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Estimating the time scale of chemical exchange of proteins from measurements of transverse relaxation rates in solution

Rieko Ishima & Dennis A. Torchia*
Molecular Structural Biology Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, U.S.A.

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Abstract

Chemical (conformational) exchange on the ms-μs time scale is reliably identified by the observation of transverse relaxation rates, R_{ex}, that depend upon the strength of the effective field ($\omega_{1eff} = \gamma B_{1eff}$) used in spin lock or CPMG experiments. In order to determine if the exchange correlation time, τ_{ex}, is the fast or slow limit, measurements of (i) signal line shape and (ii) temperature dependence of R_{ex} have been commonly used in studies of stable, small molecules. However, these approaches are often not applicable to proteins, because sample stability and solubility, respectively, limit the temperature range and signal sensitivity of experiments. Herein we use a complex, but general, two-site exchange equation to show when the simple fast exchange equations for R_{ex} are good approximations, in the case of proteins. We then present a simple empirical equation that approximately predicts R_{ex} in all exchange regimes, and explains these results in a clear, straightforward manner. Finally we show how one can reliably determine whether τ_{ex} is in the fast or slow exchange limit.

Local conformational changes in proteins on the ms-μs time scale often contribute to the rate of transverse spin relaxation, R_2, via the chemical exchange mechanism. In the case of two-site exchange, the correlation time, τ_{ex}, and relative populations of the exchanging species, p_1 and p_2, are the physical parameters of interest that characterize the exchange process. In the case of highly stable, small molecules, these parameters have been determined by analysis of line shapes recorded over a wide range of temperature (Johnson, 1965; Farrar and Becker, 1971). Alternatively, they have been derived from relaxation rates, measured as a function of the effective field strength, $\omega_{1eff} = \gamma B_{1eff}$, using Carr–Purcell–Meiboom–Gill (CPMG) (Luz and Meiboom, 1963) and/or spin-lock (Douglass and Jones, 1966) pulse sequences. When exchange between sites is in the fast limit, $(\delta\omega\tau_{ex})^2 \ll 1$ ($\delta\omega$ is the chemical shift difference between the two sites), the exchange contribution to the transverse relaxation rate is given by

$$R_{ex} = (\delta\omega)^2 p_1 p_2 \tau_{ex}/(1 + \omega_{1eff}^2 \tau_{ex}^2) \quad \text{spin-lock} \quad (1)$$

$$R_{ex} = (\delta\omega)^2 p_1 p_2 \tau_{ex}\{1 - (\omega_{1eff}\tau_{ex}/\sqrt{3}) \tanh(\omega_{1eff}\tau_{ex}/\sqrt{3})\} \quad \text{CPMG} \quad (2)$$

In spite of the different appearances of the right sides of Equations 1 and 2, numerical simulations show that R_{ex} has virtually the same dependence on ω_{1eff} and τ_{ex} for both spin-lock and CPMG experiments (Szyperski et al., 1993; Mulder, 1999). Hence it is convenient and useful to think of both experiments as being carried out in effective rotating frame fields; (i) $\omega_{1eff} = \gamma\sqrt{(B_1^2 + B_{off}^2)}$, where B_{off} is the off-resonance field in the spin-lock case, and (ii) $\omega_{1eff} = \sqrt{3}/\tau_{CPMG}$, where τ_{CPMG} is one-half of the duration between π pulses in the CPMG case. Note that the correspondence $\omega_{1eff} = \sqrt{3}/\tau_{CPMG}$ insures that Equations 1 and 2 have the same form when $(\tau_{ex}/\tau_{CPMG})^2 \gg 1$, i.e., $R_{ex} = (\delta\omega/\omega_{1eff})^2 p_1 p_2/\tau_{ex}$. A generalization of

*To whom correspondence should be addressed. E-mail: dtorchia@dir.nidcr.nih.gov.

Equation 2, that applies to multi-site exchange in the fast exchange limit, has been presented (Allerhand and Thiele, 1966).

Equations 1 and 2 relate R_{ex} to the parameters of interest in a clear, simple manner. It is therefore desirable to determine if one is in the fast exchange limit where these equations are applicable. In the case of small, stable molecules one typically employs a wide temperature range to study exchange in both the slow and fast limits. Because the signal-to-noise ratio is large, detailed line shape analysis is well suited for studies in slow or intermediate exchange, while CPMG and spin-lock experiments are better suited for investigations in the fast exchange limit (Farrar and Becker, 1971). In the case of proteins, solubility is limited and the accessible temperature range is typically restricted to 10–35 °C. The actual lower and upper limits are determined by slow tumbling and sample stability, respectively. Furthermore, changing the temperature of a protein sample can significantly alter site populations and chemical shifts in unpredictable ways.

These circumstances make it difficult to ascertain if the fast exchange condition is satisfied. Observation of one NMR signal per exchanging spin does not necessarily justify the assumption of fast exchange. In proteins, site populations will generally be quite different because conformational substates have different free energies. Note that if the free energy of site 1 excedes that of site 2 by only 1 kcal M^{-1}, $p_1/p_2 < 0.15$. (Herein, for consistency and without loss of generality, $p_1/p_2 \leq 1$ is assumed throughout.) In the case of either slow or intermediate exchange, it will typically not be possible to observe the signal from site 1 because its signal intensity may be as much as $(p_1/p_2)^2$ times smaller than that of site 2. The relative intensity of site 1 is diminished both by its smaller population and by its larger line width.

In the event that the fast exchange limit is not satisfied, a closed form expression for R_{ex}, Equation 3 (Bloom et al., 1965; Carver and Richards, 1972; Davis et al., 1994), that applies for all values of $\delta\omega\tau_{ex}$ in the case of the CPMG experiment may be required to analyze relaxation data:

$$R_{ex} = 0.5/\tau_{ex} - (0.25/\tau_{CPMG}) \operatorname{acosh}(D_+ \cosh\xi_+ - D_- \cos\xi_-) \quad (3)$$

where

$$D_\pm = 0.5[\pm 1 + (\Psi + 2(\delta\omega)^2/(\Psi^2 + \zeta^2)^{1/2}]$$

$$\xi_\pm = (\tau_{CPMG}\sqrt{2})[\pm\Psi + (\Psi^2 + \zeta^2)^{1/2}]^{1/2}$$

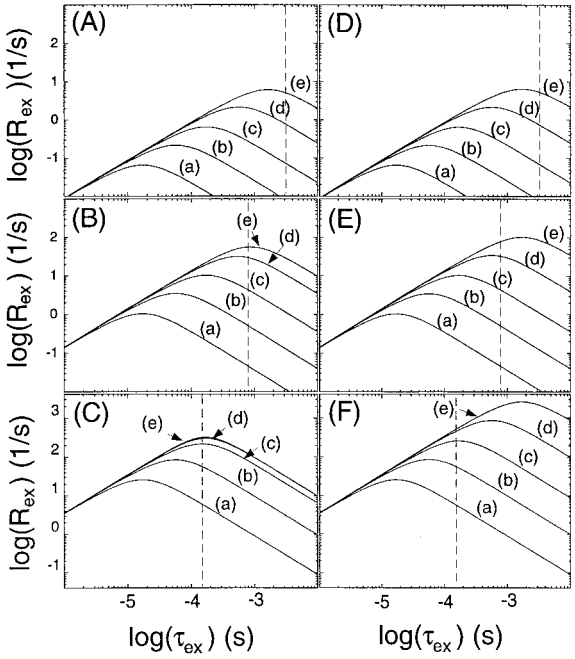

Figure 1. Comparison of plots of R_{ex} (CPMG) vs. τ_{ex}, obtained using the general equation (Equation 3) and fast limit equation (Equation 2) to calculate curves in panels A, B, C and D, E, F respectively. Comparisons are shown for three values of $\delta\omega/2\pi$; A and D: 50 Hz, B and E: 200 Hz and C and F: 1000 Hz. The five curves in each panel were calculated for $\omega_{1eff}/2\pi$ equal to (a) 10 kHz, (b) 3 kHz, (c) 1 kHz, (d) 0.3 kHz, and (e) 0.1 kHz, with $p_1/p_2 = 1/9$. The dashed vertical line in each panel is drawn at $\tau_{ex} = 1/\delta\omega$. In panels A, B, and C, R_{ex} attains its maximum value when $\omega_a\tau_{ex} =$ ca. 1, see Equation 4.

$$\Psi = \tau_{ex}^{-2} - (\delta\omega)^2; \zeta = 2\delta\omega(p_1 - p_2)\tau_{ex}^{-1}$$

In contrast with Equations 1 and 2, the relationship between R_{ex} and the parameters of interest is difficult to ascertain from Equation 3. However, Figure 1 provides a visual comparison of the dependence of R_{ex} on (τ_{ex}, $\delta\omega^2$, ω_{1eff}) predicted by Equations 2 and 3. As expected, the two equations make indistinguishable predictions in the fast exchange limit, $(\delta\omega\tau_{ex})^2 \ll 1$ (i.e., to the left of each vertical dashed line), otherwise they typically predict different values of R_{ex}. However, examination of Figure 1 shows that the latter is not always the case. For example, the curves (a) and (b) generated by both equations are indistinguishable even on the right sides of the dashed lines.

To explain the latter result, we present a simple empirical expression for R_{ex}, Equation 4 that is applicable to the CPMG experiment. This is an approximate equation derived on the basis of physical intuition and extensive computer simulation:

$$R_{ex} \approx (\delta\omega)^2 p_1 p_2 \tau_{ex}/(1 + \omega_a^2\tau_{ex}^2) \quad (4)$$

$$\omega_a^2 = \sqrt{[\omega_{1\mathrm{eff}}^4 + p_2^2(\delta\omega)^4]}$$

In contrast with Equation 3, Equation 4 clearly reveals the relationship between R_{ex} and the parameters of interest. Equation 4 predicts values of R_{ex} that agree with those calculated using Equation 3, within 15%, for all values of $\delta\omega\tau_{ex}$, when $p_1/p_2 < 0.15$. In particular, Equation 4 predicts that R_{ex} attains its maximum value when $\omega_a^2\tau_{ex}^2 =$ ca. 1, in agreement with the curves calculated using Equation 3 and plotted in Figure 1.

Equation 4 is of particular interest in two limiting cases, (*i*) $p_2^2(\delta\omega)^4 \gg \omega_{1\mathrm{eff}}^4$ and (*ii*) $\omega_{1\mathrm{eff}}^4 \gg p_2^2(\delta\omega)^4$. In the former case, $R_{ex} \approx p_1 p_2(\delta\omega)^2\tau_{ex}$ assuming fast exchange ($\delta\omega^2\tau_{ex}^2 \ll 1$), whereas $R_{ex} \approx p_1/\tau_{ex}$ assuming slow exchange ($\delta\omega^2\tau_{ex}^2 \ll 1$). This corresponds to the low field, lifetime broadening limit. Note that in the high field limit ($\omega_{1\mathrm{eff}}^4 \gg p_2^2(\delta\omega)^4$), Equation 4 becomes nearly identical to Equations 1 and 2 for all values of τ_{ex}. This is so because, in the limit of a large $\omega_{1\mathrm{eff}}$, second order perturbation theory, which is used to derive Equations 1 and 2, applies. Hence, the simple fast limit equations are recovered and Equations 1–4 all predict essentially the same results. Therefore, in Figure 1, curves (a) and (b) calculated using Equation 3 and plotted in panels (A)–(C), are indistinguishable from their fast limit counterparts, plotted in panels (D)–(F). It has been noted elsewhere, that second order perturbation theory can be used to calculate R_1 and $R_{1\rho}$ outside the fast-exchange limit (Vold and Vold, 1991; Szyperski et al., 1993; Goldman, 1994) provided that the laboratory or rotating frame field is greater than the local field of the perturbation.

In the case of diamagnetic protein spectra, $\delta\omega$ is expected to be less than 2 ppm for ^{1}H and 20 ppm for ^{15}N. Hence, the fast-limit exchange Equations 1 and 2 are good approximations for spin-lock experiments recorded with $\omega_{1\mathrm{eff}}/2\pi$ greater than ca. 2 kHz and 3 kHz at 500 MHz and 800 MHz, respectively. On the other hand, protein CPMG experiments are typically recorded with $\omega_{1\mathrm{eff}}/2\pi < 1$ kHz. When spectra are acquired using such small effective fields, and the validity of the fast exchange assumption has not been experimentally demonstrated, analysis of relaxation data should be carried out using Equation 3. We recommend this approach even if the fast limit equations provide satisfactory fits to the data.

A final point that deserves attention is that it is not trivial to determine if a dynamic process is in the fast and slow exchange limit, even when Equation 3 is used

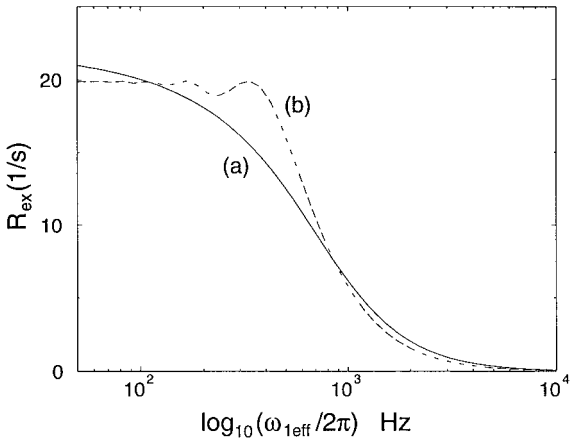

Figure 2. Comparison of the dependence of R_{ex} vs. $\omega_{1\mathrm{eff}}/2\pi$ in the fast (a) and slow (b) exchange limits. Both curves were calculated using Equation 3 with $p_1/p_2 = 1/9$ and (a) $\tau_{ex}^f = 0.25$ ms and $(\delta\omega)^f/2\pi = 160$ Hz, fast exchange, and (b) $\tau_{ex}^s = 5$ ms, $(\delta\omega)^s/2\pi = 600$ Hz, slow exchange. As discussed by Allerhand and Gutowsky (1965), R_{ex} is an oscillatory function of $\delta\omega/\omega_{1\mathrm{eff}}$ in the slow exchange limit. This is the source of the damped modulation seen in (b).

to analyze relaxation data acquired as a function of $\omega_{1\mathrm{eff}}$. This is demonstrated by the plots of R_{ex} vs. $\omega_{1\mathrm{eff}}$ in Figure 2, which were calculated using Equation 3. The parameter sets, listed in the caption of Figure 2, show that curves (a) and (b) correspond to fast and slow exchange, respectively. The similarity of the curves is striking when one considers that $\tau_{ex}^s = 20\tau_{ex}^f$. The parameter sets used to generate the curves were chosen with the aid of Equation 4 which predicts the following approximate expressions for R_{ex} in the fast and slow exchange limits respectively:

$$R_{ex}^f \approx (\delta\omega^f)^2 p_1^f p_2^f \tau_{ex}^f/(1 + \omega_{1\mathrm{eff}}^2\tau_{ex}^{f2}) \qquad (5)$$

$$R_{ex}^s \approx (p_1^s/\tau_{ex}^s)/\sqrt{(1 + \omega_{1\mathrm{eff}}^4/(p_2^{s2}(\delta\omega^s)^4))} \qquad (6)$$

Using these equations one readily finds parameter sets that satisfy the conditions, $(\delta\omega^f)^2 p_1^f p_2^f \tau_{ex}^f \approx (p_1^s/\tau_{ex}^s)$, $\tau_{ex}^{f2} \approx 1/(p_2^s(\delta\omega^s)^2$ and $\tau_{ex}^f \ll \tau_{ex}^s$ which insure that R_{ex} has a similar dependence on $\omega_{1\mathrm{eff}}$ in both the fast and slow exchange limits. In spite of the similarity of the curves in Figure 2, careful measurement of R_{ex} over a 10-fold range of ^{15}N and/or ^{1}H effective fields ($\omega_{1\mathrm{eff}}$) should reliably identify the time regime of the exchange process. In this regard, it has been noted that ratios of R_{ex} measured at different values of $\omega_{1\mathrm{eff}}$ (Szyperski et al., 1993; Ishima et al., 1998) amplify the difference in slopes in the fast and slow exchange limits, and are therefore well suited to

distinguish the fast and slow exchange regimes. Finally, we note that CPMG measurements at two values of the external field, B_0, may distinguish fast from slow exchange in the low ω_{1eff} limit, $\omega_{1eff}^4 \ll \delta\omega^4$. In this limit, Equation 4 shows that $R_{ex} \propto \delta\omega^2$ in fast exchange, but R_{ex} is independent of $\delta\omega$ in slow exchange.

Taken together, these considerations show that careful analysis of R_{ex} data is necessary to derive correct estimates of τ_{ex} from rotating frame relaxation experiments in proteins.

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