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EFFECTS OF SOLVENTS ON THE SPECTROPHOTOMETRIC AND HYDRODYNAMIC BEHAVIOR OF AMYLOSE AND ITS IODINE COMPLEX

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ABSTRACT

Aqueous solutions of the amylose–iodine complex, dialyzed against KI solution and benzene, show exceptional stability. In the extreme case, only 20% of the total iodine is extracted by benzene. Dialysis against anionic surfactant (sodium dodecyl sulfate) and nonionic surfactant (Triton X-100) solutions (at, and above, their critical, micelle concentrations) appreciably affects the iodine-retention capacity of amylose, as well as causing change in the spectral behavior of the complex. Addition of cosolvents makes the biopolymer chain more compact, except for $HCONMe_2$ and urea, which induce an open conformation at higher concentrations, after an initial induction of compactness in the lower range of concentration. Upon complexation with iodine, the amylose chain assumes considerable rigidity. A correlation between the stability of the complex and the polarity of the medium is not straightforward; neither of the solvent compositions (mole fractions) is the same at equal levels of the chain conformations, suggesting accountable roles of specific solvent-effects. The hydrodynamic parameters $[\eta]$, $\langle S^2 \rangle$, α, and the Huggins constant k are presented, to give a greater understanding of the biopolymer and its iodine complex.

INTRODUCTION

The conformation of the amylose–iodine complex has been considered to be almost comparable to that of amylose itself. Information gathered on either of them can thus be useful for the other. In the solid state, the complex has been proved to be that of V-amylose[1–4], but the conformation in solution has not been conclusively decided. In solution, amylose has been considered to have such conformations as (*1*) random coil[5–8], (*2*) tight helix[9–11], and (*3*) alternate segments of random coil and helix[12]. Whatever the conformation, its distortion would be expected to lessen the complexation with iodine; both "squeezing" and elongation of the native conformation may provide the same final effect.

Very recently, we described the stability of the amylose–iodine complex under varied experimental conditions[13], and showed that, although nonaqueous solvents in general can destabilize the system, a correlation with regard to polarity of the medium cannot be obtained. Similarly, comparison on an equimolar-fractional basis

also showed poor correlation. From a hydrodynamic study of amylose in 1,4-dioxane, and a colorimetric study of the complex in this medium, it was indicated that some kind of conformational change is involved in the decomposition of the complex. To correlate the effects of different solvents, we have since undertaken a determination of the intrinsic viscosity of an amylose sample of low viscosity (average molecular weight 14,090; rigid according to the definition of Burchard[14]) in various proportions of cosolvents to observe the extent to which the conformation and related parameters are affected. For comparison, the intrinsic viscosity of the amylose–iodine complex has been also studied. Along with these, a complementary, spectrophotometric study of the stability of the complex under varied conditions has been made. Besides some specific, solvent effects, significant change of the amylose conformation has been observed. The iodine complex of amylose has been found to be remarkably stable under normal conditions.

EXPERIMENTAL

Materials. — The amylose-2 was the same product (Aldrich Chemicals, U.S.A.; viscosity average molecular weight 163,900) described earlier[13]. Amylose-3 was a product of E. Merck, India (viscosity average molecular weight 14,090) and was used as such. The nonaqueous solvents and other additives were of pure grade, and were further purified by standard procedures prior to use, except for urea, which was Analar grade (B.D.H.) and was used without further purification. The potassium iodide and surfactants were the same materials as reported earlier[13]. Double-distilled, conductivity water was used for preparing solutions.

Methods. — Spectrophotometric measurements were recorded with a Perkin–Elmer–Hitachi-200 Digital Spectrophotometer, using cuvets of 1-cm path-length. Viscosity measurements were made in the Ostwald viscometer described earlier[13]. Dialysis tubing (Arthur Thomas and Company, Philadelphia, PA) of diameter (inflated) 22.2 mm (7/8 in.) and molecular-weight cut-off 12,000 was used for the dialysis experiments. All solutions were thermostated at 30 $\pm 0.1°$, if not otherwise mentioned.

The blue complex was prepared by mixing an amylose solution with iodine in KI solution, and equal portions of it were placed in dialysis tubings that were then closed securely by tying with a stout thread. After carefully washing the outer surface of the bag with water, the outside was dried with filter paper. The bag was immersed in a container holding the chosen dialyzate solution, and the well-stoppered container was thermostated for 2 h with intermittent shaking. The bag was then removed, the solution was collected in a spectrophotometer cuvet, and the spectrum recorded.

In the viscosity experiments, each solution was thoroughly thermostated, and the viscosity was determined (relative to that of water). The density of the amylose solution was determined in a pycnometer. The intrinsic viscosity $[\eta]$ was found by plotting η_{sp}/c against c according to the equation (*1*) of Huggins,

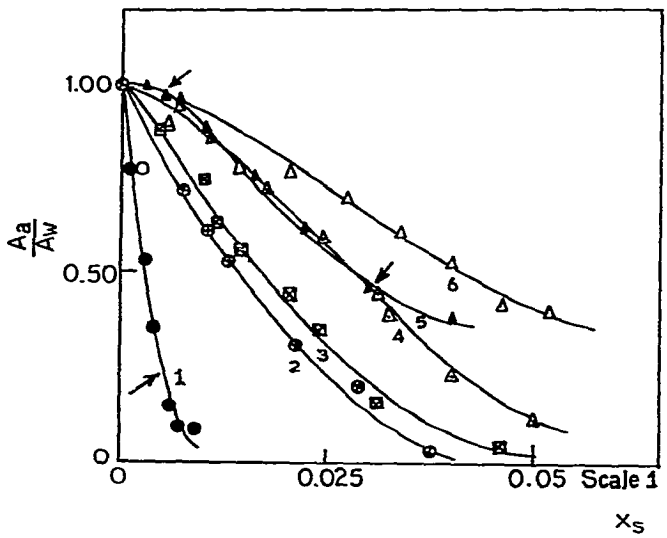

Fig. 1. Effect of solvents on the relative absorbance of the iodine–amylose-3 complex at 580 nm. [Composition: Am-3, 0.02%; I_2, 0.08mM; KI, 0.01M. Key: curve 1, Am-3–acetone; 2, Am-3–Me_2SO; 3, Am-3–$HCONMe_2$; 4, Am-3–1,4-dioxane; 5, Am-3–methanol; 6, Am-3–urea. Mole fraction (X_S). Scale 1 for curve 3; scale 1 × 2 for curves 2, 4, and 6; scale 1 × 10 for curves 1 and 5.]

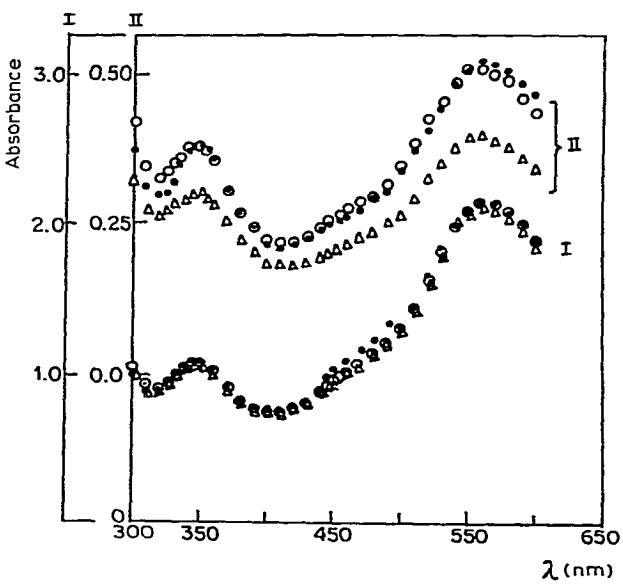

Fig. 2. Spectra of iodine–Am-3 complex after dialysis for 2 h at 30° against KI and benzene. [Composition: (Set I) low iodine concentration: Am-3, 0.08%; I_2, 1.2 mM; KI, 0.01M. (Set II) moderate iodine concentration: Am-3, 0.04%; I_2, 1.2 mM; KI, 0.01M. Key: open circle, complex not dialyzed; closed circle, complex dialyzed against 0.01M KI; open triangle, complex dialyzed against benzene. Scale I for Set I, scale II for Set II.]

$$\eta_{sp}/c = [\eta] + [\eta]^2 kc \qquad (1)$$

where η_{sp}, k, and c are, respectively, the specific viscosity, the Huggins constant, and the concentration of amylose (expressed in g/dL). The intrinsic viscosity of the amylose–iodine complex in solutions undersaturated and saturated with iodine was also determined by following similar procedures.

RESULTS

(a) *Dialysis of the complex under various conditions.* — The spectra of the iodine–amylose-3 complex after dialysis against the same strength of aqueous KI solutions as in the complex, and against benzene, are exemplified in Fig. 1. Set I refers to insufficient iodine added to the amylose, and Set II, to a moderate concentration of iodine. Samples undialyzed, and dialyzed against KI, for both sets exhibited practically no change in the spectra. When benzene was used as the dialyzand (see Fig. 2), the set having a moderate concentration of iodine showed a decrease in the absorbance, possibly due to extraction of part of the iodine by the nonaqueous solvent. The same observations were made for the iodine–amylose-2 complex.

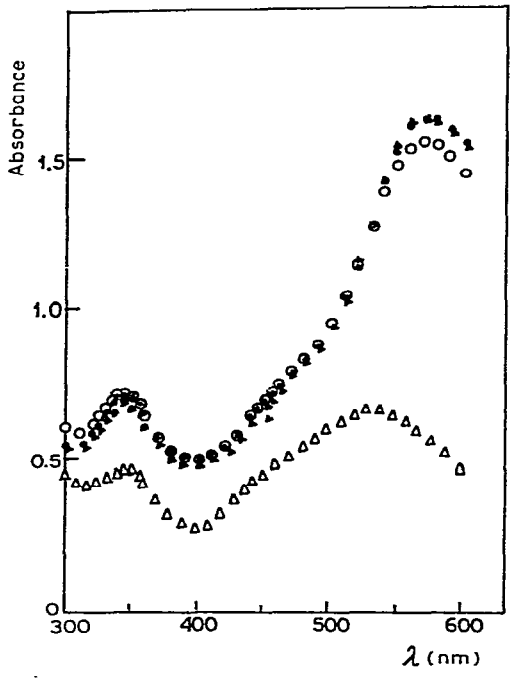

Fig. 3. Spectra of iodine–Am-2 complex after dialysis for 2 h at 30° against KI, benzene, and SDS. [Composition: Am-2, 0.0165%; I_2, 1 mM; KI, 0.01M. Key: open circle, complex not dialyzed; closed circle, complex dialyzed against 0.01M KI; closed triangle, complex dialyzed against benzene; open triangle, complex dialyzed against 0.01M SDS.]

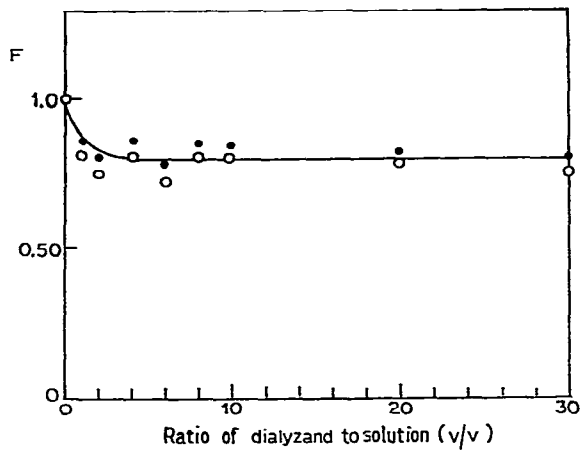

Fig. 4. Effect of different proportions of benzene as dialyzand on the relative absorbance of iodine–Am-3 complex. [Composition: Am-3, 0.04%; I_2, 1.2 mM; KI, 0.01M (moderate iodine concentration). Key: open circle, 560 nm; closed circle, 350 nm.]

Remarkable retention of iodine (a reflection of high stability of the complex) was observed.

When the dialyzate contained, in addition to KI, such surfactants as SDS and TX-100, at and above the critical micelle concentration (c.m.c.), the spectra changed. Low concentrations of CTAB (below the c.m.c.) had no effect; higher concentrations could not be used, because of precipitation. Both SDS and TX-100, in addition to lowering the absorbance, shifted the λ_{max} towards the blue. Interaction of these, leading to depletion of iodine in the complex, was anticipated. Some of these results are depicted in Fig. 3.

In Fig. 4, the efficiency of benzene in extracting iodine from the complex is presented. The results were obtained by dialyzing constant-volume samples from the same mixture against various proportions of benzene ranging from zero to twenty. The absorbance obtained at λ_{max} of triiodide (350 nm) and of the complex (580 nm), when plotted as ratios with the absorbance of the undialyzed sample, helped in the construction of the Figure. An equal extent of loss of only 20% was found for all of the samples. A similar loss was revealed on examining Set II of Fig. 1. This was a striking observation, to be discussed later.

(b) *Intrinsic viscosity and other physicochemical parameters of amylose and its iodine complex.* — The intrinsic viscosity of amylose-3 was found to decrease initially with all of the additives. Except for $HCONMe_2$ and urea, it continued to decrease with increase in the additive, whereas, in the exceptional cases, $[\eta]$ increased, from mole fractions of ~0.025 and ~0.035, respectively. The results are shown in Fig. 5. From $[\eta]$, values for $\langle S^2 \rangle$ were calculated from the relationship[15,16]

$$[\eta] = [6^{3/2}\, \phi\, \langle S^2 \rangle^{3/2}/M, \qquad (2)$$

where ϕ is a constant, $\langle S^2 \rangle$ is the mean-square radius of gyration of the polymer coil, and M is the molecular weight of the macromolecule.

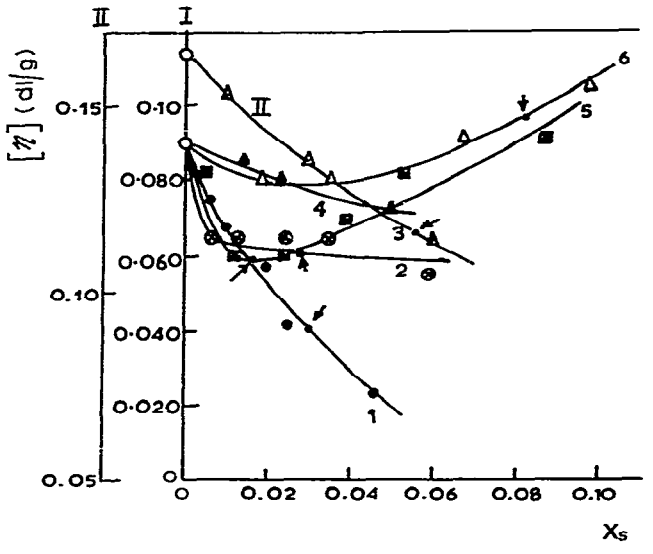

Fig. 5. Variation of intrinsic viscosity of Am-3 and Am-1 in different cosolvents at 30°. [Key: curve 1, Am-3–acetone; 2, Am-3–Me_2SO; 3, Am-1–1,4-dioxane; 4, Am-3–methanol; 5, Am-3–$HCONMe_2$; 6, Am-3–urea. Mole fraction (X_s). Scale I for Am-3, scale II for Am-1.]

The results are given in Table I. The Huggins constant k of eq. *1* is a measure of the physical states of the biopolymer. These were calculated, and are plotted in Fig. 6 against the mole fraction of the additives. Both decrease and increase were noted, in accordance with the variation of $[\eta]$ and $\langle S^2 \rangle$. An account of the segment–segment and solvent–segment interactions was obtained by calculating the Flory–Fox parameter[17] α from the relationship

$$\alpha^3 = \{[\eta] \text{ (in dL/g)} \times M_0^{3/2}\}/M^{1/2}\beta^3\phi \qquad (3)$$

where M is the molecular weight of the macromolecule, M_0 is the molecular weight of each of the D-glucosyl residues, and β is the effective bond-length. The values are also given in Table I.

A value of $\alpha = 1$ defines the solvent (in which the polymer is dispersed) to be ideal. When $\alpha > 1$, the solvent is good, and when $\alpha < 1$, it is considered to be poor. The α-values obtained were all less than one. The solvent environments used were all of the poor class; the variations then showed the degree (level).

In Fig. 7, the variations of $[\eta]$ with the dielectric constant of the media are presented. Both increase and decrease of dielectric constant had almost the same effect, namely, of decreasing $[\eta]$, except for $HCONMe_2$ and urea, which, although they have reverse polarity-direction, both increased the value of $[\eta]$ after an initial decrease.

(c) *Intrinsic viscosity and related parameters of iodine–amylose-3 complex in water, and of amylose-1 and amylose-3 in alkaline medium.* — The intrinsic viscosity and other parameters mentioned in the previous section were evaluated for amylose-1

TABLE I

HYDRODYNAMIC PARAMETERS OF AMYLOSE-1[a] AND AMYLOSE-3[a] IN DIFFERENT COSOLVENT MEDIA

mMole fraction	*Dielectric constant*[b]	$[\eta]$ *(in dL/g)*	k	$\langle S^2 \rangle \times 10^{14}\ cm^2$	α
Acetone					
6.3	75.0	0.075	0.8889	7.36222	0.6755
9.5	75.0	0.068	1.2255	6.8966	0.6537
19.4	73.0	0.057	2.2571	6.1312	0.6164
24.5	72.50	0.041	5.9488	4.9220	0.5523
Dimethyl sulfoxide					
5.7	78.50*	0.065	2.0512	6.6922	0.6440
12.0	78.50*	0.065	2.0512	6.6922	0.6440
23.6	78.40*	0.065	2.0512	6.6922	0.6440
34.7	78.20*	0.065	2.0512	6.6922	0.6440
59.4	77.80*	0.055	1.9834	5.9869	0.6091
169.7	75.70*	0.047	1.9617	5.3913	0.5780
Methanol					
14.2	76.50	0.085	0.6920	8.0029	0.7043
22.9	75.50	0.080	0.7813	7.6858	0.6901
49.6	73.00	0.072	1.0288	7.1645	0.6664
1,4-Dioxane					
10	75.00	0.153	0.7287	15.325	0.7445
30	69.20	0.135	0.7316	14.216	0.7141
60	60.00	0.115	0.9093	12.912	0.6769
Urea					
17.7	81.60*	0.080	0.7800	7.6858	0.6901
34.7	83.80*	0.080	0.7800	7.6858	0.6901
67.2	87.60*	0.092	0.9846	8.4364	0.7230
97.5	91.00*	0.105	0.6349	9.2136	0.7557
N,N-*Dimethylformamide*					
5.0	78.00*	0.082	0.9915	7.8134	0.6959
11.5	77.50*	0.060	2.3148	6.3445	0.6271
23.8	77.00*	0.060	2.3148	6.3445	0.6271
38.5	75.50*	0.070	1.3605	7.0312	0.6601
52.9	75.00*	0.082	0.9915	7.8134	0.6959
87.4	72.50*	0.090	0.7407	8.3137	0.7178

[a]M.W. of amylose-1, 32,695; of amylose-3, 14,090; temp., 30°; $\phi = 3.6 \times 10^{21}$; $\beta = 1.055$ nm.
[b]Asterisks denote the dielectric constant at 25°.

and amylose-3 in 0.5M KOH medium, and also for iodine–amylose-3 complex at two levels of iodine composition in aqueous medium. The results are presented in Table II. Although giving an increase in $[\eta]$, $\langle S^2 \rangle$, and α, and a decrease in k in an alkaline environment, complexation with iodine caused a reverse effect in aqueous medium.

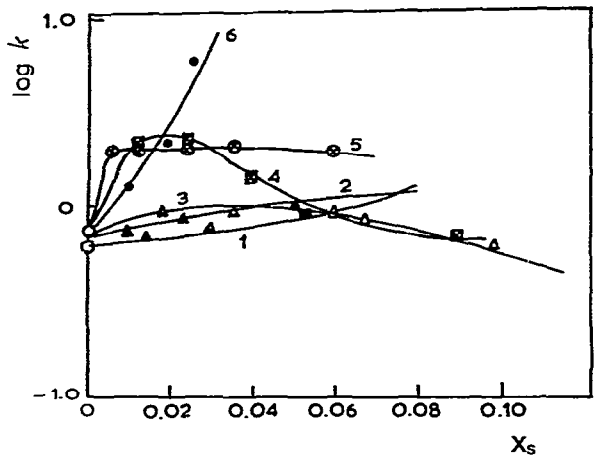

Fig. 6. Variation of log k of Am-3 and Am-1 in different cosolvents at 30°. [Key: curve 1, Am-1–1,4-dioxane; 2, Am-3–methanol; 3, Am-3–urea; 4, Am-3–$HCONMe_2$; 5, Am-3–Me_2SO; 6, Am-3–acetone. Mole fraction (X_s).]

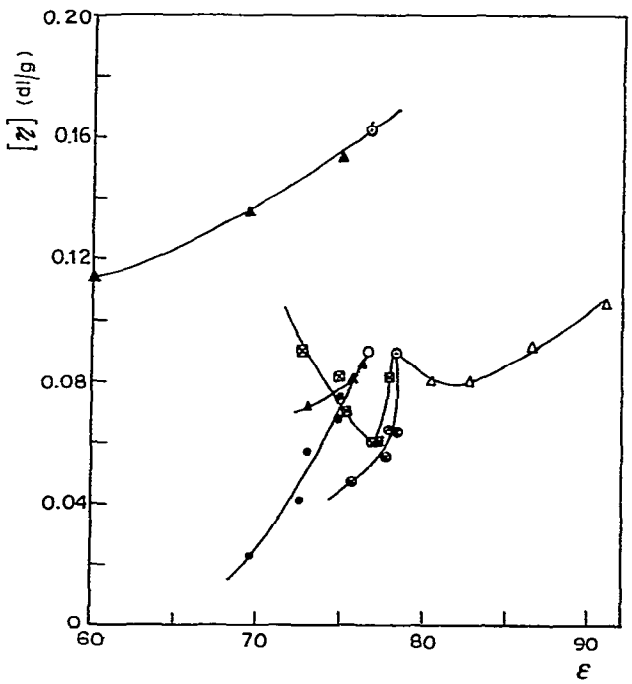

Fig. 7. Variation of intrinsic viscosity of Am-3 and Am-1 with dielectric constant of the medium. [Key: triangle with cross, Am-1–dioxane; open circle with prime, Am-1–water; open circle, Am-3–water at 25 and at 30°; closed circle, Am-3–acetone; closed triangle, Am-3–methanol; open square with cross, Am-3–$HCONMe_2$; open circle with cross, Am-3–Me_2SO; open triangle, Am-3–urea.]

TABLE II

HYDRODYNAMIC PARAMETERS OF AMYLOSE-1 (Am-1), AMYLOSE-3 (Am-3), AND IODINE–AMYLOSE-3 COMPLEX IN AQUEOUS MEDIUM[a]

System	$[\eta]$ *(in dL/g)*	k	$\langle S^2 \rangle \times 10^{13}$ *cm*^{2}	α
Am-3 in water	0.090	0.7407	0.8314	0.7178
in 0.5M KOH	0.100	0.4333	0.8919	0.7434
Am-1 in water	0.163	0.6022	1.5918	0.7604
in 0.5M KOH	0.229	0.5911	2.7160	0.8517
Iodine–Am-3 complex [Amylose]:[iodine] = 0.0916:1 (iodine in excess)	0.070	2.2449	0.7031	0.6601
Iodine–Am-3 complex [Amylose]:[iodine] = 0.3623:1 (iodine-deficient)	0.074	2.8610	0.7297	0.6725

[a]M.W. of Am-1, 32,695; of Am-3, 14,090; temp., 30°.

Like nonaqueous solvents in general, addition of iodine made the amylose conformation compact. In evaluating the $[\eta]$ value, the concentration of the complex was calculated by taking the amount of iodine bound to it; as the stability of the complex was excellent (*cf.*, Figs. 2–4), the whole of the iodine was considered to be associated with the polymer. In the case of an excess of iodine, the saturation concentration, determined from the iodine-binding experiment, was added to the amylose, and the rest was neglected (and treated as part of the solvent medium).

DISCUSSION

At low proportions of iodine, both the smaller and larger molecular-weight amylose samples showed a good degree of stability in aqueous medium. Dialysis against KI solution (of the same strength as in the complex) and benzene corroborated this finding; although almost the same spectra were obtained in an aqueous environment, partition between the water and benzene phases decreased the complex by ~20% when the proportion of iodine in the complex was moderate. Both SDS and TX-100, above the c.m.c., were effective decomplexing agents, as reported earlier[13]. The nonextractability of iodine by benzene was a remarkable feature. A high degree of stability of the complex was envisaged, which, by the action of surfactants, and of a suitable salt ($Na_2S_2O_3$), could be appreciably disturbed. Blocking of membrane pores by the macrodimensional complex inside the bag was considered meager, as such large species as tetramethyl ions were found to be transported freely through the membrane in the presence, and the absence, of the complex. The easy action of the large, surfactant molecules, instead of the barrier presented by the membrane, also did not argue in favor of the obstruction of the pores for the unusual retention of the complex when benzene was the extractant (having a partition coefficient for

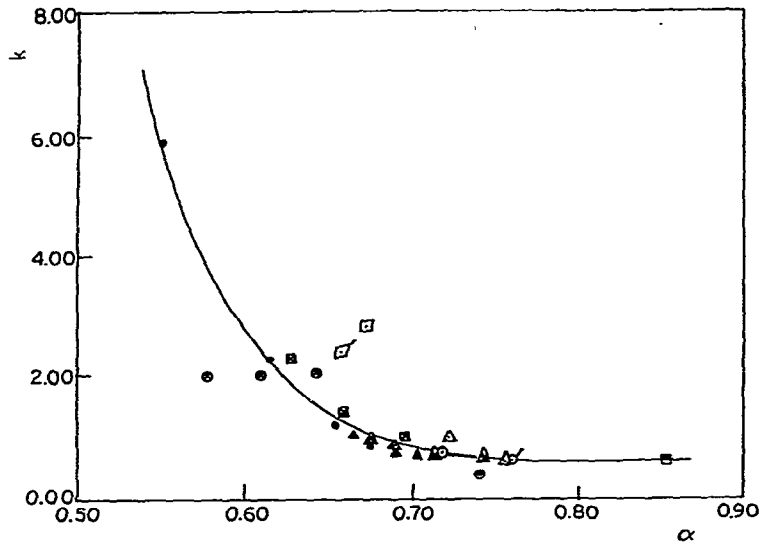

Fig. 8. Variation of k with α for Am-3 and AM-1 in aqueous and cosolvent media. [Key: open circle, Am-3–water; open circle with prime, Am-1–water; half-filled circle, Am-3–0.5M KOH; half-filled square, Am-1–0.5M KOH; open triangle, Am-3–urea; closed triangle, Am-3–methanol; open triangle with cross, Am-1–1,4-dioxane; open square with cross, Am-3–$HCONMe_2$; open circle with cross, Am-3–Me_2SO; closed circle, Am-3–acetone; open square, iodine–Am-3 complex (iodine in excess); open square with prime, iodine–Am-3 complex (iodine-deficient).]

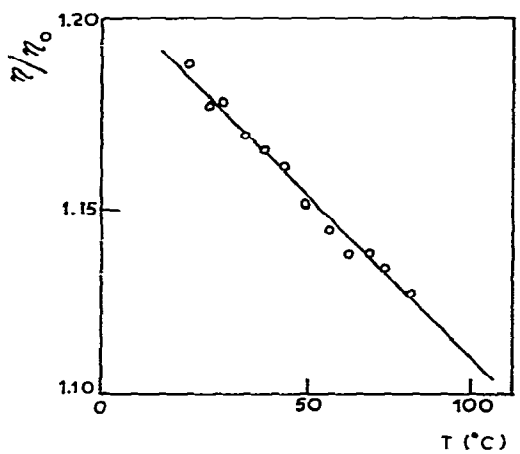

Fig. 9. Variation of relative viscosity of Am-3 with temperature.

iodine against water as high as ~360 at room temperature). The constant decrease of 20% in the absorbance of the complex, irrespective of the amount of benzene used as the dialyzand (results of Fig. 4), suggested a different mode of binding to the amylose of a part of the total iodine.

Intrinsic-viscosity measurements indicated that the amylose chain becomes extended upon addition of KOH. On the other hand, the chain is contracted on being

complexed with iodine. The stability of the complex was expected to depend on its helicity; either "squeezing" of the helix or its expansion might decomplex iodine. Blocking of the chain sites by complexation with other materials might also hinder iodine association. Spectrophotometric observations revealed that progressive addition of nonaqueous solvents lessened the extent of complexation, and, at sufficient proportion, in some cases it brought the level to zero. Viscosity measurements performed at a relatively higher concentration of amylose were of no value at higher proportions of nonaqueous solvents, owing to precipitation of the biopolymer.

Such experiments had eventually to be conducted at relatively low proportions of the solvents, *i.e.*, lower than the level producing zero absorption in colorimetry. For all of the solvents added, a significant lowering of $[\eta]$ was observed at low proportions, which, in the case of $HCONMe_2$ and urea, increased above the mole fractions of 0.025 and 0.035, respectively; this was ascribed to the complexation[18–21] of these compounds with the amylose chain, thereby endowing greater randomness at high proportions. The amide groups of these compounds were susceptible to formation of hydrogen bonds with the hydroxyl groups of the biopolymer. Complexation of Me_2SO with amylose is well known; the nonsignificant change of $[\eta]$ after addition of a certain proportion of this solvent is a characteristic feature in regard to the intrinsic-viscosity property. At a level of 50% decrease of absorbance (equivalent to 50% loss of complexation), the order of the solvents was $HCONMe_2 > Me_2SO > Me_2CO >$ MeOH. In this series, acetone caused the chain to contract the most, and methyl alcohol, the least. Whatever the cause, the effect was reflected in the $[\eta]$ value, and a parallel conclusion could be reached concerning the effects of these solvents on the amylose–iodine complex, both of which may be considered to be helical coils differing mainly in the degree of helicity.

Such nonaqueous solvents as acetone, 1,4-dioxane, and methanol lessened the solubility of amylose, whereas urea, $HCONMe_2$, and Me_2SO increased it. The first group of compounds made the environment a poor solvent, enriching segment–segment contact and compactness in the conformation, and ultimately affording a separate phase. The second group of compounds formed a complex with the biopolymer, lessening the segment contacts and, comparatively, induced "good-solvent" environments. The compactness of the chain was reflected in the values of the Huggins constant k, calculated from eq. *1*. The k values increased with the first group of compounds, and, besides acetone, approached the value of 2.0 expected for rigid spheres[22]. A low value of 0.33 is assigned to the random-coil[23] conformation, which was not found in the present work, thus justifying the conclusion that the chain was not a random coil at any stage (in all probability, it was a compact helix).

A plot of the Huggins constant k against α (see Fig. 8) revealed a correlation between the two, irrespective of the types and amounts of the cosolvents used. In this trend, the amylose–iodine complex deviated appreciably, supporting the foregoing contention of non-exact comparison of the solvent-induced chain-conformation of pure amylose with the amylose–iodine complex. The direction of $\langle S^2 \rangle$ was the same as for $[\eta]$, supporting more rigidity of the system (rather than much randomness).

The values of α were indicative of the fact of increasing degree of poor-solvent environment with the addition of cosolvents (except urea and $HCONMe_2$, which provided comparatively good environments above certain levels). The value of α was always <1, showing that it never crossed the level of ideality ($\alpha = 1$).

The variation of $[\eta]$ with the dielectric constant was peculiar. Although ε varied in opposite directions, urea and $HCONMe_2$ showed a final increase in $[\eta]$ after an initial fall. Some sort of specificity was operative, and this was anticipated to be the complexation of amylose with these additives. Dipolar interactions did not play a major role in the formation of the complex, or on the chain conformation. The percentage changes in $[\eta]$ at 50% lessening of the absorbance of the complex were 34.44, 32.22, and 55.55% in $HCONMe_2$, Me_2SO, and acetone, respectively. For $HCONMe_2$ and urea, the 50% level appeared twice.

The reappearance of the same $[\eta]$ at higher mole fractions did not show increased absorbance of the complex, signifying that, although the chain became more random, complexation with the additives expelled iodine from the field of interaction. Although $HCONMe_2$ and Me_2SO, at low concentrations, specifically complex with amylose, such effects were considered minor compared to the conformational change on decomplexing iodine. In this regard, their behavior was close to that of acetone. Dimethyl sulfoxide showed the peculiarity of holding the $[\eta]$ value almost constant, after a rapid fall at significantly low concentrations.

The intrinsic-viscosity values of amylose in excess and at below-saturation levels of iodine were almost identical, and 22.22% less than that of a sample of pure amylose in water. Shortening of the helical conformation was evident, and it was equivalent to 6.0, 4.0, and 8 mmole-fractional levels of additions of $HCONMe_2$, Me_2SO, and acetone, respectively. Additives at these levels lowered the optical absorbance (decomposed the complex) in different proportions (see Fig. 1), signifying additional factors responsible for the stability of the complex, and not only the chain conformation.

According to the work of Burchard[14] and Kodama and Noda[23], our amylose-3 sample should be a double helix. The effect of temperature, within the range of 20–80°, on the viscosity of this amylose solution showed a systematic, linear decrease, without any abruptness. The biopolymer was considered to be inherently stable; the double helix (if present) did not "melt" in the range of temperature studied.

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