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HELIX-COIL TRANSITION OF POLY-α,L-GLUTAMIC ACID IN AQUEOUS SOLUTION STUDIED BY THE DISSOCIATION FIELD EFFECT RELAXATION METHOD

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The measurements of dissociation field effect relaxation in aqueous poly-α,L-glutamic acid were carried out. From the feature of pH dependency of relaxation time, the observed relaxation was attributed to the helix-coil transition. The helical growth rate constant was calculated to be $(2.9 \pm 2) \times 10^7$ sec^{-1} from Schwarz's equation.

The helix-coil transition of synthetic polypeptides has long served as a useful model for cooperative structural changes found in more complex biological systems such as denaturation and enzyme-substrate binding. About the equilibrium properties of the transition there exists a wealth of information. However very little is known about the kinetics since the elementary steps of the transition are presumably fast processes with rate in a range inaccessible to classical kinetic method. Complete kinetic data have been taken for only a few systems by ultrasonic absorption measurements.[1,2,3] As for the poly-α-glutamic acid, Inoue has investigated the ultrasonic absorption of the poly-α,D-glutamic acid(PDGA) solution and has estimated the rate constant for the helix-coil transition.[2] Stuehr and his co-worker[3] have recently carried out the ultrasonic absorption measurements of poly-α,L-glutamic acid(PLGA) and have obtained the similar results as in the case of PDGA.

Although it is well known that the helix-coil transition is induced by the ionizing side chain, the charge-induced transition in aqueous solution has received even less attention in the kinetic study. The degree of ionization concomitant with conformational change such as helix-coil transition is expected to be very high.[4]

On the basis of this expectation, we carried out the measurements of dissociation field effect(DFE) relaxation of PLGA. The DFE method apparatus utilized here was similar to that invented by Ilgenfritz[5] and was constructed to follow transients by a conductance change measurable with a Wheatstone bridge. The instrument will be described in detail elsewhere. Poly-α,L-glutamic acid sodium salt was purchased from Protein Research Foundation, Osaka. The manufacturer listed the molecular weight as 142,000. The material was characterized and was used without further purification and fractionation. The concentration of hydrogen ion was determined with a Hitachi-Horiba Type F-5 pH meter, and the pH was adjusted to the desired values by an addition of sodium hydroxide or hydrochloric acid. The measurements were carried out at the two PLGA concentrations 1×10^{-4} and 5×10^{-5} mole residue/l as a function of pH at 20 ± 1°C.

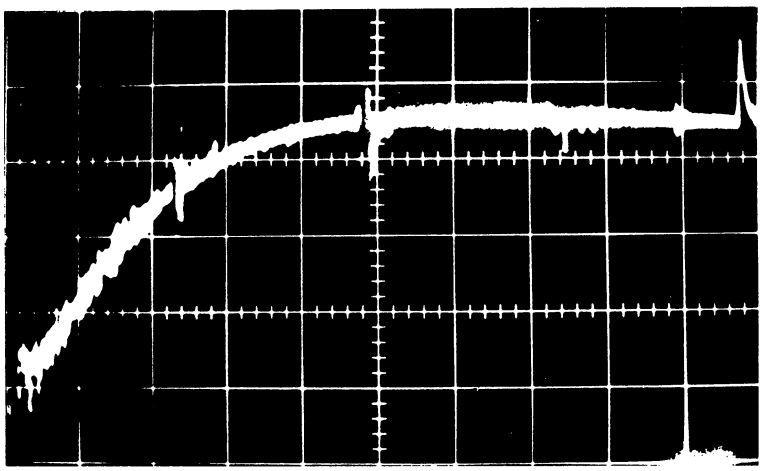

Fig. 1. Photograph of an oscilloscope trace for a dissociation field effect experiment with aqueous PLGA solution at 20°C. PLGA concentration 1×10^{-4} mole residue/l, pH 6.95, major divisions on the abscissa correspond to a time of 2.0 μsec, and major divisions on the vertical axis correspond to 50 mV. A square pulse of 20 μsec duration was applied to a sample cell.

The typical oscilloscope trace is shown in Fig. 1. The relaxation was observed only in the pH range 5.1 - 7.2. The dependency of the relaxation time on the pH and PLGA

concentration is shown in Fig. 2. As can be seen in Fig. 2, the relaxation time shows maximum at pH 5.6 and is independent of concentration. These features are predicted for the helix-coil transition. In order to examine the relationship between the relaxation time and the fraction of helicity, the optical rotatory

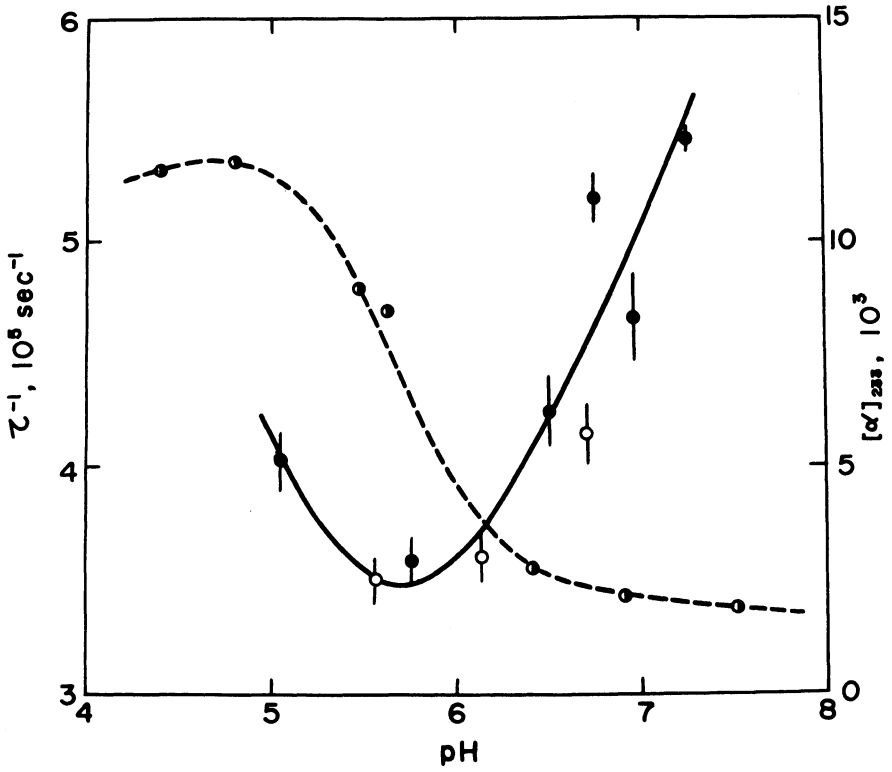

Fig. 2. pH dependence of specific rotation at 28°C (dashed curve) and reciprocal relaxation time at 20°C (solid curve).
◐, 5×10^{-4} mole residue/l; ●, 1×10^{-4} mole residue/l;
○, 5×10^{-5} mole residue/l.

dispersion was measured at the concentration 5×10^{-4} mole residue/l as a function of pH at 28°C. Specific rotation $[\alpha]_{233}$ at 233 nm is also plotted against pH in Fig. 2. From Fig. 2, it was found that the helix-coil transition is balanced at pH 5.6 under the present experimental conditions which are not kept at the constant

ionic strength. Further we carried out the DFE relaxation measurements for PDGA (molecular weight 60,000) and glutamic acid monomer under the same condition as for PLGA. For the PDGA, the similar relaxations were observed, but no relaxation was observed for the glutamic acid monomer in which relaxation would be faster than the time range measurable by the apparatus used here. From these experiments, it may be deduced that the observed relaxations for PLGA are associated with the helix-coil transition.

Schwarz[6] has deviced a model for the close-to-equilibrium kinetics of the helix-coil transition. The relaxation time in the vicinity of the transition midpoint is given by

$$1/\tau = k_f\{(s' - 1)^2 + 4\sigma\}$$

where k_f and s' are the forward rate constant and the equilibrium constant for helical growth respectively, and σ, the nucleation parameter which is $(3 \pm 2) \times 10^{-3}$ for PLGA. At the midpoint of the transition $s' = 1$. Therefore the rate constant k_f can be calculated from the relationship $\tau^{-1} = 4k_f\sigma$. The value of k_f was estimated to be $(2.9 \pm 2) \times 10^7$ sec^{-1} by use of $\tau^{-1} = 3.5 \times 10^5$ sec^{-1} at the midpoint. This value is comparable to those obtained by other methods, e.g. 4.4×10^7 sec^{-1} for PDGA[2], and $(8 \pm 5) \times 10^7$ sec^{-1} for PLGA.[3] This indicates that the DFE method is very available for the kinetics of the charge induced helix-coil transition. Further studies of the dependency of the relaxation phenomenon on the molecular weight, temperature, ionic strength, and solvent are now in progress.

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