Diffusional limits to the speed of protein folding: fact or friction?

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Abstract
Proteins fold by diffusional motion, driven by molecular collisions but limited by frictional drag. We then expect that the timescale of simple diffusional motions of the polypeptide chain defines the minimum time, or the maximum rate, for folding phenomena in general. However, such 'speed limits' are very rapid. They far exceed the rate of folding that is observed in even the fastest-folding small proteins. Why do proteins fold much more slowly than the diffusional limits predict? We present experimental evidence that, in addition to solvent friction, internal dissipative forces within a protein can slow the dynamics. These internal friction forces may ultimately set a much more restrictive limit on the speed of folding.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The study of protein folding dynamics has progressed rapidly in recent years. With the development of new experimental techniques for triggering and probing the fastest folding events, advances in the computational methods for simulating folding, and the discovery (or design) of many new fast-folding proteins and peptides, researchers have found that important steps in the folding pathway can occur on timescales much faster than previously believed possible. Although Anfinsen observed the spontaneous folding of ribonuclease to occur over a period of hours [1], many proteins are now known to make the transition from the unfolded to the native configuration within a few microseconds. At this writing, the fastest folding rates seen in the laboratory approach $\sim 10^6$ s^{-1} [2–7], a rate that scarcely seemed possible just a few years ago. Many important elementary steps in folding—such as helix formation—occur even faster [8].

This progressive shortening of the 'interesting' timescale for folding raises the question of what sets the physical limit to the speed of protein folding. The rate of diffusional motion

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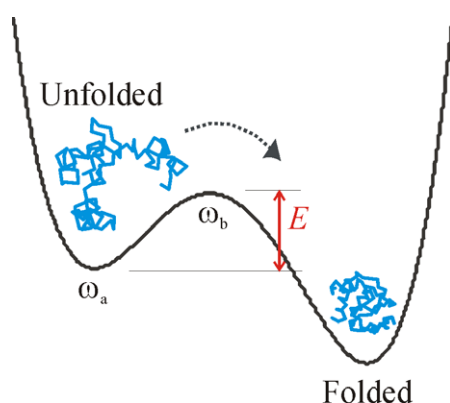[1] Author to whom any correspondence should be addressed.

Figure 1. Two-state, barrier-crossing model for diffusional transition from the unfolded to the folded configuration of a protein [10, 11, 28]. The curvature of the free energy surface is given by ω_a (for the unfolded states) and by ω_b at the barrier top.

of a polypeptide chain is an obvious candidate. However, this diffusional 'speed limit' is quite rapid—it is not at all clear that it represents the most stringent upper limit on folding speed. Here I will present evidence that interactions internal to a polypeptide chain—or 'internal friction' phenomena—set a lower and more significant limit on the speed of fast protein folding.

From an experimentalist's perspective, the dynamics of protein folding can often be interpreted as a two-state, barrier crossing process. That is, we can model the transition to the folded state as the passage of a particle, in one dimension, over a free energy barrier that divides the unfolded configurations from the folded state (figure 1). Furthermore, because the dynamics of a polypeptide chain are subject to very strong frictional damping from the solvent, the passage of this particle across the barrier top is driven by Brownian motion. Then there is no 'transition state' as such, and the rate of crossing is not described by the usual transition state theory. Rather it is more appropriately described by the reaction rate theory of Kramers [9–11]. Kramers theory states that, in the limit of strong frictional damping, the particle may diffuse repeatedly across the top of the barrier, but on average it transits the barrier (of height E) and arrives at the final state at a rate

$$k = (\omega_a\omega_b/2\pi\gamma)\exp(-E/k_BT). \qquad (1)$$

Here ω_a is the curvature of the unfolded well, ω_b is the curvature of the barrier top, and γ is the frictional drag coefficient that appears in the Langevin equation for the particle's motion. If we interpret γ as arising from the dynamic viscosity η_s of the solvent, $\gamma \propto \eta_s$, then Kramers yields the unsurprising prediction that $k \propto 1/\eta_s$, or the folding time for a protein will scale with the solvent viscosity: $k_f^{-1} \propto \eta_s$ [11].

The fact that Brownian motion drives folding suggests that the finite speed of diffusion itself sets the ultimate limit to the speed of protein folding: a protein cannot fold any faster than it can explore (by diffusion) its various configurations [12]. This has led a number of authors to estimate an upper limit on folding speed by measuring the timescale of the simplest reconfigurations of disordered polypeptides. For example, the rate at which end-to-end contacts form in a disordered polypeptide has been measured by several groups [13–17]. This rate depends on the number of residues in the loop, but for short loops (<10 residues) it appears to exceed 10^7 s^{-1} and may approach 10^8 s^{-1}. This implies a diffusional 'speed limit' for protein folding rates, of order $k_f \leqslant \sim 10^8$ s^{-1}.

This is a plausible upper limit, if only because no proteins have yet been found to exceed it. In fact, it may be too generous. It exceeds by $\sim 100\times$ even the very fastest known folding
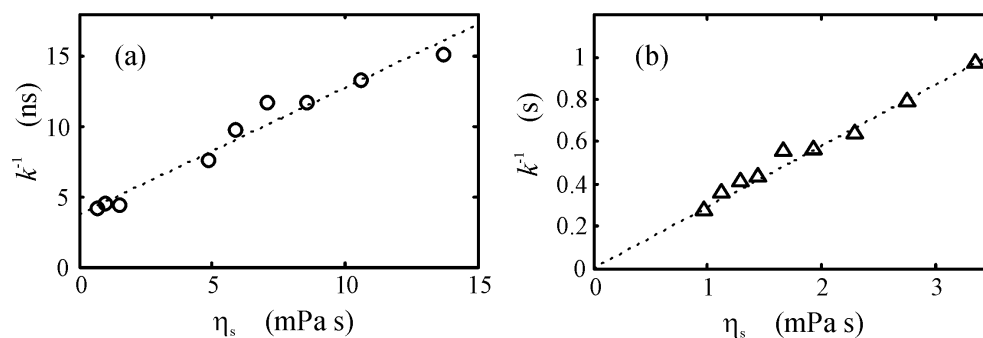
Figure 2. (a) Relaxation time $(1/k)$ for the conformational relaxation of sperm whale myoglobin, following photodissociation of a haem ligand, adapted from Ansari *et al* [20]. Extrapolating the dashed line to the horizontal axis indicates $\sigma \approx 4.1$ mPa s for this relaxation (equation (2)). (b) Folding time $(1/k_{\rm f})$ for *peptostreptococcal* protein L versus solvent viscosity, and linear fit. Data are from Plaxco and Baker [23]. Unlike the myoglobin relaxation data, these folding data are consistent only with a very small $\sigma \approx -0.1 \pm 0.2$ mPa s.

rates. Furthermore, extrapolation from simple correlations, like that between contact order and folding rate [18], suggests that folding rates of small proteins are unlikely to exceed $\sim 10^6$ s^{-1}. Even the collapse of a disordered polypeptide to a molten-globule configuration requires $\sim 10^{-5}$ s [19]. This raises the question of what other physical phenomena may set more stringent—and therefore more relevant—limitations on protein folding speed. Although early studies suggested otherwise, there is now experimental evidence to suggest that internal friction within a folding protein plays such a role.

2. Internal friction in protein dynamics

In 1992, Ansari and coworkers studied a conformational relaxation that occurs in myoglobin after photodissociation of a ligand from the haem iron [20]. They measured the effect of the solvent dynamic viscosity $(\eta_{\rm s})$ on the rate k of this relaxation. Figure 2(a) summarizes their findings: although the relaxation time k^{-1} varies linearly with $\eta_{\rm s}$ (as expected for a Kramers-like, diffusion-controlled barrier crossing), the relaxation time does not vanish in the limit of low viscosity $\eta_{\rm s} \to 0$. Rather, it approaches a finite value $k^{-1} > 0$. The authors therefore suggested that, for conformational relaxations deep within a protein, the 'normal' Kramers-like relation $k^{-1} \propto \eta_{\rm s}$ should be replaced by the empirical formula

$$k^{-1} \propto \eta_{\rm s} + \sigma. \tag{2}$$

That is, the total viscosity contributing to the Kramers friction γ is the sum of $\eta_{\rm s}$ and σ. Here the extra term σ is interpreted as an 'internal viscosity' which strongly limits the speed of the dynamics as the solvent viscosity $\eta_{\rm s}$ declines. A fit to the data then gives $\sigma \approx 4$ mPa s, indicating that the viscosity of the protein interior is significantly larger than the viscosity of water ($\eta_{\rm H_2O} = 1$ mPa s at 20.2 °C).

It is then natural to expect that a similar internal viscosity influences the dynamics of protein folding. A number of authors have measured the influence of solvent viscosity on protein folding rates [21–29]. Unfortunately, the viscosity of aqueous solvent cannot be altered without changing other properties of the solvent. At the very least, the addition of viscogenic cosolutes, such as polyols, tends to stabilize proteins through a preferential hydration effect: the exclusion of the cosolute molecules from the immediate environment of the polypeptide chain imposes a large entropic cost on the exposure of the chain to the solvent. This destabilizes

the unfolded state. The combination of rising viscosity and shifting protein stability may lead to very complicated behaviour of the folding rate.

Some experimental studies have corrected for these effects. Figure 2(b) summarizes the results of Plaxco and Baker [23], who studied the influence of a glucose cosolute on the folding kinetics of *peptostreptococcal* protein *L*. By simultaneously adding GdnHCl with glucose, these authors could maintain isostability conditions while raising the solvent viscosity to ≈3.5 mPa s. The figure shows that folding time k^{-1} is highly linear in η_s in this case, and it extrapolates quite neatly through the origin. The authors reported that the best fit to the Ansari formulation (equation (2)) gives an insignificant internal viscosity $\sigma \approx -0.1 \pm 0.2$ mPa s. A similar puzzling result was also found in several other studies; there was no evidence of a significant internal viscosity influencing protein folding dynamics. Such results are surprising, especially considering that many of the proteins studied fold through highly compact transition states, where internal intrachain interactions would be expected to be significant. If the relaxations of folded myoglobin show such a large internal viscosity, how can the internal viscosity of a protein folding reaction be virtually undetectable?

3. Origins of internal friction

The idea that polymer chains are subject to internal frictional forces has a long history in polymer dynamics [30]. Internal friction was originally introduced to explain experimental data on the high frequency rheological and dielectric relaxation properties of polymers. A number of theoretical approaches and models have addressed dissipative mechanisms in polymers that may not directly arise from the solvent viscosity [31]. Manke and Williams [31] point out that essentially all of these arise from the imperfect approximation of describing a polymer chain as a purely elastic or flexible object immersed in a continuum solvent (with a given macroscopic viscosity η_s). Some effects cannot be accurately represented as just a solvent drag, even though they give rise to dissipation or slower dynamics in chain motion. These include the work required to drive backbone bonds across their rotational energy barriers, interactions between side chain atoms, the requirement for void volume to appear in order for the chain to move through a discrete solvent, and potential nonequilibrium effects. As for the application to protein folding, several theoretical studies have implicitly posited a role for internal viscosity in protein folding dynamics [32–34]; however, there remains no clear theoretical prediction for the magnitude of any effect or its possible experimental signature in folding studies. But if many potential mechanisms for internal friction have been proposed in the polymer dynamics literature, one may suspect that the experimental manifestation of internal friction in protein folding could depend on the properties of the folding process, such as whether the molecule is in an expanded or compact configuration, whether one is observing folding versus unfolding, etc. As a simple example, the internal friction resistance that is encountered in pulling apart the endpoints of a long polymer chain is expected to diminish as chain length increases (the 'Kuhn theorem') [30]. By contrast, in compact configurations the friction due to intra-residue side-chain interactions presumably grows with chain length. It is not immediately obvious how one would parametrize internal friction in folding, and—for example—whether the Ansari formulation (equation (2)) is actually appropriate.

An ordinary homopolymer relaxes towards its equilibrium configuration (i.e. towards a random coil) with a relaxation time that is controlled by the solvent viscosity. De Gennes proposed that internal friction would slow this solvent-controlled relaxation time τ_s by adding a solvent-independent timescale τ_0. The relaxation time of the chain, following a perturbation, then becomes

$$\tau = \tau_s + \tau_0. \tag{3}$$

Because $\tau_s \propto \eta_s$, de Gennes suggested that experimental measurements of τ at several different values of η_s would allow one to extrapolate to $\eta_s \to 0$ and find the internal friction term τ_0. (Of course this does not mean that one actually performs experiments in the low friction limit; the extrapolation is the mathematical method for extracting the τ_0 contribution under normal solvent, high friction conditions.)

If we apply equation (3) to the time $\tau_f = k_f^{-1}$ for a protein folding reaction, it predicts the same viscosity dependence as does Ansari's equation (2): the folding time should increase linearly with η_s, with a finite intercept at $\eta_s \to 0$. The interpretation is very different, however, because equation (3) suggests that a timescale τ_0, rather than an internal viscosity σ, is the appropriate measure of the internal friction effect in protein folding. Folding reactions are rate limited by their slowest step, and if that slow step is strongly solvent-controlled and occurs on a timescale that far exceeds τ_0 the contribution from τ_0 may not be observable. Although internal friction may prevent a protein from folding in a time less than τ_0, this timescale may be so rapid that it limits only the fastest folding phenomena. A very rapid $\tau_0 \sim$ ns–μs would virtually never be detected in kinetics studies on proteins whose folding is limited by slow ($\sim$ms–s) processes. Along these lines, the myoglobin data of figure 2(a) and the protein L data of figure 2(b) indicate very different values for σ, but they are not inconsistent if interpreted, within equation (3), in terms of a very rapid τ_0.

4. Fast folding from a compact state

This argument suggests that the internal friction should primarily be observable in proteins that fold extremely rapidly, $k_f^{-1} \sim \tau_0$, or in the rapid, late stage folding events that follow the slower, large scale reorganization of the polypeptide chain in the solvent. We had the opportunity to study such a late stage process in horse cytochrome c [35]. Roder and coworkers have extensively characterized the equilibrium and kinetic properties of the MCO state of cytochrome c, a highly collapsed denatured globule from which folding to the native state can be exceedingly rapid [36–38]. This state is formed by chemically denaturing horse ferrocytochrome c, adding carbon monoxide (which binds to the haem iron), and then diluting the denaturant. The dilution causes the protein to collapse to a compact, near folded form, but with the CO molecule at the haem preventing the formation of the native iron–Met80 ligation. This MCO state therefore possesses largely native-like structure, but with some significant distortion [36]. The molecule is metastable: photodissociation of the CO from the haem iron allows the molecule to fold to the native state, and it does so within $\sim$10 μs at room temperature.

This folding transition is readily monitored through the visible optical absorbance of the haem. Dissociation of the CO from the iron generates a characteristic five-coordinate deoxyhaem absorbance spectrum, which is then replaced by a six-coordinate spectrum as the protein folds and the native methionine ligand reattaches to the haem. We used laser photolysis to trigger the folding, and we monitored its progress through transient absorption spectroscopy [39–41]. The viscosity was adjusted through the addition of glucose, glycerol, or ethylene glycol cosolutes. It is preferable in such studies to use these low molecular weight viscogens because they actually raise the microscopic viscosity experienced by the polypeptide, e.g. slowing processes like ligand escape and rotational diffusion. By contrast, high molecular weight cosolutes (like PEG) appear primarily to affect only the macroviscosity at the laboratory scale, leaving the microscopic dynamics unchanged [42, 43].

We directly measured the kinematic viscosity ν and density ρ of all samples at all experimental temperatures, and calculated the dynamic viscosity from $\eta_s = \nu/\rho$. Interestingly, the folding rate of MCO is insensitive to denaturant concentration up to $\sim$3 M GdnHCl, at which point MCO is itself destabilized in favour of more fully unfolded states. That is, the
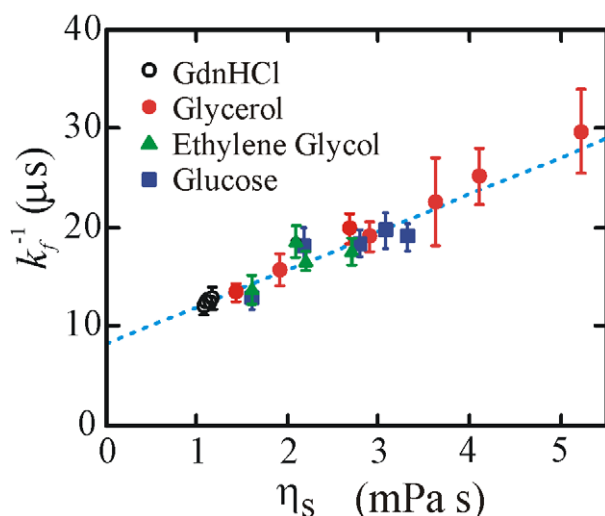
Figure 3. Folding time ($1/k_f$) versus solvent dynamic viscosity η_s for horse-heart ferrocytochrome *c* (pH 7, 20 °C), in the presence of various low molecular weight viscogenic cosolutes. The linear fit extrapolates to a time 8.1 ± 0.6 μs at $\eta_s \to 0$. The protein is initially in a highly collapsed, denatured configuration (MCO); its folding is triggered optically by photodissociation of the CO ligand with a $\sim$5–7 ns Nd:YAG laser pulse at 532 nm. Folding is probed by transient optical absorbance: after the photolysis flash, a xenon flashlamp and imaging spectrometer project the visible absorbance spectrum onto a gated, intensified CCD camera [35].

dynamics of MCO folding are unaffected by solvent changes that shift the stability of the native state. For this reason no correction was required for stabilizing effects of the viscogenic cosolutes.

A single relaxation is observed in the transient spectra as MCO folds. Because of the overwhelmingly greater stability (17 kJ mol^{-1}) of the native state relative to the initial MCO state [35, 36], the back unfolding reaction is virtually irrelevant and the time constant extracted from the transient spectra gives the folding rate k_f. Figure 3 shows that k_f shows the same behaviour regardless of the choice of viscogen added to the solvent: the folding time $k_f^{-1} \sim 10^{-5}$ s increases linearly with η_s, as expected in a diffusion-controlled Kramers-like process. However, the folding time also clearly extrapolates to a positive value $k_f^{-1} \to 8.1 \pm 0.6$ μs in the limit of vanishing solvent viscosity, $\eta_s \to 0$ at 20 °C. The fact that this limiting rate is barely faster than the rate in aqueous solvent ($\approx$12 μs) provides clear evidence that the speed of the protein's reorganization is only influenced by—not controlled by—solvent viscosity. Dissipative phenomena arising within the polypeptide evidently play a large role in setting the timescale for folding.

The temperature dependence of this behaviour is even more surprising. Figure 4 shows that k_f^{-1} versus η_s remains linear as the temperature of the solvent rises. However the slope does not change greatly. The temperature dependence is dominated by the systematic increase in the *y*-intercept. That is, if we use the simple approach of equation (3),

$$k_f^{-1} = \tau_f(\eta_s, T) = \tau_s + \tau_0$$

where $\tau_s \propto \eta_s$, then the internal friction contribution τ_0 exhibits a strong temperature dependence that accounts for most of the temperature dependence of the folding rate. This indicates a fairly large scale for the energetics of those interactions that generate the internal friction. A simple Arrhenius fit to the *y*-intercept τ_0 (obtained from a linear fit to the data at
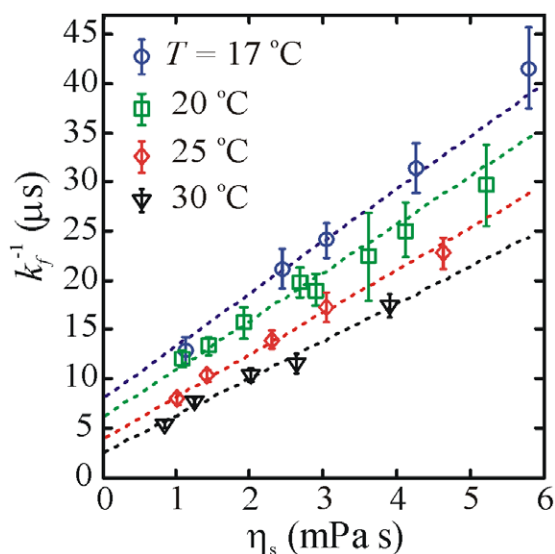
Figure 4. Temperature dependence of folding time $(1/k_f)$ for the collapsed state of cytochrome c. The dashed lines indicate linear fits to the data at each temperature, in accordance with equation (3), where τ_s and τ_0 are both constrained to an Arrhenius temperature dependence [35]. Because the indicated η_s is the dynamic viscosity measured at the indicated temperature, the temperature dependence of solvent viscosity does not contribute to the temperature dependence seen here.

each temperature in figure 4) gives an activation energy $\partial \ln \tau_0 / \partial (1/k_B T) \approx 67 \pm 16 \, \text{kJ mol}^{-1}$. (Because the abscissa in the figures refers to the actual η_s of each sample at the indicated temperature, the temperature dependence of η_s itself does not affect the apparent activation energies for τ_s or τ_0.)

The data of figures 3 and 4 show that internal friction can significantly limit the speed of protein folding. They also suggest a very simple explanation for the apparently discrepant results of figures 2(a) and (b): the 'internal viscosity' parameter σ suggested by Ansari and coworkers [20] is perhaps not the most useful way to characterize internal friction. It should rather be thought of as setting a lower limit, τ_0, to the folding dynamics. This limiting timescale may be present in all protein folding experiments, but it only plays a significant rate-limiting role in proteins that fold on timescales comparable to τ_0. Protein L [23] is not such a protein, although cytochrome c is. That is, the protein L and cytochrome c data would give very different values for σ in a fit to the Ansari model equation (2), but both experimental studies are consistent with a value τ_0 that is of the order of microseconds.

5. Internal friction in ultrafast folding

The suggestion that internal friction sets a limiting timescale ($\sim \mu$s) for folding implies that it could significantly affect the folding of some of the proteins and peptides that have been recently discovered or designed to fold on the 'ultrafast', microsecond timescale. One of the best known examples of such proteins is the tryptophan cage Tc5b (TrpCage), a polypeptide that has been described as the smallest true miniprotein, containing only normal amino acids [44]. TrpCage, only 20 residues in length, contains an alpha helical region, a 3_{10} helix, a polyproline II helix, and a compact hydrophobic core: it folds through a two-state transition at a rate $k_f > 250\,000 \, \text{s}^{-1}$ at 25 °C that makes it one of the fastest folding proteins known [2].
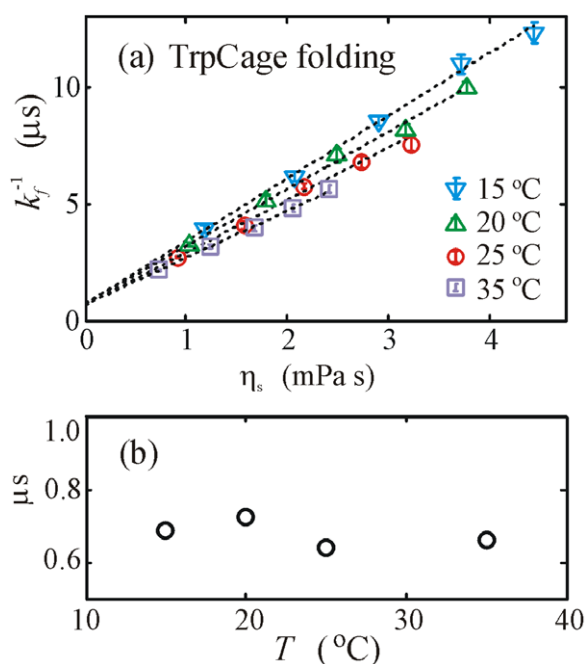
Figure 5. (a) Folding time $(1/k_f)$ of the TrpCage Tc5b, as a function of solvent viscosity and temperature, at pH 7. A linear fit at each temperature is also shown. The thermodynamic stability at each temperature is independent of η_s. Folding rates are obtained by laser temperature-jump fluorescence spectroscopy, as described previously [2]. A nanosecond laser pulse at 1.06 μm is shifted to 1.89 μm by stimulated Raman conversion in H_2 gas; this shifted pulse causes a rapid (20–30 ns) temperature rise in the aqueous solvent of the peptide, triggering re-equilibration between the folded and unfolded configurations of the peptide. These relaxation kinetics are probed by the fluorescence emission of the single tryptophan residue, with 266 nm laser pulse excitation. (b) Extrapolated value of the folding time at $\eta_s = 0$, from the linear fits. The limiting unfolding time is seen to be virtually independent of temperature.

The folding of TrpCage is readily observed through the increase in the fluorescence of the sole tryptophan (W6) upon unfolding. We therefore used laser temperature-jump spectroscopy to trigger folding/unfolding kinetics in TrpCage and measure the effect of solvent viscosity on these kinetics [45].

In this system it is necessary to compensate for stability shifts induced by the viscogen (glucose). Equilibrium experiments established that adding GdnHCl with the glucose in the ratio [GdnHCl]/[glucose] $\approx$ 0.61 was sufficient to provide isostability conditions over the range of temperatures studied. That is, for each temperature, the folding equilibrium constant $K_{eq} = k_f/k_{unfold}$ takes a fixed value, independent of the solvent dynamic viscosity η_s.

Figure 5 shows the results for k_f versus η_s. The folding time k_f^{-1} is seen to scale linearly with η_s over the entire range of viscosities studied. In fact, although k_f for TrpCage is strongly temperature dependent, plotting the folding time against the solvent viscosity almost causes the data for different temperatures to overlap. That is, the folding rate of any given sample of TrpCage varies strongly with temperature, but this variation largely disappears when the rates at different temperatures are compared at fixed solvent viscosity. Folding in this system therefore appears diffusion controlled, and lacks any enthalpic barrier; it is largely an entropic search. Further, as in the folding of the compact denatured state of cytochrome c we find that k_f^{-1} extrapolates to a small but clearly nonzero limiting value as $\eta_s \to 0$. As in the compact
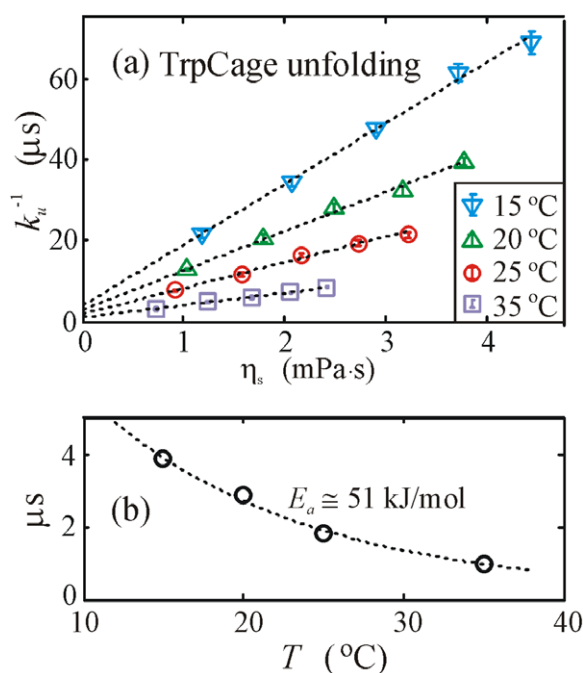
Figure 6. (a) Unfolding time ($1/k_u$) of TrpCage, with linear fit at each temperature. (b) Extrapolated value of $1/k_u$ at $\eta_s = 0$, from the linear fits. The dotted curve shows an Arrhenius fit to the data, giving an activation energy ≈ 51 kJ mol^{-1} for the limiting unfolding time of TrpCage.

cytochrome c, the folding dynamics approach a finite limiting speed at low solvent viscosity. This provides further evidence that internal friction influences protein folding, although the limiting timescale for TrpCage is significantly smaller than for the MCO state of cytochrome c. Fitting the data of figure 5(a) to a straight line at each temperature gives the y-intercept or τ_0 values shown in figure 5(b); these are of order 0.7 μs for all measurements over the temperature range 15–35 °C.

Therefore we find that the folding times of both the 20-residue TrpCage and the 104-residue MCO state of cytochrome c approach a finite limiting value at low solvent friction. The internal friction in these two systems differs however in its temperature dependence: a strongly activated τ_0 in the compact globule MCO is replaced by a T-independent τ_0 in the smaller TrpCage. This is not entirely unexpected. As suggested above, the existence of many conceivable physical mechanisms for internal friction in folding suggests that this could be seen as a class of phenomena, which could appear different in different experimental systems.

As evidence for this view, consider the unfolding rate (k_u) data for TrpCage (figure 6(a)). As with folding, the unfolding time scales with solvent viscosity. It also extrapolates towards a finite value $k_u^{-1} \sim 1$–4 μs in the limit of low solvent viscosity. However, this limiting timescale for unfolding varies strongly with temperature; an Arrhenius fit to the intercept $\tau_0(T)$ implies a fairly large activation energy of 51 kJ mol^{-1}.

Finally, it is quite interesting to note recent molecular dynamic simulations of the viscosity dependence of TrpCage folding [46]. Simulations allow the 'experimenter' to explore the regime $\eta_s \ll 1$ mPa s that is inaccessible in the laboratory. Figure 7 shows that Zagrovic and Pande observed a deviation from simple $k_f \sim 1/\eta_s$ behaviour at low η_s. However, this is a different kind of deviation from that behaviour than appears in the laboratory data. The
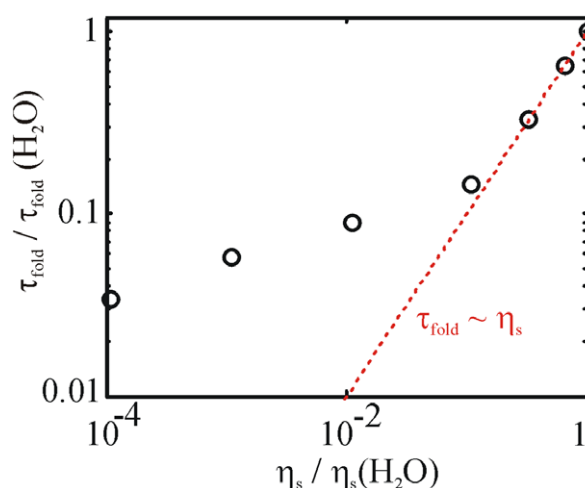
Figure 7. Folding time of TrpCage versus solvent viscosity, from molecular dynamics simulation. The dashed line indicates the behaviour for a simple Kramers rate, $k_f^{-1} \sim \eta_s$. Data are from [46].

origin of this discrepancy is unknown. One may speculate that it is associated with the implicit or continuum solvent approximation that was used in the simulation studies. This kind of experimental/simulation comparison may shed light on the microscopic origin of internal friction, and it will likely be an interesting direction for future studies.

In summary, the TrpCage data show that, for an ultra-fast folding protein, folding and unfolding are not necessarily controlled only by the viscosity of the solvent. The response of these rates to changes in η_s suggests that additional, internal friction mechanisms within the protein dissipate energy from the reaction coordinate during folding. Internal friction does play a role in fast protein folding dynamics.

6. Conclusions

The evidence for internal friction in TrpCage, when none was observed in protein *L*, CspB, or several other slower folding systems, supports the hypothesis that internal friction could set a limiting timescale for folding phenomena. We suggest that the phenomenon will be most evident—in fact it will perhaps only be visible—in the fastest folding dynamics. Based on the data currently available for cytochrome *c* and TrpCage, the relevant timescale appears to be of order $\sim \mu$s. This is of course the same order of magnitude as the very fastest known folding rates. However, the roughly tenfold difference in the magnitude of the internal friction parameter τ_0 for the two proteins studied here—and the differences in the temperature dependence of τ_0—suggest that internal friction could arise from different mechanisms in different systems, or in different types of dynamics, or in different scenarios of folding versus unfolding.

Some elementary steps in folding show evidence of internal friction effects. Deviations from a simple Kramers relation, $k \sim 1/\eta_s$ were observed in the kinetics of formation of an α-helix ($\sim$ns) but not in the formation of a β-hairpin ($\sim \mu$s) [47]. Similarly, in a study of the rate of formation of intrachain, end-to-end loops in disordered polypeptides as a function of solvent viscosity, the rate of this relatively simple process was found to exhibit an 'excess' temperature dependence, beyond that associated with the solvent viscosity $\eta_s(T)$ [48]. This is consistent with the idea within some internal friction models that even simple reconfigurations of a polypeptide backbone require thermal activation across energetic barriers.

Overall the internal friction-limited folding rates seen here, $\sim 10^6$ s^{-1}, are comparable to the very fastest protein folding rates that have been observed to date. They are also consistent with estimates for the maximum possible rates that would be derived empirically from contact order and other such experimental correlations. However they fall far short of the very rapid rates $\sim 10^7$–10^8 s^{-1} observed for simple diffusional processes in simple chains. This suggests that the diffusional 'speed limits' to protein folding, which consider only the free motion of a disordered polypeptide chain, may rather substantially overestimate the upper limits to protein folding speed. It will be interesting to see whether experimental studies can further clarify and characterize internal friction mechanisms. At the simplest level, for example, is there a simple chain length dependence of τ_0? If so we may expect that shorter proteins may ultimately be capable of faster folding. Is there a more appropriate way to measure an 'internal friction parameter' for a protein folding reaction? These questions will become increasingly important as increasing numbers of small, ultrafast folding proteins are designed and discovered.

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