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EFFECTS OF ACETONE, ETHANOL, ISOPROPANOL AND DIMETHYL SULFOXIDE ON AMYLOSE-IODINE COMPLEX†

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Key Words: Starch-iodine (Amylose-iodine) Complex Formation, Role of Non-aqueous Solvents, Peak Shift from 615 nm to 550 nm

ABSTRACT

The amylose-iodine (AI) complex formation was studied by absorption spectra in water and water containing varying proportions of ethanol, acetone, isopropanol and dimethyl sulfoxide (DMSO). Complex formation is most favored in pure water and decreases as the proportion of nonaqueous solvent is increased. A decrease in the absorbance intensity at around 615 nm (for AI complex) is accompanied by a peak shift towards 550 nm and an increased absorbance at around 350 nm (for unbound iodine). The amount of the nonaqueous solvent added, as well as the order in which it is added relative to amylose and iodine solution, change remarkably the extent of the AI complex formation. A mechanism of the complex formation is proposed.

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INTRODUCTION

The iodine complexes of carbohydrates have been known to chemists for [1-8] over a century and considered to be useful in the development of qualitative or semi-quantitative methods for determining the α-amylose [9, 10] (enzyme) activity. In recent years, the possible use of iodine complexes in the treatment of a female disease has been seriously [11] considered. In a number of studies it was shown that the molecular iodine is highly effective in curing breast lesions or other related [12- diseases in women. [12-14]. Since the carbohydrate-iodine complexes bind molecular iodine (References 15-21 and references therein) within the helix cavity and release iodine when the temperature is increased beyond 15°C, they have been seriously considered for this new drug. Among the iodine complexes, the amylose - iodine complex (AI), more commonly known as the starch-iodine complex, has the highest iodine binding [15, 17, 19] energy and thus, is the most stable carbohydrate-iodine complex. Since iodine crystals are not highly soluble in pure water, the conventional method of the complex preparation involves the addition of iodide ions (which increase the solubility of iodine crystals) in water. Unfortunately, these iodide ions are known to give undesirable side [13, 14] effects on human subjects. One, therefore, needs to examine whether the AI complex can be synthesized in pure water or in a nonaqueous solvent without the addition of iodide ions. There is already an ongoing effort to synthesize the AI complex in a nonaqueous solvent, like alcohol (Reference 11), as iodine crystals are highly soluble in it. The idea is to first synthesize the complex in an iodide free condition and then, to obtain the solid AI complex by evaporating the solvent. The present study primarily examines the effect of alcohols, acetone and DMSO on the AI complex formation and thus, aims at identifying a solvent and experimental conditions under which the complex formation can be maximum. Since amylose is only slightly soluble in alcohols and acetone, the AI complex formation in water containing a varying proportions of ethanol, isopropanol and acetone was considered in the experiment. Even though DMSO is a good solvent for both iodine and amylose, for a comparison with the other results, a DMSO-water mixture that contained varying proportions of DMSO was used.

EXPERIMENTAL

Preparation of Solutions

Iodine Solution

5 mL of cold N/10 iodine (Fisher Scientific) solution was added to 5 mL of

cold KI solution (5% W/V, Fisher Scientific) and 490 mL of cold water (around 6°C). This gives an iodine concentration of around 127 mg/L.

Amylose Solution

An amylose solution of 1.50 g/L and pH 4.0 was made by first dissolving the solid (A-0512, Lot 96H3797, Sigma Chemical) in 2M NaOH, followed by the addition of 2M HCl and pH 4.0 solution. During experiments a diluted solution of 588 mg/L was made. It should be mentioned that the solid amylose had about 3.1% butanol, and in our final dilutions (used in experiments), the concentrations of butanol were negligibly small and hence, its effect on spectrum can be neglected.

Nonaqueous Solvents

Each of the nonaqueous solvent, ethanol, isopropanol, acetone, and DMSO of high purity (around 100%) was used in the present study. Different concentrations (v/v) of these solvents were made by adding appropriate volumes of water.

Solutions for Spectrum

The first set of four experiments (Figures 1-3) involved the following preparations:

Solution A

For solutions 1-3, a diluted amylose of 196 mg/L was made by adding 50mL of water to 25 mL of 588 mg/L amylose.

Solution B

For solution 4, a 50 mL of 40% ethanol, isopropanol, acetone or DMSO solution was added to 25 mL of 588 mg/L of amylose to obtain the amylose concentration of 196 mg/L.

Solution 1

5 mL of solution A was added to 5 mL of water, 5 mL of iodine (127 mg/L) and then another 5 mL of water, exactly in that order. This mixed solution was then placed in a thermostated bath (25°C) for 10 minutes before taking absorbance readings from 310-700 nm. It should be noted that this solution does not contain any nonaqueous solvent. The final concentrations of amylose and iodine in this solution were 49 and 31.7 mg/L, repectively.

Solution 2

5 mL of solution A (196 mg/L amylose) was added to 5 mL of water, 5

mL of iodine (127 mg/L) and then 5 mL of 40% nonaqueous solvent, exactly in that order. As before, this solution (25°C) was used for taking absorbance readings from 310-700 nm. The final concentrations of amylose and iodine were the same as solution 1 (49 and 31.7 mg/L, respectively) and the nonaqueous solvent concentration was around 10%(v/v).

Solution 3

5 mL of solution A (196 mg/L amylose) was added to 5 mL of 40% nonaqueous solvent, 5 mL of iodine (127 mg/L) and then 5 mL of water, in that order. As before, this solution (25°C) was used for taking absorbance readings from 310-700 nm. The final concentrations of amylose, iodine and the nonaqueous solvent were the same as solution 2.

Solution 4

5 mL of solution B (196 mg/L amylose and 40% nonaqueous solvent) wasadded to 5 mL of water, 5 mL of iodine (127 mg/L) and then 5 mL of water, in that order. As before, this solution (25°C) was used for taking absorbance readings from 310-700 nm. The final concentrations of amylose, iodine and the nonaqueous solvent were the same as solution 2. For the second set of experiments (Figures 4 and 5), the solutions were added in the same order as in solution 4, except the percent of the nonaqueous solvent was varied to obtain a final solvent concentration of 10%, 17%, 25% and 37%. In each preparation we made sure that there was no precipitate formation in the solution. The final concentrations of amylose and iodine were the same as in the other solutions, and the absorbance readings were taken from 310-700 nm at 25°C.

RESULTS AND DISCUSSION

Order of Solvent Addition and Change in Spectrum

Figures 1-3 show the variation in the absorbance spectra due to a change in the order of addition of ethanol, acetone and DMSO, respectively. The most remarkable changes in the spectrum was noticed for alcohol solutions (Figure 1), followed by acetone (Figure 2) and DMSO solutions(Figure 3). Even though the amount of spectral changes were different from one solvent type to another, the trends were the same in each case. When the AI complex was made in pure water (uppermost curve, solution 1), the absorbance peak intensity (at 615 nm) due to the complex was maximum, suggesting a maximum concentration for the AI complex.

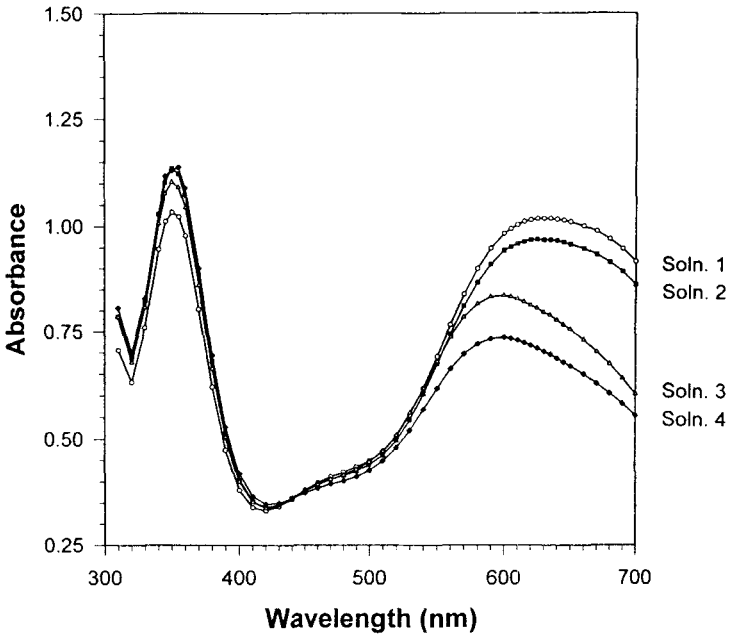

Figure 1. Spectra (25°C) are shown for the AI complex formation in pure water (solution 1), and in a 10% ethanol-water mixture (v/v) in which the nonaqueous solvent is added in different orders. In solution 2 the nonaqueous solvent is added after the formation of the AI complex, in solutions 3 and 4 the solvent is added prior to the addition of iodine. In addition, in solution 4 amylose is first exposed to a more concentrated solvent before its final dilution with water and iodine solution. In each of the solutions the amylose and iodine concentrations were 49 and 31.7 mg/L respectively.

When the nonaqueous solvent was added (solution 2) after the formation of the AI complex, the absorbance values around 615 nm were slightly lowered. However, when the nonaqueous solvent was added prior to the addition of iodine (solutions 3 and 4), the intensity values around 615 nm were remarkably reduced with a peak shift towards a smaller wavelength. The difference in the absorbance values for solutions 3 and 4 was quite unexpected. It should be pointed out that the preparation of solution 4 involved an amylose solution of 196 mg/L containing 40% of the nonaqueous solvent. The preparation of solution 3, on the other hand, involved the amylose concentration of 196 mg/L added to an equal volume of 40% nonaqueous solvent (giving 98 mg/L of amylose in 20% nonaqueous solvent).

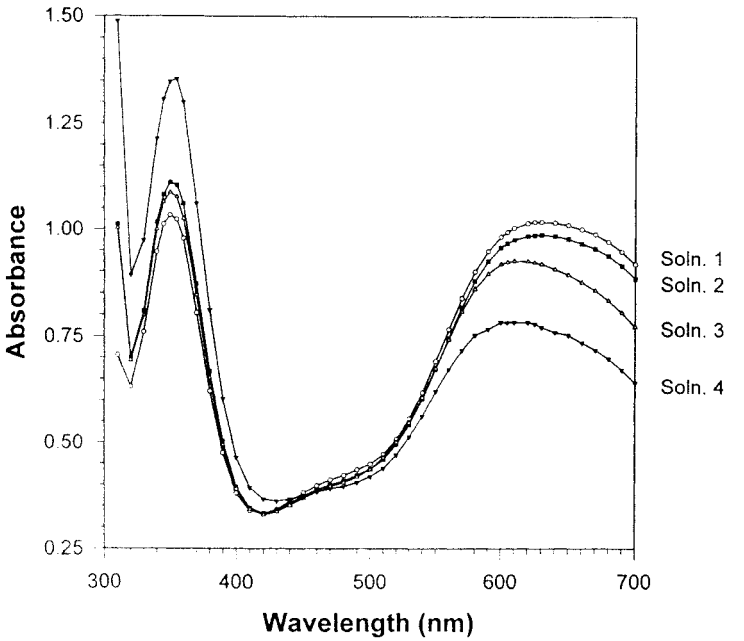

Figure 2. Figure 2 represents experiments similar to those in Figure 1, except this involves 10% acetone in water (v/v).

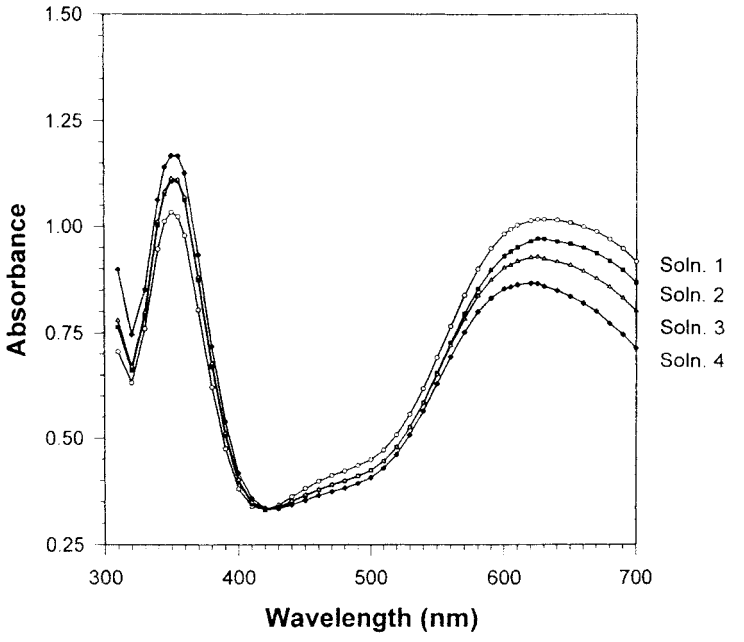

Figure 3. Figure 3 represents experiments similar to those in Figure 1, except this involves 10% DMSO in water (v/v).

In other words, in solution 4 the amylose chains were exposed to a higher concentration of nonaqueous solvent before the addition of iodine and water for the same final concentrations of amylose, iodine and the nonaqueous solvent. The observed decrease in peak intensity between 500-700 nm together with a remarkable peak shift for solution 4 is presumably caused by a larger number of nonaqueous solvents that remained bonded to the amylose chain during its early exposure and was not removed during the dilution with water. It should be noted that the formation of a complex between amylose and alcohols is already known and has been reported in several earlier papers (References 22, 23 and references therein). The mechanism of iodine binding in presence of nonaqueous solvents has been discussed in more details in the last section of this paper.

In Figures 1-3, only the effect of the addition of 10% nonaqueous solvent is shown. The obvious question is, how do the absorbance values change at higher concentrations of the solvent when the order of mixing (as in solution 4) and the final concentrations of amylose and iodine remain unchanged? This motivated us to examine the effect of increased nonaq-ueous solvent concentration on the AI spectrum

Nonaqueous Solvent Concentration and Change in Spectrum

Figures 4 and 5 (for ethanol and DMSO, respectively) are the representative plots in which an increased concentration of nonaqueous solvent shows a decreased absorbance within 500-700 nm. Both the peak shift towards 550 nm and the intensity decrease at 615 nm are quite noticeable as the nonaqueous solvent concentration increases from 0% (no nonaqueous solvent) to 37%. A highly reduced absorb-ance value at 615 nm suggests that the AI complex formation is not favored in a nonaqueous solvent. Interestingly, the 550 nm peak represents a characteristic wavelength for the amylopectin-iodine (API) complex in which an I_4 unit binds a shorter amylopectin chain of 11 anhydroglucose units (AGU,$C_6H_{10}O_5$) (References 16, 17). In addition, a similar peak shift from 615 nm to 550 nm was also noticed when amylose was hydrolyzed to shorter chainlengths by α-amylase (Reference 15). Thus, in the present experiment, the peak shift to 550 nm at a high nonaqueous solvent concentration can be explained on the basis of the involvement of smaller polyoiodine units (I_4) bonded within a shorter chain segment of amylose. This result implies that a larger segment of amylose is not available for iodine binding at high concentrations of nonaqueous solvents. As expected, a decrease in absorbance at around 615 nm is accompanied by an increased peak intensity at around 350 nm.It should be pointed out that the 350 nm peak is due to iodine molecules that are not bonded to carbohydrate molecules in the solution [15, 20, 21]. In Figures 4 and

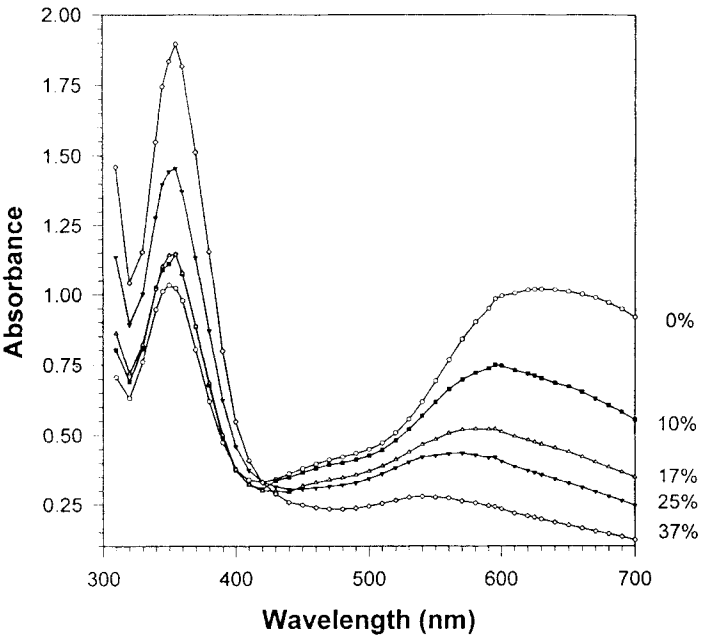

Figure 4. The spectra for the AI complex formation are presented for different concentrations of ethanol in water. The absorbance values between 300-400 nm are sharply increased, 500-700 nm reduced with a peak shift from 615 nm to 550 nm as the nonaqueous solvent concentration is increased from 0% to 37% (v/v). Similar spectral changes are also noticed for isopropanol-water.

5, the 0% nonaqueous solvent curve shows a maximum intensity at 615 nm and a minimum intensity at 350 nm. On the other hand, the 37% nonaqueous solvent curve has a minimum intensity at around 615 nm and a maximum intensity at 350 nm. This observation suggests that a reduced amount of AI complex formation (reduced intensity at around 615 nm) leaves behind a larger number of iodine molecules in the solution (increased absorbance peak at 350 nm). The above observation also suggests that the iodine binding by carbohydrates becomes increasingly unfavorable as the nonaqueous solvent concentration is increased. Even though the DMSO is a good solvent for both amylose and iodine, the spectra in DMSO solutions look quite similar to those of acetone (not a good solvent for amylose) but remarkably different from those of alcohols (also, not a solvent for amylose). It appears that the structural difference among these nonaqueous solvents and their variation in amylose binding properties (for solvent-amylose complex formation) may be responsible for the observed spectral features.

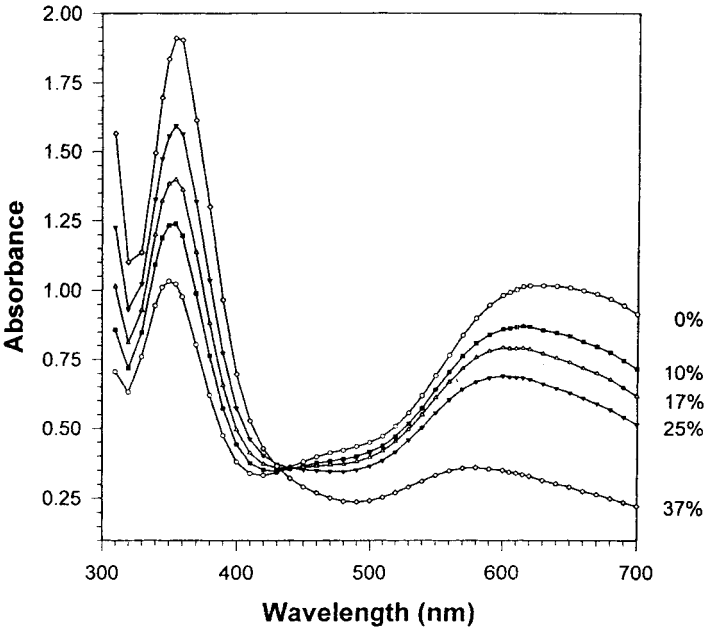

Figure 5. The spectra for the AI complex formation are presented for different concentrations of DMSO in water. As in Figure 4, the absorbance values between 300-400 nm are sharply increased, 500-700 nm reduced with a peak shift from 615 nm to 550 nm as the nonaqueous solvent concentration is increased from 0% to 37% (v/v). The acetone-water curves are similar to these, except that there is a larger decrease in 615 nm peak intensity when the solvent concentration changes from 0% to 25%. The 37% DMSO curve is very similar to 37% curves for other solvents.

Possible Mechanism of the AI Complex Formation

The following mechnism of the AI complex formation appears to explain the solvent effects that we observed in this study.

$$\text{Coil} \rightleftharpoons \text{Uncoil (Flexible coils)} \quad (1)$$

$$\text{Uncoil} + 3I_2 \rightleftharpoons \text{Helix-}I_6 \text{ (AI complex}^{15}\text{)} \quad (2)$$

$$\text{Uncoil} + \text{Solvent} \rightleftharpoons \text{Helix-Solvent complex} \quad (3)$$

The first step represents an equilibrium between the formation and the breakdown of amylose coils within the polymeric chain. This step is consistent with

the theoretical results of Brant and Dimpfl [24] suggesting that the amylose chains in an aqueous solution behave more like statistical coils rather than rigid helical coils in the absence of complexing agents. The iodine binding presumably happens (step 2) during the uncoiled state of the polymer which then promotes the formation of a rigid helix (Reference 7 and references therein). In our previous studies, we have shown that the AI complex formation requires three iodine molecules (I_6 unit) within the amylose helix of at least 17 AGU's (absorbance maximum at around 615 nm, Reference 15). Once the AI complex is formed, the nonaqueous solvent molecules cannot form a complex (step 3) by displacing iodine from the helix cavity and thus, there is a minimum change in the spectrum. On the other hand, when amylose is exposed to nonaqueous solvents prior to the addition of iodine, the solvent molecules, especially alcohols [22, 23], are likely to form rigid complexes (step 3). Once the nonaqueous solvent-amylose complex is formed, the iodine molecules cannot displace the nonaqueous solvents from the solvent-amylose complex and hence, a remarkable change in the AI spectrum is noticed. As the nonaqueous solvent concentration is increased from a small value, an increasing proportion of amylose chain segments become bonded to the solvent, and hence, only the smaller segments of amylose chains remain available to form bonds with shorter chromophore units (I_4) and give absorbance maximum at around 550 nm (References 16, 17). Thus, a peak shift towards 550 nm is expected as the nonaqueous solvent proportion is increased (Figures 4 and 5). At a fairly large nonaqueous solvent concentration (37%), most of the amylose becomes bonded and thus, only a very small amount of iodine complex can form at this point giving a very small intensity between 500-700 nm. It is interesting to point out that the DMSO solution (Figure 5) shows a minimum change in the AI absorbance peak (around 615 nm) when the DMSO concentration changes from 0% to 25%. However, when the solvent concentration increases to 37%, the absorbance change becomes significant and comparable to those observed for 37% alcohols (Figure 4) or acetone. This observation can be explained if a weak bonding within amylose helix-DMSO complex is considered Because of a weaker bonding there will be a larger concentration of unbound DMSO and amylose (Equation 3) in the solution. Only when the DMSO concentration becomes significant (37%) in the solution, the equilibrium Equation 3 is shifted far to the right (Le Chatlier's principle) forming a substantial concentration of the helix-DMSO complex and thus, the AI complex formation will be suppressed.

CONCLUSION

On the basis of this study, we can say that the AI complex formation is most favored in pure water, and the nonaqueous solvents prevent its formation. At the molecular level, one can theorize that a solvent that does not form a complex with the amylose chain will allow structural flexibility of amylose and thus, favor iodine binding.

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