Solvent Viscosity and Friction in Protein Folding Dynamics

Stephen J. Hagen*

Physics Department, University of Florida, P.O. Box 118440 Gainesville FL 32611-8440, USA

Abstract: The famous Kramers rate theory for diffusion-controlled reactions has been extended in numerous ways and successfully applied to many types of reactions. Its application to protein folding reactions has been of particular interest in recent years, as many researchers have performed experiments and simulations to test whether folding reactions are diffusion-controlled, whether the solvent is the source of the reaction friction, and whether the friction-dependence of folding rates generally can provide insight into folding dynamics. These experiments involve many practical difficulties, however. They have also produced some unexpected results. Here we briefly review the Kramers theory for reactions in the presence of strong friction and summarize some of the subtle problems that arise in the application of the theory to protein folding. We discuss how the results of these experiments ultimately point to a significant role for internal friction in protein folding dynamics. Studies of friction in protein folding, far from revealing any weakness in Kramers theory, may actually lead to new approaches for probing diffusional dynamics and energy landscapes in protein folding.

Keywords: Protein folding, Kramers theory, Viscosity, Dynamics, Friction, Energy Landscape.

1. INTRODUCTION

Even though folding of even a modest-sized protein involves reconfiguration of hundreds of bonds and interactions between thousands of atoms, energy landscape theory asserts that protein folding actually can be understood rather simply in terms of diffusion [1,2]: folding can be represented in terms of a system executing stochastic diffusive motion on a free energy surface with just a few important coordinates. Even aside from theory, it is standard practice in the experimental community to set aside the full complexity of the problem and model protein folding reactions as most other solution-phase reactions are modeled - in terms of diffusion-driven passage over a free energy barrier, along a single reaction coordinate. Such diffusion-controlled processes are the domain of Kramers reaction rate theory [3-5]. The recognition that the conformational dynamics of a protein molecule are very strongly damped by the surrounding solvent has motivated many applications of Kramers rate theory to protein dynamics, beginning with classic studies of the effect of solvent viscosity on ligand binding kinetics and conformational relaxations in myoglobin [6,7]. As these early studies have led into similar studies of protein folding, a number of authors have invoked Kramers theory as a tool for probing the diffusional character of protein folding. Jacob & Schmid [8] and Bilsel & Matthews [9] reviewed the application of Kramers theory to protein folding dynamics nearly a decade ago. At that time, one recurring question was simply whether the diffusional description of folding was in fact appropriate, or whether an alternative reaction theory (e.g. transition state theory, TST) could be relevant instead. However, experimental and theoretical evidence both indicated that the dynamics of a folding polypeptide chain are diffusional under any realistic experimental condition (excepting, perhaps, during folding in vacuo [10]). Kramers reaction theory is an appropriate starting point for modeling folding dynamics.

In subsequent years, however, a number of curious experimental results have come to light. Kramers theory is very often interpreted to mean that folding rates should scale inversely with solvent viscosity; experiments on both globular proteins and small peptides have often shown deviations from this so-called “Kramers-like” relationship between folding rate and solvent friction. These deviations have been observed not only in experiments but also in molecular dynamics simulations of folding. As more examples of such "anomalous" behavior are observed, it becomes important to review the experimental and simulation results from a critical perspective and determine what these findings say about the role of friction in folding dynamics. In fact, a careful examination of experiment and simulation clearly suggests that studies of friction in protein folding offer significant potential as a novel probe of energy surfaces in protein folding and of the character of diffusion along those surfaces.

This review focuses on the application of Kramers reaction theory to protein folding dynamics, and especially to the nature of friction in folding reactions. Section II briefly reviews the Kramers theory in its most basic form - for a one-dimensional reaction coordinate in the presence of strong friction. Section III discusses some of the practical problems that complicate experimental studies of friction in protein folding. Section IV then reviews some of the main findings from recent attempts to interpret the friction dependence of protein folding kinetics and dynamics, both in experiment and simulation. Section V considers internal friction, including its microscopic origin, its implication for naïve applications of Kramers theory to folding experiments, and its consequences for folding on more complex free energy surfaces.
2. REACTION DYNAMICS IN THE PRESENCE OF FRICTION

The landmark 1940 paper by Hendrik Kramers [3] provided a theory for chemical reaction rates by analyzing the dynamics of a simple model process: a particle, subject to frictional forces, is driven across a potential energy barrier by thermal fluctuations. Kramers calculated the rate of the particle’s escape over the barrier in three situations: (1) weak friction, where the particle is carried across the barrier by its inertia; (2) strong friction, where the motion is entirely diffusional and inertia is not important; and (3) moderate friction, where both inertia and diffusion play a role. The resulting rate theory provides a model for many types of reactions that occur in the presence of friction and it has been applied broadly to chemical reactions in the solution phase, including isomerization, tunneling, atom-transfer and charge-transfer reactions [4,5]. It has been extended to many other cases, including multidimensional free energy surfaces, where the one-dimensional barrier becomes a saddle-point [5,11,12], and to frequency-dependent friction (“memory friction”) [13]. Kramers’ strong friction case is widely held to apply to protein conformational dynamics and protein folding reactions [1,14,15], as the dynamics and the low frequency vibrations of proteins and peptides are very heavily damped by the viscous environment in the liquid phase [16]. Detailed simulations seem to confirm that folding dynamics are diffusional - never ballistic - under experimentally accessible conditions [17,18]: protein folding occurs within the strong-friction limit of Kramers theory.

The Einstein relation connects the friction coefficient $\gamma$ to the particle’s diffusion constant $D = k_BT/m\gamma$, where $k_B$ is Boltzmann’s constant ($1.38 \times 10^{-23}$ J/K). Passage to the folded state requires that the particle diffuse across a barrier of height $AG$ (Fig. 1). The shape of $G(x)$ is characterized by the curvature $\omega_b$ of the broad minimum in $G$ at the initial, unfolded state (near $x = 0$),

$$G(x) \approx (1/2)m\omega_a^2x^2 \quad (\text{for } x \text{ near } 0)$$

and by the curvature $\omega_b$ of the maximum in $G$ at the transition state (at $x_b$) at the top of the barrier,

$$G(x) \approx AG - (1/2)m\omega_b^2(x-x_b)^2 \quad (\text{for } x \text{ near } x_b).$$

Kramers theory gives the average time $\tau$ required for the particle to diffuse across the barrier. In the case of strong friction, the average rate $k = 1/\tau$ of barrier crossing is found to be [3,19,4]:

$$k = (\omega_a\omega_b m D/ k_BT 2\pi) \exp(-AG/k_BT) \quad (1)$$

This can be rewritten in terms of the friction coefficient $\gamma$,

$$k = (\omega_a\omega_b / \gamma 2\pi) \exp(-AG/k_BT) \quad (2)$$

It can also be written in terms of a prefactor $\tau_0 = \gamma/\omega_a^2$, which is a characteristic relaxation time for the particle moving in the initial well:

$$k = (2\pi \tau_0)^{-1} (\omega_a/\omega_b) \exp(-AG/k_BT) \quad (3)$$

The inverse dependence on the friction $\gamma$ in Eq. (2) is the hallmark of the high-friction Kramers rate theory: It distinguishes the Kramers rate theory from other theories, such as Eyring transition-state theory (TST). For the barrier crossing problem in Fig. (1), TST gives [4]:

$$k_{TST} = (\omega_a / 2\pi) \exp(-AG/k_BT), \quad (4)$$

where the prefactor ($\omega_a / 2\pi$) is the frequency of barrier-crossing attempts in the absence of friction. (The prefactor in TST is occasionally - incorrectly - given as simply $k_BT/\hbar$.) Both Eq. (2) and Eq. (4) share the Arrhenius exponential term $\exp(-AG/k_BT)$, but the similarity ends there, as the two rate equations derive from distinctly different physical models of the reaction. The TST expression describes barrier crossing where the initial state ($x = 0$) and the transition state ($x_b$) are in thermal equilibrium, and where the particle crosses the barrier top exactly once on its way from the reactant to the product side of the barrier. By contrast, the diffusive mechanism of Kramers theory allows for multiple, random recrossings of the barrier. These recrossings reduce the flux quite considerably, ie by a factor $\gamma/\omega_b$, which (by definition) is large in strong friction: The TST expression invariably overestimates the reaction rate.

It is noteworthy also that, unlike TST, the Kramers rate Eq. (2) has no universal prefactor or “attempt frequency”: the prefactor of the Arrhenius term depends on the friction $\gamma$ and the shape of the free energy barrier, through $\omega_b$. (However, folding simulations have suggested that $\omega_a$ and $\omega_b$ can be of comparable magnitude [17]; this simplifies Eq. (3) so

1. The friction coefficient $\gamma$ has units of inverse seconds. For a spherical particle moving through a fluid, it is proportional to the dynamic viscosity $\eta$ of the fluid. Therefore, atomic-level simulations of folding often fix the solvent viscosity by selecting a value for $\gamma$. 

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Fig. (1). The diffusional barrier crossing problem for a one-dimensional reaction coordinate. The barrier height is $AG$. $\omega_a$ and $\omega_b$ are the curvature of the free energy function $G(x)$ at the bottom and top of the barrier, respectively.

For the case of strong friction, Kramers theory gives a particularly simple expression for the rate of barrier crossing. Here we briefly summarize this theory, as it may apply to protein folding. The reaction is modeled in terms of a hypothetical particle of mass $m$ that diffuses along a one-dimensional reaction coordinate ($x$) on a free energy surface $G(x)$ (Fig. 1), from an initial position ($x = 0$), representing the unfolded state, over a barrier (at $x_b$), to a final position that represents the folded state. Friction generates a backward force $f$ on the particle when it moves at speed $v$:

$$f/m = -\gamma v.$$
that $\tau_0$ acts as a rate prefactor [18]. However, $\tau_0$ still depends on $\gamma$. The overall rate in Kramers theory depends on the shape of the free energy surface, because smaller values of $\omega_b$ make the barrier broader, flatter, and more difficult to traverse rapidly by diffusion. Greater curvature (larger $\omega_b$) allows less recrossing and enhances the reaction rate. The importance of $\omega_b$ reflects the central role of diffusion in the rate theory.

3. REACTION FRICTION IN PROTEIN FOLDING: CAVEATS

What does the Kramers theory predict for protein folding dynamics? The Stokes law asserts that the frictional drag on a small particle moving through a fluid scales in proportion to the dynamic viscosity $\eta$ of the solvent. This suggests that the relevant reaction friction for a polypeptide in solvent is also proportional to $\eta$. If the reaction friction $\gamma$ varies directly with $\eta$, then Kramers (ie Eq. (2)) implies a simple relationship between $k$ and $\eta$,

$$k = (A/\eta) \exp(-AG/k_B T)$$

(5)

where $A$ is a constant (that depends on $\omega_a$, $\omega_b$, etc.). However, Eq. (5) is not a statement of Kramers theory; by equating $\gamma$ with $\eta$, one goes one step further. Kramers theory requires that the friction coefficient $\gamma$ is simply related to any one physical parameter, such as the bulk viscosity of the solvent. In fact, the friction parameter is an abstraction that discards a tremendous amount of microscopic detail, in exchange for a handful of "effective" parameters ($\gamma$, $x$, $AG$, etc.). The friction $\gamma$ in Eq. (2) therefore stands in for all of the mechanisms by which energy can be dissipated out of the reaction coordinate. This includes direct dissipation into the solvent, although other mechanisms also exist. As we discuss below, there is abundant evidence from experiment and simulation that other mechanisms of friction, independent of $\eta$, can play a significant role in folding dynamics. Any deficiency of Eq. (5) in the laboratory does not imply that Kramers theory itself has failed.

Still, Eq. (5) has often been identified as the Kramers rate expression. It has motivated numerous experimental and simulation studies of the effect of solvent viscosity on protein folding. The aims of such studies have included determining whether rate-limiting steps of protein folding involve diffusive motion of the polypeptide - as in diffusion-collision models [20-25]; detecting the diffusive stages of folding [26]; probing late-stage (ie not rate-limiting) and non-solvent-controlled conformational dynamics [27,28]; seeking more accurate measurements of energetic barriers to folding [29,30]; and reducing computation time in folding simulations by manipulating the solvent viscosity parameter [29,31-33].

Ideally, experiments that test or exploit Eq. (5) would be as simple as measuring the rate of protein folding while adding various cosolutes or cosolvents to adjust the viscosity of the solvent. The dynamic viscosity of water ($\eta = 1$ millipascal second = 1 centipoise at 20 ºC) is readily increased by the addition of sugars, alcohols, or polymers: Addition of 50% glycerol (by weight) increases the viscosity of water by a factor of six at 20 ºC. At 100% glycerol the viscosity is increased by a factor $> 10^3$ [34]. In fact, however, experiments based on Eq. (5) are subject to many practical difficulties, including microviscosity and stability effects, as discussed below. More importantly, the very assumption leading to Eq (5), ie that the solvent viscosity is the origin of reaction friction, oversimplifies a potentially complex connection. Here we consider some of the practical problems that arise in viscosity studies of protein folding; later we critically review some of the findings that illustrate the actual complexity of the relationship between solvent viscosity and folding dynamics.

a. Stability Shifts

A variety of different viscogens have been put to use in viscosity studies of protein dynamics and folding: the list includes glucose, sucrose, xylose, trehalose, ethylene glycol, glycerol, Ficoll, polyvinylpyrrolidone, and others. However, it is only in a computer simulation that one can alter the viscosity of a solvent without changing any of its other physical properties. The addition of cosolvent alters numerous properties of a folding buffer, including its dielectric constant, surface tension, pH, ionic strength, and density. An immediate consequence of adding any viscogen—aside from the desired viscosity change—is a shift in the equilibrium stability of the protein and (assuming a linear free energy relationship) a proportionate change in the height of the free energy barrier in Eq. (2). Although solutes such as polyvinylpyrrolidone can destabilize the folded state of the protein [26], the small sugars and alcohols commonly used in viscosity studies usually stabilize the folded state. This occurs through a preferential hydration mechanism [35-37]: The cosolvent is excluded from the immediate surface of the protein molecule, and the entropic cost of this exclusion is greater when the protein is unfolded and its solvent-accessible surface area is greater. If the resulting shift of the denaturation equilibrium toward the folded state involves an accelerated rate of folding, that acceleration may compensate to some extent any slowing that is induced by the rise in viscosity. An overall increase in the folding rate, or at least an initial increase, with the addition of viscogen has been observed in at least some instances [26,38].

Chrunyk and Matthews [39] suggested that one might counteract this stabilizing effect by simultaneously adding a chemical denaturant. In a study of unfolding kinetics of the $\alpha$-subunit of tryptophan synthase, they correctly predicted for the stabilizing effects of the viscogens (glycerol and ethylene glycol) by adding urea. Under isostability conditions, the unfolding rate varied inversely with the solvent viscosity [39], exactly as Eq. (5) predicts. Of course, it is important to measure the viscosities of such mixed solvents directly; empirical viscosity formulas can be unreliable for viscogen/denaturant mixtures [40].

Isostability at variable viscosity can sometimes be accomplished without the addition of any denaturant. Jacob et al. studied the folding of the cold shock protein CspB and found that although ethylene glycol stabilized the protein, it also increased the cooperativity of folding. Hence the stability of CspB at 54 ºC was virtually independent of ethylene viscosity.
glycol concentration. At that temperature the folding rate of CspB was inversely proportional to the viscosity of the ethylene glycol mixture [23], again as predicted by Eq. (5).

Some authors have nevertheless suggested that the isostability condition, when accomplished with solvent mixtures, is unreliable, as the denaturant and the viscogen do not necessarily change the stability by the same mechanism [26]. The validity of this criticism is not so clear if the folding process is well-described by a simple, two-state model as in Fig. (1), where the dynamics involve rather few additional parameters beyond the energetics and the friction. However, it should raise an important flag if the protein folds via a series of intermediate states, or if it folds on a multidimensional free energy surface. There the equilibrium stability is insensitive to many of the possible ways that the solvent could perturb the free energy surface (See Heterogeneous friction, below). A solvent compensation scheme that fixes the equilibrium energetics of the initial and final states cannot ensure that all properties of the free energy surface or the trajectory are unchanged.

The many instances in which Eq. (5) has found experimental confirmation in isostability studies of folding kinetics can be taken as evidence that the isostability approach is sound [8]. It is possible however to go further and test for signs that the folding mechanism itself is perturbed by the viscogen or the compensating denaturant. Some authors have explored the effect of an added viscogen on the chevron curve (folding and unfolding rate vs denaturant concentration) of a protein [22,24]. The isostability method essentially assumes that addition of viscogen displaces or translates the chevron curve to slower rates and higher (typically) stability, without significantly changing its shape - ie without changing the slope of the folding/unfolding branches. Experimental data indicate that this is the case, at least for some systems. Bhattacharya & Sosnick [22] showed that the addition of glycerol simply displaces the chevron of an α-helical coiled coil, GCN4-p2', toward higher stability. Similarly, Ramos et al. [24] showed that sucrose and glycerol displaced the chevron curve of the apomyoglobin molten globule. This supports the hypothesis that an overall stability shift was the only important side effect of adding viscogen - other than the viscosity shift. There are, however, some strongly perturbing cosolvents (e.g. trifluoroethanol [22]) that directly alter the shape of the chevron. Obviously, using such additives to alter the viscosity invites trouble.

In summary, the experimenter should not ignore stability shifts induced by addition of viscogen. The isostability method is a practical although imperfect solution to this problem. Seeking out novel viscogens that do not alter protein stability is not necessarily a better approach: if one accepts the premise that an additive can have obscure (ie going beyond γ and ΔG) effects on the folding dynamics, then the addition of any viscogen or other cosolute is presumably suspect and it becomes difficult to study any viscosity effects in the laboratory.

b. Microviscosity

The distinction between the macroviscosity and microviscosity of the solvent presents another practical problem in the laboratory [41]. Macroviscosity is a bulk property, measured with a standard laboratory viscometer; it describes the diffusion of momentum within a fluid at the macroscopic length scale. Microviscosity describes the resistance encountered by an object of molecular size moving through the fluid [41]. Microviscosity and macroviscosity are not necessarily equal: Although one can readily increase the macroviscosity of water by adding soluble polymers, for example, such additives do not significantly affect the rate of small molecule diffusion in the fluid [41,42]. This distinction is clearly important to protein dynamics: Blacklow et al. found that monomeric polypeptides like glycerol and sucrose slowed the catalytic rate of an enzyme, while polymeric viscogens (polyethylene glycol, polyacrylamide, Ficoll) had virtually no such effect [41]. Numerous other authors have also observed a strong inverse correlation between the molecular weight of a viscogen and its effectiveness in slowing microscopic dynamics, such as diffusional escape of ligands [42], protein conformational relaxations [43], and protein refolding [44]. The data suggest that, for a given value of the macroviscosity, large polymeric cosolvents (e.g. dextran or polyethylene glycol) interact less with the protein exterior than do small cosolvents like glycerol or ethylene glycol [42]. Lower MW viscogens (glucose, glyceral, ethylene glycol, etc.) do influence the microviscosity, however, and are therefore preferred in studies of protein reaction friction.

c. Molecular-Level Effects

Even aside from the complications introduced by the addition of viscogens, one must proceed cautiously when interpreting the reaction friction (or diffusion constant D) in terms of the solvent viscosity η. It has long been known, for example, that diffusion constants for truly microscopic particles (such as glycine and urea molecules [45]) do not scale precisely as 1/η. The Stokes-Einstein relation (D = k_BT/6πμa) for the diffusion constant of a small spherical particle (a = particle radius) assumes that the solvent can be described as a viscous continuum, whereas at molecular length scales the discrete or molecular character of the solvent becomes important. Even for roughly spherical reactants, the Smoluchowski expression k = 4πDa for the diffusion-controlled reaction rate is accurate only if D is measured empirically, rather than predicted from solvent viscosity data [5,46]. It is also likely that the very presence of the polypeptide chain perturbs the microscopic dynamics of the solvent (e.g. solvent reorientation time [47]). At microscopic length scales, the magnitude of the viscosity η is simply not sufficient to capture all the physical properties of the solvent that may affect diffusional dynamics. This fact potentially limits our ability to interpret solvent-viscosity studies of protein folding.

4. RESULTS FROM EXPERIMENT AND SIMULATION

Experimental findings consistent with Eq. (5) (k ~ 1/η) [21,23,24,30] have led to broader acknowledgment of Kramers theory (as opposed to TST) as an appropriate model for a rate-limiting barrier-crossing process in protein folding, along with a clearer understanding that folding rates do not share a universal or absolute rate prefactor (as TST rates do). Although there have been some studies (including simula-
tions [17]) aimed at confirming the very applicability of the high-friction Kramers theory to folding reactions, most experimental work in the last decade has started with Kramers theory and then focused on such questions as whether rate-limiting stages of folding are controlled by bulk diffusion through the solvent, whether deviations from Eq. (5) reflect limitations of Kramers theory, and whether internal or non-solvent reaction friction plays a significant role in the dynamics.

Use of the isostability method led to many of the initial experimental findings in support of Eq. (5), suggesting that the solvent viscosity is the source of friction in a folding reaction. Folding rates of cold shock protein B (in ethylene glycol/water) [23] and the IgG binding domain of protein L (in glucose/water) [21] were found to scale inversely with η. These studies were both performed under isostability conditions. Bhattacharya and Sosnick found that the folding rate of GCN4-p2’ (in glycerol/water) [23] and the IgG binding domain of protein L [21], CspB [23], TrpCage [49], an α-helical peptide [29], and a glycine-serine repeat peptide [50]. Dashed lines show the fit to $q = q_0 \exp(E_0/kT)$ for each dataset. For faster folding systems, a deviation from Eq. (5) is often apparent, indicating a weakening dependence of $T$ on $q$.

Fig. (2). Sampling of experimental data on folding time $q$ vs. solvent viscosity for various proteins and peptides. Data are shown for cytochrome $c$ [48], protein L [21], CspB [23], TrpCage [49], an α-helical peptide [29], and a glycine-serine repeat peptide [50]. The data were more consistent with a power law $k \sim \eta^{-\alpha}$, over a hundredfold range of $\eta$, with $\alpha = 0.81 - 0.96$ [50]. Different viscosity dependence was observed in the folding/unfolding relaxation of a 16-residue β-hairpin derived from streptococcal protein G and in a 21-residue α-helical peptide [29]. Jas and coworkers found that the hairpin relaxation rate was $k \sim (3 \mu s)^{-1}$, and the helix relaxation rate was $k \sim (290 ns)^{-1}$ in water at $T = 20 ^\circ C$. The hairpin folding/unfolding relaxation rate at different temperatures and viscosities was consistent with $k \sim \eta^{-\alpha}$, with $\alpha = 1.07 \pm 0.25$, i.e. consistent with Eq. (5). By contrast, the relaxation rate for helix folding varied more weakly, in agreement with a power law, $k \sim \eta^{-\alpha}$ with $\alpha = 0.64 \pm 0.07$.

For intact proteins folding at microsecond (or faster) rates, the viscosity dependence of the rate may be noticeably weaker than $1/\eta$. Fig. (2) shows that this is especially true for small peptides and for the most rapidly folding proteins ($k > 10^5 - 10^6 /s$). Bieri et al. [50] found that the rate of end-to-end intrachain loop formation in some short disordered peptides (3-9 peptide bonds in length) showed a viscosity dependence weaker than $1/\eta$. The data were more consistent with a power law $k \sim \eta^{-\alpha}$, over a hundredfold range of $\eta$, with $\alpha = 0.81 - 0.96$ [50]. Different viscosity dependence was observed in the folding/unfolding relaxation of a 16-residue β-hairpin derived from streptococcal protein G and in a 21-residue α-helical peptide [29]. Jas and coworkers found that the hairpin relaxation rate was $k \sim (3 \mu s)^{-1}$, and the helix relaxation rate was $k \sim (290 ns)^{-1}$ in water at $T = 20 ^\circ C$. The hairpin folding/unfolding relaxation rate at different temperatures and viscosities was consistent with $k \sim \eta^{-\alpha}$, with $\alpha = 1.07 \pm 0.25$, i.e. consistent with Eq. (5). By contrast, the relaxation rate for helix folding varied more weakly, in agreement with a power law, $k \sim \eta^{-\alpha}$ with $\alpha = 0.64 \pm 0.07$.

One may then anticipate that the diffusional dynamics of a disordered polypeptide chain would also exhibit the same activation enthalpy $E_0$, as they should also be governed by solvent viscosity. In fact, however, experimental studies of intrachain contact formation rates in disordered peptides have often shown an energy of activation greater than $E_0$ [27,51]. Huang and coworkers studied the temperature dependence of end-to-end contact formation rates (loop formation rates) in peptides derived from the 17-residue N-terminal hairpin of ubiquitin [27]. They found that the rate of end-to-end collision did vary with solvent viscosity as expected. However, the activation energy for the collision rate was found to be 17-25 kJ/mol, significantly larger than that of the solvent. The excess activation energy of diffusional motion indicates that the conformational diffusion of a polypeptide is limited by internal energy barriers, in addition to solvent friction.

In fact, abundant experimental evidence indicates that solvent viscosity alone does not control conformational diffusion and folding of polypeptides. Rates of diffusion and folding often show a viscosity dependence that is noticeably weaker than $1/\eta$. The data were more consistent with a power law $k \sim \eta^{-\alpha}$, over a hundredfold range of $\eta$, with $\alpha = 0.81 - 0.96$ [50]. Different viscosity dependence was observed in the folding/unfolding relaxation of a 16-residue β-hairpin derived from streptococcal protein G and in a 21-residue α-helical peptide [29]. Jas and coworkers found that the hairpin relaxation rate was $k \sim (3 \mu s)^{-1}$, and the helix relaxation rate was $k \sim (290 ns)^{-1}$ in water at $T = 20 ^\circ C$. The hairpin folding/unfolding relaxation rate at different temperatures and viscosities was consistent with $k \sim \eta^{-\alpha}$, with $\alpha = 1.07 \pm 0.25$, i.e. consistent with Eq. (5). By contrast, the relaxation rate for helix folding varied more weakly, in agreement with a power law, $k \sim \eta^{-\alpha}$ with $\alpha = 0.64 \pm 0.07$.

For intact proteins folding at microsecond (or faster) rates, the viscosity dependence of the rate may be noticeably weaker than $1/\eta$. That is often seen in millisecond (or slower) folding molecules. Pabit et al. [48] measured the rate of folding of a compact, molten-globule intermediate of cytochrome $c$, as triggered by photodissociation of a CO ligand. Over a roughly fivefold range of solvent viscosity, the folding time $1/k$ showed a linear dependence on $\eta$, consistent with Eq. (5), but $1/k$ tended toward a positive limit at low viscosity:

$$1/k = \tau_0 + a \eta$$

Here $\tau_0 \approx 8.1 \mu s$ and $a \approx 6 \mu s/(mPa s)$ are constants at $T = 20 ^\circ C$, independent of the viscogen (guanidine HCl, glycerol, ethylene glycol, or glucose) (Fig. 3). Qiu et al. [28,49] studied the folding of the 20-residue TrpCage miniprotein and found again that Eq. (6) described the folding and unfolding rates under isostability conditions. For the TrpCage, how-
ever, the value of $\tau_0$ (ie the limiting value of $1/k$ at low viscosity) was very fast: $\tau_0 \approx 700 \text{ ns}$ for the folding reaction (independent of $T$) and $\tau_0 \approx 1-4 \text{ ms}$ (depending on $T$) for the unfolding reaction [52].

Fig. (3). Folding time $\tau = 1/k$ of a compact, molten-globule intermediate of cytochrome c [48]. The folding time shows a linear dependence on $\eta$, consistent with Eq. (5), but $1/k$ tends toward a positive limiting value (roughly 8 ms at $T = 20 \,^\circ\text{C}$) at low viscosity. While the slope is nearly independent of $T$, the limiting value of $\tau$ varies strongly with $T$. Dashed line is a fit to Eq. 8 [48].

Of course it is not easy to establish definitely whether the data are better fit by a power law or by a linear relation like Eq. (6) (or even by another relation), especially since folding data may only be available over a limited (e.g. 2-5 fold) range of solvent viscosities. Both models may fit the data equally well in some cases [28,52]. These experimental findings of a weak viscosity dependence, and the problem of identifying the "correct" mathematical form of the viscosity dependence, recall early studies on the viscosity dependence of protein conformational dynamics, in which deviations from simple $1/\eta$ scaling were also observed [6,7]. Beece et al. described a power-law behavior (with exponent $\kappa \sim 0.4$-0.8) in the viscosity-dependence of ligand binding in myoglobin [6], while Ansari et al. found that the rate $k$ of conformational relaxation in myoglobin could be described by:

$$k = C (\sigma + \eta)^{-1} \exp(-E_0/kT) \quad (7)$$

where $C$, $E_0$, and $\sigma$ are constants [7]. Because $\sigma + \eta$ plays the same role in Eq. (7) that $\eta$ plays in Eq. (5), $\sigma$ was interpreted as an “internal viscosity” of the protein, of magnitude $\sigma \approx 4 \text{ mPa s}$, which adds to the solvent viscosity. This idea of internal viscosity has been cited in subsequent studies of protein folding. Pradeep et al. used Eq. (7) to analyze the folding of barstar in xylose and glycerol viscogens [25]. However, although Eq. (6) and Eq. (7) are mathematically similar – ie they both describe a linear relationship between $1/k$ and $\eta$ (although with $1/k \neq 0$ at $\eta = 0$) – the two expressions place a different physical interpretation on that behavior. Eq. (7) suggests the existence of a single barrier and a reaction friction $\gamma$ with two additive components (internal and external to the protein); Eq. (6) suggests a more complex free energy surface, with a heterogeneous reaction friction, as discussed below.

In summary, experimental studies of protein folding dynamics have shown deviations from $k \sim 1/\eta$ just as often as they have shown agreement with that relation, even though all experiments lie within the high friction regime. The variability of experimental behavior is not easily dismissed as an artifact: The viscosity dependence of $k$ is often independent of the particular viscogens used [48], it is consistently a weaker (rather than stronger) dependence on $\eta$ than Eq. (5) predicts, and – most surprisingly – it is also observed in computer simulations of folding. Simulations allow solvent viscosity to be altered without introducing artifacts such as stability shifts or changes in other physical properties of the solvent. For example, Klimov and Thirumalai used a coarse, off-lattice simulation to examine the solvent viscosity dependence of the folding rates of simplified $\alpha$-helix and $\beta$-sheet peptides [17]. Over a range of solvent viscosities large enough to span the moderate-to-high friction regime, the folding kinetics showed excellent agreement with $k \sim 1/\eta$ (Eq. (5)) in strong friction. However a different result was found by Zagrovic and Pande, who performed an atomistic simulation of the folding of the 20-residue TrpCage miniprotein [32] in implicit solvent, whose viscosity is represented by a damping parameter $\gamma_s$. They found that the folding time scaled directly with $\gamma_s$ (ie $1/k \sim \gamma_s$) when the solvent viscosity was roughly comparable to that of water, $\gamma_s/\gamma_{\text{water}} > 0.1$. But at lower viscosity, $\gamma_s/\gamma_{\text{water}} \sim 10^{-3} - 10^{-2}$, the folding time varied much more weakly with solvent viscosity. Simulations by Feig also showed a weaker than expected relationship between solvent friction and conformational transition rates [53]. Best and Hummer studied the effect of $\gamma_s$ on folding in a detailed Gō model simulation of the three-helix bundle prb7-53 [18]. However, while the folding rate scaled inversely with $\gamma_s$ at high friction, it showed much weaker dependence on solvent at low friction. In all of these simulations, the folding dynamics remained diffusional over a wide range in solvent friction.

5. INTERNAL FRICTION

a. Mechanisms of Non-Solvent Reaction Friction

If the relevance of the high-friction Kramers theory (Eq. 2) in protein folding is widely accepted, then power laws $k \sim 1/\eta^n$ and other deviations from Eq. (5) require explanation. Frauenfelder has proposed, for example, that particular interactions of the viscogen with the protein could be responsible for the power-law (with $\kappa < 1$) [54]. This could explain some experimental findings [25], but it does not explain the results of simulations. Jas et al. [29] invoked the extension of Kramers theory by Grote & Hynes [13], who predicted a reduced viscosity dependence of the reaction rate for a system with “memory friction”: If the barrier crossing is so rapid that it occurs on roughly the same time scale as the interaction and equilibration with the solvent, then the solvent cannot respond instantaneously and the reaction friction is effectively frequency-dependent. This leads to a weaker influence of solvent viscosity on the reaction rate [13,15]. However, aqueous solvents have very fast microscopic dynamics (> 1 ps$^{-1}$) and are expected to exert their full frictional effect on barrier crossings that occur slower than about
1 ps [5, 14]. The primary barrier-crossing event in protein folding is almost certainly far slower than these rapid solvent reorganizations. This discrepancy argues against the Grote-Hynes picture as a general explanation for the observed viscosity behavior [28].

Therefore the disagreement between Eq. (5) and the findings of many experiments and simulations should raise questions about the core assumption that the solvent viscosity is the only source of reaction friction. As discussed in section II, Kramers theory does not require the reaction friction $\gamma$ in Eq. (2) to be identical to the dynamic viscosity $\eta$ of the solvent. The friction $\gamma$ and the coordinate $x$ stand in for the many actual coordinates, forces, and interactions that control folding; this tremendous simplification sidesteps the problem of providing a complete atomic level description. Since the friction $\gamma$ generically represents any fluctuating force that acts along, or removes energy from, the reaction coordinate, it is naïve to interpret $\gamma$ only as a measure of the solvent viscosity. Energy may be lost through interaction with the viscosity (ie solvent friction), or it may be dissipated into internal coordinates of the protein that do not contribute to progress along $x$. This latter process is called internal friction. Internal friction was first discussed in the context of dissipative dynamics of polymer solutions [55, 56], and it has been measured in biopolymers and proteins by various methods. It plays a role in mechanical oscillator and ultrasound absorption experiments in proteins [57, 58], as well as in mechanical pulling and unfolding studies that probe the viscoelastic properties of proteins [59-62]. For example, Kawakami et al. used atomic force microscopy to measure the elastic response of individual titin I27 domains, and found that the viscoelasticity of the protein is dominated by an internal friction (rather than solvent friction) that falls sharply as each domain unfolds [62].

Internal friction is occasionally interpreted literally as the rubbing or sliding of side chains; more correctly it refers to many different and often subtle mechanisms of dissipation [56], arising because the polymer chain always has some internal resistance to changing its configuration. One oft-cited example is the dihedral angle rotation of the polypeptide backbone [12, 18]. The phi/psi angles have several possible configurations corresponding to local energy minima, separated by activation barriers. Diffusional motions of the backbone that involve local rotational isomerization therefore come at the cost of an activation energy. Such configurations remove energy from the polypeptide dynamics, and yet may do nothing to advance progress toward the folded state. Hence dihedral isomerization contributes internal friction.

Theoretical studies indicate that internal friction may alter the solvent-viscosity dependence of protein dynamics and folding. In the analysis of Schlitter [15], the many internal vibrational modes of the protein together act as an additional fluctuating force (ie friction) in conformational dynamics, where these modes may interact either strongly or weakly with the solvent environment. Residues in the interior of the protein interact weakly with the solvent, while residues at the protein surface interact more strongly [63, 64]. The high-frequency vibrational modes that are localized to interior residues therefore contribute a reaction friction that is fairly independent of $\eta$, while low frequency modes that involve the protein surface contribute a friction that scales with $\eta$. Therefore, while the protein conformational dynamics are expected to become slower with rising solvent viscosity, different modes respond differently. Some relaxations slow as $\eta^{-1}$, while others respond more weakly. The overall relaxation dynamics of the protein are then expected to vary with viscosity, but – on average – more weakly than $\eta^{-1}$. In practice the general behavior might resemble a power law $\eta^{-\kappa}$ with $\kappa < 1$ [15]. However, since the exponent $\kappa$ quite possibly depends on the value of $\eta$, it would be a mistake to interpret such theories as firmly predicting a power-law relationship.

Portman et al. presented both theory and simulations of the effect of internal friction in barrier crossing by a folding protein [12]. Much like Schlitter, they argued that higher frequency modes of the protein, which depend more on large-amplitude local reconfigurations of the backbone, are controlled by internal friction from dihedral isomerizations, whereas lower frequency modes involve large scale motions that are more influenced by solvent friction. They found that, for a compact or partially-ordered molecule, barrier crossing on a multidimensional free energy surface may depend sensitively on the higher frequency modes. In this case, even small internal friction may have large consequences for the dynamics. Then the prefactor in the Kramers rate expression Eq. (2) is nearly proportional to solvent viscosity for large $\eta$, but it may level off at smaller values of $\eta$. That is, folding speed becomes limited by internal friction at low solvent viscosities. This suggests a finite limit to the folding rate at low viscosity, consistent with experiments and simulations cited above.

Recent simulations by Best and Hummer [18] provide more insight into the microscopic origins of internal friction in folding. Progress toward the folded state relies on a large number of interdependent dihedral angle isomerizations, each of which can be thought of as a single, elemental barrier-crossing event. Simulations showed that the dynamics of these individual events are quite complex. Dihedral isomerizations in longer (eg 12-residue) peptides remain diffusional as friction decreases, but with a rate that did not vary inversely with the solvent damping parameter, $\gamma_s$. This occurs because dihedral angle transitions in neighboring residues are interdependent processes that occur on roughly similar time scales. This leads to complex (ie non-Markovian [63]) diffusional dynamics, which do not obey Kramers theory. In short, although a protein folding transition can be modeled as a diffusional barrier crossing along a single reaction coordinate, each small step along that reaction coordinate actually requires a large number of individual conformational transitions. These elementary transitions have their own unusual dynamics, involving “memory friction” etc., and consequently their own relationship with the solvent friction. Therefore the progress toward the folded state is diffusional, but the friction $\gamma$ does not necessarily scale in a simple way with $\eta$. The experimental observation of internal friction in folding is ultimately the signature of very complex dynamics occurring with each underlying microscopic step along the reaction coordinate.
b. Heterogeneous Friction

If both solvent and internal friction play a role, then the relative importance of the two contributions may vary at different stages in a complex folding process. It is entirely possible that different stages of folding, or different microscopic barrier crossing events, respond differently to changes in solvent friction, with interesting consequences for the folding pathway. Recent simulations appear to demonstrate this effect [53, 66], as do very recent experimental studies of the fast folding of the villin subdomain [67] and the tryptophan zipper [68]. Changes in solvent-viscosity could then perhaps be useful as a probe of the reaction surface. For example, we can consider the hypothetical folding process shown in Fig. (4), where the molecule encounters two major barriers - a large free energy barrier at \( x_1 \), where solvent friction dominates, and a smaller barrier at \( x_2 \), where internal friction dominates. That is, friction \( \gamma_1 \) comes from the solvent \( \eta \), whereas \( \gamma_2 \) is primarily internal friction. At high solvent viscosity, passage over the primary barrier is rate-limiting and the second barrier has negligible effect on the overall folding rate. At low solvent viscosity the folding time is affected by both barriers. Ignoring, under native conditions, the reverse (unfolding) reactions, the kinetics can be modeled as,

\[
A \rightarrow B \rightarrow C
\]

\[
k_1 \quad k_2
\]

where \( k_1 \) and \( k_2 \) are the rates for passage over the respective barriers (per Kramers),

\[
k_1 = 1/\tau_1 = \omega_0 \omega_1 / (2 \pi \gamma_1 \exp(-\Delta G_1/k_B T)) = (1/\tau_1^0) \exp(-\Delta G_1/k_B T)
\]

\[
k_2 = 1/\tau_2 = \omega_0 \omega_2 / (2 \pi \gamma_2 \exp(-\Delta G_2/k_B T)) = (1/\tau_2^0) \exp(-\Delta G_2/k_B T)
\]

and

\[
\tau_1^0 = 2 \pi \gamma_1 / \omega_0 \omega_1
\]

\[
\tau_2^0 = 2 \pi \gamma_2 / \omega_0 \omega_2
\]

are two time scales. Solving the chemical kinetic equations for this 3-state model leads to the average time required for the molecule to cross both barriers and reach state \( C \):

\[
\tau = 1/k_1 + 1/k_2 = \tau_1 + \tau_2 = \tau_1^0 \exp(\Delta G_1/k_B T) + \tau_2^0 \exp(\Delta G_2/k_B T)
\]

The folding time is the sum of two parts: \( \tau_1 \) is strongly affected by \( \eta \), while \( \tau_2 \) is insensitive to \( \eta \). A graph of the folding time \( \tau \) versus \( \eta \) will then show a linear behavior, with a positive intercept (Fig. 4) at \( \tau_2 \). This is exactly the form of the empirical Eq. (6), which describes the folding kinetics of several fast-folding proteins. Eq. (8) is also very similar to the theoretical rate of reaction between two idealized, spherical particles (radius \( a \)) in a viscous medium:

\[
1/k = 1/k_D + 1/k_{coll}
\]

[19] where \( k_{coll} = \sqrt{2 \pi a^2 n} \) is the rate of ballistic collisions in the absence of any friction, and \( k_D = 4 \pi DOa \) is the familiar rate for diffusion-driven collision and depends strongly on solvent friction (through the diffusion constant \( D \sim 1/\eta \)). (In fact the equivalent collision rate for a biomolecular folding reaction is estimated at roughly \( 10^6/s \), consistent with experimental findings for \( \tau_2 \) [19]). The form of Eq. (8) is characteristic of a process that slows down in strong solvent friction, but reaches a finite limiting rate as the solvent friction decreases.

Fig. (4). Folding with two consecutive barriers on a one-dimensional reaction coordinate: (A) The barriers have height \( \Delta G_1 \) and \( \Delta G_2 \), reaction friction \( \gamma_1 \) and \( \gamma_2 \), and curvature \( \omega_0 \) and \( \omega_2 \) respectively, and are characterized by individual crossing rates \( k_1 \) and \( k_2 \). (B) The average time to reach the folded state is \( \tau = 1/k_1 + 1/k_2 \). If \( \gamma_2 \) is primarily internal (rather than solvent) friction, then the folding time \( \tau \) approaches a nonzero limit \( 1/k_2 \) at low solvent viscosity \( \eta \).

In Eq. (8) the temperature dependence of the solvent-dependent \( (1/k_1) \) and solvent-independent \( (1/k_2) \) parts of the folding time actually provides some information about the two different barriers \( \Delta G_1 \) and \( \Delta G_2 \). Pabit et al. found for cytochrome c that \( 1/k_1 \) had little if any temperature dependence, indicating that the primary (solvent-dependent) folding barrier is essentially entropic. By contrast, \( 1/k_2 \) was very strongly thermally activated, indicating a large enthalpic component for \( \Delta G_2 \); the later (internally-controlled) dynamics involve a substantial energetic barrier [48]. In this sense a solvent viscosity study of the folding rate provides a window into the energetics and dynamics of early vs. late events in folding.

Fig. (4) and Eq. (8) may provide some insight into why some fast-folding proteins and peptides deviate from \( 1/\eta \) while many slower-folding systems do not (Fig. 2). In experiments, the limiting value of \( 1/k \) at low solvent viscosity (ie the \( \eta \)-independent part of the folding time \( \tau \)) is quite small - of order microseconds or nanoseconds. This implies that crossing of the second barrier, under control of internal friction, occurs rapidly. The presence of this fast, solvent-independent process will not be detected in proteins that require long times (milliseconds or seconds) to cross the primary (solvent-controlled) barrier \( \Delta G_1 \). Most of the proteins that exhibit \( k \sim 1/\eta \) behavior fold on relatively slow time scales of milliseconds or longer. For such proteins, the first term in Eq. (8) is sufficiently large \( (1/k_1 \sim \text{ms-s}) \) that the second process \( (1/k_2 \sim \text{ns -us}) \), which generates the deviation from \( k \sim 1/\eta \), is simply undetectable, even if present.
Heterogeneous reaction friction, for which the contributions from solvent and internal friction vary in relative strength, may have other experimental consequences as well. If the free energy surface for folding is multidimensional and some folding pathways couple more strongly to the solvent (i.e., less internal friction) than do others, then changes in solvent viscosity may alter the pathway - rather than just the rate - of the folding process. A decrease in the external (solvent) friction would favor trajectories that encounter less internal friction (Fig. 5) [48, 53, 66]. There is some evidence for this phenomenon in simulations by Klimov and Thirumalai, who found that changes in the solvent friction altered the conformational pathways by which the peptides reached their native state [17]. More recent simulations by Rhee and Pande [66] have explored this in detail. These authors simulated the folding of the 23-residue miniprotein BBA5 in an implicit solvent model with variable solvent friction. They found that changes in $\gamma$ within the range $(0.01 - 2) \times \gamma_{\text{water}}$ (≈ $91 \text{ ps}^{-1}$) not only altered the speed of folding, but also shifted the folding probability $p$-fold of specific conformations; even a twofold increase in solvent friction produced a noticeable change in $p$-fold values, indicating that solvent viscosity shifts the trajectory taken by the molecule en route to the folded state.

CONCLUSIONS

Despite a large amount of work on friction and viscosity in protein folding, some simple questions still remain unanswered. Simulations and theory provide some insight into possible physical mechanisms of internal friction, but there are no experimental demonstrations of these ideas. Experiments and simulations to date have also led to phenomenological descriptions of the viscosity dependence of folding rates, but there is no consensus on which of these mathematical relationships has a stronger basis in physical theory. Reasonable physical arguments can be made for power laws as well as for linear models such as Eqs. (6) and (7). Our perspective here has been that Eq. (6) has the advantage of explaining why faster folding proteins more often exhibit an “anomalous” viscosity dependence, and it leads naturally into a discussion of heterogeneity in the friction that is encountered along the reaction coordinate. However, other interpretations of the data are certainly possible.

In proteins that have multidimensional energy surfaces and heterogeneous reaction friction, one cannot expect the folding rate to scale in simple ways with the solvent viscosity. This suggests that observations of unusual viscosity dependence are, at least in some cases, hinting at an interesting complexity in the underlying free energy surface. Current data and simulations both suggest that solvent viscosity can be an important tool for exploring those complexities. Future experiments may use solvent viscosity effects to map out previously hidden characteristics, such as multidimensionalality, late-stage barriers to folding, or the heterogeneity of reaction friction in such proteins. Rhee and Pande have proposed that proteins with high contact order may be of particular interest for such studies, as the formation of long-range contacts is presumably more sensitive to solvent friction than is the formation of short range contacts in (e.g.) an $\alpha$-helix: rising solvent viscosity may produce a noticeable shift toward folding pathways dominated by internal friction, if suitable probes are available to observe such trajectories.

Whether protein folding is a diffusional process - for which Kramers theory and its variants provide an appropriate theoretical framework - is not much in question at the present time. More important is whether a better microscopic understanding of internal friction can be gained, how this friction is most appropriately characterized or modeled, and how this understanding can be put to use in exploring the transition to the folded state.

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