Kinetics of the Solubilization of Pinacyanol Chloride into a Complex between Bovine Serum Albumin and Sodium Dodecyl Sulfate

Kunio TAKEDA,* Tohru OHTAWA, Kazuaki HACHIYA, and Koichiro AOKI†
Department of Applied Chemistry, Faculty of Technology, Okayama University of Science, 1-1 Ridai-cho, Okayama 700
(Received July 19, 1986)

Synopsis. The stopped-flow method was applied to the solubilization of pinacyanol chloride into a complex formed by bovine serum albumin and sodium dodecyl sulfate. The rate constant of the solubilization became larger with the step-by-step formation of the complex and finally attained values compatible with those for the pure surfactant micelle.

Various studies have been made of the interaction of bovine serum albumin (BSA) with sodium dodecyl sulfate (SDS).[1-3] However, most attention has been focused on both the binding nature of the surfactant and the conformational change in the protein. Few attempts have been made to find the nature of the bound surfactant ions themselves. The solubilization phenomenon is believed to be convenient for investigating the bound state of the surfactants on the protein. Equilibrium studies have been done on the solubilization of several dyes into the protein-surfactant complex,[4,5] but kinetic studies have not yet been done on this phenomenon.

Pinacyanol chloride is well-known as a useful dye for determining spectrophotometrically the critical micelle concentration (cmc) of anionic surfactants such as SDS.[6,7] In the present study, the solubilization of the pinacyanol chloride into the BSA–SDS complex was studied by the stopped-flow method. This paper shows a kinetic similarity of the complex to the SDS micelle in the solubilization of the dye.

Experimental

The sources of crystalline BSA and SDS have been described elsewhere.[8] The cmc of SDS by the electrical conductance method was 8.3×10^{-3} M at 25 °C (1 M=1 mol dm^{-3}).[8] The BSA concentration was determined spectrophotometrically using $E^{1\%}_{1cm}=6.8$ at 280 nm.[9] The protein concentration was kept at 1.0×10^{-5} M. The pinacyanol chloride was purchased from the Eastman Kodak Co. The absorption coefficient of the dye was assumed to be 88,000 at 600 nm at concentrations around 1.0×10^{-5} M.[7] The dye concentration was kept at 1.0×10^{-5} M. We previously experienced that the presence of an electrolyte accelerated the fading of the dye somewhat.[10-12] Thus, 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.[13]

The absorbance measurements were carried out with a Hitachi double-beam spectrophotometer, Model 220. Measurements of stopped-flow and rapid-scanning were made with a stopped-flow apparatus, RA-401, of the Union Giken Co. equipped with a kinetic data processor, RA-415. All the measurements were made at 25 °C.

†Professor Emeritus of Gifu University, Gifu 501-11.

Results and Discussion

The color change of pinacyanol chloride from red to blue occurs in a concentration range not very far from the cmc of the anionic surfactant.[6,7] The blue color above the cmc is characterized by two absorption bands, α- and β-bands at 607 and 562 nm, and the red color below the cmc, by the γ-band around 480 nm. The γ-band is due to the aggregate formed by the dye and the dodecylsulfate ion, while the α- and β-bands are attributable to the solubilization of the dye into the micelle.[6] (In the absence of the surfactant, the α- and β-bands appear at 600 and 550 nm respectively, probably because of the monomer-dimer equilibrium of the dye,[6] while the γ-band does not appear). Figure 1 shows the SDS concentration dependences of the absorbance at these three bands in the absence (dotted curve) and in the presence of BSA. In a SDS solution without the protein, the absorbance at the γ-band disappeared at SDS concentrations higher than 7 mM, which is slightly below the cmc. Simultaneously, the absorbances at α- and β-bands began to increase at this concentration. However, the absorbances at the α- and β-bands began to increase at much lower SDS concentrations in the presence of BSA than in the absence of BSA. This is probably due to the solubilization of the dye into the complex formed by BSA and SDS.[4,5] In the presence of BSA, the

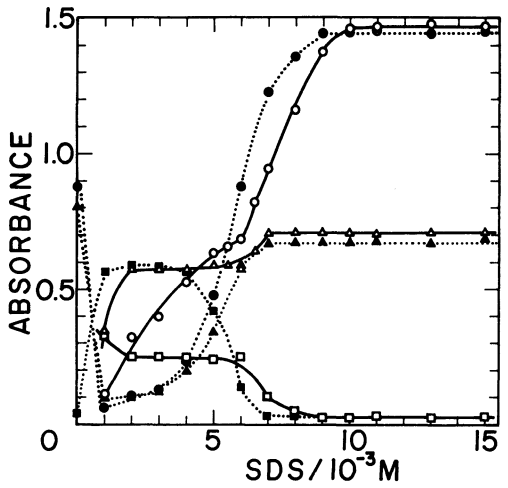

Fig. 1. The SDS concentration dependences of absorbance of pinacyanol chloride at α-(○, ●), β-(△, ▲), and γ-bands (□, ■) in the absence (dotted curve) and the presence (solid curve) of 1.0×10^{-5} M BSA.

absorption at α-band increased step-by-step until 10 mM SDS, making a clear break around 6 mM SDS, as is shown in Fig. 1. The helical structure of BSA is unfolded step-by-step by SDS both in water[13] and in a dilute phosphate buffer.[8] The major structural change is completed at 6 mM SDS in water,[13] where the break of absorbance appears at the α-band in Fig. 1.

Rapid-scanning experiments were made in the wavelength region between 450 and 620 nm, using the stopped-flow mixer to mix the dye solution with the BSA–SDS complex solution. When the pinacyanol was solubilized into the BSA–SDS complex, the absorption increased with time around the α-band in the rapid-scanning measurements, as seen in the inserted figure in Fig. 2. Since the dye has been considered to be solubilized in a state of the dye-surfactant aggregate, the mechanism of the solubilization may be expressed as follows:[12]

$$\mathrm{DS} + \mathrm{M} \longrightarrow \mathrm{MDS}, \qquad (1)$$

where DS is an aggregate formed by the dye and SDS and M is one of the solubilizing sites of the micelle or of the surfactant-protein complex. The formation of the DS aggregate has been considered to be very fast compared with the time range of this work.[12] The stopped-flow measurements were made at the α-band in order to follow the solubilization of the DS aggregate. The solubilization of the dye into the SDS micelle has been observed as a pseudo-unimolecular reaction.[12] The first-order plot gave a good linear relationship also in the present kinetic study of the solubilization of the dye into the surfactant-protein complex.[14] These findings suggest that fairly many DS aggregates are solubilized into one micelle or into one surfactant-protein complex. It is generally accepted in the application of the stopped-flow method that only forward reactions can be observed in most of the time range in which the absorbance changes appreciably with the progress of the reaction.[15] Therefore, Reaction (1) can be rewritten as the following pseudo-unimolecular reaction;

$$\mathrm{DS} \xrightarrow{k} \mathrm{MDS} \quad \text{(in excess of M)} \qquad (2)$$

where k is the forward rate constant.

The SDS concentration dependence of the rate constant, k_{complex}, of the solubilization of pinacyanol into the complex is shown in Fig. 2, together with the SDS concentration dependence of the rate constant, k_{micelle}, of the solubilization of the dye into the SDS micelle. The rate constant, k_{complex}, increased step-by-step with the increase in the SDS concentration, while k_{micelle} did so gradually and monotonously. On the other hand, since the BSA–SDS complexes are formed in a concentration range of SDS where the concentration of unbound surfactants is lower than the cmc, ordinary micelles do not exist in the total SDS concentrations below about 10.3 mM (=8.3 mM (cmc) of unbound SDS + about 2 mM SDS bound to 1.0×10^{-5} M BSA).[8,13] Therefore, the step-by-step change in k_{complex} below 10 mM SDS must reflect the nature of the BSA–SDS complex. In other words, k_{complex} depends on the binding number of SDS to the protein. After the sudden increase in the k_{complex}, it gave a plateau in spite of the increase in the binding number, as may be seen in Fig. 2. It appears that k_{complex} begins to increase again when a binding of about 120 SDS is attained; this can be said in view of the binding isotherm of the BSA–SDS system in a dilute phosphate buffer of an ionic strength of 0.014[8] and the dependence of the residue ellipticity, $[\theta]_{222}$, on the SDS concentration in the phosphate buffer[8] and in water.[13] Finally, it increased sharply and approached the values of k_{micelle}. However, it is not certain whether the increase in k_{complex} in the narrow concentration range of 8—11 mM SDS means the solubilization of the dye into the complex with a larger number of SDS or into the SDS micelle itself. The present results indicate that the more the surfactants bind to the protein, the faster the solubilization occurs.

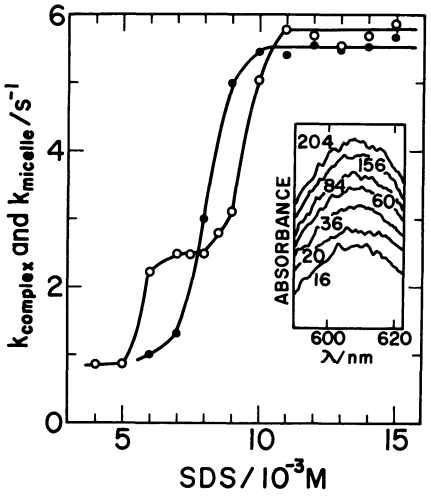

Fig. 2. The SDS concentration dependences of k_{complex} (○) and k_{micelle}(●). The rapid scanning spectra around α-band are inserted in this figure. The numerical values designate time in millisecond unit (including dead time) after mixing dye and complex solutions.

Recently, a single relaxation has been found in the same BSA–SDS system by means of the pressure-jump method with conductivity detection.[13] The relaxation time of the BSA–SDS system approaches those in the SDS micelle system, just as the k_{complex} value becomes compatible with the k_{micelle} value with an increase in the SDS concentrations. This relaxation has been considered to be due to the partial breakdown and reformation of the complex.[13] The surfactant monomers participate in the partial breakdown and reformation of the complex, while pinacyanol participates in the present solubilization process.

It is worth noting that a similarity of the protein-surfactant complex to the surfactant micelle also appears in these kinetic phenomena. Both the kinetic phenomena reflect the fact that the bound states of dodecyl sulfate ions on the BSA molecule are very close to those of the SDS micelle.

References

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

2) M. N. Jones, "Biological Interfaces," Elsevier Scientific Publishing Co., Amsterdam (1975), pp. 101—130.

3) S. Lapanje, "Physicochemical Aspects of Protein Denaturation," John Wiley & Sons, New York (1978), pp. 156—179.

4) J. Steinhardt, N. Stocker, D. Carroll, and K. S. Birdi, *Biochemistry*, **13**, 4461 (1974).

5) J. Steinhardt, J. R. Scott, and K. S. Birdi, *Biochemistry*, **16**, 718 (1977).

6) H. B. Klevens, *Chem. Rev.*, **47**, 1 (1950).

7) P. Mukerjee and K. J. Mysels, *J. Am. Chem. Soc.*, **77**, 2937 (1955).

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

9) "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.

10) T. Yasunaga, K. Takeda, and S. Harada, *J. Colloid Interface Sci.*, **42**, 457 (1973).

11) K. Takeda and T. Yasunaga, *J. Colloid Interface Sci.*, **45**, 406 (1973).

12) K. Takeda, N. Tatsumoto, and T. Yasunaga, *J. Colloid Interface Sci.*, **47**, 128 (1974).

13) K. Takeda, *Bull. Chem. Soc. Jpn.*, **55**, 2547 (1982).

14) For the first-order plot and the determination of the rate constant, see Refs. 10 and 12.

15) B. Chance, "Investigation of Rates and Mechanisms of Reactions (Part II)," ed by G. G. Hammes, John Wiley & Sons, New York (1974), pp. 5—62.