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Cooperativity in the Binding of Sodium Dodecyl Sulfate to Amylose

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The binding isotherm of sodium dodecyl sulfate to amylose was potentiometrically investigated by using amyloses of various degrees of polymerization from 32 to 1100. From the Scatchard plots, the binding was found to be cooperative for the amyloses of DP≧76 and Langmuir type for those of DP≦57. By analogous experiments using a series of sodium alkyl sulfates, it was clarified that (1) eight carbon atoms of surfactant are required for the complexation and (2) more than twelve carbon atoms of surfactant are required for the cooperative binding. Thus, the amylose has an interrupted helical structure in solution and the cooperativity arises from the hydrophobic interaction between the surfactants included partly in the helic of amylose.

A number of investigations[1-7] have been performed on the complex formation of amylose with various reagents, in connection with the biological significance of the amylose structure in solution. Three representative models have been proposed, *i.e.*, an interrupted helix,[8,9] an entirely random coil,[10] and a deformed helix.[11,12] In previous papers,[13,14] we have proposed the interrupted helical structure as the most adequate model for amylose conformation in solution from the kinetic and static investigations on the amylose–iodine complex formation by using the amyloses of various degrees of polymerization, DP.

In extension of our studies on the structure of amylose, the binding isotherm of sodium dodecyl sulfate, SDS, to amylose was investigated with use of amyloses of various DPs. Further, the same experiment was performed for the homologue of SDS using the amylose of DP 1100, and the effect of the chain length of alkyl group of surfactant on the binding isotherm was examined.

Experimental

Materials. Potato amylose, purchased from Pierce Chemical, was purified by precipitation three times from 1-butanol. Amyloses of various DPs were prepared as described in a previous paper.[13] The molecular weight, $\bar{M}_w$, of the amylose was estimated from the elution profile of the gel chromatography on a Sepharose 4B column (2.5×62 cm), using the following relationship obtained by Dextran T10, T20, T70, T150, and T500.

$$\log \bar{M}_w = 7.05 - 3.19 K_{av}, \quad (1)$$

with

$$K_{av} = \frac{V_e - V_v}{V_t - V_v}, \quad (2)$$

where K_{av} is the partition coefficient;[15] V_v, V_t, and V_e are the void volume, total volume, and elution volume, respectively. The amylose was used in the form of the wet 1-butanol complex.[10] Sodium alkyl sulfates, $CH_3(CH_2)_{n-1}SO_4Na$ with n=6, 8, 10, 12, and 14, were synthesized according to the method of Dreger.[16] Dodecyltrimethylammonium dodecyl sulfate was prepared according to the method of Satake and Yang.[17]

Measurements. The concentration of bound SDS was determined by its electromotive force (emf) using a cell constructed as follows:[17,18]

|reference electrode|agar bridge|reference solution; 0.6×10^{-4} M** SDS|nitrobenzene; 1×10^{-4} M $(C_{12}H_{25}(CH_3)_3N)^+(SO_4C_{12}H_{25})^-$|sample solution; 5×10^{-3} M amylose in AGU, SDS|agar bridge|reference electrode|.

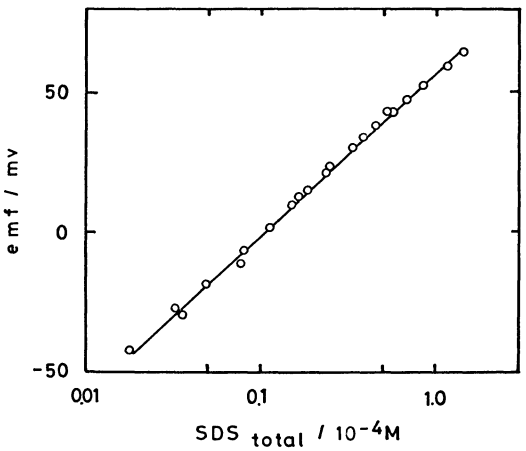

Fig. 1. Plot of emf *vs.* the logarithm of the concentration of SDS at 30 °C.

Calibration of the apparatus was performed by measuring the emf of the SDS solution without amylose. To attain a stable and accurate responce of the liquid membrane, 21.3 mM of sodium chloride was added to each solution. Figure 1 shows the semilogarithmic plot of the emf *vs.* SDS concentration. The slope of the line, 59 mV at 30 °C, agrees well with the ideal Nernst slope (60 mV at 30 °C), which implies that the liquid membrane responds exclusively to a surfactant ion.[19] The pH of the solutions ranged from 5.5 to 6.2, since no buffer solution was used. Amylose solution was freshly prepared immediately before the measurements and the concentration in anhydroglucose residue unit was determined by the phenol-sulfuric acid method.[20] To rule out any effect due to the micellization, the concentration of surfactant was kept below the critical micelle concentration. All measurements were carried out at 30 °C.

Results and Discussion

Typical potentiometric titration curves obtained with SDS in the presence and absence of amylose are shown in Fig. 2. Since the SDS bound to amylose can not contribute to the emf, the concentration of the bound SDS is equal to the difference in the SDS concentrations required to give the same emf on curves a and b in Fig. 2. The concentration of the bound SDS estimated for the various DPs of amylose is plotted against the free SDS concentration in Fig. 3. As seen

** 1 M=1 mol dm^{-3}.

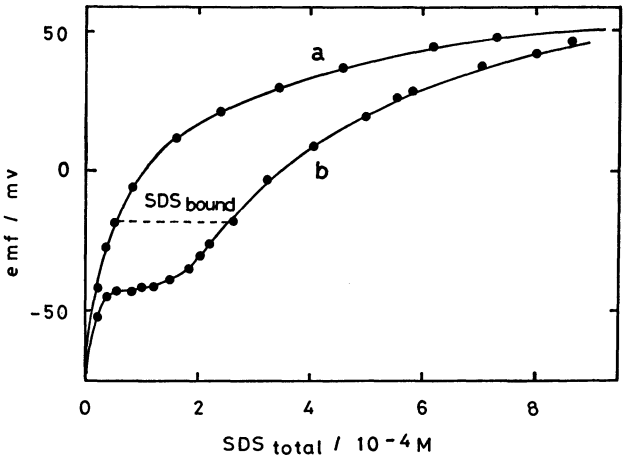

Fig. 2. Potentiometric titration curves of amylose (DP 1100) with SDS. (a); Without amylose, (b); with amylose of 5 mM in anhydroglucose residue unit.

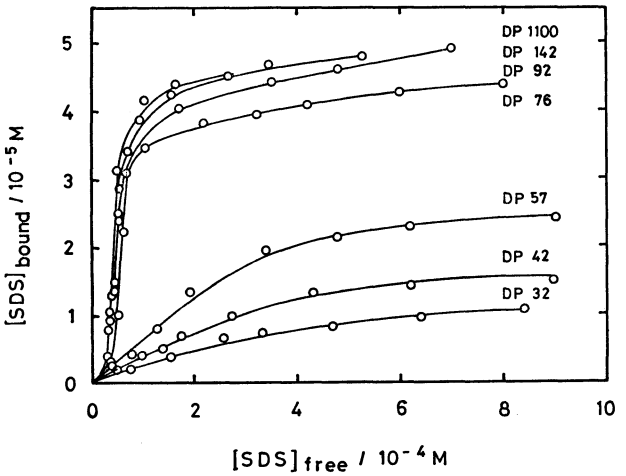

Fig. 3. Binding isotherms of SDS for a series of amyloses at 30 °C.

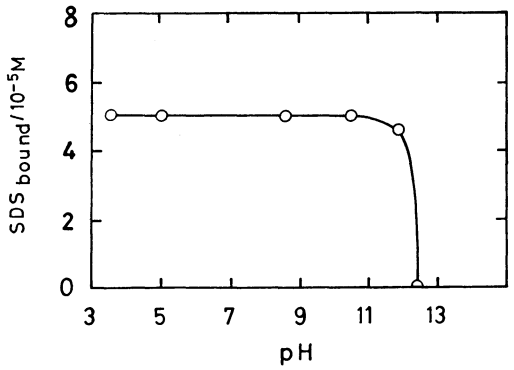

Fig. 4. pH dependence of the total amount of bound SDS to amylose (DP 1100, 1 mM) at 30 °C.

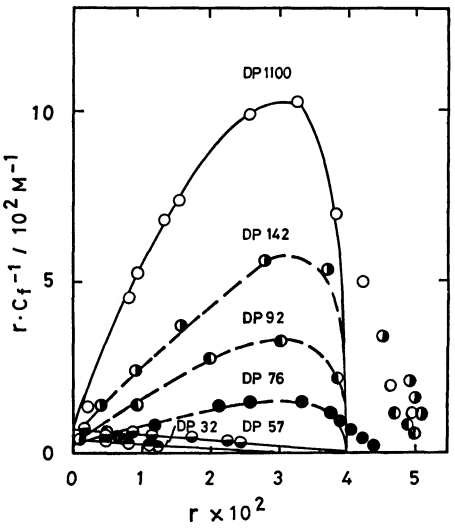

Fig. 5. Representative Scatchard plots of binding isotherms of SDS to amyloses. Solid lines show theoretical curves obtained by Eqs. 2 and 3. The dotted lines have no theoretical meaning. (○); DP 1100, (◑); DP 142, (◐); DP 92, (●); DP 76, (◒); DP 57, (◓); DP 32.

in this figure, the total amount of the bound SDS increases with the DP of amylose.

As for the pH dependence of the concentration of bound SDS, no alteration takes place up to pH 10.6, but a sudden decrease appears at around pH 12, as shown in Fig. 4. A similar drastic change in the conformation of amylose with pH has been reported; this was ascribed to the break-down of the helical structure.[4] From a consideration of these two experimental results, it can be deduced that the SDS molecule binds to the helical part of amylose.

The binding equilibrium of amylose–SDS system is written as

$$A + SDS_f \rightleftharpoons SDS_b, \quad (3)$$

where A is the binding site of amylose and SDS_f and SDS_b are the free and bound SDS, respectively. The Scatchard equation for Reaction 3 is given by

$$r/C_f = K(n-r), \quad (4)$$

where r is the ratio of bound SDS to total amylose in anhydroglucose residue units, n is the maximum amount of bound SDS per anhydroglucose residue, C_f is the concentration of free SDS, and K is the apparent equilibrium constant. The representative Scatchard plots for the amyloses of various DPs are shown in Fig. 5. At first glance, an obvious difference can be seen in the plots, *i.e.*, straight lines with negative slope for the amyloses of DP≦57, and curvature with upward convex for those of DP≧76. The straight lines indicate the Langmuir type binding of SDS to amylose, while the curvature indicates the cooperative binding. A similar change in the binding profile around DP 60 has been also recognized in the amylose–iodine complex formations, as previously reported.[13] These facts give support for the interrupted helix model of the conformation of amylose in solution.

For the Langmuir type binding, n and K_L (the subscript L means the Langmuir type binding) were evaluated from the intercept and the slope of the straight line, respectively. These values are listed in Table 1 together with the number of binding sites of SDS on one amylose molecule, m. The value of m increases with DP of amylose, whereas the equilibrium constant, K_L, remains constant.

As for the cooperative binding, the equilibrium con-

Table 1. The binding parameters of the complex formation

DP	m	$n\times10^2$	Langmuir type binding	Cooperative binding	
			K_L / $10^3\ M^{-1}$	K_0 / $10^3\ M^{-1}$	K_c
32	0.94	2.95	1.7±0.3	—	—
42	1.22	2.90	1.4±0.6	—	—
57	2.30	4.00	2.0±0.2	—	—
1100	59	4.00	—	2.0±0.7	22±2

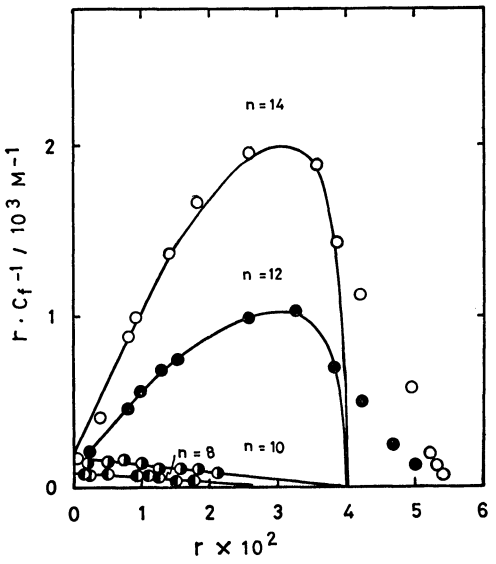

Fig. 6. Scatchard plots of binding isotherms of surfactants to amylose (DP 1100). Solid lines show theoretical curves obtained by Eqs. 2 and 3. (○); $n=14$, (●); $n=12$, (◑); $n=10$, (◐); $n=8$.

stant K in Eq. 4 can be rewritten according to the one-dimensional Ising model by McGhee and von Hippel;[22]

$$K = K_0\left\{\frac{n-2r+R}{2(n-r)}\right\}^2, \quad (5)$$

with

$$R = [(n-2r)^2 + 4K_c r(n-r)]^{1/2}, \quad (6)$$

where K_0 is the equilibrium constant for the intrinsic binding of SDS to amylose (intrinsic equilibrium constant) and K_c is the cooperative parameter. The values of K_0 and K_c were determined for the amylose of DP 1100 so as to give the best fit of the data to the Scatchard equation, and are listed in Table 1. As can be seen in Fig. 5, experimental points deviate from the theoretical curve in the region of $r\geqq0.04$ for DP 1100. These data were disregarded for analysis because of their low reliability. A good agreement between values of K_0 for DP 1100 and K_L for DP$\leqq$57 in Table 1 implies the similarity in the mechanism of the isolated binding and the Langmuir binding. Meanwhile, the value of K_c for the amylose of DP 1100 means positive cooperativity, *i.e.*, the cooperative interaction contributes to the stabilization of the complex. In the cases of amyloses of DP 76 to 142, the Scatchard plots are also indicative of the cooperativity, as seen in Fig. 5. Further quantitative analysis of these plots, however, was not carried out because Eq. 5 can not be applied to these small amyloses.[22]

Table 2. Effect of the chain length of alkyl group of surfactants on the binding parameters of the complex formation

Alkyl group	$n\times10^2$	Langmuir type binding	Cooperative binding	
		K_L / $10^3\ M^{-1}$	K_0 / $10^3\ M^{-1}$	K_c
n-C_8H_{17}	2.1	2.1±0.2		
n-$C_{10}H_{21}$	4.0	2.2±0.2		
n-$C_{12}H_{25}$	4.0		2.0±0.7	22±2
n-$C_{14}H_{29}$	4.0		2.2±0.9	36±8

In order to clarify the effect of the chain length of alkyl group of surfactant on the complexation, the binding isotherms of a series of sodium alkyl sulfates $CH_3(CH_2)_{n-1}SO_4Na$ with $n=8$, 10, 12, and 14 to amylose of DP 1100 were investigated. The Scatchard plots for these surfactants are shown in Fig. 6. These plots showed large differences in the binding profiles; the binding of surfactants of $n=8$ and 10 is Langmuir type, whereas that of $n=12$ and 14 is cooperative. These results suggest that a certain length of the alkyl chain of surfactant ($n\geqq12$) is necessary for the cooperative binding. Meanwhile, sodium alkyl sulfate with $n=6$ did not bind to amylose. Consequently at least eight carbon atoms of surfactant molecule should be required for the complex formation. Table 2 summarizes the binding parameters obtained for each surfactant system. As can be seen from this table, the values of K_L for surfactants of $n=8$ and 10 are in good agreement with that of K_0 for $n=12$ and 14, which implies that the alkyl chain length included in a helix of amylose is approximately equal for each surfactant system.

The binding parameters of SDS to the amylose of DP 1100 were obtained at various temperatures; their temperature dependences are shown in Fig. 7. From these, thermodynamic parameters were obtained and are listed in Table 3. It is seen that the intrinsic complex formation is enthalpy-determined while the cooperative interaction between bound SDS is entropy-determined.

Finally, the possibility of two other models for amylose structure was examined. Three experimental facts already noted above: noncooperative binding of two SDS molecules to an amylose of DP 57, sudden appearance of the cooperative binding of SDS to the amylose of DP 76, and noncooperative binding of

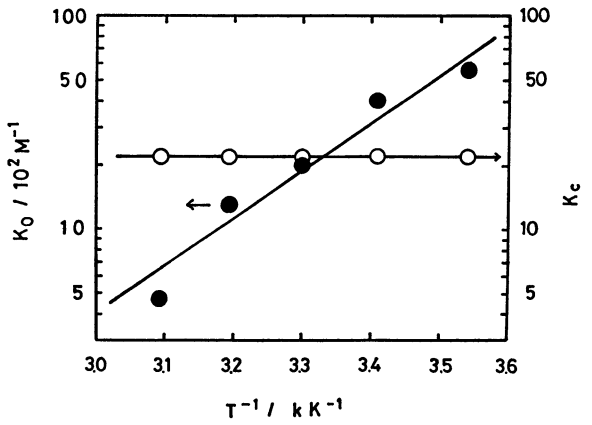

Fig. 7. The temperature dependence of the binding parameters of SDS to the amylose of DP 1100.

TABLE 3. THERMODYNAMIC PARAMETERS OF THE COMPLEX FORMATION

ΔH_0/kJ mol^{-1}	−40.5
ΔG_0/kJ mol^{-1}	−19.1
ΔS_0/J K^{-1} mol^{-1}	−70.5
ΔH_c/kJ mol^{-1}	0
ΔG_c/kJ mol^{-1}	−7.8
ΔS_c/J K^{-1} mol^{-1}	26

alkyl sulfates of n=8 and 10 to the amylose of DP 1100, exclude the possibility of two models: that of the entirely random coil and that of the deformed helix, for the structure of amylose in solution.

We have revealed the existence of two types of binding of SDS to amylose, as shown schematically in Fig. 8. One was ascribed to the Langmuir type binding due to an inclusion of SDS at the end of a helical segment of amylose, and the other to the cooperative binding due to the interaction of the outer part of alkyl chain of SDS bound to the interrupted helix of amylose. Furthermore, on the basis of the present work, the binding isotherms of fatty acids and alcohols with amylose, which have been hitherto reported as showing only Langmuir adsorption, should be reinvestigated from the standpoint of the cooperative binding with use of amylose of various DPs.

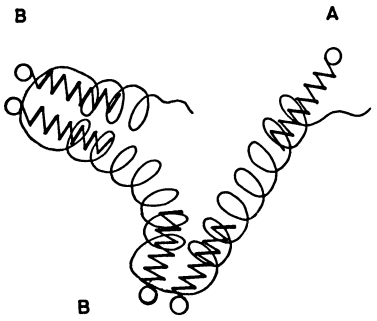

Fig. 8. Schematic picture of the structure of amylose–SDS complex. A; Langmuir type binding, B; cooperative binding.

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18) The ion-exchanger $(C_{12}H_{25}(CH_3)_3N)^+(SO_4R)^-$ corresponding to each R was used for the measurements of RSO_4Na. For the measurement of these surfactants of R=n-C_6H_{13} and n-C_8H_{17}, the ion-exchanger of R=n-$C_{10}H_{21}$ was used.
19) From the slope of the line, the values in the range from 59 to 60 mV were obtained for these surfactants of $n \geqq 10$, but those in the cases of R=n-C_6H_{13} and n-C_8H_{17} decreased.
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