Adding Harmonic Motion to the Karplus Relation for Spin−Spin Coupling

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Progress in NMR methodology and instrumentation now allows the measurement of a large number of NMR parameters of molecules in solution with increasingly high precision. Their quantitative interpretation often requires the inclusion of molecular dynamical properties, as for example in the case of dipolar relaxation data.[1] In this communication, the effect of Gaussian dihedral angle distributions, resulting from harmonic dynamics, on scalar ^3J-coupling constants is treated and an analytical expression for a modified Karplus-type equation is given. The validity of the expression is discussed on the basis of a 1.5 ns molecular dynamics simulation of myoglobin in solution. The relationship suggests that different experimental parametrizations of the Karplus equation reflect differences in the average dihedral angle fluctuations. Inversion of the relationship yields modified Karplus parameters, which may be of practical importance for the back-calculation of J-coupling constants using molecular force field based methods.

Scalar ^3J-coupling constants can be measured by a variety of techniques, depending upon the size of the molecule.[2−5] Although extracted J-coupling constants are in certain cases affected by systematic errors,[6] their interpretation continues to become more quantitative, leading to a better understanding of motional contributions as well. The effect of molecular structure and dynamics on a vicinal scalar J-coupling constant between two spins is related to the distribution of the intervening dihedral angle θ. For a rigid molecule the J-coupling constant can be parametrized by the Karplus relationship[7]

$$J(\theta) = A\cos^2\theta + B\cos\theta + C = \frac{A}{2}\cos 2\theta + B\cos\theta + \frac{A}{2} + C \quad (1)$$

with empirical parameters A, B, and C expressed in units of hertz. In the presence of motion J is averaged according to $\langle J\rangle = \int_{-\pi}^{\pi} J(\theta)\, p(\theta)\, d\theta$, where $p(\theta)$ is the probability distribution of θ. This averaging relationship holds for motional time scales which are faster than the inverse of the J-coupling fluctuation amplitude, ranging typically between femto- and milliseconds.

The importance of conformational averaging on J-coupling constants in biomolecules is well-known and has been addressed in numerous studies. Population-weighted contributions of discrete rotamers to fit the NMR data have been introduced by Pachler.[8a,b] Averaging of homonuclear ^3J-coupling values in peptides has been investigated theoretically for isolated molecule potentials[8c,d] and in the protein lysozyme using a solvated molecular dynamics simulation.[8e] Recently, a method (CUPID) has been introduced to fit continuous dihedral angle distributions to experimental ^3J-coupling and cross-relaxation data.[8f,g] Such distributions have also been derived in carbohydrates using a maximum entropy approach.[8h] Valine side-chain conformations of ribonuclease T_1 have been analyzed assuming discrete and continuous χ_1 dihedral angle distributions.[8i] ^3J-coupling constants of the four proline residues of the cyclic peptide antamanide have been back-calculated from Langevin dynamics simulations for different force fields[8j,k] and compared with experimental data.[8l]

We focus here on classical harmonic motion resulting in a Gaussian distribution of the dihedral angle θ centered about θ_0 with standard deviation σ. For $\sigma \ll \pi$ averaging effects can be expressed analytically using the Fourier relationship

$$\langle\cos(m\theta)\rangle \cong \int_{-\infty}^{\infty}(2\pi\sigma^2)^{-1/2} e^{-(\theta-\theta_0)^2/(2\sigma^2)}\cos(m\theta)\, d\theta = e^{-m^2\sigma^2/2}\cos(m\theta_0) \quad (2)$$

and substitution of eq 2 into eq 1 yields

$$\langle J(\theta)\rangle = Ae^{-2\sigma^2}\cos^2\theta_0 + Be^{-\sigma^2/2}\cos\theta_0 + \frac{A}{2}(1 - e^{-2\sigma^2}) + C \quad (3)$$

which retains the general form of the Karplus equation of eq 1 with modified coefficients

$$A' = Ae^{-2\sigma^2},\quad B' = Be^{-\sigma^2/2},\quad \text{and}\quad C' = C + \frac{A}{2}(1 - e^{-2\sigma^2}) \quad (4)$$

(σ is expressed in units of radians). For small σ, A' is about 4 times more sensitive to changes in σ^2 than B', and for large fluctuations $\langle J\rangle$ becomes independent of θ_0 approaching $\langle J\rangle = A/2 + C$.[10] Similarly, harmonic motion can be "removed" from the parameters A, B, and C by inversion of eq 4, which corresponds formally to a replacement of σ^2 by $-\sigma^2$ in eq 4.

The assumption of Gaussian distributed dihedral angles can be tested on the basis of molecular dynamics simulations, as in ref 8e. Here, we use a 1.5 ns MD simulation of myoglobin,[11a] solvated in a box with 4300 water molecules at 312 K using the CHARMM19 polar hydrogen parameter set, to study the effect of fluctuations in the φ dihedral angles on ^3J-coupling constants. The simulation protocol is the same as that described

(1) For a recent review, see: Brüschweiler, R.; Case, D. A. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 27.
(2) (a) Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Chem. Phys.* **1986**, *85*, 6837. (b) Brüschweiler, R.; Madsen, J.; Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Magn. Reson.* **1987**, *73*, 380. (c) Montelione, G. T.; Winkler, M. E.; Rauenbühler, P.; Wagner, G. *J. Magn. Reson.* **1989**, *82*, 198. (d) Wider, G.; Neri, D.; Otting, G.; Wüthrich, K. *J. Magn. Reson.* **1989**, *85*, 426. (e) Sørensen, O. W. *J. Magn. Reson.* **1990**, *90*, 433. (f) Wagner, G.; Schmieder, P.; Thanabal, V. *J. Magn. Reson.* **1991**, *93*, 436. (g) Griesinger, G.; Eggenberger, U. *J. Magn. Reson.* **1992**, *97*, 426. (h) Madsen, J. C.; Sørensen, O. W.; Sørensen, P.; Poulsen, F. *J. Biomol. NMR* **1993**, *3*, 239.
(3) Kim, Y.; Prestegard, J. H. *J. Magn. Reson.* **1989**, *84*, 9.
(4) Ludvigsen, S.; Andersen, K. V.; Poulsen, F. M. *J. Mol. Biol.* **1991**, *217*, 731.
(5) (a) Bax, A.; Max, D.; Zax, D. *J. Am. Chem. Soc.* **1992**, *114*, 6923. (b) Vuister, G. W.; Delaglio, F.; Bax, A. *J. Biomol. NMR* **1993**, *3*, 67.
(6) Harbison, G. S. *J. Am. Chem. Soc.* **1993**, *115*, 3026.
(7) (a) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11. (b) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.
(8) (a) Pachler, K. G. R. *Spectrochim. Acta* **1963**, *19*, 2085. (b) Pachler, K. G. R. *Spectrochim. Acta* **1964**, *20*, 581. (c) Gibbons, W. A.; Nemethy, G.; Stern, A.; Craig, L. C. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 239. (d) Tonelli, A. E.; Brewster, A. I.; Bovey, F. A. *Macromolecules* **1970**, *3*, 412. (e) Hoch, J. Ç.; Dobson, C. M.; Karplus, M. *Biochemistry* **1985**, *24*, 3831. (f) Džakula, Ž.; Westler, W. M.; Edison, A. S.; Markley, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 6195. (g) Džakula, Ž.; Edison, A. S.; Westler, W. M.; Markley, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 6200. (h) Poppe, L. *J. Am. Chem. Soc.* **1993**, *115*, 8421. (i) Karimi-Nejad, Y.; Schmidt, J. M.; Rüterjans, H.; Schwalbe, H.; Griesinger, C. *Biochemistry* **1994**, *33*, 5481. (j) Brunne, R. M.; van Gunsteren, W. F.; Brüschweiler, R.; Ernst, R. R. *J. Am. Chem. Soc.* **1993**, *114*, 4764. (k) Schmidt, J. M.; Brüschweiler, R.; Ernst, R. R.; Dunbrack, R. L.; Joseph, D.; Karplus, M. *J. Am. Chem. Soc.* **1993**, *115*, 8747. (l) Mádi, Z. L.; Griesinger, C.; Ernst, R. R. *J. Am. Chem. Soc.* **1990**, *112*, 2908.
(9) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
(10) If the dihedral angle moves in a square-well potential with limits $\theta_0 \pm \Delta$ and standard deviation $\sigma = \Delta/\sqrt{3}$, the Karplus relationship also retains its form with modified coefficients $A' = A\sin(\sqrt{12}\sigma)/(\sqrt{12}\sigma)$, $B' = B\sin(\sqrt{3}\sigma)/(\sqrt{3}\sigma)$, and $C' = C + A/2(1 - \sin(\sqrt{12}\sigma)/(\sqrt{12}\sigma)$, which are very similar to the Gaussian case (eq 4 for $\sigma < 25°$.
(11) (a) Hirst, J.; Brooks, C. L., III. *J. Mol. Biol.*, in press. (b) Brooks, C. L., III. *J. Mol. Biol.* **1992**, *227*, 375.
(12) For side-chain χ_1 dihedral angles the situation is less regular, since numberous side chains exhibit transitions between different rotamers leading to a larger discrepancy between the exact J-average and the estimate based solely on the center and the variance of the distribution. In these cases, a superposition of the Gaussian fluctuation model with Pachler's treatment of exchange between discrete rotamers[8a,b] provides a better description.[8i]

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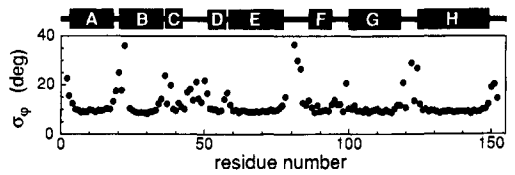

Figure 1. Backbone φ-angle fluctuations σ_φ of myoglobin calculated from a 1.5 ns MD trajectory[11] using CHARMM.[9] The σ_φ values of residues 23, 79, 80, 121, and 153 lie off scale and are 88°, 79°, 60°, 72°, and 88°, respectively. The bars on top of the figure give the locations and the names of the α-helices.

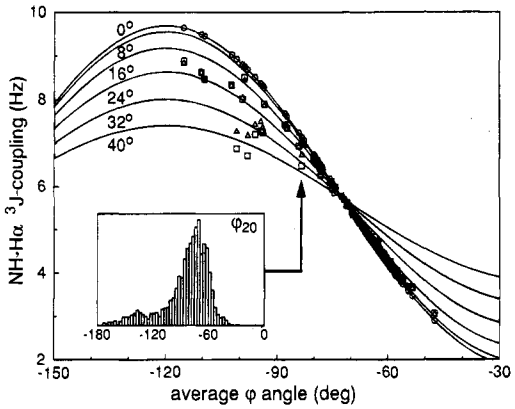

Figure 2. HN−Hα ^3H-coupling constants of myoglobin evaluated from a 1.5 ns MD simulation using the Karplus relationship with parameters $A = 6.4$, $B = -1.4$, and $C = 1.9$ and $\theta = \varphi - 60°$ given by Pardi et al.[13] J values have been calculated on the basis of averaged angles $\langle\varphi\rangle$ (circles), by averaging J directly over the trajectory (squares), and by using eq 3 with σ_φ evaluated from the trajectory (triangles). Superimposed are the curves of the modified Karplus relationships according to eq 3 with $\sigma_\varphi = 0°$, 8°, 16°, 24°, 32°, and 40°, respectively. The inset shows the φ distribution of Asp20 with an average indicated by the arrow.

earlier for apomyoglobin,[11b] and further analysis will be published elsewhere. Figure 1 shows the resulting standard deviations σ_φ, which are typically around 10° for the α-helices (black bars) and noticeably larger at the ends and in the loop regions of the polypeptide chain. Figure 2 shows $J_{HN-H\alpha}$-coupling constants evaluated using the Karplus relationship with standard parameters $A = 6.4$, $B = -1.4$, and $C = 1.9$ (ref 13) and different ways of motional averaging. The largest motional effects are observed for average φ angles below −80° belonging to loop regions where the effective J-coupling constant can be reduced by as much as 2 Hz, corresponding to a change of the apparent average φ angle by about 20°. It can be seen that the exact J-averages (squares) and the estimates from eq 3 (triangles), which are based on the assumption that the distributions of dihedral angles are Gaussian, yield very similar results for almost all residues, reflecting the fact that most φ angles in the MD simulation are nearly Gaussian distributed.[12] The inset shows the φ distribution of Asp20, where the presence of a small population around $\varphi = -150°$ lowers $\langle J\rangle$ significantly and leads to an overestimation of $\langle J\rangle$ by the Gaussian model. Also shown in Figure 1 are Karplus relationships modified according to eq 3 with variable σ_φ.

Most commonly the coefficients A, B, and C are obtained by empirical calibrations based on measured J-coupling constants of dihedral angles known from averaged X-ray or NMR structures. As has been noted before,[8e] the coefficients A, B, and C then already include contributions from thermal dihedral angle fluctuations. For $J_{HN-H\alpha}$ scalar J-coupling constants several Karplus parameter sets have been reported using different molecules under different conditions for calibration[4,13-18] (Table 1). It is instructive to correlate the seven distinct parameter sets by assuming that the differences originate from different amounts of motion parametrizable by σ in eq 4. Using

Table 1. Interrelations between Different Karplus Parameter Sets for the $J_{HN-H\alpha}$ Coupling Constant Based on Eq 4

parameter set	A_{exp}	B_{exp}	C_{exp}	A_{back}[a]	B_{back}[a]	C_{back}[a]	σ_{fit} (deg)[b]
set 1[15]	8.0	−0.9	0.9	8.1	−1.3	1.0	16 (16)
set 2[16]	7.9	−1.55	1.35	7.8	−1.3	1.2	18 (18)
set 3[18]	5.4	−1.3	2.2	5.5	−1.2	2.3	30 (29)
set 4[17c]	9.4	−1.1	0.4	9.4	−1.4	0.4	4 (6)
set 5[13]	6.4	−1.4	1.9	6.4	−1.3	1.9	25 (25)
set 6[14]	6.0	−1.4	2.4	5.9	−1.2	2.2	28 (27)
set 7[4]	6.7	−1.3	1.5	6.8	−1.3	1.7	23 (23)
static set (best fit)[b]				9.5	−1.4	0.3	0

[a] Back-calculated Karplus parameters from best fit (static set, bottom line) and σ_{fit} values using eq 4. [b] Nonlinear least-squares fit of three Karplus parameters (static set) and seven fluctuation amplitudes σ_{fit} (=10 fit parameters) to seven experimental parameter sets (=21 data points). Very similar results for the σ_{fit} values are obtained for the square-well potential (values in parentheses).[10] [c] This parameter set was obtained using mainly constrained (cyclic) peptides, which may explain the small σ_{fit} value.

a least-squares fit, an individual effective σ value can be assigned to each of the sets, and the results in Table 1 indicate that the parameter sets belong to the same family of curves spanned by the parameter σ. For all seven sets, parameter A can be reproduced within 0.1 Hz, B within 0.4 Hz, and C within 0.2 Hz. Comparison of the three protein studies (sets 5−7) shows that σ is largest for the study carried out at the highest temperature[14] and smallest for the one at lowest temperature,[4] although a high-temperature analysis on BPTI at 341 K resulted in parameters which remained close to the ones obtained at 309 K[13]. The σ values for the three protein studies are around 25°, which is higher than the average σ_φ fluctuations of the helices seen in the MD simulation of myoglobin (~10°, Figure 1) and would correspond to $^{15}N-H$ S^2 relaxation order parameters around 0.7 according to the GAF model.[19] It should be noted that experimental order parameters and current MD simulations are sensitive only to fast time scale motion with correlation times typically below 1 ns, yielding σ values that are equal to or smaller than the ones experienced by J-couplings. Although motional effects are the most likely cause for the observed differences between the Karplus parameter sets, other contributions may be present as well.[20] Further analysis could rely on scalar J-coupling measurements made over a wider range of temperatures and taking into account explicitly the dependence of σ on secondary structure.

Finally, the fact that experimentally extracted Karplus parameters A, B, and C intrinsically include motional averaging imposes a problem for the back-calculation of J-coupling constants from MD, Monte Carlo, or normal mode simulations, since the use of the original parameters would overemphasize thermal fluctuations by *de facto* taking them into account twice (once during calibration and once during the simulation). This problem can be addressed by using corrected parameters A', B', and C' for the theoretical calculations, obtained by a fitting approach as described above or by application of the inverted form of eq 4 with a representative σ estimated from simulations themselves or from complementary experimental sources, such as relaxation data.

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(13) Pardi, A.; Billeter, M.; Wüthrich, K. *J. Mol. Biol.* **1984**, *180*, 741.
(14) Smith, L. J.; Sutcliffe, M. J.; Redfield, C.; Dobson, C. M. *Biochemistry* **1991**, *30*, 986.
(15) Bystrov, V. F.; Portnova, S. L.; Tsetlin, V. I.; Ivanonv, V. T.; Ovchinnikov, Y. A. *Tetrahedron* **1969**, *25*, 493.
(16) Ramachandran, G. N.; Chandrasekaran, R.; Kopple, K. D. *Biopolymers* **1971**, *10*, 2113.
(17) Bystrov, V. F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41.
(18) DeMarco, A.; Llinás, M.; Wüthrich, K. *Biopolymers* **1978**, *17*, 637.
(19) Brüschweiler, R.; Wright, P. E. *J. Am. Chem. Soc.*, in press.
(20) Edison, A. S.; Markley, J. L.; Weinhold, F. *J. Biomol. NMR* **1994**, *4*, 519.