Note

Behavior of amylose in ethylenediamine and formamide

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(Received July 11th, 1970; accepted September 2nd, 1970)

In the past 10 years the properties of amylose in solution have been studied in various aqueous and nonaqueous solvent systems. We report here some observations and measurements concerning the properties of amylose in ethylenediamine, which we consider a good solvent, and in formamide, which we consider a poor one. As reviewed by Creeth and Knight[1], the approximate hydrodynamic shape in solution may be measured by the ratio of the concentration-dependence coefficient of sedimentation (K_s) to the intrinsic viscosity $[\eta]$. The sedimentation behavior of amylose in both solvents may be represented by the equation $1/S = 1/S_0\ (1+K_s\ C)$, where S_0 is the sedimentation constant extrapolated to zero concentration, and C is the concentration in g/ml.

Viscosity measurements have been made by Cowie[2], who found the Mark–Houwink relation for amylose in ethylenediamine to be: $[\eta] = 1.55 \times 10^{-2} \overline{M}_w^{0.70}$, where $\overline{M}_w$ is the weight-average molecular weight. Some problems encountered in using ethylenediamine as a solvent for amylose have been noted by Cowie[3]. Amylose fractions have also been examined by Burchard[4], who found the radius of gyration of a given amylose fraction to be slightly higher in ethylenediamine than in dimethyl sulfoxide. A value of 0.70 for the exponent of $\overline{M}_w$ was determined recently by Banks and Greenwood[5] for amylose in dimethyl sulfoxide.

According to Flory's theory[6], the $\overline{M}_w$ exponent of 0.70 indicates that amylose is random coil-like in both ethylenediamine and dimethyl sulfoxide. However, ethylenediamine is a basic solvent, and it is well known that in aqueous solutions at pH values of 11 and greater, amylose becomes negatively charged and its specific viscosity increases as the pH increases[7]. We considered it worthwhile to estimate the shape of amylose in ethylenediamine by the $K_s/[\eta]$ ratio method, to determine if asymmetry would be detected.

Samples of amylose were prepared freshly as the *n*-butanol complex from *n*-butanol-saturated aqueous solution and were treated according to the procedures of Banks and Greenwood[5]. After stirring gently for a few h at room temperature the air-dried samples dissolved readily in ethylenediamine to give an apparently clear solution. Since measurements of viscosity and sedimentation for solutions less than 4 h old sometimes yielded erratic values, solutions of amylose in ethylenediamine were allowed to age overnight before being measured.

Results of the sedimentation and viscosity measurements with corn amylose in ethylenediamine are summarized in part A of Table I. The $K_s/[\eta]$ ratio is within the range of 1.5 to 1.7, indicating a spherical shape in solution. Hence, gross asymmetry, which could be caused by extension resulting from negative charges on the amylose backbone, is not detected by this measurement.

TABLE I

HYDRODYNAMIC FACTORS RELATED TO SHAPE OF AMYLOSE MOLECULES

Dent corn amylose, preparation of solutions	$[\eta]$ *ml/g*	K_s *ml/g*[a]	$K_s/[\eta]$
A In ethylenediamine			
1 Fraction of $\overline{M}_w = 2.8 \times 10^5$	100.6 ±1.0	168 ±4	1.68 ±0.07
2 Unfractionated sample $\overline{M}_w = 2.3 \times 10^5$	80.5 ±0.5	126 ±4	1.57 ±0.06
B In formamide			
1 Fraction of $\overline{M}_w = 1.35 \times 10^5$ Concentrations freshly prepared from stock solutions. Not dialyzed. Concentrations contained up to 5% of dimethyl sulfoxide.	52.0 ±1.0	124 ±15	2.39 ±0.33
2 Solutions dialyzed against formamide at least 18 h.		Sedimentation constant values of dialyzed solutions showed high deviations from linear behavior.	Range of 1 to 1.3

[a]The error in K_s for sample B-1 was taken from a least-squares fit of eight measurements of sedimentation constant. All other errors are estimated. All viscosity and sedimentation measurements were made at 25.0°. Sedimentation values are corrected for cell dilution. Viscosity measurements were made in series 75 Cannon semimicro viscometers and sedimentation measurements were made at 47,660 r.p.m. with a Spinco Model E ultracentrifuge.

Reports of the solubility of amylose in formamide are conflicting. Banks and Greenwood[5] stated that their samples dissolved in formamide with gentle shaking overnight at room temperature. They considered formamide to be a thermodynamically good solvent for amylose. Cowie[8] found it necessary to heat samples for 5–10 min at about 50° to dissolve them. Burchard[4] heated samples of amylose with formamide for 8 h at 100° and considered some of the resulting solutions to be metastable. We were unable to dissolve amylose in formamide under mild conditions. Heating amylose in formamide overnight on a steam bath does cause significant dissolution, but this procedure may cause degradation.

Samples of corn amylose were dissolved in dimethyl sulfoxide by gentle stirring overnight at room temperature. The dissolved sample was placed in a Visking*

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

bag and dialyzed against formamide for at least 18 h with several changes of solvent. However, these amylose–formamide solutions were metastable. At concentrations from about 0.3 to 0.5% of amylose, the solutions could show a precipitate or cloudiness at times varying from 18 h to 5 days after the dialysis was started. Measurements of viscosity and sedimentation were made on some dialyzed solutions that appeared perfectly clear to the unaided eye, although light-scattering measurements showed evidence of aggregation. Significant increases in both turbidity and dissymmetry were observed on dialyzed solutions within 5 to 20 h after they had been subjected to preparative centrifugation. To minimize effects of aggregation, solutions were prepared freshly at each concentration by dilution with formamide of an 8% stock solution of amylose in dimethyl sulfoxide. Thus, at the 0.5 to 0.1% concentrations of amylose used in this study, the solutions contained from 95 to 98% of formamide, a trace of water, and up to 5% of dimethyl sulfoxide.

The results of sedimentation and viscosity measurements on samples of corn amylose in formamide (mixed solvent containing up to 5% of dimethyl sulfoxide) are summarized in part B of Table I. Measurements were made on the "fresh" solutions (B-1) within 5 h after the stock solution was diluted. Measurements were made on three dialyzed solutions (B-2) within 5 days after the start of dialysis. If these dialyzed solutions showed a precipitate or cloudiness, they were heated for a few min to 90–100° until they became clear. The $K_s/[\eta]$ value of 2.39 for the fresh solutions is higher than the accepted values for the spherical shape in solution. The $K_s/[\eta]$ values obtained for dialyzed solutions may indicate an asymmetric shape of particles in aged solutions of amylose in formamide. Since aggregation occurs in formamide, no conclusions should be made with respect to the hydrodynamic shape of individual amylose molecules in this solvent.

Also investigated was the solubility in formamide of the *n*-butanol complex of potato amylose. Potato starch was extracted freshly from Idaho potatoes and fractionated with *n*-butanol to separate the amylose from amylopectin. The precipitated butanol complex was recrystallized once and then dehydrated with *n*-butanol according to the procedure of Banks and Greenwood[5]. The *n*-butanol-wet amylose complex appeared to dissolve quickly in formamide at room temperature with gentle shaking. However, mild preparative centrifugation, $15 \times 10^3 \times g$ for 15 min, removed a major portion of material from solution. The clarified solution was placed in a light-scattering cell, where evidence of aggregation was observed within 24 h. Some of the butanol complex was air-dried overnight at room temperature and ground to a fine powder with a mortar and pestle. The powdered amylose was screened through a 60-mesh sieve and shaken overnight in formamide at room temperature. Again, mild preparative centrifugation removed much, apparently dissolved, material. Measurements of optical rotation showed a concentration of 0.08% of dissolved amylose in the clarified solution, or about 15% by weight of the original sample. The 0.08% solution of amylose precipitated within 72 h at room temperature (*ca.* 22°). It is concluded that, in general, formamide is a poor solvent for amylose.

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