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INTRAMOLECULAR α-HELIX–β-STRUCTURE–RANDOM COIL TRANSITION IN POLYPEPTIDES

II. DYNAMIC CASE

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The conformational changes of polypeptides which are capable of forming the α-helix, β-structure and random coil (or the unordered) conformations are discussed. The kinetics of this system are studied as the time evolution of the probabilities describing the conformational states of the system. The time behavior of the average numbers of the α-helix and the β-structure reveals the existence of intermediate states which are not found and not stable at equilibrium. These intermediates make the kinetics of this system more complex. Such situations can occur in protein folding and unfolding processes in such a way that a conformation absent in the tertiary structure appears in the intermediate stages and disappears finally, and the time course of the reaction is described by the sum of two or more exponential terms, in other words, the protein folding and unfolding processes display multiphasic kinetics. These intermediates, which are formed by short-range interactions, may usually be destroyed but sometimes can be stabilized by medium- and long-range interactions and remain stable for a fairly long time in the process of renaturation in real proteins.

1. Introduction

The first step in the folding process of a protein is considered as the formation of secondary structures such as α-helices and β-structures. Therefore, the helix–coil and β-structure–coil transitions are acceptable as simple models for understanding protein folding and unfolding processes. In addition to this property, there is experimental evidence suggesting that a local structure absent in the native state appears sometimes in the intermediate stage of protein folding and disappears finally [1]. The existence of these local structures will influence the kinetic properties of protein folding.

The phenomena described above cannot be explained by usually investigated conformational transition models, which regard the ordered states always as those found in the native state. We have to consider a model which has two or more kinds of ordered states; one is the same as the native state and the others are different. We present here a simple model which undergoes a α-helix–β-structure–random coil transition and can exhibit an intermediate local structure which disappears at the final stage of the renaturation process. The treatment for the equilibrium state has been discussed in ref. 2. In the present paper, we present kinetic studies of this model by treating the conformational transitions in terms of the time evolution of the conformational probability distributions of the states for a finite perturbation of the environmental conditions (a preliminary report has been published recently [3]). The kinetic method adopted here is the same as that used by Kanehisa and Tsong [4,5] in their cluster model.

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2. Method

Consider a one-dimensional array of N sites. Each site is assumed to take one of the three states, i.e., α-helix, β-structure in antiparallel pleated sheet and random coil. The two ordered regions of the α-helix and β-structure are assumed not to be in direct contact with each other, a random coil region being located between them. As mentioned in the previous paper [2], we assume the statistical weights of these three states as follows. The statistical weight of the α-helical region consisting of m sites is v for $m = 1$ and $v^2 w^{m-2}$ for $m \geqq 2$, which is the same definition as that in the usually investigated helix–coil transition [6]. The statistical weight of antiparallel β-structure region consisting of m sites is $\alpha\mu^{\sqrt{m}}\lambda^m$ as assigned by Kanō [7]. This statistical weight expresses the existence of the long-range interaction, which is characteristic of the β-structure, and gives rise to cooperativity in this system. In the transition region there exist approximately two free energy minima, each of which is thermodynamically stable on one side of the transition region and metastable on the other. In the case of an infinitely long chain system, there occurs a first-order transition mentioned in the previous paper. The statistical weight of a random coil region is u^m, but this can be set to unity without loss of generality.

Now we consider the time evolution of the conformational change from the initial equilibrium conformation to a final one for a finite perturbation of the environmental conditions. It is assumed that one step of the conformational change is a single flip between the ordered state (α-helix or β-structure) and the random coil state. Thus, the conformational change is assumed to be described by the following master equation, the solution of which gives the time-dependent behavior of the conformational probability distribution of the system:

$$\frac{\partial P_N(m_\alpha,m_\beta,t)}{\partial t} = \sum_{\substack{\Delta m_\alpha=\pm 1\\ \text{or } \Delta m_\beta=\pm 1}} W(m_\alpha-\Delta m_\alpha, m_\beta-\Delta m_\beta;\Delta m_\alpha,\Delta m_\beta) \times P_N(m_\alpha-\Delta m_\alpha, m_\beta-\Delta m_\beta, t) - \sum_{\substack{\Delta m_\alpha=\pm 1\\ \text{or } \Delta m_\beta=\pm 1}} W(m_\alpha,m_\beta;\Delta m_\alpha,\Delta m_\beta) \times P_N(m_\alpha,m_\beta,t), \qquad (1)$$

where $P_N(m_\alpha, m_\beta, t)$ is the conformational probability of all conformations with m_α α-helical sites and m_β β-structure sites, regardless of the number of these islands included and their locations. Here, $W(m_\alpha, m_\beta; \Delta m_\alpha, \Delta m_\beta)$ is the transition probability from the (m_α, m_β) state to the $(m_\alpha+\Delta m_\alpha, m_\beta+\Delta m_\beta)$ state, and (m_α, m_β) represents the conformational state with m_α α-helical sites and m_β β-structure sites. The transition probability W is assumed to be independent of time irrespective of the environmental condition. At equilibrium, the transition probability W must satisfy the detailed balance condition:

$$W(m_\alpha,m_\beta;\Delta m_\alpha,\Delta m_\beta)P_N^{\text{eq}}(m_\alpha,m_\beta) = W(m_\alpha+\Delta m_\alpha,m_\beta+\Delta m_\beta;-\Delta m_\alpha,-\Delta m_\beta) \times P_N^{\text{eq}}(m_\alpha+\Delta m_\alpha,m_\beta+\Delta m_\beta), \qquad (2)$$

where P_N^{eq} is the conformational probability at equilibrium, which is determined by the final environmental condition and has been calculated already in the preceding paper [2] in terms of the statistical weights mentioned above. There is some uncertainty in determining the transition probability from the detailed balance condition. For simplicity we take

$$W(m_\alpha,m_\beta;\Delta m_\alpha,\Delta m_\beta) = \sqrt{\frac{P_N^{\text{eq}}(m_\alpha+\Delta m_\alpha,m_\beta+\Delta m_\beta)}{P_N^{\text{eq}}(m_\alpha,m_\beta)}} \qquad (3)$$

as was done by Kanehisa in his cluster model. Since the present theory assumes a single-flip transition, we have to consider only a transition probability of the type: $W(m_\alpha, m_\beta; \pm 1, 0)$ and $W(m_\alpha, m_\beta; 0, \pm 1)$.

From the master equation, eq. 1, the relevant expression is given by

$$P_N(m_\alpha,m_\beta,t) = P_N^{\text{eq}}(m_\alpha,m_\beta) + \sum_{j=1}^{M-1} C_j(m_\alpha,m_\beta)e^{-\lambda_j t}. \qquad (4)$$

where $c_j(m_\alpha, m_\beta)$ are the time-independent coefficients, which are dependent on the states (m_α, m_β), and λ_j (> 0) are the eigenvalues of the transition matrix $-W$. These $c_j(m_\alpha, m_\beta)$ can be obtained by the initial condition. Here, M is the number of available (m_α, m_β) states, i.e., $M = N(N+1)/2 + 2$. (For studying the kinetics of the conformational change, some physical properties of the system are usually calculated as functions of time.) We follow the time behavior of the average numbers of the ordered states after a finite perturbation of the environmental condition is imposed. The time-dependent average numbers $\theta_\alpha(t)$ and $\theta_\beta(t)$ of the ordered states, i.e., α-helix and β-structure, can be expressed, respectively, as

$$\theta_\alpha(t) = \sum_{(m_\alpha, m_\beta) \in \Lambda} m_\alpha P_N(m_\alpha, m_\beta, t),$$

$$\theta_\beta(t) = \sum_{(m_\alpha, m_\beta) \in \Lambda} m_\beta P_N(m_\alpha, m_\beta, t), \tag{5}$$

where Λ is the set with available states (m_α, m_β). Therefore, the average number $\theta_c(t)$ of the random coil state is expressed as

$$\theta_c(t) = N - \theta_\alpha(t) - \theta_\beta(t). \tag{6}$$

From the solution of the master equation (eq. 4), $\theta(t)$ are given by

$$\theta_\alpha(t) = \theta_\alpha^\infty + \sum_{j=1}^{M-1} \alpha_j e^{-\lambda_j t}, \tag{7}$$

$$\theta_\beta(t) = \theta_\beta^\infty + \sum_{j=1}^{M-1} \beta_j e^{-\lambda_j t}, \tag{8}$$

where

$$\theta_\alpha^\infty = \sum_{(m_\alpha, m_\beta) \in \Lambda} m_\alpha P_N^{\mathrm{eq}}(m_\alpha, m_\beta), \tag{9}$$

$$\theta_\beta^\infty = \sum_{(m_\alpha, m_\beta) \in \Lambda} m_\beta P_N^{\mathrm{eq}}(m_\alpha, m_\beta), \tag{10}$$

$$\alpha_j = \sum_{(m_\alpha, m_\beta) \in \Lambda} m_\alpha C_j(m_\alpha, m_\beta), \tag{11}$$

and

$$\beta_j = \sum_{(m_\alpha, m_\beta) \in \Lambda} m_\beta C_j(m_\alpha, m_\beta). \tag{12}$$

After a finite perturbation, α_j and β_j are given at $t = 0$, and $\theta(t)$ approach to equilibrium θ^∞. It has the form of a sum of simple exponentials with several decay rates λ_j. The characteristic properties of the time course of the system are given by the smallest decay rates (λ_j). The relaxation behavior of these average numbers of the ordered states, θ_α and θ_β, and of the conformational probabilities P_N, expresses well the kinetic properties of this system.

3. Conformational changes from α-helical to random coil states and vice versa

First we study conformational changes from the almost α-helical structure to almost random coil state and vice versa. To show qualitatively the relations between each kinetic experiment and an equilibrium curve, a schematic depiction is given in fig. 1. This equilibrium curve is a qualitative drawing of the CD experimental curve given by Satake et al. [8]. They have detected the stepwise conformational changes of poly(L-lysine), in aqueous 1-octanesulfonate solution, by CD measurements from random coil to β-form and from β-

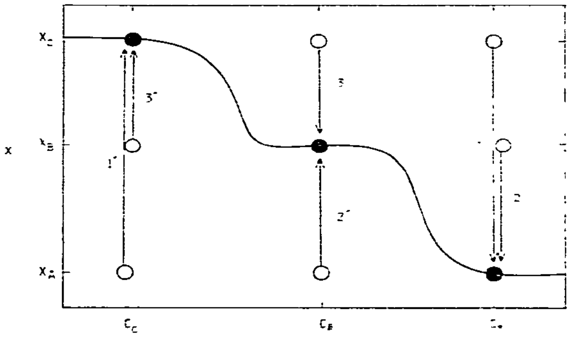

Fig. 1. Typical kinetic experiments in relation to an equilibrium curve for the conformational change of a polypeptide chain as followed by some optical property X. Equilibrium values of X are indicated by the heavy line, and X_A, X_B and X_C correspond to the equilibrium values of a certain optical experiment typical of α-helix, β-sheet and random coil, respectively. Vertical lines are representative of typical kinetic experiments, open circles represent initial points and filled circles represent final equilibrium values of the kinetic experiment. This figure is modified from Tanford's drawing [9].

form to helical structure with increase in surfactant concentration. Fig. 1 shows some optical property, X, vs. the environmental condition. In the case of Satake's experiment, the ellipticity at 222 nm is plotted vs. surfactant concentration. Here, X_A, X_B and X_C correspond to the values of the optical property typical of α-helix, β-structure and random coil, respectively. C_α, C_β and C_c are representative of the typical environmental conditions of α-helix, β-structure and random coil, respectively. Equilibrium values of X are indicated by the thick line. Vertical arrows are the changes of states for kinetic studies. Open circles represent initial states constructed by a jump of the environmental condition at a particular time, and the filled circles represent the final state, i.e., the equilibrium state defined by the environmental condition after a jump.

In the present model the environmental conditions are defined by five parameters, i.e., v and w arising from the statistical weights of the α-helix, and α, μ and λ from the statistical weights of the β-structure. The distribution functions P_N, the transition matrix W and the other statistical mechanical properties of this system depend on the values of these parameters. The equilibrium transition curve is sharper for smaller v, α and μ. In the usually investigated helix–coil transition model, v is fixed at a value from 10^{-1} to 10^{-4}. Since the parameters v, α and μ are more insensitive than w and λ for a change of the environmental condition, the values of these parameters v, α and μ are assumed to be fixed for change of the environmental condition, and are put tentatively as $v = 0.01$, $\alpha = 1.5$ and $\mu = 0.05$.

If we take $w = 2.0$ and $\lambda = 1.477$, the calculated average numbers are $\theta_\alpha = 14.56$ and $\theta_\beta = 0.93$ at equilibrium with $N = 20$, therefore, the system is in almost α-helical structure. Thus, $w = 2.0$ and $\lambda = 1.477$ correspond to the environmental condition C_α in fig.1. Similarly, $w = 0.6$ and $\lambda = 0.651$ correspond to C_c, since $\theta_\alpha = 0.17$ and $\theta_\beta = 1.22$, and the system is in the almost random coil (unordered) state. That is, the changes in the environmental condition from C_c to C_α and vice versa give rise to conformational transitions from almost random coil state to almost α-helical structure and vice versa, respectively. They are represented by arrows 1 and 1′ in fig. 1, respectively. The difference from the usually investigated helix–coil transition model is that this system can contain three types of states simultaneously during the conformational changes.

In fig. 2, conformational changes in the present model between almost helical and almost random coil structure are described graphically by the trajectories of the average numbers $\theta_\alpha(t)$ and $\theta_\beta(t)$ of the α-helix and β-structure, respectively. Here, A and C represent the states of polypeptides at equilibrium for the environmental condition C_α and C_c, respectively. The time courses of the averages $\theta_\alpha(t)$ and $\theta_\beta(t)$ apparently indicate that the pathways are not the same for different directions of the conformational changes, and renaturation from the almost random coil to the almost helical state proceeds in two steps. That is, first, the β-structure which is not stable and not found at equilibrium is created rapidly from a random coil region. Second, the β-structure becomes broken slowly and the α-helical structure begins to be formed. Subsequently, renaturation of the helical structure is completed and it reaches the equilibrium state. This conformational rearrangement occurs more slowly than the first step of this renaturation. That is, it indicates that the force which has constructed the β-structure cannot keep this intermediate conformation stable under inces-

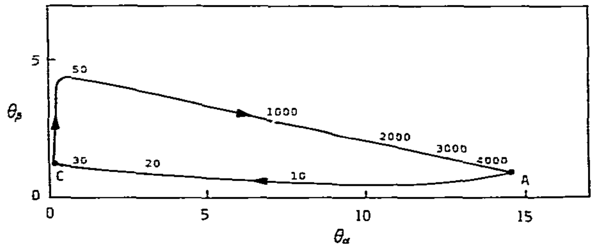

Fig. 2. The trajectory of the average numbers of α-helical sites and β-structure sites for the conformational change from the random coil to α-helical structure and vice versa, with $N = 20$, $v = 0.01$, $\alpha = 1.5$ and $\mu = 0.05$. Two filled circles, A and C, represent the average numbers of the α-helix and β-structure at two equilibrium states. Two equilibrium states are: A, $\theta_\alpha = 14.56$, $\theta_\beta = 0.93$ with $w = 2.0$ and $\lambda = 1.477$; and C, $\theta_\alpha = 0.17$, $\theta_\beta = 1.22$ with $w = 0.6$ and $\lambda = 0.651$. The values on the curves indicate the time(s).

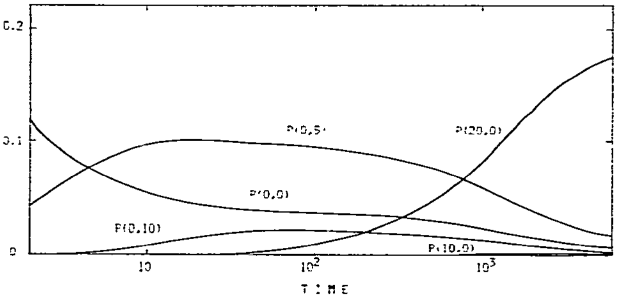

Fig. 3. Time dependence of $P_N(m_\alpha, m_\beta)$, the probabilities of individual conformations for the same conformational changes as shown in fig. 2.

sant thermal fluctuations and leaves it destroyed finally.

The time dependence of several conformational probabilities, $P_N(m_\alpha, m_\beta)$, is shown in fig. 3 for the renaturation process from almost random coil to almost α-helical structure as already shown in fig. 2. Here, $P_N(0, 0)$, which is the conformational probability for the complete random coil state with no α-helical and β-structure states, is shown to decrease through this process. In contrast, $P_N(20, 0)$ for the complete α-helical state is increasing. As shown also in fig. 2, the conformational probabilities $P_N(0, 5)$ and $P_N(0, 10)$, which indicate the existence of the β-structure, increase at the intermediate stage and disappear finally. Then we find that the α-helical structure is created after construction of the intermediate β-structure together with its successively occurring destruction.

Conversely, the denaturation from the almost helical state to almost random coil state is simpler and faster than the former conformational change, as shown in fig. 2. It follows that the renaturation occurs biphasically but the denaturation is mostly monophasic. Furthermore, the conformational changes of different directions exhibit themselves in different pathways. The result that the renaturation has more complex and slower kinetics than denaturation is what one can intuitively anticipate.

4. Conformational changes from β-structure to α-helical structure and vice versa

The time behavior of this system is shown in figs. 4 and 5 for the rearrangement process between almost β-structure and α-helical structure. If we take $w = 2.1$ and $\lambda = 2.859$, we have $\theta_\alpha = 0.27$ and $\theta_\beta = 16.12$, and the system is in almost β-structure. If we take $w = 2.8$ and $\lambda = 2.271$, we have $\theta_\alpha = 18.14$ and $\theta_\beta = 0.44$, and the system is in almost α-helical structure. We take the same values of the parameters v, α, μ as we took in section 3. The values of (w, λ) chosen above correspond to the environmental conditions C_β and C_α, respectively, in fig. 1. Therefore, the changes of the environmental condition from C_β to C_α and vice versa give rise to conformational changes from almost β-structure to almost α-helical structure and vice versa, which are represented by arrows 2 and 2′ in fig. 1, respectively.

The time course of the system indicates that the rearrangement from almost β-structure to almost α-helical structure proceeds in two steps. As shown in figs. 4 and 5, some of the β-structures are destroyed, first being rapidly converted into the

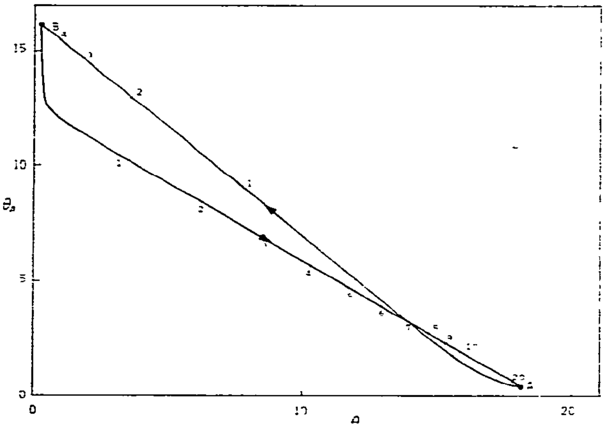

Fig. 4. The trajectory of the average numbers θ_α and θ_β for the rearrangement between the helical structure and β-structure with $v = 0.01$, $\alpha = 1.5$ and $\mu = 0.05$. Two equilibrium states are: A, $\theta_\alpha = 18.14$, $\theta_\beta = 0.44$ with $w = 2.8$, $\lambda = 2.271$; and B, $\theta_\alpha = 0.27$, $\theta_\beta = 16.12$ with $w = 2.1$, $\lambda = 2.859$. The values on the curves indicate the time (s) ($\times 10^{-3}$).

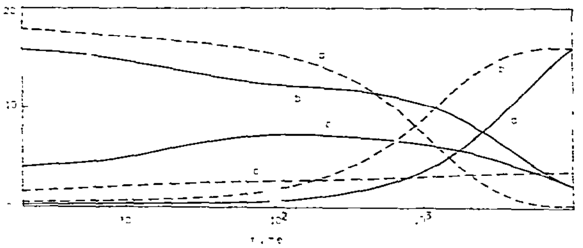

Fig. 5. Time dependence of the average numbers, $\theta_\alpha(t)$, $\theta_\beta(t)$ and $\theta_c(t)$, of α-helix, β-structure and random coil, a, b and c, respectively, for the same rearrangements as shown in fig. 4, i.e., the rearrangements for helix-to-β-structure (— — —) and for β-structure-to-helix (———). Time in s.

random coil, and the α-helical nuclei begin to appear in the resulting coil regions. Second, small helical nuclei grow or merge with each other and finally the chain reaches the almost helical structure. In fig. 5 we find that at the intermediate stage of this rearrangement the average number of the random coil state increases and finally decreases. The increase in the α-helical state follows the decrease in the random coil state. On the other hand, the conformational change from almost α-helical structure to almost β-structure is just the rearrangement between α-helix and β-structure states. That is, as shown in fig. 5, the average number of the β-structure is increasing with the decrease in the average number of the α-helix. During this conformational change the average number of the random coil state does not increase as much.

These schemes of the rearrangements of this system depend on the values of the parameters, v, α and μ. The different values of these parameters will give rise to the different time behavior. In fig. 6, we take different values of α and μ, $\alpha = 1.822$ and $\mu = 0.212$, for the β-structure with weaker cooperativity. In this system the rearrangement from almost β-structure to almost α-helical structure has the same property as that of the system shown in figs. 4 and 5. But, in contrast to the case in fig. 4, the rearrangement from almost α-helical structure to almost β-structure takes place in two steps. This is interpreted as follows: firstly, the β-structure nuclei are created rapidly with the decrease in the α-helical regions and the random coil regions. Secondly, as the α-helical regions decrease more slowly, the β-structure regions and the random coil regions increase, and finally the chains reach the almost β-structure. Therefore, the rearrangements between the α-helical state and the β-structure state occur biphasically in both ways, but the rearrangements in different directions exhibit themselves in different pathways.

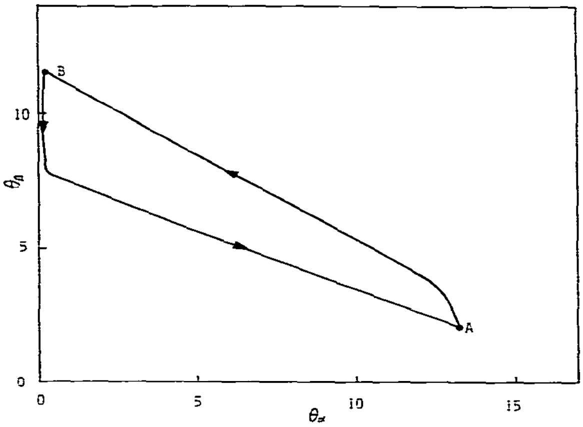

Fig. 6. The trajectory of the average numbers θ_α, θ_β in the same rearrangement as in fig. 4, with $v = 0.01$, $\alpha = 1.822$ and $\mu = 0.212$. Two equilibrium states are: A, $\theta_\alpha = 13.69$, $\theta_\beta = 2.27$ with $w = 2.75$ and $\lambda = 1.25$; and B, $\theta_\alpha = 0.31$, $\theta_\beta = 11.68$ with $w = 2.60$ and $\lambda = 2.00$.

5. Conformational changes from random coil to β-structure and vice versa

In section 3 we have shown that the β-structure is formed as an intermediate of the conformational change from the random coil to α-helical structure and finally disappears. In contrast, in the conformational change from the random coil state to β-structure state with the same values of parameters, the distinct intermediate conformation (α-helix) does not appear and the conformational change exhibits simple kinetic behavior.

Thus, we try to study the time behavior of the conformational change in which the α-helical intermediate exists, for different values of the

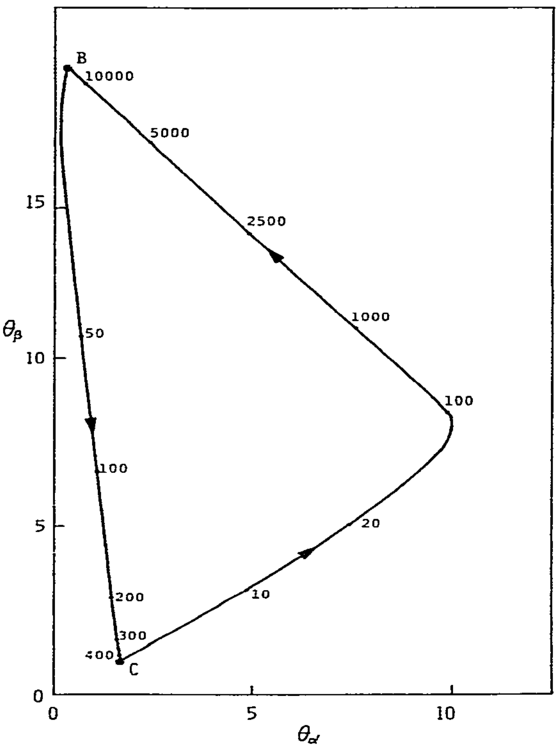

Fig. 7. The trajectory of the average numbers θ_α and θ_β for the conformational change from the random coil to β-structure and vice versa with $v = 0.1$, $\alpha = 0.03$ and $\mu = 0.05$. Two equilibrium states; C, $\theta_\alpha = 1.679$, $\theta_\beta = 0.987$ with $w = 0.02$, $\lambda = 1.90$; and B, $\theta_\alpha = 0.288$, $\theta_\beta = 18.686$ with $w = 2.00$, $\lambda = 4.43$. The values on the curves indicate the time (s).

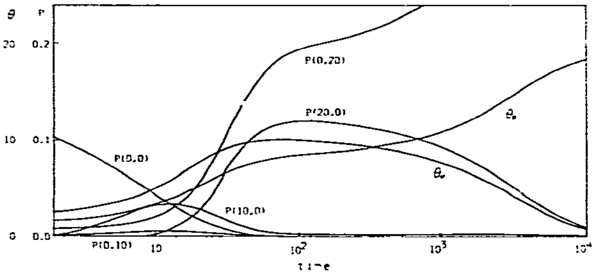

Fig. 8. Time dependence of θ_α and θ_β, the average numbers of α-helix and β-structure, respectively, and $P_N(m_\alpha, m_\beta)$, the probabilities of individual conformational states in the same conformational change as in fig. 7.

parameters, $v = 0.1$ and $\alpha = 0.03$. The larger value of v (in the previous case we put $v = 0.01$) makes formation of the α-helix easier, and the smaller value of α (in the previous case, $\alpha = 1.5$) makes nucleation of the β-structure more difficult than that in the previous case. The conformational changes from the random coil state to β-structure and vice versa are represented by arrows 3 and 3′ in fig. 1, respectively.

The time course of the average numbers of the α-helix and β-structure and of several conformational probabilities $P_N(m_\alpha, m_\beta, t)$ is shown in figs. 7 and 8. Then we can find the different rearrangement type in these figures.

In the renaturation process from the random coil to the almost β-structure, the average number of α-helical sites increases temporarily as that of β-structure sites increases, and finally the α-helical sites disappear. That is, in this case the α-helical structure is an intermediate of the conformational change. In addition to this property, fig. 8 indicates that the conformational probabilities $P_N(10, 0)$ and $P_N(20, 0)$ which show the existence of the α-helical chain in the absence of the β-structure increase temporarily and disappear finally. Therefore, it indicates that at the early stages of this renaturation process the α-helical chain and the β-structure chain coexist, subsequently with time the α-helical segments are destroyed and finally almost all chains reach the β-structure state. Also, in this case formation of the intermediate, the α-helical segments, occurs faster than their destruction, and the renaturation process occurs biphasically. In contrast, the denaturation is much simpler and faster, and it follows that the conformational changes of different directions exhibit themselves in different pathways.

6. Discussion

The model which is capable of forming three types of conformational states, α-helix, β-structure, random coil, is developed here with the consideration of the mechanism of protein folding. We have studied the time behavior of the system after a finite perturbation of the environmental condition by following the average numbers of α-helices and β-structure. We have found some dynamic properties different from those envisaged by the equilibrium properties of the conformational transitions. Detailed features depend strongly on the environmental conditions.

First, at the early stages of the renaturation process the intermediate which is not found and not stable at equilibrium is formed and finally disappears. On the other hand, in the denaturation process the intermediate does not appear, and the conformational changes proceed monophasically (figs. 2 and 7).

Second, due to the temporal existence of the intermediate, the conformational change exhibits biphasic kinetics. That is, the conformational change from the random coil state to the intermediate occurs very rapidly and the rearrangement from the intermediate to the conformational state which is stable at equilibrium occurs more slowly. This kinetic scheme indicates the existence of the fast phase and the slow phase in this conformational transition (figs. 2 and 7).

Third, the conformational changes of different directions, i.e., denaturation and renaturation, exhibit themselves in different pathways and the renaturation occurs more slowly and more complexly than the denaturation (figs. 2 and 7). These kinetic properties are what one can intuitively anticipate for the conformational change of polypeptides and globular proteins.

Fourth, the rearrangements between the α-helical structure and β-structure occur almost biphasically without an intermediate such as that mentioned above. This is attributed to the differences in the speed of formation and destruction of the α-helix and β-structure (fig. 4).

Fifth, the dynamic properties of the conformational change are dependent on how the system passes through, on the way to equilibrium, the intermediate states in the conformational space. In effect, the path of a dynamic system avoids the intermediate states of high free energy and thus affects the dynamic properties. For this reason, the dynamic approach to the conformational transition is necessary in order to understand the dynamic aspect of protein folding.

In the process of protein folding, it is usually emphasized that nucleation, in the early stages of the folding, is rate limiting. As shown in the present model, however, it is quite possible that if nucleation of the certain local ordered structure is difficult, some of the segments in the chain search for another local structure which is easily formed as a nucleus although such a local structure cannot be stabilized by itself. Therefore, the local structure is destroyed by thermal fluctuations. In one case of the present calculations, the intermediate is the β-structure which is easily formed as a nucleus and is destroyed finally. As Richards and Richmond [10] have pointed out, the β-structure has less solvent accessibility than the α-helix has in globular proteins, therefore, it seems that hydrophobic interaction acts as the force constructing this local β-structure.

If one regards the polypeptide chain treated in the present model as a part of the segments in the protein molecule, it is likely that the intermediate which is not stable at equilibrium through the 'intra-segment' interaction acquires stability owing to medium- and the long-range interactions resulting from the close contact between segments in its vicinity. In this way, the local ordered structure can be formed in the kinetic pathway rather than being due to the stability of its structure. If such a situation exists in real proteins, the accuracy of predicting the secondary structure depends on whether the secondary structure can be constructed with or without the medium- and the long-range interactions.

The simple model discussed here offers a possibility of interpreting the multistate kinetics of protein folding by the existence of kinetic intermediates which are not observed in the native conformation of globular proteins. Furthermore, it is evident that the renaturation process takes a characteristically different pathway from that of the denaturation process. We can carry out similar calculations on the effects of compositional heterogeneity using the same kinetic model, but the details of these studies will be left for future investigations.

References

1 T.E. Creighton, J. Mol. Biol. 113 (1977) 295.
2 H. Wakana, T. Shigaki and N. Saitô, Biophys. Chem. 16 (1982) 275.

3 H. Wakana and N. Saitô, in Proceeding of the 6th Taniguchi International Symposium, ed. S. Ishiwata (Taniguchi Foundation, Osaka, 1980).
4 M.I. Kanehisa and T.Y. Tsong, J. Mol. Biol. 124 (1978) 177.
5 M.I. Kanehisa and T.Y. Tsong, Biopolymers 18 (1979) 2913.
6 D. Poland and H.A. Scheraga, Theory of helix–coil transitions in biopolymers (Academic Press, New York, 1970).
7 F. Kanô, J. Phys. Soc. Jap. 41 (1976) 219.
8 K. Hayakawa, K. Ohara and I. Satake, Chem. Lett. (1980) 647.
9 C. Tanford, Ciba Found. Symp. 7 (1972) 125.
10 F.M. Richards and T.J. Richmond, Ciba Found. Symp. (1978) 23.