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Conformational Change of Delta-chymotrypsin Caused by Sodium Dodecyl Sulfate as Studied by Stopped-flow Circular Dichroic Method

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The conformational change of delta-chymotrypsin was studied in a sodium dodecyl sulfate (SDS) solution containing 3.33 mmol/dm^3 NaH_2PO_4 and 3.56 mmol/dm^3 Na_2HPO_4 (pH 7.0, μ=0.014) by means of the circular dichroism (CD) and absorbance stopped-flow methods. The protein adopts a conformation with a higher helix content than the native state upon the addition of SDS. The rate of the conformational change strongly depends on the SDS concentration. The corresponding rate constant sharply increases in the range of 5—12 mmol/dm^3 SDS, indicating that the higher the helix content, the faster the coil to α-helix transition. The nature of the conformational change was examined by parallel measurements of the CD, the difference spectra, and the binding isotherm. As a result, it was concluded that, at a certain SDS concentration above 5 mmol/dm^3, the native conformation of the protein is directly transformed to another conformation without passing through any intermediate one which may be caused when the SDS concentration is below the one mentioned above.

Sodium dodecyl sulfate (SDS) is now widely used in the purification and characterization of proteins.[1] The interactions of surfactants with proteins *per se* are interesting enough, and so have been extensively investigated.[2,3] However, the kinetic aspects of the interactions have been little studied, although many kinetic studies have been done on the conformational changes of proteins induced by other agents.[4] As is well known, several proteins clearly adopt conformations with high helix contents than their native states upon the addition of anionic surfactants such as SDS.[5,6] This is in marked contrast with the denaturation processes induced by the other denaturants. In this work, delta-chymotrypsin has been used as an example of a protein which can be converted in part to the α-helical conformation. As is also well known, the conformation of protein in an aqueous solution can be more clearly detected by the circular dichroism (CD) measurements. Although a few CD stopped-flow studies of conformational changes of proteins have recently been carried out, most of them have been done in the visible and near-ultraviolet regions.[7–11] Since the ellipticity in the far-ultraviolet region reflects the change of the protein backbone structure, such kinetic studies should be done in this region as well. The present author wishes to report on the SDS-induced conformational change of the protein followed by means of the far-ultraviolet CD and the near-ultraviolet absorbance stopped-flow methods. The main purpose of this paper is to discuss the characteristic process of the conformational change of the protein in the presence of the surfactant.

Experimental

Crystalline delta-chymotrypsin from beef pancreas (MW: 25400) was obtained from Miles Laboratories. The SDS and the phosphate buffer used have been described in a previous paper.[12]

The CD measurements were carried out with a JASCO J-500A spectropolarimeter equipped with a JASCO DP-501 data processor. The details of CD and CD stopped-flow measurements have been stated elsewhere.[13] The absorbance stopped-flow measurements were made with a rapid mixer of a stopped-flow apparatus, RA-401 of Union Giken Co., combined with a single-beam spectrophotometer, Unispec A of the same company, the time constant of which was modified to be less than 1.0×10^{-3} s. The static measurements of the ultraviolet absorbance were made with a Hitachi double-beam spectrophotometer, Model 200-10.

The binding isotherm of SDS to the protein was obtained by the use of gel chromatography, as has been reported before.[12]

Throughout the experiments, the protein concentration was constant, 1.0×10^{-5} M (as a concentration unit, 1 M= 1 mol/dm^3 was used), and its solution was used within 6 hours after preparation. The spectra of the CD and the absorbance of the protein were measured within 5 min after mixing the solutions of the protein with those of SDS.

Results and Discussion

The SDS-induced conformational change of delta-chymotrypsin was studied primarily by observing the changes in the CD spectra in the far-ultraviolet region in order to make clear the changes of the backbone structure of the protein polypeptide. Figure 1 shows the typical CD spectra of the protein in the absence (I) and in the presence (II) of 20 mM SDS and the difference CD spectrum(III) between them. The difference CD spectrum clearly indicates an increase in the α-helix content upon the addition of SDS, as in the case of alpha-chymotrypsin.[6] The SDS concentration dependence of the residue ellipticity at 206 nm, $[\theta]_{206}$, is shown in Fig. 2. The value of $[\theta]_{206}$ sharply increases at first and then attains a plateau at about 12 mM SDS, indicating that the helix content of the protein increases in such a concentration range of SDS.

Figure 3 shows the effect of added SDS on the absorbance spectra of delta-chymotrypsin. It is well known that the denaturation blue shift observed here is attributable to the exposure of chromophores, such as tryptophan and tyrosine, to an aqueous environment (the protein contains 9 tryptophan residues and 3 tyrosine ones). The difference spectrum of absorbance shows a blue shift and has a positive peak around 255 nm and a major negative peak at 295 nm. The SDS-induced difference absorption coefficient at 295 nm, $\Delta\varepsilon_{295}$, is plotted against SDS in Fig. 2. The

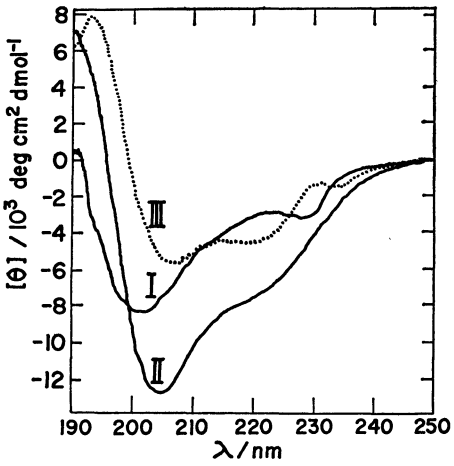


Fig. 1. Typical CD spectra of delta-chymotrypsin in the absence (curve I) and the presence (curve II) of 20 mM SDS and the difference CD spectrum between them (curve III) at 25 °C.
The difference spectrum was directly obtained by the use of the data processor. The concentration of the protein was 1.0×10^{-5} M. The thickness of cell used was 1.0 mm. These spectra are averaged over 8 repetitions.

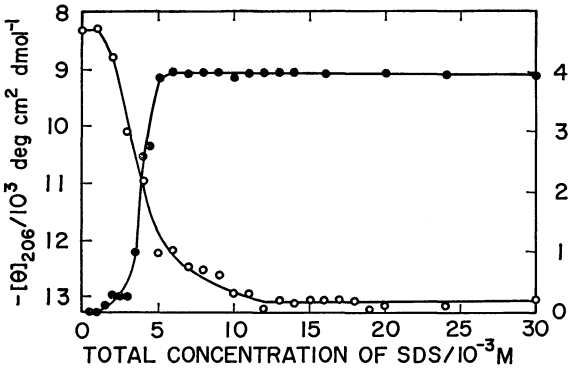


Fig. 2. The effects of total SDS concentration on the residue ellipticity at 206 nm, $[\theta]_{206}$ (○) and the difference absorption coefficient at 295 nm, $\Delta\varepsilon_{295}$ (●) at 25 °C.
The both values were obtained on the basis of the results at 1.0×10^{-5} M delta-chymotrypsin.

value of $\Delta\varepsilon_{295}$ increases just like that of $[\theta]_{206}$, but flattens out at a lower SDS concentration, 5 mM. Their importance will be referred to later in discussion of a reaction process and a transition state of the conformational change.

It is characteristic of the conformational change of a protein in a surfactant solution that large amounts of surfactant molecules bind to the protein. The binding isotherm of SDS to delta-chymotrypsin, as obtained by the gel chromatography, is shown in Fig. 4. The amounts of SDS bound to the protein (the protein concentration$=1.0\times10^{-5}$ M) are also plotted against the total SDS concentration (solid curve) in order to compare them with the total SDS concentration dependences of $[\theta]_{206}$ and $\Delta\varepsilon_{295}$. The amounts of SDS bound to the protein increase discontinuously at 1—2 and 4—5 mM of total SDS, and finally reach

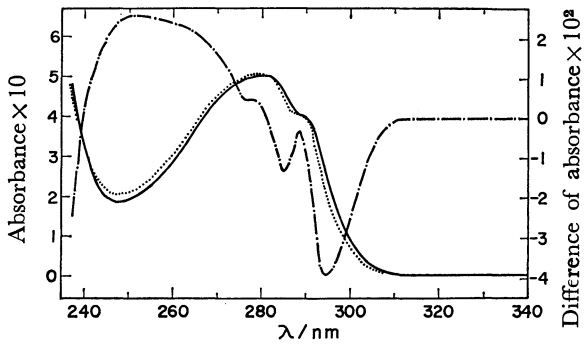


Fig. 3. Typical absorbance spectra of delta-chymotrypsin in the absence (solid curve) and the presence (dotted-curve) of 20 mM SDS and the difference spectrum of them (dot-dashed curve) at 25 °C.
The concentration of the protein was 1.0×10^{-5} M.

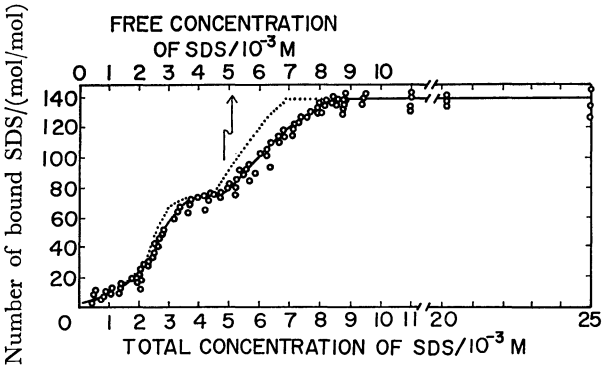


Fig. 4. Binding curve of SDS to delta-chymotrypsin *vs.* total concentration of SDS at 25 °C.
The dotted line indicates the binding isotherm plotted against free concentration of SDS. The total concentration of SDS was determined by the addition of the molar concentration of bound SDS per 1.0×10^{-5} M protein to its free concentration on the basis of the plot of number of bound SDS (mol/mol) *vs.* its free concentration.

a maximum, 140 mol of SDS per mole of the protein, in the vicinity of 8.5 mM SDS. This trend is the same as in the case of the system of SDS and bovine serum albumin.[12] We can approximately divide the binding curve into two stages, that is, the first binding stage of 70 mol of SDS per mole of the protein below 5 mM SDS and the second binding stage of a further 70 mol of SDS. In comparing the stepwise binding curve in Fig. 4 with the SDS concentration dependences of $[\theta]_{206}$ and $\Delta\varepsilon_{295}$, the exposure of chromophores to an aqueous environment completes in the first binding stage and the helix content also predominantly increases in this stage. The second binding of 70 mol of SDS can occur without a large-scale conformational change of the polypeptide chain.[14]

The CD stopped-flow measurements were carried out at 192 and 206 nm, where the positive and the negative maxima appeared in the difference spectrum of CD. The representative time courses of the conformational change are presented in Fig. 5. As is to be expected from the CD spectra in Fig. 1, the

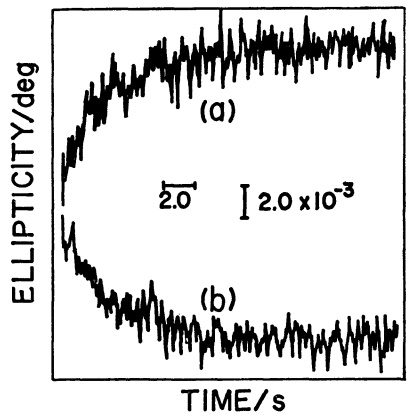


Fig. 5. Representative time courses of ellipticity changes at 192 (a) and 206 nm (b) at 25 °C.
The traces (a) and (b) are the averages of 32 and 2 repetitions, respectively. The final concentrations of SDS and delta-chymotrypsin were 20 mM and 1.0×10^{-5} M, respectively.

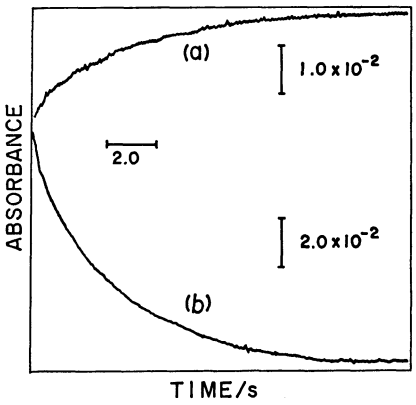


Fig. 6. Representative time courses of absorbance changes at 255 (a) and 295 nm (b) at 25 °C.
The experimental conditions were identical with those in Fig. 5.

ellipticity at 192 nm changed from the negative ellipticity to the positive one, while the negative ellipticity at 206 nm increased with time. The observed process, therefore, seems to correspond to an increase in the helix content. The absorbance stopped-flow measurements were made at 255 and 295 nm. The representative time courses are shown in Fig. 6. The directions of absorbance changes with time are in accordance with those expected from the difference spectrum of absorbance, as in the case of the CD stopped-flow measurements.

By the analysis of the curves shown in Figs. 5 and 6, the first-order rate constant, k, of the SDS-induced conformational change of the protein was obtained. The analysis was made by the ordinary treatment.[13] The magnitude of k is of the same order as those of the thermal denaturations of alpha-chymotrypsin[15] and lysozyme[16] and the denaturation of lysozyme by guanidine,[17] although the conformational changes caused by these agents are essentially different from that caused by SDS by which the helix content increases. The SDS concentration dependence of k is

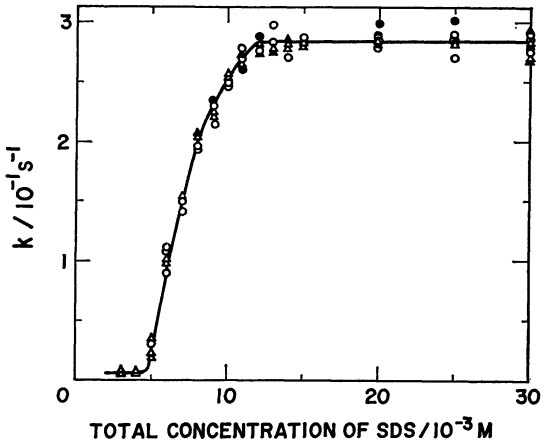


Fig. 7. Plot of rate constant, k *vs.* total concentration of SDS at 25 °C.
The values of k were obtained from time courses of ellipticity changes at 195 (●) and 206 nm (○), and those of absorbance changes at 255 (▲) and 295 nm (△).

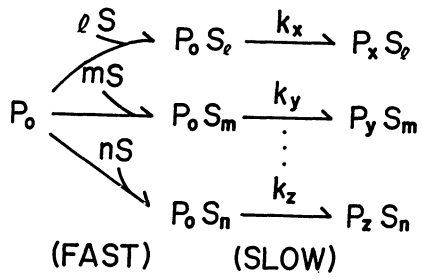
presented in Fig. 7. It is noticeable that the same values of k are obtained, irrespective of the method of detection, and that the behavior of chromophores is useful in following the conformational changes of the protein. The rate constant, k, begins to increase around 5 mM, reaching a constant value around 12 mM SDS. The sharp increase of the rate constant in the range of 5—12 mM SDS indicates that the higher the helix content, the faster the rate of coil to α-helix transition. The rate constant, k, is independent of the SDS concentration in the range below 5 mM,[18] where the values of $[\theta]_{206}$ and $\Delta\varepsilon_{295}$ change as is shown in Fig. 2. Thus, the discussion given below will be limited to the conformational change in the range of SDS concentrations above 5 mM.

It is expected that the binding ratio of surfactants to protein determines the conformation of the surfactant-protein complex. In reference to the results of the interactions of bovine serum albumin with some ionic surfactants,[3,19–24] there may be some limited particular binding ratios (surfactants/protein) in the present system. Therefore, the following process may be easily assumed for the complex formation and the accompanying conformational change:

$$P_0 \xrightarrow{a_1 S} \cdots \xrightarrow{} P_x S_l \xrightarrow{a_2 S} P_y S_m \xrightarrow{} \cdots \xrightarrow{a_i S} P_z S_n \qquad (1)$$

where P_0 is the native protein, S is the surfactant molecule, a_i is a particular number, and $\cdots P_xS_l$ and $P_yS_m\cdots P_zS_n$ denote the complexes (the helix content increases in the order $\cdots$ x, y, $\cdots$ z; the binding number of surfactants, $70\leqq l<m<\cdots<n$). The reaction model (1) indicates that the longest time is required for the transition from P_0 to P_zS_n with the highest helix content. However, the transition rate becomes faster as the final helix content increases as seen in Fig. 7. On the other hand, with reference to many results of micellization kinetics,[25] the binding rate of

SDS to a protein seems to be rapid compared to the time range of the conformational change, since it should be of approximately the same order as the time of the association of surfactant molecules in a micelle formation. This suggests that the protein cannot adopt various different conformations as reaction intermediates in the presence of a certain quantity of SDS, because they would rapidly bind to the protein, causing the transition only to a particular conformation of the protein. The features of the conformational change may be drawn schematically as follows,



(2)

where k_x, k_y, and k_z denote rate constants of the corresponding processes ($\cdots k_x < k_y < \cdots < k_z$). This reaction model means that the binding of the surfactants completes before the conformational change of the native protein is completed. The conformation of the protein polypeptide must change together with many of the surfactant molecules bound to it. Through the particular conformational change of the protein polypeptide, the bound surfactants must be rearranged; that is, they may be forced to move from the initial binding sites to more favorable ones. Through this process, the bulky hydrophobic groups of the surfactant should come into maximum contact with the hydrophobic parts of the polypeptide, reconciling with the structure formation to reach a minimal free energy. Reaction 2 seems to be more suitable as an explanation of the present kinetic results, although Reaction 1 may be favorable for an understanding of the growth of the complex. Reaction 2 is also reminiscent of conformational changes of poly(L-lysine) in an octyl sulfate solution: the polypeptide, which adopts a disordered structure in the absence of the surfactant, takes an α-helix and a β-structure at low and high concentrations of the surfactant respectively, but the disordered structure of the polypeptide directly turns to the β-structure.[13] The author considers that, at a certain SDS concentration above 5 mM, the native conformation of the protein is directly transformed into another conformation without passing through any intermediate conformation such as may be caused when the SDS concentration is below the one mentioned above.

The temperature dependence of k was examined at 20 mM SDS in order to evaluate the activation parameters of the corresponding conformational change. Table 1 shows the temperature dependence of k and the activation parameters, ΔH^* and ΔS^*, of the process evaluated in a usual manner.[26] In the case of the conformational change induced by the surfactant, the entropic contributions must be due not only to the folding and unfolding of the polypeptide chain, but also to the rearrangement of the surfactant molecules bound to the polypeptide and the solvent-ordering process around the hydrophobic groups of the surfactant molecule exposed to an aqueous environment. The latter two contributions must be negative. If the new ordered structure is merely added to the original structure of the native protein, the value of ΔS must be negative, while that of ΔS^* may also be negative. However, the observed value of ΔS^* is positive and large. It is interesting to compare the present results with the activation parameters of the conformational change of lysozyme caused by other agents.[16,17] The values of ΔH^* and ΔS^* are similar to those of the unfolding process of the lysozyme. It may be noted here that the value of ΔS^* in the present study is similar to that of the conformational change in which the ordered structure decreases. As has been mentioned above, the helix content increases through the conformational change caused by SDS, while the degree of exposure of chromophores becomes greater in the denatured conformation than in the native one. Subsequently, the polypeptide chains must have been partially refolded in the transition process; that is, part of the polypeptide chains must have been unfolded, followed by a continuous refolding together with the bound surfactants in the transition process. As a result of the conformational change of the protein, more chromophores are exposed to the aqueous environment in the denatured state than in the native one. Therefore, the degree of unfolding becomes significant in the transition state relative to the native state, and the value of ΔS^* may reflect such a large-scale conformational change of the complex.

Table 1. Kinetic parameters of conformational change of delta-chymotrypsin by SDS

Temperature/°C	15	20	25	30	35
$k/10^{-1}\,s^{-1}$ a)	0.40	0.85	2.9	7.0	15
ΔH^*/kJ mol^{-1}			140		
ΔS^*/J K^{-1} mol^{-1}			160		

a) These values were calculated from the results at 1.0×10^{-5} M protein and 20 mM SDS.

Further studies comparing the kinetic parameters of the same conformational change of proteins similar to the protein used here are in progress and will be reported in due course.

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