

Measurement of Chemical Exchange Rate Constants with Solvent Protons Using Radiation Damping

Jin-Hong Chen and Xi-An Mao¹

Laboratory of Magnetic Resonance and Atomic Molecular Physics, Wuhan Institute of Physics and Mathematics,
The Chinese Academy of Sciences, P.O. Box 71010, Wuhan, 430071, China

E-mail: maoxian@nmr.whenc.ac.cn

Received May 23, 1997; revised October 3, 1997

When chemical exchange occurs between a concentrated and a diluted species like the proton exchange between water and proteins in biochemical systems, it is difficult to use the conventional methods for a quantitative dynamical study. In this paper, a new approach which utilizes radiation damping of the strong water magnetization exchanging with the weak resonance of the mobile protons in biomolecules is proposed. It is demonstrated with a dilute guanidine solution, using the simple inversion-recovery pulse sequence, that the exchange rate constants between water and guanidine protons are evaluated by solving the Bloch equations modified with both the radiation damping and the chemical exchange effects. © 1998 Academic Press

In NMR experiments on samples containing biomolecules (proteins, polypeptides, and nucleic acids), attention has been paid to chemical exchange of protons between biomolecules and water (1). Thus, quantitative study of the exchange dynamics is of great importance (1, 2). Various NMR techniques have been available for such a purpose, among which three methods are most widely used. The traditional one is via the lineshape analysis of the exchanging signals in variable temperature experiments (3). Saturation transfer effect can be applied under favorable conditions (4). The most popular one is the two-dimensional exchange spectroscopy (2D EXSY) (5). However, when the exchanging system consists of a weak signal and a strong water resonance that is under radiation damping (6), it is difficult to use any of the established methods. It is impossible to analyze the lineshapes because of the great disparity between the weak biomolecular signals and the strong water signals. Radiation damping may interfere with the saturation when saturation transfer method is used. If a 2D EXSY experiment is performed, before the read pulse the water magnetization is already back to the equilibrium state, since the mixing time (usually hundreds of milliseconds) is long enough to allow the radiation damping process to be complete, and the EXSY

spectrum will not contain any exchange information. As a result, other techniques for avoiding radiation damping have been proposed in recent years (1). The present Communication describes an approach that benefits from the radiation damping of water. When the radiation damping effect is transferred from water to the mobile solute protons through chemical exchange, the exchange rate constants can be quantitatively evaluated. On one hand, we suggest a new method for studying the exchange dynamics; on the other hand we report for the first time the phenomenon of radiation damping involved chemical exchange, which may be of general interest in biomolecular NMR studies.

The sample studied was 0.17 M aqueous solution of guanidine chloride ($\text{NH}_2\text{C}(\text{NH}_2) = \text{NH} \cdot \text{HCl}$), where the five equivalent NH protons have physico-chemical properties similar to those in biomolecules. D_2O (30%) was used for lock purpose. The pulse scheme was the simple inversion-recovery sequence $\pi - \tau - \pi/2 - \text{FID}$, where τ is the variable recovery delay. According to the Bloch equations (7), the recovery of the guanidine magnetization, which could not show appreciable radiation damping effects, should obey the simple exponential law

$$M_z = M_0[1 - 2 \exp(-\tau/T_1)], \quad [1]$$

where T_1 is the spin-lattice relaxation time. However, when the water magnetization recovers quickly through the radiation damping pathway (7), the radiation damping effect must be transferred from water to guanidine due to chemical exchange, much like the process of saturation transfer (4). As a result, the exponential law is not valid. Figure 1 shows the recovery data (circles) of the guanidine protons, which agree with the dotted curve (calculated using Eq. [1] with the measured T_1 value 1.5 s) only when the recovery time is extremely short ($\tau < 50$ ms).

In order to understand the unusual recovery data in Fig. 1, the Bloch equations modified by both radiation damping

¹ To whom correspondence should be addressed.

and chemical exchange should be solved, which consists of six coupled differential equations. While the numerical solution can always be obtained with the help of a computer, analytical solutions are more desirable because they can provide a more intuitive insight into the physics of the problem. The radiation damping time (6) T_{rd} of water under the given experimental conditions is about 20 ms, while the relaxation times are $T_1 = 2.1$ s and $T_2^* = 0.3$ s. So the relaxation terms in the Bloch equations regarding the water protons can be neglected, as is usually done in radiation damping studies (6, 9, 10). This greatly simplifies the Bloch equations. Further simplification can be achieved by noting the fact that the molar ratio between the water and the NH protons is more than 100 (the concentration of the water protons is 77

with $t_0 = T_{rd} \ln[\tan(\theta_0/2)]$, where θ_0 is the initial angle of the magnetization with respect to the B_0 field. By substitution of Eq. [3] into Eq. [2b], and using the relation $M_A^0/\tau_A = M_B^0/\tau_B$, Eq. [2b] can be changed into

$$dM_{Bz}/dt = (1/T_{1B})M_{B0} - (1/T_{1B} + 1/\tau_B)M_{Bz} + (1/\tau_B)M_{B0} \tanh[(t - t_0)/T_{rd}]. \quad [4]$$

An analytical solution of Eq. [4] is not difficult to obtain and different forms of solutions could be found, but the mathematical derivation is somewhat tedious and is considered for publication elsewhere (8). Here we give straightforward the result which consists of two parts

$$\frac{M_{Bz}}{M_B^0} = \begin{cases} \frac{1/T_{1B} - 1/\tau_B}{1/T_{1B} + 1/\tau_B} + c \exp(-t/T_{1B} - t/\tau_B) \\ - (2/\tau_B) \sum_{n=1}^{\infty} \frac{(-1)^n \exp(2nt/T_{rd})}{2n/T_{rd} + 1/T_{1B} + 1/\tau_B} [\cot(\theta_0/2)]^{2n} \\ \text{(for } 0 \leq t \leq T_{rd} \ln[\tan(\theta_0/2)]) \end{cases} \quad [5a]$$

$$\begin{cases} 1 + c' \exp(-t/T_{1B} - t/\tau_B) \\ + (2/\tau_B) \sum_{n=1}^{\infty} \frac{(-1)^n \exp(-2nt/T_{rd})}{-2n/T_{rd} + 1/T_{1B} + 1/\tau_B} [\tan(\theta_0/2)]^{2n} \\ \text{(for } t \geq T_{rd} \ln[\tan(\theta_0/2)]) \end{cases} \quad [5b]$$

M, while that of the guanidine protons is 0.65 M when the H^1-H^2 exchange reaches equilibrium). Because of the large molar excess of the water protons, no matter how fast or how slow the exchange is, the weak guanidine magnetization cannot make an important influence on water. Hence as far as the water magnetization is concerned, exchange can also be neglected. For an inversion-recovery experiment, we are interested only in the z components during the recovery period. The transverse magnetization, if any, can be averaged away by a proper phase cycling or by pulsed field gradients. We then have a pair of simplified Bloch equations for the two-site exchanging system with water labeled by A and guanidine by B

$$dM_{Az}/dt = (M_{A0}^2 - M_{Az}^2)/M_{A0}T_{rd} \quad [2a]$$

$$dM_{Bz}/dt = (1/T_{1B})M_{B0} - (1/T_{1B} + 1/\tau_B)M_{Bz} + (1/\tau_A)M_{Az}, \quad [2b]$$

where τ_A and τ_B denote the lifetime of the proton at species A and B, respectively, the reciprocal of which are just the exchange rate constants. The solution of Eq. [2a] is well known (6c)

$$M_{Az} = M_{A0} \tanh[(t - t_0)/T_{rd}] \quad [3]$$

where c and c' are the integration constants. The constant c is easily determined with the initial condition that $M_{Bz} = -M_{B0}$ at $t = 0$ and we have $c = -(2/T_{1B})/(1/T_{1B} + 1/\tau_B)$. With the continuous condition that Eq. [5a] and Eq. [5b] are equal at $t = t_0$, the constant c' can be determined

$$c'(1/T_{1B} + 1/\tau_B) = -2/T_{1B} - \frac{2}{\tau_B} \exp\left[\left(\frac{T_{rd}}{T_{1B}} + \frac{T_{rd}}{\tau_B}\right) \ln\left(\tan \frac{\theta_0}{2}\right)\right] \times \left[1 + 2 \sum_{n=1}^{\infty} \frac{(-1)^n (1/T_{1B} + 1/\tau_B)^2}{(1/T_{1B} + 1/\tau_B)^2 - (2n/T_{rd})^2}\right].$$

Although the result seems rather complicated, it is obvious that when the lifetimes τ_B is extremely long (the exchange is extremely slow), both c and c' are reduced to -2 and Eq. [5] is correspondingly reduced to Eq. [1]. In the inversion-recovery experiment, the information that we wish to have is the value of M_z before the read pulse, which is just the initial value of the FID and can be measured by the integration of the Fourier transformed signal.

When the inversion-recovery experiment is quantitatively discussed using Eq. [5], however, the radiation damping effect after spin inversion (10) should be taken into account, due to which the initial angle θ_0 cannot be π . Hence a small

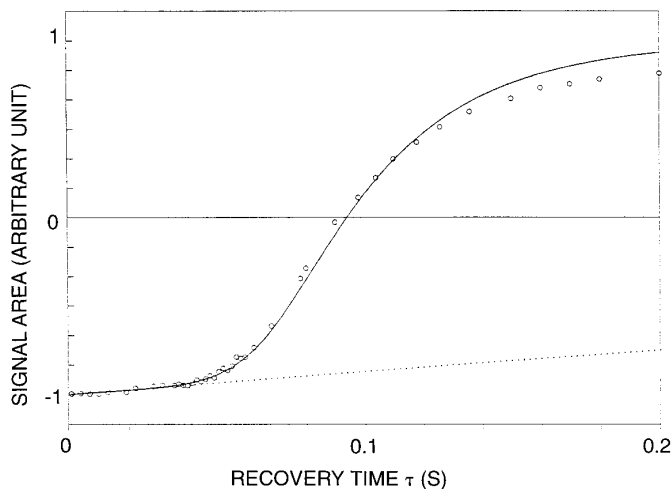


FIG. 1. Plot of the inversion-recovery intensities (the signal areas) of the NH protons in guanidine as a function of recovery time (τ). The inversion-recovery experiment was performed on an ARX-500 NMR spectrometer and 52 τ values were used (only 43 data between 0 and 0.2 s are shown). In order to cancel the transverse components during the recovery period, four transients were accumulated using the phase cycling of $\phi_1 = 0, \pi, 0, \pi$; $\phi_2 = \phi_3 = 0, 0, \pi, \pi$, where ϕ_1, ϕ_2 , and ϕ_3 are the phases of the inversion pulse, the read pulse, and the receiver, respectively. Circles: the experimental integration data. Solid curve: computational result based on Eq. [5] using $\theta_0 = 175^\circ$ (i.e., $\delta = 5^\circ$), $T_{rd} = 20$ ms, $T_{1B} = 1.5$ s, and $\tau_B = 0.041$ s. Dotted curve: the simple exponential relation (Eq. [1]) with $T_1 = 1.5$ s.

angle $\delta \ll \pi$ must be introduced, just as done in the quantitative discussion of the radiation damping induced half-frequency harmonic peaks in the J -resolved spectrum (10). We then use $\pi - \delta$ instead of π for θ_0 , and t_0 becomes $T_{rd} \ln \{ \tan [(\pi - \delta)/2] \}$. Since t can be shorter or longer than t_0 , both Eqs. [5a] and [5b] are needed for a quantitative discussion. The calculated inversion-recovery data based on Eq. [5] are shown in Fig. 1 as a solid curve, which closely fits the experimental results. In the fitting, $\delta = 5^\circ$, $T_{1B} = 1.5$ s and $T_{rd} = 20$ ms have been assumed. As a result, $\tau_B = 0.041$ s and $\tau_A = 4.88$ s are simultaneously determined. Therefore, the fitting of the inversion-recovery data provides a useful method to measure the exchange rates k ($k_{AB} = 1/\tau_A = 24.4$ s $^{-1}$, $k_{BA} = 1/\tau_B = 0.2$ s $^{-1}$).

For a comparison, in Fig. 2 we show the inversion-recovery data of water which are simulated by the solid curve using Eq. [3] with $T_{rd} = 20$ ms. The similarity of the two solid curves in Fig. 1 and Fig. 2 clearly indicates that radiation damping effect has been transferred from water to guanidine.

If water were free of radiation damping, much slower recoveries would be expected, since the exchange is not fast enough to influence the lineshape, and after 0.2-s recovery both the water and the NH magnetizations would remain negative, far from the equilibrium state, as indicated by the two dotted curves in the figures. However, the strong radia-

tion damping field drives the water magnetization quickly to equilibrium and at the same time accelerates the recovery of the NH magnetization through chemical exchange. This enables the exchange rate constants to be evaluated. Although the proposed method is applied to the simple water-guanidine exchanging system, it can be easily generalized to multiple-site exchanging systems which are common in biological studies.

People may note that in Fig. 2 when the recovery time is longer (from 0.08 s up), the agreement between the simulation and the measurement becomes poor. This can be understood that in simulation using Eq. [3], the relaxation has been completely ignored. In fact, the inhomogeneous relaxation T_2^* can be 0.1 s. When the recovery time is short enough not to allow T_2^* to be effective, the simulation is good, but when the recovery time is long, T_2^* effect begins. So neglecting T_2^* effect cannot get good results for the whole recovery range in Fig. 2. This has also affected the simulation in Fig. 1. It is possible to consider the relaxation and radiation damping effects at the same time using the established lineshape theory (6d). However, analytical solution could not be obtained.

In summary, when chemical exchange occurs between a concentrated and a diluted species like the proton exchange between water and proteins in biochemical systems, the traditional methods, mainly the lineshape analysis, saturation transfer, and the two-dimensional exchange spectroscopy, prove not to be useful for a dynamic study. A new approach has been proposed and demonstrated, which utilizes radiation damping of the strong water magnetization exchanging with the weak resonance of the mobile protons in biomolecules. Using the simple inversion-recovery pulse sequence, the exchange rate constants between water and guanidine

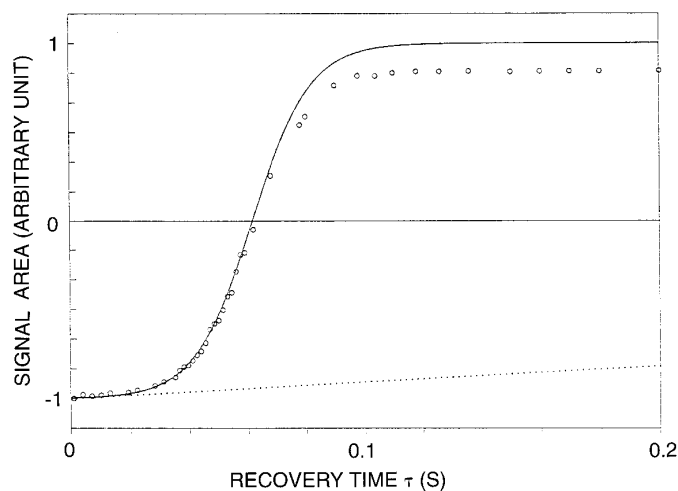


FIG. 2. Circles: the inversion-recovery integration data of water in the same experiment as Fig. 1. Solid curve: computational result based on Eq. [3] with $\theta_0 = 175^\circ$ and $T_{rd} = 20$ ms. Dotted curve: the simple exponential relation (Eq. [1]) with $T_1 = 2.1$ s.

have been evaluated by solving the Bloch equations modified with both the radiation damping and the chemical exchange effects. Radiation damping has so far been regarded as a negative factor in high field NMR experiments and should be suppressed in most cases (11). However, we have shown in this Communication that radiation damping can be used to solve the quantitative problem in exchange dynamic studies as an alternative to the newly developed methods (1).

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China.

REFERENCES

1. (a) S. Grzesiek and A. Bax, *J. Am. Chem. Soc.* **115**, 12,593 (1993); *J. Biomol. NMR* **3**, 627 (1993). (b) S. Mori, C. Abergunawardana, P. C. M. van Zijl, and J. M. Berg, *J. Magn. Reson. B* **110**, 96 (1996).
2. V. Doetsch and G. Wider, *J. Am. Chem. Soc.* **117**, 6064 (1995).
3. J. I. Kaplan and G. Fraenkel, "NMR of Chemically Exchanging Systems," Academic Press, London (1980).
4. (a) A. G. Redfield and S. Waelder, *J. Am. Chem. Soc.* **101**, 6151 (1979). (b) R. A. Bednar and W. P. Jencks, *J. Am. Chem. Soc.* **107**, 7117 (1985).
5. R. R. Ernst, G. Bodenhausen, and A. Wokaun, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," Chap. 9, Oxford Press, Oxford, (1987).
6. (a) N. Bloembergen and R. V. Pound, *Phys. Rev.* **95**, 8 (1954). (b) A. Abragam, "The Principles of Nuclear Magnetism," p. 73, Clarendon, Oxford, (1961). (c) X.-A. Mao and C.-H. Ye, *J. Chem. Phys.* **99**, 7455 (1993). (d) X.-A. Mao, J.-X. Guo, and C.-H. Ye, *Phys. Rev. B* **49**, 15702 (1994). (e) X.-A. Mao and C. H. Ye, *Concepts Magn. Reson.* **9**, 173 (1997).
7. F. Bloch, *Phys. Rev.* **70**, 460 (1946).
8. J. H. Chen and X.-A. Mao, *J. Chem. Phys.* **107**, 7120 (1997).
9. (a) W. S. Warren, S. L. Hames, and J. L. Bates, *J. Chem. Phys.* **91**, 5895 (1989). (b) A. Vlassenbroek, J. Jeener, and P. Broekaert, *J. Chem. Phys.* **103**, 5886 (1995). (c) X.-A. Mao, J.-X. Guo, and J.-H. Chen, *J. Phys. D: Appl. Phys.* **29**, 1595 (1996).
10. (a) J.-X. Guo and X.-A. Mao, *J. Phys. II France* **6**, 1183 (1996). (b) J.-H. Chen, X.-A. Mao, and C.-H. Ye, *J. Magn. Reson. A* **123**, 126 (1996).
11. (a) P. Broekaert, J. Jeener, *J. Magn. Reson. A* **113**, 274 (1995). (b) C. Anklin, M. Rindlisbacher, G. Otting, and F. H. Laukien, *J. Magn. Reson. B* **109**, 218 (1995). (c) A. Boeckmann and E. Guttet, *J. Biomol. NMR* **8**, 87 (1996). (d) S. Zhang, and G. Gorenstein, *J. Magn. Reson. A* **118**, 291 (1996).