Note

Stopped-Flow Circular Dichroism Study on Conformational Change of Alpha-Chymotrypsin by Sodium Dodecyl Sulfate

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Conformations of Protein in aqueous solution can be more intrinsically detected by circular dichroism (CD) measurements. Recently, a few stopped-flow CD studies have been made in visible and near ultraviolet regions.¹⁻⁵⁾ In the case of detection of the conformational change of protein, however, the kinetic measurements should be done around 200 nm in far ultraviolet region, the ellipticity at which most reflects the change in protein backbone structure. On the other hand, it has been found that α -chymotrypsin adopts a conformation of a higher helix content than the native protein by the addition of sodium dodecyl sulfate (SDS).⁶⁾ Now, the authors wish to report the application of stopped-flow CD spectroscopy in far ultraviolet region to conformational change of the protein.

Crystalline α -chymotrypsin from beef pancreas was purchased from Miles Laboratories. The concentration of the protein was determined spectrophotometrically using $E_{1\text{cm}}^{1\%} = 20.6$ at 282 nm. The surfactant, SDS was purchased from BDH Chemicals Ltd. and the critical micelle concentration (CMC) of it in pure water was determined to be 8.3×10^{-3} mol/liter at 25°C by the electrical conductivity method. The α -chymotrypsin-SDS interaction was studied in a buffer solution of pH 7.0, containing 3.33×10^{-3} and 3.56×10^{-3} mol/liter of Na_2HPO_4 and NaH_2PO_4 , respectively.*

* The rather dilute buffer was used because the higher the salt concentration, the lower the SDS concentration at which the final conformational change of protein is attained and thus detailed studies on the conformational change become difficult. This change in the buffer concentration shows no significant effects on the mode of circular dichroic spectra and the binding isotherm (not shown). The CMC of SDS in the buffer was 5.6×10^{-3} mol/liter at 25°C by the electrical conductivity method.

The measurements of CD were carried out with a JASCO J-500A spectropolarimeter of Japan Spectroscopic Co., equipped with a DP-501 data processor of the same company. The data processor has been partially modified to follow rapid reactions. The time constant and the scanning speed of the apparatus are variable from 2.5×10^{-4} to 0.64 sec and from 1.0 to 1000 nm/min, respectively. The sampling time required for 5 data points/nm on chart for both X and Y directions can be selected from 1.0×10^{-4} to 0.10 sec for kinetic measurements. The stopped-flow measurements by CD detection were made with a rapid mixer, model MX-70 of Union Giken Co. and a handmade observation cell. The pathlength and the incident area of the cell were 1 mm and 1 cm², respectively. The system of stopped-flow was derived at about 2.0 kg/cm² pressure of gas. Under these conditions, the dead volume beyond the mixer and the dead time were calculated to be 250 μ l and 2 sec, respectively.

The CD spectra were measured within 5 min after mixing the solution of the protein with those of SDS.** Figure 1 shows the representative CD spectra in the absence and the presence of 2.0×10^{-2} mol/liter SDS. Although the observed spectrum does not show a double negative maximum at 206 and 222 nm characterizing α -helical structure, the content of such a structure increases as mentioned by Hunt and Jirgensons.⁶⁾ In the CD stopped-flow measurements, ellipticity changes were observed with time above 5.0×10^{-3} mol/liter of SDS (the concentration of the protein was 1.0×10^{-5} mol/liter). The rate was dependent on the SDS concentration up to 2.0×10^{-2} mol/liter. The representative time courses at 195 (at which the positive maximum appears in the difference CD spectrum in Fig. 1), 206 and 222 nm are shown in Fig. 2 (a-c). Half of the total ellipticity change were observed with time at each wavelength in comparing with the spectra in Fig. 1. However, as anticipated from the results in Fig. 1, the ellipticity at 195 nm and those at 206 and 222 nm changed upward and downward with time, respectively, in Fig. 2. These results together with the fact that no ellipticity changes occurred at 195 nm ((d) in Fig. 2) and the other wavelengths in mixing the same 2.0×10^{-2} mol/liter SDS solution with 1.0×10^{-5} mol/liter bovine serum albumin solution, support the determination that the observed time course is not an artificial one which is occasionally observed in stopped-flow measurements. In addition, no ellipticity changes were observed at any wavelength adopted here in mixing the α -chymotrypsin solution with the other electrolyte solutions or solution of lower SDS concentrations, indicating that heat of mixing is negligible. Therefore, the observed process was concluded

** The CD spectra of the solution containing only α -chymotrypsin begin to change when leaving for 10 hr at 25°C. Especially, in the presence of $0.5 \sim 4.0 \times 10^{-3}$ mol/liter SDS, the CD spectra of the protein change within 1 to 2 hr.

FIG. 1. The Typical Circular Dichroic Spectra of Solution of α -Chymotrypsin in the Absence (I) and the Presence (II) of 2.0×10^{-2} mol/liter SDS and the Difference Circular Dichroic Spectrum (III) of Them at 25°C .

The concentration of the protein was 1.0×10^{-5} mol/liter and the thickness of the cell used was 1.0 mm. The time constant and the scanning speed of the spectropolarimeter were set to be 1.0 sec and 20 nm/min, respectively. These spectra are the averages of 8 repetitions.

to correspond the conformational change of α -chymotrypsin induced by the addition of SDS. As seen in Fig. 3, a semilogarithmic plot of $|\theta_t - [\theta]_{t=\infty}|$ vs. time, t , gave a linear relationship between them over the time range in which ellipticity clearly changed. So, the conformational change of the protein may be treated as a first-order reaction. The forward rate constants of the conformational change, probably increase of helix content were calculated from the slopes in such plots and tabulated in Table I. The time courses at the three wavelengths approximately gave the same rate constant as easily anticipated from the plots in Fig. 3. The values of the rate constant are one order of magnitude greater than those obtained by thermal denaturation of the protein,⁷⁾ while

FIG. 2. The Typical Traces of Ellipticity Changes of 195 (a), 206 (b) and 222 nm (c) at 25°C .

The final concentrations of SDS and α -chymotrypsin were 2.0×10^{-2} and 1.0×10^{-5} mol/liter, respectively. The trace (d) presents the case of no ellipticity change observed at 195 nm in the same time range, in mixing SDS solution and bovine serum albumin solution. The final concentrations of SDS and the protein in this case were 2.0×10^{-2} and 1.0×10^{-5} mol/liter, respectively. The traces (a) and (d) are the averages of 32 repetitions and the traces (b) and (c) are the averages of 8 repetitions.

FIG. 3. Semilogarithmic First-Order Plots for the Approach to Equilibrium on the Three Time Courses (a-c) in Fig. 2.

The quantities representing $|\theta_t - [\theta]_{t=\infty}|$ are taken as a unit in 4×10^{-3} (a) and 2×10^{-3} degree (b, c) on the basis of best fit curves drawn on time course in each case.

TABLE I. FORWARD RATE CONSTANTS, k OF CONFORMATIONAL CHANGE OF α -CHYMOTRYPSIN AT SEVERAL SDS CONCENTRATIONS

SDS/ 10^{-3} mol/liter	5.0	6.0	8.0	14	18	20	24	30
$k^*/10^{-1} \text{ s}^{-1}$	0.40	1.3	2.5	3.4	3.7	3.8	3.8	3.8

* The values were obtained from the results at 206 nm. The time courses at 195 and 222 nm gave the rate constant to be 4.1 and $3.5 \times 10^{-1} \text{ s}^{-1}$ at 2.0×10^{-2} mol/liter SDS. Experimental error at 206 nm is within 10%.

the helix content decreases in the thermal denaturation. In the CD stopped-flow measurements, the ellipticity changes are observed above 5.0×10^{-3} mol/liter SDS as stated above. However, this concentration of SDS is not directly correlated with the value of the CMC in the buffer, because the SDS molecules bind to protein as free monomers.^{8,9)} The dependence of the rate constant on the SDS concentration seems to reflect the extent of denaturation induced by binding of SDS.

It is concluded that the CD stopped-flow spectroscopy in far ultraviolet region can be effectively applied to conformational change of polypeptide containing no aromatic amino acids.

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