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Measuring the χ_1 torsion angle in protein by CH-CH cross-correlated relaxation: A new resolution-optimised experiment

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Abstract

Here we introduce an experiment with high sensitivity and resolution for the measurement of CH-CH dipolar-dipolar cross-correlated relaxation rates (CCRR) in protein side-chains. The new methodology aims to the determination of structural and dynamical parameters around the torsion angle χ_1 by measuring $C_\alpha H_\alpha$-$C_\beta H_\beta$ cross-correlated relaxation rates. The method is validated on the protein ubiquitin: the χ_1 angles determined from the CCRR data are compared with the χ_1 angles of a previously determined NMR structure. The agreement between the two data sets is excellent for most residues. The few discrepancies that were found between the CCR-derived χ_1 angles and the angles of the previously determined NMR structure could be explained by taking internal motion into account. The new methodology represents a very powerful tool to determine both structure and dynamics of protein side-chains in only one experiment.

Abbreviations: NOE – Nuclear Overhauser Enhancement, CCRR – Cross-Correlated Relaxation Rate.

Introduction

The torsion angle χ_1 in proteins is typically obtained by measuring J_{HH} coupling constants between the H_α and the H_β spins, J_{NC} coupling constants between the amide nitrogen and the C_γ and J_{CC} coupling constants between the carbonyl and the C_γ spins (Griesinger and Eggenberger, 1992; Eggenberger et al., 1992; Bartik and Redfield, 1993; Vuister et al., 1993; Konrat et al., 1997; Hu and Bax, 1997a, b). However, the feasibility of scalar coupling measurements decreases with increasing protein size, due to both large line-widths and resonance overlap. Alternatively, torsion angles can be accessed by cross-correlated relaxation rates (CCRR), as it has been extensively demonstrated in the past years (Reif et al., 1997; Yang et al., 1998; Yang and Kay, 1998; Boisbouvier et al., 1999; Felli et al., 1999; Carlomagno et al., 1999; Chiaparin et al., 2000; Carlomagno et al., 2001). As opposed to scalar coupling constants, CCRR effects increase with the protein size and can be measured also for molecules with broad NMR lines, provided that the resonances are well resolved. The χ_1 torsion angle can be derived by measuring dipolar-dipolar cross-correlated relaxation between the C_α-H_α and the C_β-H_β dipolar interactions. CH-CH dipolar-dipolar cross-correlated relaxation rates ($\Gamma_{CH,CH}$) are easily measured in a HCCH correlation experiment, as has been shown in nucleic acids (Felli et al., 1999; Carlomagno et al., 1999, 2001). However, the HCCH spectrum of large proteins is poorly resolved, which severely limits the usefulness of this approach. Here, we propose a new pulse sequence that is an extension of the HBHA(CBCACO)NH (Grzesiek and Bax, 1993) experiment and allows to measure $\Gamma_{C\alpha H\alpha,C\beta H\beta}$ CCRRs in a H_β-N-H_N correlation, with considerably higher resolution than in a HCCH correlation for large proteins. The method belongs to the class of the Γ-quantitative experiments, where CCRRs are measured by the intensity ratio of peaks in a *cross* and in a

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reference experiment. The pulse sequence utilizes the same couplings as the HBHA(CBCACO)NH experiment for the magnetization transfer steps that do not take place through CH-CH cross-correlated relaxation. This makes each transfer step fast enough to be applicable to large proteins. The measurement of the CCRRs and the extraction of the torsion angles χ_1 will be demonstrated on the protein ubiquitin.

Methods

The dipolar-dipolar cross correlated relaxation between the C_α-H_α and the C_β-H_β vectors depends on the projection angle θ between the two vectors, according to the following equation:

$$\Gamma_{C\alpha H\alpha, C\beta H\beta} = \frac{\gamma_H^2 \gamma_C^2 \mu_0^2 \hbar^2}{40\pi^2 r_{C\alpha H\alpha}^3 r_{C\beta H\beta}^3} S_{\alpha\beta}^2 \frac{3\cos^2\theta - 1}{2} \tau_c, \quad (1)$$

where $r_{C\alpha H\alpha / C\beta H\beta}$ is the length of the C_α-H_α/C_β-H_β bond vectors, τ_c is the correlation time of the molecule and $S_{\alpha\beta}^2$ is the order parameter which takes into account possible internal motion around the C_α-C_β bond. The projection angle θ is correlated to the dihedral angle χ_1 by the following equation:

$$\cos\theta = -\cos^2(109^\circ) + \sin^2(109^\circ)\cos(\chi_1 - \alpha) \quad (2)$$

for the amino acids with only one H_β proton (α is equal to 120° for threonine and isoleucine and to 0° for valine) and for the amino acids with two H_β protons by the equations:

$$\cos\theta_1 = -\cos^2(109^\circ) + \sin^2(109^\circ)\cos(\chi_1 + 120^\circ)$$

$$\cos\theta_2 = -\cos^2(109^\circ) + \sin^2(109^\circ)\cos(\chi_1 - 120^\circ) \quad (3)$$

for the H_β proton in pro-R and in pro-S, respectively.

The pulse sequence used to measure the $\Gamma_{C\alpha H\alpha, C\beta H\beta}$ rate is shown in Figure 1. The experiment is very similar to a HBHA(CBCACO)NH correlation with an additional building block (in the frame in Figure 1) responsible for the magnetization transfer via CCRR. After evolution of the chemical shifts of the H_α/H_β protons in t_1, the magnetization is transferred to the C_α/C_β carbons at point *a*. In the following $2\Delta_1$ delay of 7 ms the $^1J_{CC}$ scalar coupling evolves generating the terms $4C_{\beta x}C_{\alpha z}H_{\beta z}$ and $2C_{\alpha y}H_{\alpha z}$ at point *b*. Two experiments are recorded, a *cross* and a *reference* spectrum. In the cross experiment the magnetization term $4C_{\beta x}C_{\alpha y}H_{\beta z}$ present at point *c* is transferred to the term $4C_{\beta y}C_{\alpha x}H_{\alpha z}$ at point *d* via $\Gamma_{C\alpha H\alpha, C\beta H\beta}$ cross-correlated relaxation, while the term $2C_{\alpha y}H_{\alpha z}$ at point *b* is transformed into $2C_{\alpha z}H_{\alpha z}$ and therefore remains unvaried. The delay τ_1 is equal to τ_2 and $2\tau_1 + 2\tau_2 = 1/J_{CC}$ to ensure refocusing of the CC scalar coupling. In the reference experiment $\tau_1 = 1/(4J_{CC}) + 1/(13.3J_{CH})$ and $\tau_2 = 1/4J_{CC} - 1/(13.3J_{CH})$ and the same magnetization transfer $4C_{\beta x}C_{\alpha y}H_{\beta z} \rightarrow 4C_{\beta y}C_{\alpha x}H_{\alpha z}$ is achieved via evolution of the J_{CH} scalar coupling. The second part of the pulse sequence is equivalent to that of a standard HBHA(CBCACO)NH experiment with evolution of the amide nitrogen chemical shifts in t_2 and of the amide proton chemical shifts in t_3. The cross correlated relaxation rate is extracted by the ratio of the $H_{\beta,i}$-N_{i+1}-$H_{N,i+1}$ peak intensities I_{cross} and $I_{ref.}$ in the cross and reference experiment, respectively, according to the formula:

$$\frac{I_{cross}}{I_{ref.}} = \frac{\sinh\left(\Gamma_{C\alpha H\alpha, C\beta H\beta} T\right)}{\cosh\left(\Gamma_{C\alpha H\alpha, C\beta H\beta} T\right)\sin^2\left(\frac{\pi}{3.33}\right) + \sinh\left(\Gamma_{C\alpha H\alpha, C\beta H\beta} T\right)\cos^2\left(\frac{\pi}{3.33}\right)} \quad (4)$$

for the amino acids with one H_β proton only. The time T in Equation 4 is equal to $2\tau_1 + 2\tau_2$. The second term at the denominator of Equation 4 arises from the fact that in the reference experiment the J_{CH} coupling evolves for a total time of $1/(3.33J_{CH})$ instead of $1/(2J_{CH})$ and therefore the transfer $4C_{\beta x}C_{\alpha y}H_{\beta z} \rightarrow 4C_{\beta y}C_{\alpha x}H_{\alpha z}$ in the reference experiment takes place through both J-coupling (first term at the denominator of Equation 4) and cross-correlated relaxation (second term at the denominator of Equation 4). The J_{CH} coupling total evolution time of $1/(3.33J_{CH})$ was chosen to optimise transfer of magnetization for both $C_\alpha H$-$C_\beta H$ and $C_\alpha H$-$C_\beta H_2$ moieties.

For the amino acids with two H_β protons the rates cannot be extracted directly from the ratio between peak intensities in the cross and reference experiments with a similar formula as in Equation 4, due to the presence of the cross-correlated relaxation rate $\Gamma_{C\beta H\beta 1, C\beta H\beta 2}$ and of the strong NOE between the $H_{\beta 1}$ and $H_{\beta 2}$ protons, that cause the mutual inter-conversion of the terms $4C_{\beta x}C_{\alpha y}H_{\beta_1 z} \rightleftarrows 4C_{\beta x}C_{\alpha y}H_{\beta_2 z}$, as previously described (Carlomagno et al., 2001). For a CH-CH_2, moiety the two $\Gamma_{C\alpha H\alpha, C\beta H\beta}$ rates are obtained by fitting the experimental data to simulated intensity ratios, calculated taking full auto- and cross-correlated relaxation of

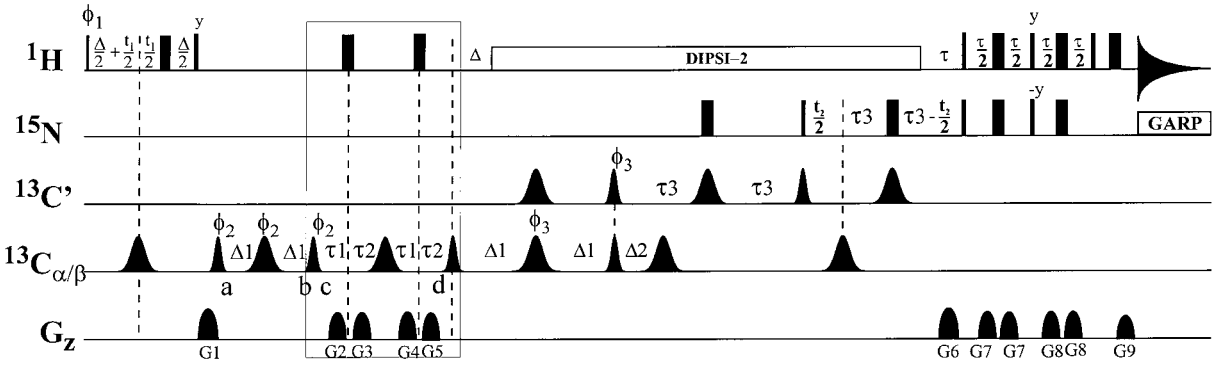

Figure 1. Pulse sequence for the measurement of the $\Gamma_{C\alpha H\alpha, C\beta H\beta}$ CCRRs in proteins. The experiment is a HBHA(CBCACO)NH correlation with an additional building block (in the frame) for the magnetization transfer via CCR. Two experiments are necessary for the measurement of the CCRRs, a *cross* and a *reference* experiment. $\Delta = 1/(2J_{CH})$; $\Delta_1 = 1/(8J_{CC})$; $\Delta_2 = 1/(4J_{C\alpha C'})$; $\tau = 1/(2J_{NH})$; $\tau_1 = \tau_2$ and $2\tau_1 + 2\tau_2 = 1/J_{CC}$ in the *cross* experiment while $\tau_1 = 1/(4J_{CC}) + 1/(13.3J_{CH})$ and $\tau_2 = 1/4J_{CC} - 1/(13.3J_{CH})$ and $2\tau_1 + 2\tau_2 = 1/J_{CC}$ in the *reference* experiment; $\tau 3 = 1/(4J_{C\alpha N})$. The shaped pulses on carbon are Q5 of 320 μs (90° pulse) and Q3 of 256 μs (180° pulse). G6 = 4G9; G2+G3 = G4+G5. The pulses for which the phase is not specifically given are along x. ϕ_1 = x, −x; ϕ_2 = 2(x), 2(y), 2(−x), 2(−y); ϕ_3 = 8(x), 8(−x); $\phi_{rec.}$ = x, 2(−x), 2(x), 2(−x), x, −x, 2(x), 2(−x), 2(x), −x. Proton and nitrogen were decoupled with DIPSI2 and GARP sequences of 3550 Hz and 1225 Hz field strength, respectively.

the CH-CH_2 group into account (Carlomagno et al., 2001). For most amino acids containing a $C_\alpha H$-$C_\beta H_2$ moiety, the experimental CCRRs could not be explained assuming a single χ_1 value in absence of internal motions; the data were thus interpreted assuming gaussian axial fluctuations around the C_α-C_β bond, with the parameter σ representing the amplitude of the motion (Brüschweiler and Wright, 1994; Bremi et al., 1997). The fitting procedure described in Carlomagno et al. (2001) was then incorporated in a grid search where the χ_1 angle was varied between −180° and 180° and σ between 0° and 30°. The order parameter for the $\Gamma_{C\beta H\beta 1, C\beta H\beta 2}$ CCR rate was calculated at each step from the correspondent σ value.

Cross and reference spectra were acquired with the pulse program of Figure 1 on a Bruker 600 MHz spectrometer for a 2 mM sample of the $^{13}C/^{15}N$ labelled protein ubiquitin in a H_2O (90%)/D_2O (10%) mixture at pH 6.5. Stripes from the cross and reference experiments are shown in Figure 2. Two or three peaks are observable for each amino acid, at the H_α and H_β frequencies, corresponding to the $4C_{\beta y}C_{\alpha x}H_{\alpha z}$ and $2C_{\alpha z}H_{\alpha z}$ magnetization terms present at point *d*. The peaks of interest are the $H_\beta(\omega 1)$-$N(\omega 2)$-$H_N(\omega 3)$ peaks that represent the $4C_{\beta y}C_{\alpha x}H_{\alpha z}$ term of point *d*.

Results and discussion

The χ_1 angles for 13 of the 18 residues of ubiquitin that have only one H_β proton were calculated from the CCRR values under the assumption of an order parameter $S^2_{\alpha\beta}$ equal to 1 (Table 1, column 2). To verify the validity of the new method we compare the CCRR derived χ_1 angles with the χ_1 angles from the solution structure of ubiquitin calculated from NOE, scalar couplings and dipolar couplings data (Tjandra et al., 1997) (Table 1, column 3). From now on we will refer to this structure as the 'NMR structure' of ubiquitin. The correlation between the two data sets (Figure 3) is very good and, as expected, the χ_1 angles cluster around the three staggered conformations. The maximum deviation of the CCRR derived χ_1 angles from the χ_1 angles of the NMR structure is found for residues 13, 14 and 70, the side-chain of which are exposed to the solvent and therefore more prone to internal motions. Interestingly, the agreement between the CCRR derived χ_1 angles and the one extracted from the NMR structure is excellent for the side-chains of buried residues, as Val17, Val26 and Ile30, indicating that the small discrepancy between the two data sets can be fully attributed to the presence of internal motions, which invalidates the assumption of $S^2_{\alpha\beta} = 1$.

One CH-CH dipolar-dipolar cross-correlated relaxation rate around a C-C bond is not sufficient to derive both the χ_1 and the S^2 values. However, if the conformation around the C-C bond is obtained from coupling constants or NOE data, CCRRs can be used to describe the internal dynamics of the C-C segment. Here we calculated the order parameters $S^2_{\alpha\beta}$ in a best fit procedure of the experimental CCRRs to the χ_1

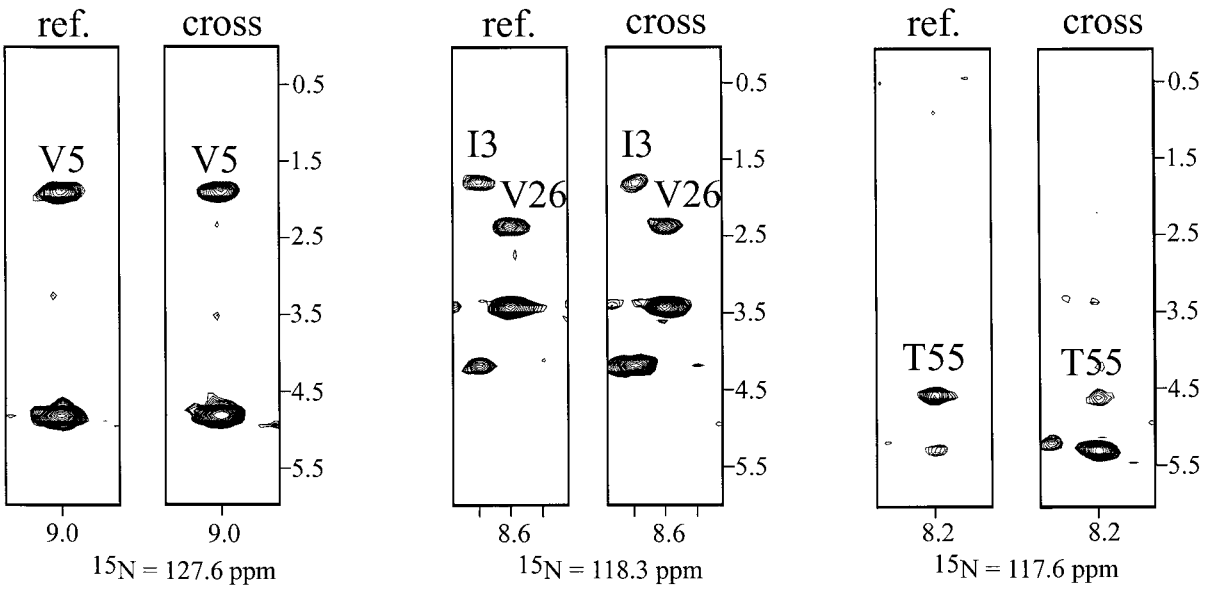

Figure 2. Strips from the *reference* and *cross* spectra acquired with the pulse sequence of Figure 1. The peaks in the H_β region in ω_1 correspond to the term $4C_{\beta y}C_{\alpha x}H_{\alpha z}$ at point *d* and are the peaks of interest; those in the H_α region correspond to the term $2C_{\alpha y}H_{\alpha z}$ at point *d* and can be ignored.

Table 1. Torsion angles χ_1 derived from CCRRs for amino-acids with one H_β proton only

Amino acid #	χ_1 from CCRR assuming a $S^2_{\alpha\beta}$ of 1	χ_1 from the NMR structure	Best fit $S^2_{\alpha\beta}$	$^3J_{NC\gamma}$ (Hz)	$^3J_{C'C\gamma}$ (Hz)
Ile3	55.0 ± 3.0	65.3	1.59	0.43	1.0
Val5	−158.0 ± 2.0	−175.1	0.81	1.70	<0.6
Thr9	62.0 ± 1.0	66.3	0.80	0.66	2.8
Thr12	−33.0 ± 2.0	−58.6	0.72	1.43	<0.4
Ile13	−18.0 ± 1.0	−38.2	0.48	0.53	2.3
Thr14	−23.0 ± 2.0	−58.7	0.51	1.29	0.7
Val17	−58.0 ± 2.0	−62.0	0.83	0.32	3.6
Val26	166.0 ± 5.0	173.9	0.92	1.84	0.8
Ile30	−68.0 ± 10.0	−71.0	1.01	0.31	–
Ile44	−86.0 ± 4.0	−69.2	0.77	0.29	3.1
Thr55	67.0 ± 1.0	65.4	0.86	0.73	–
Ile61	−75.0 ± 6.0	−67.1	0.92	0.36	3.3
Thr66	−40.0 ± 3.0	−58.4	0.83	1.58	–
Val70	136.0 ± 10.0	152.4	0.48	1.12	–

The χ_1 torsion angles for the side-chain of the amino acids of ubiquitin containing a $C_\alpha H_\alpha$-$C_\beta H_\beta$ moiety are reported in column 2 and compared with the corresponding torsion angles extracted from the NMR structure (Tjandra et al., 1997). Column 4 contains the values of the order parameter that reproduce the experimental CCRRs better assuming the χ_1 torsion angles of column 3. Column 5 and 6 contain the values of the $^3J_{NC\gamma}$ and $^3J_{C'C\gamma}$ coupling constants measured in Hu and Bax (1997a) and Hu and Bax (1997b), respectively. For valine and isoleucine we report only the coupling constant with the Cγ that defines the χ_1 angle.

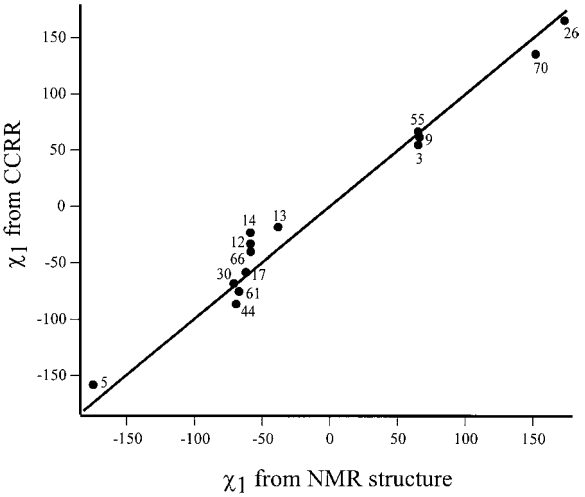

Figure 3. Correlation plot of the χ_1 values extracted from CCRRs for the residues of ubiquitin that contain one H_β proton vs. the χ_1 torsion angles of the NMR structure (Tjandra et al., 1997). As expected, the values cluster around the three staggered conformations.

angles of the NMR structure (Table 1, column 4) according to Equation 1: The $S^2_{\alpha\beta}$ are in the typical range of 0.7–1.0 for most residues. In general, for an order parameter $S^2_{\alpha\beta}$ of 0.8, the error on the χ_1 angle, calculated from CCR data assuming $S^2_{\alpha\beta} = 1$, is less than 10%, the exact value depending on the slope of the function of Equation 1. Only residues 13, 14 and 70 are affected by extensive dynamics ($S^2_{\alpha\beta} \leq 0.5$). Noticeably, the CCR data for Thr14 could be explained assuming conformational averaging between the NMR (gauche$^-$) and the X-Ray (gauche$^+$) (Vijay-Kumar et al., 1987) conformations in a 4:1 ratio. Evidence for conformational averaging in the side-chain of Thr14 is found also in the $^3J_{NC\gamma}$ coupling constant (Table 1, column 5). While for most amino acids the $^3J_{NC\gamma}$ and the $^3J_{C'C\gamma}$ values qualitatively agree with the χ_1 values, the somehow reduced size of the $^3J_{NC\gamma}$ of Thr14 with respect to the typical value for the gauche$^-$ conformation ($^3J_{NC\gamma} \geq 1.7$ Hz) suggests the presence of a second conformation around the C_α-C_β bond.

Table 2. Torsion angles χ_1 derived from CCRRs for amino-acids with two H_β protons

Amino acid #	$\Gamma_{C\alpha H\alpha C\beta H\beta 1}$	$\Gamma_{C\alpha H\alpha C\beta H\beta 2}$	Average χ_1	σ	χ_1 from	$^3J_{NC\gamma}$ (Hz)	$^3J_{C'C\gamma}$ (Hz)
Gln2	−6.4	12.4	*8 ± 1 or −137 ± 2*	*9 ± 4 or 22 ± 2*	−70.4	0.92	2.4
Lys6	5.05	5.95	*no*	*no*	−90.8	1.10	2.0
Pro19	−6.4	12.4	**8 ± 1**	9 ± 4	−19.8	–	2.2
Asp21	23.6	−10.4	**− 78 ± 1**	19 ± 1	−77.4	–	5.5
Asn25	−8.4	20.4	−157 ± 1(X-ray)	22 ± 1	−95.9	–	3.4
Lys27	15.7	−3.6	**− 85 ± 5**	30 ± 1	−66.0	0.29	–
Lys29	35.2	−10.9	**− 63 ± 7**	11 ± 11	−69.6	0.40	4.0
Gln31	−14.4	30.8	**−167 ± 3**	8 ± 8	176.9	–	0.6
Glu34	−5.7	14.1	*−142 ± 3*	*23 ± 1*	−70.1	0.42	2.9
Gln40	25.8	−9.7	**− 71 ± 3**	18 ± 2	−67.4	0.39	3.4
Leu43	28.4	−7.0	**− 58 ± 4**	11 ± 11	−69.0	0.35	3.0
Phe45	−9.7	0.2	**−161 ± 3**	20 ± 2	174.9	–	–
Leu50	25.1	0.2	**− 40 ± 8**	10 ± 10	−52.1	0.37	2.4
Glu51	29.6	−11.8	**− 70 ± 4**	14 ± 4	−69.7	0.47	2.9
Leu56	21.8	−8.5	**− 78 ± 6**	23 ± 2	−60.4	<0.3	2.8
Asp58	27.6	−10.4	**− 69 ± 2**	17 ± 1	−70.3	–	5.6
Tyr59	25.5	−6.8	**− 62 ± 3**	21 ± 1	−68.0	–	3.6
Asn60	2.00	9.20	*no*	*no*	−62.3	–	2.0
Ser65	27.6	−16.9	**− 83 ± 2**	4 ± 4	−71.4	–	–
Leu69	−6.6	17.2	**−151 ± 5**	23 ± 4	−179.1	1.75	0.8
Arg72	2.30	3.90	*no*	*no*	179.6	–	–

Table 3. Torsion angles χ_1 derived from CCRRs for five amino acids with two H_β protons

Amino acid #	$\Gamma_{C\alpha H\alpha, C\beta H\beta_1}$	$\Gamma_{C\alpha H\alpha, C\beta H\beta_2}$	% gauche +	% trans	% gauche −	χ_1 from the NMR structure
Gln 2	−6.4	12.4	12	37	51	−70.4
Lys 6	5.05	5.95	28	35	37	−90.8
Glu 34	−5.7	14.1	32	12	56	−70.1
Asn 60	2.00	9.20	27	29	44	−62.3
Arg 72	2.30	3.90	29	38	33	179.6

The population distribution percentages, obtained by fitting the CCRR values of columns 2 and 3 to a motion model that assumes conformational averaging of the three staggered conformations around the χ_1 angle, are reported in columns 4, 5 and 6 for the amino acid side-chains of ubiquitin that have a $C_\alpha HC_\beta H_2$ moiety and for which the fitting of the CCRR data with the axial gaussian fluctuation model of motion failed. For comparison the corresponding χ_1 values from the NMR structure (Tjandra et al., 1997) are reported in column 7.

The cross-correlated relaxation rates of the amino acids in ubiquitin with two H_β protons are reported in Table 2. The attempt to fit the CCR rates to a static χ_1 torsion angle, assuming no internal motions, mostly failed. Hence the experimental data were fitted to a motional model that assumes gaussian axial fluctuations around the C_α-C_β bond, with the parameter σ representing the amplitude of the motion. In this case, due to the availability of two CCR rates, both the χ_1 and the S^2 values can be derived from the CCRR data. The average χ_1 angle of the gaussian distribution and the amplitude of motion σ that best fit the experimental rates are reported in the fourth and fifth column of Table 2. Generally, two values of χ_1 are possible for each couple of CCR rates if the stereospecific assignment of the H_β protons is not known. Whenever more than one χ_1 satisfies the CCR rates, the torsion angle closer to the one of the previously determined NMR structure is reported in Table 2.

Seventy percent of the CCRR-derived χ_1 angles (reported in bold) agree well with the χ_1 angles of the NMR structure and with the $^3J_{NC\gamma}$ and the $^3J_{C'C\gamma}$ coupling constants (Table 2, column 7 and 8): The amplitude of motions varies from ±4° for Ser65 to ±30° for Lys27 (Table 2, column 5). For Asn25 no $^3J_{NC\gamma}$ coupling-derived constraint is available for the χ_1 angle: the CCR rates restrain this torsion close to the *trans* conformation, which is also found in the X-ray structure. However, a value of the $^3J_{C'C\gamma}$ coupling smaller than 1 Hz would be expected for the *trans* conformation. For Gln2 and Glu34 the average χ_1 angle does not correspond to that found in the NMR structure. Lys6, Asn60 and Arg72 show small, positive CCR rates that are clearly indicative of an extensive conformational averaging that cannot be described with the gaussian axial fluctuation model of motion. It should be noted that no angular structural information from coupling constants was used for either Gln2 and Glu34 or Lys6, Asn60 and Arg72 in the calculation of the NMR structure although $^3J_{NC\gamma}$ and $^3J_{C'C\gamma}$ coupling constants are available for Gln2, Lys6 and Gln34 and Asn60. Moreover, for Gln2 and Lys6 the $^3J_{NC\gamma}$ values are larger than what is expected from the χ_1 angles of the NMR structure.

In order to describe the CCR rates observed for Gln2, Lys6, Glu34, Asn60 and Arg72, we applied an alternative model of motion, that assumes the simultaneous presence in solution of the three staggered conformations with different populations (Table 3). Interestingly, for the solvent exposed side-chains of Lys6, Asn60 and Arg72 the CCR rates are best explained by assuming an almost uniform distribution of populations over the three staggered conformations. The $^3J_{NC\gamma}$ and $^3J_{C'C\gamma}$ values can be reproduced on the basis of the population distribution derived from the CCRR data for Gln2, Glu34 and Lys6.

Cross-correlated relaxation represents an extremely valuable tool to derive both structural and dynamical information in C-C chains. The examples of Gln2, Glu34, Lys6, Asn60 and Arg72, where the torsion angle χ_1 of the previously calculated NMR structure is in disagreement with the CCRR data, stress the importance of accessing an NMR parameter that is sensitive to internal motion, in order to detect the flexibility of the various structural elements in proteins.

Conclusions

We have presented a new method for the measurement of $\Gamma_{C\alpha H\alpha, C\beta H\beta}$ cross-correlated relaxation rates in proteins with optimal resolution. The new experiment can be applied to proteins of both large and small size

and is used to determine the χ_1 torsion angle. For protein of large sizes the values of the delays need to be readjusted to compensate for the increase in auto- and cross-correlated relaxation rates. In particular the delay used for the magnetization transfer via CCR ($2\tau_1 + 2\tau_2 = 28$ ms) needs to be shortened. This will not decrease the efficiency of the CCR mediated magnetization transfer, as the CCR rates increase with the protein size and therefore a shorter delay is needed to obtain an efficient transfer. However, if $2\tau_1 + 2\tau_2$ differs from $1/J_{CC}$, the C_β have to be decoupled from the C_γ to conserve the full intensity of the signal.

The method was validated by measuring the $\Gamma_{C\alpha H\alpha, C\beta H\beta}$ CCRRs and the corresponding χ_1 torsion angle for the protein ubiquitin. The χ_1 values obtained from the CCRRs optimally reproduce the χ_1 values of a previously determined NMR structure for all residues where these torsion angles were restrained by coupling constants data in the structure calculation. For most of the residues for which no coupling constants data are available, the CCRR data are indicative of a high flexibility of the side-chains, although in the NMR structure only one conformation is found. On the basis of these results it can be concluded that CCRRs constitute a valuable tool to obtain both structural parameters and information on the internal dynamics of protein side-chains.

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References

Bartik, K. and Redfield, C. (1993) *J. Biomol. NMR*, **3**, 415–428.
Boisbouvier, J., Brutscher, B., Pardi, A., Marion, D. and Simorre, J.-P. (2000) *J. Am. Chem. Soc.*, **122**, 6779–6780.
Bremi, T., Bruschweiler, R. and Ernst, R.R. (1997) *J. Am. Chem. Soc.*, **119**, 4272–4284.
Brüschweiler, R. and Wright, P.E. (1994) *J. Am. Chem. Soc.*, **116**, 8426–8427.
Carlomagno, T., Blommers, M.J.J., Meiler, J., Cuenoud, B. and Griesinger, C. (2001) *J. Am. Chem. Soc.*, **123**, 7364–7370.
Carlomagno, T., Felli, I.C., Czech, M., Fischer, R., Sprinzl, M. and Griesinger, C. (1999) *J. Am. Chem. Soc.*, **121**, 1945–1948.
Chiarparin, E., Pelupessy, P., Ghose, R. and Bodenhausen, G. (2000) *J. Am. Chem. Soc.*, **122**, 1758–1771.
Felli, I.C., Richter, C., Griesinger, C. and Schwalbe, H. (1999) *J. Am. Chem. Soc.*, **121**, 1956–1957.
Griesinger, C. and Eggenberger, U. (1992) *J. Magn. Reson.*, **97**, 426–434.
Grzesiek, S. and Bax, A. (1993) *J. Biomol. NMR*, **3**, 185–204.
Eggenberger, U., Karim-Nejad, Y., Thüring, H., Rüterjans, H. and Griesinger, C. (1992) *J. Biomol. NMR*, **2**, 583–590.
Hu, J.-S. and Bax, A. (1997a) *J. Biomol. NMR*, **9**, 323–328.
Hu, J.-S. and Bax, A. (1997b) *J. Am. Chem. Soc.*, **119**, 6360–6368.
Konrat, R., Muhandiram, D.R., Farrow, N.A. and Kay, L.E. (1997) *J. Biomol. NMR*, **9**, 409–422.
Reif, B., Hennig, M. and Griesinger, C. (1997) *Science*, **276**, 1230–1233.
Tjandra, N., Omichinski, J.G., Gronenborn, A.M., Clore, G.M. and Bax, A. (1997) *Nat. Struct. Biol.*, **4**, 732–738.
Yang, D. and Kay, L.E. (1998) *J. Am. Chem. Soc.*, **120**, 9880–9887.
Yang, D., Konrat, R. and Kay, L.E. (1997) *J. Am. Chem. Soc.*, **119**, 11938–11940.
Vijay-Kumar, S., Bugg, C.E. and Cook, W.J. (1987) *J. Mol. Biol.*, **194**, 531–544.
Vuister, G.W., Wang, A.C. and Bax, A. (1993) *J. Am. Chem. Soc.*, **115**, 5334–5335.