Differential multiple quantum relaxation caused by chemical exchange outside the fast exchange limit

Chunyu Wang & Arthur G. Palmer, III

Department of Biochemistry and Molecular Biophysics, Columbia University, 630 W. 168th Street, New York, NY 10032, U.S.A.

Abstract

Differential relaxation of multiple quantum coherences is a signature for chemical exchange processes in proteins. Previous analyses of experimental data have used theoretical descriptions applicable only in the limit of fast exchange. Theoretical expressions for differential relaxation rate constants that are accurate outside fast exchange are presented for two-spin-system subject to two-site chemical exchange. The theoretical expressions are validated using experimental results for ^{15}N-^1H relaxation in basic pancreatic trypsin inhibitor. The new theoretical expression is valuable for identification and characterization of exchange processes in proteins using differential relaxation of multiple quantum coherences.

Recently, the relaxation behavior of multiple quantum (MQ) coherences subject to chemical exchange processes has attracted renewed interest. Differential relaxation of zero and double quantum coherences, e.g., in backbone amide HN (Kloiber and Konrat, 2000) or $C^\alpha C^\beta$ spin systems (Fruh et al., 2001), can be a signature of chemical exchange in proteins, although dipole-dipole and chemical shift anisotropy (CSA)–CSA cross correlations also contribute to differential relaxation (Tessari and Vuister, 2000). In addition, the differential relaxation rate between multiple quantum operators of different coherence orders can be used to compensate for the line-broadening effect of chemical exchange (Pervushin, 2001; Rance, 1988).

Differential relaxation of multiple quantum coherences arises because isotropic multiple quantum chemical shifts are affected differently by chemical exchange processes. For illustration, a pair of covalently bonded backbone amide nitrogen and proton spins subject to the same chemical or conformational exchange process in a protein will be considered. The Larmor frequency differences between the two exchanging sites for nitrogen and proton spins are $\Delta\omega_N$ and $\Delta\omega_H$, respectively. For zero quantum (ZQ) coherence (H^+N^- and H^-N^+) the chemical shift modulation due to exchange is therefore:

$$|\Delta\omega_{ZQ}| = |\Delta\omega_H - \Delta\omega_N|; \tag{1}$$

while for double quantum (DQ) coherence (H^+N^+ and H^-N^-):

$$|\Delta\omega_{DQ}| = |\Delta\omega_H + \Delta\omega_N|. \tag{2}$$

Existing expressions for chemical exchange broadening of single quantum (SQ) coherences are appropriate for MQ coherences simply by replacing the SQ $\Delta\omega$ with the appropriate MQ $\Delta\omega$. However, Equations 1 and 2 show that if $\Delta\omega_H$ and $\Delta\omega_N$ have the same (opposite) sign, then exchange is slower on chemical shift time scale for DQ (ZQ) than for ZQ (DQ) coherences. In the fast exchange limit, the SQ exchange contribution to transverse relaxation R_{ex} is well known:

$$R_{ex} = p_1 p_2 \Delta\omega^2 \tau_{ex}, \tag{3}$$

where p_1 and p_2 are the fractional populations of the two sites ($p_1 \geq p_2$), $\tau_{ex} = 1/k_{ex} = 1/(k_1 + k_2)$ is the correlation time of the exchange process, k_1 and

*To whom correspondence should be addressed. E-mail: agp6@columbia.edu

k_2 are the forward and reverse rate constants for the exchange process, and $\Delta\omega$ is the chemical shift modulation due to exchange. Substituting Equations 1 and 2 into Equation 3 yields:

$$
\begin{aligned}
R_{ex(DQ)} &= p_1 p_2 \Delta\omega^2_{DQ}\tau_{ex} \\
&= p_1 p_2 (\Delta\omega_H + \Delta\omega_N)^2 \tau_{ex}
\end{aligned} \tag{4}
$$

$$
\begin{aligned}
R_{ex(ZQ)} &= p_1 p_2 \Delta\omega^2_{ZQ}\tau_{ex} \\
&= p_1 p_2 (\Delta\omega_H - \Delta\omega_N)^2 \tau_{ex},
\end{aligned} \tag{5}
$$

under the assumption that both the ZQ and the DQ coherences satisfy the requirements for fast exchange. The chemical exchange contribution to differential relaxation rate of double and zero quantum coherences in the fast exchange limit is the difference between Equations 4 and 5:

$$
\begin{aligned}
\Delta R^{MQ}(\text{exchange}) &= R_{ex(DQ)} - R_{ex(ZQ)} \\
&= 4p_1 p_2 \Delta\omega_H \Delta\omega_N \tau_{ex}.
\end{aligned} \tag{6}
$$

This is equivalent to expressions presented by Kloiber and Konrat (2000) and Pervushin (2001). When $\Delta\omega_H = \pm\Delta\omega_N$, depending on the relative sign of $\Delta\omega_H$ and $\Delta\omega_N$, either $R_{ex(ZQ)}$ or $R_{ex(DQ)}$ will vanish in the fast exchange limit, which is the basis for suppression of conformational exchange line broadening of MQ coherences (Pervushin, 2001; Rance, 1988). In addition, when $\Delta\omega_H$ and $\Delta\omega_N$ are of similar magnitude, comparing Equations 3 and 6 shows that the exchange contribution to ΔR^{MQ} is four times greater than R_{ex} for single quantum coherences. Although faster relaxation of MQ coherences makes measurement of ΔR^{MQ} more challenging, in the fast exchange limit, ΔR^{MQ} can be more sensitive to exchange than the transverse relaxation rate of SQ coherences.

Equation 6 does not hold outside the fast exchange limit. For example, if exchange is slow ($\Delta\omega \gg k_{ex}$) and the site populations are asymmetric ($p_1 \gg p_2$), only spins in site 1 contribute to the observed signal (Ishima and Torchia, 1999). In this case, the relaxation rate constant is the sum of intrinsic transverse relaxation rate, due to dipolar and CSA interactions, and k_1, which is not sensitive to chemical shift modulation (Woessner, 1961). Therefore, the exchange contributions to $R_{ex(DQ)}$ and $R_{ex(ZQ)}$ will be identical and ΔR^{MQ} (exchange) $= 0$. Thus, even when no appreciable differential relaxation of multiple quantum coherences is observed, significant chemical exchange processes can still exist and contribute to linebroadening of individual SQ and MQ coherences.

ΔR^{MQ} is measured by observing the time evolution of the spin echo amplitude of multiple quantum coherences (Kloiber and Konrat, 2000). The spin echo signal amplitude E(t) in the presence of exchange is described by Woessner (1961) and in general is the sum of four terms. However, in a large subset of conditions, e.g. when k_{ex} is large compared with spin echo delay or when the site populations are skewed ($p_1 \gg p_2$), only one exponential decay term yields observable signal. This also is a necessary condition for measuring ΔR^{MQ} with the elegant spin echo experiments proposed by Kloiber and Konrat (2000), because each multiple quantum operator is assumed to relax monoexponentially during the spin echo period. Under such conditions, the 180° pulses applied to ^{1}H and ^{15}N spins in the middle of the spin echo period do not affect exchange contributions to relaxation. Assuming identical intrinsic transverse relaxation rate constants, R_2, for the two exchanging sites, the relaxation rate of a transverse operator affected by chemical exchange is simply the free precession rate given by Woessner (1961):

$$
\begin{aligned}
R(\Delta\omega) = R_2 + \frac{k_{ex}}{2} - \frac{1}{\sqrt{8}}\Big\{ k^2_{ex} - \Delta\omega^2 + \\
\big[(k^2_{ex} + \Delta\omega^2)^2 - 16\Delta\omega^2 k^2_{ex} p_1 p_2\big]^{\frac{1}{2}} \Big\}^{\frac{1}{2}}.
\end{aligned} \tag{7}
$$

The exchange contribution to differential relaxation rate for double and zero quantum coherence is then:

$$
\begin{aligned}
\Delta R^{MQ} \,(exchange) = \frac{1}{\sqrt{8}}\Big\{ k^2_{ex} - \Delta\omega^2_{ZQ} + \\
\big[(k^2_{ex} + \Delta\omega^2_{ZQ})^2 - 16\Delta\omega^2_{ZQ} k^2_{ex} p_1 p_2\big]^{\frac{1}{2}} \Big\}^{\frac{1}{2}} \\
- \frac{1}{\sqrt{8}}\Big\{ k^2_{ex} - \Delta\omega^2_{DQ} + \\
\big[(k^2_{ex} + \Delta\omega^2_{DQ})^2 - 16\Delta\omega^2_{DQ} k^2_{ex} p_1 p_2\big]^{\frac{1}{2}} \Big\}^{\frac{1}{2}}.
\end{aligned} \tag{8}
$$

The difference in the intrinsic transverse relaxation rate for double and zero quantum coherence also contributes to ΔR^{MQ}, which originates from cross correlation of the two CSA interactions and from dipole-dipole cross correlation to remote spins. These effects have been discussed elsewhere (Kloiber and Konrat, 2000; Tessari and Vuister, 2000).

When the site populations are very skewed, that is, $p_1 \gg p_2$, the product $p_1 p_2$ approaches zero, and Equation 7 can be expanded to the first order with respect
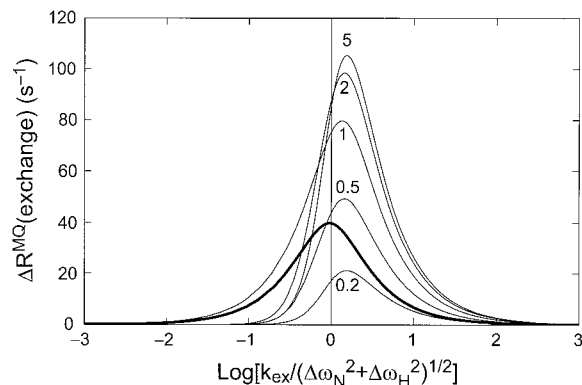
Figure 1. Theoretical results for ΔR^{MQ} (exchange). Shown is the dependence of ΔR^{MQ} (exchange) upon exchange time scale, k_{ex}, and the proton and nitrogen chemical shift modulations, $\Delta\omega_H$ and $\Delta\omega_N$, respectively. The family of thin curves correspond to ΔR^{MQ} (exchange) calculated from Equation 8 with $\Delta\omega_N = 2$ ppm at 600 MHz, $p_1 = 0.9$ and $\Delta\omega_H/\Delta\omega_N$ ratios equal to 0.2, 0.5, 1, 2, 5. The curves are labeled with the ratio near each maximum. The plotted results would be multiplied by -1 if $\Delta\omega_H$ and $\Delta\omega_N$ had opposite signs. The bold curve shows the exchange contribution to SQ ^{15}N relaxation, $R_{ex} = R(\Delta\omega) - R_2$, evaluated from Equation 7, plotted versus $\log(k_{ex}/\Delta\omega_N)$.

to $p_1 p_2$ to yield:

$$R(\Delta\omega) = R_2 + \frac{\Delta\omega^2 k_{ex} p_1 p_2}{k_{ex}^2 + \Delta\omega^2}. \qquad (9)$$

In this limit, the exchange contribution to differential relaxation rate for double and zero quantum coherence is given by:

$$
\begin{aligned}
&\Delta R^{MQ} \text{ (exchange)} \\
&= p_1 p_2 k_{ex}\left(\frac{\Delta\omega_{DQ}^2}{k_{ex}^2 + \Delta\omega_{DQ}^2} - \frac{\Delta\omega_{ZQ}^2}{k_{ex}^2 + \Delta\omega_{ZQ}^2}\right) \qquad (10) \\
&= \frac{4 p_1 p_2 \Delta\omega_N \Delta\omega_H k_{ex}^3}{(k_{ex}^2 + \Delta\omega_N^2 + \Delta\omega_H^2)^2 - 4\Delta\omega_N^2\Delta\omega_H^2},
\end{aligned}
$$

which is accurate even if k_{ex} is comparable to the spin echo delay.

Figure 1 shows the theoretical dependence of ΔR^{MQ} (exchange) upon the exchange time scale and the relative sizes of $\Delta\omega_H$ and $\Delta\omega_N$. In performing these calculations, $\Delta\omega_N$ was kept constant at 2 ppm (at a proton Larmor frequency of 600 MHz), while $\Delta\omega_H$ was varied from $0.2\Delta\omega_N$ to $5\Delta\omega_N$. When $k_{ex}/(\Delta\omega_H^2 + \Delta\omega_N^2)^{1/2} \gg 1$ (fast exchange), ΔR^{MQ} (exchange) increases with increasing $\Delta\omega_H$, as predicted by Equation 6. However, when $k_{ex}/(\Delta\omega_H^2 + \Delta\omega_N^2)^{1/2} < 0.3$, corresponding to $\log[k_{ex}/(\Delta\omega_H^2 + \Delta\omega_N^2)^{1/2}] < -0.5$ in Figure 1 (slow to intermediate exchange),

ΔR^{MQ} (exchange) is sensitive to the relative sizes of $\Delta\omega_N$ and $\Delta\omega_H$: ΔR^{MQ} (exchange) is large when $\Delta\omega_H \approx \Delta\omega_N$, and small when $\Delta\omega_H <> \Delta\omega_N$. For comparison, the R_{ex} contribution to ^{15}N single quantum is plotted as a bold curve in Figure 1 as well. As the exchange time scale approaches the intermediate to slow regime, ΔR^{MQ} (exchange) becomes less than R_{ex} unless $\Delta\omega_H \approx \Delta\omega_N$. Most importantly, when ΔR^{MQ} is close to zero, significant chemical exchange can still exist if the exchange time scale is not fast and $\Delta\omega_N$ and $\Delta\omega_H$ different by more than a factor of two. Thus, while ΔR^{MQ} (exchange) can be a powerful tool for detecting and characterizing chemical exchange, in some time regimes, SQ experiments will be preferable.

The above theoretical results were tested in basic pancreatic trypsin inhibitor (BPTI). All NMR experiments were performed on a 2.6 mM [U-98% ^{15}N] sample (Huang et al., 1997) at pH 5.1, 300 K using Bruker DRX600 and DRX500 spectrometers equipped with triple-resonance probes with three-axis gradients. Backbone assignments of BPTI were obtained from Glushka et al. (1989). The pulse sequence developed by Kloiber and Konrat (2000) was used to measure differential relaxation rate of zero and double quantum coherences in BPTI backbone amide HN moieties. Two-dimensional (F1 $\times$ F2) spectra were acquired using (64 $\times$ 2048) complex points and spectral widths of (2500 $\times$ 10 000) Hz at both 600 MHz and 500 MHz. The recycle delay was 1.5 s and the spin echo delays (T_c) for the multiple quantum coherence relaxation period were 10, 20, 30, 40, 50 ms. A 2 ms RE-BURP (Geen and Freeman, 1991) pulse centered on amide protons with a bandwidth of 4 ppm was used to refocus amide proton chemical shifts during T_c. The number of scans for each time point is adjusted according to the sensitivity of the particular experiment, with 8 scans for all $2H_x N_x$ experiments and 256, 128, 64, 64, 64 scans for $2H_y N_y$ experiments with mixing times of 10, 20, 30, 40, 50 ms, respectively. Spectra were processed with nmrPipe (Delaglio et al. 1995). Both t_1 and t_2 dimension interferograms were apodized with a cosine-bell window function, zero filled by a factor of two and Fourier transformed. Peak volumes were obtained using nlinLS (Delaglio et al. 1995). The differential relaxation rates ΔR^{MQ} between double and zero quantum coherences were obtained by fitting the ratio of the $2N_y H_y$ signal intensity to the $2N_x H_x$ signal intensity using the function $\tanh(\Delta R^{MQ} T_c/2)$ (Kloiber and Konrat, 2000).

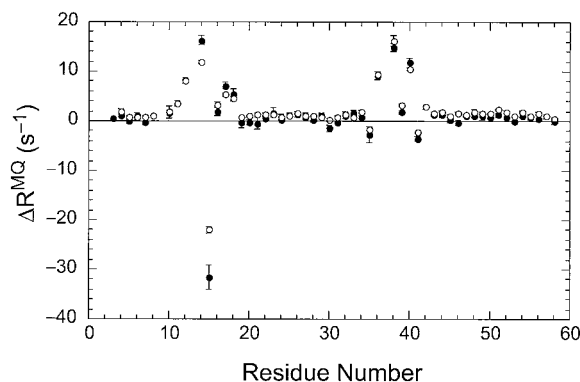Figure 2 shows ΔR^{MQ} measured at 500 and 600 MHz versus the BPTI sequence. A chemical ex-

Figure 2. ΔR^{MQ} versus BPTI sequence at 600 MHz (filled circles) and 500 MHz (open circles). The residues with significant ΔR^{MQ} are clustered around the location of disulfide bond between C14 and C38. ΔR^{MQ} for R39 is small even though SQ coherences are significantly broadened by disulfide isomerization (see text). The negative value of ΔR^{MQ} for K15 indicates that $\Delta \omega_H$ and $\Delta \omega_N$ have opposite signs.

change process in BPTI results from isomerization of the C14 to C38 disulfide bond (Otting et al., 1993). Large values of ΔR^{MQ} are observed for residues near C14 and C38, as expected from the location of disulfide bond, while ΔR^{MQ} values of the remaining residues are close to zero (reflecting small contributions from dipole-dipole and CSA-CSA cross correlation). Interestingly, although R39 has a large ^{15}N SQ $R_{ex} = 13.2 \pm 0.1$ s^{-1} at 600 MHz (Millet et al., 2000), the ΔR^{MQ} of R39 is only 1.8 ± 0.4 s^{-1}, comparable to the average $\Delta R^{MQ} = 0.7 \pm 1$ s^{-1} for residues unaffected by exchange process. This example clearly demonstrates that significant chemical exchange processes can exist even when measured ΔR^{MQ} is small.

Theoretical values of ΔR^{MQ} (exchange) were calculated for the disulfide isomerization using Equations 6, 8 and 10. Exchange parameters were taken from Millet et al. (2000) as the average of the exchange parameters for C38 and R39: $k_{ex} = 440 \pm 60$ s^{-1}, $p_1 = 0.959 \pm 0.003$, $p_2 = 0.041 \pm 0.003$ (Millet et al., 2000). Chemical shift changes due to isomerization were measured from the 2D exchange spectrum obtained by Otting et al. (1993). Uncertainties in calculated values of ΔR^{MQ} (exchange) were generated by Monte Carlo simulations with 1000 random trials, assuming normal distributions of errors associated with the exchange parameters. For many residues, the Larmor frequency differences due to disulfide isomerization are similar to the magnitude
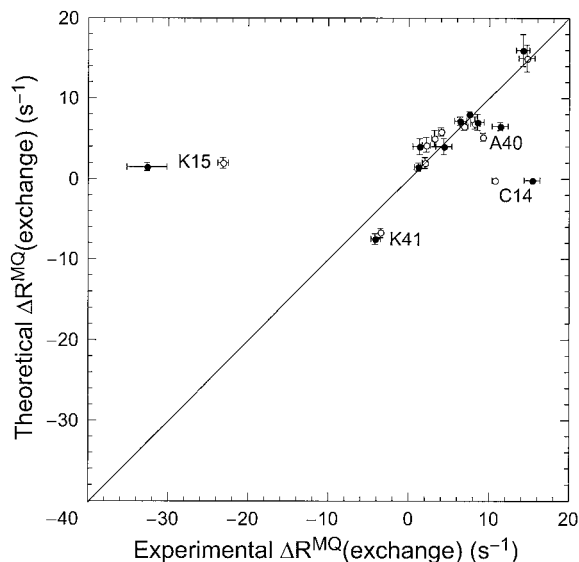

Figure 3. Comparison between the calculated and experimental ΔR^{MQ} (exchange) in BPTI at 600 MHz (filled circles) and 500 MHz (open circles). The experimental ΔR^{MQ} (exchange) was calculated by subtracting the average of ΔR^{MQ} for residues unaffected by exchange processes from ΔR^{MQ} for residues subject to exchange. Theoretical values of ΔR^{MQ} (exchange) were calculated by Equation 8. The discrepancy between measured and calculated values suggests the presence of additional exchange processes for C14, K15, A40 and K41 (see text). Data points for these residues are labeled. The line was drawn with slope of unity and an intercept of zero to guide the eye.

of k_{ex}. Therefore, BPTI provides an ideal system for studying ΔR^{MQ} outside the fast exchange limit.

Theoretical and experimental results are compared in Table 1 and Figure 3. When the experimental results were interpreted using Equation 6, assuming the fast exchange limit, marked discrepancies were found (Table 1, compare columns 2 and 5). In many cases, the measured and the calculated values differ by an order of magnitude. In contrast, general agreement was found between experimental ΔR^{MQ} and theoretical results calculated using Equation 8 (Figure 3 and Table 1, compare columns 2 and 3). The measured and predicted ΔR^{MQ} value shows very good agreement for residues G12, A16, R17, I18, G36, C38 and R39. For the disulfide isomerization process in BPTI, $p_1p_2 = 0.039$, consequently, the skewed population approximation of Equation 10 also is quite accurate (Table 1, compare columns 3 and 4). The small value of ΔR^{MQ} of R39 observed experimentally is predicted accurately by Equation 8 and 10 (see Table 1). For R39, $\Delta \omega_N = 1400$ s^{-1} and $\Delta \omega_H = 380$ s^{-1}; thus exchange is slow on the ^{15}N chemical shift time scale, intermediate on the ^1H chemical shift

Table 1. Comparison of measured and theoretical ΔR^{MQ} at 500 MHz[a]

Residues	ΔR^{MQ} (exchange) (s^{-1})			
	Experimental	Theoretical		
		Equation 8	Skewed populations, Equation 10	Fast exchange, Equation 6
G12	6.8 ± 0.5	6.4 ± 0.4	6.0 ± 0.4	12
C14	10.6 ± 0.3	-0.1 ± 0.1	-0.3 ± 0.1	-147
K15	-23.2 ± 0.7	2.0 ± 0.6	1.4 ± 0.6	64
A16	2.1 ± 0.5	4.2 ± 0.9	3.3 ± 0.8	49
R17	4 ± 0.4	5.8 ± 0.5	5.3 ± 0.4	17
I18	3.2 ± 0.3	5 ± 1	3.9 ± 0.8	43
G36	8.2 ± 0.6	6.9 ± 0.8	6.1 ± 0.7	35
C38	15 ± 1	15.6 ± 1.6	14 ± 1.4	175
R39	2.0 ± 0.3	2.0 ± 0.7	1.3 ± 0.7	201
A40	9.2 ± 0.2	5.1 ± 0.5	4.6 ± 0.4	23
K41	-3.5 ± 0.1	-6.7 ± 0.6	-7.3 ± 0.5	-17

[a]The experimental exchange contribution was calculated by subtracting the average of ΔR^{MQ} for residues unaffected by exchange processes from ΔR^{MQ} for residues subject to exchange. Theoretical values of ΔR^{MQ} (exchange) were calculated by Equation 8.

time scale, and the two shift changes are quite different ($\Delta\omega_H/\Delta\omega_N = 0.27$). As illustrated by Figure 1, small exchange contributions to ΔR^{MQ} are predicted for such parameters.

The discrepancies between predicted and measured values of ΔR^{MQ} for C14, K15, A40 and K41 indicates the existence of additional exchange processes, other than the disulfide isomerization, that affect these residues. Complete ^{15}N CPMG dispersion curves for these residues are also inconsistent with those expected for disulfide bond isomerization (data not shown). C14 and K15 are affected by an exchange process with a rate constant 10 fold faster than the disulfide isomerization kinetics. The origins of the exchange processes experienced by these residues are being investigated further. Thus, using the theoretical expressions presented above to interpret experimental measurements of ΔR^{MQ} can be helpful in identifying exchange processes not easily detected by other techniques.

In similar fashion as for SQ coherences (Millet et al., 2000; Wang et al., 2001), information regarding the time scale of the exchange process can be obtained directly if ΔR^{MQ} is measured at two or more field strengths. In the fast exchange limit, ΔR^{MQ} (exchange) scales quadratically with B_0, according to Equation 6. Thus, for C14 and K15, two residues in fast exchange due to a process other than disulfide isomerization, the ratios of ΔR^{MQ} (exchange) at 600 MHz to that of 500 MHz are both 1.4 ± 0.1, in

good agreement with the ratio of the square of the magnetic fields, $(14.1 \text{ T}/11.7 \text{ T})^2 = 1.44$. In slow exchange, ΔR^{MQ}(exchange) is independent of B_0 if $\Delta\omega_N \approx \Delta\omega_H$. Thus, for C38, $\Delta\omega_N = 630 \text{ s}^{-1}$ and $\Delta\omega_H = 650 \text{ s}^{-1}$, and the ratio of ΔR^{MQ} (exchange) at 600 MHz to that of 500 MHz is 1.0 ± 0.1. This result agrees well with theoretical calculations yielding a ratio of 1.1. Both of these theoretical limits for the static magnetic field dependence of ΔR^{MQ} (exchange) are identical to the limits obtained for SQ coherences. In contrast, ΔR^{MQ} (exchange) scales as $1/B_0^2$ when exchange is slow and $\Delta\omega_N <> \Delta\omega_H$, as can be deduced from Equation 10. This surprising result suggests that ΔR^{MQ} (exchange) can be observed to decrease with increasing values of B_0. For R39, $\Delta\omega_N = 1400 \text{ s}^{-1}$ and $\Delta\omega_H = 380 \text{ s}^{-1}$, and the ratio of ΔR^{MQ} (exchange) at 600 MHz to that of 500 MHz is 0.6 ± 0.1. This result, which is less than unity, is in reasonable agreement with theoretical calculations yielding a ratio of 0.75. Therefore, measuring ΔR^{MQ} at two field strengths is a simple way for confirming that observed exchange broadening results from a fast-limit process.

In conclusion, simple theoretical expressions for the exchange contribution to differential relaxation of zero and double quantum coherences, ΔR^{MQ}, have been presented and validated using BPTI. The expressions are accurate for all exchange time scales provided that the exchange process is fast compared to the spin echo delay used in the experimental measurement of ΔR^{MQ}, or provided that the populations of

exchanging sites are highly asymmetric. These more general theoretical expressions facilitate use of experimental measurements of differential relaxation of multiple quantum coherences to identify and characterize exchange processes in proteins. Although only zero and double quantum relaxation of backbone amide HN moieties in proteins have been discussed here, the same approach is applicable generally to other spin systems and other multiple quantum coherences.

Acknowledgements

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