Interaction of Surface Active Agent with Amylose

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In the previous papers, one of the authors have reported the interaction of surface active agents with non-ionizable polymers[1] as well as with proteins[2,3]. It was found that the aspect of the interaction of surface active agents with non-ionizable polymers is entirely different from that of the interaction of surface active agents with proteins. Accidently, the authors have found that there is another way of the interaction of the surface active agent with polymer. Actually, they wished to examine the inactivation of Taka-amylase A by surface active agent using amylose as a substrate. As a matter of fact, it was found that amylose is precipitated by sodium dodecyl sulfate (SDS) even in a concentration as low as 0.007% from 0.05% amylose solution in 0.05 M sodium chloride. Accordingly, the cause of this precipitation of amylose was investigated thoroughly.

The precipitate was examined by the X-ray diffraction method and compared with the amorphous amylose precipitated by ethanol. A complex might be formed between amylose and SDS. It was interesting to examine whether such a complex might be formed even in a dissolved state under proper condition. So the possibility was examined from the two standpoints of view as will be described below. The effect of SDS on the coloration of amylose-iodine complex was investigated spectrophotometrically. As the viscosity of polymer solution is a function of the configuration assumed by the dissolved polymer molecules, the solution of amylose containing SDS was studied also by viscosity measurement. From all these experimental findings, the authors have concluded that the precipitation of amylose by the surface active agent should be due to the formation of an adduct between amylose and the surface active agent. The details of the present investigations and inferences based on them will be described hereafter.

Experimental

Amylose.—Amylose was prepared from potato starch by Schoch's butanol precipitating method[4] and was recrystallized three times. The purified amylose was dehydrated by methanol and then dried over calcium chloride in vacuo.

Amylopectin. — Amylopectin was precipitated by adding a large amount of methanol to the supernatant separated from amylose prepared by Schoch's method. The precipitate was suspended in methanol for some time to remove butanol and then dried over calcium chloride in vacuo.

Sodium Dodecyl Sulfate.—Sodium dodecyl sulfate was prepared by Dreger's method[5] and was recrystallized three times from aqueous butanol.

X-ray Diffraction Measurement. — The sample for X-ray diffraction study was prepared as will be described below. One gram of amylose was dissolved in 10 ml. of 1 N sodium hydroxide and kept in a refrigerator overnight to dissolve the amylose completely, and then diluted to about 50 ml. The solution was neutralized with 1 N hydrochloric acid and diluted with distilled water just up to 100 ml. A precipitate was formed by adding 25 ml. of 0.5% SDS solution to the amylose solution and the solution was stood overnight. The precipitate was gathered by centrifugation and dried over calcium chloride in vacuo. On the other hand, amylose was precipitated by adding excess ethanol to the amylose solution prepared just in the same way without the addition of SDS and after washing the precipitate with ether, it was dried over calcium chloride in vacuo. The amylose thus prepared was used as a reference sample without SDS for X-ray diffraction study.

X-ray diffraction patterns were taken by the powder method using "Norelco" recording X-ray diffractometer (Cu-K_α line was used).

Measurement of Absorption Spectrum of Amylose-iodine Complex.—Five milliliters of 0.1% solution of amylose which was prepared by Fuwa's method[6] was diluted to about 200 ml. with distilled water, to which the necessary volume of SDS solution was added to assure a desired final concentration and shaken thoroughly. After bringing out the color by the addition of 5 ml. of 0.2% iodine solution (containing 2% potassium iodide), the solution was diluted just up to 250 ml. After standing for about 30 min., the absorption spectra of the solutions were measured by a Hitachi spectrophotometer model EPU-2 or by a Beckman recording spectrophotometer model DK-2.

Measurement of Viscosity.—One gram of amylose was suspended in 10 ml. of 1 N sodium hydroxide, was kept in a refrigerator for 2 days and then was diluted with distilled water just up to 100 ml. This solution was diluted with 0.1 N sodium

1) T. Isemura and A. Imanishi, *J. Polymer Sci.*, **33**, 337 (1958).
2) T. Isemura and A. Imanishi, *Mem. Inst. Sci. Ind. Res., Osaka Univ.*, **15**, 173 (1958).
3) T. Isemura and T. Takagi, *J. Biochem.*, **46**, 1637 (1959).
4) T. J. Schoch, *J. Am. Chem. Soc.*, **64**, 2957 (1942).
5) E. E. Dreger et al., *Ind. Eng. Chem.*, **36**, 610 (1944).
6) H. Fuwa, *J. Biochem.*, **41**, 583 (1954).

hydroxide to obtain the solution of various concentrations in respect to amylose. Each solution was mixed with an equal volume of SDS solution of various concentrations. The solutions of various compositions were heated on a water bath at 60~80°C for about 30 min. and kept for 3 hr. in a thermostat at 30±0.05°C. Then, the viscosity of amylose solutions was measured by a viscosimeter of the Ostwald type in the same thermostat.

Viscosity of the amylopectin solutions was measured just in the same manner.

Results

Effect of SDS on the X-ray Diffraction Pattern of Amylose.—The X-ray diffraction pattern of amylose precipitated by SDS is shown in Fig. 1a, while that for amylose precipitated by ethanol in Fig. 1b. In the pattern for the former, two distinct diffraction peaks were found at $2\theta=13$ and 20° (θ=incident angle) which might be attributed to the helical structure of amylose[7-9], whereas in the pattern for the latter, only a broad peak was found which suggests an amorphous structure of amylose.

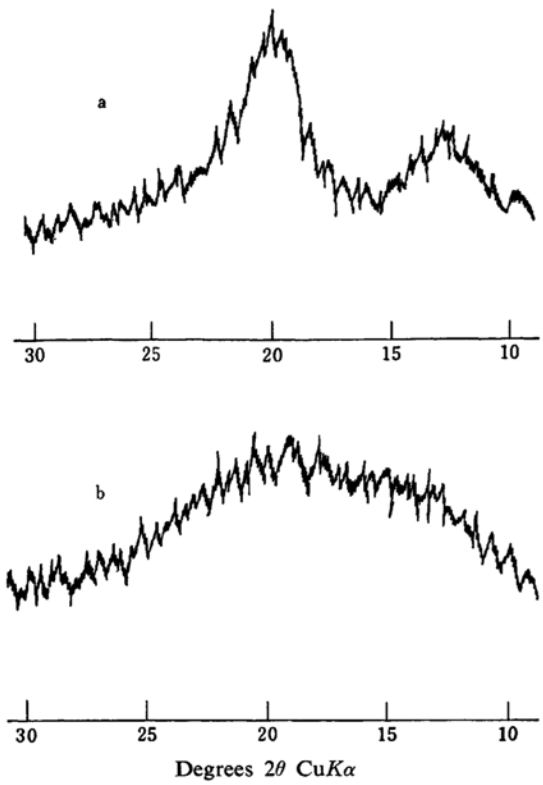

Fig. 1. X-ray diffractometer traces of SDS-treated and non-treated specimens of amylose.
a, precipitated by the addition of SDS.
b, precipitated by the addition of ethanol.

Effect of SDS on the Coloration of Amylose-iodine Complex.—If a helical complex is formed between amylose and SDS even in a dissolved state, it is expected that the coloration of amylose-iodine complex may be considerably affected in the presence of SDS. When an aqueous iodine solution was added to the amylose solution to which SDS was added previously, the solution appears at first light yellow assuming the color of I_3^- ion. Then the blue coloration of amylose-iodine complex comes out gradually. It needs about 30 min. to attain the steady coloration. All curves of

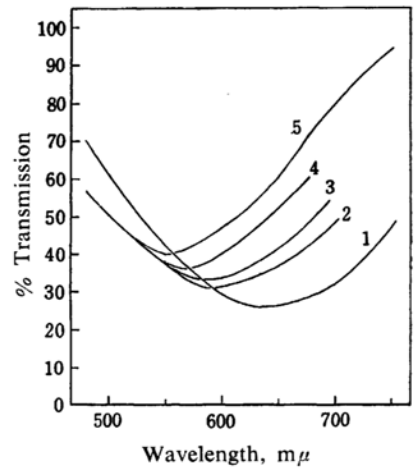

Fig. 2. The effect of the addition of SDS on the absorption spectrum of amylose-iodine complex.
1, 0 M; 2, 2.0×10^{-5}; 3, 1.0×10^{-4} M; 4, 6.0×10^{-4} M; 5, 4.2×10^{-3} M. Amylose concentration, 2.0×10^{-3} g./l.

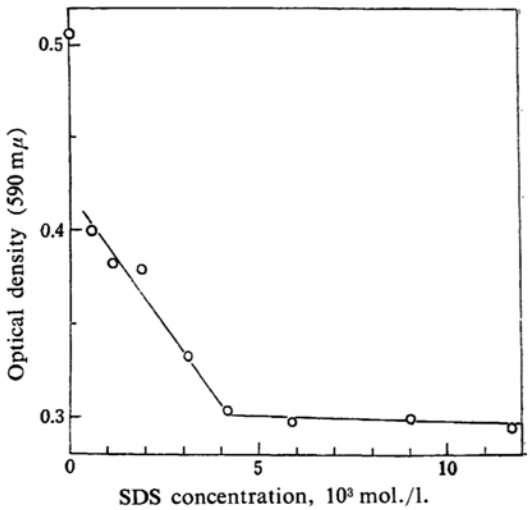

Fig. 3. The inhibitory effect of SDS on the coloration of amylose-iodine complex.

7) R. E. Rundle and D. French, *J. Am. Chem. Soc.*, **65**, 1707 (1943).
8) F. F. Mikus, R. M. Hixon and R. E. Rundle, ibid., **68**, 1115 (1946).
9) R. E. Rundle, ibid., **69**, 1769 (1947).

absorption spectra in Fig. 2 were measured after this steady state was attained. With the increase in the amount of added SDS, the absorption maximum shifts to a shorter wavelength side and the absorption decreases. If one examines the effect of the concentration of SDS by measuring the absorption at 590 mμ over a wide range of the concentration, the effect of SDS increases with the concentration up to 4×10^{-3} M, beyond which the change of the absorption spectra was not found as shown in Fig. 3. The concentration of SDS corresponds nearly to the critical micelle concentration of this surface active agent.

Effect of SDS on the Viscosity of Amylose Solution.—As is well known, the viscosity of polymer solution is a function of polymer configuration in solution. So the viscosity of an amylose solution might be profoundly affected by the addition of a small amount of SDS, if amylose changes its configuration from a random coil to a helical structure. The viscosity of amylose solution was measured in the presence of SDS in alkaline solution which is known as a good solvent for amylose and compared with that of amylose solution in the absence of SDS. As shown in Fig. 4., the effect of SDS on the viscosity of amylose solution is very remarkable. On the other hand, amylopectin which can not assume a helical structure because of its branched structure is expected to be scarcely affected by the addition of SDS. The experiment verifies that this is the case, as shown in Fig. 5.

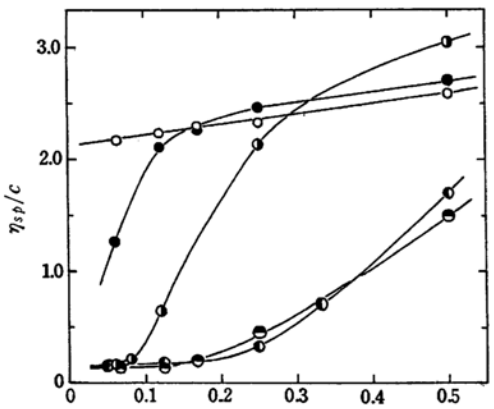

Fig. 4. The effect of the addition of SDS on the viscosity of amylose in 0.1 N KOH. SDS concentration, ○, 0 M; ●, 1.7×10^{-4} M; ◑, 3.5×10^{-4} M; ◐, 5.6×10^{-4}; ◓, 7.0×10^{-4} M.

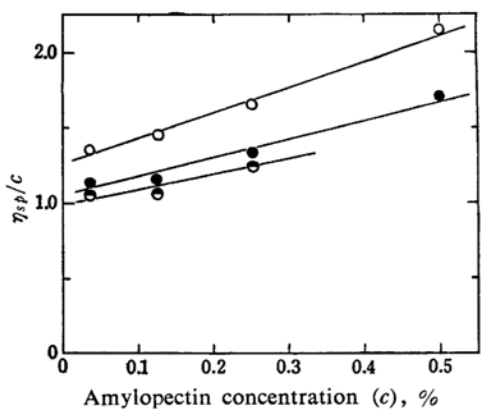

Fig. 5. The effect of the addition of SDS on the viscosity of amylopectin in 0.1 N KOH. SDS concentration, ○, 0 M; ◓, 3.5×10^{-4} M; ●, 7.0×10^{-4} M.

Discussion

As one of the authors have already reported[1], some surface active agents interact with some polymers causing oriented adsorption on the polymer molecules, often making insoluble polymers soluble. On the other hand, proteins interact with an ionic surface active agent at their ionic sites of the opposite sign[10]. Surface active agents precipitate proteins having opposite electric net charge, changing hydrophilic sites by covering with hydrocarbon chains. However, in the presence of excess surface active agents, surfactant ions are built up on the hydrocarbon tails of surfactant previously combined with protein molecules. Thus, the hydrophobic protein molecules which have been separated from the solution are turned to be hydrophilic again.

In the case of the interaction of amylose with an ionic surface active agent, the ion-ion interaction as in the case of proteins does not occur because of the lack of ionic sites. The oriented adsorption of surfactant ions as in the case of polyvinyl alcohol, as previously reported[1], might be excluded. Also, the dipole-ion interaction between hydroxyl groups of amylose and SDS ions as suggested by Fava and Eyring[11] in the case of adsorption of SDS on cellulose will be refused for the following reasons. In the present case, only an ion of SDS is sufficient to precipitate about 12 glucose residues in amylose. Moreover, it was found in the previous investigation[1] that the interaction of SDS with polyvinyl alcohol which has many hydroxyl groups in a molecule like amylose showed no sign of the insolubilization of the polymer. Further, the insolubilization of amylose can not be ascribed to the adsorption of SDS extending the hydrocarbon tails toward water as in the case of proteins, because of the

10) F. W. Putnam, *Advances in Protein Chem.*, **4**, 83 (1948).
11) A. Fava and H. Eyring, *J. Phys. Chem.*, **60**, 890 (1956).

lack of ionic sites as mentioned above. The precipitation of amylose is also caused by a cationic surface active agent such as cetylbenzyldimethyl amine bromide as well as SDS. On the other hand, the large excess of SDS, even as high as 12% in total concentration does not cause the redissolution of the precipitate. It is also to be noticed that no precipitate was formed by SDS with amylopectin which has a number of branched chains in the molecule.

According to X-ray diffraction study, the amylose precipitate formed by the interaction with SDS gave two distinct peaks at $2\theta=13$ and 20° as shown in Fig. 1a. Rundle and his collaborators[7] concluded from their studies on amylose-iodine complex by X-ray diffraction that amylose forms an adduct with iodine, where amylose assumes a helical structure in which six glucose residues make a turn and iodine molecules lie along the axis of helix, which gives two distinct peaks in X-ray diffraction pattern at $2\theta=13$ and 20°.

Later, they found that amylose forms similar adducts with some higher alcohols and normal fatty acids of moderate chain length[8,9]. As these two diffraction peaks are characteristic of the helical structure of amylose, the amylose precipitate formed by the addition of SDS should be an adduct of amylose including SDS ions in its helical structure. Although the mixture of amylose solution must be heated with higher fatty acid or alcohol at about 70°C and then cooled to obtain the adduct, only the addition of SDS to amylose solution at room temperature is enough in the present case. This difference might originate from the fact that SDS is much more soluble than higher fatty acid or alcohol.

There is another evidence for the formation of the adduct having a helical structure between amylose and surfactant. It is well known that when iodine was added to amylose solution, intensive blue color appears instantaneously. Now it is generally accepted that this characteristic coloration of amylose solution by iodine is caused by the change of an electronic state of iodine molecules lying along the axis of helix of the amylose-iodine complex[12]. The change of the electronic state should be caused by the large polarity of amylose in a helical structure due to the orientation of a large number of hydroxyl groups in glucose residues in a similar direction. As mentioned above, when iodine solution was added to amylose solution containing SDS, the blue color of amylose-iodine complex appears gradually instead of instantaneous coloration as in the case without SDS. It may be expected that it needs some time to exchange SDS ions with iodine in a helix of amylose.

As the authors have mentioned above, the absorption maximum shifts to shorter wavelength-side and absorption decreases with the concentration of SDS. The decrease in absorption is remarkable at the longer wavelength-side. Rundle et al.[13] studied the effect of the molecular weight of amylose on the coloration of amylose with iodine. They found that with the decrease in the molecular weight or the chain length of amylose, the absorption maximum shifts to the shorter wavelength side and the absorption decreases. This change of absorption is remarkable at the longer wavelength side. As iodine competes with SDS in intruding into a helix of amylose, the linear array of iodine molecules which intervened between SDS ions might become shorter with the increase of SDS concentration. The increase of SDS concentration might be assumed to have the same effect as the decrease of the chain length of the amylose molecule forming a helical complex with iodine. Parallel changes in the shift of absorption maximum and the decrease in absorption in both cases mentioned above might be explained in terms of the chain length concerning one continuous unit of the amylose-iodine-complex.

No more change was found in the absorption spectra of amylose-iodine complex, when the concentration of SDS exceeds about 4×10^{-3} M. This concentration almost exactly corresponds to the critical micelle concentration of this surfactant, so that this fact suggests that only the SDS ions are concerned with the formation of the helical complex.

According to the study of the absorption spectra mentioned above, amylose seems to assume a helical configuration in the presence of SDS even in a dissolved state. Viscosity measurments, as well known, give some insights into the configuration of polymer molecules in solution. As shown in Fig. 4., when the reduced viscosity η_{sp}/c of amylose solution in the absence of SDS was plotted against amylose concentration, the same linear relation was obtained as in the case of a typical non-electrolytic polymers. However, if SDS is present in the solution, η_{sp}/c decreases rapidly with the decrease in amylose concentration and converges to a definite value independent of the concentration of SDS in the low concentration of amylose. The larger the ratio of SDS to amylose is, the larger is the concentration of amylose from which the reduced viscosity begins to decrease. Accordingly, the ratio of SDS to amylose is evidently an important factor to the decrease of the reduced viscosity.

12) R. S. Stein and R. E. Rundle, *J. Chem. Phys.*, **16**, 195 (1948).

13) R. R. Baldwin. R. S. Bear and R. E. Rundle, *J. Am. Chem. Soc.*, **66**, 111 (1949).

In the case of amylopectin, the linear relation between η_{sp}/c and c was retained even in the presence of SDS. The line moves parallel, a little downward. Since the intrinsic viscosity of the polymers should be an indication of their volume in the dissolved state, the decrease in the reduced viscosity of amylose suggests the decrease in the effective volume of amylose when SDS was added to the solution. The cause of the decrease in viscosity should be attributed to the formation of a helical complex between amylose and SDS. Owing to the mutual compensation of the polarity of hydroxyl groups in the helical complex, the hydration of amylose might be decreased. This might be one of the reasons why the viscosity of amylose-surfactant-complex decreased.

The minor decrease in viscosity found with amylopectin in the presence of SDS might be caused by the contamination of a small amount of amylose, since the decrease of viscosity is almost the same with the solution containing 0.01% SDS and that containing 0.02% SDS.

In conclusion, it was found experimentally that there are at least three different ways of interaction between surface active agents and polymer, namely, (1) only by van der Waals' force, (2) by electrovalent bonding only or together with van der Waals' force, and (3) by a special combination found in the case of adduct formation. In the case of the interaction of a surface active agent with non-ionizable polymers such as polyvinyl formal or polyvinyl acetate[1], cooperative force between chains of surfactant is very significant. Accordingly, hydrophilic and lypophilic properties of surfactant must be appropriately balanced in this case. The cationic detergent shows poor interaction with polymers, probably because of its stronger hydrophility than that of the anionic detergent.

The electrovalent bonding of the surfactant with polymer is of course encountered with the polymers such as proteins which have ionic sites in the molecules[10]. The surface active agents which can act on the polymers should have the charge of the opposite sign to the polymer. After forming the envelope of the surface active agent of the thickness of a molecule, more layers of surface active agents can be built up on it by van der Waals' force.

The third class of interaction such as these encountered in the case of amylose and surface active agents, some specific steric conditions must be fulfilled. In this case, whether the surface active agent is anion or cation is out of the question. However, non-ionic detergents were found to have scarce interaction with the polymers throughout all these three types of interactions[14].

Summary

It was found that a specific precipitate is formed when some ionic surface active agents are added to amylose solution and it was confirmed by X-ray diffraction study that this phenomenon is due to the formation of the helical complex between amylose and a surface active agent.

It is also inferred that the same complex is formed even in the dissolved state from the study on the effect of the surface active agent on the viscosity of amylose solution and on the absorption spectrum of amylose-iodine complex.

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14) Preliminary experiments.