# Hydrodynamic Characterization of Amylose-SDS Complex by Viscosity Measurement

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The hydrodynamic behavior of amylose with and without sodium dodecyl sulfate (SDS) has been examined by viscosity measurements. Two distinct viscosity phenomena have been observed by complexation; one is the increase at the first stage of binding of SDS, which disappears by an addition of salt, and the other is the subsequent decrease in the region of further binding of SDS. These results are interpreted by an idea that the expansion of the complex occurs by an electrostatic repulsion of the bound SDS molecules and the contraction of the complex by a hydrophobic interaction of cooperatively bound SDS molecules. This idea was confirmed further by experiments using amyloses of different length and homologous surfactants of SDS.

It is known that surfactants interact with amylose in the same way as iodine, aliphatic acids, and alcohols, forming complexes where these guest molecules are included in the amylose helix.[1-4] The helical conformation of amylose has been established in the solid state, while the conformation of amylose in solution still remains uncertain. Three models have so far been proposed for the structure of amylose in solution, i.e., (1) entirely random coil model, (2) interrupted helical model, and (3) entirely helical model.[5,6] Banks and Greenwood claimed that the conformation of amylose is the model (1)[7] and Szejtli et al. proposed the model (2),[8] and Senior et al. proposed a model which is characterized by the regions of loose and extended helices which alternate with shorter random coil regions.[9] We also conducted a series of studies on the complex formation of amylose and proposed a model similar to the model (2) and revealed the properties of the cooperative binding of guest molecules to amylose.[10-13] These results indicate a probability of structural change of the amylose by the complexation.

In the present study, the viscosity of the amylose solution with and without SDS was investigated in connection with the cooperative binding and is discussed from a viewpoint of the structural changes of the amylose by the complexation.

## Experimental

**Materials.** Potato amylose, purchased from Pierce Chemicals, was purified by precipitation three times from 1-butanol. Its average degree of polymerization (DP) was estimated to be 1100 by gel filtration. Amyloses of small DPs were prepared as described in the previous paper.[10] Wet 1-butanol complex of amylose was dissolved in water and 1-butanol was subsequently removed by distillation for 30 min in a boiling water bath with bubbling nitrogen gas. After cooling, the solution was filtered through a G3 sintered glass filter. The concentration was determined by the phenol-sulfuric acid method.[14] Sodium alkyl sulfates were synthesized according to the method of Dreger et al.[15] Other chemicals used were of guaranteed grade.

**Measurements.** Amylose solutions were prepared in the concentration range of 0.10—1.50 mg ml$^{-1}$ for the amyloses of DP 100, 300, and 1100 and 10—15 mg ml$^{-1}$ for those of DP 42 and 57. Fresh amylose solutions were pipetted into the viscometer and the same volume of 2 mM surfactant solution (1 M=1 mol dm$^{-3}$) was added. The mixed solution was held for 30 min in the water bath before runs. Since the amylose-surfactant complexation is rapid all the reactions were presumed to be fully equilibrated on this time scale.[16] The viscosity was measured by Ubbelohde type viscometers at 20.0±0.1 °C. The efflux times of pure water were around 180—240 s and the kinetic energy corrections were negligible. The measurements were repeated for solutions prepared by subsequent addition of surfactant solution. The concentration of bound surfactants was determined potentiometrically as described in the previous paper.[12] To rule out any effect due to the micellization, the concentration of surfactant was kept below the critical micelle concentration.

## Results

The specific viscosity, $\eta_{sp}$, was measured for the amylose solution in the absence or in the presence of SDS and the reduced viscosity, $\eta_{sp}/C_p$, was obtained for the concentration of amylose, $C_p$. The reduced viscosity was constant for free amylose or SDS solutions. For the amylose-SDS system, on the other hand, the value of $\eta_{sp}/C_p$ increases rapidly in the first stage and then decreases with the SDS concentration as seen in Fig. 1.

These results are indicative of two-step hydrodynamic changes of the amylose complex, and systematic experiments were carried out by changing various experimental factors as follows. Firstly, the viscosity of the SDS-amylose solution was measured by changing the concentration of amylose under the constant concentration of SDS. As seen in Fig. 2, the $\eta_{sp}/C_p$ value was independent of the concentration of amylose in the concentration range of 0.10 to 0.50 mg ml$^{-1}$ for various concentrations of SDS. Secondly, the viscosity was measured for the SDS-amylose solution in the presence of NaCl and the results are shown in Fig. 3. Here, the experiments were carried out at relatively low NaCl concentration in order to avoid

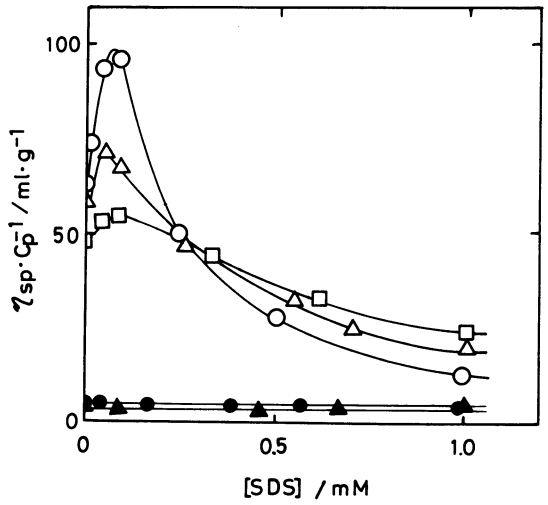


Fig. 1. SDS concentration dependence of reduced viscosity. (○) DP 1100; 0.42 mg ml$^{-1}$, (△) DP 300; 0.50 mg ml$^{-1}$, (□) DP 100; 1.00 mg ml$^{-1}$, (●) DP 57; 5.0 mg ml$^{-1}$, (▲) DP 42; 5.0 mg ml$^{-1}$.

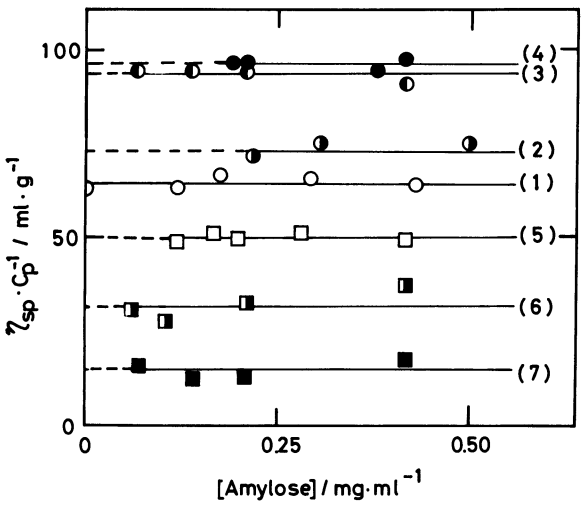


Fig. 2. Amylose concentration dependence of reduced viscosity at various SDS concentrations. (1) 0 mM, (2) 0.01 mM, (3) 0.05 mM, (4) 0.1 mM, (5) 0.2 mM, (6) 0.5 mM, (7) 1.0 mM.

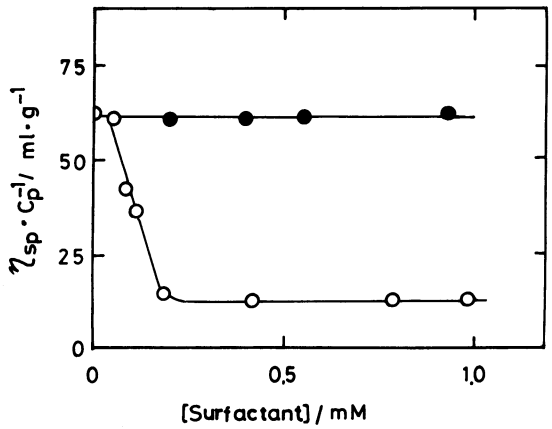


Fig. 3. Surfactant concentration dependence of reduced viscosity in the presence of 10.7 mM NaCl. (●) $C_8$, (○) $C_{12}$ (=SDS). Amylose concentration was in the range of 0.46 to 1.38 mg ml$^{-1}$.

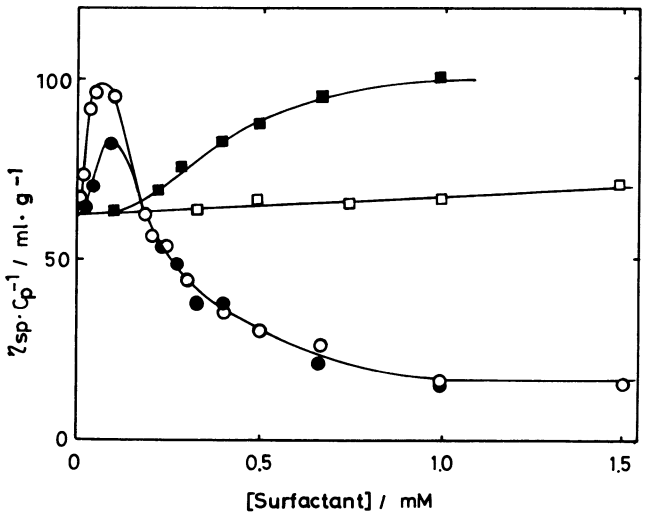


Fig. 4. Surfactant concentration dependence of reduced viscosity for sodium alkyl sulfate-amylose systems in the absence of NaCl. (□) $C_8$, (■) $C_{10}$, (○) $C_{12}$ (=SDS), (●) $C_{14}$. Amylose concentration was in the range of 0.46 to 1.38 mg ml$^{-1}$.

aggregation of the complex. As seen in the figure, the characteristic increase of the viscosity in the first stage disappeared, i.e., the viscosity was constant in the range of [SDS]<0.03 mM, and above this concentration, the viscosity decreased uniformly with increase of SDS. Thirdly, the viscosity was measured by changing the surfactant to the homologues of SDS, i.e., $C_nH_{2n+1}SO_4Na$ ($n$=8—14). As seen in Fig. 4, the concentration dependency behavior of $\eta_{sp}/C_p$ differs greatly between the surfactants of $C_8$ and $C_{10}$ and those of $C_{12}$ and $C_{14}$. In the former systems, only the increase of the viscosity was observed with increase of the surfactant concentration and the viscosity decreasing process is absent. While in the latter systems, the viscosity increases at first and then decreases. The increasing behavior observed for the surfactant-amylose systems disappeared on an addition of salt. Fourthly, the viscosity of the solutions of SDS and amyloses of small DP was examined in the same way as that of amylose of DP 1100 system studied above. The values of $\eta_{sp}/C_p$ for the amyloses of DP 42 and DP 57 were independent of the SDS concentration (Fig. 1), and the result is in contrast to the behavior of the amylose of DP 1100. Those of the amyloses of DP 100 and 300 are similar.

The amount of bound surfactant was measured by potentiometric titration. As seen in Fig. 5, the amount of bound SDS does not change on the addition of salt under the present experimental concentration range.

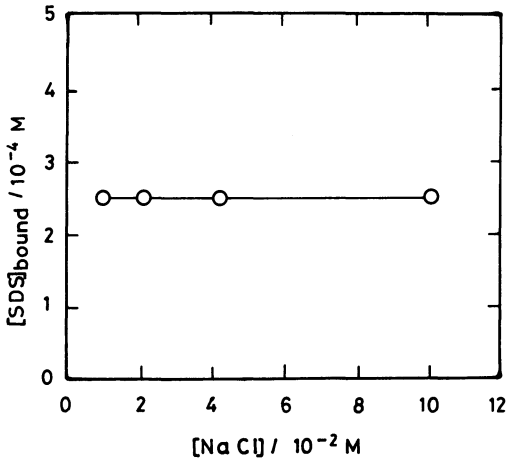


Fig. 5. NaCl concentration dependence of the maximum amount of bound SDS to amylose (DP 1100). [Amylose]=0.46 mg ml$^{-1}$.

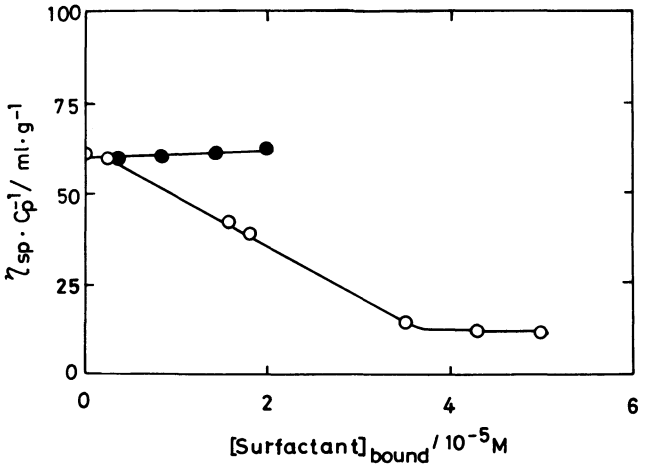


Fig. 6. The dependence of the viscosity on the amount of bound surfactant. (●) $C_8$, (○) $C_{12}$ (=SDS). Amylose concentration was in the range of 0.46 to 1.38 mg ml$^{-1}$.

## Discussion

As the origin of the characteristic viscosity behavior observed above, two effects may be considered. One is the intermolecular effect of amylose or its SDS complex. This may be discounted, because the viscosity of the solution of amylose or its complex was independent of their concentration. The result suggests that the amylose retains the same binding state irrespective of the amylose concentration as long as the SDS concentration is constant. This suggestion was supported by the fact that the amount of bound SDS was proportional only to the amylose concentration under the present experimental condition. The other possibility is the intramolecular effect which has been proposed by some workers.

Rao et al. have observed the viscosity decrease on complexation for the SDS-amylose system and ascribed it to the propagation of the helix.[17] According to them, SDS first binds to the originally helical part of amylose, which is not accompanied by any change of viscosity. Further binding induces a helix propagation accompanying a decrease of viscosity. Their idea is applicable to our data for SDS-amylose system (Fig. 6) indicating the original helical part of amylose corresponds to about 5% of the total amount of SDS binding. If their interpretation is applied to the binding of $C_8$ surfactant system, similar viscosity behavior to those of SDS system is expected since the amount of its binding exceeds fully that for the originally helical part of amylose.[12] However, this expectation was not satisfied as seen in Fig. 6 and the idea of Rao et al., therefore, is questionable. For a complexation of amylose with iodine, Banks et al. observed a decrease of viscosity and ascribed it to the conformational change of amylose from a random coil to a helix.[7] While, Senior et al. insisted that the decrease is due to a shortening of the linear dimension of the polymer chain.[9] All these experiments have been performed in the presence of electrolyte, and only the decrease in viscosity by complexation has been observed. Therefore, these interpretations cannot be applied satisfactorily to either the lack of decrease of viscosity in $C_8$ and $C_{10}$ surfactant systems or the increase of viscosity in $C_{12}$ and $C_{14}$ surfactant systems. Recently, Francois et al. observed an increase in viscosity on the complexation of poly(oxyethylene) with SDS and claimed that the polymer complexes with SDS micelles.[18] However, this idea is not applicable to the present system where the surfactant concentration is much lower than the critical micelle concentration. As a consequence, we developed our own idea in the following way.

It is said that amylose is composed of several segments which are composed of about 60 anhydroglucose units; there is however some discrepancy in the literature values.[10,19-21] Taking this fact into consideration, the amyloses of DP 42 and 57 are composed of a single segment and DP 100, 300, and 1100 of several segments. These facts show that the characteristic viscosity behavior appears when the amylose is composed of more than two segments. So, segment-segment interactions of the SDS complex are the origins of the increase and/or the decrease of viscosity.

Further information on these interactions are derived from the salt effects on the viscosity. Since the binding of SDS was not affected appreciablely by the presence of salt, the disappearance of the viscosity increasing behavior on the addition of salt should not be ascribed to the decomposition of the complex. Instead it implies that the segment-segment interaction contributing to the viscosity increase is interrupted by the addition of salt, i.e., the interaction may

be an electrostatic one. Therefore, the viscosity increase indicates an expansion of the complex by electrostatic repulsion among the segments charged by the initial binding of SDS. On the other hand, the interaction contributing to the viscosity decrease may be nonelectrostatic since the viscosity decrease process was not affected by the addition of salt.

We have investigated the binding isotherm of surfactant to amylose by the potentiometric titration and clarified that (i) SDS binds to isolated binding sites in the region of [SDS]$<$0.03 mM while it binds cooperatively in the region of 0.03 mM$\leqq$[SDS]$\leqq$0.20 mM, (ii) the cooperativity is approved for the bindings of $C_{12}$ and $C_{14}$ surfactants but not for those of $C_8$ and $C_{10}$ surfactants, (iii) the cooperativity is approved for amyloses composed of more than two segments, i.e., it is related to the segment-segment interaction.[12] These facts, together with the viscosity data, indicate that the boundary concentrations of SDS in the viscosity behavior shown in Fig. 3 are in good agreement with those observed in the binding curve, respectively. The viscosity constant region in the first stage of SDS binding also corresponds to the binding on the isolated sites while the decreasing behavior in the subsequent stage corresponds to the cooperative binding. Therefore, it is clear that the viscosity decrease indicates a contraction of the complex by the nonelectrostatic segment-segment interaction induced by the additional binding of SDS. In accordance with this conclusion, it is interesting to see that the cooperativity is observed also for the iodine-amylose system, and the viscosity decrease observed by Banks et al.[7] and Senior et al.[9] will be due to the contraction of the complex induced by the cooperative binding of iodine. Furthermore, lack of the viscosity decrease process in the binding of the $C_8$ and $C_{10}$ surfactants to amylose (DP 1100) is also compatible with the lack of the cooperativity in their bindings.

These characterization of viscosity behavior supports a model previously proposed for the SDS-amylose complex, where SDS molecules are binding at each end of the helical segment and the cooperativity is ascribed to the hydrophobic interaction between the alkyl chains of bound SDS.[12] Based on this model, we conclude that the viscosity increase observed at the first stage of the SDS binding is due to the expansion of the complex by electrostatic repulsion between the charged head groups of SDS bound to the isolated sites of the helical segments. The viscosity decrease observed in the second stage is due to the contraction of the amylose complex induced by the hydrophobic interaction of the bound SDS.

The above interpretation is also applicable to the results for the $C_8$ and $C_{10}$ surfactant systems and to those for SDS-amyloses of low DPs. The $C_8$ and $C_{10}$ surfactants bind to amylose non-cooperatively due to their somewhat shorter alkyl chain, and the bindings do not induce the contract of the complex because the electrostatic repulsion among the segments with charged surfactants is predominant. Hence, only the viscosity increase process is observed and the decrease process is not observed in their bindings. In the binding of SDS to amyloses of DP 42 and 57, the interaction between the segments in the complex and the cooperativity are not expected because they consist of a single segment. Therefore, any characteristic hydrodynamic behavior is not observed.

**References**

1) F. F. Mikus, R. M. Hixon, and R. E. Rundle, *J. Am. Chem. Soc.*, **68**, 1115 (1946).
2) T. Takagi and T. Isemura, *Bull. Chem. Soc. Jpn.*, **33**, 437 (1960).
3) Y. J. Kim and R. J. Robinson, *Staerke* **31**, 293 (1979).
4) Y. Hui, J. C. Russell, and D. G. Whitten, *J. Am. Chem. Soc.*, **105**, 1374 (1983).
5) O. Kratky and G. Porod, *Recl. Trav. Chim. Phys-Bas*, **68**, 1106 (1949).
6) Q. T. Nguyen, P. Aptel, and J. Neel, *Biopolymers*, **15**, 2097 (1976).
7) W. Banks and C. T. Greenwood, *Staerke*, **23**, 300 (1971).
8) J. Szejtli, M. Richter, and S. Augustat, *Biopolymers*, **5**, 5 (1967).
9) M. B. Senior and E. Hamori, *Biopolymers*, **12**, 65 (1973).
10) M. Yamamoto, T. Sano, and T. Yasunaga, *Bull. Chem. Soc. Jpn.*, **55**, 1886 (1982).
11) M. Yamamoto, T. Sano, S. Harada, and T. Yasunaga, *Bull. Chem. Soc. Jpn.*, **55**, 3702 (1982).
12) M. Yamamoto, T. Sano, S. Harada, and T. Yasunaga, *Bull. Chem. Soc. Jpn.*, **56**, 2643 (1983).
13) M. Yamamoto, S. Harada, T. Sano, T. Yasunaga, and N. Tatsumoto, *Biopolymers*, **23**, 2082 (1984).
14) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
15) E. E. Dreger, G. I. Keim, G. D. Miles, L. Shedlovsky, and J. Ross, *Ind. Eng. Chem.*, **36**, 610 (1944).
16) S, V, Bhide, M, S, Karve, and N, R, Kale, *Am. Chem. Soc. Symp. Ser.*, No. 50, pp. 491—511 (1981).
17) V. S. R. Rao and J. F. Foster, *Biopolymers*, **1**, 527 (1963).
18) J. Francois, J. Dayantis and J. Sabbadin, *Eur. Polym. J.*, **21**, 165 (1985).
19) J. Szejtli, M. Richter, and S. Augustat, *Biopolymers*, **6**, 27 (1968).
20) T. Watanabe, K. Ogawa, and S. Ono, *Bull. Chem. Soc. Jpn.*, **43**, 950 (1970).
21) K. Hiromi, T. Shibaoka, and S. Ono, *J. Biochem.*, **68**, 205 (1970).