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KINETIC STUDY ON DENATURATION OF TOBACCO MOSAIC VIRUS COAT PROTEIN BY THE RAPID CIRCULAR DICHROIC SPECTRA MEASUREMENT

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The kinetics of denaturation of tobacco mosaic virus coat protein has been studied by a stopped-flow spectropolarimeter. The time courses of ellipticity change at 222 nm suggest the denaturation of TMV protein by guanidinium chloride or urea to be a pseudo-first order reaction.

Kinetic studies of denaturation of a protein are made by measuring the change of an appropriate physical property after the denaturant has been added to the protein. Optical properties such as ultraviolet (uv) difference spectra, optical rotation[1-5] and ultrasonic absorption[6] are measured for this purpose. It may be more useful to observe a variety of physical properties in the time course of denaturation with a stopped-flow apparatus for the purpose of obtaining the detailed information. We may expect to pursue the denaturation kinetics by the measurement of CD spectra, if we are able to record the ellipticity change at a sufficiently high speed. Especially, the change in the far-uv region yields a direct information about the rates of denaturation processes which involve conformational changes of proteins, for example, from α-helical to random coil structures. However, few detailed studies have been reported on these reactions.

In this paper we describe the kinetic study of denaturation process of TMV coat protein with a new technique of rapid CD spectra measurements in the far-uv region at 222 nm. It was observed that the interconversion of the native and denatured states of TMV coat protein follows a first-order reaction in both directions under 4M guanidinium chloride or 5M urea.

TMV, Japanese common strain OM was purified and coat protein was prepared by the acetic acid method[7,8]. Guanidinium chloride and urea were purchased from Nakarai Chemicals (special pure grade for the biochemical use).

The static CD measurements were performed on a Jasco Model J-40 spectropolarimeter (Nihon Bunko Co.,Ltd.) utilizing 2 mm cell. CD data are reported as molar ellipticity, $[\theta]$, in units of deg.cm^2/dmol. The mean residual weight, 111, was used for TMV protein.

The time course of a CD spectral change was measured with the combination of a flow-type cell, a jet-type rapid mixer and a Jasco Model J-40 equipped with a rapid CD data processor. The sample chamber is composed of a flow-type quartz cell (T-70, Fujihara Co.,Ltd.) of 10 mm wide and of 2 mm optical path length being surrounded with a water-jacket. The stopped-flow system is derived pneumatically at about 3.5 kg/cm^2 pressure of nitrogen gas. Equal volumes of TMV protein solution dissolved in 0.1M sodium phosphate buffer (pH 7.2) and of 8M guanidinium chloride or 10M urea

solution dissolved in 0.1M sodium phosphate buffer (pH 7.2) was rapidly mixed with the mixer (model MX-7, union Giken Co.,Ltd.), and then the signals in the ellipticity at 222 nm were memorized by the rapid CD data processor. The memory accomodates 2,000 of data points and the sampling time per data point ranges from 6 ms to 200 ms. It is possible to accumulate the signals on the processor up to 64 times, if necessary, in order to raise the signal-to-noise ratio. The signals in the data processor were then recorded on the X-Y recorder. The dead time of this apparatus was about 10 ms.

As shown in the previous paper[7], the native TMV protein has two negative CD bands in the far-uv region at 222 nm and 210 nm, indicating that the TMV protein in the native state has both α-helix and β-structure. An addition of denaturant up to a concentration of 5M urea or 4M guanidinium chloride induces the decrease of both CD bands at 222 nm and 210 nm, and TMV protein behaves as a random coil.

Figure 1 shows the time course of CD band at 222 nm by the addition of denaturants. The ellipticity at the starting point was the same as that in the native state. The ellipticity at the end of time course agreed well with that obtained from the static measurement, where both protein and denaturant solutions were mixed and measured with the usual CD cell (optical path length of 2 mm) after being left standing for 24 hr at room temperature (state 2 or 3 in Fig. 1). These curves (a) and (b) seem to be expressed by first-order kinetics.

The results recorded in Fig. 1 may be treated formally in terms of a simple equilibrium[9-10]

$$N \underset{k_N}{\overset{k_D}{\rightleftharpoons}} D \qquad (1)$$

between native (N) and unfolded (D) forms of the protein. With the assumption that Eq.(1) holds, an apparent equilibrium constant for the reaction may be obtained[9-10]

$$K = [D]_\infty / [N]_\infty = k_D / k_N \qquad (2a)$$

$$= (\theta_\infty - \theta_N) / (\theta_D - \theta_\infty) \qquad (2b)$$

$[N]_\infty$ and $[D]_\infty$ are the concentrations of native and unfolded forms at time $t=\infty$. The θ_o ($= \theta_N$), θ_∞ and θ_D are the ellipticities at the initial (native), final and unfolded state, respectively. The θ_D may be taken as independent of denaturant concentration and may be assumed to be equal to zero, because the θ_D for the TMV protein denatured at about 8M guanidinium chloride was almost zero. The apparent equilibrium constants obtained from Eq.(2a) were 4.13 for 5M urea and 8.34 for 4M guanidinium chloride.

The rate constants in the interconversion of the native and denatured states were then obtained by applying the CD data to Eq.(3) which a single first order process obeys

$$(\theta_t - \theta_\infty) / (\theta_o - \theta_\infty) = \exp(-\lambda t) \qquad (3)$$

where λ is the phenomenological rate constant and is equal to $(k_D + k_N)$. Figure 2 shows the semilogarithmic plot of CD changes at 222 nm of TMV protein. All data fit well on a straight line. The λ is obtained from the inclination of these straight lines and the values are 0.142 for 5M urea and 0.785 for 4M guanidinium chloride. The rate constants of both the reactions, k_N and k_D, are calculated from the values of k and λ. The calculated values are k_D=0.114 s^{-1} and k_N=0.028 s^{-1} for 5M urea,

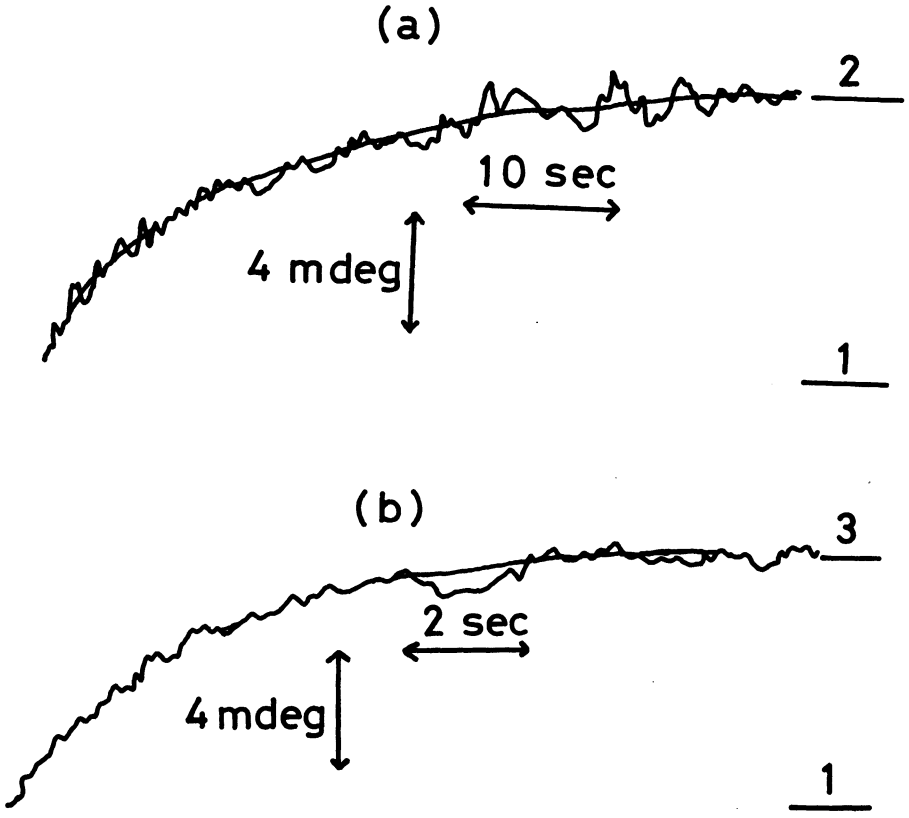

Fig. 1. Time courses of CD of TMV protein at 222 nm, in (a) 5M urea and (b) 4M guanidinium chloride in 0.1M sodium phosphate buffer (pH 7.2). Concentrations of TMV protein was 0.248 g/l. Line 1 represents the ellipticity at 222 nm in the native state, and line 2 and 3 represents, respectively, those in the denatured states induced by 5M urea and by 4M guanidinium chloride.

k_D=0.70 s^{-1} and k_N=0.084 s^{-1} for 4M guanidinium chloride.

The present paper reported the results of the studies on unfolding of TMV protein, obtained by the rapid measurements of CD in the far-uv region. It is very interesting to study the CD spectral change around 222 nm, because it reflects the change in protein backbone structures. The stopped-flow CD system in this paper is suitable for such a short time resolution as in the denaturation process of TMV protein. The effect of the denaturant concentration on the denaturation process of TMV protein will be presented elsewhere.

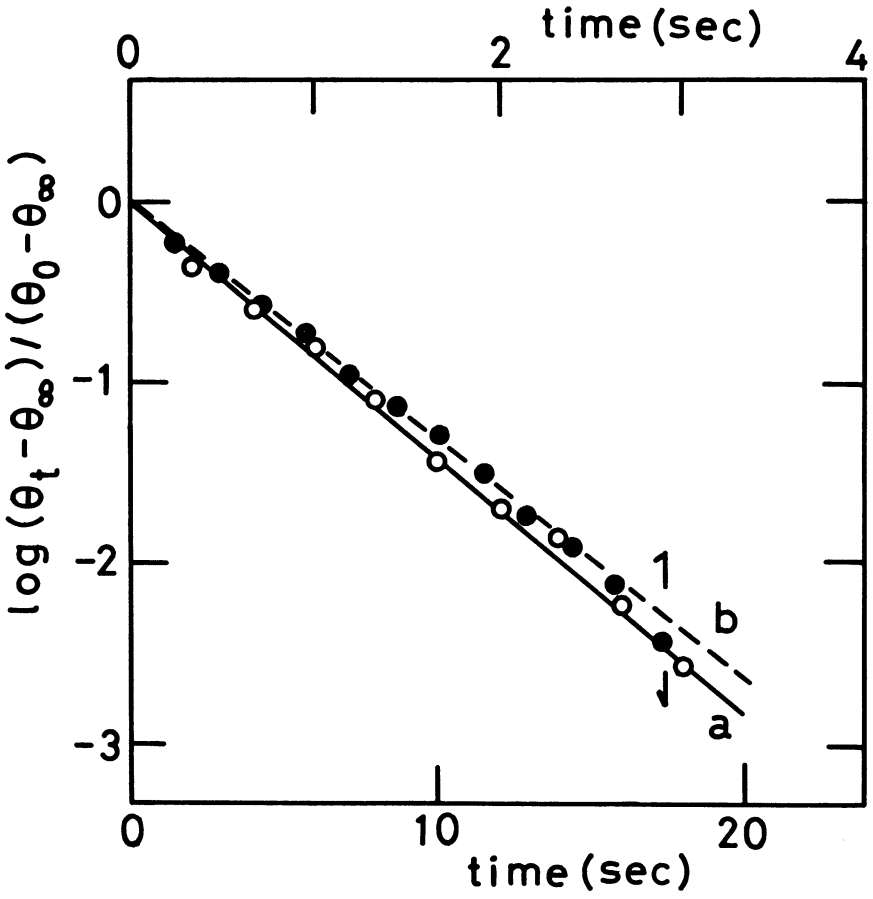

Fig. 2. Semilogarithmic plot of CD changes at 222 nm of TMV protein solution due to denaturation indicated in Fig. 1. a: 5M urea and b: 4M guanidinium chloride.

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