A Limiting Speed for Protein Folding at Low Solvent Viscosity

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The increasing numbers of proteins that are discovered to fold at "ultrafast" rates ($\sim 10^4$ s^{-1} or greater) raise the question of what physical phenomena set the ultimate limits to the speed of folding. Because folding involves microscopic motions in a viscous environment, it is necessarily a heavily damped, diffusional process. Many researchers have therefore investigated "speed limits" for folding that arise from the finite rate of bulk diffusional motions (e.g., contact formation) in disordered or unfolded polypeptides.[1] These studies find the simplest structures forming at rates exceeding 10^7 s^{-1}. Here we show that internal interactions within the polypeptide may introduce additional limits to folding speed.

Kramers theory for unimolecular reactions asserts that the rate k for a barrier-crossing process subject to strong damping friction will vary as $k \propto \gamma^{-1} \exp(-\Delta G_a/RT)$, where ΔG_a is the barrier height and γ is the friction.[2] If the friction in protein folding arises from η_s (the dynamic viscosity of the solvent) then folding rates should scale as $k_f \propto 1/\gamma \propto 1/\eta_s$. Yet one cannot expect k_f to grow without bound as η_s declines: although folding would remain strongly damped even if η_s fell by orders of magnitude, dissipative intrachain interactions within the polypeptide should eventually take control of the dynamics. These "internal friction" effects[3] would limit the overall folding rate and cause a deviation from simple $k_f \propto \eta_s^{-1}$ behavior. Although some deviation may occur in nanosecond helix formation,[4] several experimental studies found no significant deviation in folding dynamics.[5] This has led to some puzzlement over why internal friction does not appear to influence protein folding dynamics, even in proteins that fold through rather compact transition states.

If internal friction places a limit—although perhaps a very weak one—on folding speed, we expect it to become more evident in the fastest folding proteins. We used laser temperature-jump spectroscopy to measure the effect of solvent viscosity on the folding kinetics of the "Tryptophan Cage" TC5b (PDB 1L2Y). TrpCage is a designed protein that, despite having only 20 residues, folds to a compact native state with α-helical secondary structure and a hydrophobic core.[6] By adding glucose (0–2 M) to the folding buffer, we can raise the dynamic viscosity η_s of the solvent by ~ 1–$4\times$ and examine the coupling between solvent friction and the speed of the exceedingly rapid two-state folding of the TrpCage ($k_f > 2.5 \times 10^5$ s^{-1} at room temperature[7]). To compensate for the stabilizing effects of viscogen on the protein, we simultaneously add denaturant (GdnHCl) with the glucose, to maintain a constant unfolding ΔG over the range of viscosities studied.

By observing the fluorescence of Trp-6 that follows a thermal perturbation, we detect the folding/unfolding reequilibration of the protein. Figure 1 shows that, for a fixed value of T and ΔG, the relaxation time k^{-1} after perturbation grows linearly with η_s. Although this accords with expectations from Kramers theory, the k^{-1} vs η_s data clearly do not extrapolate through the origin. Instead, the small positive offset or y-intercept in $1/k$ indicates that the relaxation accelerates as solvent viscosity declines, but it ultimately tends toward a finite limit $1/k \approx (500$ ns$)^{-1}$. That is, the barrier-
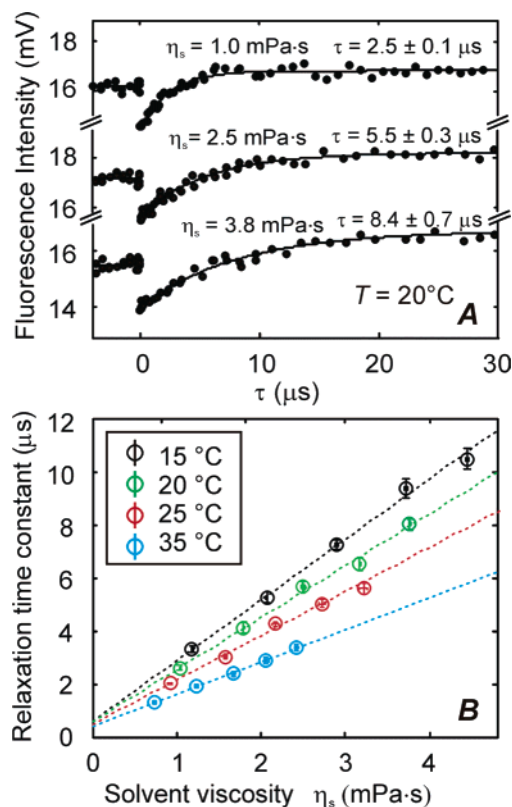

Figure 1. Folding/unfolding kinetics of TrpCage induced by laser T-jump. (A) Trp-fluorescence relaxation after 5 °C jump to 20 °C, for different solvent viscosities η_s. T-jump is induced by a 5–7 ns laser pulse at $t = 0$. Protein is 50 μM in 50 mM phosphate, pH 7, with glucose and GdnHCl to control viscosity and stability ($\Delta G = 3.4$ kJ/mol at 20 °C). Solid curves show exponential fits for relaxation times τ. (B) Relaxation τ (averaged from repeated traces) versus solvent viscosity. Dotted lines are linear fits, indicating finite relaxation rate $k = 1/\tau$ for $\eta_s \rightarrow 0$. Viscosity was directly measured at all conditions: η_s (water) = 1 mPa·s = 1 centipoise at 20 °C. TrpCage TC5b was prepared by solid-phase FMOC synthesis.[7] The laser T-jump instrument has been described previously.[7]

crossing time may scale with the reaction friction γ, but η_s is not the primary contributor to γ when η_s becomes small.

These data have interesting implications for the actual folding (k_f) and unfolding (k_u) rates. Because each of the four k^{-1} vs η_s curves in Figure 1 is collected under isostability conditions, each is characterized by a fixed equilibrium constant $K_{eq} = k_f/k_u$, while $k = k_f + k_u$ (for two-state folding). Therefore the folding and unfolding rates are proportional to k: $k_f = k/(1 + K_{eq}^{-1})$ and $k_u = k_f/K_{eq}$. The folding/unfolding times must, like $1/k$, vary linearly with η_s and extrapolate toward a positive intercept as $\eta_s \rightarrow 0$. Figure 2 shows this behavior in both $1/k_f$ and $1/k_u$. Unlike in previous studies with slower ($\sim$ms) time resolution,[5] a finite limit to both the folding and unfolding rates is clearly evident.

One simple interpretation for the linearity of the folding time $\tau_f = 1/k_f$ is that it reflects two time scales, $\tau_f = \tau_{f1}(\eta_s) + \tau_{f0}$. Here the
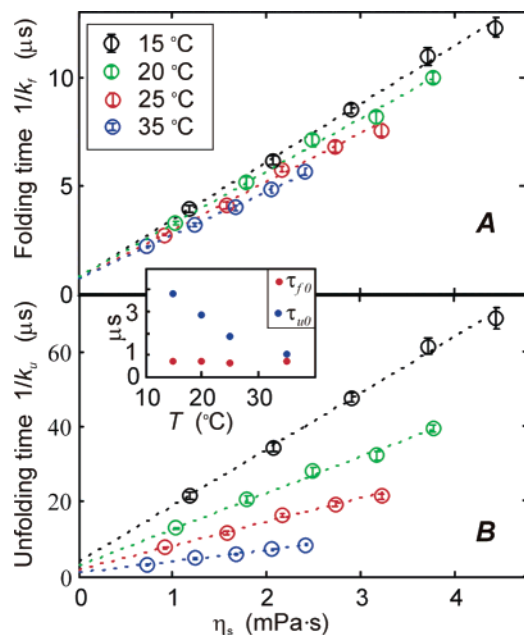
Figure 2. TrpCage folding ($1/k_f$) and unfolding ($1/k_u$) times versus solvent viscosity. (A) Folding time is linear in viscosity, with intercept at $\eta_s \rightarrow 0$ implying a limiting value $\tau_{f0} \approx 680 \pm 40$ ns, independent of T. (B) The unfolding time also extrapolates to a nonzero limit τ_{u0} at low viscosity. Dotted lines are linear fits. (Inset) Limiting time scales τ_{f0} and τ_{u0}, vs T.

first time scale $\tau_{f1}(\eta_s) \propto \eta_s$ represents the diffusive folding dynamics that are primarily controlled by solvent friction. This term shows a Kramers-like dependence on solvent viscosity, and it dominates the folding rate when $\eta_s \geq 1$ mPa·s. The second time scale τ_{f0} indicates a stage of folding that is controlled by frictional interactions that have a physical origin distinct from bulk solvent friction. These could include side-chain interactions, energetic barriers to backbone bond rotations, or the limited accessibility of void space for chain motion through the solvent.[3] The additivity of time scales (rather than rates) implies that the τ_{f0} and τ_{f1} dynamics are primarily sequential, rather than parallel: As solvent viscosity decreases, the process associated with τ_{f0} becomes rate-limiting to folding.

In Figure 2 the limiting time scale for folding, $\tau_{f0} \approx 680 \pm 40$ ns, varies little for $T = 15-35$ °C. This implies a weak activation energy for the associated friction. By contrast, the unfolding time k_u^{-1} tends toward a strongly T-dependent limit τ_{u0} at low viscosity.

An Arrhenius fit gives a substantial activation enthalpy of 50 kJ/mol for τ_{u0} (inset to Figure 2). These results indicate that energetic interactions do give rise to an internal friction that slows the conformational dynamics, but this primarily affects passage out of the compact or folded configuration. The internal friction that affects the folding pathway is qualitatively different.

We find that rapid two-state folding can deviate from a Kramers-like dependence on solvent viscosity. Although the speed of chain diffusion through solvent is often viewed as the physical "speed limit" for folding, nanosecond dynamics that are independent of solvent friction contribute substantially to the folding time when folding becomes sufficiently rapid. This implies that internal friction may impose a rather stringent (i.e., physically significant) limit on folding rates: the practical limits to folding speed may be substantially slower than idealized diffusional limits.[8] Future work, both experiment and simulation,[9] will be needed to understand the microscopic origin of this friction.

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