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ENVIRONMENT-INDUCED, PHYSICOCHEMICAL BEHAVIOR OF AMYLOSE–IODINE COMPLEXES

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ABSTRACT

Effects of salts, surfactants, co-solvents, and temperature on the formation and stability of the iodine complexes of two amylose samples (differing five-fold in molecular weight) are reported Differences in the characteristics of the complexes formed by the two amylose samples have been observed with regard to spectral features, and surfactant and temperature effects An optimal ionic strength was found necessary for the maximum formation of the complex under otherwise identical, experimental conditions Surfactants and co-solvents mainly destabilize the complex, and the overall polarity of the mixed medium is insufficient to systematize the solvent effects An attempt has been made to explain the effect on the basis of conformational aspects of the amylose–iodine complex in 1,4-dioxane–water The iodine complex of the amylose sample of lower molecular weight showed permanent hysteresis in the heating and cooling curves, a phenomenon not observed for the iodine complex of the amylose sample of higher molecular weight Enthalpy considerations showed the complexation process to consist of two distinct. cooperative interactions, with a fair degree of environmental, order-producing effect

INTRODUCTION

Although the blue complex of amylose with iodine has been well studied[1–10], many salient features thereof still remain unexamined For instance, a clear understanding of its formation and stability in solution is as yet incomplete The physicochemical properties of the complex in aqueous medium depend on various factors, such as the degree of polymerization of the biopolymer[11 12], the pH[13 14], and the temperature[10 12 15 16] Studies in these areas made in the past stressed only one or two of these aspects, and observations made on the basis of the effects of the various factors on a particular system are very limited

In the present study, we selected two amylose samples having a five-fold difference in molecular weight, the formation of a complex with iodine in the presence of potassium iodide was investigated under various conditions with regard to salts, surfactants, co-solvents, and temperature, by employing such physicochemical methods as spectrophotometry, colorimetry, and viscometry In the present study,

a wide range of temperature was used, and the effects of ionic and nonionic surfactants are presented for the first time

EXPERIMENTAL

Materials and methods — The sample (source unknown) of amylose (A R grade) of low molecular weight was obtained from E Merck, Darmstadt, and the sample (potato amylose) of higher molecular weight was a pure variety obtained from Aldrich, U S A , by courtesy of Dr A R Das of Clarkson College of Technology, Brookline, MA, U S A The molecular weights were determined from viscosity measurements, the media being 0 5M aqueous potassium hydroxide for the lower, and dimethyl sulfoxide for the higher, molecular-weight samples, respectively The viscosity average molecular weights were 32,695 and 163,900, respectively

The salts, KI and KCl, and the iodine were of A R grade (BDH) The surfactants, sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), and *p-tert*-alkylphenoxy-poly(oxyethylene ether) (Triton X-100), had the specifications described earlier[17]

The co-solvents, acetone, 1,4-dioxane, and ethanol, and cosolutes D-glucose and urea, were either of E. Merck *pro analysi* or BDH AnalaR grade They were distilled, or otherwise purified, prior to use, by standard procedures

Spectral measurements were made with a UV Chem Hilger–Watts digital spectrophotometer using cuvets 0 5 cm wide Colorimetric measurements were made with a Hilger-pattern Biochem Absorptometer of Associated Instrumenters Private, Limited, India Its calibration was checked against the spectrophotometer in the wavelength range studied

An Ostwald viscometer of flow times 200 s at 30° for water was used for viscosity measurements, densities were measured in a pycnometer

The temperature used for the measurements was 30 ±0 02°, if not otherwise stated

Preparation of solutions — The amylose (Am_1) of lower molecular weight was dissolved in conductivity water (specific conductance, 1 6–2 0 × 10^{-6} mho cm^{-1} at 30°) by heating on a boiling-water bath The suspension was filtered through a sintered-glass septum (G-4), and the concentration was determined by carefully evaporating a fixed volume in an oven at 90°, and drying to constant weight over sulfuric acid in a desiccator

The amylose (Am_2) of higher molecular weight was similarly dissolved, but with heating for 30 min Stock solutions of amylose were never used for more than four consecutive days

Iodine was dissolved in potassium iodide solution by adding a known weight of it, and then estimating it with dichromate-standardized, thiosulfate solution. For storage, the container was wrapped in black paper and kept in the dark

Spectrophotometric measurements. — Amylose and iodine were mixed in the

requisite proportions, and the mixture was kept for 2 h for attainment of equilibrium The spectra were then determined with the spectrophotometer

Colorimetric measurements — Amylose was titrated with iodine in potassium iodide solution A fixed concentration of amylose was maintained in several test-tubes and the absorbance was measured after adding different amounts of iodine solution, mixing thoroughly, and allowing 2 h for attainment of equilibrium A reverse set of experiments was also performed, in which various amounts of amylose were added to a fixed concentration of iodine, and the absorbance was measured

To observe the effects of salts, surfactants, and co-solvents, the material was added in various proportions to samples having a fixed amylose iodine composition, in the presence of a constant concentration (0 01M) of potassium iodide

To study the effect of temperature on the complex, the reactants were mixed at a fixed ratio in the colorimeter tube The tube was completely filled with the solution, and closed with a glass stopper so as to leave no air gap Finally, the top was sealed with a heat-resistant adhesive, to prevent loss due to evaporation during heating, the pressure generated by volume expansion of the liquid was observed to be insufficient to loosen the stopper during heating The whole sample was immersed in a thermostat whose temperature was raised, in steps, from 2° to 75° At each temperature of measurement, the sample was normally allowed 1 h, the optimum observed for the system to reach equilibrium, and reading was taken in the colorimeter within 5 sec, a time interval considered insufficient to cause any change in the absorbance (due to temperature fluctuations experienced by the complex system between the bath and the colorimeter) The data were found to be reproducible within the limits of experimental accuracy, and so the procedure was adopted Experiments in the directions of both stepwise heating and cooling were performed A cooling experiment was also performed, without allowing 1 h at the temperature of measurement, on the assumption that the system reached equilibrium at each measured temperature, as the cooling was slow, 0 1°/min

RESULTS

Spectra of iodine in the presence of amylose — The characteristic peaks for the complex appeared at 580 and 640 nm for Am_1 and Am_2, respectively (see Fig 1) The blue-shifted peak for Am_1 was attributed to its lower degree of polymerization compared to that of Am_2 In this Figure, the spectra of the Am_1–I_2 complex in the presence of a low concentration of 1,4-dioxane is given, because nonaqueous solvents have been reported to form a complex with the amylose–iodine system[10] Additional spectra recorded in the presence of SDS and CTAB have also been incorporated in the Figure, these will be discussed in subsequent sections

Colorimetric titration of amylose with iodine in potassium iodide — The results of colorimetric titrations for both Am_1 and Am_2 are shown in Fig 2 The results, plotted as absorbance *versus* molar concentration of I_2, showed Langmuirian behavior The difference in heights for the two sets with Am_1 were due to the difference in

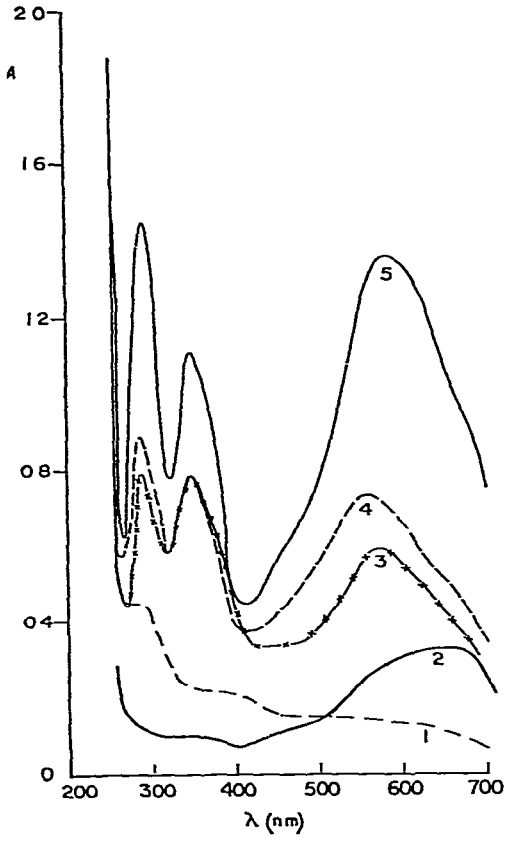

Fig 1 Spectra of amylose–iodine complex in various environments at 25° [Key curve 1, Am_1 (0 02%)–I_2 (0 08 mM)–KI (0 01M) in CTAB (0 01mM), curve 2, Am_2 (0 0001%)–I_2 (13 3μM)–KI (1.7mM), curve 3, Am_1 (0 01%)–I_2 (0 04mM)–KI (5 0 mM) in 0 56M 1,4-dioxane, curve 4, Am_1 (0 02%)–I_2 (0 08mM)–KI (0 01M) in SDS (1 5mM), curve 5, Am_1 (0 02%)–I_2 (0 08mM)–KI (0 01M)]

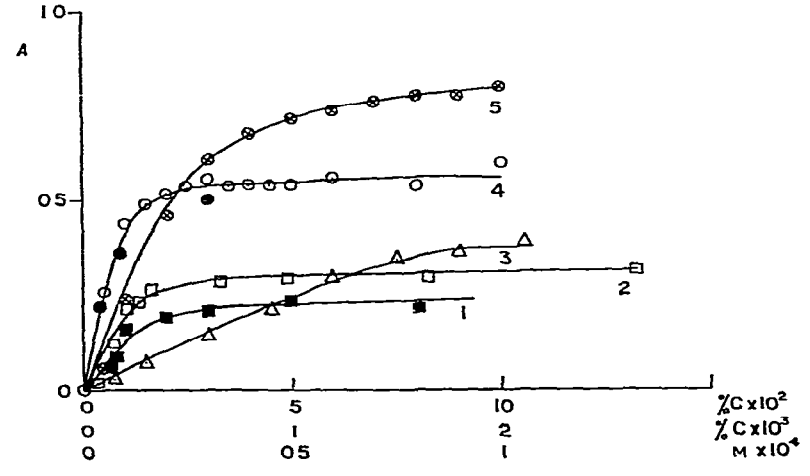

Fig. 2 Colorimetric titration of amylose with iodine in 0 01M KI at 580 nm [Key curve 1, constant Am_2 (0 0001%) with varied I_2, curve 2, constant Am_2 (0 0002%) with varied I_2, curve 3, constant I_2 (0 02mM) with varied Am_2, curve 4, constant I_2 (0 02mM) with varied Am_1, curve 5, constant Am_1 (0 02%) with varied I_2]

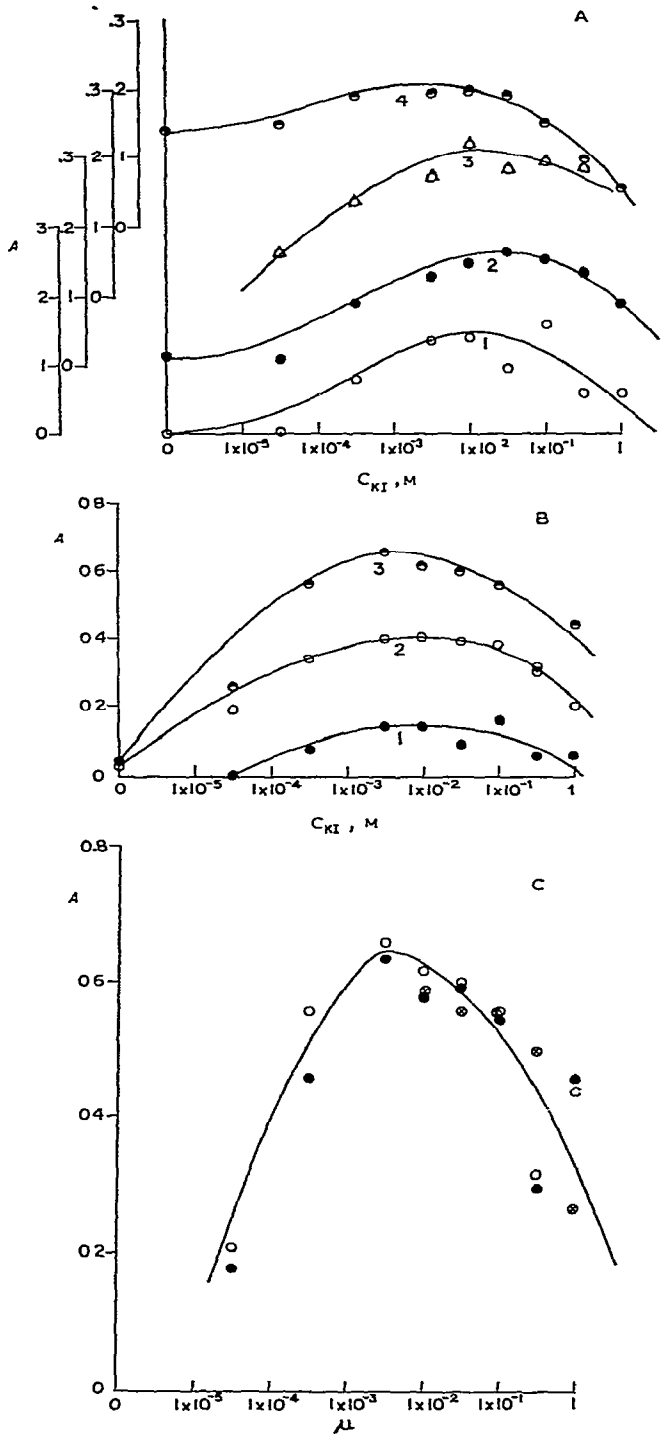

Fig 3A Effect of KI on Am_1–I_2 complex having various iodine–amylose ratios (Key curve 1, 0 02mM I_2 plus 0 002% Am_1, curve 2, 0 04mM I_2 plus 0 002% Am_1, curve 3, 0 06mM I_2 plus 0 002% Am_1, curve 4, 0 08mM I_2 plus 0 002% Am_1)

Fig 3B Effect of KI on Am_1–I_2 complex having various amylose–iodine ratios (Key curve 1, 0 002% Am_1 plus 0 02mM I_2, curve 2, 0 01% Am_1 plus 0 02mM I_2, curve 3, 0 05% Am_1 plus 0 02mM I_2]

Fig 3C Effect of ionic strength on the formation of the complex, measured at 580 nm (Key 0 05% Am_1 plus 0 02mM I_2, open circle, in KI, closed circle, in KI, measured after 1 h, circle with cross, in KCl plus KI.)

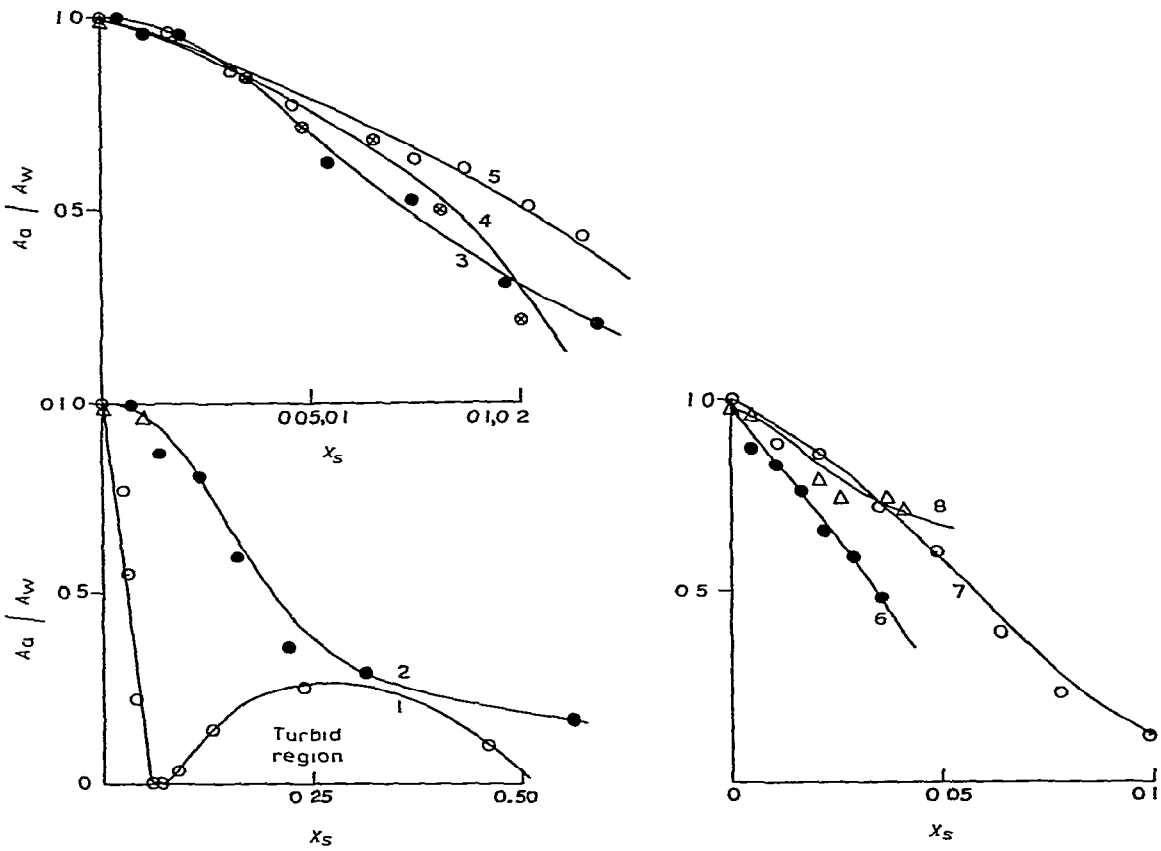

Fig 4 Effect of solvents on the relative absorbance of the amylose-iodine complex at 580 nm [Composition 0·02% Am_1–0·08mM I_2–0·01M KI, and 0·0002% Am_2–0·08mM I_2–0·01M KI Key curve 1, Am_1–acetone, curve 2, Am_1–ethanol, curve 3, Am_1–acetonitrile, curve 4, Am_2–urea, curve 5, Am_1–urea, curve 6, Am_2–1,4-dioxane, curve 7, Am_1–1,4-dioxane, curve 8, Am_1–D-glucose Mole fraction (X_s), scale 0–0·2 for curve 4]

overall concentration of the two sets From the linear portion of the curves, the molar extinctions of I_2 in bound form at 580 nm were found to be 16,533 and 8098 for Am_1 and Am_2, respectively, and 13,497 at 610 nm for Am_2 For amylose, Kuge and Ono[5] reported an extinction of 36,300 at 640 nm In Fig 2, results obtained at a constant concentration of iodine with various proportions of amylose are also shown, the nature of the curves was the same

Effect of KI *and* KCl *on the formation of the complex* — Such effects, measured colorimetrically with Am_1, are illustrated in Figs 3 and 4 All of these results show an optimal concentration of salt for maximization of the complex at a fixed ratio of the reactants Thus, at any amylose·iodine ratio, the ionic strength plays a definite role which is optimal between 0·005 and 0·01M. It was, therefore, concluded that, for the complexation, only a small amount of KI is necessary, and, where no KI was added externally[10], it may normally come from hydrolysis of I_2, the excess of

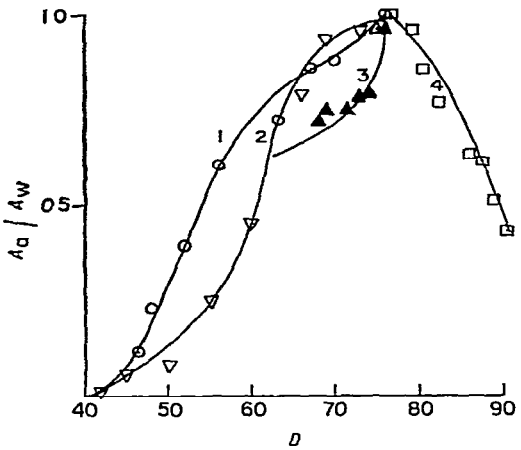

Fig 5 Effect of dielectric constant (D) on the relative absorbance of the Am_1–I_2 complex at 580 nm (Composition 0 02% Am_1–0 08mM I_2–0 01M KI, Key curves 1–4, 1,4-dioxane, ethanol, D-glucose, and urea)

it only acts to maintain the ionic strength, and, in this regard, KI and KCl are equally effective, either separately or mixed Our results only partly support the salt effects reported by Ono *et al* [14], who found a difference between the effect of KI and that of KCl

Effect of co-solvents — A co-solvent normally diminished the absorbance of the complex, but no systematic trend was observed in this regard A considerable decrease in the absorbance of the complex in the presence of 1,4-dioxane, without any shift of the λ_{max}, indicated decomplexing of I_2 by 1,4-dioxane without the latter itself being bound (see Fig 1) The decomplexing effects of the solvents with regard to both mole fraction and dielectric constant are shown in Figs 4 and 5, in neither of these was any correlative feature observed As a particular example, 1,4-dioxane imparted a low dielectric constant, whereas urea, which imparted an increment in dielectric constant, also diminished the absorbance significantly The reasons could be quite different, but it is certain that the dielectric effect, alone, is an insufficient explanation A general conclusion concerning the effects of co-solvents could not, therefore, be reached The decrease in absorbance was not due to any instability of the complex or of the amylose in the nonaqueous environments, because insolubility would lead to turbidity and precipitation, leading in most cases to an increase in the absorbance One example is acetone, which showed turbidity after a mole fraction of 0 08 was reached Based on the observations, the following orders of decomplexing by the agents, with regard to mole fraction and dielectric constant, were recognized, at 0 25 mole fraction D-glucose > urea > 1,4-dioxane > acetonitrile; at 0 05 mole fraction acetone > urea > 1,4-dioxane > acetonitrile > ethylene glycol > ethanol > methanol, at 0 25 mole fraction acetone > methanol > ethanol> ethylene glycol, at dielectric constant 50 ethylene glycol > methanol > ethanol >

1,4-dioxane, and at dielectric constant 70 acetone > acetonitrile > D-glucose > ethylene glycol > 1,4-dioxane > methanol > ethanol

Effect of surfactants — The effects of both ionic and nonionic surfactants on the formation of the amylose–iodine complex are reported. CTAB (cationic), SDS (anionic), and Triton X-100 (nonionic) surfactants were found to be effective decomplexing agents for both Am_1 and Am_2 Very low concentrations of CTAB [much less than the critical micelle concentration (c m c)] were observed to diminish the complexation in the case of Am_1, and, for Am_2, it caused precipitation at all concentrations Formation of turbidity (in a certain range) and partial, as well as total, precipitation were observed both below and above the c m c for Am_1. The results are shown in Fig 6 It was noted that CTAB is effective below the c m c , whereas, both SDS and Triton X-100 are effective above the c m c

Effect of temperature on complexation — A detailed study of the effect of temperature on the complexation process was made, the temperature being varied from 2 to 75° In Fig 7, the heating and cooling curves, and the results of repeated cycling on the Am_1 complex are shown For the Am_1 complex, the heating and cooling curves did not coincide, permanent hysteresis occurred, but this was not observed for the Am_2 complex There had been some earlier reports on the effect of temperature on amylose of relatively high molecular weight, but studies made for such a wide range of temperature have seldom been documented We observed that the Am_1–I_2 complex was relatively less heat-resistant than the Am_2–I_2 complex, indeed, the Am_2 complex was practically reversible with respect to the effect of temperature

To investigate the enthalpy change for the process, log (absorbance) was plotted

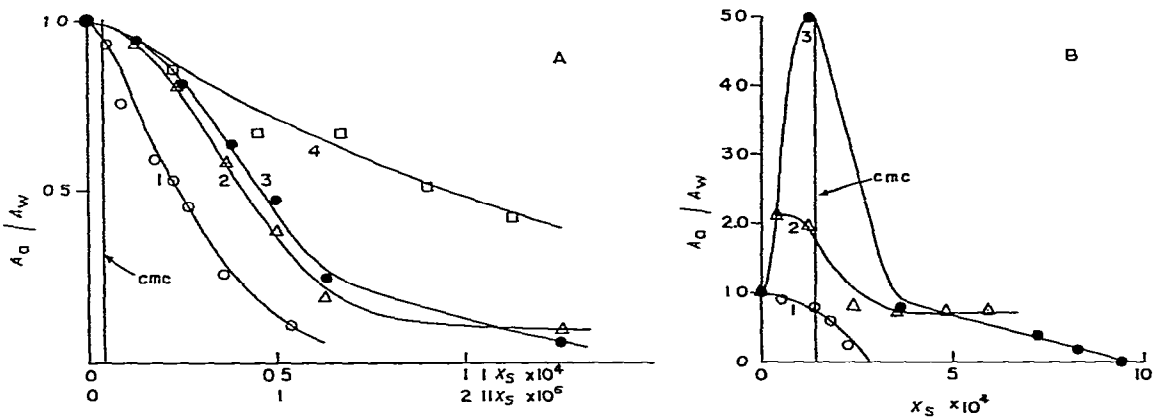

Fig 6A Effect of CTAB and TX-100 on the relative absorbance of the amylose–iodine complex at 580 nm (Key Am_1–TX-100 system, composition, 0 02% Am_1–0 08mM I_2–0 01M KI Scale I, curve 2, Am_2–TX-100 system, composition, 0 0004% Am_2–0 08mM I_2–0 01M KI Scale II, curve 3, Am_2–TX-100 system, composition, 0 0008% Am_2–0 08mM I_2–0 01M KI Scale I, curve 4, Am_1–CTAB system, composition, 0 02% Am_1–0 08mM I_2–0 01M KI Scale II)

Fig 6B Effect of SDS on the relative absorbance of the amylose–iodine complex at 580 nm (Key curve 1, Am_1–SDS system, composition, 0 02% Am_1–0 08 mM I_2–0 01M KI, curve 2, Am_2–SDS system, composition, 0 0004% Am_2–0 08mM I_2–0 01M KI, curve 3, Am_2–SDS system, composition, 0 0008% Am_2–0 08mM I_2–0 01M KI)

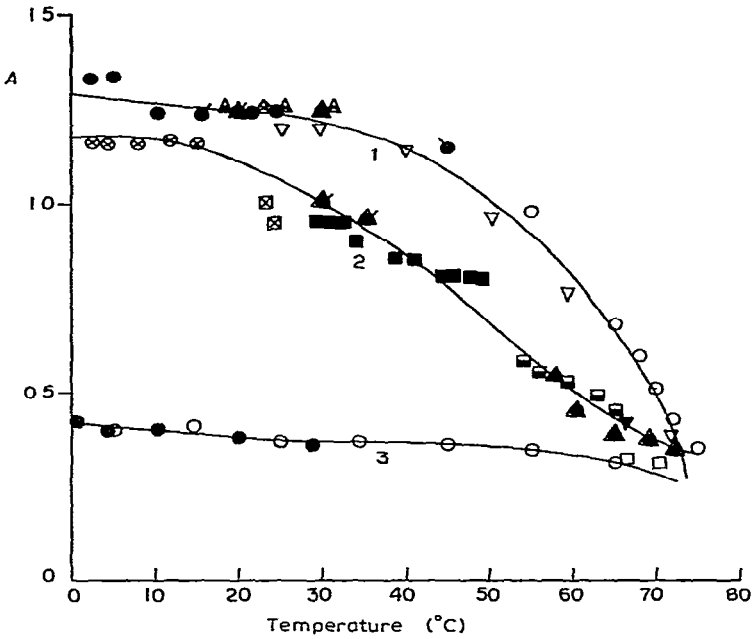

Fig 7 Effect of temperature on the stability of the amylose–iodine complex (Composition 0·02% Am_1–0·08mM I_2–0·01M KI, and 0·0023% Am_2–0·08mM I_2–0·01M KI Key curve 1, Am_2–I_2 complex, open and full circles represent heating and cooling runs, curve 2, Am_1–I_2 complex, cooling runs, curve 3, Am_1–I_2 complex, heating runs plus cooling runs in the range 2–50°, signs for curves 2 and 3, measurements taken allowing 1 h at the temperature of measurement, triangle with cross, cooling below room temperature and warming to 20·4°, open triangle, sample kept for 20 h, and heated from 25 to 72°, closed triangle, sample cooled from 72 to 66°, open square, after 21 h, the sample was heated to 70° and cooled, measurements taken at random time-intervals, half-filled square, same, after further 21 h, closed square, same, after further 23 h, square with cross, same, after further 19 h, circle with cross, same, after further 20 h, closed circle, a separate cooling run, from below room temperature down to 10°, at random time-intervals, closed circle with prime, same, then heated to 45°, open circle, the sample was kept for 22 h at 45° and then heated to 75°, circle-enclosed triangle, the sample was cooled to 57°, circle-enclosed triangle with prime, the sample was kept for 18 h at 57°, allowed to cool to room temperature, and then cooled below room temperature down to 20°)

against T^{-1} A curve was obtained that could be resolved into at least two, distinct, straight lines in the temperature range of 0–50° and 50–75°, respectively, from the slopes of which the enthalpy changes could be obtained (see Fig 8) The justification for this procedure is given next.

For the reaction,

$$\mathrm{Am} + n\,\mathrm{I_2} \overset{K_a}{\rightleftharpoons} \underset{\text{complex}}{\mathrm{Am(I_2)_n}}, \qquad (1)$$

the association constant,

$$K_a = [\text{Complex}]/[\mathrm{Am}]_{\text{free}}\,[\mathrm{I_2}]^n_{\text{free}} \qquad (2)$$

In equation 2, concentrations have been used for the activities

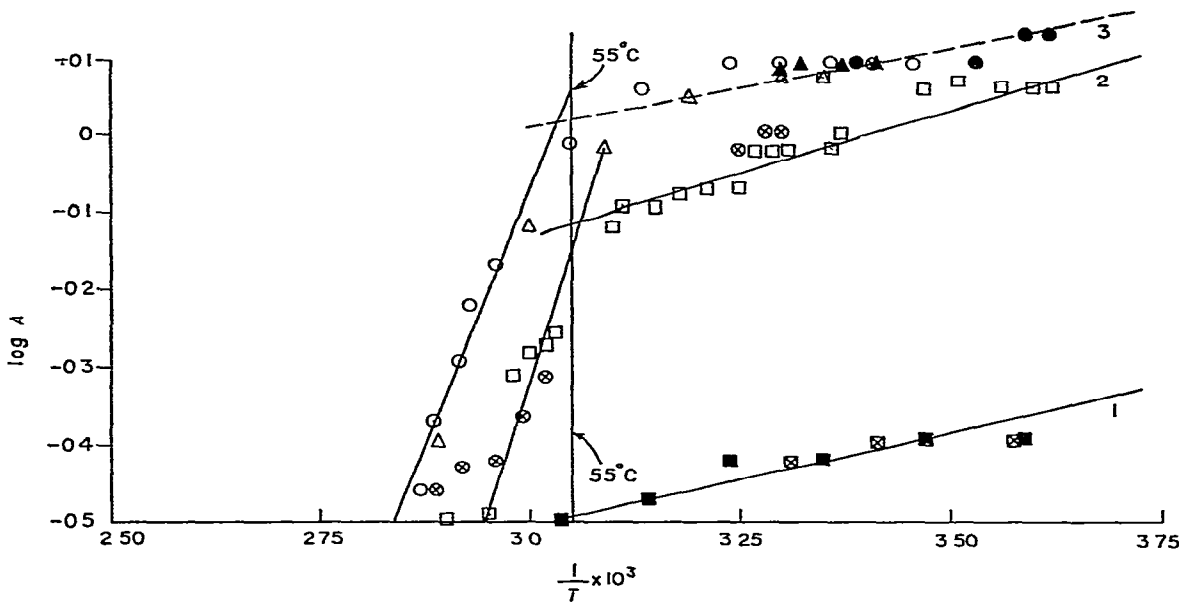

Fig 8 Plot of log A *versus* $1/T$ for the Am_1–I_2 and Am_2–I_2 complexes (Key curve 1, Am_2–I_2 system, heating and cooling runs, curve 2, Am_1–I_2 system, cooling runs, curve 3, Am_2–I_2 system, heating runs From the data of Fig. 7)

Now,

$$\log K_a = \log A - \log \varepsilon_c[\mathrm{Am}][\mathrm{I_2}]^n. \qquad (3)$$

In equation *3*, the second term is highly positive compared to the first term, as the concentrations of both the amylose used and the iodine were very low, and n is expected to be somewhat larger than unity Therefore, a small variation in the free concentration of both amylose and iodine due to the changed temperature would hardly affect the high positive value of the second term Taking this term as equivalent to a constant, equation *3* may be written as

$$\log K_a = \log A - \log (\text{constant}),$$

and the corresponding, integrated, Van 't Hoff equation is

$$\log K_a = \log A - \log (\text{constant}) = -\Delta H^0/2\,303\,RT + I, \qquad (4)$$

where ΔH, R, T, and I are the enthalpy change, the gas constant, the absolute temperature, and the integration constant, respectively

Equation *4* is equivalent to

$$\log A = (-\Delta H^0/2\,303\,RT) + I' \qquad (5)$$

where $I' = I + \log (\text{constant})$

This equation can then yield ΔH^0, which was, therefore, calculated (see Fig 8) for the present systems In the temperature range 0 to 50°, the mean enthalpy change for both of the amylose samples corresponded to −8 37 kJ mol^{-1} (−2 0 kcal mol^{-1})

In the higher range, the mean enthalpy change for the heating and cooling experiments on the Am_1 complex became -64.9 kJ mol^{-1} (-15.5 kcal mol^{-1}) The value for the lower temperature range is the lowest so far reported [-25.12 kJ mol^{-1} (-6.0 kcal mol^{-1}) was reported by Eliassaf and Lewin[18]] The present -64.90 kJ mol^{-1} (-15.5 kcal mol^{-1}) is very close to the enthalpy value usually reported for the amylose–iodine complex[10,12] The amylose samples of low and high molecular weight did not exhibit much difference in the enthalpy change in the lower range of temperature

DISCUSSION

The characteristic absorption maxima at 580 and 640 nm observed for the two amylose samples were in agreement with the red-shifted peaks observed for the increased degree of polymerization of the biopolymer[12]

The dependency of the complexation on the ionic strength only, and not on the kind of electrolyte (KCl and KI), as well as the fact of formation of the complex even in the absence of externally added[10] KI, supported the agglomeration of the negatively charged, amylose–poly(iodine)–iodide complex by the charge-neutralization principle holding for hydrophobic colloids[19] *via* the actions of the counter-cations After the optimal ionic strength had been reached, charge reversal by the adsorption of K^+ ions on the surface of the biopolymer caused a dispersing effect, with disaggregation of the colloid-like bodies producing a decrease in absorbance at the higher concentrations of salt It was observed that, beyond the optimal concentration of salt, the complex precipitated out in the container, but it could be partly redissolved by dialyzing the whole mass, a colloid type of behavior of the complex was thus further supported In this connection, it should be mentioned that the concept of colloid-aggregation was suggested by Ono *et al*[14] many years ago

The action of surfactants differed from the effects of the salts, a decomplexing action was mainly exhibited by them At concentrations lower than the c m c , the cationic surfactant CTAB became bound to the negatively charged complex, iodine was released, and the whole product became insoluble by aggregation and precipitated out as a white mass Increased concentrations of CTAB (above the c m c) did not help to solubilize the complex, iodine was extracted into the micelles, and the amylose was precipitated as a white mass The negatively charged surfactant SDS was not particularly effective below the c m c , but it showed maximum absorbance at the c m c , with a sharp decomplexing effect afterwards The SDS acted like a salt at lower concentrations, and helped to aggregate the complex, yielding an increased absorbance; after the c m c , it extracted iodine in the micelles, lessening the blue color Triton X-100, on the other hand, only became effective above the c m c , and decomplexed the iodine thereafter, indicating extraction of iodine from the complex inside the micelles of these detergents, or loosening of the binding of iodine with the amylose moiety, thereby disrupting the kind of helical arrangements necessary for them to have polarizing effects on the polyiodine chain, to afford the blue color

The actions of the nonaqueous solvents on the complexation process were difficult to reconcile All of the solvents used, including solutions of urea and D-glucose, showed decomplexing effects It was speculated that (*1*) the nonaqueous solvent molecules at the microscopic level abstract iodine from the complex, (*2*) they cause deformation of the helical conformation required for the blue coloration, and (*3*) transition of the helix to the coil took place in their presence, one or more of these could be the operative factor(s) Now, the nonaqueous solvents are, in general, poor solvents for amylose, and ready precipitation from them would indicate formation of a compact configuration of the biopolymer in them (However, urea may act in the opposite direction, and keep mostly the extended-coil conformation in aqueous medium, and solubility may be favored in its presence The decomplexing effect of urea would then be due to helix-to-coil transition, and inability to polarize the iodine by the elongated amylose chain) As 1,4-dioxane showed significant, decomplexing effects at relatively low concentration, and the conformational aspect may become an important factor in the complexing process, measurement of the intrinsic viscosity of Am_1 in the presence of 1,4-dioxane was undertaken The intrinsic viscosity was found to decrease with increase of the proportion of 1,4-dioxane The Huggins constant, which relates the intrinsic viscosity with concentration[20], and is given by equation *6*, was also calculated

$$\eta_{sp}/C = [\eta] + [\eta]^2 k C, \tag{6}$$

where η_{sp}, k, and C are the specific viscosity, the Huggins constant, and the concentration of amylose (g/ml), respectively

The constant k was found to increase with increase in the proportion of 1,4-dioxane (see Table I) When plotted, both $[\eta]$ and k showed linear variation This observation suggested that the amylose chain becomes more compact with the addition of dioxane, the Huggins constant is 2 for a perfect sphere[20] Therefore, the deformed, helical shape of the complex assumed a more compact conformation, and the characteristic blue color was decreased in the presence of 1,4-dioxane and other nonaqueous solvents in general, as already mentioned, solutions of urea and D-glucose were exceptions At all concentrations of 1,4-dioxane, hydration of the amylose molecule was calculated[20] from the equation of Simha, assuming the axial-ratio-dependent factor to be 2 5 (Einstein's coefficient for spheres)

$$[\eta] = \nu(v_2 + \delta v_1), \tag{7}$$

where ν, v_1, v_2, and δ are Simha's factor, the specific volume of water, the specific volume of amylose, and the water (g/g) bound with amylose, respectively Solvation by 1,4-dioxane was neglected, as it is a poor solvent for the biopolymer, and no complexation of it was found spectrophotometrically Again, assuming the hydration to be zero, the Simha factor ν for the Am_1 was calculated at all of the mole fractions of 1,4-dioxane, an estimate of the dissymmetry of the amylose molecule in aqueous 1,4-dioxane was thus obtained, the results are given in Table I The high intrinsic-viscosity of Am_1 and its slow decrease with addition of 1,4-dioxane, as well as k

TABLE I

MOLECULAR DIMENSIONS OF AMYLOSE IN 1,4-DIOXANE–WATER, FROM THE INTRINSIC VISCOSITY

Mole fraction of 1,4-dioxane X_s	*Intrinsic viscosity (ml/g)* [η]	k	*Maximum asymmetry* (ν *at* $\delta = 0$)	*Maximum solvation (g/g)* (δ *at* $\nu = 2.5$)
0.00	16.3	0.60	27.06	6.4
0.01	15.3	0.73	25.40	6.0
0.03	13.5	0.73	22.41	5.3
0.06	11.5	0.93	18.76	4.4

values smaller than 2.0, suggested that the biopolymer is present in a state of significant elongation. Assumption of the spherical shape ($\nu = 2.5$) resulted in values of the hydration number much higher than that normally observed for similar biopolymers. The axial-ratio-dependent ν, observed for the zero state of hydration, was sufficiently large, and could not be significantly altered by taking a small value of hydration, *e.g.*, 1.0 g/g. All of these results indicated that the 1,4-dioxane added decomplexed the iodine with only a small change in the helical conformation.

The effect of temperature became abrupt above 50°, particularly for the Am_1–I_2 complex. Below this temperature, dissociation of the complex was not significant for either Am_1 or Am_2. The difference between the heating and cooling curves was a special feature of the Am_1 sample. In the temperature range below 50°, heating and cooling caused practically no difference, but, once the sample was heated to 75°, the cooling curve followed a different course, and was essentially independent of the elapsed time. Such a difference has seldom been observed in the past, most probably because of the use of samples of amylose mainly of high molecular weight, and not performing the experiments well above 50°. A permanent change in the amylose molecule was envisaged, but its type cannot yet be predicted. It is possible that the aggregated molecules are loosened during heating, and do not revert back to the original form during cooling, thus causing a permanent decrease in the absorbance. For Am_2, this phenomenon was minimal, possibly due to the use of a significantly lower concentration of it in solution (at least a tenth of that of Am_1, because of its lower solubility). From a consideration of the enthalpy, the value of -8.37 kJ mol^{-1} for the formation of the complex in the lower range of temperature was not consonant with the earlier findings[12,15,16,21] (-25.12 to -87.92 kJ mol^{-1}), but the value for the high-temperature range (-64.90 kJ mol^{-1}) was very close to most of the earlier findings, although results for temperatures above 50° have seldom been reported. This heat term was greater than the hydrogen bonding or weak Van der Waals type of interaction (physical adsorption). The sharp change in the lines for log A *versus* $1/T$ indicated a sharp change in the exothermic behavior upon complexation at temperatures above 50°, this suggested that two distinct processes are involved in the formation of the amylose–iodine complex, and they are likely to be co-operative.

Szejtli *et al* [12] suggested an equation for the amylose–iodine complex, namely,

$$(I_f)_v = \exp(-\Delta H/RT + A), \quad (8)$$

where $(I_f)_v$ is the concentration of free iodine at half saturation, A is an empirical constant, and the other terms have the usual significance The value of $(I_f)_v$ was also shown to be related to the apparent equilibrium constant, K_a, as

$$(I_f)_v = 1/K_a \quad (9)$$

Using the present enthalpy value, −64 90 kJ.mol^{-1} in equation 9, and taking A as equal to 19 62, as given by Szejtli *et al* [12], the value of $(I_f)_v$ and, hence, the equilibrium constant, was calculated for 60° The enthalpy value in the high-temperature range only was utilized, as equation 8 was considered to be valid in this region. As a consequence, ΔG^0 and ΔS^0 were computed to be −9 88 kJ mol^{-1} and −165 79 J mol^{-1} deg^{-1}, respectively The small change in the free energy suggested a non-chemical type of interaction, and the large, negative change in entropy indicated an appreciably ordered environment upon complexation

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REFERENCES

1 R E Rundle and F C Edwards, *J Am Chem Soc* , 68 (1943) 2200–2203
2 J Hollo' and J Szejtli, *Staerke*, 10 (1958) 49–52
3 W Banks and C T. Greenwood, *Carbohydr Res* , 21 (1972) 229–234
4 E Hamori and M B Senior, *Ann N Y Acad Sci* , 210 (1973) 34–37
5 W W Everett and J F Foster, *J Am Chem Soc* , 81 (1959) 3459–3464
6 F R Dintzis, A C Beckwith, G E Babcock, and R Tobin, *Macromolecules*, 9 (1976) 471–478
7 C D Cronan, F W Schnieder, and S K Podder, *J Phys Chem* , 72 (1968) 4563–4568
8 Q T Nguyen, P Aptel, and J Neel, *Biopolymers*, 15 (1976) 2097–2100
9 W Banks, C T Greenwood, and K M Khan, *Carbohydr Res* , 17 (1971) 25–33
10 S V Bhide and N R Kale, *Biochim Biophys Acta*, 444 (1976) 719–726
11 F L Bates, D French, and R E Rundle, *J Am Chem Soc* , 65 (1943) 142–148
12 J Szejtli, M Richter, and S Augustat, *Biopolymers*, 6 (1968) 27–41, and references cited therein
13 T Kuge and S Ono, *Bull Chem Soc Jpn* , 33 (1960) 1269–1272
14 S Ono, T Watanabe, K Ogawa, and N. Okazaki, *Bull Chem Soc Jpn* , 38 (1965) 643–648
15 T Kuge and S Ono, *Bull Chem Soc. Jpn* , 33 (1960) 1273–1278
16 J F Foster, in R L Whistler and E F Paschall (Eds), *Starch Chemistry and Technology*, Academic Press, New York, 1965, p 349
17 S P Moulik, S Ray, and A R Das, *J Phys Chem* , 81 (1977) 1766–1769
18 J Eliassaf and M Lewin, *Nature*, 201 (1964) 1023–1024
19 B Jirgensons and M E Straumanis, *A Short Textbook of Colloid Chemistry*, 2nd edn , Pergamon Press, New York, 1962, p 329
20 C Tanford, *Physical Properties of Macromolecules*, Wiley, New York, 1961, pp 391–392
21 K Takahashi and S Ono, *J. Biochem (Tokyo)*, 73 (1972) 1041–1043