J|A|C|S
COMMUNICATIONS

Published on Web 05/11/2002

Effect of Chemical Exchange on Radiation Damping in Aqueous Solutions of the Osmolyte Glycine

Juan Carlos Rodríguez, Patricia A. Jennings, and Giuseppe Melacini*

University of California San Diego, 9500 Gilman Drive, 92093-0314, La Jolla, California

Received January 24, 2002

The nuclear magnetic resonance properties of uncoupled spins belonging to solutes dissolved at millimolar concentrations are adequately described by the phenomenological Bloch equations.[1] However, for highly concentrated molecular species, such as water, nonlinear terms must be included into the Bloch equations to account for an additional magnetic field ($\mathbf{B}_{rd}$), which is created by the transverse components of the magnetization, according to Lenz's law. The $\mathbf{B}_{rd}$ field exerts a torque that rotates the water magnetization toward the *z*-axis in a tuned coil, thus giving rise to the phenomenon of radiation damping.[1,2] As a result of the ~110 M concentration of water protons and of the typically high Q-factor of modern NMR probes, radiation damping occurs in a time scale of tens of milliseconds, and it often interferes with solvent suppression schemes.[3−5] The time constant for radiation damping is expected to become even shorter with the advent of ultrahigh-field magnets (i.e. 900 MHz) and the design of supersensitive probes, thus making radiation damping one of the important problems in current NMR spectroscopy.[6,7] In addition, it has been recently shown[7] that the joint action of the distant dipole field and of radiation damping causes the resurrection of water magnetization crushed by pulsed-field gradients. This process leads to the creation of an undesired macroscopic signal characterized by chaotic dynamics, which affects the reproducibility of the experiments. These observations therefore emphasize the importance of a thorough understanding of the factors involved in radiation damping of water during NMR experiments.

The effect of pulsed-field gradients (PFG)[8] and of active-feedback[9] on radiation damping has been extensively characterized. Here we show how radiation damping can be dramatically affected without hardware adjustments or application of pulsed-field gradients. Specifically, we demonstrate that radiation damping is attenuated by the incoherent dephasing of the transverse water magnetization caused by chemical exchange with the NH_3^+ group of a well-known osmolyte, glycine.[10] In addition, it is shown that this effect suppresses the resurrection of unwanted macroscopic water signals.[7]

Osmolytes, including glycine, are frequently used as co-solutes for biochemical samples to stabilize proteins in aqueous solutions.[10,11] The concentration of Gly NH_3^+ protons is only one order of magnitude smaller than that of water protons at the molarity necessary for this stabilizing effect. Thus, significant dephasing of the water transverse magnetization and consequent reduction of radiation damping is expected under the pH and temperature conditions for which the exchange rate between the Gly NH_3^+ and water protons is close to the intermediate exchange regime.[12] For instance, at pH 6.0 and a temperature of 313 K, the Gly NH_3^+/water exchange is anticipated to fall within the intermediate kinetic limits,[13] at a field of 500 MHz and a dramatic suppression of radiation damping is observed in water inversion−recovery experiments (Figure 1, Top). The observed effect under these conditions

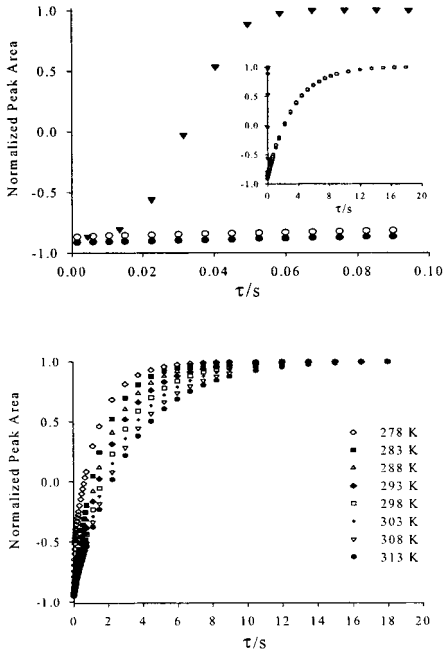

Figure 1. (Top) The inversion−recovery (I−R) profile of the water magnetization at 313 K is shown for samples containing 2 mM hen-egg white lysozyme in 90% H_2O/10% D_2O at pH 6.0 in the presence (●) or absence (▼ and ○) of 2 M Gly-d_5. Data denoted with (○) was obtained from experiments in which radiation damping was suppressed through application of pulsed-field gradients. The inset shows a longer time course of the same inversion−recovery profiles. (Bottom) The I−R data of the water magnetization in samples containing 2 mM hen-egg white lysozyme and 2 M Gly-d_5 obtained at different temperatures. A total of 49 τ intervals were acquired for each experiment. See Supporting Information for further experimental details.

is comparable to that of a chain of purging gradients applied during the recovery period, both in the millisecond (Figure 1, Top) and in the second time scales (Inset of Figure 1, Top).

The role of the Gly-induced dephasing at pH 6.0 is further supported by the more rapid water inversion observed when the temperature is decreased stepwise from 313 to 278 K. The dephasing becomes less pronounced and radiation damping is more effective as the exchange kinetics moves from the intermediate toward the slow regime at low temperatures (Figure 1, Bottom). This result is fully confirmed by a thorough monitoring of zero-crossing times in water inversion−recovery profiles measured without gradients in the pH range 4−8 and at temperatures in the interval 278−313 K (Figure 2, Top). As the pH and temperature are increased in a stepwise fashion, the Gly NH_3^+/water exchange rate shifts from a minimum at pH 4 and 278 K to a maximum at pH 8 and 313 K. Thus, the exchange kinetics moves gradually from the slow regime through the intermediate regime (Figure 2, Bottom)

* Address correspondence to this author. E-mail: gm@chem.ucsd.edu.

10.1021/ja0256966 CCC: $22.00

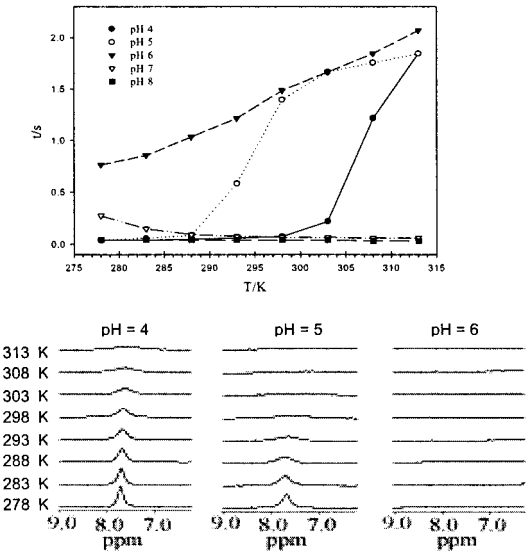

Figure 2. (Top) Temperature and pH dependence of the zero-crossing time in inversion−recovery experiments performed on samples containing hen-egg white lysozyme (2 mM) and 2 M Gly-d_5. The time values plotted represent the instant at which a change of sign of the water magnetization occurs. A total of 128 τ increments were sampled from 2.5 ms to 18 s, each with one scan and a 2408 Hz spectral width. A relaxation delay of 30 s between scans was used. (Bottom) Stacked 1D plots showing the effect of temperature and pH on the ^{1}H NMR resonance of glycine ammonium protons.

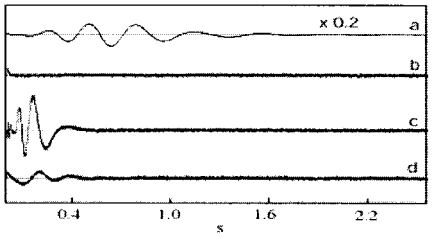

Figure 3. Water ^{1}H FIDs measured after the sequence $[(\pi/2)_x - (GT)_z]$, where $(GT)_z$ represents a squared *z*-gradient of 3.5 G/cm with a 1 ms duration, followed by a 0.51 ms delay. A single transient was recorded with an acquisition time of 2.56 s. The temperature was stabilized at 298 K for at least 2 h before data acquisition. All samples were optimally shimmed and deuterium-locked. (a) A sample of 90% H_2O and 10% D_2O at pH 6; (b) as (a) with 1 M glycine added; (c) as (b) with the pH adjusted to 4; (d) as (b) with the pH adjusted to 8.

to reach the fast regime. These shifts in exchange regimes are highly correlated with the observed inversion−recovery rates: when the system is close to the intermediate exchange regime, a dramatic delay of the zero-crossing time occurs (Figure 2, Top), while no significant attenuation of radiation damping is detected in either the slow or fast exchange limits because the dephasing of the transverse water magnetization is only minimal. This confirms that the attenuation of radiation damping is not caused by changes in ionic strength occurring upon addition of glycine. Measurements at 600 MHz further support the interpretation of zero-crossing times in terms of exchange broadening (Supporting Information).

The attenuation of radiation damping obtained by using glycine is very useful to control and suppress the resurrection process that leads to the creation of unwanted macroscopic water magnetization crushed by a PFG.[7] The resurrected signal observed in the absence of glycine (Figure 3, a) is suppressed in the presence of 1 M glycine at pH 6 and 298 K (Figure 3, b), as expected on the basis of the dramatic attenuation of radiation damping occurring under these experimental conditions (Figure 2). When the exchange kinetics is brought into the slow regime at pH 4 (Figure 3, c) or into the fast regime at pH 8 (Figure 3, d), radiation damping becomes effective again, and a resurrected macroscopic signal reappears.

In conclusion, we have shown that the dephasing resulting from the intermediate chemical exchange between water and the protein-stabilizing osmolyte glycine has a dramatic effect on radiation damping, similar to that of a series of repeated PFGs. This effect is particularly relevant because it suppresses the resurrection of undesired water magnetization crushed by pulsed-field gradients.[7] When used in combination with PFGs and water flip-back schemes,[14] glycine is therefore expected to tame chaotic dynamics and thus improve the reproducibility of the NMR experiments affected by it.

Acknowledgment. We thank Drs. S. J. Opella, S. S. Taylor, E. A. Komives, M. Goodman, J. M. Wright, A. Nevzorov, M. Mesleh, and M. Tessari and the National Institutes of Health (Grant DK 54441 to P.A.J) for financial support. We are grateful to the Keck Foundation for funding the KeckII computer center.

Supporting Information Available: Experimental details, zero-crossing times at 600 MHz, solute (protein) spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Skelton, N. J. *Protein NMR: Principles and Practice*; Academic Press: London, 1996.
(2) (a) Szantay, C.; Demeter, A. *Concepts Magn. Reson.* **1999**, *11*, 121. (b) Otting, G.; Liepinsh, E. *J. Biomol. NMR* **1995**, *5*, 420. (c) Chen, J. H.; Mao, X. A. *J. Magn. Res.* **1998**, *131*, 358. (d) Cutting, B.; Chen, J. H.; Moskau, D.; Bodenhausen, G. *J. Biomol. NMR* **2000,** *17*, 323. (e) Augustine, M. P. *Prog. Nucl. Magn. Reson. Spectrosc.* **2002**, *40*, 111.
(3) Mao, X.; Guo, J.; Ye, C. *Chem. Phys. Lett.* **1994**, *222*, 417.
(4) Gueron, M.; Leroy, J. L. *J. Magn. Reson.* **1989**, *85*, 209.
(5) He, Q.; Richter, W.; Vathyam, S.; Warren, W. S. *J. Chem. Phys.* **1993**, *98*, 6779.
(6) (a) Sobol, A. G.; Wider, G.; Iwai, H.; Wuthrich, K. *J. Magn. Reson.* **1998**, *130*, 262. (b) Hwang, T.-L.; Mori, S.; Shaka, A. J.; van Zijl, P. C. M. *J. Am. Chem. Soc.* **1997**, *119*, 6203. (c) Gueron, M.; Plateau, P.; Decorps, M. *Prog. Nucl. Magn. Reson. Spectrosc.* **1991**, *23*, 135. (d) Price, W. S.; Stilbs, P.; Jonsson, B.; Soderman, O. *J. Magn. Reson.* **2001**, 150, 49.
(7) (a) Lin Y. Y.; Lisitza, N.; Ahn, S.; Warren, W. S. *Science* **2000**, *290*, 118. (b) Warren, W. S.; Richter, W.; Andreotti, A. H.; Farmer, B. T. *Science* **1993**, 262, 2005.
(8) (a) Dalvit, C.; Hommel, U. *J. Magn. Res.* B **1995**, *109*, 334. (b) Dalvit, C.; Hommel, U. *J. Biomol. NMR* **1995**, *5*, 306. (c) Dalvit, C. *J. Magn. Res. A* **1995**, *113*, 120. (d) Sklenar, V. *J. Magn. Res. A* **1995**, *114*, 132. (e) Zhang, S. M.; Gorenstein, D. G. *J. Magn. Res. A* **1996**, *118*, 291. (f) Dalvit, C. *J. Magn. Res. B* **1996**, *112*, 282. (g) Dalvit, C.; Bohlen, J. M. *J. Magn. Res. B* **1996**, *113*, 195. (h) Dalvit, C.; Bohlen, J. M. *J. Magn. Res.* **1997**, *126*, 149. (i) Mattiello, D. I.; Warren, W. S.; Mueller, L.; Farmer B. T. *J. Am. Chem. Soc.* **1996**, *118*, 3253. (j) Dalvit, C. *J. Biomol. NMR* **1998**, *11*, 437. (k) Melacini, G.; Boelens, R.; Kaptein, R. *J. Biomol. NMR* **1999**, *13*, 67. (l) Melacini, G.; Kaptein, R.; Boelens, R. *J. Magn. Res.* **1999**, *136*, 214.
(9) (a) Louisjoseph, A.; Abergel, D.; Lallemand, J. Y. *J. Biomol. NMR* **1995**, *5*, 212. (b) Broekaert, P.; Jeener, J. *J. Magn. Res. A* **1995**, *113*, 60. (c) Otting, G.; Liepinsh, E. *J. Magn. Res. B* **1995**, *107*, 192. (d) Abergel, D.; Louisjoseph, A.; Lallemand, J. Y. *J. Biomol. NMR* **1996**, *8*, 15.
(10) (a) Yancey, P. H.; Clark, M. E.; Hand, S. C.; Bowlus, R. D.; Somero, G. N. *Science* **1982**, *217*, 1214. (b) Creighton, T. E. *Protein Structure: A Practical Approach*; Oxford IRL Press: Washington, DC, 1989; p 331. (c) Arakawa, T.; Timasheff, S. N. *Biophys. J.* **1985**, *47*, 411. (d) Santoro, M. M.; Liu, Y.; Khan, S. M. A.; Hou, L.; Bolen, D. W. *Biochemistry* **1992**, *31*, 5278.
(11) Matthews, S. J.; Leatherbarrow, R. J. *J. Biomol. NMR* **1993**, *3*, 597.
(12) (a) Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307. (b) Ni, F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 517. (c) Millet, O.; Loria, J. P.; Kroenke, C. D.; Pons, M.; Palmer, A. G. *J. Am. Chem. Soc.* **2000**, *122*, 2867.
(13) (a) Rabenstein, D. L.; Fan, S.; Nakashima, T. T. *J. Magn. Res.* **1985**, *64*, 541. (b) Emerson, M. T.; Grunwald, E.; Kromhout, R. A. *J. Chem. Phys.* **1960**, *33*, 547.
(14) (a) Grzesiek, S.; Bax, A. *J. Am. Chem. Soc.* **1993**, *115*, 12593. (b) Jahnke, W.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, (4), 469.

JA0256966