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Modulation of Folding Internal Friction by Local and Global Barrier Heights

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Abstract

Recent experiments have revealed an unexpected deviation from a first power dependence of protein relaxation times on solvent viscosity, an effect which has been attributed to “internal friction”. One clear source of internal friction in protein dynamics is the isomerization of dihedral angles. A key outstanding question is whether the global folding barrier height influences the measured internal friction, based on the observation that the folding rates of fast-folding proteins, with smaller folding free energy barriers, tend to exhibit larger internal friction. Here, by studying two alanine-based peptides we find that systematic variation of global folding barrier heights has little effect on the internal friction for folding rates. On the other hand, increasing local torsion angle barriers leads to increased internal friction, which is consistent with solvent memory effects being the origin of the viscosity dependence. Thus, it appears that local torsion transitions determine the viscosity dependence of the diffusion coefficient on the global coordinate, and in turn internal friction effects on the folding rate.

Graphical abstract

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Supporting Information Available

Supporting methods, figures and tables.

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Introduction

Protein folding has been successfully characterized using a funneled energy landscape, in which native interactions are the main driving force towards the folded, functional, state due to the evolutionary requirement for efficient folding.^{1–4} In this framework, the projection of the folding dynamics onto a well-chosen reaction coordinate (e.g. the fraction of native contacts, Q^5) can be described as diffusion on a one-dimensional free energy surface, in the same picture as the description of chemical reactions in solution using Kramers theory.⁶ The required (position-dependent) diffusion coefficient for such a model will be influenced by the local features of the energy landscape.¹ Most experiments only give direct information on the relative free energy of the stable states, or changes in barrier height, while probing contributions from local barriers on the energy landscape is much more difficult. One type of experiment which can potentially yield insights into the local dynamics on the free energy landscape involves measuring the dependence of protein relaxation times on solvent viscosity.^{7–16} Although the translational diffusion coefficients of small solutes have a simple negative first power (Stokes-like) dependence on solvent viscosity,¹⁷ protein relaxation times often deviate from a simple linear dependence on solvent viscosity with zero intercept. This phenomenon has been termed “internal friction” in the literature, in reference to the idea that the protein itself may act as a source of friction. As we discuss below, the actual origin of this effect may be different, but we adopt the experimental terminology of internal friction to describe this anomalous viscosity dependence. Such internal friction has been observed in native state dynamics,⁷ protein folding rates,^{8–14,16} folding transition-path times^{14,16} and unfolded state dynamics.¹⁵ In addition, internal friction has been inferred from the molecular phase observed in temperature-jump experiments, which is related to transition-state transit times.¹⁸

In theoretical work, the isomerization of local torsion barriers has been proposed to be one cause of internal friction.^{19–22} Recent explicit solvent simulations have demonstrated the

link between torsion transitions and internal friction in both protein folding¹⁷ and unfolded dynamics.²³ It has also been suggested that hydrogen bonding^{24,25} and salt bridges¹⁴ may play a role. In earlier work, we have found that the insensitivity of torsional transitions to solvent viscosity is most likely due to solvent memory effects: there is not a clear separation between the time scales for crossing a torsion barrier and the time over which the solvent loses memory of its previous configuration, and so the solvent friction is effectively lowered;¹⁷ a similar mechanism probably explains the role of salt-bridges in internal friction.^{14,26} While still related to friction, these possible origins of the observed viscosity dependence do differ somewhat from the intention of the original “internal friction” terminology,⁷ but we retain the term for consistency with the literature.

We have recently extended our work on the origin of internal friction to explaining the variation of internal friction effects from protein to protein and across the energy landscape of the same protein.²⁷ Specifically, we showed that internal friction arises when torsion angle changes dominate the folding mechanism near the folding free energy barrier. In that work, however, it was not possible to separate clearly the effects of local and global barriers on the results. Changing the height of a local torsion barrier can change the importance of solvent memory effects on the rate of barrier crossing, and hence internal friction. But changing the height of the global folding barrier may also affect internal friction. One might expect that lower barriers, where a larger region of the energy landscape effectively contributes to the viscosity dependence of the rate, would be more likely to show internal friction effects. Indeed, internal friction is more often seen in fast-folding proteins^{8,10,11,14,18,28} than in slower-folding ones.^{29,30}

Here, we set out to address the relative effect of local and global barriers on internal friction in protein folding dynamics by using two simple alanine-based peptides, alanine dipeptide and alanine-8. We choose alanine dipeptide because it is the smallest molecule exhibiting internal friction effects, and alanine-8 because it can form a small population of α -helix, a simple prototype for protein folding. An important consideration is computational cost, because we need to obtain accurate rates in a two-dimensional space of barrier height and solvent viscosity. While it would have been desirable to study model two-state folders such as the GB1 hairpin and Trp cage as in our earlier work,²⁷ the GB1 hairpin does not exhibit any internal friction effect, and internal friction is only observed for the first barrier of Trp cage, which essentially corresponds to formation of the native helix, similar to the process we study here. We show a schematic of the local and global barriers we try to vary in Figure 1. Varying these two types of barriers independently is much more challenging in experiment, whereas in simulation we are able to vary the barriers systematically to cover a wide range of folding time scales in these two peptides. We find that the internal friction is reduced when lowering the torsion barriers, consistent with the reduction of barrier curvature (and vice versa for increasing barrier height). On the other hand, for variations of the global free energy barrier, we find no changes (within statistical uncertainty) of the internal friction.

Local barrier of alanine dipeptide

The simplest example for which internal friction has been observed is the isomerization of alanine dipeptide between α_R and extended minima.¹⁷ Here we systematically test the

dependence of internal friction on barrier height, by introducing five different dihedral barrier heights covering a wide range of time scales. The method of varying the dihedral barrier is similar to our previous work,¹⁷ and to recent work²³ showing that internal friction associated with unfolded chain end-end reconfiguration time is weaker when rescaling the dihedral barrier by a factor of 0.5.

The local barrier we vary is that for the Ψ torsion angle, which separates the extended (β and polyproline II) and α -helical states of the backbone. Changing the height of the barrier has little effect on the helical propensity of the peptide, but a large effect on the torsion angle relaxation time scale. We implement the variation of barrier height by introducing an extra torsion term

$$U(\Psi) = k_{\Psi}(1 + \cos(2\Psi - \Psi_s)) \quad (1)$$

in which $\Psi_s = 150^\circ$ and $k_{\Psi} = -5, -2.5, 0, 2.5$, and 5 kJ/mol, noting that a positive k_{Ψ} would increase the torsion barrier and vice versa. Details of molecular dynamics methods can be found in Supporting Methods 1.1.

The resulting free energy profile along Ψ is shown in Figure 2A. The torsion barrier height

G_{Ψ} is estimated from the free energy difference between the extended states and the top of the barrier at $\Psi = 70^\circ$. It varies roughly from 2.5 to 21.2 kJ/mol, introducing a variation of torsion relaxation times from 5.5 ps to 1.6 ns at normal water viscosity (Fig. 2D). We study the torsion relaxation time in different solvent viscosities via rescaling of the solvent mass³¹ in explicit solvent simulations, so that full solvent dynamics are included, as done in previous work on internal friction.^{17,23,24,27} We note that in simulations with implicit solvent, friction can also be varied directly but usually via a simple δ -correlated friction model.^{32,33} Finally, we calculate the relaxation time τ of torsion transitions by integrating the cosine autocorrelation function (ACF) for Ψ (Figure S1 and Supporting Methods 1.2), similar to previous work.²³ As a cross validation on the results, we have also built a two-state Markov-state model (MSM)^{34–36} using the discretization described in Supporting Methods 1.3.

The relaxation time as a function of solvent viscosity and barrier height is shown in Figure 2D. We have obtained essentially identical results from the Ψ correlation time and the two-state MSM for all but the lowest barriers of alanine dipeptide; that discrepancy is most likely due to the break-down, for these low barriers, of the two-state approximation implicit in the MSM construction performed here. The deviation of the relaxation times from simple proportionality to viscosity has been quantified in experiments and simulations using two procedures. The first is a fit to a power law dependence on viscosity, $\tau(\eta) = \tau_0(\eta/\eta_0)^\beta$, where η is the viscosity, τ the relaxation time, and τ_0 and η_0 the relaxation time and viscosity corresponding to pure water under the conditions chosen. A power law exponent less than unity indicates internal friction. This relation fits well data at low viscosities that are primarily accessible in simulation. Internal friction can also be quantified from the τ -intercept of a linear fit to the data, known as the internal friction time τ_i .¹⁵ The linear fit describes the data at higher solvent viscosities better than a power law in both experiments¹⁵

and simulations.^{17,23} To characterize the internal friction, we report both the power law exponent β , as well as a parameter α , namely the intercept normalized by the relaxation time at normal viscosity ($\alpha = \tau/\tau_0$), to correct for the contribution to the relaxation time coming from varying the barrier height (a similar approach has been used previously²³). The fitting methods and the applicability of β and α are discussed in Supporting methods 1.4. Here, we note only that both fits are phenomenological, but that they yield similar conclusions. A value of $\beta = 1$ or $\alpha = 0$ corresponds to a linear relation between the relaxation time and solvent friction and therefore no internal friction, while smaller β values or larger α values suggest a strong internal friction.

There is a clear trend of increasing internal friction (larger β , or smaller α) in alanine dipeptide with increasing local torsion barrier height, whether the relaxation time is estimated from the ACF or the MSM (Figure 3). This emphasizes the major contribution of the torsion barrier itself to the internal friction of this simple peptide, consistent with the argument that solvent memory effects are the cause of the internal friction: since barrier curvature increases with barrier height, while the solvent friction kernel should remain the same, Grote-Hynes theory would predict a stronger deviation from a Kramers-like dependence on friction, as is indeed observed.^{37,38} To quantify the dependence of α or β on barrier height in Figure 3, we have determined the slope m of a linear fit to the dependence of α or β on barrier height, with errors δm estimated by a bootstrapping method. In Table 1, we report error bounds on the slope ($m - \delta m$, $m + \delta m$), as well as the overall probability of the true slope being positive.

An interesting feature of the results is that the exponent β does not reach a limiting value of unity for a vanishing barrier in alanine dipeptide. However, this limit is very much an exception in the data set, because the absence of a barrier means that barrier curvature in reaction rate theory cannot be used to illuminate the result. Some of the insensitivity to viscosity in this case may arise because alanine dipeptide is small enough to make some torsion transitions without displacing any water molecules, limiting the contribution from solvent viscosity to the torsion relaxation time. To test this hypothesis, we have frozen all water molecules by applying an harmonic position restraint to each cartesian component of the position of the oxygen atom of the water molecules, with a spring constant of 1000 kJ/mol/nm² (corresponding to a mean-square displacement of 0.05 nm at 300 K). We show the Ramachandran plot and the ACF in both the non-restrained and restrained cases in the normal solvent viscosity in Figure S2. Both α_R and extended minima are still present in the free energy surface with the restraints (although with slightly different energies, as expected).

Local barriers of alanine-8—In order to compare the dependence of internal friction on local barriers with the dependence on the global folding barrier, we need a minimal folding model. For this purpose, we have chosen alanine-8, which has a weak propensity for helix formation,^{39,40} and no clear global barriers in the force field we use. Therefore it serves as a good model for testing the effect of varying both the local and global barriers on the internal friction. First we introduce the same additional torsion term (Eq. 1) as used in alanine dipeptide to adjust the local torsion barrier, with $k_{\Psi} = -3, -1.5, 0, 1$, and 2 kJ/mol, and $\Psi_s = 130^\circ$ (note that Ψ_s is different from that used for alanine dipeptide, to capture a slight shift

of the free energy surface $F(\Psi)$ in the context of the longer peptide). As shown in the free energy for Ψ (averaged over all eight Ψ angles) in Figure 2B, the torsion barrier height G_{Ψ} , defined as the free energy difference between the extended states and the top of the barrier at $\Psi = 70^{\circ}$, varies from 6.0 to 15.5 kJ/mol (Figure 2B).

We probe the viscosity dependence of both local relaxation times, from the cosine ACF of Ψ averaged over all Ψ angles (individual torsion ACF is shown in Figure S3, S4, S5, S6 and S7), and of the global relaxation time, from the correlation time of the fraction of native contacts Q (Figure S8), similar to a previous study using the ACF of end-end reconfiguration time to study the global relaxation of an unfolded protein.²³ The definition of Q is given in the Supporting Methods 1.5. Native contacts are defined relative to a fully helical structure for alanine-8. We obtain qualitatively similar results for the correlation time of the radius of gyration (Figure S9). We find about one order of magnitude variation of the global relaxation time when changing the local torsion barriers. Again, there is a clear correlation (Figure 3 and S1) between the local barrier height and the internal friction of global relaxation time, with β_Q changing from 1.00 to 0.71 and α_Q changing from 0.03 to 0.35 from the smallest to the largest torsion barriers. The internal friction of local relaxation time ($\beta_{\Psi ACF}$ and $\alpha_{\Psi ACF}$) become less sensitive to the barrier height for barriers above the normal dihedral barrier height. However a decreasing trend of internal friction from local relaxation time when increasing barrier height is still statistically most likely (Table 1). These results demonstrate that torsion barriers are a major contribution to internal friction effects, as probed by both local and global relaxation times.

We have also estimated the global relaxation time by using the torsion-discretized MSM. The relaxation time from the slowest mode in the MSM is consistent with that of the ACF, except for the three largest viscosities, for the highest barrier height (Figure 2E). This is probably because of sampling limitations for the highest barrier, in which the relaxation time is only one order of magnitude smaller than the trajectory length. This is also seen in the large error bar of $\beta_{\Psi MSM}$ and $\alpha_{\Psi MSM}$ for the highest barrier using the MSM (Figure 3). However for the first four barrier heights, there is still a clear correlation between local barrier height and internal friction of relaxation time from the slowest mode of MSM. The discrepancy for the highest barrier may arise from limited sampling of transitions for such large barrier heights.

Qualitatively, our result of decreasing internal friction for lower barrier heights is consistent with earlier work showing a decrease of internal friction in unfolded proteins when the barrier height was scaled by a factor of 0.5.²³ However, our interpretation is different in that we relate the internal friction to the intrinsic viscosity dependence of torsion isomerization, rather than to the internal friction inferred from the fit of a suitable polymer model.²³

The internal friction of alanine-8 almost vanishes for the lowest torsion barrier we have tried, different from alanine dipeptide. This is presumably because, unlike alanine dipeptide, significant solvent displacements are required for torsion isomerization in the larger alanine-8 molecule. Thus, if the water is restrained in a similar fashion to what was done for alanine dipeptide, no global transitions are possible (in Q) and torsional transitions are only possible for terminal residues, as might be anticipated.

Global barrier of alanine-8—Having established the strong impact of local dihedral barriers on the internal friction of local and global relaxation time, the remaining question we want to address is whether there is an impact of the height of the global folding barrier on internal friction observed in the global relaxation time. The reason that some variation might be expected is that higher global barriers will make the global dynamics sensitive to a smaller region of the energy landscape, and hence may reduce the apparent internal friction. We revisit alanine-8 by varying the global barrier along the fraction of native contacts Q , which is known to be a reasonable coordinate for protein folding.^{41,42} The global barrier is tuned by introducing an external Gaussian potential

$$U = k_Q \exp(-(Q - 0.695)^2 / 0.02), \quad (2)$$

in which k_Q is varied to be 6.27, 12.54 and 18.81 kJ/mol. This is a convenient, although slightly contrived, way of tuning the global barrier whilst exactly preserving the local features of the energy landscape. Changing the barrier height as in real proteins, by varying sequence, (or by varying contact strength in a computational model) would invariably change both the local and global barriers, making their effects on internal friction hard to disentangle.

When increasing global barrier height, sampling becomes challenging. Here we use a variant⁴³ of transition-path sampling (TPS),⁴⁴ in which transition paths are obtained by running trajectories from a dividing surface on a reaction coordinate, close to the top of the barrier on that reaction coordinate (Q here). It has been successfully used to study internal friction in the folding of the GB1 hairpin and Trp cage mini protein.²⁷ TPS focuses on sampling rare transitions between stable states (here: unfolded and folded), avoiding wandering in each of these free energy basins between transitions, and therefore tremendously reduces the statistical error in the rate estimate, with orders of magnitude more folding events than in conventional brute force simulation.

In Figure 2C, we show four different barrier heights, including the case without an external bias on Q . Since the unbiased potential does not have a clear folding barrier on the free energy along Q , TPS would not give any advantage relative to brute force simulations. Therefore we still use the relaxation time from the ACF along Q as in the previous section. In the other cases, where TPS was performed, we have estimated β in two ways: firstly, from the folding relaxation rate computed from TPS, and secondly from the transition-path times obtained from the same calculation. It is clear that in all the four cases we have tested, the exponential fitting exponent β for the relaxation of Q is close to 0.75 (Figure 3), suggesting no significant change in the internal friction when changing global folding barriers. The internal friction for the transition path time has a similar trend, albeit with larger errors in the largest barrier. Consideration of statistical errors suggests no evidence of increasing internal friction when increasing global barrier height for both folding relaxation time and transition-path time (Table 1). Albeit for a simple peptide folding case, this suggests that the lower internal friction observed in slow folders is not intrinsic to their large folding barrier height.

The simplest interpretation of the results of the global barrier variation is by applying Kramers rate theory to the global transition. At least in the systems we have studied, local torsion barriers determine the viscosity dependence of the diffusion coefficient on Q and therefore the internal friction of the folding rate. Varying the global barrier on Q via an external potential scales the folding rate without varying the diffusion coefficient viscosity dependence. This is a non-trivial result because the folding diffusion coefficient is position-dependent and therefore its viscosity dependence will be too; varying the global barrier height could in principle have altered the sensitivity of rate to viscosity. A caveat is the somewhat contrived way in which the folding barrier has been varied, however alternative methods would not have yielded a clear conclusion. In all cases we have tested, including our previous work on two mini-proteins,²⁷ torsion transitions remain the key factor in determining the viscosity dependence of the diffusion coefficient, and therefore the internal friction of folding.

In summary, our results from variation of local (dihedral) barrier heights confirm the importance of these barriers in determining internal friction, in both alanine dipeptide and alanine-8. Furthermore, the strong variation of solvent viscosity dependence with barrier curvature in alanine dipeptide strongly suggests solvent memory effects to be an origin of internal friction. Other effects may of course be important in different systems. Variation of the global folding barrier on Q , however, results in no change in internal friction of alanine-8. Though the current study is on short peptides, it still suggests that the larger internal friction of fast-folding proteins is related to the fact that many of them are α -helical and that their folding mechanisms are intrinsically more likely to exhibit internal friction,²⁷ rather than to their low folding barrier heights.

A subject not addressed by the present work is the origin of internal friction in unfolded chains. In that case, the variation of solvent quality (by changing denaturant concentration) has been shown to strongly influence the degree of internal friction. Both torsional transitions²³ as well as other factors such as solvent exclusion may play a role here, but further investigation on internal friction in disordered states is needed to address this.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic showing two types of barrier varied in the simulations. (A) Torsional barrier for α/β isomerization about the Ψ torsion angle. (B) Global free energy barrier on the fraction of native contacts Q for folding of a helical structure. Structures illustrate the type of transition occurring, and red arrows the barriers which are varied.

Figure 2.

Effect of local and global barrier height on internal friction. Upper row: (A) variation of backbone Ψ torsion barrier for alanine dipeptide; (B) variation of local Ψ torsion barriers in alanine-8 (figure shows potential of mean force averaged over all internal Ψ angles of alanine-8); (C) variation of global free energy barrier on fraction of native contacts Q for alanine-8. Lower row shows the solvent-viscosity dependence for different barrier heights (colors as in (A)–(C)) for (D) alanine dipeptide (E) alanine-8 local barrier variation and (F) alanine-8 global barrier variation. Dash lines show an exponential fit and dotted lines a linear fit.

Figure 3.

Normalized linear fitting intercept α ((A), (B), and (C)) and power law fitting exponent β ((D), (E), and (F)) of relaxation time dependence on solvent viscosity in alanine dipeptide and alanine-8. (A), (B), (D) and (E) show α and β changes for variation of local barriers in alanine dipeptide and alanine-8, and (C) and (F) for variation of global barrier height in alanine-8.

Table 1Trends of β with variation in barrier height.

	$P_{\alpha+}^{a)}$	$[m_{\alpha} - \delta m_{\alpha}, m_{\alpha} + \delta m_{\alpha}]^{b)}$	$P_{\beta+}^{a)}$	$[m_{\beta} - \delta m_{\beta}, m_{\beta} + \delta m_{\beta}]^{b)}$
Ala-dipeptide Ψ_{ACF}	1.00	[0.018,0.020]	0.00	[-0.022,-0.019]
Ala-dipeptide Ψ_{MSM}	1.00	[0.008,0.010]	0.00	[-0.008,-0.006]
Alanine-8 Q	0.95	[0.009,0.040]	0.02	[-0.038, -0.014]
Alanine-8 Ψ_{ACF}	0.87	[0.001,0.021]	0.11	[-0.018, -0.002]
Alanine-8 Ψ_{MSM}	0.72	[-0.008,0.034]	0.55	[-0.013, 0.017]
Alanine-8 ^{c)} Q	0.75	[-0.009,0.040]	0.04	[-0.049,-0.013]
Alanine-8 ^{c)} Ψ_{ACF}	0.92	[0.006,0.040]	0.12	[-0.028, -0.003]
Alanine-8 ^{c)} Ψ_{MSM}	0.92	[0.006,0.039]	0.11	[-0.042,-0.004]
Alanine-8 τ	0.58	[-0.006,0.009]	0.52	[-0.007,0.008]
Alanine-8 τ_{ip}	0.98	[0.01,0.04]	0.19	[-0.022,0.001]

^{a)} The probability of a positive dependence of β on barrier height.^{b)} m is the slope between β and barrier height and δm the standard error.^{c)} Estimation without the highest barrier.