storage Duration (h)	Thawing Rate (°C/min)	
1.0	−12	1	~3	13.6 ± 1.6
			0.30	78.8 ± 0.3
			0.03	90.3 ± 0.4
		18	~3	50.1 ± 4.2
			0.30	84.4 ± 1.2
			0.03	93.0 ± 0.4
10.0	−12	1	~3	22.4 ± 0.5
			0.30	84.2 ± 0.3
			0.03	94.2 ± 0.3
		18	~3	66.6 ± 0.4
			0.30	90.4 ± 1.0
			0.03	95.1 ± 0.6

tralized, and then frozen–thawed. In this case, the rather extensive swelling of the resultant AL cryoprecipitates was not only due to the low initial polymer concentration in the cryogenically treated system, but also due to the presence of 0.35*M* NaCl in the composition of the solvent. The salt, present as result of the neutralization reaction, caused the volume of the unfrozen liquid microphase to increase, thus reducing the polymer concentration. In other words, a "high enough" negative temperature and a high initial solute concentration (concentration of a gelling polymer together with other solutes, if they are present, as MD itself in the case of ≥10-g/dL MD solutions, or NaCl in the case of AL systems) were the factors responsible for diluting the polymer in the unfrozen phase and, as a consequence, decreasing the efficiency of the cryostructuration.

Role of the Thawing Step in the MD Cryotropic Structuration

Earlier, it was shown that the efficiency of cryotropic gelation occurring during formation of noncovalent thermoreversible cryogels (for instance, those based on PVA, starch polysaccharides, or locust bean gum[12,13,16,22–24]) depended significantly on the thawing conditions. Therefore, in this study, we explored this dependency for MD-containing systems as well.

The experiments were carried out using the following temperature/time protocol: Low-concentration (1.0 g/dL) and moderate-concentration (10.0 g/dL) MD solutions were initially frozen at −12°C for 1 or 18 h, and then the samples were heated at the rates of either ~3, 0.3, or 0.03°C/min. Such a protocol allowed us to trace the influence of two factors: the thawing rate and, at a certain extent, the duration of the frozen storage on the efficiency of freeze–thaw-induced MD insolubilization. The results of the experiments on the influence of thawing rate on the cryostructuration yield are presented in Table V.

The following trends were shown: First, even a short-term freezing and frozen storage (1 h) of the samples at −12°C (as well as at −6 or −24°C, not presented in Table V) and subsequent to their fast thawing resulted in a partial cryoprecipitation of MD and the *Y* values increased with rising polymer concentration. Apparently, the formation of similar "initial" cryoprecipitates was induced by freezing the system only, which could be considered as the first stage of MD cryostructuring.

Second, extending the duration of frozen storage to 18 h caused a marked growth of the cryostructuration yield, from 13.6–22.4% for the 1-h samples to 50.1–66.6% for the 18-h samples (the entries in Table V for the respective fast-thawed specimens). This evidently meant that MD association phenomena took place at a "moderate rate" when held at −12°C, whereas at lower temperature, for example, −24°C, we detected only an insignificant increase (by 2–3%) in *Y* after the

18-h incubation and subsequent fast thawing as compared with the 1-h samples.

Third, the decrease in the thawing rate gave rise to a considerable increase in the cryostructuration yield for both 1-h frozen and 18-h frozen species. This testified to the significant intensification of the cryostructuration processes occurring in the course of thawing at a slow rate. In fact, more than a 90% yield was reached as a result of prolonged residence of the samples at subzero temperatures during the slowest thawing. Therefore, virtually nonchanging Y values obtained from freezing at temperatures -12 and -24°C as presented above (Tables I and III) were invariant due mainly to employing a slow-thawing regime. Hence, the thawing step could be considered as the second stage of MD cryostructuring. This second stage was responsible for the high efficiency of the process, when the majority of the MD association events in the unfrozen liquid microphase of the still macrofrozen system occurred during the slow increase in temperature. An analogous two-stage pattern in the "development" of cryotropic gelation was found earlier for other polymer systems: AP,[12] AP–AL mixtures,[13] and locust bean gum.[22] With these materials, it was necessary only to freeze the initial solution and then thaw it rapidly to obtain partial insolubilization of the polymer. Slow thawing or thermostating at one of the "high" subzero temperatures resulted in an "additional" growth of Y values (e.g., in the case of dilute AP solutions when the highest cryoprecipitation yield was reached at -2 and -1°C).[12]

In this study, when the 10-g/dL MD-containing system was initially frozen at -24°C for 1 h and then rapidly (at the rate of ~1.5°C/min) heated to -2°C, the cryoprecipitation yield was found to be about 56% after a 1-h incubation frozen at this "high" negative temperature and more than 96% after the 18-h incubation. For the incubation temperatures of -3 and -1°C, corresponding Y values were as follows: 38 and 57% (1 h) and 92 and 97% (18 h). Recognizing that this incubation protocol was somewhat equivalent to a very slow thaw rate and taking also into consideration the data on AP and AP/AL systems,[12,13] we were able to deduce that the v_{th} variation controls the efficiency of the cryostructuration of starch polysaccharides, in general, over a very wide range of their molecular weights.

The latter results explain why in the course of slow thawing an increase in the cryoprecipitation yield was observed. When the fast-thawing regime (3°C/min) was used, one could easily compute that the frozen system "passed through" the temperature interval from -3 to 0°C in only 1 min. At v_{th} = 0.3°C/min, this time was lengthened up to 10 min, and at v_{th} = 0.03°C/min, the system resided at these temperatures for 100 min. Apparently, this latter time (a little bit less than 2 h) was long enough for the significant growth of the cryoprecipitation yield, which is why the very slight dependency of the Y values on the frozen-storage temperatures was observed (Table I). This, certainly, was due to the use of a sufficiently slow thawing rate (0.03°C/min), which virtually smoothed over the influence of the freezing and frozen storage (at -6, -12, or 24°C) prehistory.

Thermal Properties of MD Cryostructurates

In our previous article,[12] some thermal characteristics of AP cryoprecipitates were described and the influence of a decrease in thawing rate on the increase of their fusion temperature and fusion enthalpy was demonstrated. Similar studies were also carried out with MD cryostructurates formed from the 10-g/dL initial solutions upon freezing–thawing under the same conditions as were used for the samples listed in Table V. DSC traces for these samples are shown in Figure 1, and Table VI contains data on their thermal characteristics.

For the MD cryostructurates thawed at different rates, DSC traces (Fig. 1) show that in common with AP cryoprecipitates[12] composed of the AP macromolecules only MD specimens exhibited an identical trend in their thermal behavior dependence on the v_{th} values. The slower the thawing rate, the higher was the fusion enthalpy (ΔH, square between the DSC curve and the baseline) for the respective samples, and for the sample thawed-out rapidly, virtually no cooperative thermal effect was detected at all (trace 1). However, the position of the melting points corresponding to these MD cryostructurates (temperature maxima of the traces 2 and 3) did not coincide with the order of ΔH magnitudes (see Table VI). Samples thawed with v_{th} = 0.3°C/min fused at higher temperatures as compared to the cryoprecipitates thawed 10 times slower. The reason for this experimental finding is not yet clear, so further investigation of the effect is required. In addition, it is necessary to point out the presence of the second, small area, higher-temperature endothermic peaks ("shoulders") on traces 2 and 3 at ~338K (65°C) and ~335K (62°C), correspondingly. One reason for this could be that these

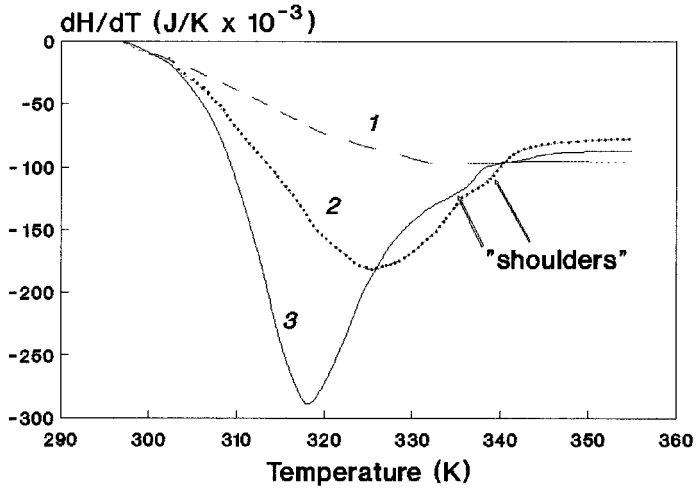

Figure 1 DSC traces for the fusion of MD cryostructurates formed from the 10 g/dL MD–water solutions, when they were frozen at −12°C for 18 h and thawed at v_{th} of (1) 3.0°C/min, (2) 0.3°C/min, or (3) 0.03°C/min.

shoulders are related to the melting of more ordered microcrystallites, possibly consisting of short AL chains. Another reason for these shoulders in DCS traces could, obviously, be the melting of retrograded polysaccharide molecules of higher (as compared to the MD main fraction of 8 kDa) molecular weight, since the presence of such "longer" chains in the content of the initial MD was recorded (see Materials), and they could contribute separately in the fusion pattern observed.

The values of the fusion enthalpies of the MD cryostructurates were different from the thermal properties of the AP cryoprecipitates described earlier.[12] Although the molecular weight of MD (enzyme-degraded starch) was considerably less than that of native AP, the ΔH values of the respective MD cryostructurates were much greater: about 11–15 J/g (Table VI) in contrast to only ~0.1–0.4 J/g for the AP samples.[12] It is thought that this significant difference is the consequence of linear oligomeric AL fragments (their polymerization degree is about 50 glucose units per a chain) present in this starch derivative. Even such short "AL macromolecules" present in MD were able to increase the fusion enthalpy of the cryoprecipitates by more than 10 times.

In general, the experimental data on the influence of thawing regimes on the efficiency of MD cryostructuration testified to the universal character of the trends observed. The tendencies revealed for MD–water solutions were the same as those described for other polymers capable of gelling as a result of cryogenic treatment of their solutions [poly(vinyl alcohol), starch polysaccharides, locust bean gum]. Typically, use of a slow as possible thawing rate led to the promotion of as-

Table VI Influence of Cryogenic Treatment Regimes on the Thermal Characteristics of MD Cryostructurates

	Conditions of the Cryogenic Treatment			Thermal Properties of the Cryostructurates	
Initial MD Concentration (g/dL)	Freezing Temperature (°C)	Frozen-storage Duration (h)	Thawing Rate (°C/min)	Fusing Temperature (°C)	Fusing Enthalpy (J/g)
10.0	−12	18	~3	ND	<0.1
			0.30	52 ± 1	10.9
			0.03	45 ± 1	14.9

ND, not determined (no possibility for the correct determination).

sociative phenomena that gave rise to increasing yield values and a more ordered structure of the cryostructurates obtained (higher orders of supermolecular packing were reflected in the higher ΔH values inherent in the fusion characteristics of the respective specimens). This certainly meant that the longer a frozen system resided at definite subzero negative temperatures during thawing, the more the possibilities for polymer–polymer interactions were realized.

CONCLUSIONS

The studies performed in this work showed that dilute solutions (0.1–5 g/dL) of the low molecular weight starch derivative MD (MW 8,000 Da) were stable at room temperature for at least 24 h, whereas their cryogenic treatment caused cryoprecipitation events, thus demonstrating the extreme cryoinstability of even dilute solutions of low molecular weight starch polysaccharides. More concentrated MD–water solutions (7.5–15 g/dL), which remained fluid (but became opaque) over the same time scale at positive temperatures, transformed into weak cryogels when subjected to a freeze–thaw cycle. The main features of MD cryostructuration turned out to be very similar to those inherent in other gelling polysaccharide systems, for example, AP or locust bean gum. Thus, one may conclude that, in general, the characteristics of freeze–thaw behavior of similar systems are rather close and governed by analogous mechanisms.

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