$$1.3K_e = \frac{\theta^2 f_\pm Z^2 1.3}{2M_{p'}M(1-\bar{v}\rho)} = \frac{\theta^2 f_\pm \nu^2 N}{2 \times 1.3(1-\bar{v}\rho)}$$

where

$$f_\pm = (1/2)(f_{K^+} + f_{I^-}) \sim 2 \times 10^{-9}$$

Z is the valence of an AI polyion = $\nu n M_A = \nu M/1.3$, and $M_{p'}$ is the mass of an AI polyion. Thus $1.3K_e \sim 5 \times 10^8\theta^2$.

This estimate of the coefficient of C_A/m_{KI} taken from experimental conditions is equated to the value obtained from the slope of the line in Figure 5 to yield the result: $\theta^2 \sim 40$. Since by definition $0 < \theta < 1$, this result indicates that the Alexandrowicz and Daniel treatment is not valid for AI complexes.

References and Notes

(1) Presented in part at the Midwest Regional Meeting of the American Chemical Society, Iowa City, Iowa, November 7–8, 1974.
(2) For reviews of the complex see: (a) C. T. Greenwood, *Adv. Carbohydr. Chem.*, **11**, 367 (1956); (b) J. F. Foster, "Starch: Chemistry and Technology", Vol. I, R. L. Whistler and E. F. Paschall, Ed., Academic Press, New York, N.Y., 1965, Chapter XV; (c) H. Morawetz, "Macromolecules in Solution", Interscience, New York, N.Y., 1965; (d) J. Szejtli and S. Augustat, *Staerke*, **18**, 38 (1966).
(3) R. L. Whistler and C. Johnson, *Cereal Chem.*, **25**, 418 (1948).
(4) S. Lanzky, M. Kooi, and T. J. Schoch, *J. Am. Chem. Soc.*, **71**, 4066 (1949).
(5) J. F. Foster and D. Zucker, *J. Phys. Chem.*, **56**, 170 (1952).
(6) J. F. Foster and E. F. Paschall, *J. Am. Chem. Soc.*, **74**, 2105 (1952).
(7) K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **24**, 389 (1941).
(8) T. Kuge and S. Ono, *Bull. Chem. Soc. Jpn.*, **33**, 1269 (1960).
(9) K. Takahashi and S. Ono, *J. Biochem.*, **72**, 1041 (1972).
(10) B. Pfannemüller, H. Mayerhöfer, and R. C. Schulz, *Biopolymers*, **10**, 243 (1971).
(11) M. B. Senior and E. Hamori, *Biopolymers*, **12**, 65 (1973).
(12) K. O. Pedersen, *J. Phys. Chem.*, **62**, 1282 (1958).
(13) P. F. Mijnlieff, "Ultracentrifugal Analysis in Theory and Experiment", J. W. Williams, Ed., Academic Press, New York, N.Y., 1963.
(14) Z. Alexandrowicz and E. Daniel, *Biopolymers*, **1**, 447 (1963).
(15) T. J. Schoch, *Adv. Carbohydr. Chem.*, **1**, 247 (1945).
(16) W. W. Everett and J. F. Foster, *J. Am. Chem. Soc.*, **81**, 3460 (1959).
(17) F. R. Dintzis and R. Tobin, *Biopolymers*, **7**, 581 (1969).
(18) W. Banks, C. T. Greenwood, and D. D. Muir, *Staerke*, **23**, 118 (1971).
(19) A. C. Beckwith, H. C. Nielson, and R. O. Butterfield, *Anal. Chem.*, **43**, 1471 (1971).
(20) C. Tanford, "Physical Chemistry of Macromolecules", Wiley, New York, N.Y., 1961.
(21) J. W. Williams, K. E. VanHolde, R. L. Baldwin, and H. Fujita, *Chem. Rev.*, **58**, 715 (1958).
(22) R. Trautman, "Ultracentrifugal Analysis in Theory and Experiment", J. W. Williams, Ed., Academic Press, New York, N.Y., 1963.
(23) R. J. Goldberg, *J. Phys. Chem.*, **57**, 194 (1953).
(24) A. Savitsky and M. J. E. Goley, *Anal. Chem.*, **36**, 1627 (1964).
(25) J. A. Thoma and D. French, *J. Am. Chem. Soc.*, **80**, 6142 (1958).
(26) Z. Alexandrowicz and E. Daniel, *Biopolymers*, **6**, 1500 (1968).
(27) J. T. Edsall and J. Wyman, "Biophysical Chemistry", Vol. I, Academic Press, New York, N.Y., 1958, p 272.
(28) J. C. Thompson and E. Hamori, *Biopolymers*, **8**, 689 (1969).
(29) R. S. Higginbotham, *J. Text. Inst., Trans.*, **40**, 783 (1949).
(30) C. L. Cronan and F. W. Schneider, *J. Phys. Chem.*, **73**, 3990 (1969).
(31) R. E. Rundle and D. French, *J. Am. Chem. Soc.*, **65**, 1709 (1943).
(32) H. G. Elias and J. Gerber, *Makromol. Chem.*, **112**, 122 (1968).
(33) J. Gerber and H. G. Elias, *Makromol. Chem.*, **112**, 142 (1968).
(34) The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Amylose–Iodine Complex. II. Molecular Weight Estimates[1]

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ABSTRACT: Ultracentrifugation measurements made by the Archibald method on solutions of amylose–iodine–iodide (AI) complexes, containing 0.003% amylose of weight average molecular weight 4.0×10^5 at 3.6×10^{-3} M KI, yield an apparent molecular weight at the meniscus of 8×10^5 when measurements are extrapolated to 1200 rpm. Sedimentation equilibrium measurements at 1200 rpm yield apparent molecular weight at the meniscus of 6×10^5 and at the cell bottom of 2.4×10^6. Heterogeneity and aggregation are major features of AI complex solution behavior. Apparent molecular weights increase as a function of increasing potassium iodide concentration and with time. This behavior directly correlates with AI complex sedimentation coefficient behavior previously reported. Molecular weight estimates are of the same order for AI complex solutions saturated and 65–70% saturated with respect to the iodine-binding capacity of amylose. Qualitative estimates of net macroion charge effects upon apparent molecular weights are presented.

This paper continues a study of amylose–iodine–iodide (AI) complexes in very dilute solutions in which measurements are made on many of the same stock solutions for which sedimentation data were presented previously.[2] Molecular weight estimates from ultracentrifuge measurements were calculated mainly by the transient method of Archibald.[3] We report a few estimates based on sedimentation equilibrium measurements. The purpose of this paper is to obtain molecular weight estimates on AI complexes in solution and to determine whether or not the increase in sedimentation coefficient observed with increasing potassium iodide concentration represents aggregation.

Many practical and theoretical aspects of molecular weights determined with an ultracentrifuge are now well established and documented. An ample bibliography is provided by Creeth and Pain[4] in their useful general review. Because of the uncertainties that exist in our experimental data, we believe a detailed consideration of theory is not warranted. Therefore, data will be discussed qualitatively in terms of existing simple theory.

Theory

Molecular weights estimated from the transient method of Archibald are calculated by a conventional formulation for the upper meniscus:

$$\left(\frac{1}{rC}\frac{dC}{dr}\right)_{r=r_m} = \frac{M_{(r_m)}^{app}(1-\bar{v}\rho)\omega^2}{RT} \quad (1)$$

where C = count number, which is proportional to AI concentration and is measured with absorption optics; r = distance from center of rotation; r_m = distance from center of rotation to upper meniscus; $\bar{v}$ = partial specific volume of solute; ρ = density of solution; ω = angular velocity of rotor; R = gas constant; T = absolute temperature; and $M_{(r_m)}^{app}$, the apparent molecular weight at the meniscus, is expressed as:

$$M_{(r_m)}^{app} = M/[1 + (\mathrm{d} \ln y/\mathrm{d} \ln c)] \quad (2)$$

where: M = molecular weight of AI complex; y = activity coefficient on the c scale; c = concentration.

When r is not restricted to r_m, eq 1 is a general fundamental sedimentation equilibrium expression for a two-component system of homogeneous uncharged solute in an incompressible solvent. Equation 1 may be transformed to obtain:

$$M_{(r_m)}^{app} = \left(\frac{\partial \ln C}{\partial r^2}\right) 2RT/[\omega^2(1 - \bar{v}\rho)] \quad (3)$$

where $\partial \ln C/\partial r^2$ is estimated from a plot of ln C vs. r^2 as the limiting slope extrapolated into the meniscus. Molecular weights based on the Archibald method are calculated in this paper from eq 3. We expect these molecular weight estimates to be crude, because our AI complexes are heterogeneous and are likely to be charged.

For an uncharged polymer of n components capable of different molecular weights, eq 1 is not appropriate. Fujita's book[5] presents theoretical development and interpretations of equations derived for multicomponent systems. Sedimentation equilibrium measurements on AI complexes yield a molecular weight representation across the entire cell and demonstrate heterogeneity expected for multicomponent systems. In our work, identical partial specific volumes and absorption coefficients are assumed for various components of AI complexes in solution. Presumably, the partial specific volume of the complex in solution is properly estimated from the measured density of 1.7 g/cm^3 reported for the solid state complex.[6] Beckwith et al.[7] have presented methods of data processing and calculation of dC/dr across the cell.

We further tentatively assume the validity of a concept used by Alexandrowicz and Daniel,[8] that a fixed fraction of counterions is bound to the macroion while those counterions in the remaining fraction, θ, are free to move about in solution as independently as do other small ions. Given this condition, one may use the treatments of either Johnson et al.[9] or Williams et al.[10] to estimate effects of net macroion charge, $Z_{p'}$, as a function of θ on the apparent molecular weight of the AI complex. The most significant correction term to M^{app} is presented[10] as (in our notation):

$$M^{app} \propto M\left\{1 + \frac{Z_{p'}{}^2 M_{KI} C_p}{2MC_{KI}}\right\}^{-1} \quad (4)$$

where M_{KI} = formula weight of KI; C_p = g of AI complex/ml; and C_{KI} = g of KI/ml.

If all the I_2 complexed by amylose is in the form of I_3^- according to the reaction $I_2 + I^- \rightarrow I_3^-$, then the total valence of the AI complex would be high.

Experimental Section

Methods of preparation, handling, and storage of AI solutions are given in the accompanying paper.[2] A new requirement encountered was the extended time of contact between AI solution and ultracentrifuge cell components during a sedimentation equilibrium run. We were dismayed to note that our AI complex solutions were likely to be unstable, i.e., lose color during extended containment in an ultracentrifuge cell. This behavior occurred in double sector cell centerpieces made from Epon resin and Kel F. Careful washing and rinsing of cell components that are in contact with AI solution did not solve the problem.

A coating of paraffin wax on walls of cell sectors seemed to improve AI solution stability. A solution of 2% (w/v) of paraffin wax in n-hexane was prepared and placed in a separatory funnel with concentrated H_2SO_4. The contents were shaken occasionally and allowed to remain in contact overnight to remove possible unsaturation centers in the paraffin. After concentrated H_2SO_4 was separated, the remaining solution was washed first with a $NaHCO_3$ solution to remove all acid and then washed several times with distilled water. Cell centerpieces were immersed into the 2% paraffin wax–n-hexane solution, removed, and allowed to dry overnight.

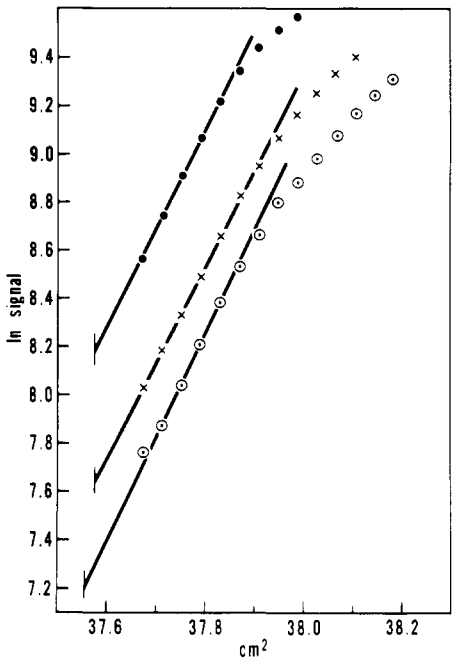

Figure 1. Approach to equilibrium measurements made at 12 000 rpm on a solution of 0.003% amylose–iodine (AI) complex of fraction B amylose at 3.6×10^{-3} M KI. Three minutes were required to reach 12 000 rpm: (●) 8 min after start of rotation; (×) 17 min after start of rotation; (⊙) 26 min after start of rotation.

Even with this procedure, sometimes only 40% of our AI solutions maintained a blue intensity upon reaching equilibrium. Because of such behavior, we chose the Archibald method to determine effects of KI concentration and amylose concentration upon apparent molecular weight of the AI complex. Rotor speeds for these measurements were chosen so as to minimize containment time of AI solution in the cell while maintaining a well-behaved concentration gradient during depletion of the meniscus region.

Almost all measurements reported here were made on solutions of AI complex prepared from a fraction of dent corn amylose, fraction B, of weight average molecular weight determined by light-scattering measurements, $\bar{M}_w = 4.0 \times 10^5$. Measurements were made on a few solutions of AI complex made from a fraction of dent corn amylose, designated F_2, of $\bar{M}_w = 3.4 \times 10^4$. Details of materials are given in ref 2.

Results and Discussion

Archibald Method. Unless otherwise specified, our remarks refer to AI complex solutions saturated with respect to the iodine-binding capacity of amylose. Sample data from absorption scan measurements on an AI complex solution using the approach to equilibrium method are graphed in Figure 1. The last scan (⊙) was taken when the meniscus was about two-thirds depleted. Data in Figure 1 are sufficiently linear to permit a reasonable extrapolation into the meniscus. Other examples of valid data may not yield limiting slopes as precise as those shown. Limiting slopes at a given rpm usually are not time dependent. At a given rpm, $\bar{M}_w^{app}$ seems to be constant during the times used to perform measurements.

However, apparent molecular weights estimated by the Archibald method are dependent upon rotor speed. Two examples of this dependence are presented in Table I for AI complexes of fraction B amylose. Both sets of data, 0.003% AI and 0.001% AI, represent linear functions of rpm vs. $\bar{M}_w^{app}$ (cf. Figure 2). $\bar{M}_w^{app}$ values are obtained from an average of three scans on at least three ultracentrifuge cells. Values at 8000 rpm in the first set have been displayed to indicate variation between cells. If it is valid to extrapolate to 0 rpm to reach a "true" $\bar{M}_w^{app}$ at the meniscus, then at 3.6×10^{-3} M KI complexes at 0.003% AI yield a value of about 8×10^5 and 0.001%

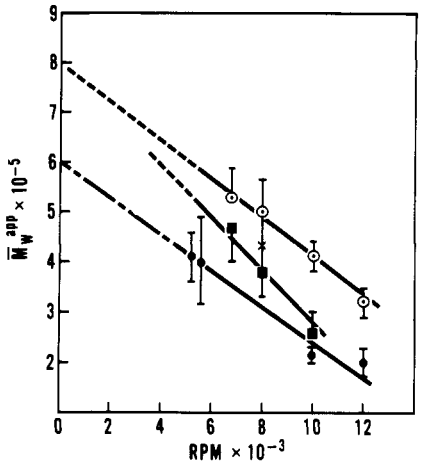

Figure 2. Effect of rotation rate upon apparent molecular weight by the Archibald method. Vertical lines represent standard deviations. At some points only the positive or negative deviation is shown. Solution ages are: >3 days and <2 weeks. (○) 0.003% AI at 3.6 × 10^{-3} M KI; (■) 0.001% AI at 3.6 × 10^{-3} M KI; (●) 0.001% AI at 1.2 × 10^{-3} M KI.

Table I
Approach to Equilibrium-Rotor Speed Dependence

Amylose Iodine (AI), "Aged" 3.6 × 10^{-3} M KI		
Rpm		$\bar{M}_w^{app} \times 10^{-5}$
6 800	0.003% AI	5.3 ± 0.6[a]
8 000		4.6 ± 0.2 (2 cells)
		5.0 ± 0.7 (3 cells)
10 000		4.1 ± 0.3
12 000		3.2 ± 0.3
6 800	0.001% AI	4.7 ± 0.7
8 000		3.8 ± 0.5
10 000		2.6 ± 0.4

[a] Standard deviation.

AI complexes could do likewise. It appears resonable to estimate that amylose increases its mass by about 30% upon complexing with I_3^- so that our amylose fraction of $\bar{M}_w$ = 4.0 × 10^5 would yield an AI complex "monomer" of about 5.2 × 10^5. Therefore, our measurements indicate that at the meniscus these AI complexes (Figure 2, ⊙, ■) exist on the average as a monomer–dimer distribution. AI complex solutions at 1.2 × 10^{-3} M KI (Figure 2, ○) yield an $\bar{M}_w^{app}$ at a 0 rpm of 6 × 10^5. This apparent molecular weight indicates a monomer average distribution at the meniscus.

Interpretation of additional results (Table II, Part A) is complicated by dependence on at least three variables: percent AI, rpm, and KI concentration. Nevertheless, some trends are discernible. Higher apparent molecular weights at 8.3 × 10^{-3} M KI are a major feature that seems to be insensitive to a sevenfold change in AI concentration (last three examples). Yet at 3.6 × 10^{-3} M KI and 10 000 rpm, there does exist a dependence of $\bar{M}_w^{app}$ upon AI concentration. Clearly shown in Figure 2 is this dependence upon percent AI concentration or the ratio % AI/salt concentration at higher rotor speeds. An increase in $\bar{M}_w^{app}$ with an increase in KI concentration is confirmed (Figure 2 and Table II) and therefore correlated with the previously reported increase in sedimentation coefficient as a function of salt concentration.

At 12 000 rpm the iodine-unsaturated AI solution yields (Table II, part B) an apparent molecular weight of the same order as that exhibited by the iodine-saturated KI solution

Table II
Approach to Equilibrium, Complexes of Fraction B Amylose

% AI	Rpm	M KI × 10^3	$\bar{M}_w^{app} \times 10^{-5}$
A. Saturated with Respect to Iodine-Binding Capacity of Amylose			
0.001	12 000	1.2	2.0 ± 0.3[a]
0.001	10 000	1.2	2.16 ± 0.15
0.001	5 600	1.2	4.0 ± 0.9
0.001	5 200	1.2	4.1 ± 0.5
0.001	10 000	3.6	2.6 ± 0.4
0.003	10 000	3.6	4.1 ± 0.3
0.007	7 200	8.3	11 ± 2
0.001	5 600	8.3	11 ± 2
0.001	4 800	8.3	13 + 1
B. Unsaturated with Respect to Iodine-Binding Capacity of Amylose (wt of I_2 added/wt of I_2 bound at saturation = 0.69[b])			
0.004	12 000	0.82	2.83 ± 0.07 (aged)[c]
0.004	12 000	0.82	2.38 ± 0.38 (fresh)[d]

[a] Standard deviation. [b] Calculated on the basis of 19.5 mg of I_2 bound per 100 mg of amylose. [c] Aged = >3 days after preparation. [d] Fresh = <6 h after preparation.

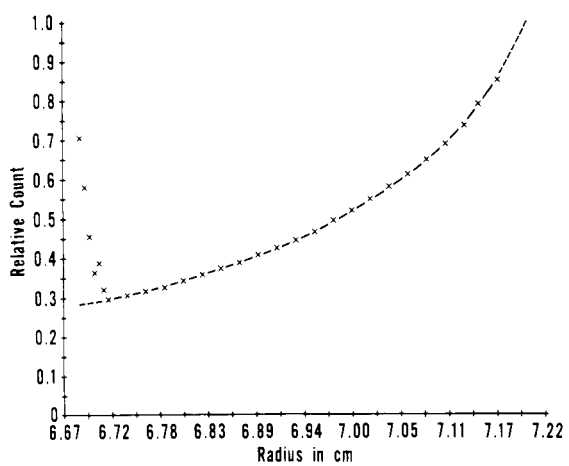

Figure 3. Sedimentation equilibrium concentration profile in cell 4 of 0.003% AI complex solution of fraction B amylose at 3.6 × 10^{-3} M KI after 6 days at 1200 rpm: (x) experimental count values; (- - -) profile extension obtained by calculation from polynomial fit to measured count values.

in Table I. The increase in $\bar{M}_w^{app}$ upon aging is confirmed, by analysis of data variation, to be significant. This increase with age also is observed in AI solutions saturated with respect to the iodine-binding capacity of amylose. Hence, the increase in sedimentation coefficient of the complex with age of AI solution correlates with an increase of $\bar{M}_w^{app}$ of the complex.

It is worth comparing $\bar{M}_w^{app}$ of these aged iodine-unsaturated and iodine-saturated AI complexes with their respective sedimentation coefficient values (cf. ref 2, Tables II and III, last examples). We observe that both $\bar{M}_w^{app}$ and sedimentation coefficients, S, are similar for the two kinds of AI complexes ($S \sim 21 \times 10^{-13}$). This rough comparison indicates to us that AI complexes in iodine-unsaturated solutions might be aggregated. Evidently, our understanding of iodine-unsaturated AI solutions is primitive.

Sedimentation Equilibrium Measurements. A valid sedimentation equilibrium measurement is graphed in Figure

Table III
Sedimentation Equilibrium Results

0.003% AI complex of B amylose monomer $\bar{M}_w \sim 4.0 \times 10^5$		3.6×10^{-3} M Ki, 1200 rpm,
Cell no.	Time in days	$\bar{M}_w^{app} \times 10^{-6}$
1	3	1.6
	6	1.4
2	3	1.3
	6	0.80[a]
3	3	1.1
	6	0.78[a]
4	3	1.6
	6	1.7
5	3	1.1
	6	0.7[a]

[a] Blue intensity loss visible to observer.

3. Experimental count values representing concentration (×) are extended to the meniscus and cell bottom, at the left and right, respectively, by calculation involving a polynomial fit to the data (dashed lines). A portion of the meniscus region is evident on the left of the figure. Molecular weight heterogeneity is demonstrated by a continuously increasing concentration gradient across the cell. This successful example of an equilibrium measurement occurred in cell 4 of the series represented in Table III.

Measurements at sedimentation equilibrium were attempted using a six-place rotor with five cells and a reference counterweight. At best only about 40% of the AI solutions appeared to remain stable during the run (Table III). After initial overspeeding at 8000 rpm for 4 h followed by 3 days at 1200 rpm, cells 1 and 4 show the same $\bar{M}_w^{app}$ of 1.6×10^6. After 6 days AI solution in cell 4 has remained stable while that in cell 1 may not have. Solutions in cells 2, 3, and 5 suffered marked loss of color at the end of the run and even after 3 days; AI solutions in those cells have $\bar{M}_w^{app}$ values significantly lower than solutions in cells 1 and 4.

An $\bar{M}_w^{app}$ value of 1.6×10^6 across the cell represents a trimer complex. In cell 4 after 6 days, $\bar{M}_w^{app}$ at the meniscus is of the order of 6×10^5. This value compares with about 8×10^5 estimated by the Archibald method. At the cell bottom, $\bar{M}_w^{app}$ is about 2.4×10^6. Therefore, our 0.003% AI complexes of fraction B amylose at 3.6×10^{-3} M KI appear to exist in solution as aggregates with a distribution range from mainly monomer–dimer to tetramer–heptamer.

A few equilibrium measurements made at 4800 rpm on a 0.002% AI complex solution of fraction F_2 amylose at 2.4×10^{-3} M KI yield a $\bar{M}_w^{app}$ across the cell of $(3.0 \pm 0.3) \times 10^5$. Since a monomer of the complex is estimated to have a molecular weight of ~43 000, these results would represent an average of a hexamer–heptamer aggregate across the cell. Apparent molecular weights averaging 1.2×10^5 and 4.8×10^5 were calculated for the meniscus and cell bottom, respectively. These estimates correspond to a distribution in solution of dimer–trimer at the meniscus and undecamer at the cell bottom. The important influence of parent amylose upon the state of the resulting AI aggregate can be seen by comparing apparent molecular weights of AI complexes prepared from amylose fractions B and F_2.

Charge Effects and Molecular Weight. Possible effects of charge upon apparent molecular weight are examined according to expression 4. Values of $\bar{M}_w^{app}$ are chosen to be 1.6×10^6 for a 0.003% AI complex solution of fraction B amylose at 3.6×10^{-3} M KI and 3.0×10^5 for a 0.002% AI complex solution of fraction F_2 amylose at 2.4×10^{-3} M KI. Net macroion charge, $Z_{p'}$, is calculated by:

$$Z_{p'} = \theta \nu n M_A \quad (5)$$

where n is the number of amylose chains that may associate to form an aggregate, M_A is molecular weight of parent amylose, and ν is the mol of I_3^- bound/g of amylose (cf. ref 2, Table IV, and Appendix for further details).

Given these solution conditions and values of $\bar{M}_w^{app}$, $Z_{p'}$ is $\sim 950\theta$ for AI complexes of fraction B amylose and is $\sim 164\theta$ for AI complexes of fraction F_2 amylose. In other words, about 950 and 164 I_3^- ions or the equivalent thereof are estimated to be complexed with the respective average AI aggregate. These results correspond to approximately 8.0 and 9.0 anhydroglucose units (AGU) per I_3^- for the respective amylose fractions B and F_2. X-ray investigations of Rundle and French[6] indicate a solid state structure of six AGU per helix turn that contains one I_2. Baldwin et al.[11] noted a decrease in iodine content from one I_2 per 6 AGU to one I_2 per about 8 AGU as KI concentration was increased in their solution studies of AI complexes. They concluded that some I_2 had been replaced by I^- or I_3^-. Theoretical considerations and experimental work of Cronan and Schneider[12] lead to a proposed bound specie in solution of approximate overall composition, $I_2 \cdot I_b^-$, where, $0 \leq b \leq 1$. On the basis of iodine–iodide adsorption isotherms of potato amylose determined from spectrophotometric measurements taken during titrations, Cronan and Schneider postulate: at low KI concentrations, $\sim 10^{-5}$ M, the bound species is predominantly I_2; at high KI concentrations, $\sim 10^{-2}$ M, the bound species is predominately I_3^-.

The correction factor in expression 4, $1 + (Z_{p'}^2 M_{KI} C_p / 2MC_{KI})$, is $1 + 0.35 \times 10^{-5} Z_{p'}^2$ and $1 + 1.8 \times 10^{-5} Z_{p'}^2$ for average AI complex aggregates of amylose fractions B and F_2, respectively. In Table IV we display estimated values of Z and correction factors as a function of θ. The values would be high if composition of the bound iodine species is that postulated by Cronan and Schneider.[12] In addition, if iodine were bound as a species of composition between I_2 and I_3^-, then the molecular weight of an AI complex monomer would be less than estimated, and therefore, estimates of average aggregation numbers, n, would be greater.

Table IV
Estimated Corrections to $\bar{M}_w^{app}$

	AI aggregates of fraction B (3.6×10^{-6})		AI aggregates of fraction F_2 (2.4×10^{-6})	
KI (mol/ml)/θ	$Z_{p'}$[a]	$1 + 0.35 \times 10^{-5} Z_{p'}^2$	$Z_{p'}$[a]	$1 + 1.8 \times 10^{-5} Z_{p'}^2$
0.1	95	1.03	16	1.005
0.2	190	1.13	33	1.02
0.3	285	1.28	49	1.04
0.4	380	1.51	66	1.08
0.5	475	1.79	82	1.12

[a] Estimated on the basis that I_2 is bound according to the reaction $I_2 + I^- \rightarrow I_3^-$.

According to Table IV, when $\theta = 0.2$, $\bar{M}_w^{app}$ is estimated to be about 13 and 2% low for AI aggregates of B and F_2 amylose, respectively. Note that effects of charge on $\bar{M}_w^{app}$ appear to be dependent upon both θ and molecular weight of the parent amylose. According to this model, an AI aggregate could be a highly charged particle in solution if $\theta \gtrsim 0.2$.

Conclusion

Experimental results here and earlier[2] prove that aggre-

gation is a major features of AI complexes in aqueous solution. Increases in values of sedimentation coefficients and apparent molecular weights correlate as a function of increase in salt concentration. Effective mass properties of AI complexes in our solutions saturated with respect to iodine-binding capacity of amylose are demonstrated to depend upon molecular weight of parent amylose and either KI concentrations or the ratio, amylose concentration/KI concentration, or perhaps both of these. AI complex solutions approximately 69 to 85% iodine saturated with respect to the iodine-binding capacity of amylose yield apparent molecular weights and sedimentation coefficient values similar to solutions saturated with respect to the iodine-binding capacity of amylose. From this result we infer that aggregation of iodine-unsaturated AI complexes may occur.

A major unresolved question raised by these studies is: what are the values of effective net charges on AI complex aggregates (i.e., $Z_{p'} = ?$)? For polylysine hydrochloride, Daniel and Alexandrowicz[13] calculate a dynamic value for θ of 0.57 from sedimentation measurements and an equilibrium value of 0.18 from osmotic pressure measurements. Consequently, molecules that can become highly charged may have significant θ values. A further unknown: although Alexandrowicz and Daniel[8] propose a model in which θ is invariant with ionic strength in their polypeptide solutions, effects of aggregation upon θ have not been determined. One might suppose in these experiments that there is sufficient KI in solution to effectively "salt out" major charge effects. For example, for 0.003% AI complexes of fraction B amylose, even though total ionic strength is low, $\sim 3.6 \times 10^{-3}$ M KI, the ratio, mol of KI/mol of average AI complex aggregate, is $\sim 1.9 \times 10^5$. According to the Svedberg–Pederson model, these conditions eliminate primary charge effects on the sedimentation coefficient.[2] However, according to expression 4 and the correction terms listed in Table IV, there could be substantial corrections to $\bar{M}_w^{app}$ if $\theta > 0.2$. Determinations of θ for AI complex aggregates, perhaps by electrophoresis experiments, would be most helpful to our understanding of the system.

Kuge and Ono[14] have demonstrated that inorganic salts such as KCl and $(NH_4)_2SO_4$ can enhance AI complex absorbance provided some I_3^- is present. Therefore, the generality of our results to AI complexes in the presence of other electrolytes remains to be determined. Bittiger and Husemann[15] used the electron microscope to observe rodlike structures of AI complexes prepared from narrow distributions of phosphorylase-synthesized amyloses. Some individual rods, in addition to rod clusters, are noted in a micrograph prepared from a 0.0025% solution of amylose ($\bar{M}_w \sim 3.1 \times 10^5$) containing 10^{-3} N iodine. When concentration of amylose is increased, a network of threadlike structures or fibrils is observed in the micrograph. Bittiger and Husemann[15] note that under their conditions, the precipitate of rods associates end to end rather than side to side.

Other workers have observed time dependence of optical rotatory dispersion and circular dichroism for iodine complexes of starch, amylose, and amylopectin[16,17] and also for iodine complexes of some narrow molecular weight amylose distributions produced by enzymatic synthesis.[18,19] Such increases with time of specific rotation and molecular ellipticity might correlate with increases in sedimentation coefficient and apparent molecular weight of our AI aggregates. Since time scale is approximately the same for both types of change, optical summetry properties of the AI complex might be affected, in part, by gross changes in effective mass properties of the dissolved aggregate.

Some attempts have been made to deduce AI complex chain configuration in solution from intrinsic viscosity measurements[20] and also pulsed electric dichroism studies.[21] Neither of these two attempts considered the possibility of AI species in solution existing as aggregates under the experimental conditions used. Our results show that AI aggregates can exist under conditions which overlap the higher concentrations used by Senior and Hamori[20] (i.e., AI complex solutions not saturated with respect to the iodine-binding capacity of amylose). The dichroism studies of Foweraker and Jennings[21] were done on freshly prepared AI solutions in which concentrations of amylose, I_2, and KI were similar to those we used. If sufficient aggregation of AI complexes occurs under conditions as used by these workers,[20,21] their experimental data would need to be reinterpreted.

Experience leads us to suggest that future work on AI complexes should be done on systems of greater stability. Perhaps fractions of lightly derivatized hydroxyethyl amylose could be used. In the presence of I_2–KI, hydroxyethyl potato amylose, degree of substitution 0.028, yields the deep blue color of an AI complex and has significantly greater stability in concentrated $CaCl_2$ than do AI complexes of natural amyloses.[22] Another aid to this type of research probably would be use of an all-glass ultracentrifuge cell, for our systems showed greatest stability when in contact with glass. We conclude these studies with the opinion that a great deal remains to be learned about AI complexes in solution.

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References and Notes

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(2) F. R. Dintzis, A. C. Beckwith, G. E. Babcock, and R. Tobin, *Macromolecules*, preceding paper in this issue.
(3) W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).
(4) J. M. Creeth and R. H. Pain, "Progress in Biophysics and Molecular Biology", Vol. 17, Pergamon Press, New York, N.Y. 1967, pp 217–287.
(5) H. Fujita, "Mathematical Theory of Sedimentation Analysis", Academic Press, New York, N.Y., 1962.
(6) R. E. Rundle and D. French, *J. Am. Chem. Soc.*, **65**, 1709 (1943).
(7) A. C. Beckwith, H. C. Nielsen, and R. O. Butterfield, *Anal. Chem.*, **43**, 1471 (1971).
(8) Z. Alexandrowicz and E. Daniel, *Biopolymers*, **1**, 447 (1963).
(9) J. S. Johnson, K. A. Kraus, and G. Scatchard, *J. Phys. Chem.*, **58**, 1034 (1954).
(10) J. W. Williams, K. E. VanHolde, R. L. Baldwin, and H. Fujita, *Chem. Rev.*, **58**, 715 (1958).
(11) R. R. Baldwin R. S. Bear, and R. E. Rundle, *J. Am. Chem. Soc.*, **66**, 112 (1944).
(12) C. L. Cronan and F. W. Schneider, *J. Phys. Chem.*, **73**, 3990 (1969).
(13) E. Daniel and Z. Alexandrowicz, *Biopolymers*, **1**, 473 (1963).
(14) T. Kuge and S. Ono, *Bull. Chem. Soc. Jpn.* **33**, 1269 (1960).
(15) H. Bittiger and E. Husemann, *Chem. Soc., Spec. Publ.*, **No. 23**, 159 (1968).
(16) R. C. Schulz, R. Wolf, and H. Mayerhöfer, *Kolloid Z. Z. Polym.*, 65 (1968).
(17) R. Wolf and R. C. Schulz, *J. Macromol. Sci., Chem.*, **2**, 821 (1968).
(18) B. Pfannemüller, H. Mayerhöfer, and R. C. Schulz, *Makromol. Chem.*, **121**, 147 (1969).
(19) B. Pfannemüller, H. Mayerhöfer, and R. C. Schulz, *Biopolymers*, **10**, 243 (1971).
(20) M. B. Senior and E. Hamori, *Biopolymers*, **12**, 65 (1973).
(21) A. R. Foweraker and B. R. Jennings, *Adv. Mol. Relaxation Processes*, **6**, 241 (1974).
(22) F. R. Dintzis, *Staerke*, **26**, 56 (1974).