The Binding of Sodium Dodecyl Sulfate to Lysozyme in Aqueous Solutions. II. The Effect of Added NaCl

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The isotherms for the binding of sodium dodecyl sulfate (SDS) to lysozyme have been investigated as a function of the concentration of added NaCl at pH 5.8 and 25 °C by a potentiometric method utilizing a surfactant-selective electrode. The equilibrium surfactant concentration at which the binding begins to occur increases with increasing NaCl concentration. The equilibrium surfactant concentration giving the same binding number above 10, on the contrary, decreases with increasing NaCl concentration. Thermodynamic analysis shows that there takes place not only the exchange between chloride ion (Cl^- ion) and dodecyl sulfate ion (DS^- ion) but also the binding of DS^- ion to the positively charged site unoccupied by Cl^- ion. The addition of NaCl does not change the equilibrium SDS concentration at which the binding number begins to exceed 10. This equilibrium concentration is regarded as a characteristic one at which the affinity between lysozyme and SDS can be evaluated. The value of standard free energy change upon DS^- ion binding and the number of Na^+ ions bound to a DS^- ion which forms a cluster on lysozyme were estimated from a thermodynamic equation based on the phase separation model.

In the previous paper, we discussed the binding mode of SDS to lysozyme and the accompanying structural change of lysozyme.[1] It was suggested from the previous experiments that the driving force of the initial binding (the binding number, $\bar{\nu}<10$) was mainly electrostatic, and that of the subsequent binding ($\bar{\nu}\geq 10$) was hydrophobic. If the binding force is different in the two regions, *i.e.*, regions of $\bar{\nu}<10$ and $\bar{\nu}\geq 10$, the addition of NaCl must give different effects on the binding in the respective regions. The effect of NaCl on the binding isotherm, therefore, is expected to give more detailed information about the binding mechanism of SDS to lysozyme.

A surfactant-selective electrode was used for potentiometry.[2-4] In this work, a poly(vinyl chloride) gel membrane was used as the ion exchange membrane instead of the nitrobenzene membrane, to prevent the solubilization of nitrobenzene.

Experimental

The solutions of hen egg-white lysozyme and other chemicals were prepared in the same way as in the previous paper.[1] The binding isotherms were made by means of potentiometry, as described previously,[1] except that the poly(vinyl chloride) gel membrane was used instead of the nitrobenzene liquid membrane. The carrier used in the surfactant-selective electrode was prepared by mixing equivalent amounts of SDS and dimethyldioctadecylammonium chloride (Kaō Soap Co. Ltd.,) in water. The details of the preparation of poly(vinyl chloride) gel membrane have been mentioned in the paper by Shirahama *et al.*[2] In the present case, a voltage follower amplifier was connected to the digital multimeter (Takeda Riken TR 6885) in order to raise the input impedance.

All measurements were carried out at pH 5.8 and 25 °C. NaCl concentrations were 10, 20, 30, 50, and 100×10^{-3} mol dm^{-3}. Every calibration curve of the electromotive force (emf) of SDS in NaCl solutions showed a good linearity over the concentration range from 3×10^{-5} mol dm^{-3} to the concentration near its critical micelle concentration(CMC) and gave a slope which was very close to a Nernstian response (55.2 ± 0.6 mV per decade of change in SDS at 25 °C).

In order to examine the accuracy of each emf value for the sample solution, emf's for several SDS solutions of known concentration were also measured after measurements of the three sample solutions.

Results and Discussion

As is shown in Fig. 1, the equilibrium concentration of SDS in the initial binding region ($\bar{\nu}<10$) increases with increasing concentration of NaCl. On the contrary, the equilibrium concentration of SDS in the region of subsequent binding ($\bar{\nu}\geq 10$) decreases with increasing concentration of NaCl. One should pay attention to the fact that all isotherms intersect each other at the equilibrium concentration of SDS at which $\bar{\nu}=10$.

The flat region in Fig. 1, where $\bar{\nu}$ could be kept at 10, becomes narrower with increasing NaCl concentration. The free energy difference of DS^- ion

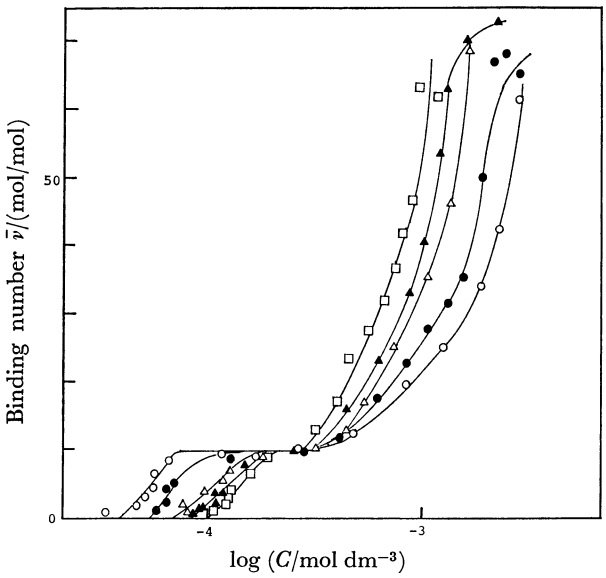

Fig. 1. Binding isotherms of lysozyme–SDS system at 25 °C and pH 5.8.
$\bar{\nu}$: Binding number, C: equilibrium SDS concentration, NaCl concentration: ○ 10, ● 20, △ 30, ▲ 50, □ 100 mmol dm^{-3}.

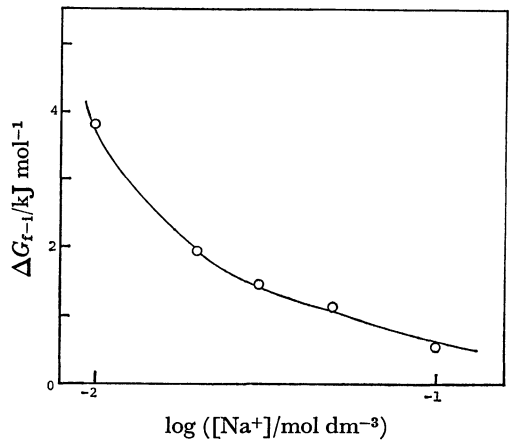

Fig. 2. ΔG_{f-1} *vs.* log [Na$^+$] plot evaluated from Eq. 1.

between the states of the initial and final points at this region can be obtained from the respective equilibrium SDS concentration as follows:

$$\Delta G_{f-1} = RT \ln \frac{a_f}{a_1} \simeq RT \ln \frac{C_f}{C_1}, \quad (1)$$

where a_1 and a_f are the activities of DS^- ions in solution at the initial and final points of the region, respectively, and C_1 and C_f are the respective equilibrium SDS concentrations. The results obtained from Eq. 1 are shown in Fig. 2. ΔG_{f-1} is considered to be the free energy accumulation for the subsequent binding. ΔG_{f-1} decreases with increasing NaCl concentration and seems to approach zero. When ΔG_{f-1} becomes zero, the binding isotherm will show an inflection at the binding number of 10.

The plots of log $C_{\bar{\nu}}$ *vs.* log [Na$^+$] are given in Fig. 3. $C_{\bar{\nu}}$ and [Na$^+$], here, represent the equilibrium SDS concentration where $\bar{\nu}$ ions of DS^- are bound to lysozyme and the total Na^+ ion concentration originating in NaCl and SDS, respectively. The plot of log $C_{0.5}$ *vs.* log [Na$^+$] gives a straight line with the positive slope of 0.5.

In the initiation of the binding of SDS to lysozyme, the Cl^- ion must be taken into account along with the dodecyl sulfate ion (DS^- ion), because both DS^- and Cl^- ions may be bound to the positively charged sites on lysozyme.[5] The initiation of SDS binding to the lysozyme–Cl^- complex can be treated as the exchange reaction between Cl^- and DS^- ions, since lysozyme has been dissolved in respective NaCl solutions before the addition of SDS. The reaction scheme for the first step of initial binding of DS^- ion to lysozyme is written as

$$P\cdot Cl_m^- + DS^- \overset{K}{\rightleftharpoons} P\cdot Cl_{m-n}^-\cdot DS^- + nCl^-, \quad (2)$$

$$K = \frac{[P\cdot Cl_{m-n}^-\cdot DS^-][Cl^-]^n}{[P\cdot Cl_m^-][DS^-]}, \quad (3)$$

where P represents lysozyme and n is the number of Cl^- ions excluded by the first binding of one DS^- ion. Now, a special case, where $\bar{\nu}$ is 0.5 in average, is considered. The meaning is that 50% of the lysozyme molecule is bound by one DS^- ion and the rest is not. This situation is realized if the concentration of SDS is reasonably low. Under this condition,

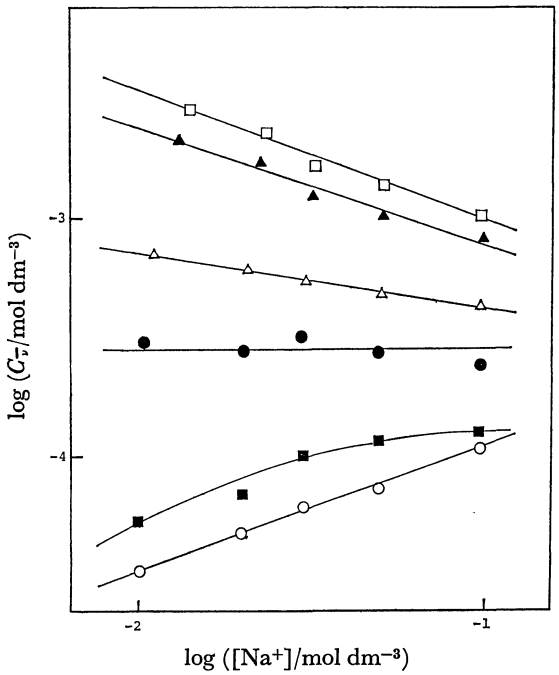

Fig. 3. Log $C_{\bar{\nu}}$ *vs.* log [Na$^+$] plots.
$C_{\bar{\nu}}$: Equilibrium SDS concentration at binding number $\bar{\nu}$, $\bar{\nu}$: ○ 0.5, ■ 5, ● 10, △ 17, ▲ 40, □ 60.

$[P\cdot Cl_{m-n}^-\cdot DS^-]$ equals $[P\cdot Cl_m^-]$. Then, Eq. 3 at the binding number of 0.5 can be rewritten as

$$\log [DS^-] = n \log [Cl^-] - \log K. \quad (4)$$

In Eq. 4, $[Cl^-]$ can be replaced by $[Na^+]$, because the concentration of NaCl is sufficiently large compared with those of SDS and lysozyme at a binding number of 0.5. Hence n is almost equal to the slope (0.5) of the plot of log $C_{0.5}$ *vs.* log [Na$^+$] in Fig. 3. As mentioned above, the slope n means the number of Cl^- ions which are excluded by a DS^- ion. The fact that n is not equal to unity but to 0.5, therefore, implies that the exchange between Cl^- and DS^- ions and the binding of DS^- ions to the positively charged sites take place at the same time. This leads to the decrease in the number of positively charged sites and hence to the precipitation of lysozyme.[1]

For $\bar{\nu}=5$, the plot of log C_5 *vs.* log [Na$^+$] gives a convex curve, as is shown in Fig. 3. In this case, the assumption used in deriving Eq. 4 from Eq. 3 cannot be used, since lysozyme–Cl^-–DS^- complexes with different numbers of Cl^- and DS^- ions distribute around the mean binding number ($\bar{\nu}=5$). It is difficult, therefore, to estimate the value of n from a log C_5 *vs.* log [Na$^+$] plot. The evaluation of n could not be made for other lysozyme–Cl^-–DS^- complexes having different binding numbers either, without estimating the concentrations of the complexes from other experiments.

As is shown in the plot of log C_{10} *vs.* log [Na$^+$] in Fig. 3, C_{10}, the equilibrium SDS concentration at which $\bar{\nu}$ begins to exceed 10, is independent of the concentration of NaCl added. This equilibrium SDS concentration corresponds to the intersection point of the isotherms in Fig. 1. At this point, the Cl^- ions on the positively charged sites are completely

replaced by DS^- ions. The lysozyme molecule has ionizable amino acid residues, *i.e.*, 6 Lys's, 11 Arg's, and 1 His, as basic residues and 7 Asp's and 2 Glu's as acidic residues. At the pH studied (pH 5.8), Lys and Arg are positively charged, since their pK values on lysozyme are within the ranges of 10.3—10.6 and 12.7—13.3[5] respectively. This means that a lysozyme molecule has 17 positive charges at this pH. On the other hand, the negatively charged residues are 7 Asp's and one Glu[7], since their pK values are within the ranges of 3.0—4.7 and 2.7—4.7[5] respectively. Therefore, the net charge of lysozyme is +9(+17−8=+9) at pH 5.8. Accordingly, a lysozyme–DS^- complex is almost electrically neutral at C_{10}. The further binding of ions to the electrically neutral lysozyme–DS^- complex will be restricted to the ions having a hydrophobic part like DS^-. From these points of view, such an equilibrium concentration as C_{10} may be looked upon as a characteristic concentration of the lysozyme–DS^- system. The other system of charged protein-ionic surfactant also might have the same characteristic concentration as that of the lysozyme–SDS system.

Plots of log C_{17}, C_{40}, and C_{60} *vs.* log [Na^+] give straight lines with negative slopes, as is shown in Fig. 3. These trends seem to reflect the effect of added NaCl on micelle formation of an ionic surfactant on the surface of lysozyme. In the previous paper,[1] the site number of the first layer at pH 5.8 was estimated from the analysis by the use of BET equation to be about 17, which corresponds to the number of positively charged residues of lysozyme,[5] and it was suggested that the hydrophobic interaction between DS^-'s was expected for the subsequent binding over the first layer. Such behavior may be considered thermodynamically the same as that of the micelle formation. The standard free energy change of the micelle formation from the phase separation model is expressed as

$$\Delta G^\circ/RT = \ln X_{CMC} + \beta \ln X_{Na^+}, \quad (5)$$

where X_{CMC} and X_{Na^+} are the mole fractions of the surfactant and Na^+ ion in the intermicellar solution at CMC, respectively, and β is the binding number of Na^+ ions per surfactant molecule in micelle.[6] If the binding of SDS is assumed to proceed like a micelle formation above the first layer of SDS on the complex, the intercept and slope of the straight line in the plot of ln $X_{\bar{\nu}}$ *vs.* ln X_{Na^+} will give $\Delta G^\circ_{\bar{\nu}}$ and β, where $\Delta G^\circ_{\bar{\nu}}$ is the difference between the standard Gibbs free energy of a mole of monomeric hydrated DS^- ion with β counterion and that of a mole of DS^- ion with β counterion bound to the complex, and $X_{\bar{\nu}}$ is the mole fraction of DS^- ion in solution at the binding number $\bar{\nu}$. Equation 5 can be applied to the plots log $C_{\bar{\nu}}$ *vs.* log [Na^+] above $\bar{\nu}=17$ in Fig. 3, if the common logarithmic concentration in mol dm^{-3} is converted into the natural logarithmic mole fraction. The linearity of the plots is maintained even in the plots of the mole fraction unit. The results obtained by applying Eq. 5 to the plots in Fig. 3 are listed in Table 1.

Table 1. Thermodynamic parameters

$\bar{\nu}$	β	ΔG°/kJ mol^{-1}
17	0.25	−33
40	0.50	−35
60	0.55	−36

The β increases with increasing binding number and seems to approach the value for the micelle of SDS. The negative charged density on the surface of the complex will increase with increasing DS^- ion binding above the binding number 10, which will cause the binding of Na^+ to the complex and hence the increment in β. The negative charge density on the surface of the complex is smaller than that of SDS micelle, because the surface area of the former is larger than that of the latter. Therefore, β of the complex must be smaller than β of the micelle.

$\Delta G^\circ_{\bar{\nu}}$'s are comparable to ΔG° (−37 kJ/mol at 25 °C estimated roughly by putting $\beta=0.7$) of SDS micelle formation. The difference between $\Delta G^\circ_{\bar{\nu}}$ and ΔG° can be attributed to the fact that the hydrocarbon part of the DS^- ion on the complex is in a less hydrophobic environment than in micelle. This suggests that lysozyme is somewhat loosely covered by DS^- ions. The results obtained here support the concept that the driving force of initial binding ($\bar{\nu}<10$) is electrostatic, and that of subsequent binding ($\bar{\nu}\geq 10$) is hydrophobic in nature.

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