COMBINED EFFECT OF RESTRICTED ROTATIONAL DIFFUSION PLUS JUMPS ON NUCLEAR MAGNETIC RESONANCE AND FLUORESCENCE PROBES OF AROMATIC RING MOTIONS IN PROTEINS

RONALD M. LEVY AND ROBERT P. SHERIDAN
Department of Chemistry, Rutgers University, New Brunswick, New Jersey 08903

ABSTRACT A simple model is presented for the motion of phenylalanine and tyrosine rings in proteins. The model consists of restricted rotational diffusion of the rings about the side chain C^{α}—C^{β} (χ_1) and C^{β}—C^{γ} (χ_2) axes combined with 180° ring flips. The model is used to evaluate order parameters for nuclear magnetic resonance relaxation and fluorescence depolarization probes of aromatic ring motions in proteins. The dependence of the order parameters on orientation in the ring plane is examined and it is demonstrated that in the presence of ring flips, additional ring librations can have a large effect on the probe order parameters.

INTRODUCTION

The motions of aromatic side chains in proteins have been the subject of several recent experimental (1–8) and theoretical investigations (8–10). Many experimental techniques can be used to study the ring motions, including ^{13}C nuclear magnetic resonance (NMR) relaxation measurements of protonated and nonprotonated carbons, and ^{1}H and ^{2}H lineshape analysis and fluorescence depolarization measurements. NMR relaxation and fluorescence depolarization rates are determined by time correlation functions. These correlation functions decay on several different time-scales. On the shortest time-scale there is a rapid initial loss of correlation in the first few picoseconds (11). The fast decay results from the combined effect of the vibrational potential of the residue containing the nucleus, and collisions between the atoms of the residue and those of the surrounding cage in the protein. For residues that are involved in larger, more complex fluctuations, the initial decay is followed by a much slower loss of correlation over the next tens to hundreds of picoseconds. If the internal motions are restricted, the correlation functions will decay to a plateau value that is equal to the inverse of the square of the order parameter, which describes the distribution of NMR or fluorescence probe orientations (8, 11, 12). Finally, for experiments in solution, the correlation functions relax to zero owing to the overall tumbling of the proteins. Several studies have demonstrated that aromatic rings in proteins can undergo large-scale motions. For phenylalanine and tyrosine rings, these motions include flips about the C^{β}—C^{γ} ring axis on a time-scale between 10^{-3} s to less than 10^{-8} s (1, 2, 5–7). It is therefore natural to idealize the motions of aromatic rings as restricted rotations about the side-chain axes on a picosecond time-scale combined with infrequent ring flips, although the exact ring dynamics undoubtedly involves additional motions. Levy et al. (8, 11, 12) have used molecular dynamics simulations of pancreatic trypsin inhibitor (PTI) to evaluate the picosecond motional averaging of order parameters for NMR and fluorescence probes. In the course of this work it was observed that the order parameters were anomolously small for certain probe orientations on two of the eight aromatic rings of PTI. A closer examination revealed that these two rings both flipped once during the 96-ps trajectory (no other rings flipped). Although the ring flips are not statistically meaningful in the short trajectory, it is of interest to analyze the combined effect of librational motion and flips on spectroscopic probes of ring motions. The analysis presented below is useful for the interpretation of experiments on proteins in solution when the ring motions are faster than the protein-tumbling relaxation time.

In the following section, we consider a simple model for the motion of phenylalanine and tyrosine rings in proteins; the model consists of restricted rotational diffusion about the side chain C^{α}—C^{β} (χ_1) and C^{β}—C^{γ} (χ_2) axes combined with 180° flips of the rings. Probe order parameters are evaluated within the model. We demonstrate that in the presence of ring flips, the thermal oscillations of the ring will have a large effect on the order parameters for some orientations of the probes.

Restricted Diffusion Plus Jump Model

It has previously been shown (11, 12) that for very rapid internal motions (less than a few picoseconds), measured

NMR and fluorescence depolarization parameters can be used to evaluate order parameters that are determined by the orientational distribution of the probe. For dipolar NMR relaxation, when the internal motions are very fast,

$$\frac{1}{T_1} \simeq \mathcal{S}^2 \frac{1}{T_1^R}. \tag{1}$$

T_1 is the measured spin-lattice relaxation time, T_1^R is the rigidly tumbling protein value, and $\mathcal{S}^2$ is the square of the probe order parameter. The order parameter satisfies $0 \leq \mathcal{S} \leq 1$ and is a measure of the spatial restriction of the motion in the sense that $\mathcal{S} = 0$ when the motion is isotropic whereas $\mathcal{S} = 1$ if it is completely restricted. Lipari and Szabo (13, 14) have developed a more general relation valid for internal motions with components that relax between 1 and 100 ps

$$\frac{1}{T_1} = \mathcal{S}^2 \frac{1}{T_1^R} + C(1 - \mathcal{S}^2)\tau_e, \tag{2}$$

where τ_e is an effective single-relaxation time for the internal motions and C is a constant. The order-parameter of Eqs. 1 and 2 is given by

$$\mathcal{S}^2 = \frac{4\pi}{5} \sum_{a=-2}^{+2} |\langle Y_a^2(\theta_r, \phi_r) \rangle|^2, \tag{3}$$

where $Y_a^2(\theta_r, \phi_r)$ is a second-rank spherical harmonics and (θ_r, ϕ_r) are the spherical polar coordinates of the probe in a coordinate system rigidly attached to the tumbling protein. The problem we are considering is shown schematically in Fig. 1. A probe is attached rigidly in the plane of an aromatic ring that undergoes restricted diffusion in an angular range $\pm \gamma_1$ about the C^α—C^β axis and $\pm \gamma_2$ about the C^β—C^γ axis. The analysis of the restricted diffusion plus jump model is accomplished by rewriting Eq. 3 in terms of the internal coordinates of the side chain. If restricted diffusion is limited to motion about χ_2 (the C^β—C^γ axis [see Fig. 1]), then Eq. 3 may be used directly; the probe coordinates are defined with the $\hat{\mathbf{z}}$ axis along the C^β—C^γ bond. For rotational motion about both χ_1 and χ_2, the expression for the order parameter is more complicated. The expression for the order parameter is derived using the Wigner rotation matrices to transform the second-rank spherical harmonics (Eq. 3) from the molecular coordinate system tumbling rigidly with the protein to a coordinate system attached to the ring. When choosing the molecular coordinate system with the z-axis along the N—C^α bond and the y-axis perpendicular to the plane formed by the N, C^α, and C^β atoms, the required transformation is

$$Y_a^2(\theta_r, \phi_r) = \sum_{bc} D_{ab}^{2*}(0, \beta_0, \chi_1 + \chi_1')\, D_{bc}^{2*}(0, \beta_1, -\alpha + \pi) \times Y_c^2(\theta, \chi_2')$$

$$= \sum_{bc} d_{ab}^2(\beta_0) e^{+ib(\chi_1 + \chi_1')}\, d_{bc}^2(\beta_1)\, e^{+ic(-\alpha+\pi)}\, Y_c^2(\theta, \chi_2') \tag{4}$$

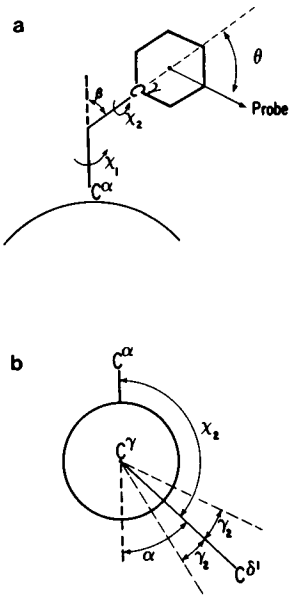

FIGURE 1 (*a*) Internal coordinates for restricted diffusion plus 180° flips of aromatic rings. The dihedral angles χ_1 and χ_2 correspond to rotations about the C^α—C^β and C^β—C^γ axes, respectively. The polar angle β_1 is the argument of the reduced Wigner functions (Eq. 4). The numbering of the ring atoms in the text increases in the counter-clockwise direction with the C^γ carbon referred to as C^1. In the figure, the probe with polar angle $\theta = 60°$ is along the C^5—H^5 bond vector. (*b*) Newman projection defining the Euler angle α of Eq. 5. The figure illustrates restricted rotations of χ_2 with angular bounds $\pm \gamma_2$.

The D_{ab}^2 are Wigner matrices, the $d_{ab}^2(\beta)$ are the real reduced Wigner matrix elements, and β_0, β_1 are the complements of the N—C^α—C^β and C^α—C^β—C^γ valence angles. The rotational angles χ_1' and χ_2' describe restricted rotations about the mean values of the side chain dihedral angles χ_1 and χ_2, α is the complement of χ_2, and θ is the angle the probe makes with the C^β—C^γ axis (Fig. 1). Using Eq. 4, the expression for the order parameter Eq. 3 can be written for uncorrelated rotations about χ_1 and χ_2 as

$$\mathcal{S}^2 = \sum_{\substack{abc \\ b'c'}} \langle (d_{ab}^2(\beta_0)\, e^{+ib(\chi_1 + \chi_1')})\, d_{bc}^2(\beta_1)\, e^{ic(-\alpha+\pi)}\, Y_c^2(\theta, \chi_2') \rangle$$
$$\times \langle (d_{ab'}^2(\beta_0) e^{-ib'(\chi_1 + \chi_1')})\, d_{b'c'}^2(\beta_1)\, e^{-ic'(-\alpha+\pi)}\, Y_{c'}^{2*}(\theta, \chi_2') \rangle \tag{5a}$$

$$= \sum_{bcc'} \langle e^{+ib\chi_1'} \rangle \langle e^{-ib\chi_1'} \rangle\, d_{bc}^2(\beta_1)\, d_{bc'}^2(\beta_1)\, (-1)^{c-c'}$$
$$\times \langle Y_c^2(\theta, \chi_2') e^{-ic\alpha} \rangle \langle Y_{c'}^{2*}(\theta, \chi_2') e^{+ic'\alpha} \rangle. \tag{5b}$$

The sum rule for the Wigner rotation matrix elements has been used to derive Eq. 5b from Eq. 5a. For the model of restricted rotational diffusion in a square-well potential with angular bounds $\pm\gamma_1$ and $\pm\gamma_2$, respectively, the equilibrium probability distributions and root mean square (rms) displacements of the side chain dihedral angles are given by:

$$P_{eq}(\chi_i') = \frac{1}{2\gamma_i} \qquad i = 1, 2 \tag{6a}$$

$$\langle(\Delta\chi_1')^2\rangle = \frac{\gamma_1}{\sqrt{3}}. \tag{6b}$$

The order parameter is then easily evaluated

$$\begin{aligned}\mathcal{S}^2 = &\sum_{bcc'}\left(\frac{1}{2\gamma_1}\int_{-\gamma_1}^{\gamma_1} e^{ib\chi_1'}d\chi_1'\right)^2 \\ &\times d^2_{bc}(\beta_1)\, d^2_{bc'}(\beta_1)(-1)^{c-c'} \\ &\times d^2_{co}(\theta)\, d^2_{c'o}(\theta)\left(\frac{1}{2\gamma_2}\int_{-\gamma_2}^{\gamma_2} e^{+ic\chi_2'}d\chi_2\right) \\ &\left(\frac{1}{2\gamma_2}\int_{-\gamma_2}^{\gamma_2} e^{-ic'\chi_2'}d\chi_2'\right)\mathrm{Re}\,[e^{-i\alpha(c-c')}] \\ = &\sum_{bcc'} d^2_{bc}(\beta_1)\, d^2_{bc'}(\beta_1)\,(-1)^{c-c'} \\ &\left[\frac{\sin(b\gamma_1)}{b\gamma_1}\right]^2\left[\frac{\sin(c\gamma_2)}{c\gamma_2}\right]\left[\frac{\sin(c'\gamma_2)}{c'\gamma_2}\right] \\ &\times d^2_{co}(\theta)\, d^2_{c'o}(\theta)\cos[(c-c')\alpha].\end{aligned} \tag{7}$$

When the ring jumps, the value of α changes; the effect of ring jumps can be incorporated into the model by summing over the allowed values of α

$$\begin{aligned}\mathcal{S}^2 = &\sum_{bcc'} d^2_{bc}(\beta_1)\, d^2_{bc'}(\beta_1)\,(-1)^{c-c'} \\ &\times\left[\frac{\sin(b\gamma_1)}{b\gamma_1}\right]^2\left[\frac{\sin(c\gamma_2)}{c\gamma_2}\right]\left[\frac{\sin(c'\gamma_2)}{c'\gamma_2}\right] \\ &\times d^2_{co}(\theta)\, d^2_{c'o}(\theta)\left[\mathrm{Re}\sum_{\alpha\alpha'} e^{-i(\alpha c-\alpha' c')}P_{eq}(\alpha)\,P_{eq}(\alpha')\right],\end{aligned} \tag{8}$$

where the sums over α and α' are over the jump orientations (χ_2) of the ring. For 180° jumps with $P_{eq}(\alpha) = P_{eq}(\alpha + \pi) = 1/2$, the order-parameter is

$$\begin{aligned}\mathcal{S}^2 = &\sum_{bcc'} d^2_{bc}(\beta_1)\, d^2_{bc'}(\beta_1)\,(-1)^{c-c'} \\ &\left[\frac{\sin(b\gamma_1)}{b\gamma_1}\right]^2\left[\frac{\sin(c\gamma_2)}{c\gamma_2}\right]\left[\frac{\sin(c'\gamma_2)}{c'\gamma_2}\right] \\ &\times d^2_{co}(\theta)\, d^2_{c'o}(\theta)\,(1/4[\cos[\alpha(c-c')] \\ &+\cos[\alpha(c-c')+\pi c] \\ &+\cos[\alpha(c-c')-\pi c'] \\ &+\cos[\alpha(c-c')+\pi(c-c')]).\end{aligned} \tag{9}$$

Eqs. 7–9 are the main results of this section.

Note especially that in the presence of restricted motion about χ_1, the order parameter depends on the value of χ_2 (α) in addition to the magnitudes of the restricted rotational diffusion γ_1 and γ_2. We have used Eqs. 7 and 9 to evaluate the order parameters as a function of restricted diffusion amplitudes γ_1 and γ_2, side-chain rotational angle α, and probe orientation θ. The results are discussed below.

RESULTS AND DISCUSSION

Fig. 2 shows the variation of the order parameter with probe polar angle for restricted diffusion in the absence of ring jumps. The results of the model calculations are expressed in the figures as the inverse of the square of the order parameters ($\mathcal{S}^{-2}$); in the limit of fast internal motions (Eq. 1), $\mathcal{S}^{-2}$ gives the increase in T_1 for the probe over the rigid protein value. The angular bounds for the restricted diffusion in Fig. 2, $\gamma = \pm 40°$, correspond to root-mean-square side-chain dihedral angle displacements of 25° (Eq. 6b). Side-chain fluctuations of this magnitude have been inferred from analysis of experimental NMR relaxation times in a variety of proteins (14). For the motional parameters of Fig. 2, $\mathcal{S}^{-2}$ varies between 1.0 and 1.6. There is a 60% increase in T_1 over the rigid ring value for a probe that is tilted at 90° with respect to the C^β—C^γ axis. The order parameters for probes with polar angles θ = 0°, 60°, 120°, 240°, and 300° are of particular interest because these orientations correspond to the directions of the C^4—H^4, C^5—H^5, C^6—H^6, C^2—H^2 and C^3—H^3 internuclear dipole vectors, respectively. The θ = 80° and 265° directions are also of interest because these angles specify the orientations of the C^1H^6 and C^1H^2 internuclear dipole vectors; ~40% of the dipolar relaxation of the nonprotonated C^1 carbon can be attributed to interactions with the H^2 and H^6 ring protons. For probe orientations on the ring that differ by 180°, the order parameters are equivalent. The motional averaging, for example, will be equivalent for the C^2—H^2 and C^5—H^5 bond vectors, even in the absence of ring flips (Fig. 1). When the ring librates about χ_1, but does not flip, the motional averaging of probes with polar angles that differ by 60° or 120° will not be equivalent so that, for example, the order parameter for the C^3—H^3 vector will be different from the value for the C^5—H^5 vector. For restricted rotational motion about χ_2 alone, the order parameter is independent of the average value of χ_2. There is a minimum at θ = 0° ($\mathcal{S}^{-2}$ = 1.0), and a maximum at θ = 90° ($\mathcal{S}^{-2} \simeq 1.6$). For restricted motion about χ_1, the results depend on the average value of χ_2. When the ring plane coincides with the C^α—C^β—C^γ plane (α = 0°), the minimum value of $\mathcal{S}^{-2}$ = 1.0 occurs at θ = 110°, and the maximum, $\mathcal{S}^{-2}$ = 1.6, is obtained when θ = 20°. The polar angles θ = 110° and θ = 20° correspond to

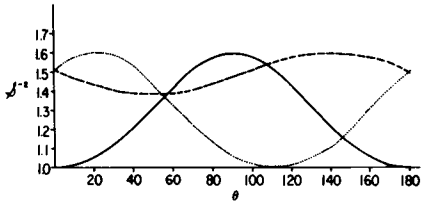

FIGURE 2 $\mathcal{S}^{-2}$ for restricted ring diffusion excluding 180° ring jumps: ——, γ_1 = 0, γ_2 = ±40°; . . . , γ_1 = ±40°, γ_2 = 0°, α = 0°; — — —, γ_1 = ±40°, γ_2 = 0°, α = 90°. The inverse of the square of the order parameter is plotted as a function of the probe polar angle θ.

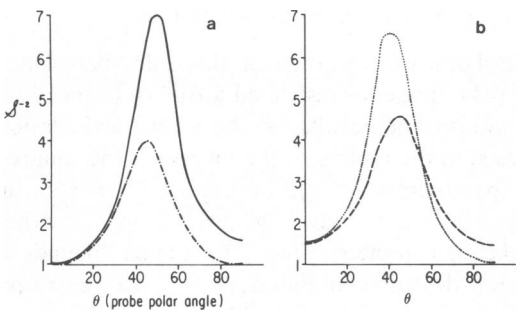

FIGURE 3 $\mathcal{S}^{-2}$ for restricted ring diffusion including 180° ring jumps. (*a*) — · — , $\gamma_1 = 0°$, $\gamma_2 = 0°$; —— , $\gamma_1 = 0°$, $\gamma_2 = \pm 40°$. (*b*) . . . , $\gamma_1 = \pm 40°$, $\gamma_2 = 0°$, $\alpha = 0°$; – – – , $\gamma_1 = \pm 40°$, $\gamma_2 = 0°$, $\alpha = 90°$.

parallel and perpendicular orientations of the probe with respect to the C^α—C^β libration axis. When the ring is 90° out of the C^α—C^β—C^γ plane, the order parameter is less sensitive to the orientation of the probe on the ring, varying between 1.4 and 1.6.

The effects of 180° ring jumps combined with restricted rotational diffusion are illustrated in Fig. 3. Fig. 3 *a* shows the variation of $\mathcal{S}^{-2}$ with polar angle for 180° jumps in the absence of ring librations ($\gamma_1 = 0$, $\gamma_2 = 0$) and in the presence of librations about χ_2 ($\gamma_1 = 0$, $\gamma_2 = \pm 40°$). Considering jumps alone, $\mathcal{S}^{-2} = 1.0$ along the jump axis ($\theta = 0°$). The maximum value, $\mathcal{S}^{-2} = 4.0$, occurs at $\theta = 45°$ and then decreases so that $\mathcal{S}^{-2} = 1.0$ at $\theta = 90°$. The somewhat surprising result that 180° flips have no effect on the order parameter for a a probe inclined at 90° to the flip axis is understood by considering the effect of jumps on the individual components of $\mathcal{S}$. For 180° jumps, the spherical harmonics components must be averaged over the polar angles (θ, ϕ), $(\theta, \phi + \pi)$ so that for $\theta = 0°$ and $\theta = 90°$, the components have the values

$$\theta = 0$$

$$\frac{4\pi}{5} \times |\langle Y_0^2(0, \phi)\rangle|^2 = 1$$

$$\frac{4\pi}{5} \times |\langle Y_{\pm 1}^2(0, \phi)\rangle|^2 = 0$$

$$\frac{4\pi}{5} \times |\langle Y_{\pm 2}^2(0, \phi)\rangle|^2 = 0$$

$$\mathcal{S}^2 = 1$$

$$\theta = 90°$$

$$\frac{4\pi}{5} \times \left|\left\langle Y_0^2\left(\frac{\pi}{2}, \phi\right)\right\rangle\right|^2 = 0.25$$

$$\frac{4\pi}{5} \times \left|\left\langle Y_{\pm 1}^2\left(\frac{\pi}{2}, \phi\right)\right\rangle\right|^2 = 0$$

$$\frac{4\pi}{5} \times \left[\left|\left\langle Y_{+2}^2\left(\frac{\pi}{2}, \phi\right)\right\rangle\right|^2 + \left|\left\langle Y_{-2}^2\left(\frac{\pi}{2}, \phi\right)\right\rangle\right|\right] = 0.75$$

$$\mathcal{S}^2 = 1$$

Since 180° jumps do not affect the order parameter for a probe inclined at $\theta = 90°$, a probe with this orientation will only be sensitive to the librational motions of the ring.

The order parameters for the four tyrosine ring C—H bond vectors are equivalent ($\mathcal{S}^{-2} \simeq 2.3$) when the ring is jumping without any other motion. The effect of additional ring librations on the motional averaging of a probe depends strongly on the probe polar angle. Fig. 3 *a* shows how $\mathcal{S}^{-2}$ changes with θ when the restricted diffusion about χ_2 ($\gamma_2 = \pm 40°$) is combined with 180° ring flips. For $0 \leq \theta \leq 30°$ the additional librational motion has a negligible effect on the order parameter. The maximum increase in $\mathcal{S}^{-2}$ occurs at $\theta \simeq 55°$; $\mathcal{S}^{-2}$ is about twice as large when both ring flips and librations contribute to the averaging as compared with ring flips alone. Fig. 3 *b* demonstrates the effect of ring flips combined with restricted diffusion about χ_1. The order parameter depends on the average value of χ_2. When the average ring orientation is 90° out of the C^α—C^β—C^γ plane, $\mathcal{S}^{-2}$ is dominated by the ring flips.

The variation in $\mathcal{S}^{-2}$ with the amplitude of restricted diffusion about χ_2 is evaluated in Fig. 4. The probe orientation chosen ($\theta = 60°$) corresponds to the C^5—H^5 bond vector of a phenylalanine or tyrosine ring. In the absence of ring flips $\mathcal{S}^{-2}$ increases from 1 to ~2.2 when the amplitude of the restricted diffusion about χ_2 is varied between 0 and 60°. In contrast, when the ring is also undergoing 180° flips, $\mathcal{S}^{-2}$ increases from ~2.3 to greater than 11 for the same variation in γ_2. Fig. 4 shows that the sensitivity of the order parameter to ring librational motions is amplified by the ring flips. The effect can be understood by considering the averaging of the components of $\mathcal{S}$. Because $Y_0^2(\theta, \phi)$ vanishes at $\theta = 54.7°$ (the magic angle for continuous rotation), probes with polar angles close to this value (e.g., 60°) will be particularly sensitive to azimuthal averaging. Both $Y_1^2(\theta, \phi)$ and $Y_2^2(\theta, \phi)$ components are averaged by restricted diffusion about χ_2; however, the extent of the averaging is greater for $Y_2^2(\theta, \phi)$.

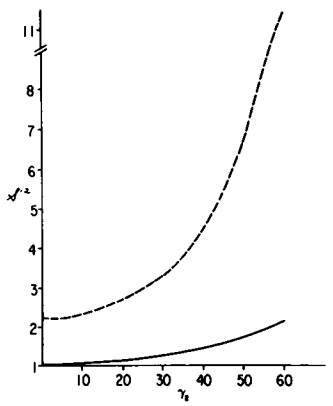

FIGURE 4 Effect of ring libration combined with 180° ring flips on $\mathcal{S}^{-2}$ for a probe polar angle $\theta = 60°$. —— , restricted diffusion about χ_2 with angular bounds $\pm \gamma_2$ excluding 180° ring flips; – – – , including 180° ring flips.

Because $Y_1^2(\theta, \phi)$ is averaged to zero by 180° ring flips, the value of $\mathcal{S}^{-2}$ increases more rapidly with the amplitude of the restricted diffusion, if the ring is also flipping.

In this communication, we have examined the combined effect of restricted rotational diffusion and 180° flips on the order parameters of aromatic rings in proteins. We have presented a formula (Eq. 8) for the order parameter that is a function of probe orientation (θ), restricted diffusion amplitude (γ_1, γ_2), and jump statistics (α_i, P_{eq} $[\alpha_i]$). Choosing reasonable values for the amplitudes of restricted ring diffusion, $\mathcal{S}^{-2}$ increases by as much as 50% in the absence of ring jumps and there are much larger increases when the rings are also jumping. The formulas we have presented can be applied directly to the interpretation of NMR relaxation and fluorescence depolarization experiments on proteins in solution when the ring motions occur on a time-scale faster than the protein tumbling. In this regard, it is of interest to note the recent report of rate constants for tyrosine ring flips as large as 5×10^8 s^{-1} in the fd bacteriophage coat protein (6); these results were obtained using solid-state NMR techniques. An analysis of the effect of restricted diffusion combined with ring jumps on NMR lineshapes in the solid state will be the subject of a future communication.

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REFERENCES

1. Campbell, I. S., C. M. Dobson, G. R. Moore, S. J. Perkins, and R. J. P. Williams. 1976. Temperature dependent molecular motion of a tyrosine residue of ferrocytochrome c. *FEBS Fed. Eur. Biochem. Soc. Lett.* 70:91–100.
2. Wagner, G., A. DeMarco, and K. Wüthrich. 1976. Dynamics of the aromatic amino acid residues in the globular conformation of the basic pancreatic trypsin inhibitor (BPTI). *Biophys. Struct. Mech.* 2:139–158.
3. Lakowicz, J. R., and G. Weber. 1980. Nanosecond segmental mobilities of tryptophan residues in proteins observed by lifetime-resolved fluorescence anisotropies. *Biophys. J.* 32:591–601.
4. Munro, I., I. Pecht, and L. Stryer. 1979. Subnanosecond motions of tryptophan motions in proteins. *Proc. Natl. Acad. Sci. USA.* 76:56–60.
5. Gall, C. M., A. DeVerdi, and S. J. Opella. 1981. Phenylalanine ring dynamics by solid state ^2H NMR. *J. Am. Chem. Soc.* 103:5039–5043.
6. Gall, C. M., T. A. Cross, J. A. DiVerdi, and S. J. Opella. 1982. Protein dynamics by solid state NMR: aromatic rings of the coat protein in fd bacteriophage. *Proc. Natl. Acad. Sci. USA.* 79:101–105.
7. Rice, D. M., R. J. Wittebort, R. G. Griffin, E. Meirovitch, E. R. Stimson, Y. C. Meinwold, J. H. Freed, and H. A. Scheraga. 1981. Rotational jumps of the tyrosine side chain in crystalline eukephalin ^2H NMR lineshapes for aromatic ring motion in solids. *J. Am. Chem. Soc.* 103:7707–7710.
8. Levy, R. M., C. M. Dobson, and M. Karplus. 1982. Dipolar NMR relaxation of non protonated aromatic carbons in proteins: structural and dynamical effects. *Biophys. J.* 39:102–113.
9. Gelin, B. R., and M. Karplus. 1975. Side chain torsional potentials and motions of amino acids in proteins: PTI. *Proc. Natl. Acad. Sci. USA.* 81:801–805.
10. McCammon, J. A., P. G. Wolynes, and M. Karplus. 1979. Picosecond dynamics of tyrosine side chains in proteins. *Biochemistry* 18:927–942.
11. Levy, R. M., M. Karplus, and J. A. McCammon. 1981. Increase of ^{13}C NMR relaxation times in proteins due to picosecond motional averaging. 1981. *J. Am. Chem. Soc.* 103:994–996.
12. Levy, R. M., and A. Szabo. 1982. Initial fluorescence depolarization of tyrosines in proteins. *J. Am. Chem. Soc.* 104:2073–2075.
13. Lipari, G., and A. Szabo. 1982. Model free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. I. Theory and range of validity. *J. Am. Chem. Soc.* 104:4546–4558.
14. Lipari, G., and A. Szabo. 1982. Model free approach in the interpretation of nuclear magnetic resonance in macromolecules. II. Analysis of experimental results. *J. Am. Chem. Soc.* 104:4558–4570.
15. Brink, D. M., and G. R. Satchler. 1971. Angular Momentum. Oxford University Press, London.