Amylose-Iodine Complex. IV. The Complex in Dimethyl Sulfoxide - Water Mixtures

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In a previous report[1] the failure of amylose to form an iodide-free iodine complex in solution has been reported. Judging from the physicochemical properties, Kuge and Ono[2] have suggested a deformed helix conformation, similar to the one proposed by Holló and Szejtli,[3] of amylose in neutral aqueous solutions. However, the dependence of the formation of blue amylose-iodine complex on both the iodide ion and the dissolved state of aqueous uncoupled amylose has not yet been perfectly demonstrated.

In the present work, in order to obtain further knowledge on the conditions of amylose-iodine complex formation, colorimetric, spectrophotometric and viscometric studies have been made of the complex in dimethyl sulfoxide - water mixtures.

Experimental

Materials. — Amylose was obtained from potato starch as follows.[4] Potato starch dispersed in a small quantity of water was poured into 1 N sodium hydroxide and dissolved by stirring them with a magnetic stirrer under nitrogen. After the starch had dissolved, the pH of the solution was adjusted to 5.4—5.6 with 2 N hydrochloric acid. The temperature of the solution was then raised to 70—80°C, and the solution was saturated with an excess of *n*-butanol while being stirred constantly, after which it was continually stirred for one day. After centrifugation of the amylose-butanol complex, the complex was recrystallized three times from hot water (about 90°C) in the presence of excess butanol. The recrystallized complex was washed thrice with absolute methanol and then once with absolute ethanol to remove butanol and water, and then dried in a vacuum over anhydrous calcium chloride.

The fractionation of amylose was carried out at

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[1] T. Kuge and S. Ono, This Bulletin, 33, 1269 (1960).

[2] T. Kuge and S. Ono, ibid., 34, 1264 (1961).

[3] J. Holló and J. Szejtli, *Die Stärke*, 10, 49 (1958).

[4] H. Azumi and T. Nakajima, *Sci. Rep. Tohoku Univ. Ser. I*, 36, 278 (1952).

room temperature. The amylose was dissolved in dimethyl sulfoxide (DMSO) and fractionated by the addition of appropriate amounts of absolute ethanol, as described by Everett and Foster.⁵⁾ Seven subfractions of amylose were prepared.

The amylose subfraction whose number average molecular weight, as determined by osmotic pressure measurements, was 6×10^5 in DMSO at 35°C was principally used in this experiment.

As DMSO, commercially-available DMSO was distilled in a closed system under reduced pressure over Drierite.⁶⁾ This procedure gave a distillate whose density and viscosity agreed well with those reported in the literature.⁶⁾

The water used was thrice distilled by using a glass apparatus.

Sample Solution.—The amylose DMSO solution was prepared as follows. Amylose was dissolved in DMSO by stirring them with a magnetic stirrer in a glass-stoppered flask for several hours and then allowing the mixture to stand for one night in a desiccator over calcium chloride in order to complete the dissolution.

Iodine purified by sublimation was dissolved in DMSO or potassium iodide aqueous solutions.

Amylose iodine DMSO solutions were prepared by mixing both the amylose and the iodine DMSO solutions.

Colorimetry. — The blue-colored amylose-iodine complex DMSO-H_2O solutions were prepared in glass-stoppered test tubes. The test tubes were immersed in a water bath whose temperature was raised at the rate of one degree per minute. Then the colored solution in the test tube changed its color from blue through green to yellow (or red brown[*1]) with the rising temperature. The temperature at which the greenish color of the solution completely faded (T_f) was determined. After the above procedure, the temperature of the bath was reversely allowed to fall slowly and the temperature was determined at which the solution perfectly developed a blue color (T_d). Generally, the values of both T_f and T_d obtained were accurate within ±2°C.

Spectrophotometry. — The spectrophotometric measurements were carried out by using a Hitachi-type EPU-2A photo-electric spectrophotometer with silica cells 1.00 cm. thick.

Viscometry. — Viscosity was determined in suspended-level dilution viscometers modelled after FitzSimons' modification of the Ubbelohde viscometer with one capillary tube. The dimensions were as follows: capillary diameter, 0.6 mm.; capillary length, 110 mm.; volume of efflux bulb, 5 ml.; volume of dilution bulb, 150 ml. The procedure used for determining the change in the viscosity of solutions with dilution by DMSO or by the addition of water to a given amylose DMSO solution, was as follows. Twenty milliliters of a given amylose DMSO solution was pipetted into the dilution bulb of the viscometer in a thermostat at a given temperature and then allowed to stand for 20 min. in order to establish the temperature equilibrium. The efflux time was determined with a stopwatch to the nearest 0.1 sec. The dilution of the solution by DMSO was made up by the repeated dilution of the charged solution in the dilution bulb. The addition of water to the DMSO solution was also made up by the repeated addition of water to the charged solution in the dilution bulb. Dilution by DMSO or the addition of water was done volumetrically. In the case of DMSO-H_2O mixtures, the specific viscosities were obtained by comparing the viscosities of the solutions with those of the corresponding DMSO-H_2O mixtures.

Results and Discussion

The formation of blue amylose-iodine complex can not be observed in an amylose iodine DMSO solution, but it is sharply observed when the concentration of water in an amylose iodine DMSO-H_2O solution reaches a critical value, as has been reported by Peticolas.⁷⁾ Figure 1 shows the dependences of T_f and T_d on the concentration of water in DMSO-H_2O mixtures. The critical concentration of water for the formation of the blue complex could not be reduced below 28 mol./l., irrespective of both the amylose and iodine concentrations. Above the critical water concentration, both T_f and T_d rise with the increase in water concentration. T_f and T_d also rise with the increase in iodine concentration, as is shown in Fig. 2. Figure 3 shows the dependence of T_f on the amylose concentration and also the results with amyloses, whose number average molecular weights were 4×10^5 [*2] and 4×10^4 [*3]

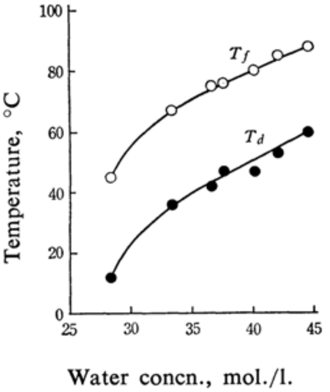

Fig. 1. Plot of T_f and T_d against water concn. in DMSO-H_2O mixture: Concn. of amylose, 2 g./l.; concn. of iodine dissolved, 0.3 g./l.

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5) W. W. Everett and J. F. Foster, *J. Am. Chem. Soc.*, **81**, 3459 (1959).

6) R. G. LeBel and D. A. I. Goring, *J. Chem. Eng. Data*, **7**, 100 (1962).

[*1] The color of this solution depends on both iodine and water concentrations.

7) W. L. Peticolas, *Nature*, **197**, 898 (1963).

[*2] Another subfractionated amylose.

[*3] Degraded amylose prepared by treating the amylose (number average molecular weight: 6×10^5) iodine DMSO solution at 70°C for 23 hr. (see *4 on the degrading action of iodine).

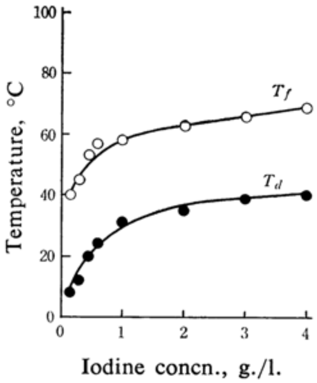

Fig. 2. Plot of T_f and T_d against iodine concn. in DMSO : H_2O=1 : 1 (v/v) mixture : Concn. of amylose, 2 g./l.

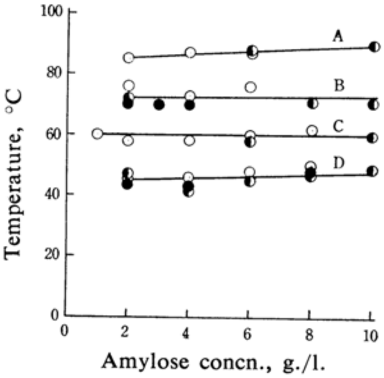

Fig. 3. Plot of T_f against amylose concn. in iodine DMSO-H_2O solution.
Curve A 1 g./l. iodine in DMSO : H_2O= 1 : 2 (v/v)
Curve B 0.3 g./l. iodine in DMSO : H_2O= 1 : 2 (v/v)
Curve C 1 g./l. iodine in DMSO : H_2O= 1 : 1 (v/v)
Curve D 0.3 g./l. iodine in DMSO : H_2O= 1 : 1 (v/v)
Molecular weight of amylose: ○, 60×10^4; ◐, 40×10^4; ●, 4×10^4

respectively. The results illustrated in Figs. 1, 2 and 3 show that the influence of amylose concentration and its molecular weight on T_f values is slight compared with that of the water or iodine concentration. This fact, together with the fact that no purple or reddish color was observed at temperatures ranging from T_d to T_f, may indicate that in this range of temperature the amylose-iodine complex has a fixed length of iodine chain and that the formation of the complex proceeds in all fashions or in none, irrespective of the concentrations and the molecular weights of amylose.

The dependence of T_f on the composition of potassium iodide-iodine aqueous solutions of a given amylose concentration is shown in Table I. A regular relationship has been found only between T_f values and triiodide ion concentrations (Fig. 4). The iodine, iodide ion and triiodide ion concentrations were calculated with the equilibrium constants[8] of the ($I_3^- \rightleftharpoons I_2+I^-$) reaction at the temperature of T_f. In these experiments T_d values could not be determined accurately because of the pale blue color development. The results shown in Fig. 4 indicate that the triiodide ion contributes to the thermal stability of the amylose-iodine complex.

TABLE I. DEPENDENCE OF T_f ON THE COMPOSITION OF POTASSIUM IODIDE-IODINE SOLUTIONS

T_f °C	Equilibrium constant* $K\times10^3$	Concn., M		
		Iodine $[I_2]\times10^5$	Iodide ion $[I^-]\times10^3$	Triiodide ion $[I_3^-]\times10^5$
50	2.0	1.7	0.3	0.26
67	2.5	0.9	3.0	1.1
75	2.8	0.2	30	1.8
81	2.9	6.0	3.0	6.0
84	3.0	0.4	30	3.6
90	3.1	10	3.0	10

* $K=[I_2][I^-][I_3^-]^{-1}$

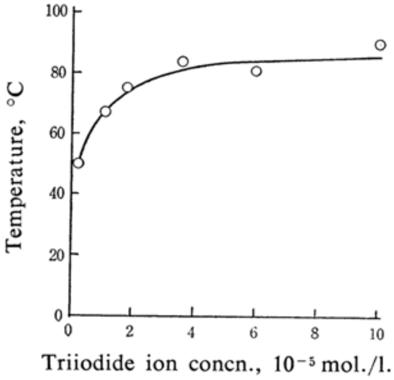

Fig. 4. Plot of T_f against triiodide ion concn. in amylose (0.1 g./l.) potassium iodide-iodine aqueous solution.

The color of the iodine DMSO solution is similar to that of the iodine-iodide aqueous solution. The iodine DMSO solution possesses two absorption peaks (at 295 and 365 mμ), similar to those of the iodine-iodide aqueous solution (at 288 and 350 mμ). When water is added to the iodine DMSO solution, the two peaks shift towards the shorter wavelengths. The addition of amylose to the iodine DMSO solution causes no change in the ultraviolet absorption spectrum of the solution. When

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[8] M. Davies and E. Gwynne, *J. Am. Chem. Soc.*, **74**, 2748 (1952).

water is added to the amylose iodine DMSO solution, the ultraviolet and visible spectra of this amylose iodine DMSO-H_2O solution show no change in nature compared with those of the corresponding iodine DMSO-H_2O solution, as Fig. 5 shows, unless the blue coloration of the amylose iodine solution has been developed. When the concentration of water has exceeded a critical value, the blue amylose-iodine complex is produced in solution and the suppression of the peak at about 290 mμ and an increase in the height of the peak at about 355 mμ are observed, contrasting to the peaks of the corresponding iodine DMSO-H_2O solution (Fig. 6). A similar change in the height of both the peaks in the ultraviolet spectrum is observed with the amylose iodine-iodide aqueous solution (Fig. 7), as reported by Bebbington et al.⁹⁾ and Mould.¹⁰⁾

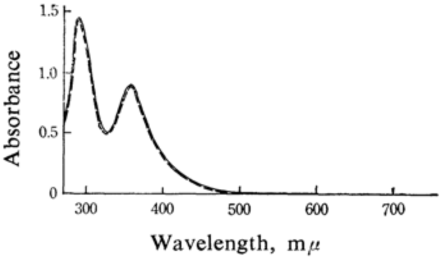

Fig. 5. Absorption spectra of 0.04 g./l. iodine in DMSO : H_2O=2 : 3 (v/v) mixture with 0.6 g./l. amylose (solid line) and without amylose (dashed line) at 24°C.

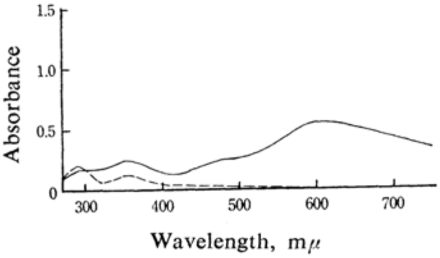

Fig. 6. Absorption spectra of 0.02 g./l. iodine in DMSO : H_2O=1 : 4 (v/v) mixture with 0.3 g./l. amylose (solid line) and without amylose (dashed line) at 24°C.

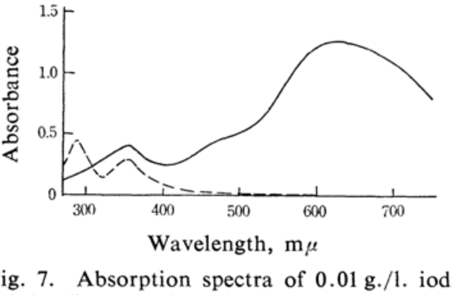

Fig. 7. Absorption spectra of 0.01 g./l. iodine 0.1 g./l. potassium iodide aqueous solution with 0.15 g./l. amylose (solid line) and without amylose (dashed line) at 24°C.

These spectrophotometric results show that the nature of the amylose-iodine complex in DMSO-H_2O mixtures is essentially the same as that of the complex in water. Both the peaks observed at the ultraviolet spectrum with a potassium iodide-iodine solution have been attributed to the triiodide ion by Awtrey and Connick.¹¹⁾ The peaks observed with an iodine DMSO-H_2O solution might also be attributed to the triiodide ion or the chemical species with an electron configuration similar to that of the triiodide ion, i. e., the polyiodide ion. Moreover, the rise in the values of T_f and T_d with the increase in iodine concentration (Fig. 2) seems to be explainable in terms of the increase in the triiodide (polyiodide) ion concentration.

Further spectrophotometric investigation of iodine DMSO-H_2O solutions with or without amylose is in progress.

Figure 8 shows the viscometric results. Curve A shows the relationship between the amounts of water added to the 20 ml. of a 1% amylose DMSO solution and the specific viscosity of

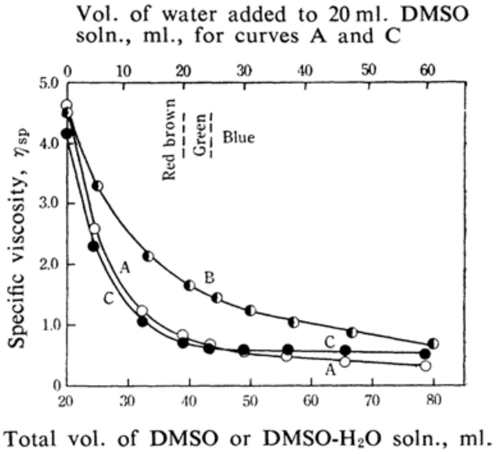

Fig. 8. The change in specific viscosity of the amylose solutions (with and without iodine) observed in the following experiments at 35°C.

Curve A	1% amylose DMSO solution (20 ml.)+water
Curve B	1% amylose DMSO solution (20 ml.)+DMSO
Curve C	1% amylose, 0.15% iodine DMSO solution (20 ml.)+water (in this case blue color development of the solution occurs at the water content shown in figure)

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9) A. Bebbington, E. J. Bourne and I. A. Wilkinson, *J. Chem. Soc.*, **1952**, 246.

10) D. L. Mould, *Biochem. J.*, **58**, 593 (1954).

11) A. D. Awtrey and R. E. Connick, *J. Am. Chem. Soc.*, **73**, 1842 (1951).

the solution. Curve B shows the change in the specific viscosity of the amylose DMSO solution in the case of dilution with DMSO. The change in the specific viscosity with the increase in the amount of water (curve A) is very sharp compared to that in the case of simple dilution with DMSO (curve B). Curve C[*4] shows the relationship between the specific viscosity of the amylose iodine solution and the amounts of water added to the 20 ml. of a 1% amylose and 0.15% iodine solution. In this case the color of the solution changes from red brown through green to blue at the water content level where the sharp decrease in the specific viscosity terminates with the addition of water. The viscosity of DMSO-H_2O mixtures shows no appreciable change with the dissolution of iodine. Therefore, the character of curve C may be determined primarily by the dissolved state of amylose in solution. No appreciable discontinuity in the change of the curvature of curve C has been observed at the range of water content where the blue amylose-iodine complex formation proceeds. This fact suggests that the formation of the complex does not accompany any appreciable conformational change of the amylose molecule. With the iodine complex, the molecular chain of amylose takes a helical conformation; therefore, the uncoupled amylose molecule in the corresponding DMSO-H_2O mixture must take a conformation which may easily be transformed into the well-ordered helix accompanying the complex formation, i. e., the deformed helix.[2,3] The critical water concentration for blue amylose-iodine complex formation, as observed by colorimetric studies, agrees with the water content level of the amylose DMSO-H_2O solution at which the sharp decrease in specific viscosity terminates. This fact suggests that the molecular chain of amylose must exist primarily as a deformed helix prior to the formation of the iodine complex.

An experiment similar to that of curve A has been made using 0.05 M iodic acid instead of water. In this case, the production of the triiodide (polyiodide) ion was repressed by the iodic acid,[*5] and no blue-colored complex was formed. The curve obtained was similar to curve A and lay parallel to and somewhat downward from it. This character of the curve shows that the amylose exists as a deformed helix at a high water content, as in the case of curve A. This result suggests that the amylose molecule existing as a deformed helix alone could not form the amylose-iodine complex without the presence of an amount of triiodide (polyiodide) ions sufficient to form the complex in solution.

The fact that the specific viscosity of the amylose-iodine complex solution is higher than that of the amylose solution at a high concentration of water in DMSO-H_2O mixture may be attributed to the rigid nature of the structure of the complex compared to that of the uncoupled amylose.

Everett and Foster[12] have demonstrated that amylose exists as a coil in DMSO. Cowie,[13] on the other hand, has suggested that the existence of a helical structure could be expected of the amylose molecule dissolved in both DMSO and DMSO-H_2O mixtures. Taking these authors' results into account, the present experimental results may be explained as follows. The amylose molecule may exist as a coil in DMSO; however, it can not be expected to exist as a completely extended linear chain because of the α-1, 4-glucosidic linkages that impose steric limitations on the molecular chain. When water is added to the amylose DMSO solution, the degree of extension of the molecular chain may decrease, and the chain tends to draw itself into a somewhat helical conformation (the deformed helix[2,3]). This change in molecular chain extension seems to reveal itself as the initial sharp decrease in specific viscosity with the addition of water shown in curve A of Fig. 8. Spectrophotometric studies show that there is no interaction between the amylose and iodine unless the blue complex has been formed. The initial sharp decrease in the specific viscosity observed with curve C of Fig. 8 may be expected to be brought about by the same conformational change in the amylose chain as in the case of curve A.

The deformed helix may be regarded as a chain conformation in which the thermal fluctuations in the conformation of the molecular chain occur between the helically-wound chain conformation and the linear-extended one in a single molecule; the helical conformation is superior to the linear. Probably the

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[*4] The amylose DMSO solution shows no change in viscosity after standing for one month after preparation. On the contrary, in the amylose iodine DMSO solution, amylose is degraded by the action of iodine, and the viscosity of the solution slowly decreases. Therefore, the specific viscosity of the amylose DMSO solution (without the addition of water) of curve A (without iodine) does not coincide with that of curve C (with iodine).

[*5] In the present case, the production of the triiodide (polyiodide) ion was not completely repressed. For example, the absorbance at 355 mμ of the iodine solution in DMSO: 0.05 M iodic acid = 1 : 3 (v/v) decreases by an order of magnitude of 10^{-2} compared to that of iodine in DMSO : H_2O = 1 : 3 (v/v); the former solution with amylose could not develop the blue color at 35°C, but it slowly developed a somewhat purple color when the solution was cooled below 20°C.

12) W. W. Everett and J. F. Foster, *J. Am. Chem. Soc.*, **81**, 3464 (1959).

13) J. M. G. Cowie, *Makromol.*, **42**, 230 (1961); **59**, 189 (1963).

formation of the amylose-iodine complex proceeds by holding the iodine molecules inside the helically-wound chain parts of the amylose molecule. Therefore, the number of iodine molecules concerned in the complex formation may be determined by the length of the helically-wound parts of the deformed helical amylose molecule. However, a stable iodine complex may be expected to be formed against the thermal fluctuations of the conformation when the length of the helically-wound parts of the amylose molecule has exceeded a certain value. Therefore, the minimum number of iodine molecules necessary for the stable amylose-iodine complex formation may be determined mainly by the conformation of the amylose molecule. As for the stable iodine complex formation, the concentration of water in a DMSO-H_2O mixture may be the primary factor in determining the amylose conformation, and the concentration of triiodide (polyiodide) ions would be a subsidiary factor contributing to the thermal stability of the iodine molecules in the helix. In these circumstances, the iodine complex with fewer iodine molecules than necessary can not exist stably but will release the combined iodine instantly. This prerequisite to the iodine complex formation reveals itself as an all or nothing feature of the complex formation. The increase in water concentration in the DMSO-H_2O mixture beyond the critical value necessary for the complex formation would cause an increase in the superiority of the helical conformation and also a rise in both T_d and T_f values. With the rise in temperature, the deformed helical amylose molecules would change their conformation to a somewhat extended one as the result of the decrease in the superiority of the helical conformation, and they would become unable to combine with the iodine molecules to form the stable iodine complex because of their deficiency in the conformational prerequisite to the holding of the minimum necessary number of iodine molecules inside the helically-wound parts of the amylose molecule.

Further physicochemical studies of the deformed helical conformation of amylose are in progress.

As for the iodine, its contribution to the formation of the complex may be made through two chemical species, i. e., the iodine molecule and the triiodide (polyiodide) ion. The existence of the triiodide (polyiodide) ion is indispensable for the formation of the complex; the minimum concentration of this ion necessary for complex formation is determined by the water concentration in the DMSO-H_2O mixture at a given temperature and increases with the rise in temperature in a given water concentration. The spectrophotometric results show that no interaction between iodine or the triiodide (polyiodide) ion and the amylose molecule occurs unless the blue complex has been formed in the solution. Therefore, both the chemical species may be said to make a co-operative contribution to the complex formation. In these circumstances, the triiodide (polyiodide) ion may be supposed not only to assist the entrance of the iodine molecules into the helically-wound chain of amylose but also to act as a stabilizer for the fixation of the iodine within the helix. Thus, the resonating iodine chain in the amylose helix may be expected to be stabilized against thermal disturbance by the triiodide (polyiodide) ions adsorbed at both ends of the iodine chain, and the amylose-iodine complex may be expected to become more rigid in nature compared to that of the uncoupled amylose molecule.

Summary

The temperature at which the blue color due to the formation of amylose-iodine complex fades completely has been determined with a system of amylose and iodine dissolved in dimethyl sulfoxide-water mixtures. This temperature rises with the increase in the concentration of iodine in mixtures, and also with the increasing concentration of water in the mixed solvents.

The spectrophotometric results show that the nature of the amylose-iodine complex in a dimethyl sulfoxide-water mixture is similar to that of one in water, and that the triiodide ion or a chemical species similar to the ion, i. e., the polyiodide ion, contributes to the formation of the complex.

The viscometric studies show that the amylose molecule takes a deformed helix conformation prior to the formation of the blue iodine complex and that this conformational prerequisite to the complex formation is fulfilled when the water concentration in a dimethyl sulfoxide-water mixture exceeds a critical value.

These results stated above may indicate that the triiodide (polyiodide) ion plays the part of "stabilizer" for the fixation of iodine molecules within the helically-wound chain of the amylose molecule when the amylose exists primarily as a deformed helix in solution.

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