J. Am. Chem. Soc. **2000,** *122,* 11523−11524

Resolving Contradictions between Overdetermined NMR Cross-Correlation Rates

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Received June 27, 2000
Revised Manuscript Received August 15, 2000

In the past few years, a rapidly expanding class of NMR experiments has been introduced that exploits the measurement of cross-correlation between the fluctuations of two interactions such as dipole−dipole couplings and chemical shift anisotropies to obtain a measure of dihedral angles in proteins, nucleic acids, and other systems.[1−3] These cross-correlation effects are typically observed by monitoring the partial interconversion of suitably excited multiple-quantum coherences or by quantifying differential line-broadening in multiple-quantum spectra. The build-up rates $R(\theta_{p,q})$ depend on the angle $\theta_{p,q}$ subtended by the principal axes of the two interactions. For example, assuming overall isotropic tumbling and fast isotropic internal motion, the cross-correlation rate between the $^{13}\mathrm{C}^{\alpha}-\mathrm{H}^{\alpha}$ and $^{15}\mathrm{N}-\mathrm{H}^{\mathrm{N}}$ dipole−dipole interactions is given by:

$$R(\theta_{p,q}) = \left(\frac{\mu_0\hbar}{4\pi}\right)^2 \frac{\gamma_C\gamma_N\gamma_{\mathrm{H}}^2}{r_p^3\, r_q^3}\,\frac{2S^2\tau_{\mathrm{c}}}{5}\,\frac{(3\cos^2\theta_{p,q}-1)}{2} \qquad (1)$$

where $\theta_{p,q}$ is the angle subtended between the $^{13}\mathrm{C}^{\alpha}-\mathrm{H}^{\alpha}$ and $^{15}\mathrm{N}-\mathrm{H}^{\mathrm{N}}$ bond vectors represented by p and q, respectively, r_p and r_q are the internuclear distances, τ_{c} is the overall correlation time for isotropic molecular rotational diffusion, S^2 is an order parameter accounting for fast internal motions, and all the other symbols have their usual meaning. In proteins, the angles $\theta_{p,q}$ depend on dihedral angles, such as ϕ, ψ, and ω, and thus are useful probes of secondary structure.

Many complementary cross-correlation rates can now be measured so that their interpretation in terms of intervening dihedral angles may lead to contradictions. In general, overdetermined measurements will not be compatible with a unique set of dihedral angles ϕ, ψ, and ω. Internal inconsistencies among the observed rates can be attributed to a number of factors: errors in bond distances, bond angles, and CSA tensor parameters used to calculate the rates, deviations from isotropic rotational diffusion, rapid local motions occurring on time scales faster than that of overall tumbling, and exchange between two or more distinct conformations affecting $\theta_{p,q}$ on time scales extending to milliseconds. Here we describe a simple approach for resolving inconsistencies by accounting for motional fluctuations about dihedral angles. In many cases this will afford a more realistic appreciation of structural parameters in the presence of complicating motional effects.

As an example, we consider the case appearing in eq 1. The angle $\theta_{p,q}$ depends only on a single intervening dihedral angle ϕ (see Figure 1), allowing an expression for the cross-correlation rate to be conveniently recast in terms of a Fourier series about the dihedral angle ϕ, for example, $R(\phi) = \mathrm{A} + \mathrm{B}\cos\phi + \mathrm{C}\cos 2\phi$. This form is familiar from the so-called Karplus relationships between vicinal scalar couplings and dihedral angles. The presence of conformational averaging about ϕ will lead to the observation of a population-weighted average over all conformations sampled. For example, in the case of exchange between two equally populated conformations, r and s, with two distinct angles $\phi^{\mathrm{r}} = \phi_0 + {}^1\!/_2\Delta\phi^{\mathrm{rs}}$ and $\phi^{\mathrm{s}} = \phi_0 - {}^1\!/_2\Delta\phi^{\mathrm{rs}}$ the function $R(\phi)$ can be simply replaced by an averaged function $\langle R(\phi)\rangle^{\mathrm{r,s}} = ({}^1\!/_2)[R(\phi^{\mathrm{r}}) + R(\phi^{\mathrm{s}})]$. The motionally averaged theoretical curve is obtained from a simple convolution ("moving average") of the function $R(\phi)$ with a pair of Dirac functions spaced by $\Delta\phi^{\mathrm{rs}}$:

$$\langle R(\phi)\rangle^{\mathrm{r,s}} = R(\phi)*\left[\delta\left(+\frac{1}{2}\Delta\phi^{\mathrm{rs}}\right) + \delta\left(-\frac{1}{2}\Delta\phi^{\mathrm{rs}}\right)\right] \qquad (2)$$

A Fourier transformation of the function $R(\phi)$ with respect to the variable ϕ (which may be regarded as a pseudo time-domain t) yields a plot with just three nonvanishing Dirac functions with amplitudes A, B, and C, a plot which may be regarded as a "stick spectrum" in a pseudofrequency-domain ω. Motional averaging can then be taken into account by multiplication with $\cos({}^1\!/_2\Delta\phi^{\mathrm{r,s}}\omega)$. This amounts to weighting the two Karplus-like coefficients B and C, while A remains unaffected. The greater the angular variation $\Delta\phi^{\mathrm{r,s}}$ associated with conformational exchange between the two sites r and s, the more the coefficients B and C are attenuated, and the more pronounced the smoothing of the function $\langle R(\phi)\rangle^{\mathrm{r,s}}$. The advantage of this conjugate representation is that it is very straightforward to derive analytical expressions that incorporate the effects of motional averaging about an intervening dihedral angle. The approach can be extended to cases with unequal populations, or where a range of conformations are accessible with a suitable probability distribution function.[4]

Figure 1 illustrates how the conformation of two consecutive peptide subunits can be determined by measurement of four complementary cross-correlation rates: $R_{\mathrm{NH}(i),\mathrm{C}\alpha\mathrm{H}\alpha(i)}$, $R_{\mathrm{C}\alpha\mathrm{H}\alpha(i-1),\mathrm{NH}(i)}$, $R_{\mathrm{C}\alpha\mathrm{H}\alpha(i-1),\mathrm{C}'(i-1),}$ and $R_{\mathrm{C}\alpha\mathrm{H}\alpha(i-1),\mathrm{C}\alpha\mathrm{H}\alpha(i)}$. Note that the angles ϕ and ψ involved refer to two consecutive amino acids, unlike the situation described in Ramanchandran plots. In addition to the dashed theoretical cross-correlation curves, the straight dotted lines in Figure 1 show experimental cross-correlation rates that have been determined for two residues (phenylalanine 4 and valine 5) in the protein ubiquitin. In the absence of motion, the intersections of the straight dotted lines with the theoretical curves should correspond to dihedral angles that are compatible. Note that the intersections with the functions $R_{\mathrm{NH}(i),\mathrm{C}\alpha\mathrm{H}\alpha(i)}$, $R_{\mathrm{C}\alpha\mathrm{H}\alpha(i-1),\mathrm{NH}(i)}$,, $R_{\mathrm{C}\alpha\mathrm{H}\alpha(i-1),\mathrm{C}'(i-1)}$ are compatible with a manifold of four pairs of dihedral angles $(\psi_4, \phi_5) \approx (150, -140)$ or $(150, -100)$, or $(90, -140)$ or $(90, -100)$. Consideration of the intersections with $R_{\mathrm{C}\alpha\mathrm{H}\alpha(i-1),\mathrm{C}\alpha\mathrm{H}\alpha(i)}$, which give $\Sigma = \psi_4 + \phi_5 \approx \pm 30°$, can reduce the number of solutions by a factor two. In this case, however, such a simplification is ambiguous. Indeed, no single pair of dihedral angles is compatible with all measurements.

One might be tempted to adjust the order parameters S^2 to bring the rates into agreement. However, cross-correlated order parameters depend on the details of the internal motion in a rather complicated manner, requiring considerably more information than

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10.1021/ja0023130 CCC: $19.00

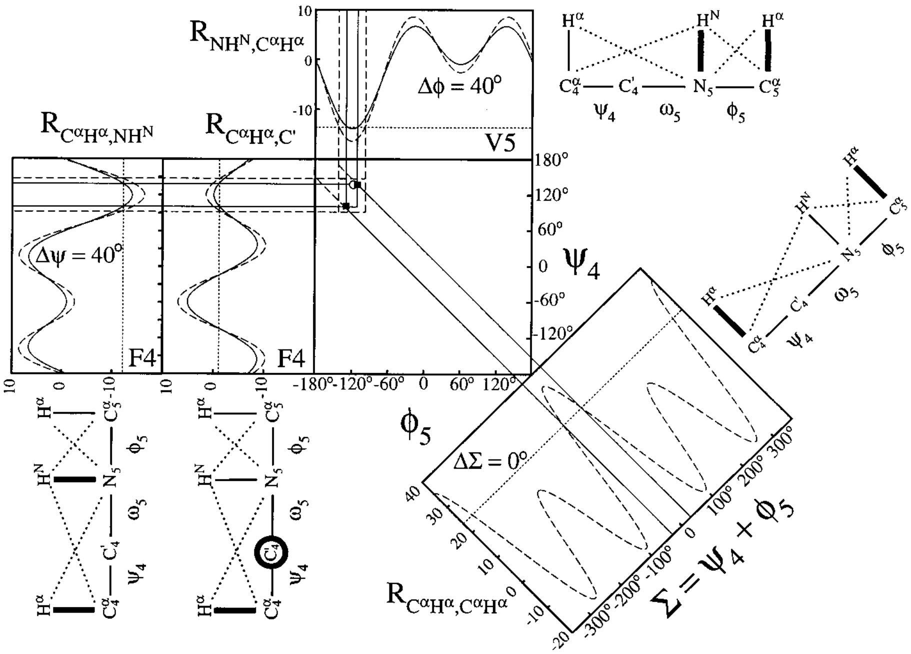

Figure 1. Contradictions between complementary cross-correlation rates measured for residues phenylalanine 4 and valine 5 in ubiquitin may be resolved by introducing simple two-site jumps about dihedral angles. The measured cross-correlation rates are indicated by straight dotted lines.[2] The dashed curves correspond to the absence of internal motion ($r_{NH} = 1.02$ Å, $r_{CH} = 1.09$ Å, CSA of C′ carbonyl: $\sigma_x = 244$ ppm, $\sigma_y = 178$ ppm, $\sigma_z = 90$ ppm). The intersections of these lines correspond to dihedral angles that are compatible with individual measurements, but significant inconsistencies between dihedral angles derived from the four complementary experiments are encountered. These inconsistencies can be resolved using a model in which the peptide plane, spanned by the atoms C'_4, N_5, and $H^N{}_5$, jumps through 40° between two sites. The solid curves and lines correspond to the motionally averaged case. The corresponding X-ray values for ϕ_5 and ψ_4 are indicated by an open circle.

we have at hand to make reasonable estimates. Further complementary cross-correlation experiments that are currently being developed in various laboratories might help in this respect. Our proposed approach is to set $S^2 = 1$ and model dynamics in terms of wobbling of the peptide plane. This allows one to account for some degree of conformational variability, as well as some of the effects of fast motions normally included in the order parameter. This situation can be described in terms of two conformers described by $(-{}^1/_2\Delta\phi = +{}^1/_2\Delta\psi)$ and $(+{}^1/_2\Delta\phi = -{}^1/_2\Delta\psi)$ relative to a mean, with Σ unaffected by this motion. A systematic search was used to find the two-site jump amplitude that maximizes consistency. As described above, we observe that the Karplus-like functions in Figure 1 are smoothed to varying degrees by introducing these motions. The solid curves and lines in Figure 1 illustrate the effect of smoothing resulting from a wobbling amplitude $\pm{}^1/_2\Delta\phi = \mp{}^1/_2\Delta\psi = 20°$ $(\Delta\Sigma = 0)$. In principle, some degree of departure from perfectly anti-correlated ϕ and ψ fluctuations could be introduced by allowing departure of $\Delta\Sigma$ from 0 as well. For this particular case, just a simple two-site averaging of the peptide plane is sufficient to resolve the contradictions, and the intersections of the measured rates with the four smoothed cross-correlation functions are compatible with mean dihedral angles $(\langle\psi_4\rangle,\langle\phi_5\rangle) = (139°, -110°)$ and $(101°, -130°)$ (X-ray values: 140.2°, −118.0°).[5] Although this sort of approach may often lift contradictions in rates, in general there will not be a unique set of motional amplitudes that can accomplish this.

To summarize, we have proposed a graphical construction that allows one to appreciate the complementarity of various cross-correlation rates for the determination of dihedral angles. The overdetermined information may often be incompatible with any particular conformation. Smoothing the cross-correlation functions by convolution to describe exchange between different conformations can often resolve these contradictions. Correction factors required to account for local order parameters and for anisotropy of rotational diffusion must be considered separately.

Acknowledgment. This work was supported by the Fonds National de la Recherche Scientifique (FNRS) and the Commission pour la Technologie et l'Innovation (CTI) of Switzerland and by the Centre National de la Recherche Scientifique (CNRS) of France.

JA0023130

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