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A Kinetic Study on the Conformational Change of Bovine Serum Albumin Induced by Sodium Dodecyl Sulfate

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Stopped-flow measurements were made on the conformational change of bovine serum albumin induced by sodium dodecyl sulfate in a buffer solution containing 3.33 mmol/dm³ NaH_2PO_4 and 3.56 mmol/dm³ Na_2HPO_4 (pH 7.0, μ=0.014). The protein adopted a conformation with a smaller helical content than the original native state (helix to coil transition) upon the addition of the surfactant. The rate of transition depended strongly on the surfactant concentration. The results were compared with those of conformational changes of other proteins, in which a helical structure increased in the surfactant solution (coil to helix transition). It is noticeable that the value of the activation entropy is negative in the helix to coil transition, as compared with its positive and large values in the coil to helix transition. It seems that the binding of the surfactant to the protein causes the transition to only a particular conformation of the protein *via* each individual process.

The interactions of surfactants with proteins are interesting *per se*, and so have been extensively investigated.[1,2] However, kinetic studies of the interactions have been neglected. Recently, the kinetic aspects of these interactions have been studied by Wyn-Jones and his coworkers.[3-5] They paid attention to the behavior of the surfactant molecules associated with their binding to macromolecules. Conformational changes of protein polypeptides induced by the binding of surfactants are also interesting. With respect to the conformational changes of proteins, the addition of ionic surfactants such as sodium dodecyl sulfate (SDS) causes either an increase or a decrease of helix content, depending on the nature of the proteins.[6-13] More recently, Takeda and his coworkers have studied the kinetic aspects of the conformational changes of a synthetic homopolypeptide[14,15] and the increase of the helix content of some proteins[16,17] in surfactant solutions. They reported characteristic processes in which the native conformation directly turns to a particular conformation without passing through other conformations.

In this work, a kinetic study was carried out on the conformational change of bovine serum albumin (BSA), which had been reported to adopt a conformation with a smaller helix content than its native state upon the addition of ionic surfactants such as SDS.[9-13]

Experimental

Crystalline BSA and SDS have been described elsewhere.[12] The concentration of the protein was determined spectrophotometrically using $E^{1\%}_{cm}$=6.8 at 280 nm.[18] A sodium phosphate buffer of pH 7.0 was exclusively used (μ=0.014). The final concentrations of NaH_2PO_4 and Na_2HPO_4 in the buffer were 3.33×10^{-3} and 3.56×10^{-3} M (1 M=1 mol/dm³). The critical micelle concentration of the SDS was determined by the electric conductance method to be 8.3×10^{-3} M in pure water and 5.6×10^{-3} M in the buffer (25 °C).

Static measurements of ultraviolet absorbance were made with a Hitachi double-beam spectrophotometer, Model 220A. The absorbance stopped-flow measurements were carried out with a stopped-flow apparatus, RA-401 of Union Giken Co. equipped with a kinetic data processor, RA-450. The circular dichroism measurements were made with a JASCO J-500A spectropolarimeter equipped with a JASCO DP-501 data processor.[12]

Throughout the measurements, the protein concentration was kept at 1.0×10^{-5} M. The aqueous solution of the protein was used within 6 h of preparation.

Results

Prior to the stopped-flow measurements, the conformational change of the BSA was examined by circular dichroism measurements. Figure 1 shows the circular dichroic spectra of the protein in the absence and the presence of SDS. All of the spectra showed a double negative maximum, which is characteristic of an α-helical structure, although the magnitude of the ellipticity decreased with an increase of SDS. The protein did not lose all of its helical structure[19] and had a constant magnitude of ellipticity above 7.0 mM SDS. However, the predominant loss of the helix content occurs up to 3.5 mM, as seen in Fig. 1. The ultraviolet absorbance change accompanying the conformational change of the protein is shown in Fig. 2. The blue-shift was observed and the difference spectrum had a positive peak around 265 nm and a negative one at 288 nm.

The stopped-flow measurements were carried out at these two wavelengths. Representative time depend-

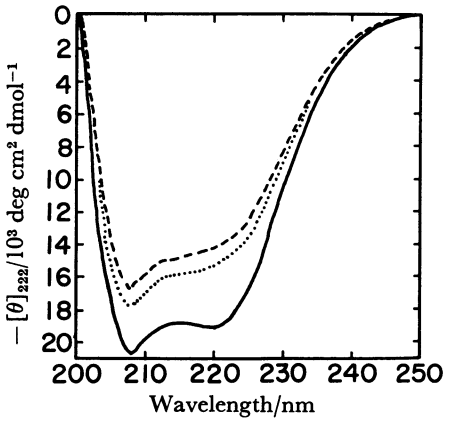


Fig. 1. Typical circular dichroic spectra of BSA in the absence (solid curve) and the presence of 3.5 mM (dotted curve) and 7.0 mM (dashed curve) SDS at 25 °C. These spectra were obtained over 8 repetitions.

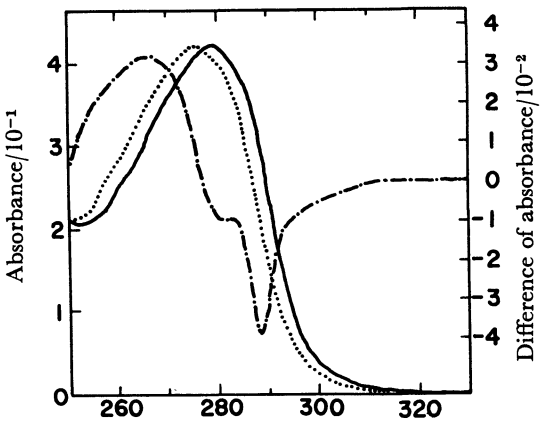


Fig. 2. Typical absorbance spectra of BSA in the absence (solid curve) and the presence of 7.0 mM SDS (dotted curve) and the difference spectrum of them (dot-dashed curve) at 25 °C.

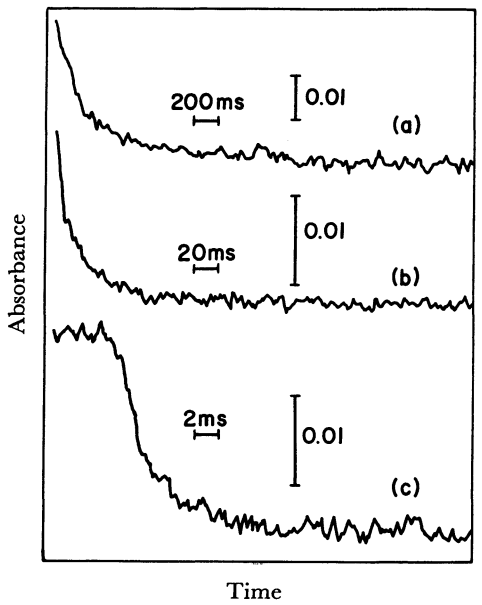


Fig. 3. Representative time courses of absorbance changes at 288 nm. Final concentrations of BSA and SDS were 1.0×10^{-5} M and 3.5 (a), 5.0 (b), and 10.0 mM (c), respectively. The trace (c) is the average of 4 repetitions.

ances of the absorbance changes are shown in Fig. 3. As expected from the absorbance change in Fig. 2, the absorbance decreased with time at 288 nm (as seen in Fig. 3) and increased at 265 nm (not shown here). As clearly recognized in Fig. 3, the rate of the absorbance change became faster with an increase of SDS. Unfortunately, the rate of the conformational change of BSA was too fast to be measured by a circular dichroism stopped-flow method.[14-17] However, it was apparent in previous work[16,17] that the absorbance stopped-flow measurements give identical rates with those obtained by the circular dichroism stopped-flow ones, and the behavior of chromophores such as tryptophan and tyrosine is useful in following the conformational

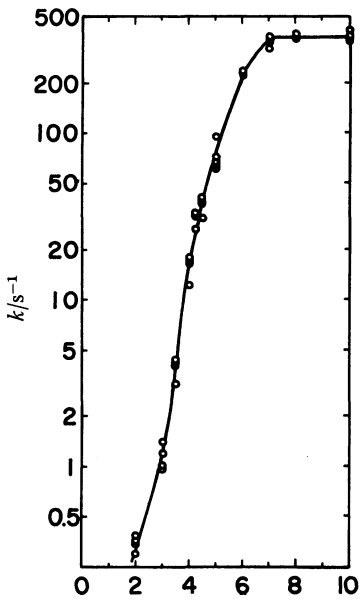


Fig. 4. SDS concentration dependance of rate constant, k, at 25 °C.

TABLE 1. KINETIC PARAMETERS FOR CONFORMATIONAL CHANGES OF PROTEINS IN SDS SOLUTION

	Temperature/°C	Helix to Coil (BSA)	Coil to Helix (δ-Chymotrypsin)
	20	240	0.085
k/s^{-1}	25	570	0.29
	30	470	0.70
ΔH^*/kJ mol^{-1}		48	140
ΔS^*/J K^{-1} mol^{-1}		−34	160

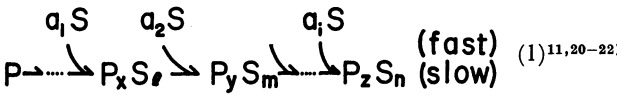
changes of proteins. This is also emphasized when we take account of a similarity of the SDS concentration dependance of the absorption coefficient of the protein at 288 nm to that of the residue ellipticity at 222 nm.[12] Therefore, the absorbance change with time is considered to correspond to the rate of the conformational change of the protein. The first-order rate constant, k, was obtained by the usual treatment.[14] The SDS concentration dependance of k is represented in Fig. 4. The rate constant, k, sharply increased with an increase of the SDS concentration and attained a constant value around 7 mM SDS. The temperature dependance of k was examined at 10 mM SDS in order to evaluate activation parameters of the conformational change. The activation parameters, ΔH^* and ΔS^*, were calculated in the usual manner.[17] Table 1 shows the activation parameters together with those for the conformational change of delta-chymotrypsin.[17]

Discussion

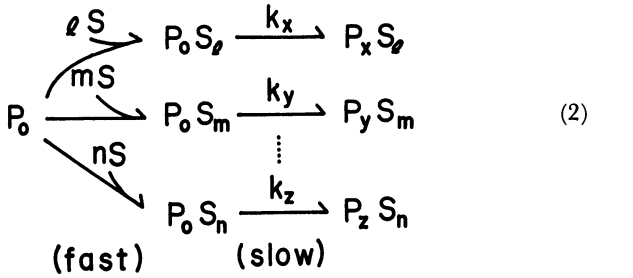
Kinetic studies were made on the conformational change of BSA induced by SDS. The protein loses some parts of the helical conformation (termed the helix to coil transition). This conformational change accompanies the change of the ultraviolet absorbance, as seen

in Fig. 2. The rate constant, k, of the conformational change was obtained by the absorbance changes with time. The value of k strongly depends on the SDS concentration, indicating that the greater the degree of the conformational change, the faster the rate of the conformational change. This tendency also appears in a conformational change of delta-chymotrypsin, in which the helix content increases upon the addition of SDS (termed the coil to helix transition).[17]

The next two processes may be expected for conformational changes of proteins in a solution of surfactant such as SDS,



(1)[11,20-22]



(2)

where P_0: a native protein, S: a surfactant molecule, a_i: a particular number, P_xS_l, P_yS_m,$\cdots$, P_zS_n: complexes (the helix content decreases in the order of x, y, $\cdots$, z in this case), l, m, n: binding numbers of surfactants to a protein, k_x, k_y, k_z: rate constants. In this case, the protein designated by P_0 has the most abundant helical conformation and the complex designated by P_zS_n has the least helix content. If the conformational change proceeds *via* reaction (1) which has been often assumed for the formation of these complexes,[11,20-22] the longest time is required for the transition from P_0 to P_zS_n. However, the transition to P_zS_n with the least helical conformation is faster than those to the intermediate species, as seen in Fig. 4. As discussed before, a protein cannot adopt various different conformations as reaction intermediates in the presence of a ceratin quantity of SDS.[17] This is because the surfactants rapidly bind to a protein,[3-5,23] causing the transition only to a particular conformation of the protein. Reaction (2) indicates that the binding of the surfactant to a protein is completed before the conformational change of the protein,[24] and that the conformation of the protein changes together with the many surfactant molecules bound to it. The bulky hydrophobic groups of the surfactant are obliged to come into maximal contact with the hydrophobic parts of the other surfactants and the protein polypeptide. The saturated binding of SDS would reduce the freedom of choice in adopting a final conformation. On the contrary, the protein with the unsaturated binding of SDS may take much time to choose a final conformation, because this situation necessarily needs more redistribution of the bound surfactants to unoccupied binding sites than in the saturated binding stage. As a result, the rate of the conformational change seems to become faster with an increase of the bound surfactant. It is considered that the conformation of a protein does not change *via* the process expressed by the reaction (1). The reaction (2) appears to be suitable for the understanding of the kinetic aspects of the conformational change of a protein induced by a surfactant such as SDS.

SDS binding to the BSA proceeds in two steps.[12] Under the present condition, the initial binding of about 115 mol of SDS per mole of BSA completes around 4 mM of total SDS and the second binding of a further 90 mol of SDS goes to completion around 7 mM of total SDS (see Fig. 5 in Ref. 12). It is noticeable that the rate constant, k, becomes constant above 7 mM SDS where the binding of SDS saturates (Fig. 4). The stepwise natures of the SDS binding and the conformational change of the protein must be correlated with the fact that the protein has at least two independent domains.[25-29] Jones *et al.* have proposed that the binding initially occurs mainly to the C-terminal section of BSA and then the more stable N-terminal part begins to be bound.[29] In the light of their proposal, it seems that the conformational change occurs only in the C-terminal part of the protein in the initial binding stage (below about 4 mM SDS under the present condition). The rate of the conformational change in the saturated binding stage is faster by two or three orders of magnitude than those in the initial binding stage, as seen in Fig. 4. However, the difference between the rates of conformational changes would not relate to the original stabilities of the C- and N-terminal sections of the protein because of the following reason. Since all of the bindings to both the C- and N-terminal parts occur at the same time in the time scale of the conformational change as mentioned above, the conformations of all of the parts are forced to change together and simultaneously in the sense of reaction (2).

One of the purposes of this work is to make clear distinctions between the helix to coil and the coil to helix transitions. The value of k for the helix to coil transition increases more sharply with an increase of the SDS concentration (Fig. 4), compared with those of k for the other transition.[16,17] In addition, the rate of the helix to coil transition is clearly faster than those of the other one as seen in Table 1, although few kinetic studies have been made on the conformational changes of proteins. The difference between both the transitions is also distinct in their kinetic parameters, as seen in Table 1. The value of ΔH^* for the helix to coil transition is about one-third of that of the other. In particular, it should be noted that the value of ΔS^* is negative in the helix to coil transition, while it is positive and large in the other one. As discussed previously,[17] in the case of the conformational changes induced by the surfactant, the entropic change must reflect not only the folding and unfolding of the protein polypeptide, but also both the rearrangement of the surfactants bound to the polypeptide and the solvent-ordering around the hydrophobic groups of the surfactant exposed to an aqueous environment. Therefore, it seems that the former contribution to the entropic change is

defeated by the latter two of the surfactants, which must be negative, in the activation state of the conformational change of the BSA. It has been considered that a large-scale conformational change of protein-surfactant complex occurs in the coil to helix transition, that is, a part of the polypeptide chain is unfolded, followed by a continuous refolding in the activation state.[17] Compared with this, the helix to coil transition may proceed in a rather simple way.

The kinetic studies of conformational changes of polypeptides made to date[14-17] have strongly suggested that, when a certain number of surfactants bind to a protein, the native conformation of the protein directly transforms into another particular conformation without passing through any intermediate conformation such as may be induced below the binding number mentioned above. This is quite a different feature from the sense of reaction (1) which may be favorable for an interpretation of the gradual growth of complexes.

References

1) C. Tanford, *Adv. Protein Chem.*, **23**, 121 (1968).

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

3) D. M. Bloor, W. Knoche, and E. Wyn-Jones, "Techniques and Applications of Fast Reactions in Solution," ed by W. J. Gettins and E. Wyn-Jones, D. Reidel Publishing Co., Holland (1979), pp. 265—270.

4) J. Gettins, C. Gould, D. G. Hall, P. L. Jobling, J. E. Rassing, and E. Wyn-Jones, *J. Chem. Soc., Faraday Trans. 2*, **76**, 1535 (1980).

5) D. M. Bloor and E. Wyn-Jones, *J. Chem. Soc., Faraday Trans. 2*, **78**, 657 (1982).

6) B. Jirgensons, *J. Biol. Chem.*, **242**, 912 (1967).

7) A. H. Hunt and B. Jirgensons, *Biochemistry*, **12**, 4435 (1973).

8) B. Jirgensons, *Macromol. Chem., Rapid Commun.*, **2**, 213 (1981).

9) W. J. Leonard, Jr., and J. F. Foster, *J. Biol. Chem.*, **236**, PC 73 (1961).

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

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

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

13) K. Aoki, H. Okabayashi, S. Maezawa, T. Mizuno, M. Murata, and K. Hiramatsu, *Biochim. Biophys. Acta*, **703**, 11 (1982).

14) K. Takeda, A. Iba, and K. Shirahama, *Bull. Chem. Soc. Jpn.*, **54**, 1793 (1981).

15) K. Takeda, A. Iba, and K. Shirahama, *Bull. Chem. Soc. Jpn.*, **55**, 985 (1982).

16) K. Takeda and S. Takagi, *Agric. Biol. Chem.*, **45**, 777 (1981).

17) K. Takeda, *Bull. Chem. Soc. Jpn.*, **55**, 1335 (1982).

18) H. A. Sober and R. A. Harte (eds), "Handbook of Biochemistry (Selected Data for Molecular Biology)," 2nd ed, CRC Press, Cleveland, Ohio (1970), p. C-71.

19) These findings are in qualitative agreement with earlier results.[13-15,17]

20) J. Oakes, *J. Chem. Soc., Faraday Trans. I*, **70**, 2200 (1974).

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

22) J. A. Reynolds, J. P. Gallagher, and J. Steinhardt, *Biochemistry*, **9**, 1232 (1970).

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

24) The binding processes of surfactants to proteins are considered to consist of successive bimolecular steps on a very short time scale. Refer to the following: N. Muller, "Solution Chemistry of Surfactants," ed by K. L. Mittal, Plenum Press, New York (1979), Vol. 1, pp. 267—295.

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

26) 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).

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

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

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