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Determination of Molecular Torsion Angles Using Nuclear Singlet Relaxation

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Nuclear magnetic resonance (NMR) is used routinely to investigate molecular conformations in solution. Bond torsion angles may be estimated from vicinal scalar coupling constants, while the nuclear Overhauser effect (NOE) provides information on internuclear distances.[1] However, in some cases, these standard methods are unavailable, ambiguous, or insufficiently accurate. In this communication, we demonstrate that the relaxation of *nuclear singlet states*[2−4] can provide complementary information. In favorable cases, a comparison of the singlet relaxation time constant T_S with the ordinary spin−lattice constant T_1 provides useful qualitative information on the molecular conformation without resorting to detailed models of the NMR relaxation.

The singlet state of a spin-1/2 pair is denoted as follows:

$$|S_0\rangle = 2^{-1/2}(|\alpha\beta\rangle - |\beta\alpha\rangle) \tag{1}$$

where the symbols α and β indicate quantum states with an angular momentum $\pm\,\hbar/2$ along the applied magnetic field. Such states can be observed for inequivalent spin-1/2 pairs by a variety of methods.[3−7] Their lifetimes may exceed the ordinary relaxation time constant T_1 by over an order of magnitude, depending on factors such as the spatial proximity of neighboring magnetic nuclei[8,9] and the relative orientations of chemical shift anisotropy tensors.[10]

The proton spin pair of an inequivalent CH_2 group is a particularly favorable case for the quantitative study of singlet relaxation. The conventional T_1 relaxation is dominated by a single mechanism, namely, the modulation of the internuclear magnetic dipole−dipole coupling by molecular tumbling in solution. In consequence T_1 provides an effective internal calibration of the rotational correlation time. The relaxation time *ratio* T_S/T_1 may depend, within plausible assumptions and to a good approximation, only on molecular geometry and the spin−spin coupling strengths and be insensitive to the rotational behavior of the molecules.

For the case where the CH_2 protons have no significant J-couplings to neighboring protons, the relaxation time ratio T_S/T_1 is only dependent on geometry, to a good approximation. As detailed in the Supporting Information (SI), the following form describes the predicted ratio T_S/T_1 in the absence of scalar spin couplings to neighboring nuclei, which is valid for small molecules in the extreme-narrowing regime:

$$\frac{T_S}{T_1} \simeq \sum_{j>2} \frac{3b_{12}^2}{2(b_{1j}^2 + b_{2j}^2 - 2b_{1j}b_{2j}P_2(\cos\theta_{1j2}))} \tag{2}$$

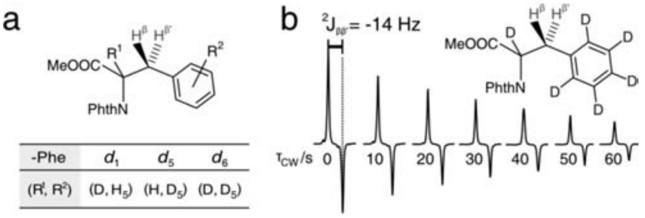

Figure 1. (a) Molecular graph of the 2× protected Phe isotopologues highlighting the diastereotopic C^β protons where singlet states are prepared. (b) The characteristic antiphase NMR signal derived from the long-lived state is shown decaying with exponential time constant $T_S = 51 \pm 2$ s for d_6-Phe (half-doublets shown, proton Larmor frequency =400 MHz).

In this expression indices 1 and 2 indicate the members of the proton pair and j those nuclei in their vicinity; $b_{jk} = -\hbar\gamma^2\mu_0/4\pi r_{jk}^3$ indicates the dipolar coupling factor, which is inversely proportional to the third power of the internuclear distance r_{jk}, $P_2(x) = 1/2(3x^2 - 1)$ is a Legendre polynomial, and θ_{1j2} is the angle between the two vectors joining spins 1 and 2 with spin j. Above $r_{1j} \approx 2$ Å, the ratio decays with the inverse eighth power of the distance from each additional spin to the center of the spin pair.[8] Magnetic nuclei at long-range therefore have a negligible effect, even if they are present in large numbers. The comparison of T_S and T_1 may hence be used to set strong geometric restraints on the immediate molecular environment of the CH_2 group.

To test this concept we prepared a series of three deuterated phenylalanine isotopologues. Substitutions were made (i) alone at C^α (referred as d_1-Phe), (ii) on only the proton sites of the phenyl ring (d_5), and (iii) both of these environments (d_6-Phe) (Figure 1a). Methyl and N-phthalimido groups, which were not deuterated, were added to block solvent-induced relaxation at the carboxyl and amino groups respectively. At a proton frequency of 400 MHz, the nuclear singlet populations were excited on the pair at C^β using the method described by Sarkar et al.[5] The singlets were locked under resonant continuous-wave RF irradiation, and the relaxation rates were measured (for details, see the SI). NMR signals not passing through the singlet states were suppressed by isotropic signal filtration using a tetrahedral phase cycle.[11]

For d_6-Phe the singlet relaxation time was $T_S = 51 \pm 2$ s, approximately 37 times longer than the conventional spin−lattice relaxation time of $T_1 = 1.38 \pm 0.05$ s (Figure 1b). In d_1-Phe, which differs only by protonation of the ring, the decay time was reduced to $T_S = 8.0 \pm 0.1$ s or only 6.8 times longer than $T_1 = 1.17 \pm 0.04$ s. This change is attributable to the spin interactions between the two β-protons and the phenyl protons, in particular, the two *ortho* protons of the ring; the data confirm that the T_1 of the β-protons is dominated by the large dipolar interaction between them and, conversely, that the singlet state in d_1-Phe is relaxed by the DD couplings to the neighboring *ortho* protons of the ring. The long singlet lifetime for the d_6 compound indicates that chemical shift anisotropy has only a small contribution to the relaxation of the methylene singlet state and that the contributions of the protonated blocking groups are also negligible.

The T_1 value of 1.38 ± 0.05 s corresponds to a rotational correlation time of $\tau_c \approx 24$ ps, which verifies that the molecular rotation is within the extreme narrowing limit.

Since the β-protons do not have significant J-couplings with the ring protons, eq 2 can be used to analyze the dependence of the singlet relaxation rate constant upon the torsional angle, $\varphi_{\beta\gamma}$, around

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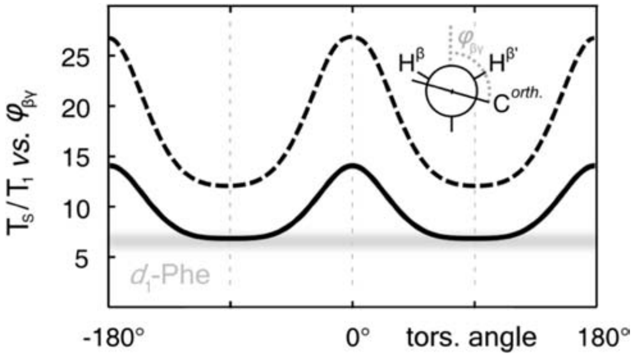

Figure 2. Theoretical ratio T_S/T_1 for d_1-Phe plotted as a function of the ring torsion angle $\varphi_{\beta\gamma}$ in the limit of static molecular geometry (solid line) and in the presence of rapid two-site jumps (dashed line). The horizontal band indicates the experimental ratio of relaxation time constants and its confidence limits.

the C^{β}/C^{γ} bond. Coordinates of the two ortho protons are constructed as a function of $\varphi_{\beta\gamma}$ by using standard bond lengths and bond angles, and the derived dipole−dipole couplings and angles θ are then used with eq 2 to predict the ratio $T_S(\varphi_{\beta\gamma})/T_1$. In our convention the angle $\varphi_{\beta\gamma} = 0$ indicates the midpoint of the vector joining the two β-protons is in the plane of the phenyl ring. The theoretical estimate of T_S/T_1 against the phenyl torsion angle is plotted in Figure 2 (solid curve). The values show a variation in the theoretical T_S/T_1 ratio between ∼14 near $\varphi_{\beta\gamma} = 0°$ and ∼6 in the vicinity of $|\varphi_{\beta\gamma}| = 90°$. The experimental ratio for d_1-Phe is $T_S/T_1 = 6.8 \pm 0.2$ indicating an angle between $|\varphi_{\beta\gamma}| = 45°$ and 135°. This orientation is consistent with the crystal structure of L-phenylalanine.[12]

Aromatic rings of amino acid side chains, such as those of phenylalanine and tyrosine, execute sporadic 180° rotations.[13,14] Ring flips that are fast compared to the rotational correlation time would have a strong effect on the CH_2 singlet relaxation as a consequence of geometrical averaging of dipolar couplings between the β- and ring *ortho*-protons. Figure 2 (dashed curve) shows the predicted φ-dependence of T_S/T_1 for the case of these rapid jumps between conformations with torsions $\varphi_{\beta\gamma}$ and $\varphi_{\beta\gamma}$+180°. This "rapid-flip" model predicts much longer singlet relaxation times, with T_S/T_1 varying between 13 and ∼26. That the experimental value of $T_S/T_1 = 6.8$ is inconsistent with this jump model for all values of $\varphi_{\beta\gamma}$ proves that 180° ring flips are infrequent on the molecular rotational time scale of ∼24 ps.

In the d_5-Phe isotopologue, the $C^{\beta}H_2$ singlet relaxation is caused predominantly by dipole−dipole interactions with the α proton. We observed $T_S = 16 \pm 0.2$ s and $T_1 = 1.33 \pm 0.04$ s to give the ratio $T_S/T_1 = 12.0 \pm 0.5$. In this case, however, eq 2 cannot be used to determine the dependence of T_S on the torsion angle $\varphi_{\alpha\beta}$, for the singlet relaxation is strongly influenced by the vicinal $^3J_{\alpha\beta}$-couplings, which are themselves dependent on $\varphi_{\alpha\beta}$. In such an instance the conformational dependence of T_S/T_1 on $\varphi_{\alpha\beta}$ is made by Liouvillian eigenvalue analysis[15] using Karplus relationships to treat the torsion angle dependence of $^3J_{\alpha\beta}$ (see Supporting Information). The predicted T_S/T_1 curve is shown in Figure 3a, with a minimum in T_S/T_1 at the eclipsed *syn* conformations ($|\varphi_{\alpha\beta}| = 60°$) and a maximum in the vicinity of the *anti* configuration ($|\varphi_{\alpha\beta}| =$ 140°−180°). The experimental ratio of the relaxation times supports an angle $|\varphi_{\alpha\beta}| \approx 100°$ which approximately agrees with the value inferred from the experimental $^3J_{\alpha\beta}$ couplings of 4.9 and 12.0 Hz. Figure 3 shows that, in general, T_S and $^3J_{\alpha\beta}$ provide complementary information. For example, the T_S measurement can easily distinguish between the 0° and 180° points, which are ambiguous in the Karplus curves. In this particular demonstration the value of T_S/T_1 merely confirms the torsional angle estimate from the vicinal *J*-couplings.

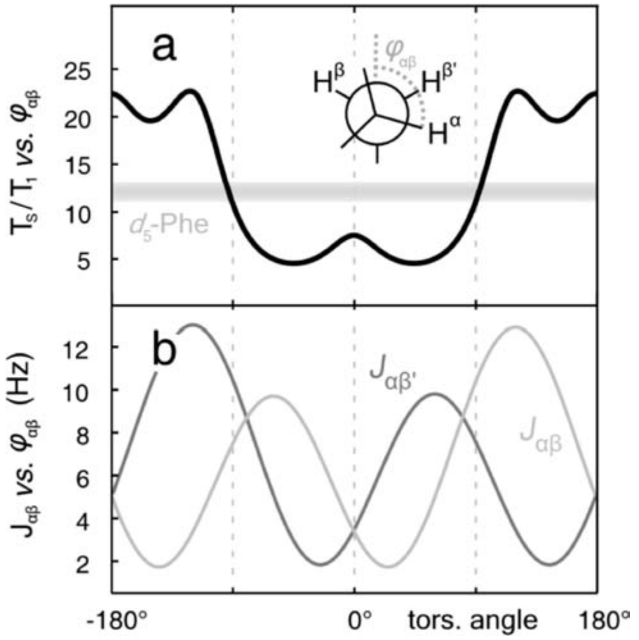

Figure 3. (a) Theoretical ratio T_S/T_1 for d_5-Phe plotted as a function of the ring torsion angle $\varphi_{\alpha\beta}$. The horizontal band indicates the experimental ratio of relaxation time constants and its confidence limits. (b) Karplus curves for the vicinal $^3J_{\alpha\beta}$-couplings,[16] emphasizing the complementary nature of the torsion angle dependence, compared to T_S/T_1.

We must stress, however, that an analysis using a single or dominant conformation will not be appropriate in all cases. In systems that are less rigid, where steric factors are not so strong, especially acyclic aliphatics, one must address the possibility of rotamer interconversion. If the conformational exchange is much slower than τ_c, the rate constants $1/T_1$ and $1/T_S$ are given by an average over their values in the relevant conformations, weighted by the equilibrium populations.

In summary, CH_2 singlet relaxation is highly sensitive to the local magnetic environment of the proton pair and depends in a predictable way on local geometric parameters, such as torsional angles. Singlet relaxation may therefore provide geometrical information that is hard to otherwise obtain and, in general, provides complementary information that is useful for verifying geometric models or for resolving ambiguities. We also anticipate that singlet relaxation is less sensitive to effects such as spin diffusion that readily confound cross-relaxation measurements. We expect numerous applications to molecular conformational studies in solution.

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Supporting Information Available: Derivation of eq 2, full details of experiments and simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G.; Skleton, N. J. *Protein NMR Spectroscopy, Principles and Practice*; Academic Press: San Diego, 1996.
(2) Levitt, M. H. In *Encyclopedia of Nuclear Magnetic Resonance*; Harris, R. K., Wasylishen, R. E., Eds.; Wiley: Chichester, U.K., 2010.
(3) Carravetta, M.; Levitt, M. H. *J. Am. Chem. Soc.* **2004**, *126*, 6228–6229.
(4) Carravetta, M.; Levitt, M. H. *J. Chem. Phys.* **2005**, *122*, 214505.
(5) Sarkar, R.; Vasos, P. R.; Bodenhausen, G. *J. Am. Chem. Soc.* **2007**, *129*, 328–334.

(6) Cavadini, S.; Dittmer, J.; Antonijevic, S.; Bodenhausen, G. *J. Am. Chem. Soc.* **2005**, *127*, 5744–5748.
(7) Sarkar, R.; Ahuja, P.; Moskau, D.; Vasos, P. R.; Bodenhausen, G. *ChemPhysChem* **2007**, *8*, 2652–2656.
(8) Grant, A. K.; Vinogradov, E. *J. Magn. Reson.* **2008**, *193*, 177–190.
(9) Pileio, G.; Concistrè, M.; Carravetta, M.; Levitt, M. H. *J. Magn. Reson.* **2006**, *182*, 353–357.
(10) Ahuja, P.; Sarkar, R.; Vasos, P. R.; Bodenhausen, G. *J. Chem. Phys.* **2007**, *127*, 134112.
(11) Pileio, G.; Levitt, M. H. *J. Magn. Reson.* **2008**, *191*, 148–155.
(12) Al-Karaghouli, A. R.; Koetzle, T. F. *Acta Crystallogr.* **1975**, *E31*, 2461–2465.
(13) Gall, C. M.; DiVerdi, J. A.; Opella, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 5039–5034.
(14) Campbell, I. D.; Dobson, C. M.; Williams, R. J. P. *Proc. R. Soc. London, Ser. B* **1975**, *189*, 503–509.
(15) Pileio, G.; Levitt, M. H. *J. Magn. Reson.* **2007**, *187*, 141–145.
(16) DeMarco, A.; Llinás, M.; Wüthrich, K. *Biopolymers* **1978**, *17*, 617–636.

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