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Separation of Chemical Exchange and Cross-Relaxation Effects in Two-Dimensional NMR Spectroscopy

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Recently, Bothner-By and co-workers described an experiment for measuring nuclear Overhauser effects under spin-locked conditions (*1*). An advantage of this NOE experiment over conventional NOE experiments is that spin-locked NOEs are always positive and monotonically increase with increasing motional correlation time. In contrast, the regular NOE effect is positive for fast motion ($\omega\tau_c < 1$) and negative for slow motion ($\omega\tau_c > 1$), where ω is the angular Larmor frequency and τ_c is the motional correlation time.

In the phase-sensitive presentation of a 2D *spin-locked* NOE spectrum, cross peaks that are due to NOE therefore have opposite phase relative to diagonal peaks, independent of τ_c (*1–3*). However, in analogy with the NOESY experiment (*4*), cross peaks due to chemical exchange can also occur in such a 2D experiment and will be in phase with the diagonal resonances (*5, 6*). For macromolecules the regular NOEs are usually negative ($\omega\tau_c > 1$), and in the NOESY spectrum cross peaks due to chemical exchange are basically indistinguishable from NOE cross peaks (*4*). We demonstrate that the phase of the cross peak in a spin-locked NOE experiment can be advantageously used to discriminate between NOE and exchange. The mechanism for distinguishing between NOE and exchange is fundamentally different from *zz* spectroscopy, recently introduced by Bodenhausen *et al.* (*7*), a technique that under certain conditions also allows one to separate NOE and exchange effects.

As an example, Fig. 1 shows 2D contour plots of the spin-locked NOE spectrum of the aromatic region of bovine trypsin inhibitor (M_r 6500) recorded at 42°C, with a spin-lock time (mixing period) of 75 ms. Figure 1a shows all resonances that are in phase with the diagonal peaks, and one can clearly see two sets of exchange cross peaks between the chemically nonequivalent δ_1 and δ_2 protons (7.76 and 6.68 ppm) and the ϵ_1 and ϵ_2 protons (6.77 and 6.83 ppm) of Tyr-35 (*8, 9*). Based on the equations given by Jeener *et al.* (*3*), one finds a two-site exchange rate, k_{ex} of 8.5 ± 0.5 s^{-1}. From rate constants determined similarly at temperatures between 17 and 57°C we estimate an activation energy of 15.3 ± 0.8 kcal/mol. These values for the rate constant and the activation energy are somewhat lower than those reported previously by Wagner *et al.* (*9*) who used lineshape analysis to estimate the exchange rates.

Figure 1b shows the negative contours of the same spectrum. Here one sees several cross peaks due to spin-locked NOE between the δ and ϵ protons of Tyr-10 (7.33 and

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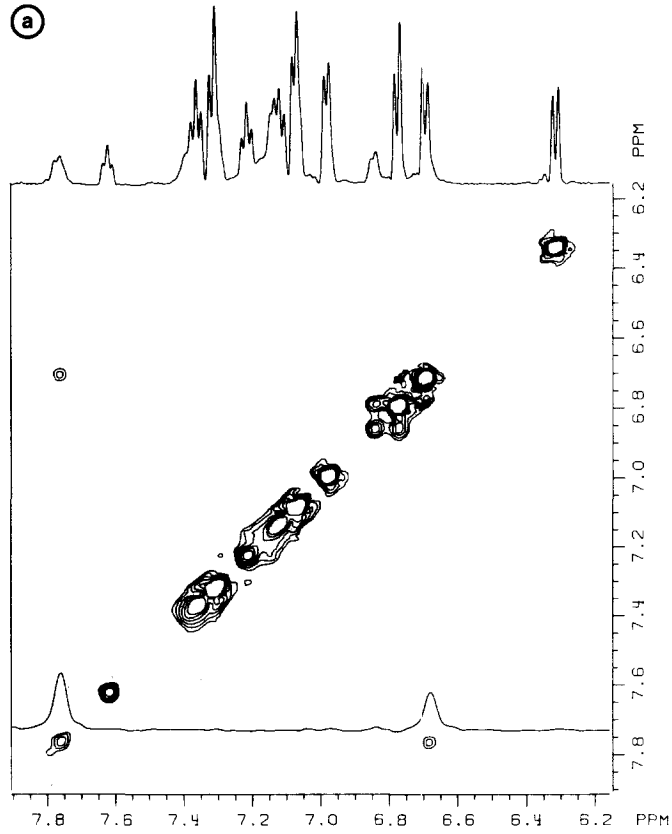

FIG. 1. Aromatic spectral region of the phase-sensitive 2D spectrum of the bovine trypsin inhibitor obtained with a spin-locked mixing period of 75 ms. (a) Resonances that are in phase with the diagonal display chemical exchange, i.e., ring flipping of Tyr-35. (b) Resonances opposite in phase relative to the diagonal, displaying spin-locked NOE between vicinal δ and ϵ protons of the fast flipping aromatic rings of the Tyr and Phe residues.

7.07 ppm), Tyr-23 (7.18 and 6.32 ppm), Tyr-21 (6.70 and 6.77 ppm), and Phe-4 (6.98 and 7.37 ppm).

Some experimental details of the spin-locked NOE experiment have been described previously (*2*). In the present example, we used a modest spin-lock field of ca. 3 kHz, with the carrier offset by 1 kHz from the center of the aromatic spectral region. These conditions were chosen to minimize coherence transfer from homonuclear Hartmann Hahn cross polarization (*2, 3*).

In summary, we have shown that the 2D version of the spin-locked NOE experiment can be usefully applied to the study of chemical-exchange phenomena in moderately large molecules with complex NMR spectra. Because magnetization transfer in the rotating frame that is due either to chemical exchange or to cross relaxation gives cross peaks that are 180° out of phase relative to one another, the origin of these cross peaks is readily identified when the phase-sensitive presentation is used. This type of experiment can be particularly useful for studying the kinetics of protein and nucleic acid unfolding and refolding, examples where one can anticipate a large number of NOE

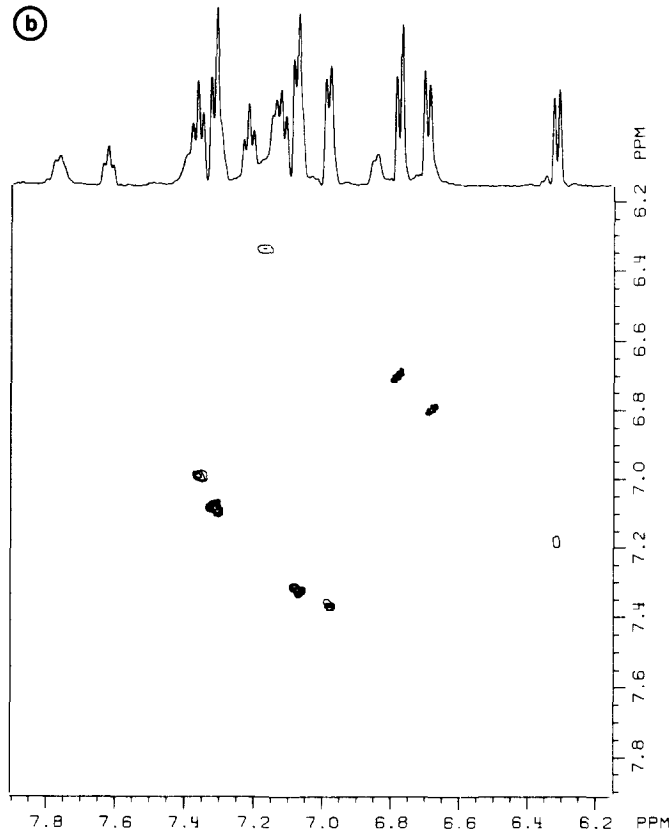

FIG. 1—*Continued.*

and chemical exchange cross peaks whose identity is often less apparent than in the bovine trypsin inhibitor. In any case, there would be no ambiguity as to their origin if examined by this technique.

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