Dynamics of the Helix—Coil Transition in Isotope Exchange of Polypeptide

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ABSTRACT: Isotope exchange experiments in the study of the helical conformation of a polypeptide suggest that the rate constant of the helix—coil change of a unit depends on its location and that it is very low in the central portion of the chain. In this paper, a model is presented to explain the low rate constant and its site dependence. When an inner residue in the helix state unwinds to the coil state, the entire helix should move in viscous medium. The mobility of unwinding in the complete helical polypeptide is calculated by the method of Kirkwood. As a result of this frictional effect, the transition rate constants obtained are in reasonable agreement with experimental results.

KEY WORDS   Helix—Coil Transition / Isotope Exchange / Rate Constant / Schwarz—Ising Model / Friction Constant / Kirkwood Theory /

The investigation of the dynamical properties governing the change of the secondary structure of a protein molecule such as a helix—coil transition, plays an important role in understanding the dynamics of denaturation and renaturation of protein molecules, in addition to its inherent interest. Consequently, the dynamics of the helix—coil transition in polypeptide has been studied both experimentally and theoretically.

In particular, Schwarz[1] has shown that the initial relaxation time of the helix content at the middle point of transition is 10^{-6}—10^{-8} sec. Poland and Scheraga[2] have also treated the subject of the initial rate and obtained similar results as Schwarz's. Their results are supported by the experiments of dielectric dispersion,[3] ultrasonic dispersion[4] and temperature jump.[5]

On the other hand, Ikegami, et al.,[6,7] have shown, in their isotope exchange experiments, that the central portions in a helical polypeptide are rather rigid and that the fluctuation time is as slow as $\sim 10^4$ sec. Similar slow relaxation times are observed in NMR experiments, whose interpretation, however, has not been agreed upon

definitely among reseachers. In fact, from the double peak observed in the transition region in NMR experiments, the relaxation time is considered to be from 10—10^{-2} sec. Ferretti, et al.,[8,9] have indicated this to be the time for the formation of a first helix unit from an all-coil chain. However, Nagayama and Wada,[10] and Ullman[11] are of the opinion that the poly-dispersity of samples is the prime cause for the double peak and the analysis of the linewidth gives the time as being from 10^{-3}—10^{-4} sec.

The relaxation times obtained by various experiments need not be in complete agreement with each other, because many kinds of modes may be expected in the transition. Different relaxation times are observed for different experiments. Theoretical attempts have been made to determine relaxation spectra.[12,13] However, the width of the relaxation times thus obtained is not so large as to serve to explain the isotope experiments.

In this paper, we propose a model which explains the slow rate phenomena observed by isotope exchange experiments by Ikegami and coworkers. This will lead to fundamental

process in the dynamics of conformational change of large molecules like polypeptides in solution.

In Section 2, the isotope exchange experiments carried out by Ikegami, *et al.*, are reviewed. The rate constants for conformational fluctuations of a complete helix were shown to depend on the portion of the chain where the fluctuation takes place. In Section 3, our model is described in comparison with the Schwarz model. In Section 4, the mobility of helical rods in solution is calculated by the method of Kirkwood for an irreversible process in macromolecular solution. A discussion is presented in Section 5.

ISOTOPE EXCHANGE EXPERIMENT

Ikegami and his coworkers have recently shown that the rate constant of the helix—coil (h—c) change of a residue in a polypeptide chain is small when it is located toward the central part of the helical chain; this was done by measuring the hydrogen—tritium (or deutrium) exchange rate of samples, and by combining the result with the knowledge of the helix content estimated from the static properties.

The scheme of the isotope exchange reaction is considered to be as follows:

$$h^* \xrightleftharpoons[K_-]{K_+} c^* \xrightarrow{K_e} c(\xrightleftharpoons{} h) \qquad (1)$$
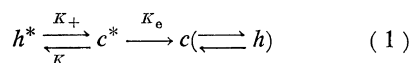
where h and c refer to the respective helix and coil state of a residue, and $*$ indicates the labeled isotopes. If the rate constants of the transition, K_+, and K_-, are much greater than the rate constant K_e of the isotope exchange which takes place in the coil form, *i.e.*,

$$K_+, K_- \gg K_e \qquad (2)$$

the total exchange rate is

$$d[c^* + h^*]/dt = -K[c^* + h^*] \qquad (3)$$

where, $K = K_e \gamma$ and γ is the coil fraction. The apparent rate constant K is dependent on the portion of the chain. If eq 2 is true, the apparent rate constant of K_j of the j-th residue from the closer terminal must be equal to the product of γ_j and K_e, *i.e.*,

$$K_j = K_e \gamma_j \qquad (4)$$

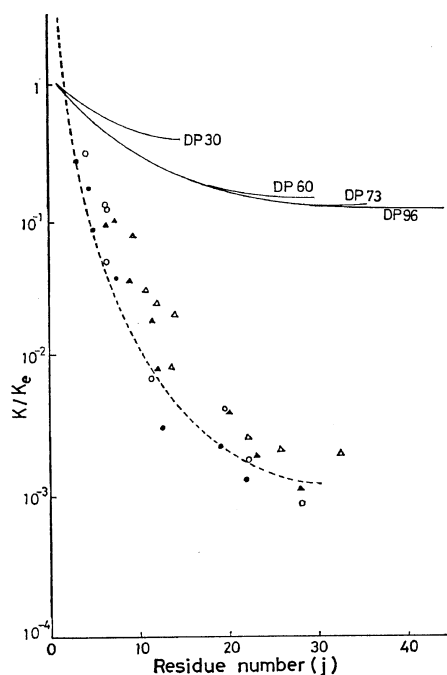In Figure 1, the experimental results for K_j/K_e

Figure 1. Semi-logarithmic plot of K_j/K_e of the exchange reaction of poly(L-glutamic acid) against j. (●) at pD 3.1; (○) at pD 3.6; (▲) at pD 4.0; and (△) at pD 4.6. The continuous curves show the coil fraction, γ, at each portion of the chain calculated using the Zimm—Bragg theory. DP is the degree of polymerization. (After Nakanishi, *et al.*, 1972.) The broken curve is the calculated result at a polymerization degree of 60, based on the model which is mentioned later.

in various solvents against j are shown by the various symbols and γ_j's values are also shown by the continuous curves for each degree of polymerization which can be calculated using the Zimm—Bragg theory.[15] It is apparent that K_j/K_e does not coincide with γ_j, contrary to eq 4.

Ikegami, *et al.*, have tried to explain the discrepancy in several ways; *e.g.*, by developing a stochastic treatment of h—c transition and by the Monte Carlo simulation, etc. To the extent that the assumption of the relation (2) and the Ising model are adopted, the slowly exchangeable portions in the α-helical structure cannot reasonably be explained. On the other hand, it is more probable with a very low pH far from the transition region that the relation (2) is not valid because the h—c transition rate is not

always very rapid. If $K_e > K_+, K_-$, we have $K = K_+$; the apparent rate is just the rate of h—c change of a unit. Then the j-dependence of K indicates that the rate of h—c change is also j-dependent, which implies that in this case also the Schwarz—Ising model for polypeptide does not hold.

Then, we make an assumption that the slow rate actually found should be attributed to a slow h—c reaction rate, and a new model is proposed in place of the Ising model to explain the j-dependence of the rate constant.

MODEL

The conventional theories of the helix—coil transition developed by Schwarz and other authors are based exclusively on the dynamical Ising model. The rate constant of the transition of a unit, *i.e.*, a residue, between the helix state and the coil state are assumed to be determined only by the states of its neighbors, and have the same value at any portion of the chain and are irrespective of whether the chain is completely helical or coil rich.

A polypeptide chain, however, is different from the Ising chain in the following respect. If a unit of polypeptide chain located in the inner part of the chain changes the conformation by virtue of bond rotations, both sides or at least one side of the chain around that unit moves in viscous solvent. Therefore, the frictional resistance caused by a conformational change of a unit cannot be the same irrespective of the location of the unit in the chain. This kind of movement is expected to occur in an almost complete α-helix with negligible fraction of coil states, as in the case of isotope exchange experiments, while in the coil rich state the movements of a chain brought about by a bond rotation are considered to be restricted to small region because in the latter case simultaneous rotations around more than two bonds can take place. Thus, consider the case when a chain is in a complete helix. If an internal helical unit is transferred to a coil, the helical rods of both sides of the unit must rotate around each other. The friction of the rods in solution may lower the rate constants greatly in the central portions, compared with the terminal portions.

In the Schwarz model the coil nucleation reaction is given as follows:

$$h\,h\,h \underset{K_-}{\overset{K_+}{\rightleftarrows}} h\,c\,h \qquad (5)$$
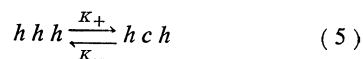
where K_+ and K_- are the forward and the backward rate constants respectively. The ratio of the rate constants must satisfy the following relation in accordance with the detailed balance condition:

$$K_+/K_- = \sigma/s \qquad (6)$$

where s is the statistical weight for the growth of a helix, σ is the cooperativity parameter in the Zimm—Bragg theory, and σ/s is the equilibrium constant of this reaction. The absolute values of the rate constants, however, cannot be determined by the detailed balance condition. Schwarz and all other researchers put

$$\begin{aligned} K_+ &= \gamma_c k\sigma \\ K_- &= \gamma_c ks \end{aligned} \qquad (7)$$

by introducing kinetic parameters γ_c and k which are same for all of portions of the chain just like the Ising model, where γ_c is considered as the mobility and ks and $k\sigma$ are the rate constants for the coil nucleation and its reverse reaction.

We may introduce the mobility $\mu(j)$ around the j-th unit in place of γ_c, and assume the rate constants of the j-th unit in the form:

$$\begin{aligned} K_+(j) &= \mu(j)k'\sigma \\ K_-(j) &= \mu(j)k's \end{aligned} \qquad (8)$$

where k' is another quantity independent of j similar to k. The rate constants of conformational change of polymer are formulated in the form of eq 8 by Helfand, who developed the Kramers theory of Brownian motion into the polymer dynamics, but has not yet applied to peptide chains. Of course the rate constants given by eq 8 satisfy the detailed balance condition as in the Schwarz's case.
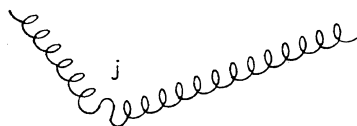
$$K(j) = \mu(j)k\sigma$$

Figure 2. The coil nuleation of an internal unit of the helical rod.

MOBILITY

Let us consider a helical polypeptide of the degree of polymerization N and the length $(N-1)b$. When the j-th unit of the inner part of a helical rod is changed to a coil state, the helix splits into two helices of lengths $l_1=(j-1)b$ and $l_2=(N-j)b$. During the h—c change of the j-th unit, the two rods l_1 and l_2 have to rotate with the center of mass of the chain unchanged. This serves to separate the over all motion into rotatory and translational motions both which are irrelevant to the h—c change and the bending motion directly caused by the h—c change. The bending motion of a once broken rod can be regarded as a two dimensional process, say, in the x—y plane. Thus we have only two degrees of freedom in describing the "shape" of the chain as shown in Figure 3. The initial orientation of the helical rod is taken as the x-axis with the center of mass at the origin. While bending, two rods make the angles θ_1, θ_2 with the x-axis. Thus the angle ϕ between the two rods is

$$\phi=\theta_1-\theta_2 \qquad (9)$$

as shown in Figure 3. Now, it is convenient to introduce another angle θ

$$\theta=A\theta_1+B\theta_2 \qquad (10)$$

$$A+B=1 \qquad (11)$$

with two constants A and B to be determined later.

Since the center of mass remains unchanged, the coordinates of the j-th unit $\boldsymbol{R}_j(x_j, y_j)$ at the
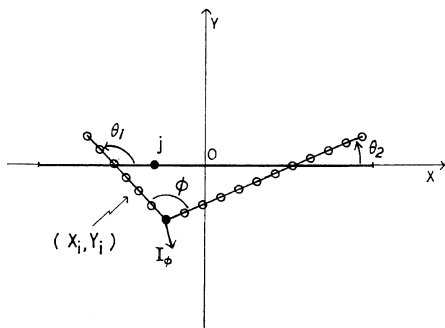

Figure 3. The coordinate system. A once broken helical rod is described in three coordinate systems respectively, $(x_i, y_i) \leftrightarrow (\theta_1, \theta_2) \leftrightarrow (\phi, \theta)$. The center of mass is at the origin.

joint of the two rods are written as,

$$X_j=-(l_1^2\cos\theta_1+l_2^2\cos\theta_2)/2L$$
$$=-\{l_1^2\cos(B\phi-\theta)+l_2^2\cos(A\phi+\theta)\}/2L$$
$$Y_j=-(l_1^2\sin\theta_1+l_2^2\sin\theta_2)/2L$$
$$=\{-l_1^2\sin(B\phi-\theta)+l_2^2\sin(A\phi+\theta)\}/2L$$
$$L=l_1+l_2=(n_1+n_2)b=(N-1)b \qquad (12)$$

In order to calculate the mobility, we can apply the Kirkwood method[16] of an irreversible process in solutions of macromolecules. To do this first consider the Riemannian space with the following metrics. The line element is written in the ϕ, θ space by transformations from (x, y) to (ϕ, θ) coordinate systems, as follows:

$$ds^2=\sum_1^{j-1}(dx_i^2+dy_i^2)$$
$$+\sum_{j+1}^N(dx_k^2+dy_k^2)+(dx_j^2+dy_j^2)$$
$$=\sum_{l_i=b}^{l_1}l_i^2 d\theta_1^2+\sum_{l_k=b}^{l_2}l_k^2 d\theta_2^2$$
$$+(l_1^4 d\theta_1^2+l_2^4 d\theta_2^2-2l_1^2 l_2^2 d\theta_1 d\theta_2)/4L^2$$
$$=E d\phi^2+F d\theta^2+H d\phi d\theta \qquad (13)$$

where the metric tensors E, F and H are given by,

$$E=A^2\sum_{l_k=b}^{l_2}l_k^2+B^2\sum_{l_i=b}^{l_1}l_i^2+\{(Al_2^2+Bl_1^2)/2L\}^2$$

$$F=\sum_b^{l_2}l_k^2+\sum_b^{l_1}l_i^2+\{(l_2^2-l_1^2)/2L\}^2$$

$$H=A\{2L^2\sum_b^{l_2}l_k^2+3l_2^2(l_2^2-l_1^2)\}$$

$$-B\{2L^2\sum_b^{l_1}l_i^2+3l_1^2(l_1^2-l_2^2)\}$$

$$A+B=1 \qquad (14)$$

Since we are interested in the bending of a complete helix, we have put ϕ equal to π in eq 14 (see Section 5, Discussion). In order to make (ϕ, θ) coordinates mutually independent, the value of A and B are determined so that the coefficient H vanishes. Then A and B become, with condition (11):

$$A=\{3l_1^2(l_1-l_2)+12L\sum_b^{l_1}l_i^2\}P$$

$$B=\{3l_2^2(l_2-l_1)+12L\sum_b^{l_2}l_k^2\}P$$

$$P^{-1}=L(L+2)\{L(4L+1)-12l_1l_2\} \qquad (15)$$

Substituting A and B into eq 14 we have the new metric tensors,

$$ds^2 = g_{\phi\phi}d\phi^2 + g_{\theta\theta}d\theta^2 \qquad (16)$$

with $g_{\theta\phi}=0$. The covariant and contravariant tensors, $g_{\phi\phi}$ and $g^{\phi\phi}$, at $\phi=\pi$ are given by,

$$g_{\phi\phi} = B^2b^2 \sum_1^{n_1} i^2 + A^2b^2 \sum_1^{n_2} k^2$$
$$+ \{b(Bn_1^2 + An_2^2)/2(N-1)\}^2 \qquad (17)$$
$$g^{\phi\phi} = (g_{\phi\phi})^{-1}$$

where A and B are given by eq 15. Therefore, $g^{\phi\phi}$ is a function of n_1, n_2. $g^{\theta\theta}$ can also be obtained, but it has nothing to do with the h—c change and thus is not given here.

According to the Kirkwood theory, the mobility tensor $\mu^{\alpha\beta}$ is given as

$$\mu^{\alpha\beta} = g^{\alpha\beta}/\zeta + T^{\alpha\beta} \qquad (18)$$

Here $T^{\alpha\beta}$ is the $\alpha\beta$ component of hydrodynamical interaction tensor, and $\zeta = 6\pi\eta(b/2)$ is the friction constant of a unit, where η is the viscosity coefficient of the solvent.

The hydrodynamical interaction between i and k units is described by the Oseen Tensor in the cartesian coordinate,

$$T^{ik} = \frac{1}{8\pi\eta R_{ik}} \left\{ I^{ik} + \frac{R_{ik}^i R_{ik}^k}{R_{ik}^2} \right\} \qquad (19)$$

where R_{ik} is the vector $R_k - R_i$ from i to k-th unit with magnitude R_{ik}, I is the unit tensor. Now, the $\alpha\beta$ component of the Oseen Tensor is given by

$$T^{\alpha\beta} = a^\alpha \cdot T \cdot a^\beta = \sum_{i,k} T_{ik}^{\alpha\beta}$$

where,

$$a^\alpha = \sum_\beta g^{\beta\alpha} a_\beta$$
$$a_\beta = \sum_i \partial R^i/\partial q^\beta, \qquad \partial q^\beta = (\partial\phi \text{ or } \partial\theta) \qquad (20)$$

Thus, $T^{\phi\phi}$ is given by,

$$T^{\phi\phi} = \frac{1}{8\pi\eta}(g^{\phi\phi})^2 \sum_{i,k} \frac{1}{R^{ik}} \left\{ \frac{\partial R^i}{\partial\phi} \cdot \frac{\partial R^k}{\partial\phi} \right.$$
$$\left. + \frac{(R^{ik} \cdot \partial R^i/\partial\phi)(R^{ik} \cdot \partial R^k/\partial\phi)}{(R^{ik})^2} \right\} \qquad (21)$$

By utilizing the relations

$$\partial R^i/\partial\phi = Bbi I_\phi, \quad 1 \leq i \leq n_1$$
$$\partial R^k/\partial\phi = Abk I_\phi, \quad 1 \leq k \leq n_2 \qquad (22)$$
$$\partial R^l/\partial\phi = b(Bn_1^2 + An_2^2)/2(N-1) I_\phi, \quad l=j$$

where I_ϕ is the unit vector of the ϕ direction (see Figure 3), $T^{\phi\phi}$ is represented as a sum of three terms; intrarod interactions and interrod interaction.

$$T^{\phi\phi} = \frac{1}{8\pi\eta}(g^{\phi\phi})^2 \left[Bb(1+\cos^2\Phi_1) \right.$$
$$\times \sum_{\substack{i=1 \\ \neq k}}^{n_1} \left\{ B \sum_{k=1}^{n_1} \frac{ik}{|i-k|} + i(Bn_1^2 + An_2^2)/2N \right\}$$
$$+ Ab(1+\cos^2\Phi_2) \sum_{\substack{i=1 \\ \neq k}}^{n_2} \left\{ A \sum_{k=1}^{n_2} \frac{ik}{|i-k|} \right.$$
$$\left. + i(Bn_1^2 + An_2^2)/2N \right\} + ABb \sum_{i=1}^{n_1} \sum_{k=1}^{n_2} ik$$
$$\left. \times (1+\cos^2\Phi_3)(i^2 + k^2 - 2ik\cos\phi)^{-1/2} \right] \qquad (23)$$

where Φ_1, Φ_2, and Φ_3 are the angles between R_{ik} and I_ϕ respectively. The angle ϕ is again put equal to π and then I_ϕ is parallel to the y-axis and R_{ik}'s are parallel to the x-axis, so that we put $\cos\Phi_1 = \cos\Phi_2 = \cos\Phi_3 = 0$. Consequently $T^{\phi\phi}$ is reduced to:

$$T^{\phi\phi} = \frac{1}{8\pi\eta}(g^{\phi\phi})^2 \left\{ B^2b \sum_{\substack{i,k \\ i \neq k}}^{n_1} \frac{ik}{|i-k|} + A^2b \sum_{\substack{i,k \\ i \neq k}}^{n_2} \frac{ik}{|i-k|} \right.$$
$$\left. + ABb \sum_k^{n_2} \sum_i^{n_1} \frac{ik}{i+k} + \frac{b}{2N}(Bn_1^2 + An_2^2)^2 \right\}$$
$$= \frac{b}{8\pi\eta}(g^{\phi\phi})^2 \cdot f(n_1, n_2) \qquad (24)$$

This shows that $T^{\phi\phi}$ is also a function of n_1 and n_2, and the mobility can be obtained from eq 18,

$$\mu^{\phi\phi} = g^{\phi\phi}/\zeta\{1 + \tfrac{3}{8}b^2 g^{\phi\phi}f(n_1, n_2)\} \qquad (25)$$

Finally the rate constant $K_+(j)$ of the j-th unit is thus written from eq 8,

$$K_+(j) = \mu^{\phi\phi}(j)k'\sigma$$

and in particular for $j=1$,

$$K_+(1) = \mu^{\phi\phi}(1)k's \qquad (26)$$

In order to make clear the dependence of the rate constant on j, the relative rate constant $K_+(j)/K_+(1)$ is calculated. The value of the $K_+(j)/K_+(1)$ at the degree of polymerization 60 is also plotted in Figure 1 as a broken curve, which decreases sharply to the extent of 10^{-3} at the portion located 30 units from the terminal. The calculated value is adjusted so as to agree with the experimental data at the central portions
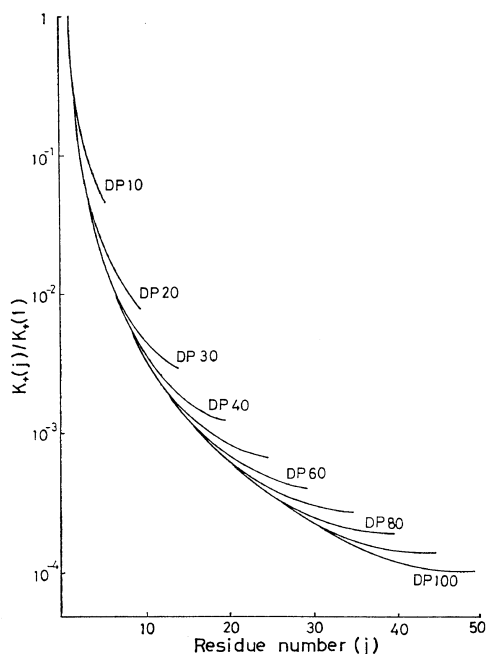
Figure 4. Semi-logarithmic plot of $K_+(j)/K_+(1)$ with different degrees of polymerization 10, 20, ..., 100 against j. DP is the degree of polymerization.

and overall agreement is fairly good except the terminal part. Some calculations with different degrees of polymerization are illustrated in Figure 4. This shows that the results from samples having different degrees of polymerization lie on a curve within a small width as the case of the experimental data in Figure 1.

DISCUSSION

As is shown in Figure 1, the agreement between the experimental and calculated values of the relative rate constants is fairly good at least in the order of magnitude. This provides evidence that our model is valid for dynamical behavior of the helical chain in solution.

In our model, a helical chain is regarded as a rod made of pearl necklace beads and the change to a coil of the j-th residues comes about by bending helical rods of both sides of the j-th unit. In this bending motion, the dihedral angles around the C_j^α atom must deviate from their values the helical state. The bending is designated as an angle ϕ, and the other variables (θ and direction consines of the x—y plane) are

independent of the angle ϕ and are regarded as irrelevant to the h—c change.

The asumption mentioned in Section 2 that the h—c change rate $K_+(j)$ is not very rapid at a low pH may be verified by our calculation. In the central portions of the chain where the rate $K_+(j)$ becomes as low as 10^{-3} in magnitude, the relation $K_+(j) < K_e$ can reasonably hold. In the Schwarz model, the rate of the coil nucleation is estimated as,

$$K_+(1) = \sigma \gamma_c k = 10^{-4} k \qquad (27)$$

where

$$\gamma_c = 1 , \qquad \sigma = 10^{-4}$$

Using the relative rate constant in the central portions,

$$K_+(j)/K_+(1) = 10^{-3} \quad (j \sim 30) \qquad (28)$$

Equation 26 is written as,

$$K_+(j) = \{K_+(j)/K_+(1)\} \cdot K_+(1) = 10^{-7} \cdot k \ (\text{sec}^{-1}) \quad (29)$$

If this value is to be smaller than K_e about 10^{-1} (sec^{-1}), k must be smaller than $10^6 \ (\text{sec}^{-1})$ and this seems reasonable at such a low pH ≤ 4 in almost helical conformations. The accurate experimental estimation of the value of $K_+(j)$ is difficult and its theoretical calculation requires further kinetic studies. The present paper only refers to the reaction of the coil nucleation through bending of the helical rod. We shall have to consider other processes for the disappearance of helix states. Kanô,[17] et al., considered the problem in the random walk model of one helical sequence. Some researchers (Jernigan, Ferretti and Weiss;[9] Miller[18]) have calculated the mean lifetimes of the j-th unit in the helix state by considering the process of the stepwise helix melting off the end of helix. The ratio of the mean lifetimes at the end and at the middle part of the chain is as large as 10^{-3} in the case of N about 100. However, the mean lifetimes have no relation with the observed rate constant in the isotope exchange experiment. Because in the scheme (1) the j-th unit once changed to a coil state is replaced irreversibly to an unlabeled one, and the rate constant is referred not to the mean lifetime but to the first passage time, i.e., the time in which the j-th unit in helix first changes to a coil state. The first passage time of the j-th may be shorter

than the mean lifetime and its j-dependence may be insufficient as an explanation for the experiment. If the few successive helical units change to coil states, colinear untwisting about the helical axis would be possible as suggested by Jernigan, et al.[19] They have discussed the position-dependence of the reaction rate by this process, but still, this is also insufficient. In the relaxation at the intermediate states which contain both helical and coil portions, the rate constants should be affected by the average conformations of the chain in the h—c transition region. de Gennes[20] claimed that the frictional effects of coil part of the chain may lower the rate of the transition. Ikegami[7] and Ferretti,[9] et al., suggest that a better theory should be considered in regard to the multiple state transition instead of two step one in conventional Ising model. In this paper, we do not take up the problems at the intermediate states.

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