Phase Changes in Amylose-Butanol Complex Solution

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The phase change for an amylose-butanol complex solution in 10% of dimethylsulfoxide was investigated as a function of temperature. The phase change was determined with measurements of the turbidity, fluorescent depolarization, and viscosity. The phase diagram obtained was qualitatively similar to that for an amylose solution. From the result, the change in solution phase for the amylose-butanol complex is suggested to be similar to that for amylose, *i.e.*, when the solution cools from a higher temperature, amylose molecules in the complex solution change the conformation from a random coil to an interrupted helix, and then separate into two phases. Coacervate particles resulting from the phase separation coalesce with each other to yield precipitates.

An adsorption of uranine on amylose was studied to ascertain its relationship with the fluorescent depolarization method used for detecting phase changes in solution. The result showed that uranine was adsorbed on amylose chains but not on the amylose-butanol complex.

In the previous work,¹⁾ the phase diagram for an amylose solution in the binary solvent system of DMSO with water was studied with the determination of changes in solution phases from sol to gel. The phase diagram obtained was of a similar type to that for agarose.^{2~4)}

From studies on the agarose gelling system,^{2~4)} Hayashi *et al.* have suggested that when the hot solution cooled, agarose molecules in a random coil form were wound into an interrupted helix and then separated into two phases, gel formation being caused by this phase separation. The phase separation temperature agreed with the gel formation temperature if the agarose concentration was high enough to make a gel.

From a comparison between the phase diagram for amylose and that for agarose, the gelling mechanism was suggested to be the same type for both amylose and agarose.

An amylose molecule makes complexes with many guest molecules such as butanol, *p*-nitrophenol, and iodine. The amylose molecule in these complexes is known to take a helical conformation, so-called V-amylose,

and forms a crystal.⁵⁾ The chain length of the helix has been calculated to be 120⁶⁾ or 66⁷⁾ glucose units. This suggests that the V-amylose form is a kind of interrupted helix. It is, therefore, expected that amylose complexes behave similarly to amylose when the hot solution cools from a higher temperature.

In this work, the phase change in the solution of an amylose-1-butanol (BuOH) complex was determined in order to form the phase diagram. The solvent was a binary system consisting of 10% DMSO and 90% water.

In the course of the experiment, the fluorescent depolarization ($1/p$) method was found to be insensitive in detecting phase changes accurately in the complex solution, although this method was effective for the amylose solution¹⁾ and agarose solution.^{2~4)} The measurement of $1/p$ was carried out for polymer solutions containing between 10^{-6} and 10^{-5} M uranine as an extrinsic, fluorescent probe. To enable the $1/p$ to reflect the micro-Brownian motion of the polymer in solution, an appreciable amount of uranine must be adsorbed on the polymer. In order to learn the reason for the insensitivity, the adsorption of uranine was

determined for both an amylose-BuOH complex and amylose.

Since the $1/p$ was not particularly effective for detecting phase changes in the amylose-BuOH complex, some alternative measurements were attempted and turbidity measurement was used to detect the phase separation point.

MATERIALS AND METHODS

The amylose sample was AVEBE Amylose-V as used in the previous works.^{1,8)} Other chemicals were of reagent grade. The instruments and methods of measurement were the same as in the previous works.^{1,8)}

The sample solution was prepared in the following way. An aliquot of a stock amylose solution dissolved in DMSO was diluted by appropriate volumes of water, DMSO, a uranine solution, and BuOH to yield a sample solution containing a given concentration of amylose, 10% of DMSO, 10^{-6} M uranine, and 7% of BuOH. Measurements as a function of temperature were carried out under decreasing temperature from a hot solution prepared at 70°C.

The adsorption of uranine on amylose and the amylose-BuOH complex was determined by precipitation from solutions aged at 25°C. Sample solutions containing 0.5 g/dl of amylose and between 5×10^{-7} and 10^{-5} M uranine were stood for a sufficient time to complete the precipitation of amylose and the amylose-BuOH complex. These solutions were then centrifuged at 10,000 rpm for 20 min and the residual uranine in the supernatant solutions was determined spectroscopically at a 488 nm wavelength. The adsorbed uranine was calculated as the difference between the initial and the residual uranine in the solutions.

RESULTS

Figures 4~7 show typical results selected from many similar experiments under varying conditions.

Figure 1 shows the apparent fluorescent depolarization ($1/p$) and fluorescent intensity (I_f) for a 0.4 g/dl amylose solution containing 10^{-6} M uranine as a function of temperature. Both curves show coincident change points at about 63 and 50°C. At this lower temperature of 50°C, the solution gradually became turbid during standing. This shows that a phase separation occurs at this temperature.

Figure 2 shows $1/p$ and I_f for a 0.1 g/dl

FIG. 1. Temperature Dependence of the Fluorescent Depolarization ($1/p$) and the Fluorescent Intensity (I_f) for a 0.4 g/dl Amylose Solution Containing 10^{-6} M Uranine, 7% BuOH and 10% DMSO.

FIG. 2. Temperature Dependence of the Fluorescent Depolarization and the Fluorescent Intensity for a 0.1 g/dl Amylose Solution Containing 10^{-6} M Uranine, 7% BuOH and 10% DMSO.

amylose solution as a function of temperature. The curves still exhibit a similar trend to that in Fig. 1, but an accurate determination of the change points is difficult.

Figure 3 shows the results of adsorption experiments for a 0.5 g/dl amylose solution containing between 5×10^{-7} and 10^{-5} M uranine, in which a linear relationship between $\log x$ and $\log c$ can be seen, where x and c are the decreased concentration and equilibrated concentration of uranine. This shows that uranine is positively adsorbed on the amylose molecule according to Freundlich's equation, $x = ac^{1/n}$, where a and $1/n$ are constants. On the other hand, adsorption on the amylose-BuOH complex was too little for accurate measurements.

The optical rotation showed a slight change

FIG. 3. Adsorption of Uranine on Amylose Molecules in the Retrogradation Product at 25°C.

Amylose, 0.5 g/dl; initial concentration of uranine, $5 \times 10^{-7} \sim 10^{-5}$ M; x , decreased uranine concentration; c , equilibrated uranine concentration.

FIG. 4. Temperature dependence of the Reduced Viscosity for a 0.4 g/dl Amylose Solution Containing 7% BuOH and 10% DMSO.

at the phase separation point, but it was not sharp enough to use for detection. The reduced viscosity, η_{sp}/c , showed a change similar to the phase separation, but an accurate determination of the change point was difficult by measuring the viscosity of the heterogeneous solution as shown in Fig. 4.

Figure 5 shows the absorbance at 400 nm wavelength for a 0.4 g/dl amylose solution as a function of temperature. The interval for each measurement is about 20 min. This curve clearly shows the starting point of turbidity and the phase separation point. However, in the case of the dilute solution, the onset of turbidity took too long to detect any change.

Figure 6 shows the absorbance at 35, 40, 45°C, after standing for several hours, from

FIG. 5. Temperature Dependence of the Absorbance at a 400 nm Wavelength for a 0.4 g/dl Amylose Solution Containing 7% BuOH and 10% DMSO.

FIG. 6. Dependence on Amylose Concentration of the Absorbance at 400 nm at 35, 40 and 45°C.

FIG. 7. Dependence on Amylose Concentration of the Absorbance, Fluorescent Depolarization, Fluorescent Intensity, and Reduced Viscosity at 57°C, and of the Reduced Viscosity at 70°C.

FIG. 8. Phase Diagram for the Amylose-BuOH Complex System Containing 10% DMSO and 7% BuOH.

sample preparations as a function of the amylose concentration. These curves give each critical concentration at which the amylose complex separated into two phases to yield precipitates.

The results from Figs. 5 and 6 for various amylose concentrations are denoted by a circle (○) in Fig. 8.

Figure 7 shows $1/p$, I_r , and η_{sp}/c at 57°C as a function of amylose concentration. This figure also contains η_{sp}/c at 70°C. The temperature of 57°C is an intermediate between the two change temperatures, 63 and 50°C in Fig. 1, and 70°C is higher than this 63°C. At 57°C, the curves show coincident changes at about 0.1 and 0.2 g/dl. At 70°C, η_{sp}/c shows no marked change at these two concentrations. The results from $1/p$ and η_{sp}/c are denoted by a triangle (△) in Fig. 8.

DISCUSSION

The phase diagram for the amylose-BuOH complex in Fig. 8 is essentially the same as that for amylose.¹⁾ Observations of solution phases noted in the figure are also the same as those for amylose. These results suggest that the phase changes for an amylose-BuOH complex are similar to those for amylose.

The precipitation of the amylose-BuOH complex occurs at the phase separation point. The rate of this precipitation is accelerated with an increase in concentration and with a

decrease in temperature. These observations resemble those for the amylose retrogradation. However, it is known that the precipitate of the complex shows a certain crystalline form but the retrogradation product of amylose is almost amorphous. Another difference between the amylose-BuOH complex and amylose is the gelling behavior at the phase separation point. The amylose solution makes a gel at concentrations higher than 1.0 g/dl, and an incomplete, partial gel phase at 0.6 g/dl. However, the amylose-BuOH complex solution makes no gel even at 1.3 g/dl.

The phase separation undoubtedly occurs in both solutions. Coacervate particles caused by the phase separation tend to coalesce with each other during standing of the solution. The difference in coalescence of the coacervate particles is thought to result in such different phenomena as the gel formation, retrogradation, and precipitation.

Another change in the solution phase, which is not included in Fig. 8, can be seen at 63°C for 0.4 g/dl of the amylose-BuOH complex solution as shown in Fig. 1. This temperature is a little higher than the 60°C for an amylose solution, but the difference is small enough to suggest that this is the helix-coil transition temperature. The amylose molecule must be in a random coil at a higher temperature than 63°C and no complex formation can be expected at this temperature.

At a temperature between the phase separation point and helix-coil transition point, the amylose conformation is believed to be in an interrupted helix. This helical solution in the phase diagram is separated into 3 subphases consisting of two homogeneous and one heterogeneous subphases in a similar manner to amylose. In the helical solution phase, there is no direct proof of whether the amylose helical segment makes a complex with BuOH or not. However, some information can be obtained from the phase diagram. The phase separation temperature, 50°C, for the complex is higher than that, 32°C, for amylose. The helix-coil transition temperature shows a similar trend as mentioned above, although the difference is

not great. The limit concentrations of the heterogeneous subphase in the helical solution are 0.1 and 0.2 g/dl and these concentrations are much lower than 0.5 and 0.9 g/dl for amylose.¹⁾ These data suggest a certain difference in the helical solution between the amylose-BuOH complex and amylose, but they are not always proof of the complex formation itself.

The adsorption of uranine was proved for amylose in this work. Calculations from the results in Fig. 3 show that about one molecule of uranine is adsorbed per one amylose macromolecule (as MW=200,000 from viscometry) and about 25% of the uranine is adsorbed on amylose under the experimental condition for 0.5 g/dl of amylose solution containing 10^{-6} M uranine. On the other hand, the amylose-BuOH complex adsorbed only a little uranine, and the $1/p$ measurement was too insensitive to determine the phase change accurately in the dilute complex solution. In this work, BuOH was used in a large excess to amylose in order

for equilibrium to lead to the complex formation. This BuOH in great excess is thought to disturb the adsorption of uranine on amylose and this is our interpretation of the insensitivity of the $1/p$ method for the amylose-BuOH complex.

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