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Correlation times and adiabatic barriers for methyl rotation in SNase

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Abstract

The relation of rotational correlation times to adiabatic rotational barriers for alanine methyl groups in staphylococcal nuclease (SNase) is investigated. The hypothesis that methyl rotational barriers may be useful probes of local packing in proteins is supported by an analysis of ten X-ray crystal structures of SNase mutants. The barrier heights are consistent across a set of ten structures of a native SNase and mutants containing single-point mutations or single or double insertions, most in a ternary SNase complex. The barriers for different methyls have a range of 7.5 kcal/mol, which at 300 K would correspond to a five-order-of-magnitude range in correlation time. It is demonstrated that adiabatic rotational barriers can fluctuate significantly during an MD simulation of hydrated SNase, but that a Boltzmann weighted average is predictive of rotational correlation times determined from correlation functions. Even if a given methyl is on average quite sterically hindered, infrequently sampled low-barrier conformations may dominate the Boltzmann distribution. This result is consistent with the observed uniformity of NMR correlation times for ^{13}C-labeled methyls. The methyl barriers in simulation fluctuate on multiple time scales, which can make the precise relationship between methyl rotational correlation time and methyl rotation barriers complicated. The implications of these issues for the interpretation of correlation times determined from NMR and simulation are discussed.

Introduction

The dynamics of methyl groups in proteins is of interest as an indicator of protein function (Wand et al., 1996; Mittermaier et al., 1999; Ishima et al., 2001a; Finerty et al., 2002) and of the contribution of side chain mobility to the conformational entropy (Li et al., 1996; Yang et al., 1997). For example, NMR characterization of the dynamics of methyl clusters has led to the postulate of a mechanism for the development of drug resistance in HIV-l protease (Ishima et al., 2001a). Methyl dynamics has been correlated with regions of structural plasticity, or 'hot spots' in several proteins, including an SH2 domain (Wand et al., 1996; Finerty et al., 2002). More generally, it has been suggested that the dynamics of methyl groups may be correlated with the conformational entropy of a protein's side chains (Akke et al., 1993; Li et al., 1996; Yang et al., 1997), which may contribute significantly to the fine balance of terms involved in protein folding. Finally, protein flexibility and the steric effects of protein packing may affect the barriers to methyl rotation, and thus measurement or calculation of rotational barriers may provide information on protein packing and flexibility.

The dynamics of methyl groups has been measured with ^{2}H and ^{13}C NMR (e.g., Muhandiram et al., 1995; Wand et al., 1996; Nicholson et al., 1996; Ishima et al., 2001a). Methods for selective labeling of $^{13}CHD_2$ isotopomers have recently been developed for ^{13}C relaxation studies of methyl dynamics (Ishima et al., 2001b). Thus the characterization of methyl dynamics is feasible and is of considerable interest.

NMR provides information about both the spatial extent of motion and the time scale. The simplest model-free analysis (Lipari and Szabo, 1982a,b) de-

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termines a squared order parameter, S^2, which is a measure of the degree of motional restriction (S^2 ranges from 0 for unrestricted motion to 1 for complete restriction), and a correlation time, τ_e. Most of the experimental analyses to date have focused on S^2. The value of S^2 for relaxation of a methyl ^{13}C, ^{1}H, or ^{2}H nucleus is usually assumed to be the product of a factor representing rotation about the methyl symmetry axis, S^2_{rot}, and a factor representing motion of the methyl symmetry axis, S^2_{axis}:

$$S^2 = S^2_{rot} S^2_{axis}. \quad (1)$$

For a methyl with perfect tetrahedral symmetry, it can be shown that S^2_{rot} is 0.111 (1/9 exactly). Thus S^2_{axis} has usually been determined by dividing S^2 by 0.111, although departures from tetrahedral symmetry have been shown to cause deviations of S^2_{rot} from the theoretical value by up to 20% (Wand et al, 1996; Chatfield et al., 1998). Most discussion of the relation between protein function and methyl dynamics has centered on S^2_{axis}.

In this paper, however, we focus on the rotational barriers of methyl groups, and in particular, their relation to correlation times. Like the order parameters, methyl correlation times reflect contributions from both motion of and rotation about the symmetry axis (Mittermaier et al., 1999). We will use molecular dynamics (MD) simulation and restrained geometry optimization to assess the extent to which methyl adiabatic rotational barriers are predictive of rotational correlation times.

This is the fourth in a series of papers (Chatfield et al., 1998, 2000, 2003) addressing methyl rotations in proteins, peptides, and amino acids. The first was a comparison of NMR and MD characterization of alanine and leucine methyl rotation in staphylococcal nuclease (SNase). We showed that while backbone order parameters were generally in good agreement, simulation had a much greater variation in methyl rotational correlation times that did NMR. It was postulated that this was due in part to incomplete conformational sampling on the MD time scale (18 ns). The next two papers compared NMR and MD motional parameters for crystalline amino acids and a crystalline dipeptide, systems whose order and small size avoid the problem of conformational sampling on the simulation time scale. We showed that, after correcting for neglect of tunneling, MD correlation times agreed with NMR to within a factor of two. Furthermore, we demonstrated that adiabatic rotational barriers are predictive of effective barriers during simulation to ± 1 kcal/mol in these crystalline systems. Thus we have confidence that molecular mechanics force fields can reproduce the steric environment of methyl groups well enough to model the methyl rotational dynamics meaningfully.

In this paper, we return to the protein SNase and examine the variability in methyl adiabatic rotational barriers in two situations: among the different conformations sampled during an MD simulation of native SNase, and among X-ray crystal structures of related SNase mutants. We address the following questions: Do adiabatic rotational barriers determine methyl rotational correlation times in proteins? How great is the variation in rotational barriers among the alanine methyls of SNase in a particular conformational substate (i.e., for a particular MD trajectory frame)? How great is the variation in rotational barriers among different conformational substates of SNase (i.e., over the course of an MD trajectory)? Do differences survive thermal averaging? How great is the variation in rotational barriers across a set of X-ray crystal structures of closely related mutants? Finally, we consider the implications for the possible use of methyl rotation as a probe of local packing in proteins.

Methods

Adiabatic methyl rotational barriers were calculated with the program CHARMM (Brooks et al., 1983) using the all-atom parameter set PARM30 (Molecular Simulation, 1990; Momany and Rone, 1992) with modifications to certain torsional parameters to make the methyl rotational barriers realistic, as described previously (Chatfield et al., 1998). With this modification, the adiabatic rotational barrier for an isolated neutral alanine is 3.40 kcal/mol, in agreement with ab initio calculations. PARM30 was the only CHARMm parameter set available for treating both peptides and polynucleotides at the time the SNase simulations were performed. Waters were represented with a modified TIP3P model (Jorgensen et al., 1983; Steinbach and Brooks, 1993). Electrostatics were treated with the force switch method with a switching range of 9–13 Å, and van der Waals forces were treated with the switch method using a 13-Å cutoff. Nonbond lists were kept to 15 Å and updated heuristically.

We calculated methyl adiabatic rotational barriers by restrained minimization. The value of ϕ, the angle of rotation about the three-fold axis, was restrained to particular values so as to sample the well minimum or the barrier maximum while the rest of

the protein was energy minimized. However, energy-minimization with the entire protein structure flexible (i.e., full minimization) has two drawbacks. First, full minimization can be computationally expensive if many minimizations need to be performed as, for example, in quenched dynamics. Second, the protein's overall structure can change significantly, altering the methyl rotational barrier. Thus the concept of adiabaticity becomes ambiguous when applied to localized motions such as methyl rotational transitions in the context of a large molecule such as a protein.

Therefore we devised a minimization protocol in which only the local environment of a given methyl is optimized during restrained minimization. We allow atoms within a sphere of radius R_{free}, centered on the carbon of the methyl under consideration, to move, and hold the coordinates of all other atoms are fixed. Thus we divide a protein conceptually, and somewhat arbitrarily, into a fixed portion whose conformation largely determines the adiabatic barrier, and a free portion postulated to sample the adiabatic barrier according to a Boltzmann distribution. This corresponds to assuming that global conformational changes related to motions of atoms fixed during the protocol are slow relative to the methyl rotational correlation time, while local conformational changes related to motions of atoms free during the protocol are fast relative to the methyl rotational correlation time.

To determine an optimum value for R_{free}, we calculated adiabatic barriers as a function of R_{free} for four alanine residues in SNase. These residues include both interior and near-surface methyls, as well as methyls with both fast and slow MD rotational correlation times. The starting structure was a trajectory frame selected randomly from an MD simulation previously described (Chatfield et al., 1998). Figure 1 shows that the barriers for all four methyls are relatively constant for R_{free} between 5 and 8 Å but not for for other values of R_{free}, particularly for larger ones. We chose the smallest value of R_{free} within the relatively steady region, 5 Å, for further use. Choosing a value of 5 rather than 8 Å for R_{free} reduces the number of free atoms by a factor of 4.3 and the computer time by 78%.

The optimization protocol was applied to selected trajectory frames from two MD simulations of SNase: an 18-ns simulation of a hydrated SNase molecule liganded with dpTp and a single Ca^{2+} ion; and a 3.75-ns simulation of hydrated but unliganded SNase. These simulations were described in detail previously (Chatfield et al., 1998). The MD trajectory frames were optimized separately for each of the twelve alanines.

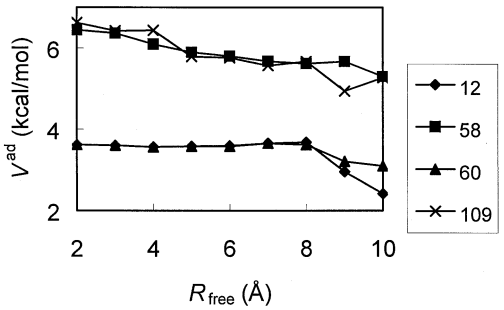

Figure 1. Methyl adiabatic rotational barrier as function of size of flexible portion of protein for alanine residues 12, 58, 60, and 109. All atoms more than R_{free} Å from the β-carbon of the given residue are fixed.

Thus a given trajectory frame was subjected to twelve different applications of the restrained optimization protocol, the starting conformation being the same in each. The optimization protocol was also applied to ten different X-ray structures of SNase (the ternary complex of the native protein, the unliganded native protein, and eight mutant structure listed below).

Correlation times for methyl rotation obtained with ^{13}C-NMR, τ_{NMR}, have been reported for alanines and leucines in SNase (Nicholson et al, 1996). These were determined by fitting the spectral density, obtained from T_1, T_2, and the NOE, to an expression in terms of τ_{NMR}, a generalized order parameter, S, and a correlation time for overall tumbling. Analogous correlation times were obtained from MD simulation of SNase (Chatfield et al., 1998) by fitting the correlation function, $C(t)$, for rotation about the methyl axis to an exponential function

$$C(t) = S^2 + (S_f^2 - S^2)\mathrm{e}^{-t/\tau_{\text{MD}}}, \qquad (2)$$

where S_{f}^2 corresponds to fast (subpicosecond) decay of the correlation function. The time scale of this fast decay is smaller than the spacing between saved trajectory frames (0.8 ps), so it is represented as instantaneous in Equation 2. This fast decay, which is due to libration of the C-H bond vector, is more than an order of magnitude faster than rotation about the methyl symmetry axis and is not pertinent to the present analysis. The details of the fitting procedure can be found in previous papers (Chatfield et al., 1998, 2000, 2003).

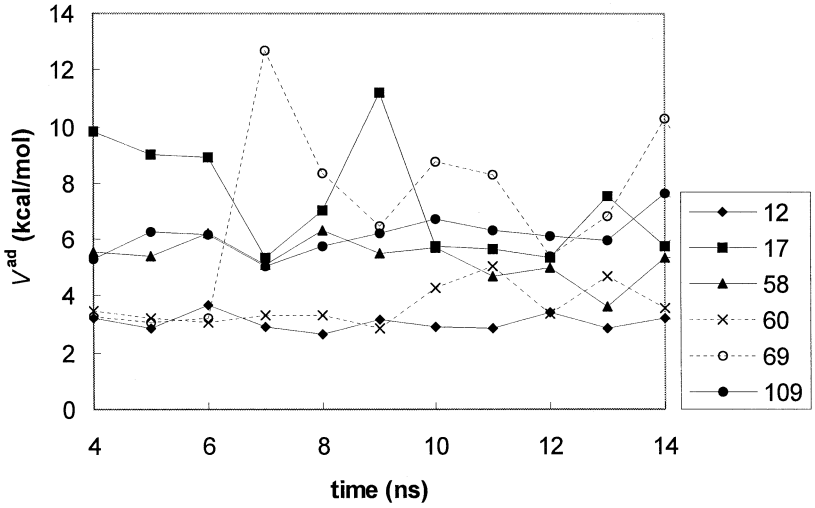

Figure 2. Methyl adiabatic rotational barrier for particular frames of the MD simulation of liganded SNase for alanine residues 12, 17, 58, 60, 69, and 109. The frames are taken every 1 ns.

Results and discussion

Barriers in MD simulations

Figure 2 shows adiabatic barriers calculated at 1-ns intervals during an 11-ns portion of the liganded SNase simulation (the portion used for analysis previously) for six representative alanine residues. Barriers for some residues such as Ala12 are relatively constant, but others, such as Ala17 and Ala69, exhibit fluctuations of more than 5 kcal/mol. This suggests that conformational changes have a large effect on the barriers. Furthermore, some conformational changes appear infrequent on the simulation time scale and are thus not well sampled. This suggests that the MD simulation is not long enough to statistically sample the relevant portion of conformational space, and it could explain the difference between NMR methyl correlation times methyl carbons and those calculated from the MD simulation. The NMR and MD correlation times for this simulation (Chatfield et al., 1998) are reproduced in Table 1.

To better assess the fluctuations in barriers to rotation, barriers were calculated every 20 ps in the interval between simulation times of 8 and 10 ns. These are shown in Figure 3 for the same six residues. In some regions, such as near 8.2 ns for Ala12 and 8.5 ns for Ala58, this sampling frequency appears to be sufficient to capture the frequency of barrier fluctuation; but in other regions such as near 8.1 ns for Ala17 or the entire 2-ns window for Ala69, even this sampling frequency is insufficient. (When the data points form a smooth curve, sampling is sufficient to represent the frequency of fluctuation; when the barriers oscillate from point to point, sampling is insufficient.) Determining the barrier for every saved trajectory frame (every 0.8 ps; data not shown) captures the frequency of barrier fluctuation in some but not all of these regions.

The fluctuation in barrier heights implies that a single structure will not be quantitatively predictive of correlation times for methyl rotation in solution. If rotational correlation times can be related to adiabatic barriers in proteins, as previous work on crystalline amino acids suggests, it will be necessary to average

Table 1. Alanine methyl rotational correlation times (ps)

Residue	Liganded		Unliganded	
	τ_{NMR}	τ_{MD}	τ_{NMR}	τ_{MD}
12	27	7.5	30	10
17	29	450	28	11
58	55	590		49
60	24	23	29	46
69	25	280	29	100
90	32	36	36	12
94	16	220	15	21
102	35	130		50
109	21	1100	31	130
112	16	72	22	47
130	21	38	22	17
132	50	28	58	35

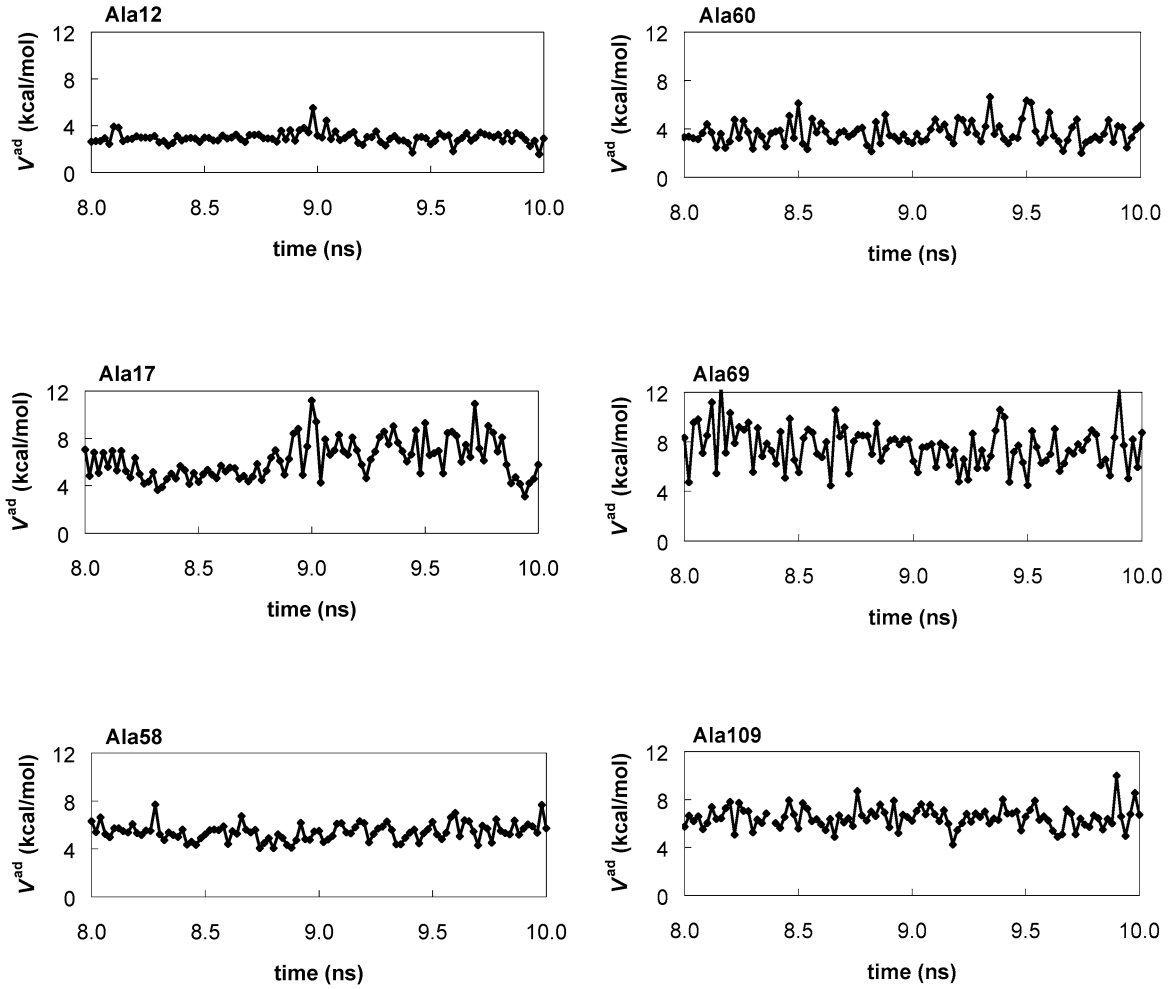

Figure 3. Like Figure 2, but for frames taken every 20 ps. Here each residue is given a separate window for clarity.

appropriately over the conformations sampled. We begin by noting that the correlation time for rotational transitions about a three-fold axis is related to the rate constant k by

$$\tau = \frac{1}{3k}. \tag{3}$$

Assuming an Arrhenius relation for k with activation energy equal to the adiabatic barrier, V^{ad}, and averaging over the trajectory frames, yields an effective correlation time, $\tau_{\mathrm{e}}^{\mathrm{ad}}$

$$\tau_{\mathrm{e}}^{\mathrm{ad}} = \left(3A\left\langle \exp(-V^{\mathrm{ad}}/RT\right\rangle\right)^{-1}, \tag{4}$$

where $<\quad>$ represents an average, and the frequency factor, A, is assumed to be the same for all methyls regardless of environment.

Table 2 compares τ_{MD} with $\tau_{\mathrm{e}}^{\mathrm{ad}}$ for the simulation of liganded SNase. Sampling for the average in Equation 4 was at 20-ps intervals. The frequency factor was determined by setting $\tau_{\mathrm{e}}^{\mathrm{ad}}$ equal to τ_{MD} for Ala12, whose methyl had the smallest correlation time, and thus presumably the best sampling statistics for rotational transitions, in the simulation. The discrepancies observed when τ_{MD} is greater than 100 ps may be due to poor sampling of rotational transitions and should not be overinterpreted. The comparison is moderately favorable, but it suggests that the adiabatic rotational barriers have a significant influence on the rotational dynamics. On the basis of this finding, we proceeded with an analysis of adiabatic rotational barriers in a set of SNase mutants.

Table 2. Alanine methyl rotational correlation times (ps) for liganded SNase calculated from correlation functions (Equation 2) and from adiabatic barriers (Equation 4)

Residue	τ_{MD}	τ_e^{ad}
12	7.5	7.5
17	450	140
58	590	170
60	23	11
69	280	45
90	36	22
94	220	127
102	130	58
109	1100	298
112	72	31
130	38	20
132	28	23

Barriers in X-ray crystal structures

We determined the variability in methyl adiabatic rotational barriers, given in Table 3, across a set of X-ray crystal structures of SNase from the Protein Data Bank (Berman et al., 2000). The structures 1SNC and 1STN were those used to start the liganded and unliganded simulations, respectively. The other structures are mutants: 2ENB (D21E), 1ENC (D21E), 1NUC (V23C), 1SNM (E43D), 1STA (INS(P11-GG)), 1STB (INS(L36-L)), 1SYD (P117G), and 1SYF (P117T). All of the mutants are ternary complexes with dpTp and a single Ca^{2+} ion, except for 2ENB, which is missing the Ca^{2+} ion. The mutants have one single-point mutation each except for 1STB and 1STA, which have one and two insertions, respectively. Variability in an adiabatic rotational barrier across the nine structures of liganded SNase could represent either sampling of different conformational substates in the minimized structures or the effect of single-point mutations. Thus we postulate that the total variability represents an upper bound to the variability due to sampling of conformational substates alone.

Methyl adiabatic rotational barriers for the ten crystal structures range from 1.5–9.0 kcal/mol. There is significant though not perfect consistency in the barrier heights across the set of liganded structures. For example, the Ala102 barrier has a range of 4.7–7.9 kcal/ mol across the set of structures, while the Ala132 and Ala60 barriers have ranges of 4.1–4.9 and 3.0–3.9 kcal/mol, respectively. In all but two structures, Ala94 has a barrier of less than 3.0 kcal/mol. These four methyls thus have largely distinct ranges of methyl rotational barrier. Across the set of nine liganded structures, the standard deviation in the barrier height for a given methyl ranges from 0.13 to 1.7 kcal/mol, with an average of 0.8 kcal/mol. These observations support the hypothesis that methyl adiabatic rotational barriers could be useful indicators of a methyl's local steric environment, or more generally, of protein packing.

The adiabatic methyl rotational barrier for an isolated alanine, when computed with our parameter set, is 3.4 kcal/mol. Comparison of the barriers for unliganded and liganded structures reveals that the former are almost always closer to 3.4 kcal/mol than are the latter; that is, both higher and lower barriers are much more common in the liganded structures. It is obvious that higher barriers can result from unfavorable steric interactions. Lower barriers result if steric interactions raise the energy of the well minimum more than the barrier maximum. Ala94 demonstrates the latter effect in most of the liganded structures. Because packing can result in both high and low barriers, the average methyl barrier for a structure is not a good measure of packing. A more useful measure is the average unsigned deviation (Ave. Dev.) of the barriers from 3.4 kcal/mol, which is 0.57 kcal/mol for the unliganded structure 1STN but in the range 0.81–1.21 kcal/mol for all of the liganded structures. This suggests that in its unliganded form, SNase has greater flexibility, enabling it to moderate unfavorable steric interactions. In this regard, we note that the radius of gyration differs only slightly between the 1SNC and 1STN crystal structures (14.3 and 14.4 Å, respectively) and between the simulation average for liganded and unliganded SNase (14.4 and 14.3 Å, respectively). Thus the differences in the methyl rotational barriers do not appear to be due to differences in overall packing.

In contrast to the variability in the rotational barriers across the set of twelve alanines in a single structure, NMR rotational correlation times are generally quite uniform for both the liganded and unliganded forms of SNase (see Table 1). This is probably due to in part to Boltzmann averaging. In solution, a great number of conformations are sampled. If conformational changes are sufficiently frequent (vide infra), the low-barrier conformations will have much greater weight than high-barrier conformations in determining the rotational correlation times. Thus the NMR methyl correlation times may not well represent a methyl's average steric environment. In other words, protein

Table 3. Alanine methyl rotational barriers (kcal/mol) in X-ray structures

Residue	1STN	1SNC	1ENC	1SNM	2ENB	1SYF	1SYD	1STA	1STB	1NUC	Ave.	Ave. Dev.[a]	St. Dev.[b]
12	3.1	2.9	2.7	3.3	2.8	3.4	3.8	8.4	3.3	3.8	3.8	0.8	1.7
17	3.2	3.1	3.6	3.0	2.4	2.8	3.1	3.4	3.5	3.0	3.1	0.4	0.4
58	3.5	5.1	4.0	5.5	3.8	5.4	4.8	6.2	4.5	5.0	4.9	1.4	0.8
60	3.3	3.5	3.7	3.7	3.6	3.4	3.0	3.7	3.6	3.9	3.6	0.2	0.3
69	5.2	6.2	2.5	3.8	1.5	3.6	4.0	4.9	5.6	4.3	4.0	1.3	1.5
90	3.6	3.9	3.7	3.6	3.8	3.5	3.7	3.9	3.9	3.7	3.7	0.3	0.1
94	2.9	1.7	1.5	1.8	1.8	1.6	2.3	3.5	3.3	2.2	2.2	1.2	0.7
102	5.9	6.4	6.9	6.4	6.0	5.9	7.9	6.0	4.7	6.3	6.3	2.8	0.8
109	3.4	5.1	4.6	3.9	4.7	4.5	5.3	5.4	9.0	4.0	5.2	1.6	1.6
112	3.9	3.1	2.5	3.2	2.0	3.3	3.4	3.2	3.6	3.0	3.0	0.4	0.5
130	3.5	4.0	3.2	3.6	3.9	3.2	3.4		3.6	4.1	3.2	0.6	1.3
132	3.9	4.7	4.8	4.3	4.1	4.4	4.1	4.7	4.6	4.9	4.5	1.0	1.4
Ave.	3.8	4.1	3.7	3.8	3.4	3.8	4.1	4.8	4.4	4.0	4.0	1.0	0.8
Ave. Dev.[a]	0.6	1.2	1.0	0.8	1.0	0.8	1.0	1.5	1.1	1.0	1.0		

[a]Average unsigned deviation from 3.4 kcal/mol, the barrier calculated for an isolated alanine methyl. The last number in the row labeled Ave. Dev. is the average of the entries in the row.
[b]Standard deviation, calculated about the average value rather than about 3.4 kcal/mol.

packing and flexibility may influence adiabatic rotational barriers, but some of this information is lost in the Boltzmann averaging inherent in NMR correlation times.

In spite of their uniformity, in certain cases NMR methyl correlation times are quite instructive. One example is Ala94 which, as mentioned above, has a barrier lower than 3.4 kcal/mol in most of the X-ray crystal structures. If such low-barrier conformations were prevalent in solution, Boltzmann weighting would lead to a very small NMR correlation time. However, the NMR correlation time for Ala94 is close to average. We conclude that in solution, the steric interactions that raise the well minimum in the crystal have been removed. It would be interesting to investigate other methyls with small adiabatic rotation barriers in the crystalline state, but this is the only one for an SNase alanine.

Barrier fluctuation and NMR correlation times

The relationship between NMR correlation times and methyl rotational barriers is not simple. NMR correlation times are determined mainly by measurements of T_1. If methyl rotational barriers fluctuate quickly (ps time scale), the longitudinal magnetization will relax primarily via the low barriers, and T_1 will reflect the low barriers due to Boltzmann weighting. On the other hand, if methyl rotational barriers fluctuate on a much longer time scale, a macroscopic sample in effect contains a mixture of conformational substates with different methyl barriers. In this case, T_1 will be determined primarily by the larger barriers. Our simulations reveal that individual methyl rotational barriers fluctuate on multiple time scales. If the slow fluctuations are well separated in time scale from the fast fluctuations, T_1 will reflect the low-barrier microstates within the high-barrier conformational substates. However, the time scales of barrier fluctuation are not always well separated, and furthermore, there may be yet longer-scale fluctuations not sampled by the simulation. The relationship between correlation times measured with NMR and calculated with MD is therefore not simple and is a fruitful area for further research.

The relative uniformity of NMR methyl correlation times shows that, in solution, low-barrier conformations are sampled in all conformational substates, even if methyls are sterically hindered on average. This provides one assessment of whether a simulation accurately represents the solution environment: long-time simulations should sample low rotational barriers for all methyls. Likewise, for a simulation representative of solution, calculated methyl barriers may provide information inaccessible from NMR correlation times alone.

Table 4. Alanine methyl rotational barriers (kcal/mol) for liganded SNase

Residue	$V^{\text{eff}}_{\text{NMR}}$	$V^{\text{eff}}_{\text{MD}}$	$\langle V^{\text{ad}}_{\text{MD}} \rangle$	$V^{\text{ad}}_{\text{1SNC}}$
12	3.6	2.8	3.1	2.9
17	3.6	5.3	6.2	3.1
58	4.0	5.4	5.8	5.1
60	3.5	3.5	3.8	3.5
69	3.5	5.0	6.4	6.2
90	3.7	3.8	3.9	3.9
94	3.3	4.8	5.6	1.7
102	3.7	4.5	4.8	6.4
109	3.4	5.8	6.6	5.1
112	3.3	4.2	4.4	3.1
130	3.4	3.8	3.6	4.0
132	3.9	3.6	4.0	4.7
Ave.	3.6	4.4	4.8	4.1
Ave. Dev.[a]	0.2	1.1	1.6	1.2

[a]Defined in Table 3.

Table 5. Alanine methyl rotational barriers (kcal/mol) for unliganded SNase

Residue	$V^{\text{eff}}_{\text{NMR}}$	$V^{\text{eff}}_{\text{MD}}$	$\langle V^{\text{ad}}_{\text{MD}} \rangle$	$V^{\text{ad}}_{\text{1STN}}$
12	3.6	3.0	3.1	3.1
17	3.6	3.0	3.7	3.2
58	[a]	3.9	5.1	3.5
60	[a]	3.9	3.7	3.3
69	3.6	4.4	6.8	5.2
90	3.6	3.1	3.7	3.6
94	3.8	3.4	4.6	2.9
102	3.2	3.9	4.9	5.9
109	[a]	4.5	5.8	3.4
112	3.7	3.9	4.2	3.9
130	3.5	3.3	3.4	3.5
132	3.5	3.7	4.9	3.9
Ave.	3.6	3.7	4.5	3.8
Ave. Dev.[b]	0.2	0.5	1.1	0.6

[a]NMR data are not available for residues 58, 60, and 102.
[b]Defined in Table 3.

Comparison of barriers from NMR, MD, and X-ray crystal structures

An alternative way to compare methyl correlation times with adiabatic barriers is to define an effective barrier, V^{eff} , in terms of τ in analogy to Equation 4

$$V^{\text{eff}} = RT \ln(3A\tau). \quad (5)$$

We add a subscript to V^{eff} to indicate whether it pertains to NMR or MD simulation. Table 4 compares $V^{\text{eff}}_{\text{NMR}}$ and $V^{\text{eff}}_{\text{MD}}$ with the average adiabatic barrier during simulation, $\langle V^{\text{ad}}_{\text{MD}} \rangle$ (an average over the 12500 frames sampled at 20-ps intervals, without Boltzmann weighting), and with the barrier in the 1SNC crystal structure, $V^{\text{ad}}_{\text{1SNC}}$, for liganded SNase. Table 5 is analogous but for unliganded SNase.

Tables 4 and 5 generally confirm points made earlier. Values of $V^{\text{eff}}_{\text{MD}}$ are usually smaller than $\langle V^{\text{ad}}_{\text{MD}} \rangle$, reflecting the Boltzmann weighting implicit in the correlation times from which $V^{\text{eff}}_{\text{MD}}$ is calculated. Generally, the $V^{\text{eff}}_{\text{NMR}}$ are more uniform and smaller than the $V^{\text{eff}}_{\text{MD}}$.This does not, however, imply that barriers tend to be lower in solution than in the hydrated structures used in simulation (which were based on X-ray crystal structures). The difference could simply be a consequence of conformational space being inadequately probed on the simulation time scale. Several of the methyls do not sample low barriers in the MD simulation. The values of $V^{\text{eff}}_{\text{MD}}$ and $\langle V^{\text{ad}}_{\text{MD}} \rangle$ generally deviate from 3.4 kcal/mol more for liganded than for unliganded SNase. This is consistent with the X-ray data in suggesting that methyls in unliganded SNase are less sterically hindered. For both liganded and unliganded SNase, the values of $\langle V^{\text{ad}}_{\text{MD}} \rangle$ are generally larger than the adiabatic barriers for the crystal structures ($V^{\text{ad}}_{\text{1SNC}}$ and $V^{\text{ad}}_{\text{1STN}}$, respectively), consistent with the idea that a crystal structure represents a free energy minimum.

Conclusion

This work was motivated by the hypothesis that the rotational dynamics of methyl groups can serve as local probes of packing in proteins. We developed a simple minimization protocol for calculating methyl adiabatic rotational barriers in proteins. The coordinates of atoms more than 5 Å from the carbon of the methyl under consideration are fixed during restrained minimization, preserving the overall conformation of the protein. With this protocol we were able to demonstrate that methyl rotational correlation times calculated from an MD simulation of SNase are correlated with the adiabatic barriers to rotation. However, the adiabatic barriers fluctuate by as much as 7.5 kcal/mol during simulation, and due to Boltzmann weighting only the lowest of these values contribute significantly to the rotational correlation time. We suggest that this is why NMR correlation times measured in solution are so uniform, and that NMR correlation times unfor-

tunately do not reflect differences in the average steric environment of methyls very well. We also note that in simulation, methyl barriers fluctuate on several time scales, some long and some short relative to the methyl rotational relaxation time. This implies a complicated relationship between rotational adiabatic barriers and relaxation times measured by NMR. Further model studies to elucidate this relationship would be useful.

A study of methyl adiabatic rotational barriers in a set of X-ray crystal structures of SNase mutants revealed that barrier heights for alanine methyls varied over a range of 1.5–9.0 kcal/mol, and that the barriers were fairly well conserved across the set of mutants. This supports our fundamental hypothesis that methyl steric hindrance reflects protein packing. In fact, if NMR correlation times reflected these barrier height differences, they would vary over a range of five orders of magnitude. Together, these observations suggest that methyl dynamics may provide a useful probe of protein packing and flexibility, provided that simulation and NMR are used in a complementary fashion.

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