Acknowledgment. We gratefully acknowledge financial support for this project from the National Science Foundation (NSF CHE 8515371 and 8818147). S.E.D. acknowledges support from the National Science Foundation (Presidential Young Investigator Award, 1985-1990) and the Alfred P. Sloan Foundation (1985-1989). B.R.H. thanks the University of Illinois for a Graduate Fellowship (1984-1988).

Supplementary Material Available: Full characterization of **1**, **2**, and **3** is provided along with general experimental and cyclization procedures (5 pages). Ordering information is given on any current masthead page.

Correlated Motion Monitored by NMR Relaxation in the Rotating Frame. A Source of Structural and Dynamic Information on Macromolecules

R. Brüschweiler, C. Griesinger, and R. R. Ernst*

Laboratorium für Physikalische Chemie
Eidgenössische Technische Hochschule
8092 Zürich, Switzerland

Received April 3, 1989

Nuclear magnetic cross relaxation has become a major tool for the investigation of macromolecular structure and dynamics.[1,2] In particular two-dimensional nuclear Overhauser effect spectroscopy (NOESY) proved to be quite informative.[3,4] Additionally, it has been shown that cross relaxation involving higher spin orders contains specific information on correlated motional processes,[5-15] useful for the description of segmental motion and conformational equilibria in biomolecules.

Recently a technique has been proposed for the observation of cross relaxation between one- and three-spin order in the laboratory frame,[16] however with applicability to small molecules ($\omega_0\tau_c \leq 1$) only. We propose in this communication an alternative method for the measurement of cross relaxation in a tilted rotating frame, called 3QF T-ROESY (T refers to Tilted frame), that does not suffer from this limitation.

For simplicity, we concentrate on the α and the two β protons in an amino acid residue of a protein. They form an AMX spin subsystem. We assume residues in which the feasible conformations are limited to the three staggered ones shown in Figure 1a that possibly may dynamically interconvert. Dipolar interaction among the three spins, modulated by overall molecular tumbling and intramolecular motion, causes correlated cross relaxation. We consider the transfer between one-spin and three-spin order:

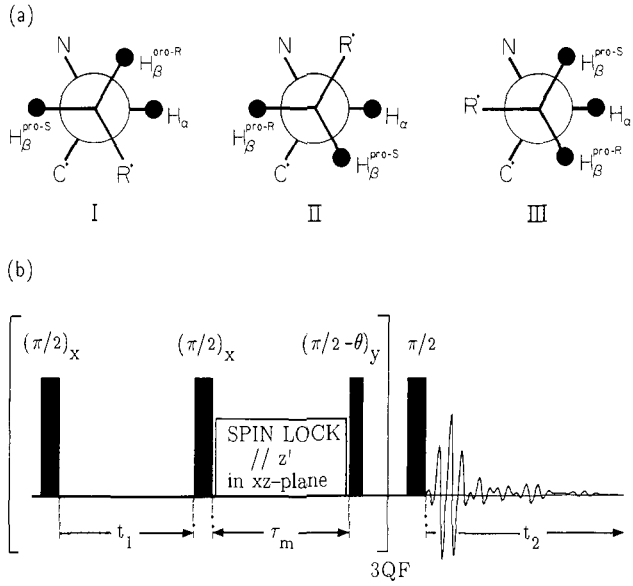

Figure 1. (a) Three staggered conformations of a $C_\alpha C_\beta$ fragment of an amino acid residue. (b) Pulse sequence for 3QF T-ROESY. The $(\pi/2)_x$ pulse before and the $(\pi/2 - \theta)_y$ pulse after the spin lock sequence ensure optimal transfer of in-phase coherence to and from the lock axis.[19] The off-resonance lock during the mixing time τ_m for $\theta = 35°$ is effected by time-proportional phase incrementation with the pulse sequence $(14^\circ_0, 14^\circ_{-20}, 14^\circ_{-40}, \ldots, 14^\circ_{-340})_n$.

$$I_{Az'} \xrightarrow{\Gamma^\theta_{AMX\,A}} 4I_{Az'}\, I_{Mz'}\, I_{Xz'}$$

We assume that during cross relaxation an rf field B_1 is applied off-resonant by $\Delta\omega$ such that the effective field in the rotating frame is oriented along z' tilted by an angle $\theta = \tan^{-1}(\gamma B_1/\Delta\omega)$ with respect to the static field. The rate constant for the creation of three-spin order is[17]

$$\Gamma^\theta_{AMX\,A} = \left(\frac{\mu_0}{4\pi}\right)^2 \gamma^4\hbar^2\frac{3}{20}[3\sin^2\theta\cos^2\theta J_{AM\,AX}(0) + (\sin^4\theta - \sin^2\theta\cos^2\theta + 2\cos^4\theta)J_{AM\,AX}(\omega_0) + (\sin^2\theta(1+\cos^2\theta)J_{AM\,AX}(2\omega_0)] \quad (1)$$

where $J_{AM\,AX}(\omega)$ is the cross power spectral density of the two dipolar interactions AM and AX, assuming equal θ values for all spins for strong rf field B_1. The first term in eq 1 disappears for laboratory frame cross relaxation (NOESY, $B_1 = 0$, and $\theta = 0$) and for on-resonance rotating frame cross relaxation (ROESY, $\Delta\omega = 0$, and $\theta = \pi/2$) for all values of τ_c, whereas for large molecules with long correlation times τ_c ($\omega_0\tau_c \gg 1$) in addition the second and third terms vanish. For large molecules, the maximum rate constant is obtained for $\theta = 45°$. Some characteristic values for the ratio of the rates in laboratory and tilted rotating frame are $\Gamma^0_{AMA\,X}/\Gamma^{45°}_{AMA\,X} = 1, 0.1, 0.01$ for $\omega_0\tau_c \cong 0, 5, 16$, respectively ($\omega_0\tau_c \cong 13$ for BPTI at room temperature and 500 MHz). In spite of a slowdown by 10% it is advisable to set $\theta = 35°$ ($\cong 90° - \cos^{-1}(1/\sqrt{3})$) as at this value cross-relaxation rate constant Γ^θ_{MA} vanishes in competitive transfers, such as in

$$I_{Az'} \xrightarrow{\Gamma^\theta_{MA}} I_{Mz'} \xrightarrow{\Gamma^\theta_{AMX\,M}} 4I_{Az'}I_{Mz'}I_{Xz'}$$

The cross power spectral density $J_{AM\,AX}(\omega)$ contains information on overall and intramolecular motional processes. We assume a random jump process between the three conformations of Figure

(1) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley-Interscience: 1986.
(2) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon Press: Oxford, 1987.
(3) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546-4553.
(4) Kumar, A.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1-6.
(5) Werbelow, L. G.; Grant, D. M. *Adv. Magn. Reson.* **1977**, *9*, 189-299.
(6) Vold, R. L.; Vold, R. R. *Prog. NMR Spectrosc.* **1978**, *12*, 79-133.
(7) Fagerness, P. E.; Grant, D. M.; Kuhlmann, K. F.; Mayne, C. L.; Parry, R. B. *J. Chem. Phys.* **1975**, *63*, 2524-2532.
(8) Böhlen, J. M.; Wimperis, S.; Bodenhausen, G. *J. Magn. Reson.* **1988**, *77*, 589-595.
(9) Brondeau, J.; Canet, D.; Millot, C.; Nery, H.; Werbelow, L. *J. Chem. Phys.* **1985**, *82*, 2212-2216.
(10) Jaccard, G.; Wimperis, S.; Bodenhausen, G.; *J. Chem. Phys.* **1986**, *85*, 6282-6293.
(11) Müller, N. *Chem. Phys. Lett.* **1986**, *31*, 218-223.
(12) Oschkinat, H.; Pastore, A.; Bodenhausen, G. *J. Am. Chem. Soc.* **1987**, *109*, 4110-4111.
(13) Oschkinat, H.; Limat, D.; Emsley, L.; Bodenhausen, G. *J. Magn. Reson.* **1988**, in press.
(14) Jaccard, G.; Wimperis, S.; Bodenhausen, G. *Chem. Phys. Lett.* **1987**, *138*, 601-606.
(15) Bull, T. E. *J. Magn. Reson.* **1987**, *72*, 397-413.
(16) Dalvit, C.; Bodenhausen, G. *J. Am. Chem. Soc.* **1988**, *110*, 7924-7926.
(17) Bull, T. E. *J. Magn. Reson.* **1988**, *80*, 470-481.

0002-7863/89/1511-8034$01.50/0

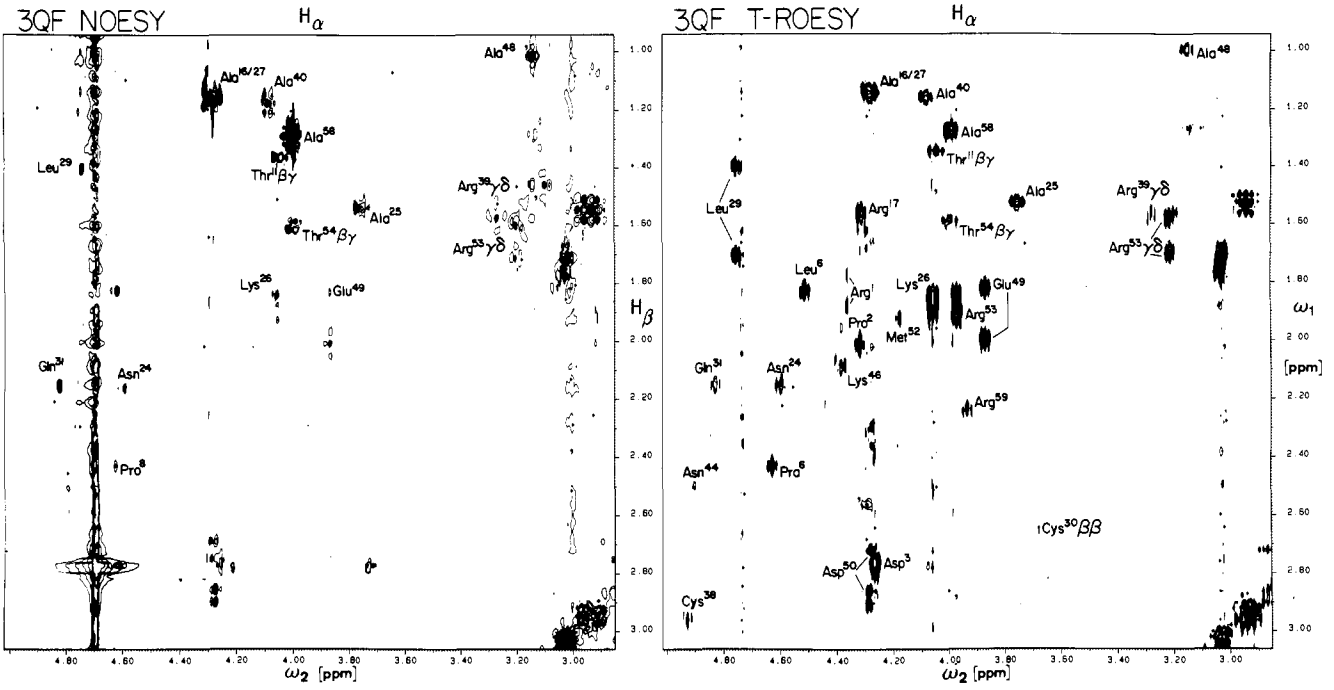

Figure 2. Proton resonance spectra of a 20 mM solution of basic pancreatic trypsin inhibitor in D_2O at pD = 4.6 recorded with the pulse sequence of Figure 1b) with an rf field strength $\gamma B_1/2\pi$ = 6100 Hz and irradiation effectively 14.3 ppm at lower field from the middle of the spectrum. 1024 experiments with 60 transients each were recorded. (a) 3QF NOESY (500 MHz), $B_1 = 0$, $\theta = 0$ at 305 K. The water resonance ridge at ω_2 = 4.7 ppm is plotted at a four times higher level than the rest of the spectrum. (b) 3QF T-ROESY with $\theta = 35°$ (600 MHz) at 300 K. The H_β–H_α cross peak region is shown, which also includes some other cross peaks. The water resonance was suppressed by presaturation.

1a, leading to an expression of the following form[18] (in units of $s(nm)^{-6}$)

$$J_{AM\ AX}(\omega) = \left[a\frac{2\tau_c}{1+\omega^2\tau_c^2} + b\frac{2\tau_t}{1+\omega^2\tau_t^2} \right] \qquad (2)$$

with $1/\tau_t = 1/\tau_c + N/\tau_e$; where τ_c is the correlation time of the isotropic molecular tumbling process, and τ_e is a characteristic correlation time of the exchange process between N (=2 or 3) of the three conformations.

The constants a and b are given in the following for three motional models. We assume transfer from one-spin order of $H_\beta^{pro\text{-}R}$ to three-spin order with use of the geometric parameters r_{CC} = 0.154 nm, r_{CH} = 0.107 nm, ∠HCC = 109.5°.

(i) For infrequent jumps ($N/\tau_e \ll 1/\tau_c$) between conformations I, II, and III with populations p_I, p_{II}, and p_{III} ($p_I + p_{II} + p_{III} = 1$), one finds for the transfer from $H_\beta^{pro\text{-}R}$ that $a = -6225p_I + 2p_{II} - 3889p_{III}$ and $b = 0$.

(ii) For equally populated conformations $p_I = p_{II} = p_{III} = 1/3$, one obtains for all jump rates $a = -3488$ and $b = 118$.

(iii) For the case that one population vanishes, one finds for the transfer the following expressions: $p_{III} = 0$: $a = -6225p_I^2 + 2p_{II}^2 - 3887p_Ip_{II}$, $b = -2336p_Ip_{II}$; $p_{II} = 0$: $a = -6225p_I^2 - 3889p_{III}^2 - 7778p_Ip_{III}$, $b = -2336p_Ip_{III}$; $p_I = 0$: $a = 2p_{II}^2 - 3889p_{III}^2 - 9613p_{II}p_{III}$, $b = 5725p_{II}p_{III}$. For the corresponding transfer starting from $H_\beta^{pro\text{-}S}$, p_I and p_{II} have to be interchanged in the expressions for a and b.

The pulse sequence of Figure 1b for the measurement of a rotating frame 2D cross-relaxation spectrum contains an off-resonance spin lock period τ_m where the offset is generated by time-proportional phase incrementation. A triple quantum filter selects the desired pathways, and a final $\pi/2$ pulse generates single quantum coherence.

A comparison of 3QF NOESY and 3QF T-ROESY ($\theta = 35°$) is given in Figure 2 for basic pancreatic trypsin inhibitor (BPTI).[20,21] The 3QF NOESY spectrum shows, as expected, much weaker cross peaks than 3QF T-ROESY, most of the visible peaks are in antiphase along ω_1 and stem from undesired and uninformative zero quantum coherence[2,22,23] that survives the mixing period due to slow T_2 relaxation. These peaks can be displaced from the interesting region by t_1-proportional incrementation of τ_m. The 3QF T-ROESY spectrum, on the other hand, shows strong in-phase cross peaks with zero quantum contributions that rapidly decay in the inhomogeneous spin lock field. Coherent TOCSY-like transfers are negligible for $\theta = 35°$ because the Zeeman level splitting is reduced only by 18%.

Pairs of $H_\beta H_\alpha$ cross peaks in T-ROESY may be weak due to an unresolved coupling $J_{\alpha\beta_1}$ or $J_{\alpha\beta_2}$. However, a strong intensity contrast between the two $H_\beta H_\alpha$ cross peaks indicates one very small cross relaxation rate. This can occur only for a dominant population of either conformation I or II (Figure 1a) or for residues with restricted mobility such as prolines. Characteristic examples of this type are Pro[2], Pro[8], Gln[31], Cys[38], and Asn[44]. Pairs of cross peaks with similar intensities indicate similar population of conformations I and II, while the population of conformation III remains unknown. This applies to the residues Leu[29], Glu[49], and Arg[53]. Further information on conformational dynamics can be obtained by a quantitative analysis of peak intensities and their build-up rates. Obtaining similar information from J couplings is often impeded by missing resolution, while cross peak intensities in NOESY spectra may suffer from rapid spin diffusion between the two β protons.

This work shows that correlated cross relaxation in molecular fragments can be exploited to investigate conformational questions for larger macromolecules provided that the measurements are performed in the rotating frame with a tilted spin lock axis.

Acknowledgment. This research has been supported by the Swiss National Science Foundation. We are indebted to Dr. W. Bermel for generous support in using AM 500 and AM 600 spectrometers at Bruker Analytische Messtechnik, Forchheim. The manuscript has been processed by H. Balsiger and I. Müller.

(18) Woessner, D. E. *J. Chem. Phys.* **1962**, *36*, 1–4; **1962**, *37*, 647–654.
(19) Griesinger, C.; Ernst, R. R. *J. Magn. Reson.* **1987**, *75*, 261–271.
(20) Deisenhofer, J.; Steigemann, W. *Acta Crystallogr. Sect. B* **1975**, *31*, 238–250.
(21) Wagner, G.; Wüthrich, K. *J. Mol. Biol.* **1982**, *155*, 347–366.
(22) Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259–281.
(23) Griesinger, C.; Ernst, R. R. *Chem. Phys. Lett.* **1988**, *152*, 239–247.