Study of Cryostructuration of Polymer Systems. XVIII. Freeze–Thaw Influence on Water-Solubilized Artificial Mixtures of Amylopectin and Amylose

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ABSTRACT: The effects caused by cryogenic treatment (freezing–frozen storage–thawing) of 0.5–2.0 g/dL solutions of individual starch polysaccharides, namely, amylopectin (solvents: water or $0.35M$ NaCl aqueous solution) and amylose (solvent: $0.35M$ NaCl aqueous solution), and also of 0.5 g/dL solutions of their artificial mixtures with various amylopectin/amylose ratios were studied. Freezing of these systems at temperatures from -6 to $-24°C$ for 18 h and subsequent thawing resulted in the formation of cryogelled or cryoprecipitated matter whose morphology and yield depended on the type of polysaccharide, initial polymer concentration, portion of each macromolecular component in the mixed systems, and conditions of cryogenic treatment. Comparison of the process efficiency in the mixed systems during the formation of precipitates (from storage at room temperature) and cryoprecipitates (as a result of cryogenic treatment) revealed the promoting effects of the freezing–thawing influence on polymer–polymer association and showed the presence of some synergism in the mutual interaction of these polysaccharides. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 78: 371–381, 2000

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INTRODUCTION

Dilute water solutions and gelatinized pastes of starches are well known[1–5] to be capable of structuring when subjected to cryogenic treatments (freezing–frozen storage–thawing). The formation of cryoprecipitates (microgel particles) or freeze-caused gels (cryogels) are routinely observed depending on the initial polymer concentration in similar water-solubilized polysaccharide systems, type of starch used, and the freezing–thawing conditions. Such freeze-thaw-induced structure formation in systems containing "natural" mixtures of solubilized starch polysaccharide macromolecules of amylose (AL) and amylopectin (AP) is a particular example of the more common phenomenon that was earlier termed as "cryostructuration"[6] or "cryotropic gelation."[7] This phenomenon includes the processes giving rise to the formation of both chemically linked (in the presence of respective cross-agents) or noncovalent (physical) cryogels. Starch cryogels are related to the latter case; they are heterogeneous thermoreversible gels, whose 3D structure is stabilized mainly by multiple inter- and intrachain hydrogen bonds in the junctions of the polymeric network (zones of microcrystallinity). In this respect starch (i.e., biopoly-

mer-based) cryostructurates closely resemble the much more well-studied cryogels on the basis of synthetic polymer, poly(vinyl alcohol) (PVA).[7–10]

Surprisingly, in spite of a very wide distribution of starches in nature (starch the second is most abundant plant biopolymer after cellulose) and various areas of their food and technical implementation where respective systems are subjected to cryogenic treatment, the fine details of the formation processes of starch cryoprecipitates and cryogels, as well as their properties, have not so far been researched sufficiently. This situation has arisen for two main reasons: On the one hand, starch polysaccharides have considerably more sophisticated molecular structures of the linear polycyclic AL and highly branched polycyclic AP as compared, for instance, to acyclic carbochain PVA, and, on the other hand, cryogelation behavior of starch-based systems strongly depends on the type of starch under use (plant source, conditions of a plant cultivation, etc.), which determines the ratio between the amylose and amylopectin and also the extent of interpolymer interactions. Therefore, the data obtained for different starches by different authors have turned out to be difficult to compare and, sometimes, to reproduce.

One of the possible ways to elucidate the general features of the freeze–thaw behavior of these biopolymers is "the simplification" of the systems under investigation, for instance, with the use of model systems instead of natural ones, namely, studying artificial AP and AL mixtures with exactly known proportions of each of the components. Such an approach allows one, in principle, to recognize the role of each of the "participants" in the processes under exploration, which, in our case, are directed to the research of processes of the cryo-induced association and aggregation of AP and AL macromolecules, resulting in the formation of cryoprecipitated or cryogelled matter.

Thus, for instance, in our previous work[10] on AP cryostructuration, it was shown that the freeze–thaw treatment of low-concentrated (0.01–0.25 g/dL) water solutions and dilute pastes (0.5–1.0 g/dL) of maize starch amylopectin led to cryoprecipitates whose yield and thermal characteristics (melting temperature and enthalpy) depended on the initial polymer concentration and conditions of freezing, frozen storage, and thawing. Research of the kinetic features of similar cryoprecipitation events revealed at least two stages for this process: (i) a rapid stage, when the precipitation of the portion of the dissolved AP occurred while the system was freezing, and (ii) a slower stage, the rate of which was dependent mainly on the thawing regimes or duration of the sample storage frozen at subzero temperatures. Cryoprecipitation phenomena were observed to be most extensive at temperatures 1–2 degrees below the melting point of the frozen system. All these observations and conclusions have formed the basis for the continuation of the studies, which further were performed over a somewhat wider polymer concentration range and included solutions of both starch polysaccharides, individual amylopectin and amylose, as well as their artificial mixtures. The results of these studies are described and discussed below.

EXPERIMENTAL

Materials

The following substances were used in the work without additional purification: Amylopectin from the maize starch, MW $\sim 10^6 – 10^7$ Da and one terminal group per 25 glucose units (the manufacturer data), was purchased from Fluka (Switzerland; Catalogue # 10120, Lot # 321672/1293). VIS-spectral analysis[1] of the amylopectin–iodine complex showed an amylose content of less than 1%. SEC analysis on a column (15 × 450 mm) filled with CL-Sepharose 6B resin (Pharmacia Fine Chemicals, Sweden), eluent 0.05N KOH, did not reveal the presence of the polymer fractions with MW less than ~ 140 kDa.

Amylose from the potato starch, MW ~ 150 kDa (the manufacturer's data), was purchased from Serva (Germany; Catalogue # 13470, Lot # not indicated). This polysaccharide batch had the viscosity-average molecular weight of 177,000 ± 2000 as determined by capillary viscometry with a Ubbelohde-type viscometer (20°C, 0.5N NaOH, $[\eta] = 1.44 \times 10^{-3} M_\eta^{0.93}$).[1]

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

Methods

Colloidal Solutions of the Polysaccharides

Colloidal solutions of the polysaccharides (0.5–2.0 g/dL) were prepared as follows:

AL. A weighed amount of dry polysaccharide powder was mixed with a known volume of a 0.5*N* NaOH water solution, and after the polymer dissolution, the sample was heated for 10 min with stirring on a boiling water bath. The solutions obtained were filtered rapidly under a vacuum through alkaline-stable paper filters. After chilling the solution to room temperature, neutralization to a pH value of about 7 was performed with a 0.5*N* HCl water solution. The final dilution of this neutralized stock solution was done with a 0.35*M* NaCl water solution in order to maintain the salt concentration at a constant level.

AP. Weighed amounts of dry AP powder were dispersed in a known volume of deionized water or a 0.35*M* NaCl solution, then heated with stirring on a boiling water bath for 20 min. The solutions thus obtained were filtered by passing the hot liquid through a polyamide (nylon) cloth with a mesh of 0.3 mm.

Quantitative Determination of Polysaccharide Concentrations

Quantitative determination of polysaccharide concentrations in the solutions under study was done spectrophotometrically for the corresponding iodine–polysaccharide complexes essentially in accordance to the known standard procedure.[1] Calibration plots were first obtained; AP calibration solutions were prepared using distilled water or a 0.35*M* NaCl solution as a solvent, and AL was dissolved in an alkaline medium in accordance with the above-described technique. In the course of analysis, 1 mL of the polysaccharide solution to be examined was mixed with 1 mL of 0.2*M* Na–acetate buffer (pH 5.3), and then 2 mL of a 0.002*M* I$_2$/KI solution was introduced. The visible-light spectra of the solutions thus prepared were recorded using a Model-557 UV-VIS spectrophotometer (Hitachi, Japan). Separate experiments were carried out no less than three times for every sample and the results obtained averaged. The peculiarities of the spectrophotometric analysis of the systems composed of the mixtures of both polysaccharides are discussed in the Results and Discussion section.

Freezing and Frozen Storage of the Samples

Freezing and frozen storage of the samples were performed in the chamber of an F 34 MH (Julabo, Germany) programmable precision cryostat (the accuracy of the 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 precipitates and cryoprecipitates was determined by placing samples between several layers of filter paper and pressing-out "free water" under a load of 0.1 MPa, weighing the wet samples, and comparing this with their weight (W) after drying at 105°C to a constant weight. $S_{w/w} = (W_{wet} - W_{dry})/W_{dry}$ (g H$_2$O/g of dry polymer).

RESULTS AND DISCUSSION

Cryoprecipitation of Individual Polysaccharides

When studying systems composed of water-solubilized amylose only, one encounters the problem of how to prepare the corresponding dilute solutions or more concentrated colloidal-type dispersions originating from the isolated dry polysaccharide. It is known[1] that AL, in contrast to AP, which dissolves easily through the gelatinization procedure, can be dissolved in pure water only by means of autoclaving at elevated temperature and excessive pressure. The water-solubilized AL systems thus obtained are extremely unstable upon storing at room temperature, with rapid precipitation of the polymer. Therefore, the solutions of AL could be prepared under moderate temperature conditions and normal pressure only with use of alkaline solvents with subsequent neutralization. The final systems are somewhat more stable, at least during nonprolonged storage times, and contain amounts of salt defined by the neutralization reaction.

Earlier, we investigated the freeze-induced precipitation of AP from the low-concentrated (no more than 1.0 g/dL) water solutions, which did not contain other solutes. In this work, the formation of cryoprecipitates from aqueous AL-containing systems, and also from the AP-containing ones with the addition of NaCl, was studied. The comparative results of these experiments showing the influence of the initial polymer concentration (in the range of 0.5–2 g/dL) and cryogenic treatment temperature (in the range from −6 to −24°C) on the appearance of the final systems and efficiency of cryoprecipitation of these two individual polysaccharides are summarized in

Table I Morphology of the Precipitates and Cryoprecipitates Formed from the Systems Containing AL or AP, When They Were Stored Unfrozen or Subjected to a Cryogenic Treatment for the Same Time (18 h)

Polysaccha-ride	Solvent	Initial Polymer Concentration (g/dL)	Morphology of the Precipitates and Cryoprecipitates Formed as a Result of the Sample Storing at Different Incubation Temperatures (°C)			
			+18	−6	−12	−24
AL	0.35M NaCl	0.5	Loose precipitate and transparent upper liquid layer	Loose cryoprecip-itate and transparent upper liquid layer	Loose cryoprecip-itate and transparent upper liquid layer	Loose swollen cryoprecipitate and transparent upper liquid layer
		1.0	The same	"Stratified" cryo-precipitate and transparent upper liquid layer	The same	Compact cryoprecip-itate and transparent upper liquid layer
		1.5	The same	The same	The same	The same
		2.0	The same	The same	Compact cryopre-cipitate and transparent upper liquid layer	The same
AP	0.35M NaCl	0.5	Jellylike precipitate and opaque upper liquid layer	Jellylike precip-itate and opaque upper liquid layer	Jellylike precipitate and opaque upper liquid layer	Jellylike precipitate and opaque upper liquid layer
		1.0	Transluent paste, no separation of the phases	Transluent jellylike cryogel	Transluent jellylike cryogel	Opaque very weak cryogel
		1.5	Transluent paste, no separation of the phases	Transluent jellylike cryogel	Transluent jellylike cryogel	Opaque very weak cryogel
		2.0	The same	Opaque weak cryogel	Opaque weak cryogel	Opaque weak cryogel
	H_2O	0.5	Jellylike precipitate and transparent upper liquid layer	A very weak cryogel occupying a part of the sample's bulk, transparent upper liquid layer	A very weak cryogel occupying a part of the sample's bulk, transparent upper liquid layer	A very weak cryogel occupying a part of the sample's bulk, transparent upper liquid layer
		1.0	Opaque very weak paste	The same	A weak cryogel	A weak cryogel
		1.5	Opaque paste	A weak cryogel	The same	The same
		2.0	The same	The same	The same	The same

Tables I and II, correspondingly. In all cases, the frozen samples were thawed at the rate of 0.3°C/min. The data for the water solutions of AP without additives of NaCl are presented to demonstrate the salt influence, while the data on the precipitation/gelation results for the same systems stored at room temperature are listed for the comparison end. In addition, for the cases where it was possible to isolate the resultant structurates separated from free liquid by filtration with subsequent pressing-out the unbound moisture (see Experimental), we measured the specific amount of water bound by these samples. The latter parameter is, in terms routinely used for the description of physical characteristics of polymeric gels, the swelling extent one ($S_{w/w}$), expressed in g H_2O per 1 g of dry polymer. Respective $S_{w/w}$ values are also presented in Table II.

Various morphological features were observed for the systems under consideration after their

Table II Yields of the Precipitates and Cryoprecipitates Formed from the Systems Containing AL or AP When They Were Stored Unfrozen or Subjected to a Cryogenic Treatment for the Same Time (18 h)

Polysaccharide	Solvent	Initial Polymer Concentration (g/dL)	Yields[a] of the Precipitates and Cryoprecipitates Formed at Different Incubation Temperatures (°C)				$S_{w/w}$ (g H$_2$O/g of Dry Polymer) for the Insoluble Matter Formed at Temperatures (°C)			
			+18	−6	−12	−24	+18	−6	−12	−24
AL	0.35M NaCl	0.5	89.5 ± 0.7	91.7 ± 0.6	90.4 ± 1.4	90.0 ± 3.7	4.30 ± 0.60	3.84 ± 0.54	3.70 ± 0.70	3.59 ± 0.21
		1.0	90.1 ± 1.4	94.1 ± 0.4	94.5 ± 1.0	95.5 ± 1.3	3.89 ± 0.47	3.79 ± 0.25	3.62 ± 0.52	3.52 ± 0.24
		1.5	91.8 ± 1.9	95.2 ± 1.3	95.3 ± 0.9	96.1 ± 0.4	3.47 ± 0.58	3.43 ± 0.07	3.36 ± 0.20	3.26 ± 0.41
		2.0	93.8 ± 0.9	95.8 ± 0.7	96.1 ± 1.1	96.4 ± 1.0	3.40 ± 0.49	3.44 ± 0.14	3.27 ± 0.27	3.11 ± 0.20
AP	0.35M NaCl	0.5	21.6 ± 2.2	34.5 ± 2.7	32.3 ± 2.7	30.9 ± 2.5	b	b	b	b
		1.0	17.1 ± 2.9	28.4 ± 2.2	31.0 ± 3.0	30.2 ± 1.6	b	b	b	b
		1.5	b	19.7 ± 3.0	25.5 ± 2.3	27.3 ± 3.7	b	b	b	b
		2.0	b	b	b	b	b	b	b	b
	H$_2$O	0.5	91.0 ± 2.0	92.3 ± 1.2	90.8 ± 1.6	86.4 ± 1.9	b	b	b	b
		1.0	83.0 ± 3.0	93.6 ± 1.4	89.2 ± 1.7	85.5 ± 2.7	b	b	b	b
		1.5	63.0 ± 4.0	94.1 ± 1.5	89.1 ± 3.1	83.3 ± 1.6	b	b	b	b
		2.0	54.7 ± 6.0	96.0 ± 2.0	88.2 ± 4.6	82.9 ± 3.9	b	b	b	b

[a] Insoluble matter was separated by the centrifugation at 4500 rpm for 30 min; yield values were computed as $(1 − C_i/C_m) \times 100\%$, where C_i was the initial AL or AP concentration, and C_m, the measured polysaccharide concentration in a supernatant after the centrifugation.

[b] The case when it was impossible to isolate the insoluble matter suitable for measurements (see the text).

incubation at room temperature or storage frozen. Depending on the type of polysaccharide, its initial concentration, salt presence, and thermal conditions of incubation, loose precipitates or somewhat higher swollen cryoprecipitates were formed in the AL-containing samples, whereas in the case of AP-containing specimens, the jellylike precipitates and cryoprecipitates, pastes, weak cryogels (which could be compacted by a gentle centrifugation), and even well-structured cryogels were obtained (Table I). In the case of weak AP cryostructurates, we were unable to determine quantitatively the $S_{w/w}$ values, since this was very difficult to do as the specimens appeared to be impossible to filter, or to press out the free water, because the jellylike matter passed through all our filters (footnote b in Table II). Therefore, for these systems, only the yields of the structuration processes were determined by the spectrophotometric quantification with iodine in the supernatants after centrifugation of the samples.

The solutions of linear AL showed a very pronounced ability to produce precipitates both at room temperature and at all negative temperatures (Table I). AL precipitation proceeded rather rapidly and with a high efficiency (yield >90%) even at +18°C, so, in most cases, cryogenic treatment was given to the AL systems in which precipitation had "already started." Consequently, the values of the cryoprecipitation yield in this case could be considered as a sum of the processes which occurred in the still unfrozen samples during their cooling and only subsequently in the course of freezing, frozen storage, and thawing. Obviously, due to similar mechanisms, the values of the AL cryoprecipitation yield were somewhat higher than the yields at room temperature. Hence, one may conclude that cryogenic treatment has additionally promoted the association of AL chains.

With increase in the AL initial concentration, the "compactness" of the cryoprecipitates and the yield of their formation was somewhat higher; an analogous influence was also exhibited by the decrease in freezing temperature. Denser samples, therefore, possessed lower swelling degrees, as seen from the values of $S_{w/w}$ (Table II). Inasmuch as AL is a high-crystalline polymer, which exhibits a very strong ability to retrograde (to form crystallites), the trends observed are rather obvious, since the increase in polymer concentration causes strengthening of polymer–polymer interactions. This may be achieved by the increase of the initial AL concentration in the system to be

frozen, and, so, by the decrease in freezing temperature, since at lower temperatures, the volume of unfrozen liquidlike inclusions is smaller and, hence, the higher the concentrating degree of solutes in these regions (they are known as the so-called unfrozen liquid microphase, where extensive polymer–polymer association and cryostructuration phenomena occur).[7]

The AP-containing systems yielded either jelly- and gel-like cryoprecipitates, or, rather, weak nonfungus cryogels (Table I), depending on the polysaccharide content. The presence of salt shifted considerably the concentration boundary of this gel formation to the higher values. This latter phenomenon was, apparently, associated with at least two factors: (i) an "inhibiting" influence of NaCl on the AP precipitation/gelation per se (Tables I and II, compare the data for positive temperature) and (ii) an increase in the volume of the unfrozen liquid microphase regions in the macrofrozen samples due to the additional presence of dissolved low molecular weight salt causing a decrease in the concentration of the polymer macromolecules in these unfrozen inclusions, thus weakening the effective polymer–polymer interactions.

At +18°C, increase in the initial AP concentration resulted in a decrease in the precipitation yield (earlier, it was explained by a certain growth in viscosity of the polymer solutions, thus influencing the diffusion rates of the interacting polysaccharide chains),[10] but for the cryoprecipitation processes, a similar decrease in yield took place only for the NaCl-containing systems. For the salt-free ones, there were no evident trends. Probably, this salt effect, along with other reasons, could be associated with the jellylike morphology of AP structurates and, as a consequence, with the experimental difficulties in phase separation. However, the yield of cryoprecipitates was usually higher than that for precipitates, thus demonstrating the facilitating influence of a cryogenic treatment on AP association, analogously to the AL-containing systems.

Cryoprecipitation of Artificial Mixtures of AL and AP

In view of the foregoing discussion and all the data obtained in respect to the precipitation and cryoprecipitation of individual starch polysaccharides, which were described above, it was of interest to trace the effect of the freeze–thaw influence on the mixtures of AL and AP, which, on the one hand, could serve as a model of natural solubi-
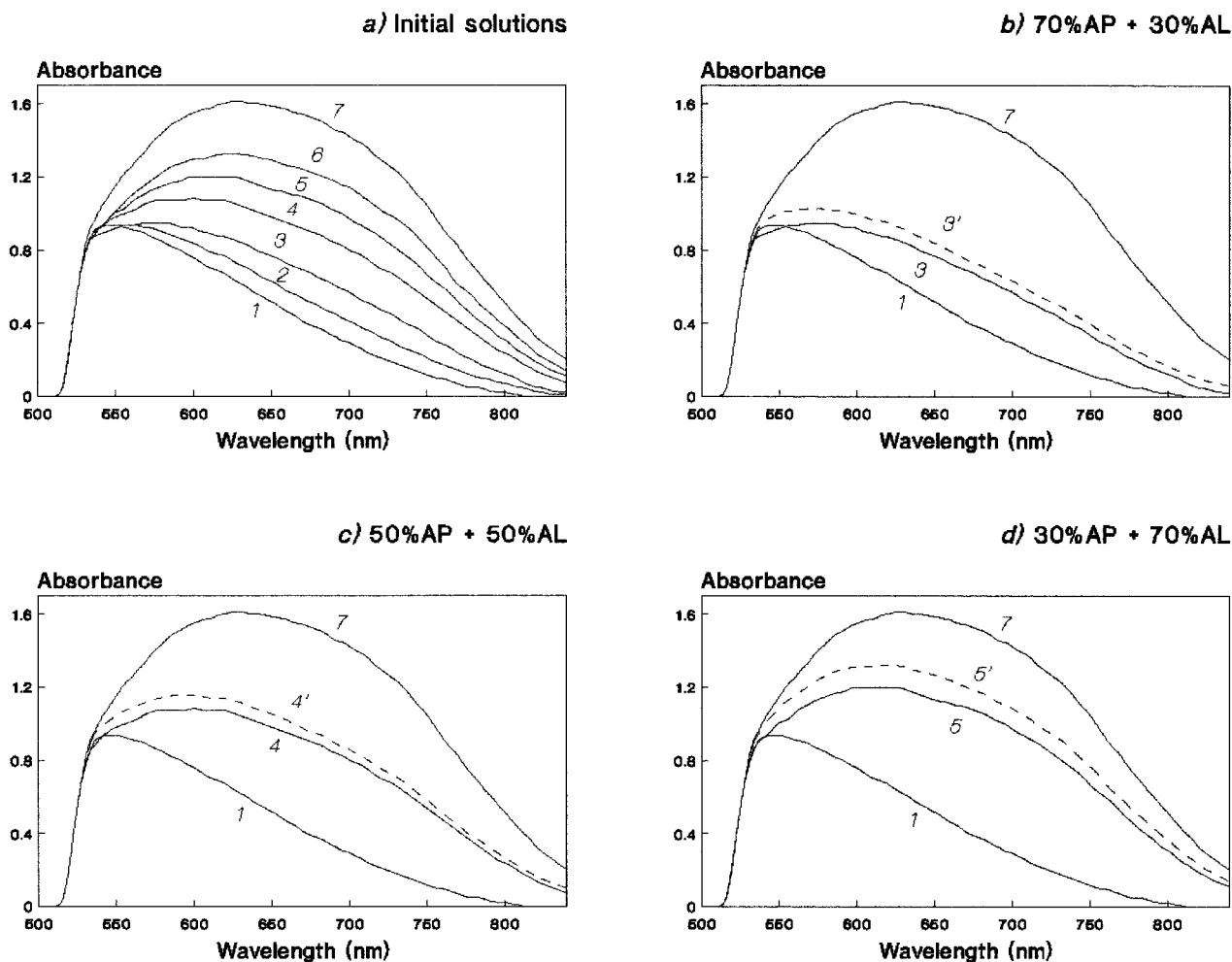
Figure 1 VIS spectra of iodine–polysaccharide complexes derived from the aliquots taken from the 0.5 g/dL solutions of individual (*1*) AP and (*7*) AL, as well as of their artificial mixtures with polymer ratios (w/w) equal to (*2*) 90/10, (*3*) 70/30, (*4*) 50/50, (*5*) 30/70, and (*6*) 10/90. [(a–d) explanations in the text.]

lized starches (see Introduction) and, on the other hand, could permit a search for possible synergism in the cryostructuraiton behavior of these biopolymers, since such a synergism in the ordinary gelation of the polysaccharides in their mixed gelatinized pastes is rather well known.[11–14] To elucidate this problem in more detail, studies of the cryoprecipitation of artificial AP–AL mixtures were accomplished. The characteristics recorded were the VIS spectra of the following systems:

(i) The iodine–polysaccharide complexes derived from the aliquots of initial solutions of the individual AP and AL;
(ii) The iodine–polysaccharide complexes derived from the aliquots of the solutions of the polysaccharide mixtures, and

(iii) The iodine–polysaccharide complexes derived from aliquots taken from the respective supernatants after centrifugation of suspensions obtained as a result of either 1-day storage unfrozen or subjected to a cryogenic treatment.

All the samples had an initial total polysaccharide concentration equal to 0.5 g/dL, and they contained NaCl in the concentration of 0.35 mol/L (see Experimental). Freezing of these samples was performed at $-12°C$ for 18 h, and thawing rates are indicated in the respective figures. Figure 1 presents the spectra for the initial solutions of iodine–polysaccharide complexes, and Figure 2 shows the changes in the spectra of the respective iodine complexes of soluble fractions induced by the precipitation (a) and formation of cryoprecipitates (b–d) un-
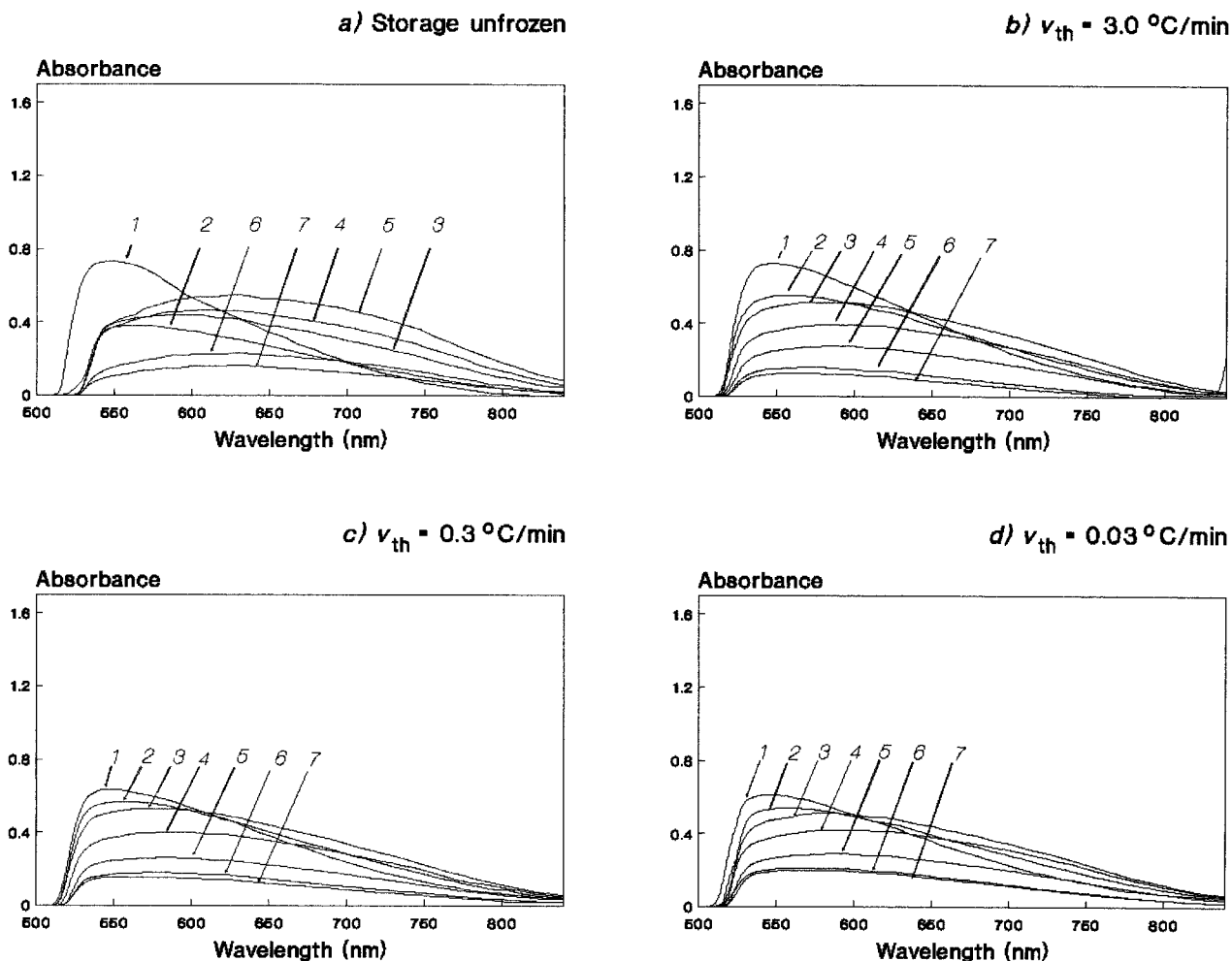
Figure 2 VIS spectra of iodine–polysaccharide complexes derived from the aliquots taken from the supernatants separated by centrifugation of the samples after their (a) storage unfrozen or (b–d) frozen for the same length of time and then thawed with various rates (labeling of the traces is the same as in Fig. 1).

der identical conditions of sample freezing, but thawed at different rates. In other words, these spectra allowed us to observe the relative changes of the amount of "residual" polysaccharides remaining in a soluble state after the precipitation or cryoprecipitation events have occurred. To obtain a better comparison of the decrease in concentration of the polysaccharides remaining in a dissolved state after the samples' incubation in either frozen or unfrozen states, the "Absorbance" axes in Figures 1 and 2 are given in the same scale.

The freeze–thaw behavior of these systems, when various defrostation regimes were used, was examined because the data obtained earlier showed a certain influence of the ν_{th} values on AP cryoprecipitation dynamics and efficiency (yield). In this earlier work, the slower the thawing, the higher was the amount of insoluble matter

formed.[10] This was due to the prolonged enough residence of the samples at the subzero temperature region in the course of their slow thawing, where the processes of association of AP polysaccharide chains proceeded with the highest intensity, whereas during fast thawing, the corresponding residence time was short and insufficient for effective kinetically controlled polymer–polymer interactions to generate significant amounts of insoluble matter.

VIS spectra of iodine complexes of individual AP and AL (traces *1* and *7,* correspondingly, in Fig. 1) differed significantly, as this is well known,[1] but the spectra of the complexes derived from the AP/AL mixtures were not simple mechanistic summations of AP–iodine and AL–iodine individual spectra. These "mixed" spectra deviated from the additivity (see below), because, ap-

parently, three kinds of the polysaccharide–polysaccharide-iodine complexes can be formed, namely, AP–iodine, AL–iodine, and AP + AL–iodine, upon the introduction of the I$_2$/KI reagent into the mixed solutions of these biopolymers. Although the trend of the incompatibility (more exactly, limited compatibility) of AP and AL in similar mixed systems is known,[15] clear evidence of the definite interactions between these two polysaccharides has also been described.[11,12] The data of Figure 1(b–d) testified that similar mixed AP + AL associates could, along with individual polysaccharides, form respective complexes with iodine. Dashed lines in these graphs depict the "apparent" spectra, which have been computed by the geometrical addition of partial contributions of the absorption of AP–iodine and AL–iodine complexes to the resultant spectra in accordance with the content of each polymer in the respective mixtures, that is, in the solutions with AP/AL ratios of 70/30, 50/50, and 30/70 (traces 3′, 4′, and 5′, correspondingly). The spectra of the complexes for the individual AP and AL are given in Figure 1(b–d) for the sake of comparison. The data for the systems with AP/AL ratios of 90/10 and 10/90 were also obtained (see below), but are not presented in the figures.

One may see that in all the cases indicated the real spectra showed lower adsorption values as compared to the computed spectra, thus demonstrating an interfering influence of AP–AL interaction for the formation of iodine complexes. The absorption maxima for the mixed systems shown in Figure 1 and the ratios between the maximum absorption values of the "computed" and real spectra were found to be different for various initial AP and AL content in these mixtures. The respective absorption values differed by the factor of 5.4% for the 90/10 system, 7.7% for the 70/30 mixture, 8.9% for the 50/50 sample, 9.8% for the 30/70 specimen, and 12.3% for the 10/90 AP/AL mixture. Therefore, we were unable (without incurring significant error) to use corresponding correlation coefficients for the quantitative determination of a relative amount of the polysaccharides in supernatants obtained after separation of the precipitates or cryoprecipitates. Moreover, it was unreasonable to hope that the ratios between the polysaccharides in the mixed sols should remain the same as the initial ones after cryogenic processing of the samples under the conditions used. That is why the data of Figure 2 can be discussed only on a qualitative level.

It was observed that a considerable decrease in the intensity of the absorption curves was due to lowering the concentration of the dissolved individual AP and AL after storing the samples in an unfrozen state [cf. traces 1, as well as 7, in Figs. 1(a) and 2(a)] and, especially, cryogenically treated [cf. traces 1 and also 7 in Figs. 1(a) and 2(b–d)]. The data of Table II show this trend quantitatively. However, clear differences were recorded in the character of the results obtained for these biopolymers. The highest reduction in the absorbance was, naturally, inherent in the AL-containing individual system, being aged unfrozen [trace 7 in Fig. 2(a)] or frozen–thawed [traces 7 in Fig. 2(b–d)]. Analogous AP samples contained higher residual polymer concentration in the corresponding supernatants (traces 1 in Fig. 2); this is also seen from the data of Table II. Note that the spectrum of iodine–polysaccharide complexes derived from the AP-containing supernatants of the samples stored unfrozen [trace 1, Fig. 2(a)] and thawed quickly [trace 1, Fig. 2(b)] were virtually identical. This testified that the room-temperature incubation of AP dissolved in the medium of 0.35M NaCl and cryogenic treatment of the same system using a fast thawing rate gave rise virtually to the same efficiency of the insoluble matter formation. Only slower thawing rates resulted in the formation of an additional amount of AP cryoprecipitates. Thus, the slower the thawing process, the lower the "residual" AP concentration, that is, the greater was the portion of the initially dissolved AP macromolecules which transformed into insoluble cryoprecipitates. This latter tendency was sufficiently obvious (see above for the speculations on the influence of thawing rate on the duration of sample residence at subzero temperatures during thawing) and confirmed the conclusions drawn earlier[10] for this polysaccharide.

At the same time, in contrast to the AP-containing solutions, we discovered that after cryogenic processing the residual concentration of soluble AL was the lowest for the conditions of fastest (3.0°C/min) thawing [trace 7, Fig. 2(d)]. This concentration was 1.44 times smaller than for the samples thawed with the rate of 0.3°C/min [trace 7, Fig. 2(c)] and 1.60 times smaller than for the samples thawed at the slowest rate [0.03°C/min; trace 7, Fig. 2(d)]. This reversal (as compared to AP systems) dependence of the AL cryoprecipitation efficacy on the thawing rate is rather unforeseen and the reasons for it are still not clear. It seems probable that this effect could be associated with the unusual kinetics of the freeze-induced crystallization (retrogradation) of the high-crystalline AL.

Without doubt, these features of cryoprecipitation behavior of individual AP and AL could contribute to the unequal contributions to cryoprecipitation processes of the polysaccharides in their mixed systems. In this respect, the trends observed for the above-mentioned synergism in the mutual influence of AP and AL on the formation of coprecipitates and cocryoprecipitates were as follows:

(i) Certain differences between the tendencies in precipitation and cryo-induced precipitation in the water-solubilized artificial mixtures of AP and AL were found. If traces *2–6* in Figure 2(a) are compared with those in Fig. 2(b–d), one may see that the order of decrease in intensity of the absorption maxima at the respective wavelengths was completely inverted ($6 < 5 < 4 < 3 < 2$) after freezing–thawing as compared to the initial order ($6 > 5 > 4 > 3 > 2$). In other words, the higher the AL portion in the mixed system to be frozen–thawed, the greater was the amount of mixed cryoprecipitates formed. On the other hand, for the precipitation at +18°C, we observed that a higher solution resistance was unexpectedly inherent in the mixture containing 30% of AP and 70% of AL [trace *5,* Fig. 2(a)]; somewhat less resistant was the 50/50 system (trace *4*) and, only then, the 70/30 and 90/10 samples (traces *3* and *2*). This obviously pointed to the absence of monotonic dependence of the storage resistance of AP/AL-mixed solutions at room temperature on the ratio between similar polymers in these systems.

(ii) It is commonly recognized for the cryostructuration phenomena resulting in the formation of noncovalent (thermoreversible) cryogels or cryoprecipitates (every "grain" of which represents little cryogel particle) that moderate freezing of the solution of a gelling agent gives rise to the promotion of polymer–polymer association events due mainly to the cryoconcentrating processes (we have already discussed this point above). Therefore, the efficiency of similar cryogenically induced gelation or precipitation turned out, as a rule, to be higher than for the same processes at positive temperatures (this was the most evidently

shown for the water–PVA systems[7,16–18] and recently for the water–AP dilute solutions[10] as well). However, for the water-solubilized artificial mixtures of starch polysaccharides, such a trend was not detected for all AP/AL ratios. Higher total polymer concentrations in the cryogenically produced sols (i.e., lower yield of insoluble fraction) were determined in the case of 90/10 and 70/30 mixtures as compared to the sol-fraction in the samples incubated unfrozen (traces *2* and *3* in Fig. 2), thus showing a certain "protecting" effect of high concentrations of AP in its mixtures with AL in respect to the cryoprecipitation of the latter polysaccharide.

(iii) When analyzing the spectra in Figure 2 thoroughly, it was also found that the decrease in the AL content in the residual sol-fraction after the incubation at a positive temperature or cryogenic treatment of the solutions of artificial AL/AP mixtures was considerably higher than was the related reduction in AP concentration, since the reduction of VIS-absorbance in the vicinity 600–700 nm (AL–iodine complexes) was significantly stronger than such a reduction in the vicinity of 500–600 nm (AP–iodine complexes). Thus, the appearance of the spectra in Figure 2 turned out closer to that of the pure AP–iodine complexes because the very strong absorption of the iodine–AL complexes at the wavelengths of 600–700 nm progressively disappeared after storage unfrozen [Fig. 2(a)] or freezing–thawing [Figs. 2(b–c)], thus demonstrating a very low resistance of solubilized AL, in general, not only in the individual systems, but also in the mixtures with AP. Among the systems examined, greater amounts of the polysaccharides were precipitated after the cryogenic treatment in the case of 10/90 and 30/70 AP/AL mixtures, since the total polymer concentration was lower in corresponding sols (traces *6* and *5* in Fig. 2). This obviously pointed to the coprecipitative ability of high AL amount in its mixtures with AP.

Therefore, based on the data obtained, we were able to draw the conclusion that synergism in the mutual influence of AP and AL was apparently exhibited both in the above-mentioned "protec-

tive" action of AP in respect to AL cryoprecipitation in the mixtures with high amylopectin content and, *vice versa,* in the coprecipitative action of AL in respect to AP in the polysaccharide artificial mixtures with a high amylose portion. Interestingly, extreme dependencies of the gel strength on the AP/AL ratios was reported[13] for the concentrated multicomponent hydrogels formed from the artificial mixtures of these biopolymers, the maximum points lying in the region of polymer ratios equal to 10/90–20/80, that is, over the same range where the most pronounced cocryoprecipitative effects of AL were observed in this research.

CONCLUSIONS

The present work was part of systematic investigations in the field of the "study of cryostructuration of polymer systems," which were performed by the authors throughout recent years. These investigations span a wide set of gelling systems and include both synthetic and natural polymers. The results obtained for natural polymers could be useful not only for the better understanding of the molecular mechanisms of the processes that occurred, but also for the choice of optimum freezing–thawing regimes of cryogenic treatment of real biopolymer-containing systems (biological fluids and tissues in the course of their cryoconservation, foodstuffs, or biochemical preparations during their frozen storage, etc.). The experimental results of the given research showed that, in the case of initial systems containing, at least, two macromolecular components possessing different association (crystallization) abilities, and depending on the composition and concentration of such gelling agents, as well as on the conditions of cryoprocessing, one may obtain final cryostructurates of various morphology (jellylike matter, cryogels, cryoprecipitates). For mixed systems similar to the water-solubilized artificial mixtures of AP and AL, a considerably more sophisticated freeze–thaw–precipitation pattern was demonstrated to be inherent in these systems as compared to the behavior of individual starch polysaccharides or simpler synthetic polymers like PVA. We believe that subsequent studies of the cryostructuration processes in other mixed gelling systems will reveal additional interesting aspects of the of cryotropic gelation phenomena. Similar studies are in progress now.

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