Chemical Exchange in Diffusion NMR Experiments

Aidi Chen,[†] Charles S. Johnson, Jr.,[‡] Melissa Lin,[†] and Michael J. Shapiro*,[†]

Department of Analytics, Preclinical Research Novartis Pharmaceuticals Corp., 556 Morris Ave. Summit, New Jersey 07901
Department of Chemistry, University of North Carolina Chapel Hill, North Carolina 27599

Received March 20, 1998
Revised Manuscript Received July 24, 1998

Diffusion NMR spectroscopy[1] which relies on Pulse Field Gradient (PFG) NMR[2] to spatially encode molecules, enables identification and even structure determination of the individual components in a mixture resulting from differences in their translational diffusion coefficients. Recently, diffusion NMR technology has been applied to the drug discovery process, and "Affinity NMR" has been developed to "separate" the bound ligands from the unbound molecules by their relative diffusion coefficients.[3]

Chemical exchange can affect the signal decay profile and its inverse Laplace transform—the final diffusion spectrum.[4] Here, we report another important consequence of chemical exchange in various diffusion NMR experiments, including longitudinal eddy current delay (LED)[5] and stimulated echo (STE).[6] Our studies demonstrate that chemical exchange can cause serious signal distortions that can lead to severe sensitivity loss and misinterpretation of diffusion ordered and diffusion edited experiments. Under certain conditions, the choice of gradient encode delay τ (between first and second 90° rf pulses) and decode delay τ (between third and fourth 90° rf pulses in LED) plays a critical role on the ability to observe chemical species involved in exchange. This is especially important in Affinity NMR experiments, because the "bound" and "free" molecules are usually involved in chemical exchange and Affinity NMR relies on the ability to observe bound ligands for the purpose of identifying active components in mixtures.

To understand the effect of chemical exchange on diffusion NMR experiments, we examined the signal observed in the LED pulse sequence (90°−(gradient encode)τ−90°−(diffusion)T−90°−(gradient decode)τ−90°−(eddy current)T_e−90°−acquisition). In case of no chemical exchange, the magnetization vector that precesses at the frequency of site A (ω_A) during the gradient encode delay τ will still precess at a frequency of ω_A during the gradient decode delay τ. The final NMR signal will be proportional to the volume integral over the sample:[7]

$$\int \cos(\pm\omega_A\tau \pm \gamma gz\delta)\cos(\pm\omega_A\tau \pm \gamma gz\delta)\,dz = \frac{1}{2} + \frac{1}{2}\int \cos(2\omega_A\tau + 2\gamma gz\delta)\,dz \quad (1)$$

where γ is gyromagnetic ratio, g is gradient amplitude, δ is gradient pulse width ($\delta \le \tau$), and z is position along the gradient direction. The first term in the right-hand side of the equation represents the echo signal. When the gradient pulses are applied, the second term (the integration term) becomes zero provided that spin distribution is uniform along the z axis. But, when the gradient amplitude is 0, this term contributes as a cosine modulation of the signal ($^1/_2\cos[2\omega_A\tau]$) as shown in Figure 1.[8] Compared to ^{1}H spectra of the Vancomycin and DDFA (tetrapeptide) mixture obtained by single pulse sequence (Figure 1a), the signal intensities in the LED experiment (Figure 1b) are cosine modulated. For example, the peaks around 2.7 ppm have disappeared because the frequencies of these peaks relative to transmitter carrier frequency are around 830 Hz and encode/decode times τ are 300 μs so that the intensities become $^1/_2 + {}^1/_2\cos(2\omega\tau) \sim 0$. Because of the existence of this modulation, it is very important that $g = 0$ should not be used as the first point in LED diffusion ordered experiments. A novel application of the LED sequence could be to suppress a broad range of unwanted signals via a careful choice of a carrier frequency and encode/decode time τ.

Now consider the situation where chemical exchange takes place during diffusion delay T in the LED experiment, and the following conditions are satisfied: (a) the chemical shifts of peaks from two exchange sites are different; and (b) during encode, decode delay and eddy current delay, the chemical exchange is negligible. The final detected signal, for example, the signal at frequency ω_A, will have contributions from two components: the fraction x_A, which has the same precession frequency ω_A during both encode and decode delay, and the fraction x_B, which has precession frequency ω_B during encode delay, but precesses at the frequency ω_A during decode and acquisition time because of chemical exchange. Obviously, $x_A + x_B = 1$. The signal at ω_A is again proportional to the integral over the sample:

$$\int [x_A\cos(\pm\omega_A\tau \pm \gamma gz\delta) + x_B\cos(\pm\omega_B\tau \pm \gamma gz\delta)]\cos(\pm\omega_A\tau \pm \gamma gz\delta)\,dz \quad (2)$$

$$= \left[\frac{x_A}{2} + \frac{x_A}{2}\int \cos(2\omega_A\tau + 2\gamma gz\delta)\,dz\right] + \left[\frac{x_B}{2}\cos(\omega_A\tau - \omega_B\tau) + \frac{x_B}{2}\int \cos(\omega_A\tau + \omega_B\tau + 2\gamma gz\delta)\,dz\right] \quad (3)$$

$$= {}^1/_2[x_A + x_B\cos(\omega_A\tau - \omega_B\tau)] \text{ (when gradient is applied)} \quad (4)$$

When the gradient is applied, the two integrals in eq 3 disappear. For systems with chemical exchange, there is an extra cosine term ($^1/_2x_B\cos(\omega_A\tau - \omega_B\tau)$) on top of the echo signal ($^1/_2x_A$) perturbing the signal intensity.

For a demonstration of this chemical exchange effect on standard LED experiments, the second and fourth 90° rf pulses following the encode and decode gradients are fired immediately after the two gradient pulses (i.e., $\tau = \delta$). The result is shown in Figure 2a. The striking effect is that the well-resolved methyl signals from free DDFA (1.30 ppm) and bound DDFA (0.55 ppm), which are involved in chemical exchange, not only decay

[†] Novartis Pharmaceuticals Corp.
[‡] University of North Carolina.

(1) (a) Morris, K. F.; Johnson, C. S., Jr. *J. Am. Chem. Soc.* **1992**, *114*, 3139. (b) Soderman, O.; Stilbs, P. *Prog. NMR Spectrosc.* **1994**, *26*, 445. (c) Morris, K. F.; Stilbs, P.; Johnson, C. S., Jr. *Anal. Chem.* **1994**, *66*, 211. (d) Chen, A.; Wu, D.; Johnson, C. S., Jr. *J. Phys. Chem.* **1995**, *99*, 828.
(2) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288.
(3) (a) Lin, M.; Shapiro, M. J.; Wareing, J. R. *J. Am. Chem. Soc.* **1997**, *119*, 5249. (b) Shapiro, M. J.; Lin, M. *J. Org. Chem.* **1996**, *61*, 7617. (c) Lin, M.; Shapiro, M. J.; Wareing, J. R. *J. Org. Chem.* **1997**, *62*, 8930. (d) Gonnella, N.; Lin, M.; Shapiro, M. J.; Wareing, J. R. *J. Magn. Reson.* **1998**, *131*, 336.
(4) Johnson, C. S., Jr. *J. Magn. Reson. Ser. A* **1993**, *102*, 214.
(5) Gibbs, S. J.; Johnson, C. S., Jr. *J. Magn. Reson.* **1991**, *93*, 395.
(6) Tanner, J. E. *J. Chem. Phys.* **1970**, *52*, 2523.

(7) We assume that both coherence number of ±1 and the cosine component of magnetization are selected by the phase cycle during the gradient encode and decode delays. The effects of relaxation and diffusion are ignored during the entire discussion for simplicity.

(8) All the experiments were done at 300 K on a Bruker DMX 500 with a 5 mm inverse triple nuclear z gradient probe. The system employed here is Vancomycin (Sigma) and DDFA (molar ratio of 1:2.8) dissolved in 99.9%-*d* D_2O.

S0002-7863(98)00941-X CCC: $15.00
Published on Web 08/19/1998

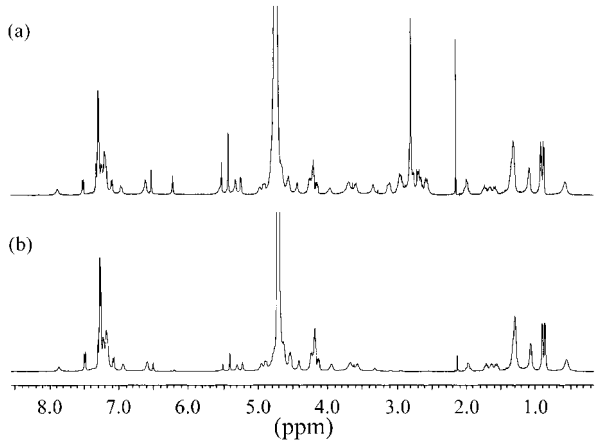

Figure 1. (a) Single pulse ^{1}H NMR spectrum of the Vancomycin and DDFA mixture (1:2.8). (b) 1D ^{1}H spectrum of the LED pulse sequence with no gradient applied, $\tau = 300$ μs, $T = 100$ ms, and $T_e = 5$ ms.

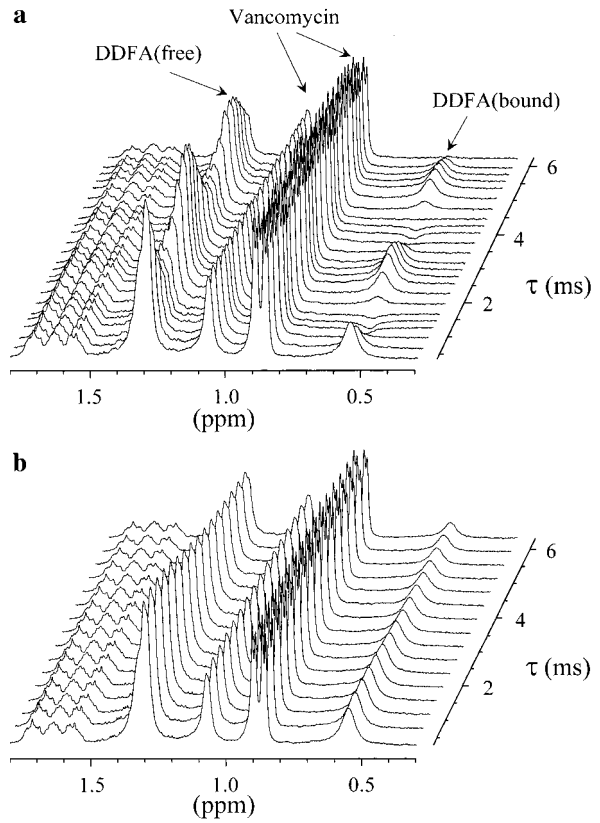

Figure 2. Stack plot of ^{1}H spectra of the Vancomycin and DDFA mixture as τ increases with (a) LED pulse sequence (τ increases from 0.3 to 6.2 ms) and (b) bipolar LED pulse sequence (τ increases from 0.4 to 6.4 ms). $T = 100$ ms, $g = 5.8$ G/cm, and $T_e = 5$ ms in both experiments.

with respect to transverse relaxation time τ (T_2 relaxation) and gradient pulse area (diffusion), but also oscillate at a frequency of 373 Hz (the chemical shift difference between the two methyl groups on the 500 MHz instrument). On the other hand, the peaks from Vancomycin are free from this kind of modulation (the J modulations are negligible because the transverse evolution time τ is kept very short).

This oscillation effect of DDFA is a direct result from chemical exchange and it can have severe impact on the diffusion experiments. In the case where τ varies during diffusion experiments, oscillation of the signal, as shown in Figure 2a, is a serious problem in diffusion measurements in addition to T_2 relaxation. In the case where τ is held constant, serious signal intensity distortion will occur (with maximum relative signal loss of $2x_B$ for resonance ω_A), and even negative signals can result

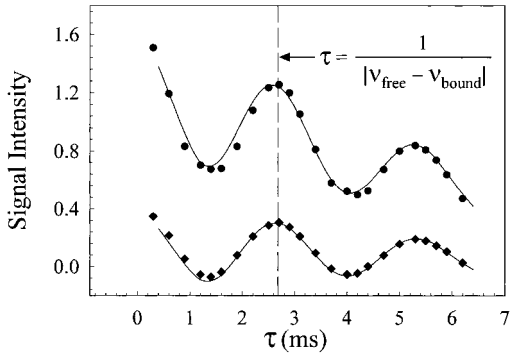

Figure 3. Simulation of signal modulation in LED experiment. Signal intensities of free (●) and bound DDFA (◆) are plotted as a function of τ. The solid line represents the simulation curve according to eq 4 with $x_{bound} = 36\%$, $x_{free} = 64\%$, and data from the bipolar LED experiment to approximate the relaxation and diffusion decay of the signal. The dashed line indicates the τ value of one oscillation, which is the reciprocal of frequency different between free and bound DDFA in hertz ($|\nu_{free} - \nu_{bound}|$).

when $x_A < x_B$. Depending on the selection of the encode and decode time τ, the NMR signal from molecules involved in exchange can totally disappear, even when a very small gradient is present, leading to misinterpretation (perceived as much faster diffusion rate). Severe sensitivity loss will also result in LED based diffusion ordered and edited experiments. These conclusions also apply to stimulated echo (STE) based experiments.

The modulation problem can be solved by using the bipolar LED pulse sequence,[9] provided that chemical exchange during encode and decode delay τ and eddy current delay can be ignored. In the bipolar LED experiment, each monopolar gradient pulse in the LED pulse sequence is replaced by a pair of gradients with opposite polarity and separated by a 180° rf pulse. During the encode and decode delay τ, the chemical shift evolution is refocused by the 180° rf pulses, and in eq 2, only those terms with gradients are left:

$$\int [x_A \cos(\pm\gamma g z \delta) + x_B \cos(\pm\gamma g z \delta)] \cos(\pm\gamma g z \delta)\, dz = {}^1/_2 + {}^1/_2 \int \cos(2\gamma g z \delta)\, dz = {}^1/_2 \quad (5)$$

and the integral approaches zero as discussed previously. The result is a pure echo signal without the cosine modulation. Figure 2b shows the NMR spectra of the mixture obtained with the bipolar LED pulse sequence as a function of τ. Signals arising from bound and free DDFA now decay with time delay τ as a result of relaxation and diffusion, in the same manner as the Vancomycin peaks, and the chemical shift modulation is completely removed. As a result, the selection of delay τ will not have significant impact on the result and interpretation of the experiments.

The intensity oscillation in the LED experiment was simulated (Figure 3) according to eq 4.[10] The excellent fit of the simulated curve to the LED experimental data confirms this exchange hypothesis. The bipolar LED sequence can totally negate this exchange effect and retain diffusion information without intensity distortion. Therefore, in diffusion experiments, the bipolar LED (or bipolar STE) pulse sequence is a much better choice to provide cleaner spectra that are free of chemical exchange modulation and less prone to misinterpretation.

JA9809410

(9) Wu, D.; Chen, A.; Johnson, C. S., Jr. *J. Magn. Reson.* **1995**, *A115*, 260.

(10) Under the experimental conditions, the chemical exchange during delay T is so fast that the bound and free DDFA are totally redistributed according to the equilibrium binding ratio between two sites. The binding ratio information was determined from ^{1}H NMR spectrum.