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A Kinetic Study of the Interaction of Sodium Dodecyl Sulfate with Bovine Serum Albumin by Means of a Pressure-jump Method

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A single relaxation was found in the system of sodium dodecyl sulfate and bovine serum albumin using a pressure-jump method with conductivity detection. The relaxation was observed in the concentration range of the surfactant below the critical micelle concentration. The relaxation time becomes fast with the stepwise formation of a complex between them and finally attains values compatible with those in the system of the pure surfactant micelle. This suggests a correlation of the relaxation phenomena with the partial breakdown and reformation of the complex. The similarity of the complex to the surfactant micelle was discussed on the basis of the kinetic aspects of the interaction of the surfactant with the protein.

The interactions between proteins and surfactants have been extensively investigated.[1] Quite recently, the present author and others have studied the interaction between sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA).[2] They have found that an electric conductivity method is very useful in detecting the formation of complexes between them. This is because the measurement is highly sensitive to changes in the concentrations of surfactant ions. On the other hand, a pressure-jump method with conductivity detection has been used in many studies of micellization kinetics.[3] Therefore, it would be interesting to apply the pressure-jump method to the study of the surfactant-protein interactions. It is believed that further studies from a new aspect would be helpful in understanding the interactions.

It is worth noting that a similarity of the complex to the surfactant micelle is also found in the kinetic phenomena of the interaction. This paper will report the presence of a single relaxation process in the SDS–BSA system, which is strongly correlated with the stepwise growth of the complexes formed between them.

Experimental

The sources of SDS and BSA have been described previously.[2] The concentration of the protein was determined spectrophotometrically using $E^{1\%}_{1cm}=6.8$ at 280 nm.[4]

Kinetic measurements were made using a handmade pressure-jump apparatus similar to that described elsewhere.[5,6] However, it has been a little improved as follows. It is driven by water-pressure and a brass diaphragm bursts at a pressure of approximately 100 atm (1 atm=101.3 kPa). The triggering of the recording is done using a piezoelectricity of $PbTiO_3$ (see Acknowledgments). Circular dichroism measurements were carried out with a JASCO J-500A spectropolarimeter equipped with a DP-501 data processor.[2] A 1.0 mm light-pass-length cell was used in the measurements. The static conductivity was measured with a Universal Bridge 4265B of Yokogawa-Hewlett-Packard. Details of the conductivity measurements have been described elsewhere.[2]

All the measurements were made at 15 °C, because the accuracy of the relaxation time obtained by the pressure-jump method was greater at lower temperatures (see Fig .3 in Ref. 6). The only available solvent in high-precision conductometry has been limited to pure water in the pressure-jump measurements,[5] and so all the measurements were carried out in a non-buffered system, using water prepared by passing redistilled water through a mixed-bed ion-exchange column. The specific conductivity of the water used was above 1×10^{-6} Ω^{-1} cm^{-1}. The BSA concentration was held constant, 1.0×10^{-5} M (as the concentration unit, 1 M=1 mol/dm^3 is used).

Results

Prior to the application of the pressure-jump method to the study of the SDS–BSA interaction, the SDS-induced conformational change of the protein was examined by the circular dichroism measurements and the conductance changes with the complex formation were also measured. Figure 1 shows the SDS concentration dependence of the difference of residue ellipticity at 222 nm, $[\theta]_{222}$, which is one of the characteristic wavelengths for the helical structure of polypeptides. The addition of SDS caused a decrease of the helix-like structure, as may be seen in the insert of Fig. 1. The helix content decreased with an increase of the SDS concentration.

A conductivity method has so far been used in order to detect the micelle formation of ionic surfactants.[7] When the method was applied to the present SDS–BSA

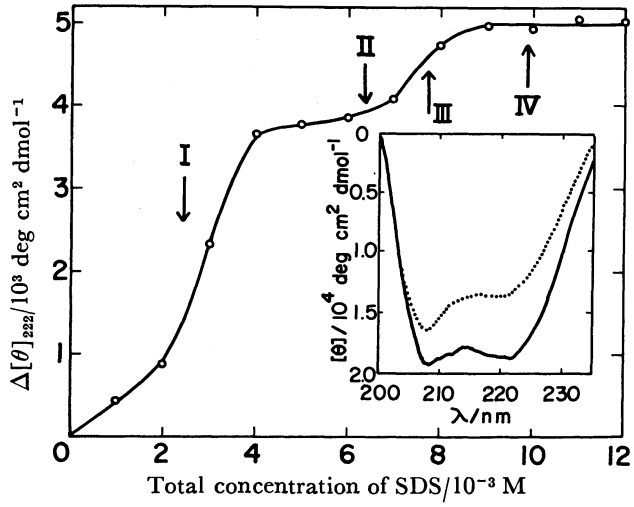

Fig. 1. The dependence of the residue ellipticity, $[\theta]_{222}$ on the SDS concentration. The typical circular dichroic spectra of BSA solutions in the absence (solid curve) and in the presence (dotted curve) of 12 mM SDS are shown in the insert. The arrows indicate the SDS concentrations where the inflection points appear in a plot such as Fig. 2.

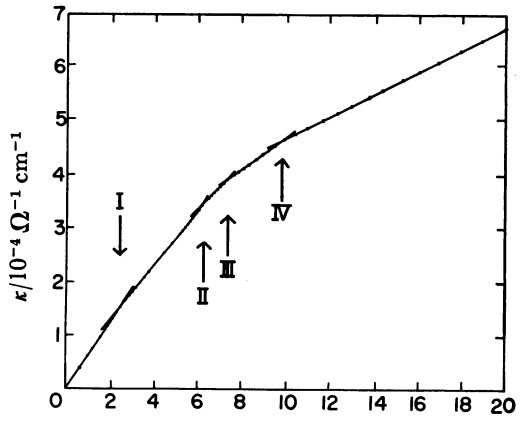

Fig. 2. The specific conductance, κ, of solutions of various SDS concentrations in the presence of 1.0×10^{-5} M BSA.

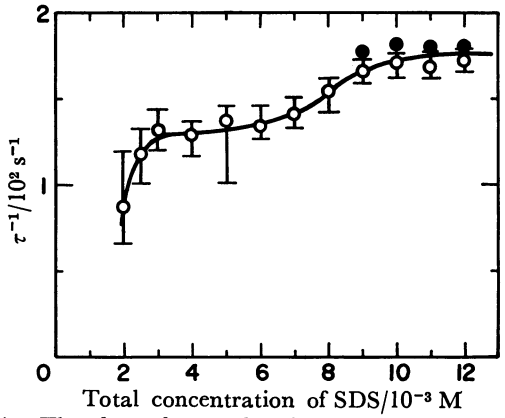

Fig. 4. The dependence of reciprocal relaxation time, τ^{-1}, on the SDS concentration (open circle). The vertical lines were drawn through the experimental points. Solid circles show the values of τ^{-1} in the pure SDS system.

system, four inflection points were observed in the plot of the specific conductance versus the concentration of SDS added to an aqueous BSA solution, as is shown in Fig. 2. These inflections can be demonstrated more clearly through an enlarged plot (not shown), as has been discussed before.[2] These inflection points appeared around 2.5, 6.5, 7.5, and 9.9 mM SDS. In comparing the SDS concentration dependence of the conformational change with the results of the conductivity measurements mentioned above, the first and the third inflections (corresponding to those at 2.5 and 7.5 mM SDS) appear in the courses of the first predominant conformational change and the subsequent one respectively, as is indicated by arrows in Fig. 1. The second and the fourth inflections (at 6.5 and 9.9 mM SDS) correspond to the beginning and the end of the second conformational change respectively (also shown by the arrows).

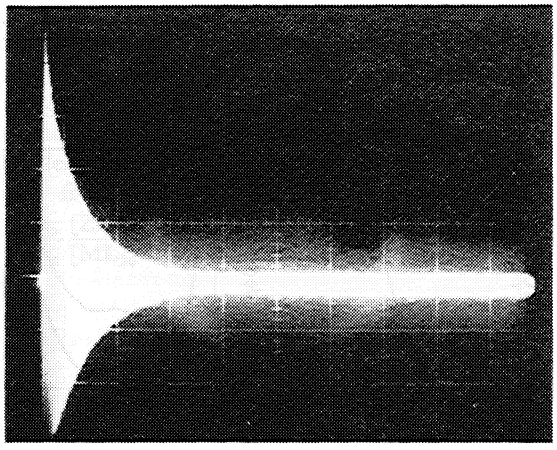

Fig. 3. The representative relaxation curve of 6.0 mM SDS solution containing 1.0×10^{-5} M BSA at 15 °C. The sweep rate is 10 m s/div.

The conductivity pressure-jump method was applied to the study of the SDS–BSA interaction. A representative relaxation curve is shown in Fig. 3. The relaxation phenomena in the presence of the protein were observed even in low SDS concentrations below the critical micelle concentration (CMC), although they had been observed only above the CMC in the pure SDS system.[3] The SDS concentration dependence of the relaxation time, τ, is presented in Fig. 4. The value of τ^{-1} gradually increased at first, attained a plateau between 3 and 7 mM SDS, and increased again up to about 9 mM SDS. The final values of τ^{-1} are compatible with those obtained in a solution containing only SDS micelles, indicating that the phenomena are not completely independent of those of micellization.

Discussion

The BSA has been proposed to have a few fairly independent domains.[8-11] The results obtained by the limited proteolysis of the protein strongly indicate that the protein consists of, at least, two independent domains.[8-10] It has been suggested by Jones *et al.*, in the light of the domain-structure theory, that the binding of surfactants initially occurs mainly to the C-terminal section of the protein molecule, bringing about its unfolding, and that the more stable N-terminal part then begins to unfold exposing new binding sites with an increase of the surfactant concentration.[11] At the same time, it is generally accepted that the surfactant molecules cooperatively bind to the protein by hydrophobic interaction following the first binding of approximately 8—10 molecules by electrostatic interaction.[1,12] In the interaction of the BSA with surfactants, the stepwise and all-or-none nature of the complex formation has been emphasized,[13] that is, there exist only complexes which consist of, at most, a few particular binding ratios (surfactants/protein), depending on the surfactant concentration ranges.[14-18] The binding isotherm has so far been explained in terms of the following expression;[12,18-20]

$$P \rightleftharpoons PS_{8-10} \rightleftharpoons PS_n \rightleftharpoons PS_{2n} \rightleftharpoons PS_{>2n} \quad (n = 40\text{—}50),$$

where P and S denote the protein and a surfactant, respectively, and n is the binding number of the surfactants (mol/mol). As has been discussed before,[2] and also as has been mentioned above, the profile of the conduc-

tance change is also reflected by the stepwise formation of complexes and the concomitant conformational changes of the protein with clearly separate domains. The binding of SDS to BSA and the accompanying conformational changes of the protein have previously been examined under experimental conditions[2] different from those of this work. In taking account of the SDS concentration dependence of the ellipticity at 222 nm[21] and the SDS concentration where the first inflection of the conductance change appears, it seems that there exist the complexes with bound surfactants above 40—50 molecules in the SDS concentration range where the relaxation has been observed. Thus, the discussion given below will be limited to the behavior of the complexes which have been considered to be formed cooperatively.

Three important facts should be taken into consideration in discussing the relaxation mechanism of the present system. The first is the fact that the contribution of the protein to the conductance is negligibly small compared with that of the surfactant, as has been stated previously.[2] This is also to be expected from the fact that the conductivity curve actually starts from the origin in Fig. 2 in spite of the existence of the protein. Naturally, the behavior of the surfactant molecules seems to be primarily related to the relaxation phenomena observed here. This may indeed be reasonable, when a similarity of the values of τ in the present system to those in the pure SDS system is taken into account. The second is that only complexes with particular binding ratios can exist at a certain SDS concentration, as has been stated above. Since the complexes are formed in the low SDS concentration range where the concentration of unbound surfactants is lower than the CMC,[1] the ordinary micelle does not exist in the total concentration range of SDS below about 10 mM (=about 8 mM (CMC) of unbound SDS+about 2 mM of SDS bound to 1.0×10^{-5} M BSA[2]). Therefore, the participating species of the surfactant may be limited to both free monomers and those bound to the protein with particular binding ratios. The nature of the latter participating species may critically change with the shift of the SDS concentration range.[11-16] Thirdly, it has been reported that the surfactant molecules bound to proteins are not easily removed from the proteins, so their removal needs specially designed techniques.[22] The difficulty of the removal of such surfactants is reminiscent of the long period necessary to attain the dialysis equilibrium which has frequently been utilized in studies of the binding of surfactants to proteins.[1,23-25] Therefore, it is improbable to assume a reversible process of a large-scale formation and complete dissociation of the complex such as $nS+P\rightleftharpoons PS_n$. This situation is also reminiscent of a previous experimental finding that only the relaxation process with relaxation times in the milli-second range exists in an emulsion system consisting of SDS and pentadecane.[26] In such an emulsion system, it would be impossible to assume a reversible process of a large-scale formation and complete dissociation of the emulsified particle. These facts might appear to contradict the previously proposed assumptions of micellization kinetics that the relaxation process with relaxation times in the milli-second range is related to the formation and complete dissociation of surfactant micelles.[3,27,28] Because of the reasons mentioned above, nevertheless, the formation and complete dissociation of the complex cannot be assumed, at least, in the present complexes system. Therefore, in the surfactant–protein system, a probable reversible process is the partial breakdown and reformation of the complex as follows;

$$mS + P_iS_{n-m} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} P_iS_n, \tag{1}$$

where $m \ll n$, P_i indicates the protein with the i-th conformation in the stepwise conformational change, and k_{12} and k_{21} are the corresponding apparent rate constants.

The rate of Reaction 1 is expressed as follows;

$$\frac{d[P_iS_n]}{dt} = k_{12}[S]^m[P_iS_{n-m}] - k_{21}[P_iS_n], \tag{2}$$

where [ ] designates instantaneous concentrations during the perturbation of the equilibrium. The following relationship can be defined between the instantaneous concentrations and the equilibrium ones designated by the subscript, e;

$$x=[S]-[S]_e, y=[P_iS_{n-m}]-[P_iS_{n-m}]_e, \text{ and}$$
$$z = [P_iS_n] - [P_iS_n]_e. \tag{3}$$

By substituting Eq. 3 into Eq. 2, and by then expanding it in a power series, we obtain;

$$\frac{d[P_iS_n]}{dt} = k_{12}([S]_e^m[P_iS_{n-m}]_e + m[S]_e^{m-1}[P_iS_{n-m}]_e x + [S]_e^m y) - k_{21}([P_iS_n]_e + z). \tag{4}$$

Here, by introducing both the definition of the equilibrium condition and these relations; $x=-mz$ and $y=-z$ obtained from the stoichiometry of Reaction 1, we obtain;

$$\frac{dz}{dt} = -\{k_{12}(m^2[S]_e^{m-1}[P_iS_{n-m}]_e + [S]_e^m) + k_{21}\}z. \tag{5}$$

Then, we obtain;

$$\tau^{-1} = k_{12}(m^2[S]_e^{m-1}[P_iS_{n-m}]_e + [S]_e^m) + k_{21}. \tag{6}$$

Since

$$K_{12} = \frac{k_{12}}{k_{21}} = \frac{[P_iS_n]_e}{[S]_e^m[P_iS_{n-m}]_e},$$

we obtain;

$$k_{12}[S]_e^m = k_{21}\frac{[P_iS_n]_e}{[P_iS_{n-m}]_e}. \tag{7}$$

By assuming

$$[P_iS_{n-m}]_e = [P_iS_n]_e, \tag{8}$$

and by neglecting the magnitude of the first term compared with the second one in parentheses of Eq. 6, we obtain;

$$k_{21} = \frac{\tau^{-1}}{2}. \tag{9}$$

Therefore, we can substantially evaluate 50—80 s^{-1} as the magnitudes of k_{21} depending on the SDS concentration. This treatment apparently indicates that the rate constant of the dissociation becomes faster with

the complex growth, as has been anticipated from Fig. 4. However, the apparent dependence of k_{21} on the SDS concentration range should be considered to be mainly due to changes in the $[P_iS_n]_e/[P_iS_{n-m}]_e$ ratio. Strictly considered, it is considered that the ratio becomes greater than 1 in higher SDS concentrations, because the average amounts of the surfactants bound to the protein increase with the total SDS concentration, as judged from the binding isotherms.[1,2] Such a change in the ratio with the complex growth leads to smaller values of k_{21} than those straightforwardly obtained by Eq. 9. Therefore, the present author considers that the magnitudes of k_{21} hardly change at all with the complex growth in the SDS concentration range discussed here.

The magnitudes of k_{21} can be connected with those of the elementary steps of Reaction 1 as follows. It should be assumed that Reaction 1 consists of these succesive bimolecular steps;

$$\begin{aligned} P_iS_{n-m} + S &\underset{k_-}{\overset{k_+}{\rightleftharpoons}} P_iS_{n-m+1} \\ &\vdots \\ P_iS_{n-2} + S &\rightleftharpoons P_iS_{n-1} \\ P_iS_{n-1} + S &\rightleftharpoons P_iS_n, \end{aligned} \tag{10}$$

where k_+ and k_- are forward and backward rate constants, respectively. If each bimolecular step has the same activation energy, we obtain;

$$\prod_{j=1}^{m} K_j = \frac{k_+^m}{k_-^m} = \frac{k_{12}}{k_{21}} = K_{12} (1 \leq j \leq m), \tag{11}$$

where K_j is the equilibrium constant of each elementary step. Then, we obtain;

$$k_+ = k_{12}^{1/m} \text{ and } k_- = k_{21}^{1/m}. \tag{12}$$

The m value has been assumed to be very small. Therefore, we can substantially evaluate the rate constant, k_-, of the elementary dissociation process to be in orders of 1 to 10.[29]

Kinetic studies have so far been neglected with regard to the surfactant-protein interactions. Needless to say, further studies are required in order to discuss the relaxation mechanism of the system in detail.

The micelle-like properties of the surfactant-protein complexes have previously been demonstrated.[30] It is worth noting that a similarity of the complexes to the surfactant micelle also appears in the kinetic aspects of the interactions.

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References

1) J. Steinhardt and J. A. Reynolds, "Multiple Equilibria in Protein," Academic Press, New York (1969), pp. 239—302.

2) K. Takeda, M. Miura, and T. Takagi, *J. Colloid Interface Sci.*, **82**, 38 (1981).

3) N. Muller, "Solution Chemistry of Surfactants," ed by K. L. Mittal, Plenum Press, New York (1979), Vol. 1, pp. 267—295.

4) "Handbook of Biochemistry (Selected Data for Molecular Biology)," 2nd ed, ed by H. A. Sober and R. A. Harte, CRC Press, Cleveland, Ohio (1973), p. C-71.

5) K. Takeda, and T. Yasunaga, *J. Colloid Interface Sci.*, **40**, 127 (1972).

6) K. Takeda, T. Yasunaga, and H. Uehara, "Micellization, Solubilization, and Microemulsions," ed by K. L. Mittal, Plenum Press, New York (1977), Vol. 1, pp. 305—327.

7) K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants," Academic Press, New York (1969).

8) T. P. King, *Arch. Biochem. Biophys.*, **156**, 509 (1973).

9) M. C. Hilak, B. J. M. Harmsen, W. G. M. Braam, J. J. M. Joordens, and G. A. J. Van Os, *Int. J. Pept. Protein Res.*, **6**, 95 (1974).

10) R. C. Feldhoff and T. Peters, Jr., *Biochemistry*, **14**, 4508 (1975).

11) M. N. Jones, H. A. Skinner, and E. Tipping, *Biochem. J.*, **147**, 229 (1975).

12) J. A. Reynolds, S. Herbert, H. Polet, and J. Steinhardt, *Biochemistry*, **6**, 937 (1967).

13) M. J. Pallansch and D. R. Briggs, *J. Am. Chem. Soc.*, **76**, 1396 (1954).

14) K. Aoki and J. F. Foster, *J. Am. Chem. Soc.*, **79**, 3385 (1957).

15) K. Aoki, *J. Am. Chem. Soc.*, **80**, 4904 (1958).

16) R. V. Decker and J. F. Foster, *Biochemistry*, **5**, 1242 (1966).

17) R. V. Decker and J. F. Foster, *J. Biol. Chem.*, **242**, 1526 (1967).

18) J. Oakes, *J. Chem. Soc., Faraday Trans. 1*, **70**, 2200 (1974).

19) A. Ray, J. A. Reynolds, H. Polet, and J. Steinhardt, *Biochemistry*, **5**, 2606 (1966).

20) J. A. Reynolds, J. P. Gallacher, and J. Steinhardt, *Biochemistry*, **9**, 1232 (1970).

21) The residue ellipticity at 222 nm sharply changes at 40—50 mol/mol binding ratios, as can be seen by comparing Fig. 7 with Fig. 5 in Ref. 2. In this work, the relaxation begins to be observed at SDS concentrations where the ellipticity changes in a manner similar to the above.

22) D. A. Hager and R. R. Burgess, *Anal. Biochem.*, **109**, 76 (1980).

23) K. Birdi, *Anal. Biochem.*, **74**, 620 (1976).

24) J. C. Dohnal and J. E. Garvin, *Biochim. Biophys. Acta*, **575**, 393 (1979).

25) B. R. Vijayendran, *J. Colloid Interface Sci.*, **60**, 418 (1977).

26) K. Takeda, *J. Sci. Hiroshima Univ., Ser. A*, **40**, 69 (1976).

27) E. A. G. Aniansson and S. N. Wall, *J. Phys., Chem.* **78**, 1024 (1974).

28) E. A. G. Aniansson and S. N. Wall, *J. Phys. Chem.*, **79**, 857 (1975).

29) Through Eq. 12, we obtain $k_-=50$—$80\ s^{-1}$ (if $m=1$), 7.1—$8.9\ s^{-1}$ ($m=2$), 3.7—$4.3\ s^{-1}$ ($m=3$), and 2.2—$2.4\ s^{-1}$ ($m=5$). Equations 11 and 12 will not hold when the m value is large, because it may become impossible for each elementary step to have the same activation energy in such a case.[6]

30) J. Steinhardt, N. Stocker, D. Carrol, and K. Birdi, *Biochemistry*, **13**, 4461 (1974).