J. Am. Chem. Soc. **1994**, *116*, 8426–8427

NMR Order Parameters of Biomolecules: A New Analytical Representation and Application to the Gaussian Axial Fluctuation Model

Rafael Brüschweiler* and Peter E. Wright*

Department of Molecular Biology
The Scripps Research Institute
La Jolla, California 92037

Received June 13, 1994

Liquid state NMR relaxation spectroscopy is a powerful tool for the investigation of fast time scale dynamical processes in biomolecules.[1-6] In the case of ^{15}N- and ^{13}C-labeled biomolecules, magnetic dipolar relaxation mediated by the directly attached proton(s) is dominant and the experimental relaxation data consisting of T_1, T_2, and NOE values are usually interpreted according to the *model free formalism*[2] in terms of an internal motional correlation time and an order parameter S^2. S^2 is a general measure of the angular motion of the corresponding internuclear vector. To gain more insight, experimentally derived S^2 values can be interpreted on the basis of analytical motional models or molecular force field based models.[6] It is shown here that $1 - S^2$ is directly proportional to the sum of the second moments of the spatial part of the dipolar interaction, revealing an analogy to crystallographic temperature factors. The relationship allows analytical treatment of the influence of Gaussian fluctuations about arbitrary axes on S^2 order parameters. This type of motion is dominant in the case of harmonic and quasi-harmonic dynamics of biomolecules.[7-13]

The S^2 order parameter corresponds to the plateau value of the normalized correlation function of the lattice part of the dipolar spin interaction,[2]

$$S^2 = \frac{4\pi}{5}\sum_{m=-2}^{2}\langle Y_{2m}{}^*(\Omega)\rangle\langle Y_{2m}(\Omega)\rangle \qquad (1)$$

where $Y_{2m}(\Omega)$ are the second-order spherical harmonics,[15] $\Omega = (\theta, \varphi)$ is the direction of the internuclear vector in a molecule fixed frame, and the angular brackets indicate time averaging.

An alternative representation of eq 1 can be derived on the basis of a fundamental relationship between the *variance* σ_f^2 of a complex time-dependent stochastic function f and its *correlation function* $C(t) = \langle f^*(0)f(t)\rangle$. Since, at $t = 0$, $C(0) = \langle f^*f\rangle$ and, for $t \rightarrow \infty$, the plateau value is $C(t) = \langle f^*\rangle\langle f\rangle$, the variance σ_f^2 corresponds to the difference between the initial and the plateau value:

$$\sigma_f^2 = \sigma_{\mathrm{Re}f}^2 + \sigma_{\mathrm{Im}f}^2 = \langle(f^* - \langle f^*\rangle)(f - \langle f\rangle)\rangle = \langle f^*f\rangle - \langle f^*\rangle\langle f\rangle = C(0) - \lim_{t\rightarrow\infty} C(t) \qquad (2)$$

Application of eq 2 to the terms in the sum of eq 1 gives

$$\langle Y_{2m}{}^*(\Omega)\rangle\langle Y_{2m}(\Omega)\rangle = \langle Y_{2m}{}^*(\Omega)(Y_{2m}(\Omega))\rangle - \sigma_{Y_{2m}}^2 \qquad (3)$$

and insertion of eq 3 into eq 1 using the addition theorem of spherical harmonics,[14]

$$\sum_{m=-2}^{2}\langle Y_{2m}{}^*(\Omega)\, Y_{2m}(\Omega)\rangle = \frac{5}{4\pi} \qquad (4)$$

yields the final result

$$S^2 = 1 - \frac{4\pi}{5}\sum_{m=-2}^{2}\sigma_{Y_{2m}}^2 \qquad (5)$$

$1 - S^2$ is thus directly proportional to the sum of the second moments of the spherical harmonics of order 2 describing the orientation of the internuclear vector. It is evident that S^2 is only sensitive to angular motion and remains unchanged under translational motion of the spin pair. S^2 is equal to 1 and maximal in the absence of motion and decreases with increasing angular mobility.[2]

Equation 5 illuminates the relationship between $1 - S^2$ and the isotropic crystallographic temperature factor (B factor) in the absence of static disorder:[16]

$$\frac{3}{8\pi^2}B = \sum_{\xi=x,y,z}\sigma_\xi^2 = \frac{4\pi}{3}\sum_{m=-1}^{1}\sigma_{rY_{1m}}^2 \qquad (6)$$

where σ_ξ^2 is the Cartesian fluctuation along direction ξ. r and $Y_{1m}(\Omega)$ are the distance and the first-order spherical harmonics of the orientation of the atomic position with respect to the equilibrium position. While $1 - S^2$ monitors the variances of the second-order spherical harmonics of atom pairs, the B factor reflects variances of distance-modulated spherical harmonics of first order of the positions of individual atoms.

The form of eq 5 suggests that the effects of certain analytical models describing the motion of the internuclear vector can also be expressed in terms of variances. Of particular interest is the case where the internuclear vector is modulated by Gaussian fluctuations about an arbitrary axis α. This type of dynamics occurs, for instance, in the practically important context of harmonic and quasi-harmonic dynamics of biomolecules.[7-13] Without loss of generality, let α point along the z axis of a molecule fixed frame and let the orientation of the normalized internuclear vector **e** be $\Omega = (\theta,\varphi)$ where θ is the angle between α and **e** and the equilibrium position of **e** is at $\varphi = 0$. The probability distribution $p(\varphi)$ is assumed to be Gaussian with variance σ_φ^2,

$$p(\varphi)\,\mathrm{d}\varphi = (2\pi\sigma_\varphi^2)^{-1/2}\mathrm{e}^{-\varphi^2/(2\sigma_\varphi^2)}\,\mathrm{d}\varphi \qquad (7)$$

resulting in the motional model shown in Figure 1, the *Gaussian axial fluctuation model* (GAF model). This model is conceptually an extension of diffusive motion about a torsion angle in a square well potential[17-19] and relies on a more realistic harmonic molecular potential. Since $p(\varphi)$ is symmetric about $\varphi = 0$, the

---

* To whom correspondence should be addressed.

(1) Abragam, A. *Principles of Nuclear Magnetism*; Clarendon Press: Oxford, 1961.

(2) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4546–4559.

(3) Kay, L. E.; Torchia, D. A.; Bax, A. *Biochemistry* **1989**, *28*, 8972–8979.

(4) Palmer, A. G.; Rance, M.; Wright, P. E. *J. Am. Chem. Soc.* **1991**, *113*, 4371–4380.

(5) Wagner, G. *Curr. Opin. Struct. Biol.* **1993**, *3*, 748–754.

(6) Brüschweiler, R.; Case, D. A. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 27–58.

(7) Noguti, T.; Gō, N. *J. Phys. Soc. Jpn.* **1983**, *52*, 3283.

(8) Brooks, B.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6571–6575.

(9) Levitt, M.; Sander, C.; Stern, P. S. *J. Mol. Biol.* **1985**, *181*, 423–447.

(10) Levy, R. M.; Karplus, M.; Kushick, J.; Perahia, D. *Macromolecules* **1984**, *17*, 1370–1374.

(11) Brüschweiler, R. *J. Am. Chem. Soc.* **1992**, *114*, 5341–5344.

(12) Palmer, A. G.; Case, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 9059–9067.

(13) Brüschweiler, R.; Case, D. A. *Phys. Rev. Lett.* **1994**, *72*, 940–943.

(14) Brink, D. M.; Satchler, C. R. *Angular Momentum*; Clarendon Press: Oxford, 1962.

(15) The second-order spherical harmonics are defined as $Y_{20} = (5/16\pi)^{1/2}(3\cos^2\theta - 1)$, $Y_{2,\pm1} = \mp(15/8\pi)^{1/2}\cos\theta\sin\theta\exp(\pm i\varphi)$, $Y_{2,\pm2} = (15/32\pi)^{1/2}\sin^2\theta\exp(\pm i2\varphi)$.

(16) Giacovazzo, E., Ed. *Fundamentals of Crystallography*; Oxford University Press: New York, 1992.

(17) London, R. E.; Avitabile, J. *J. Am. Chem. Soc.* **1978**, *100*, 7159–7165.

(18) Wittebort, R. J.; Szabo, A. *J. Chem. Phys.* **1978**, *69*, 1722–1736.

(19) McCain, D. C.; Markley, J. L. *J. Magn. Reson.* **1987**, *73*, 244–251.

0002-7863/94/1516-8426$04.50/0

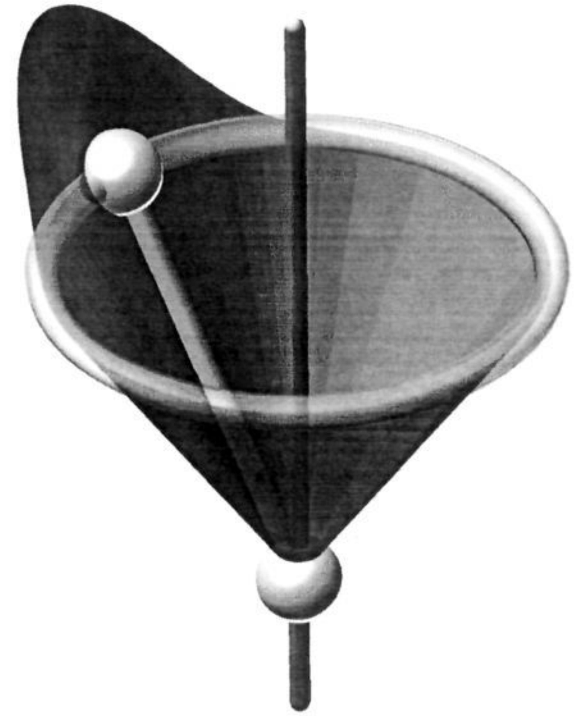

Figure 1. Gaussian axial fluctuation model of an internuclear vector moving on the surface of a cone with semiangle θ. As an example, θ = 109° for tetrahedral bond geometry.

second moments of the $\exp(im\varphi)$ terms of $Y_{2m}(\theta,\varphi)$[15] depend only on their real component:

$$\sigma^2_{Y_{2m}} = \langle e^{-im\varphi}e^{im\varphi}\rangle - \langle e^{-im\varphi}\rangle\langle e^{im\varphi}\rangle = 1 - \langle\cos(m\varphi)\rangle^2 \quad (8)$$

For sufficiently small $\sigma_\varphi \ll \pi$, which is usually well fulfilled for biomolecules for dihedral angle fluctuations in a single rotamer, the $\cos(m\varphi)$ average is readily obtained from the Fourier relationship

$$\langle\cos(m\varphi)\rangle = \int_{-\infty}^{\infty}\mathrm{d}\varphi\, p(\varphi)\cos(m\varphi) = \mathrm{e}^{-m^2\sigma_\varphi^2/2} \qquad (\langle\varphi\rangle = 0) \quad (9)$$

Insertion into eq 5 yields finally

$$S^2 = 1 - 3\sin^2\theta\left\{\cos^2\theta\,(1 - \mathrm{e}^{-\sigma_\varphi^2}) + \frac{1}{4}\sin^2\theta\,(1 - \mathrm{e}^{-4\sigma_\varphi^2})\right\} \quad (10)$$

The motional influence vanishes for $\mathbf{e} \parallel \alpha$ (θ = 0°, 180°) and is for small σ^2_φ largest when $\mathbf{e} \perp \alpha$ (θ = ±90°). For large σ^2_φ, all positions on the cone surface become equally probable and eq 10 reduces to the Woessner model ($S^2 = [P_2(\cos\theta)]^2$), which yields $S^2 = 0$ for $\theta \cong 54.7°$.[20] For $S^2 \geq 0.75$ it is sufficient to include in eq 10 only terms up to $O(\sigma^2)$ resulting in the relationship

$$S^2 \cong 1 - 3\sin^2\theta\,\sigma^2_\varphi \quad (11)$$

Local fluctuations of protein backbone φ and side-chain χ torsion angles often significantly affect S^2 values of adjacent N–H and Cβ–Hβ spin pairs, respectively. This dependence is shown in Figure 2 for the case of an N–H pair fluctuation about the φ dihedral angle (curve A) and a tetrahedral bond geometry (curve B). For instance, a backbone N–H S^2 value around 0.8 corresponds to a φ torsion angle fluctuation $\sigma_\varphi \approx 18°$ (θ = 118° [21]). Thus, eq 10 provides an intuitive and physically meaningful interpretation of S^2 in terms of local torsion angle motion. If in addition conformational exchange occurs between N different rotamers i exhibiting minimally overlapping Gaussian distributions with populations p_i and local order parameters S_i^2, the total order parameter becomes[22]

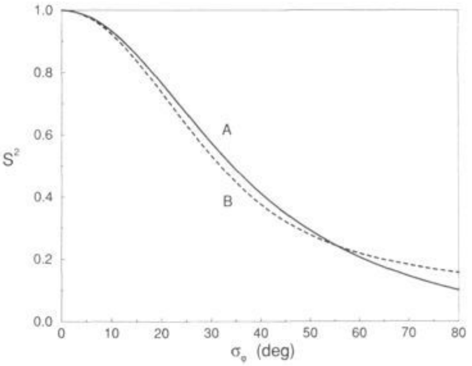

Figure 2. Dependence of S^2 order parameter on standard deviation σ_φ of the Gaussian torsion angle distribution according to eq 10. In curve A, the cone semiangle θ has been set to 118° as is the case for the backbone N–H vector fluctuating about the protein φ dihedral angle, and in curve B, θ = 109° as is the case for a Cβ–Hβ vector in an amino acid side chain fluctuating about the Cα–Cβ axis. Both curves are well approximated by Lorentzian functions $S^2 \cong 1/(1 + c\sigma^2_\varphi)$ where $c = 3.0$ rad^{-2} for curve A and $c = 3.2$ rad^{-2} for curve B (fits not shown).

$$S^2 \cong \sum_{i,j=1}^{N} p_i p_j\{\delta_{ij}S_i^2 + (1 - \delta_{ij})P_2(\cos\chi_{ij})\} \quad (12)$$

where $P_2(x) = (3x^2 - 1)/2$ and χ_{ij} is the angle between the equilibrium internuclear vector in rotamer i and the one in rotamer j.

In the case of Gaussian quasi-harmonic long-range motion about an effective three-dimensional axis α, such as hinge bending motion,[23] experimental NMR relaxation data enable the determination of the direction of α provided that σ_φ is sufficiently large to cause observable effects (typically if $\sigma_\varphi > 10°$). However, it is not possible to determine directly the absolute location of α in the molecule leaving two translational parameters undetermined, since S^2 monitors only orientational motion and not absolute fluctuations. This is in contrast to crystallographic B factors (eq 6) which display a distance dependence which rises quadratically for large distances to α.

Finally we note that eq 10 also fits the thermodynamic interpretation of order parameters with the local partition function q given by $q \propto |\sin\theta|\sigma^2_\varphi$ allowing estimates of motional contributions to the free energy difference ΔG between two different protein states as discussed by Akke et al.[24]

Acknowledgment. We thank Tom Macke for making Figure 1 using AVS.

---

(20) Woessner, D. E. *J. Chem. Phys.* **1962**, *36*, 1.

(21) Ramachandran, G. N.; Kolaskar, A. S.; Ramakrishnan, C.; Sasisekharan, V. *Biochim. Biophys. Acta* **1974**, *359*, 298–302.

(22) Brüschweiler, R. Structural dynamics in biomolecules monitored by nuclear magnetic resonance relaxation. Ph.D. Thesis, ETH, No. 9466, 1991.

(23) McCammon, J. A.; Gelin, B. R.; Karplus, M.; Wolynes, P. G. *Nature* **1976**, *262*, 325–326.

(24) Akke, M.; Brüschweiler, R.; Palmer, A. G. *J. Am. Chem. Soc.* **1993**, *115*, 9832–9833.