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Aggregation of Amylose in Aqueous Systems: The Effect of Chain Length on Phase Behavior and Aggregation Kinetics

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ABSTRACT: Nearly monodisperse amyloses having a wide range of chain lengths have been enzymically synthesized in vitro. The rate of aggregation from aqueous 0.2–5.0% solution and the physical form of extensively aggregated material have been studied as a function of chain length. Aggregation (monitored by turbidity) is found to be most rapid for chain lengths of ~100 residues in agreement with previous results (Pfannemüller, B.; Mayerhöfer, H.; Schulz, R. C. *Biopolymers* 1971, *10*, 243–261) for initial aggregation rates in 0.1% solution. Amyloses having chain lengths of <110 residues are found to precipitate from aqueous solution. Both precipitation and gelation occur for chain lengths of 250–660 residues. For longer chains (>1100 residues) gelation is found to predominate over precipitation. The variation of phase behavior and aggregation rate with chain length is rationalized on the basis of ordered polymer–polymer associations involving chain segments of typically less than 100 residues.

Introduction

Amylose is an essentially linear polysaccharide composed of α-(1→4)-linked D-glucose units. It occurs naturally in starch granules together with amylopectin, an α-(1→4)-linked D-glucan which is highly branched through additional α-(1→6) linkages. Starch granules contain densely packed polysaccharide and include regions of long-range ordering (crystallinity) which are thought to involve crystallization of amylopectin branches.[1,2] Amylose is considered to be present predominantly in the amorphous phase of starch granules.[1,2] Heating aqueous dispersions of starches causes granules to expand and amylose to be leached from the amorphous phase.[1,2] Subsequent aggregation of the amylose is thought to be responsible for the formation of viscoelastic pastes or, at higher concentrations, gels after cooling hot aqueous starch dispersions.[3,4]

The properties of amylose in aqueous solution have been widely studied.[1] Results from light-scattering experiments[1,5] show that amylose in aqueous solution has the properties of a non-free-draining coil with calculated unperturbed values of the characteristic ratio and persistence length of ~4.5 and 12.1 Å, respectively.[1,5] The aggregation of amylose in dilute (<0.5% w/v) aqueous solutions has been studied by a number of workers principally using light-scattering methods.[6–9] A major finding of such studies is that the rate of aggregation of amylose is strongly dependent on the chain length (degree of polymerization, DP). With increasing chain lengths, amylose aggregation is first slow (for DP < 50), increases rapidly to a maximum rate[9] (at DP ~ 80), and then becomes steadily slower until, for DP > 2000, only slow and limited aggregation is observed in dilute solution.[6,7] Explanations for this unusual behavior have invoked the presence of regions of rigid helical conformations (of ~80 residues[9] or 40–900 residues[8]) within chains, but there is no convincing experimental evidence for this assertion, and the relatively short (for polysaccharides[10]) persistence length of amylose in solution[1,5] would seem to preclude significant rigid helical segments.

In the present work we have extended these previous studies by examining the influence of amylose chain length on aggregation behavior in aqueous solutions of higher concentrations (0.5–5.0% w/v) than have hitherto been studied systematically. As the amylose content of starches is typically 20–25%, this concentration range corresponds to concentrations of starch (~2–20% w/v) which are used to form pastes or gels. Although amyloses from different botanical sources show molecular weight variations, they are all polydisperse (DP_w/DP_n = 1.3–3.4)[11,12] and are therefore unsuitable for detailed studies of chain length effects. Furthermore, many natural amyloses are not completely linear due to a small proportion of α-(1→6) branching linkages.[1,13] However, it is possible to synthesize enzymatically strictly linear amyloses of very narrow molecular weight distribution in vitro by using the enzyme phosphorylase.[14]

In this report, we describe the phosphorylase-mediated synthesis of amyloses of narrow molecular weight distribution and their aggregation behavior in aqueous solutions. A preliminary account of part of this work has been reported.[15] In the following two reports, we describe rheological studies on amylose gels[16] and discuss the molecular mechanisms underlying amylose aggregation.[17]

Materials and Methods

Maltoheptaose was obtained from Boehringer and glucose 1-phosphate (dipotassium salt) from Sigma. Phosphorylase activity was assayed by the method of Lee,[18] amylase activity by the method of Fischer and Stein,[19,20] and phosphate according to Lowry and Lopez.[21]

Purification of Potato Phosphorylase.[22–24] Potatoes (6 kg) were scrubbed and sliced, soaked in 0.7% sodium dithionite/0.7% sodium citrate for 1 h,[23] washed with ice-cold water, suspended in minimum 0.1 M tris(hydroxymethyl)methylamine/citrate buffer (pH 7.0), and liquidized. The crude extract was kept at 329 ± 1 K for 10 min to destroy α-amylase activity[24] and then centrifuged (4200*g*, 1 h). The supernatant was made up to 100 g/L ammonium sulfate, the precipitate was removed, and the ammonium sulfate concentration was adjusted to 250 g/L to precipitate phosphorylase.[24,25] After dissolution in 20 mM imidazole (20 mL) and dialysis against 20 mM imidazole, the solution was centrifuged (13 000*g*, 30 min) and the supernatant applied to a DEAE Sepharose column (200 mL).[22] After a washing with 20 mM imidazole (800 mL), phosphorylase was eluted with a sodium chloride

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Table I
Preparation Conditions and Chain Length Characterization of Synthetic Amyloses

maltoheptaose, mg	anhydrous dipotassium glucose 1-phosphate, g	incubation time, h	amylose yield,[a] g	% age yield[b]	intrinsic viscosity in dimethyl sulfoxide, mL/g	DP[c]	% conversion of glucose 1-phosphate to amylose[b]
300	5	0.75	1.66	97		40[d]	71
100	15	1.5	0.88	95	11.0	65 (60[d])	13
100	7.5	0.33	1.05	81	13.4	90	36
100	7.5	0.67	1.44	92	15.1	110	44
50	7.5	1.0	1.62	90	26.3	250	49
50	7.5	2.0	1.97	92	29.3	300	59
60	10	3.0	2.93	85	35.8	400	71
11	12.5	4.0	0.85	82	50.3	660	17
11	12.5	6.0	1.55	90	70.5	1100	29
11	12.5	28	3.28	82	124	2550	67
11	12.5	32	4.08	93	131	2800	73

[a] Anhydrous yield: water content of isolated amyloses was found to be 5–8%. [b] Based on DP value. [c] Mean of values derived from the Mark–Houwink–Sakurada parameters of Everett and Foster[28] and Banks and Greenwood.[31] [d] Determined by ^{1}H NMR.[32]

gradient (100 mL). Fractions containing phosphorylase activity had no detectable amylase activity and were pooled and precipitated by addition of ammonium sulfate to 700 g/L. The supernatant was removed by centrifugation (13000*g*, 90 min), and phosphorylase (~2000 units[18]) was stored as the precipitate suspended in a minimum amount of saturated ammonium sulfate. Such preparations retained activity for several weeks on storage at 277 K.

Phosphorylase-Catalyzed Amylose Synthesis. Potato phosphorylase (~200 units) prepared as above was dialyzed against 50 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 6.3) to effect solubilization and then assayed.[18] Maltoheptaose and phosphorylase solution were dissolved in 50 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 6.3) (250 mL) at 310 K. Glucose 1-phosphate was dissolved in the same buffer (250 mL), and the solutions were mixed and incubated at 310 K. Quantities of maltoheptaose and glucose 1-phosphate and the incubation times used are shown in Table I. At the end of the incubation period, an aliquot was removed for immediate phosphate analysis,[21] and the remaining solution was heated to 368 K for 30–40 min under vigorously bubbling nitrogen. Precipitated protein was immediately removed by filtration, and amylose was isolated by one of two methods depending on chain length. For anticipated chain lengths of >500 units the amylose solution was cooled to 70 °C, 1-butanol added to 10% v/v, the mixture slowly cooled, and the resulting precipitate collected, washed with ethanol (3×) and diethyl ether (3×), and air-dried. For anticipated chain lengths of <500 units, the hot amylose solution was mixed with 4 volumes of ethanol, the precipitate collected, resuspended in cold water (to solubilize unused glucose-1-phosphate), and filtered. The solid was washed with ethanol (3×) and diethyl ether (3×) and air-dried.

Characterization of Synthetic Amyloses. Molecular weights of synthesized amyloses were assessed by both phosphate analysis of incubation mixtures and intrinsic viscosity measurements of isolated amyloses in dimethyl sulfoxide. As one molecule of phosphate is produced for each glucose unit added to a synthetic amylose chain, phosphate analysis can be used to derive amylose molecular weights by assuming a monodisperse population of chains and that all the maltoheptaose primers are utilized. To assess the molecular weight distribution of synthetic amyloses, samples (~10 mg) were dissolved in 0.25 N KOH and neutralized with HCl, and gel permeation chromatography was carried out in 20 mM ammonium hydrogen carbonate buffer at 25 °C on Sephadex G-50 (two 1.5 × 95 cm columns in series) for chain lengths < 250 and Sephacryl S-400 (2.6 × 100 cm column) for chain lengths > 250. Fractions (5 mL) were collected by elution at 20 mL/h with the same buffer and analyzed by incubation with amyloglucosidase followed by glucose assay.[26] Representative chromatograms are shown in Figures 1 and 2; by this method all preparations were shown to have a narrow molecular weight distribution. Comparison of Figures 1 and 2 with previous chromatograms[27] suggests that polydispersity values (M_w/M_n) are less than 1.10 for all preparations except those of DP > 2000 for which M_w/M_n was estimated to be between 1.10 and 1.15.

Intrinsic viscosity values were determined[10] by using a Contraves Low Shear 30 viscometer. Several values of the exponent (*α*) and the preexponential factor (*K*) in the Mark–Houwink–Sakurada equation have been proposed for amylose in dimethyl sulfoxide.[28-31] Comparison of amylose molecular weights determined by phosphate analysis and intrinsic viscosity shows that the *α* and *K* values proposed by Cowie[29] (0.87 and 0.001 25, respectively) lead to an overestimation of molecular weight for all samples, and the parameters proposed by Burchard[30] (*α* = 0.82, *K* = 0.003 95) underestimate the chain lengths for samples of DP > 2000. Phosphate analysis results were, however, consistent with the *α* and *K* values proposed by Banks and Greenwood[31] (0.70

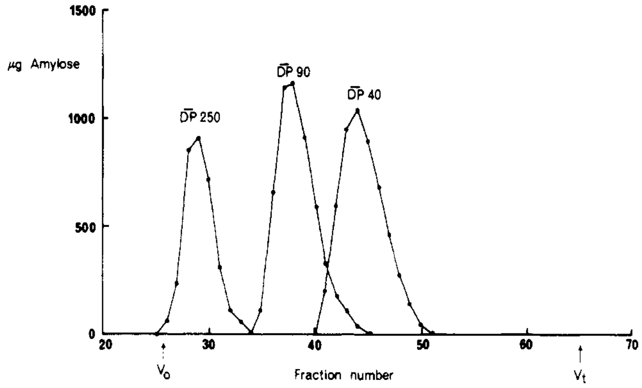

Figure 1. Gel permeation chromatography of three synthetic amyloses on Sephadex G-50 obtained in separate experiments. Chain lengths were determined viscometrically or by NMR end-group analysis (Table I) and are given above individual chromatograms. Samples were obtained by dissolving amylose (~10 mg) in 0.25 M potassium hydroxide (~0.5 mL), neutralizing the mixture with 1.0 M hydrochloric acid, and diluting it to 3 mL with 20 mM ammonium hydrogen carbonate. Sample recoveries were better than 95%.

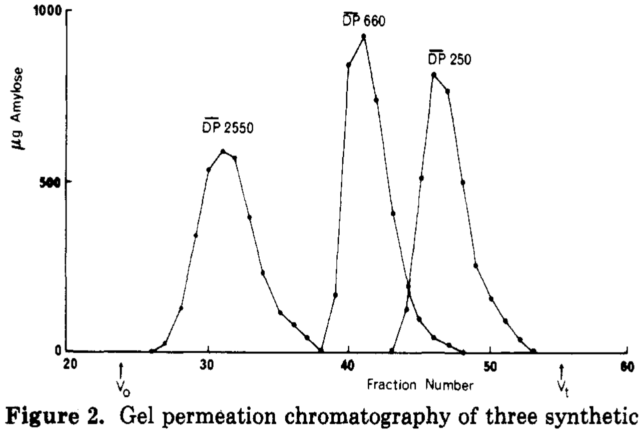

Figure 2. Gel permeation chromatography of three synthetic amyloses in separate experiments on Sephacryl S-400 having viscometrically determined chain lengths as shown. Sample preparation was as for Figure 1, and recoveries were better than 95%.

and 0.0151, respectively) and Everett and Foster[28] (0.64 and 0.0306, respectively). The molecular weight values given in Table I are the mean of values obtained by using the Mark–Houwink–Sakurada parameters due to Banks and Greenwood[31] and Everett and Foster.[28] Molecular weight values for chains of less than 80 glucose units were determined by ^{1}H NMR end-group analysis.[32]

Studies of Aggregation Behavior. To obtain aqueous solutions of synthetic amyloses, appropriate mixtures of amylose and water were sealed in a test tube under a nitrogen atmosphere, left to hydrate for several hours, and then heated at 433–443 K for 10–20 min to yield optically clear solutions. Comparisons of gel permeation chromatograms after one and two such heat treatments showed no apparent degradation for a variety of synthetic amyloses, although hydrolysis was apparent for some samples held for >30 min at 443 K. The onset of significant degradation was found to coincide with the appearance of a light-brown coloration of the solution. Amylose solutions produced at 433–443 K were cooled to 343–353 K, the sealed test tubes were opened, and solutions were subjected to various cooling regimes (see Results and Discussion). Turbidity measurements were obtained at 700 nm for a 1-cm path length by using a Beckmann DU7 spectrophotometer.

Results and Discussion

Preparation and Characterization of Synthetic Amyloses. A series of amyloses ranging in size from DP 40 (molecular weight ~6500) to DP 2800 (molecular weight ~454 000) have been synthesized by using potato phosphorylase (Table I). These preparations have been shown to be of narrow molecular weight distribution (Figures 1 and 2) and hence are suitable for studies of the effect of chain length on aggregation behavior in aqueous solutions. As found by other workers[27] long chain length synthetic amyloses (DP > 2000) have slightly broader molecular weight distributions than shorter chain materials presumably due to the increased reaction times (Table I). Syntheses were carried out at up to 73% conversion of monomer (glucose-phosphate) to polymer (amylose); higher conversions resulted in broad molecular weight distributions. This may be ascribed to a partial reversal of the enzyme reaction (shown below) due to an increase in the phosphate/glucose 1-phosphate ratio:

$$[\text{amylose}]_n + \text{glucose 1-phosphate} \xrightleftharpoons{\text{phosphorylase}} [\text{amylose}]_{n+1} + \text{phosphate}$$

Phase Behavior of Synthetic Amyloses in Aqueous Solutions. To obtain optically clear solutions of synthetic amyloses, heating to 433–443 K for 10–20 min was found to be necessary. This treatment did not cause any macromolecular degradation as evidenced by gel permeation chromatography. On cooling aqueous solutions of synthetic amyloses, phase changes occurred for all chain lengths in the concentration range 0.2–5.0%. The phase behavior was found to be dependent primarily on chain length and concentration, with cooling rate effects also being important in some cases.

Short-chain amyloses (DP < 110) were found to precipitate at all concentrations up to 5.0% upon cooling hot aqueous solutions. Amyloses of DP40 and 65 gave fine, dense precipitates whereas precipitates of DP90 and 110 were less dense, occupying a greater volume fraction of solution. Analysis of supernatants showed no detectable amylose remained in solution.

Long-chain amyloses (DP > 1100) were found to form gels on cooling solutions of >1.0%. On standing, these gels exuded a small amount (<5% by volume) of water. For starting concentration of <0.8%, a gel phase which did not occupy the total solution volume was slowly formed. Analysis of the liquid phase showed no detectable amylose. From measurement of the volumes occupied by the solvent and gel phases, the gel phase was estimated to contain 1.0–1.5% w/v amylose.

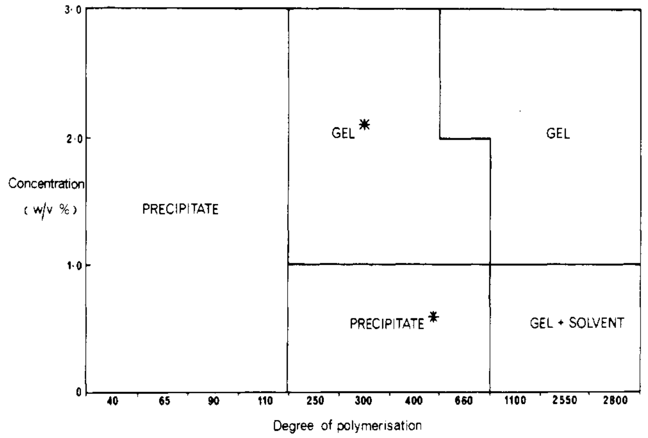

Figure 3. Partial phase diagram showing the effect of cooling aqueous amylose solutions from 433 to 298 K. In the region marked GEL*, gelation can be accompanied by precipitation particularly following slow cooling and for low concentrations and short chain lengths within the range shown. In the region marked PRECIPITATE*, gel lumps also tend to occur particularly for higher chain lengths (DP = 400 and 660) and following rapid cooling.

For chains of intermediate length (250 < DP < 660), both gelation and precipitation were observed depending on concentration and cooling rate. At concentrations of <0.8% precipitation predominated, although gel lumps were also apparent for chain lengths of 400 and 660, particularly following rapid cooling. At higher concentrations (up to 3.0%), gels occurred following rapid cooling (433 → 298 K in <10 min) whereas precipitation together with gel lump formation was favored at slower cooling rates (433 → 298 K in 30–60 min), particularly for the shorter chains within the range.

In general, therefore, precipitation is favored by shorter chain lengths, lower concentrations, and slower cooling rates and occurs for DP < 1100; gelation can occur for DP > 250 and is favored by longer chain lengths, higher concentrations, and faster cooling rates. These effects are depicted schematically in Figure 3. Agitation of solutions during cooling caused a greater tendency to precipitation compared with the behavior shown in Figure 3.

A reasonable explanation for these observed effects can be formulated based on the relative importance of chain alignment and cross-linking in cooled aqueous amylose solutions. Thus, for long chains, extensive cross-linking occurs which results in the formation of a macromolecular network eventually resulting in gelation. As the cross-linking mechanisms in polysaccharide gelation usually involve the formation of ordered structures via weak noncovalent interactions[33] (in this case presumably hydrogen bonding and hydrophobic interactions), stability can only be achieved by cooperative interactions along extended lengths of chains.[33] If the chain length over which these interactions occur is substantially shorter than the total chain length, then more than two regions within a single chain could be involved in separate interactions, thereby leading to a cross-linked network structure. Conversely, if the total chain length is not substantially longer than the interacting chain length, then extensive cross-linking will not occur and chain alignment will predominate, a process which, if followed by lateral aggregation, would eventually lead to precipitation.

Our observations show that network formation occurs for DP > 250 at >1% concentration and suggest that little cross-linking occurs for amyloses of DP = 40 and 65. The

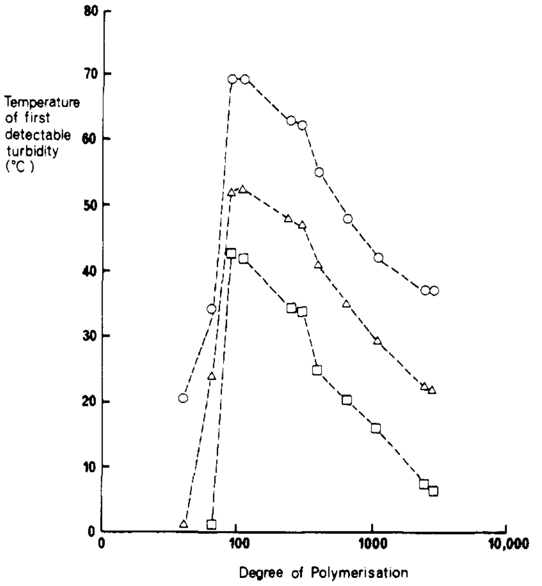

Figure 4. Effect of the amylose chain length on visually assessed turbidity onset temperatures for 2.0% (○), 1.0% (△), and 0.5% (□) w/v aqueous solutions cooled at 0.5 K/min from 343 K.

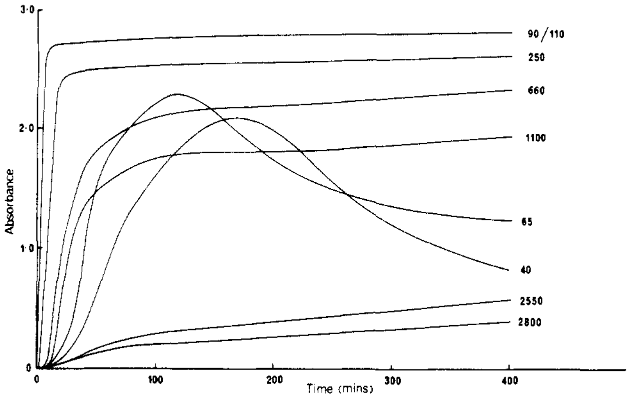

Figure 5. Development of turbidity with time for 1.5% w/v aqueous amylose solutions as a function of chain length. Amyloses with chain lengths of ~300 and 400 residues showed behavior intermediate to that of ~250- and 660-residue chains.

difference in the density of precipitates of DP 65 and 90 suggests that cross-linking may occur to a limited extent for DP 90, although chain alignment (leading to precipitation) appears to be the dominant process. The cooperatively interacting length which gives rise to an ordered chain structure in this system can therefore be significantly less than 90 residues. This is consistent with the results of Jane and Robyt,[34] who found the acid-resistant portion of aggregated amylose (presumably derived from regions of ordered chain structure) to contain chains 25–50 residues in length.[34]

For amyloses of 250 < DP < 660, both chain alignment and cross-linking can occur (Figure 3), with the latter being favored by higher concentrations and rapid cooling. Increased cooling rates would be expected to lead to increased nucleation rates, and hence cross-linked structures may be kinetically trapped whereas slower cooling might lead to greater annealing of the structure and favor chain alignment. Increases in concentration lead to greater interchain contact and hence would also be expected to favor cross-linking over chain alignment. The observation of precipitation for chain lengths of <660 residues in dilute solution suggests that, under these conditions, ordered chain structures may persist over more than one hundred residues, particularly following slow cooling.

Chain Length Effects on Amylose Aggregation Kinetics. Although cooling hot aqueous amylose solutions has been shown to lead to a variety of final states (e.g., precipitate and/or gel) depending on chain length and concentration (Figure 3), all systems studied showed increases in turbidity that preceded and/or accompanied the observed phase changes. As a qualitative probe of the effect of chain length on aggregation kinetics, we have therefore studied the turbidity of aqueous amylose solutions cooled from 433 K as a function of concentration and chain length. Two types of experiment have been performed: (a) cooling hot solutions at a controlled rate while observing the temperature at which turbidity first occurs and (b) quenching hot solutions to a fixed temperature and monitoring the evolution of turbidity with time. The results of a series of experiments of type a are shown in Figure 4. In these experiments, amylose solutions (prepared at 433 K) were cooled to 343 K, held at that temperature for 5 min, and subsequently cooled at a rate of 0.5 K/min. From Figure 4 it can be seen that for a given chain length turbidity was apparent earlier in the cooling regime (i.e., at higher temperatures) with increasing concentration. For each concentration studied, however, the temperature of turbidity onset had the same dependence on chain length, i.e., increasing up to DP = 90/110 and steadily decreasing for DP > 250.

The same series of experiments was also carried out with cooling rates of 0.15 and 1.0 K/min.[15] For all combinations of concentration (0.5%, 1.0%, 2.0%, and 3.0%) and cooling rate, the same dependence on chain length was observed; i.e., a series of temperature vs chain length plots essentially parallel to those shown in Figure 4 were obtained. Turbidity onset temperatures were found to be markedly influenced by the cooling rate. For cooling rates of 0.15 and 1.0 K/min, turbidity was observed 5–15 K higher and 10–20 K lower, respectively, than for a cooling rate of 0.5 K/min (Figure 4).

The effect of amylose chain length on turbidity development at 298 K after quenching from 353 K is shown in Figure 5. The decrease in turbidity for amyloses of DP = 40 and 65 following the initial rise was found to be due to settling of precipitate below the path of the spectrophotometer beam. Amyloses of DP = 90 and 110 also formed precipitates, but negligible settling occurred in the narrow (1-mm) cells employed, lending further support to the postulation of a low level of cross-linking for these amylose chain lengths. For amyloses of DP > 90, both the initial rate of turbidity development and the absorbance value after 400 min were found to decrease with increasing chain length (Figure 5). Following the initial rise in turbidity (0–100 min), the rate of subsequent turbidity increase was found to be slightly greater for longer chain lengths (Figure 5). After longer times (up to 10 000 mins), turbidity values showed less chain length dependence but were still lower for increasing chain lengths of DP > 90.

The effects of concentration and quench temperature on turbidity evolution were also studied: representative data are presented in Figure 6. Both decreasing concentration and increasing temperature were found to slow the evolution of turbidity for each of a range of chain lengths (DP = 65, 110, 250, 660, and 1100), although final absorbance values were found to be similar for the same chain length (Figure 6). This similarity in absorbance values suggests that similar aggregation states are achieved by a given chain length of amylose over a range of concentrations and quench temperatures.

In order to draw conclusions from the results presented above, the factors causing turbidity in this system need to be considered. Turbidity effects have their origin in

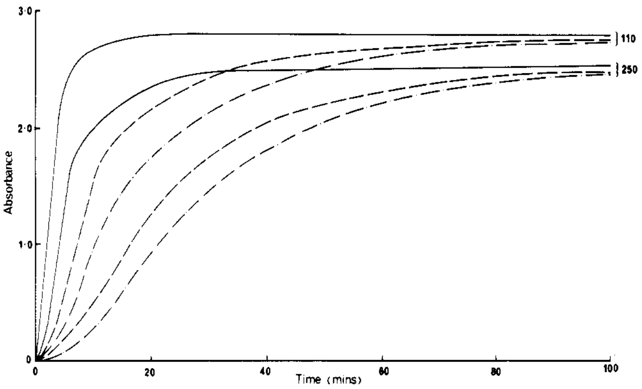

Figure 6. Time course of turbidity development for aqueous solutions of amyloses with lengths ~110 and 250 residues: at 298 K, 1.5% (—) and 1.0% (- - -) w/v concentration; at 323 K, 1.5% w/v concentration (–·–).

refractive index fluctuations over a distance scale comparable to the wavelength of observation. In a simple polymer/solvent system this is caused by density fluctuations over the same distance scale and is most likely due to extensive polymer–polymer aggregation. For the visible observation wavelengths in this study, density fluctuations in the submicron and micron range are therefore being detected, suggesting that, even at the onset of detectable turbidity (Figure 4), highly aggregated polymer structures are present. It is therefore of interest to compare the chain length effects observed in this study with those found for the initial stages of amylose aggregation by Pfannemüller et al.[9] in dilute (0.075% and 0.1%) solution by light scattering.

In their study, Pfannemüller et al.[9] showed that the time required for a doubling of the initial light scattered at an angle of 90° from aqueous amylose solutions was highly dependent on chain length.[9] In a plot of the reciprocal of the doubling time (i.e., a measure of the aggregation rate) against the degree of polymerization (Figure 5 in ref 9), a sharp maximum at DP = 75–80 was observed with a logarithmic decrease in aggregation rate with increasing chain length above this DP.[9] It is interesting that turbidity onset temperatures at 0.5–2.0% (Figure 4) show a virtually identical dependence on chain length as the doubling of 90° light scattering at <0.1%. This suggests that the same factor(s) that determine initial aggregation rates in dilute solution also determine the rate of production of the more extensively aggregated structures which we have observed by turbidity measurements.

During this study it was found that amylose aggregated from aqueous solution was not resoluble below 100 °C for any of the chain lengths studied (DP > 40). In order to achieve optically clear solution, heating at 160 °C was found to be necessary. This is in line with calorimetric studies[35,36] on aggregated amylose, which showed a solution melting transition in the range 413–433 K. All observation temperatures employed in this work (298–343 K) are substantially below the aggregate melting temperature, therefore suggesting that the aggregation processes observed are under kinetic control. Aggregation rates would therefore be expected to be greater at lower temperatures due to decreased chain mobility and to increase with increasing concentration as observed (Figure 6). Similarly, the variation of turbidity onset temperatures with cooling rate is also consistent with kinetic control of the aggregation process at temperatures below 373 K.

The variation of aggregation rates with chain length observed both in this study and previously[9] seem to be paralleled by variations in the physical state of the aggregated system (Figure 3). Thus amyloses of DP < 110, for which aggregation rates increase with chain length, precipitate from aqueous solution (showing that cross-linking is not extensive enough to lead to formation of an infinite network), whereas amyloses of DP > 250 for which aggregation rates decrease with chain length form gels at >1% concentration, demonstrating that individual chains are, on average, involved in more than two separate polymer–polymer associations. In a companion paper in this issue[17] it is shown that amylose precipitates are essentially fully ordered at the molecular level and that amylose gels contain both rigid ordered segments and mobile nonordered interconnecting segments. For short chain length amyloses (DP < 110), the observed increase in aggregation rate with DP is consistent with a single cooperative ordering process involving most of the chain length and leading eventually (following extensive aggregation) to precipitation. For longer chain length amylose (DP > 250), cross-linking becomes more important with increasing chain length, and aggregation rates decrease.

Conclusions

This study has demonstrated that two major features of the aggregation of amylose in aqueous 0.2–5.0% solution, namely, the rate of aggregation and the physical state of aggregated material, are highly dependent on chain length. The observation (by turbidity measurements) of maximal aggregation rates for amyloses of DP ~ 100 parallels the effect of chain length on initial aggregation rates in dilute (<0.1%) solution[9] and suggests that overall aggregation rates are determined by the initial stages of the process. For amyloses of DP < 110, aqueous solutions deposit precipitates at all concentrations studied. For chains of DP > 250 gelation occurs at >1.0% concentration. The effect of amylose chain length on aggregation rates and the physical state of aggregated material is rationalized on the basis of ordered amylose–amylose interactions occurring over chain segments of typically <100 residues length.

Registry No. Amylose, 9005-82-7.

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Rheological Studies of Aqueous Amylose Gels: The Effect of Chain Length and Concentration on Gel Modulus

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ABSTRACT: Aqueous amylose gels have been studied kinetically and at pseudoequilibrium as a function of polymer concentration and chain length. Pseudoequilibrium gel moduli (G') for a range of amylose chain lengths (produced by enzymatic synthesis) show the same concentration dependences as other gelling biopolymers and have been analyzed by the method of Clark and Ross-Murphy (*Br. Polym. J.* **1985**, *17*, 164–168). This treatment is shown to lead to the accurate prediction of critical gelling concentrations and suggests that the cross-linking functionality of individual amylose chains is ~DP/100 (DP = degree of polymerization). For a fixed concentration, shorter chain amylose systems are found to develop turbidity more rapidly and to attain higher final values, suggesting that decreasing amylose chain length results in more heterogeneous gel structures. The relative rates of turbidity and modulus increase for amylose gels are found to be dependent on chain length: for short chains (DP < 300) turbidity precedes gelation, whereas for longer chains (DP < 1100) gelation occurs before significant increase in turbidity.

Introduction

Amylose is a polysaccharide component of starch and, in nature, is a polydisperse α-(1→4) glucan which can be linear or lightly branched[1] (through additional α-(1→6) linkages). Fractionation of starch[1,2] yields amylose preparations that are inherently unstable in aqueous solution at room temperature.[2,3] Amylose precipitates from dilute aqueous solution, whereas gels are formed from more concentrated systems.[2,3] It is thought that gelation of the amylose component is important in starch gelation, at least during the initial stages.[4] Recently, the mechanisms involved in amylose gelation have been addressed,[5] and some of the factors which affect the gelation process have been investigated.[6]

Two of the most important variables in amylose gelation are polymer concentration and chain length.[3,6,7] In the preceding paper in this issue,[7] nearly monodisperse enzymically synthesized amyloses were used to characterize the phase change behavior and polymer aggregation kinetics of aqueous amylose systems. It was found that precipitation (and not gelation) occurred for chain lengths of DP (degree of polymerization) < 110, whereas gelation occurred for amyloses of DP > 250 at concentrations of >1.0%. In this report, we describe rheological studies of aqueous gels of enzymically synthesized amyloses with particular regard to the effect of polymer chain length and concentration. Although enzymically synthesized samples have provided much insight into chain length effects in dilute amylose solutions,[8,9] we are not aware of any studies of amylose gelation using such essentially monodisperse materials.

Methods

Synthesis and Characterization of Amylose Samples. The synthesis of amyloses using maltoheptaose as a primer, glucose 1-phosphate as monomer, and the enzyme phosphorylase as catalyst was carried out as described in the preceding paper.[7] Gel permeation chromatography was used to show that synthesized amyloses had a narrow molecular weight distribution[7] (M_w/M_n < 1.15 and typically < 1.10), and chain lengths were determined from intrinsic viscosity measurements in dimethyl sulfoxide and from analysis of polymerization solutions for inorganic phosphate.[7]

Preparation of Aqueous Amylose Gels. Gels were prepared by cooling clear aqueous solutions of amylose. Solutions were obtained by heating appropriate mixtures of amylose and deionized water in sealed tubes at 160 °C for 10–15 min[7]. After cooling to 70–80 °C, the tubes were opened, and the solutions were immediately transferred into (as appropriate) a mechanical spectrometer, a driven torsion pendulum, or a spectrophotometer cell and subjected to controlled cooling regimes as detailed below.

Rheological and Turbidimetric Measurements. The development of gel structure was monitored by small deformation mechanical measurements obtained by using a driven torsion pendulum[10] or a Rheometrics mechanical spectrometer (RMS-605).[11] Hot aqueous amylose solutions were introduced into the sample cell of the torsion pendulum or between parallel plates in the mechanical spectrometer, a thin layer of light silicone oil was added to prevent evaporative loss, and the samples were cooled from 60 to 25 °C at a rate of ~1 °C/min and held at 25 °C. For analysis of amylose concentration dependence, modulus values were recorded after 200 min at 10 rad s^{-1} and 2% strain. Sample temperatures were continuously monitored to enable turbidity measurements to be carried out under identical cooling regimes. Turbidity data were obtained for amyloses having a range of chain lengths and concentrations under temperature/time profiles directly comparable with those of rheological measurements, using a Beckman DU7 spectrophotometer at 700 nm and with a pathlength of 1 cm.

Results

Figure 1 shows the change in shear modulus (G') at 10 rad s^{-1} which takes place on cooling aqueous solutions of amylose having a DP$_n$ of ~1100. These traces follow the

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