

Viscosity Dependence of the Folding Rates of Proteins

D. K. Klimov and D. Thirumalai

Institute for Physical Science and Technology and Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742

(Received 10 February 1997)

The viscosity (η) dependence of the folding rates for four sequences (the native state of three sequences is a β sheet, while the fourth forms an α helix) is calculated for off-lattice models of proteins. Assuming that the dynamics is given by the Langevin equation, we show that the folding rates increase linearly at low viscosities η , decrease as $1/\eta$ at large η , and have a maximum at intermediate values. The Kramers' theory of barrier crossing provides a *quantitative* fit of the numerical results. By mapping the simulation results to real proteins we estimate that for optimized sequences the time scale for forming a four turn α -helix topology is about 500 ns, whereas for β sheet it is about 10 μ s. [S0031-9007(97)03573-4]

PACS numbers: 87.15.-v

Based on several theoretical studies of minimal models of proteins, a novel conceptual framework for understanding the folding kinetics of proteins [1–5] and, in general, biomolecules [6] has recently emerged. The basis of this new framework lies in the observation that the polymeric nature of proteins together with the presence of conflicting energies (arising from the differences in the structural preferences of hydrophobic and hydrophilic residues) lead to topological frustration—a situation where structures, which are favorable on relatively short length scales, are in conflict with the global free energy minimum, namely, the native state of the protein. Because of topological frustration, the underlying free energy landscape has, besides the dominant native basin of attraction (NBA), several competing basins of attraction (CBA). Theoretical studies of folding kinetics in such a complex energy landscape suggest that, in general, the folding of biomolecules takes place by multiple pathways rather than by a hierarchical organization. Recent experiments lend support to the “new view” [1–6] of the folding of biomolecules [7].

One of the important theoretical predictions is that the mechanisms of protein folding can be varied depending not only on the intrinsic sequence properties but also on external conditions (such as pH , salt concentration, viscosity, etc.) [3,5,6]. The theoretical studies to date have focused on the temperature dependence of folding rates using minimal models of proteins. The purpose of this paper is to examine the dependence of the rates of protein folding on viscosity, η (or, equivalently, the friction coefficient ζ), and to provide a picture of the folding process in terms of the free energy landscape classified using NBA and CBA. Although there are a few experimental studies that have probed the dependence of the folding rates on viscosity [8], they have not been systematic enough to reveal the underlying folding mechanisms.

We use continuum minimal model representation of the polypeptide chain and Langevin dynamics to compute the folding rates as a function of viscosity. The major results of this study, which were obtained by examining four

sequences each with either a β sheet or α helix as the native state, are as follows:

(a) The folding rate k_F for the formation of a β sheet or an α helix increases linearly with η at low viscosities reaching a maximum at moderate values of η and starts to decrease as $1/\eta$ at higher viscosities.

(b) By assuming that the typical free energy barrier to folding scales as $\sqrt{M} k_B T_s$ [5] (T_s is the simulation temperature and M is the number of beads in the sequence), we find that the Kramers' expression for barrier crossing [9] (with the frequencies at the bottom and at the barrier height of an appropriate reaction coordinate as adjustable parameters) gives a *quantitative* fit of the simulation results. This implies that, at least for small proteins with simple native state topology, a low-dimensional (or even one) reaction coordinate can adequately describe the folding process [10].

(c) For fast folding sequences, i.e., those that essentially display two-state folding kinetics, the fraction of molecules that reaches the native state rapidly without being trapped in CBA, namely, the partition factor Φ , is close to unity and is *independent* of viscosity. For slow and moderate folders, Φ depends on η , implying that viscosity can be used to alter the mechanisms of protein folding.

The polypeptide chain is modeled by a sequence of M connected beads, each of them corresponding to, perhaps, a blob of actual α carbons [11]. The chain conformation is determined by the vectors $\{\vec{r}_i\}$, $i = 1, 2, \dots, M$. Although real polypeptide sequences are made from twenty amino acids, it has been shown that three letter code sequences (i.e., sequences of residues of three types) can faithfully mimic certain properties of real proteins [11]. Accordingly, we assume that the protein sequence is made of hydrophobic (B), hydrophilic (L), and neutral (N) residues. In these models a sequence is specified by the precise way in which the B , L , and N beads are connected together.

Following our earlier work [11], the energy of a conformation is taken to be the sum of the bond-stretch

potential, bond-angle potential, potential associated with the dihedral angle degrees of freedom, and nonbonded potential, which is responsible for tertiary interactions. The details of the potentials and the compositions of the three sequences labeled E , G , and I with a β sheet as the native state are given elsewhere [11]. The potential energy function and sequence composition for sequence H with the α helix as the native conformation are the same as in a previous study [12]. The parameters in the dihedral angle potential $V(\phi) = A(1 - \cos \phi) + B(1 + \cos 3\phi) + C(1 - \sin \phi)$ are taken to be $A = 1.0\epsilon_h$, $B = 1.6\epsilon_h$, and $C = 2.0\epsilon_h$, respectively, where the parameter $\epsilon_h \approx 1-2$ kcal/mol is the average strength of the hydrophobic interaction. These parameters differ from the ones used earlier [12]. The native conformations for sequences I and H , which are determined by the methods described in [11], are displayed in Fig. 1.

The choice of sequences was dictated by the following considerations. It has been shown [11] that the kinetic accessibility and the associated thermodynamic stability of the native conformation for minimal protein models correlate extremely well with $\sigma = (T_\theta - T_F)/T_\theta$ ($0 \leq \sigma \leq 1$), where the two characteristic temperatures intrinsic to the sequence, T_θ and T_F , are the collapse and folding transition temperatures, respectively. Sequences with relatively small values of σ reach the native conformation very rapidly without being trapped in any detectable intermediates, whereas those sequences with

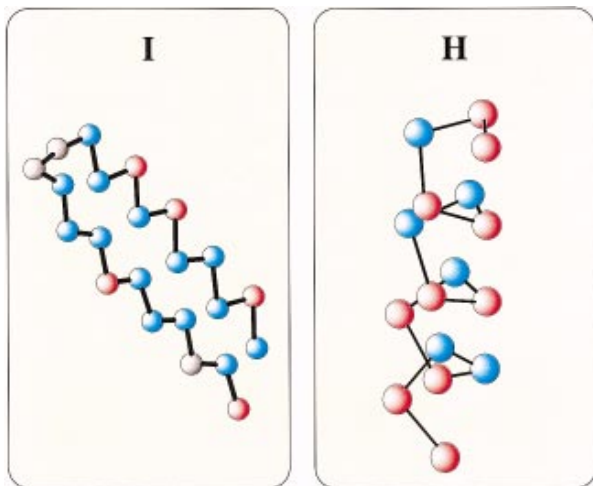


FIG. 1(color). The conformations of the native state for sequences I (β sheet, left panel) and H (α helix, right panel). The sequences consist of hydrophobic B residues (shown in blue), hydrophilic L residues (shown in red), and neutral N residues (shown in grey). In the β sheet, neutral residues are near the loop region, where the dihedral angles can adopt g^+ ($\approx 60^\circ$), t ($\approx 180^\circ$), or g^- ($\approx -60^\circ$) positions, where g^+ , t , and g^- are the three minima in the dihedral angle potential [11]. The dihedral angles in the β strands are in the t conformation. The β sheet is stabilized by the attractive hydrophobic B - B interactions. All the dihedral angles in helical structure are in g^+ positions. The number of beads (residues) in one turn of the helix is 3.9. These structural features are in accord with those seen in real proteins.

large σ have several CBA that act as kinetic traps [11]. Two of the sequences, G and I , have relatively small values of σ [11] (0.20 and 0.14, respectively) and hence are fast folders, which implies that in excess of 90% of the molecules reach the native conformation on the time scale in which collapse and formation of the native state are almost synchronous [5]. Sequence E is a moderate folder, while H is a slow folder (with $\sigma = 0.39$ and 0.75, respectively). Since these four sequences involve the two common structural motifs in proteins and span a range of σ , meaningful conclusions regarding the viscosity dependence of small proteins can be drawn.

We assume that the Langevin equation provides an adequate description of the polypeptide chain dynamics. Since our goal is to study the dependence of the folding rate as a function of viscosity over a wide range, we are forced to use different algorithms, depending on the precise value of the viscosity η or the friction coefficient $\zeta (= 6\pi\eta a)$. In the low ζ limit, corresponding to the energy diffusion regime, the inertial terms are important, and we use the noisy molecular dynamics [11]. At higher values of ζ we use the Brownian dynamics algorithm of Ermak and McCammon [13]. We have verified that both of the algorithms give *identical* results for folding rates in the intermediate range of ζ . For the sake of consistency, we measure time in units of $\tau_L = (ma^2/\epsilon_h)^{1/2}$, where m is the mass of a bead and a is the bond length between successive beads.

The external conditions in the simulations are ζ and temperature T . Since we focus here on the variation of the folding rate with ζ , it is desirable to choose sequence dependent simulation temperatures T_s so that the extent of the native conformation (given by the structural overlap function χ , which measures the similarity of a given conformation to the native state [11]) is the same for all sequences. The simulation temperature, T_s , is chosen so that (i) $T_s < T_F$ and (ii) $\langle \chi(T_s) \rangle = \alpha$ would be the same for all sequences with a given native topology. The condition $T_s < T_F$ ensures that the native conformation has the largest occupation probability, while the second condition (ii) allows us to subject the sequences to similar folding conditions. With the assumption that $\alpha = 0.26$, T_s (measured in the units of ϵ_h) turns out to be 0.29, 0.37, and 0.41 for sequences E , I , and G , respectively [11]. For sequence H we took α to be 0.32, and the resulting T_s is 0.24 (details to be published elsewhere).

The folding rate is calculated as

$$k_F = \frac{1}{N_{\max}} \sum_{i=1}^{N_{\max}} \frac{1}{\tau_{1i}}, \quad (1)$$

where τ_{1i} is the first passage time (the first time a given trajectory reaches the native conformation) for the trajectory i , and N_{\max} is the maximum number of trajectories used in the simulations. The value of N_{\max}

ranges from 200–600, depending on ζ and the sequence, which gives well-converged results for k_F . In Fig. 2 we plot the ratio k_F/k_{TST} as a function of ζ , where k_{TST} is the transition state estimate of the folding rate. The calculation of k_{TST} is described in the caption for Fig. 2. The top, middle, and lower panels correspond to sequences *G*, *E*, and *H*, respectively. It is clear that the folding rate increases (roughly linearly) at low ζ and decreases as ζ^{-1} at higher viscosity. There is a maximum at moderate values of ζ .

The remarkable similarity of the dependence of the rate of folding to the predictions of Kramers' theory for barrier crossing [9] suggests that for proteins with simple native state topology a suitable one-dimensional reaction coordinate suffices. In fact, the simple one-dimensional Kramers' theory can be used to analyze our results quantitatively. According to Kramers' theory the rate for barrier crossing in the moderate to high viscosity

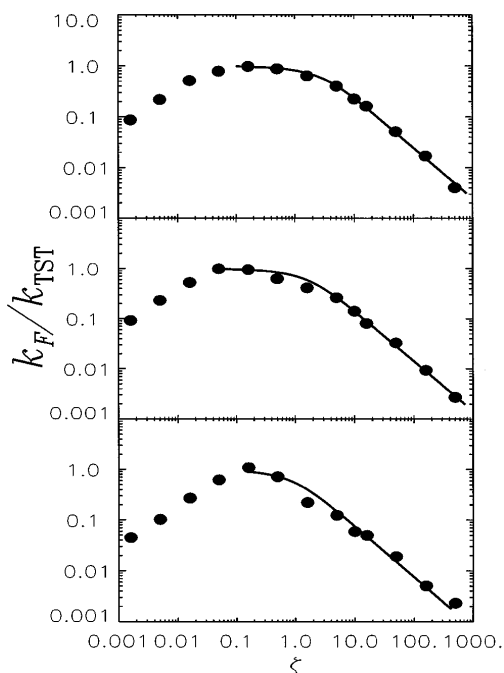


FIG. 2. The ratio of the folding rate to that of the transition state value as a function of ζ . The top, middle, and bottom panels correspond to sequences *G*, *E*, and *H*, respectively. Sequences *G* and *E* have a β sheet as a native state, and the native conformation for sequence *H* is an α helix. The solid lines are the fit using the Kramers' expression for barrier crossing [cf. Eq. (2)]. The free energy barrier in Eq. (2) is taken to be $\sqrt{M}T_s$ so that ω_A/ω_B and ω_B are used as fitting parameters. From the best fit, the transition state estimate of the folding rate is calculated using $k_{\text{TST}} = \omega_A \exp(-\sqrt{M})/2\pi$. For sequences *G* and *H*, the fit is done using nine data points with $\zeta \geq 0.16$, while for sequence *E* ten data points are used with $\zeta \geq 0.05$. The most accurate least squares fit for sequences *G*, *E*, and *H* give [1.86 (0.01), 2.47 (0.01)], [1.95 (0.08), 1.43 (0.03)], and [3.78 (0.02), 0.75 (0.002)], respectively. The set of the numbers in the square brackets corresponds to ω_A and ω_B , and the numbers in parentheses are error estimates. Viscosity for water gives $\zeta = 50$.

regime is given by [9,14]

$$k_{\text{KR}} = \frac{\omega_A}{2\pi\omega_B} \left(\sqrt{\frac{\zeta^2}{4} + \omega_B^2} - \frac{\zeta}{2} \right) \exp\left(-\frac{\Delta F}{T}\right), \quad (2)$$

where ΔF is the typical barrier height that the protein overcomes en route to the native state, and ω_A and ω_B are the frequencies at the minimum and saddle (transition state [15]) points of a suitable undetermined one-dimensional reaction coordinate describing the folding process. One of us has argued that the typical free energy barrier in the folding process scales as $\Delta F \approx \sqrt{M}T_s$ [5,16,17]. If this result is used, then there are two parameters in k_{KR} , namely, ω_A/ω_B and ω_B , which can be used to fit the simulation results. The solid lines in Fig. 2 show the results of such a fit. It is clear that Eq. (2) fits the data quantitatively. The numerical values of ω_A and ω_B (see caption to Fig. 2) for the four sequences (data for sequence *I* not shown) suggest that the barriers for slow folders are, in general, flatter than for fast folders.

The quantitative description of the simulation results by the Kramers' theory shows that (a) the assumption of a one-dimensional reaction coordinate for folding of proteins with simple native state topology is appropriate [10]. (Furthermore, it appears that in the energy diffusion regime an appropriate folding reaction coordinate couples rather weakly to the other degrees of freedom. This is supported by the notion that in nearly all the sequences examined once a critical number of native contacts is established a rapid acquisition of the native state takes place.) (b) The typical barriers in the folding process scale sublinearly with M , the number of amino acids, and is adequately given by $\sqrt{M}T_s$. (c) The transition state estimate for the folding rate in the viscosity regime of experimental interest is at least 2 orders of magnitude less than the actual rate.

In our earlier studies we have shown that, due to topological frustration, the refolding of proteins follows the kinetic partitioning mechanism (KPM) [5,6,11]. It is of interest to compute the partition factor Φ , which gives the yield of native molecules that arrive rapidly without being trapped in any intermediate, as a function of ζ . Using the distribution of first passage times, Φ can be easily obtained [11]. The partition factor Φ shows no significant variation for the fast folding sequences *G* and *I* (data not shown). The dependence of Φ on ζ for sequence *E* is displayed in Fig. 3. Similar results are obtained for the α helix. This figure shows that, as suggested earlier [5,6], the generic feature of KPM, namely, that, for foldable sequences, a fraction of molecules Φ (determined by σ) reaches the native conformation rapidly, remains valid for all values of ζ . Just as the rate of folding itself, Φ also shows a nonmonotonic behavior. Although there is no systematic trend in the variation of Φ with ζ , the sequence *E* tends to behave as a fast folder ($\Phi \geq 0.9$) at the higher and lower values of ζ .

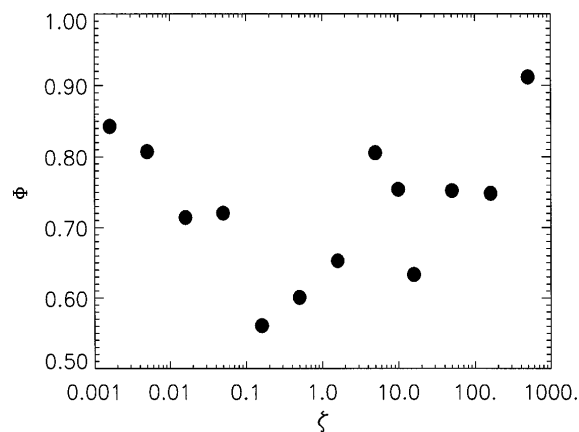


FIG. 3. The partition factor Φ , which gives the fraction of fast folding trajectories, as a function of ζ for sequence E (a moderate folder). Folding pathways strongly depend on ζ leading to a dramatic variation in Φ . For example, at $\zeta = 500$ this sequence may be classified as a fast folder ($\Phi > 0.9$), while at all other ζ it is a moderate folder. There is no dependence of Φ on ζ for fast folding sequences (data not shown).

The large variation of Φ with ζ for moderate and slow folders suggests that pathways by which the polypeptide chain reaches the native state can be altered significantly by changing η . In order to probe this we generated 200 distinct initial conditions at $\zeta = 5.0$ for sequence E . At this value of ζ we find $\Phi \approx 0.8$, and, accordingly, we determined that there are 44 trajectories that get trapped in the CBAs. Using exactly the same initial conditions, we altered ζ to 0.16 and discovered that, out of the 44 slow folding trajectories, 20 of them became fast folding, indicating the dramatic change in pathways with the alteration of viscosity. Similar results were obtained for the 156 fast folding trajectories at the higher ζ . Note that Φ is a dynamic quantity, and the conservation of the number of denatured molecules requires only that the sum of amplitudes of fast and all slow folding phases be constant. An important experimental consequence of this result is that the folding scenarios can be dramatically changed by varying ζ , so that a sequence that appears to be the fast folder at one value of ζ may be a moderate folder at a different viscosity.

The results in Fig. 2 may be used to obtain time scales of the folding of small proteins. The friction coefficient ζ corresponding to water ($\eta = 0.01$ poise at $T = 25^\circ\text{C}$) is roughly $6\pi\eta a$, and this corresponds to $\zeta \approx 50$ in reduced units with $a \approx 5 \text{ \AA}$. In this range of ζ values the inertial terms are irrelevant, and the natural measure of time is $\tau_H = \zeta a^2 / k_B T_s$, which for water turns out to be about 3 ns [11]. Using this mapping, we find that the time constant for the formation of β sheet ranges from 0.03–0.1 ms, depending on the value of σ . A similar calculation for sequence H shows that the time scale for the formation of a short α helix, containing about four turns, is about 10 μs . It should be noted that the α helix

in our study is not well optimized, since $\sigma = 0.75$ is relatively large. Well-optimized sequences have σ values in the range of 0.3 or less [11]. If we assume that folding time scales roughly as σ^3 [5], then we predict that an optimized helical sequence with four turns would fold in almost 500 ns or 0.5 μs .

Our theoretical predictions can be verified by experiments on folding kinetics in the viscosity regime $\eta_{\text{water}} \leq \eta \leq 10\eta_{\text{water}}$. The prediction that the yield of the fast folding process (namely, Φ) for moderate and slow folders can be drastically altered by changing viscosity is amenable to experimental tests.

This work was supported in part by a grant from the National Science Foundation (CHE96-29845).

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 - [15] In proteins there are multiple transition states so that ω_B (and ω_A as well) will be functions of χ . For simplicity these effects are not considered.
 - [16] It was argued in [5] that the free energy barrier scaling as \sqrt{M} holds good only for foldable sequences. This result is expected to be valid only over a temperature range in which the folding rate is optimal. At very low temperatures the free energy barrier, even for foldable sequences, could scale as M .
 - [17] We have also used ΔF as a fitting parameter and find that the resulting three parameter fit gives a value very close to the theoretical prediction $\Delta F \approx \sqrt{M} T_s$. Higher powers in M do not fit the data well.