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Studies of slow conformational equilibria in macromolecules by exchange of heteronuclear longitudinal 2-spin-order in a 2D difference correlation experiment

Gerhard Wider, Dario Neri and Kurt Wüthrich

Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule – Hönggerberg, CH-8093 Zürich, Switzerland

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SUMMARY

Observation of the exchange of heteronuclear longitudinal 2-spin-order in a 2D difference correlation experiment enables studies of slow dynamic processes in biological macromolecules with minimal interference from background signals. The experiment is used to establish relations between corresponding ^{15}N–^{1}H groups in the native globular form and an unfolded form of the protein 434 repressor(1–69) present in aqueous solution containing 4.2 M urea.

Chemical-exchange NMR spectroscopy has long been used to measure equilibrium constants and interconversion rates for chemical systems in slow equilibrium (Gutowsky and Saika, 1953); and a variety of one-dimensional and two-dimensional (2D) homonuclear exchange experiments have been proposed (e.g., Forsen and Hoffman, 1963; Macura and Ernst, 1980; Wagner et al., 1985). For work with macromolecules, these homonuclear NMR experiments are often limited by spectral overlap. More recently, 2D chemical exchange experiments combined with proton-detected heteronuclear correlation have been introduced (Montelione and Wagner, 1989), where the chemical shift of the heterospin is used to improve the spectral resolution. However, in practice these experiments tend to be limited again by the fact that the strong direct correlation signals are a source of unwanted t_1 noise and may obscure the usually much smaller exchange peaks. The present note introduces a difference experiment for observation of exchange of heteronuclear longitudinal 2-spin-order, which alleviates such limitations. As an illustration, the experiment is used to study the exchange of ^{15}N–^{1}H 2-spin-order between the native and a urea-unfolded form of a protein. This application is of practical interest, since uniformly ^{15}N-labeled proteins are often

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readily available, and the N–H entity is a suitable probe for investigations of the polypeptide backbone.

The experiment used here (Fig. 1) uses elements of the aforementioned exchange measurement by Montelione and Wagner (1989) (Fig. 1A) and of difference spectroscopy with the strategy previously described for the suppression of the diagonal peaks in homonuclear NOESY spectra (Bodenhausen and Ernst, 1982; Denk et al., 1985). In the experimental scheme of Fig. 1A, proton magnetization, I_z, is initially transferred into antiphase single-quantum coherence, I_zS_y, of the heterospin S. The term I_zS_y evolves during t_1, and is then converted to heteronuclear 2-spin-order, I_zS_z. After the mixing time, τ_m, I_zS_z is converted into antiphase proton coherence, I_yS_z, which is then refocused into in-phase proton coherence and detected. If the entity of the two spins I and S undergoes chemical exchange during the mixing time, this will be manifested in the spectrum by two cross peaks which connect the two direct correlation peaks in a rectangular pattern. This experiment differs from the chemical exchange experiment of Montelione and Wagner (1989) by the fact that heteronuclear single-quantum coherence is selected during the evolution period instead of heteronuclear multiple-quantum coherence (HMQC). As a result, proton homonuclear scalar couplings do not evolve during t_1, which normally results in narrower linewidths along ω_1. As an additional improvement, the spin lock pulse after τ_1 purges the spectrum of coherence from protons that are not coupled to a heterospin (Otting and Wüthrich, 1988), and suppresses the water resonance to such an extent that no additional water suppression is needed for short mixing times τ_m (Messerle et al., 1989).

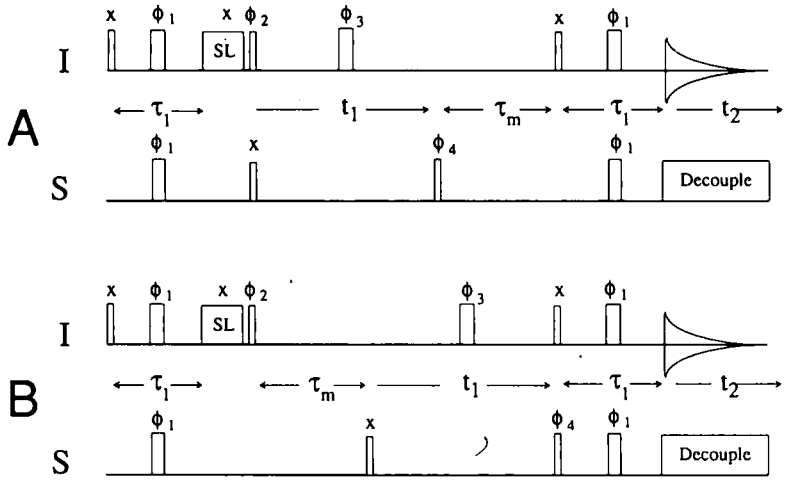

Fig. 1. Scheme used for the observation of exchange of heteronuclear longitudinal 2-spin-order in a 2D difference correlation experiment. (A) Heteronuclear COSY relayed with exchange of heteronuclear longitudinal 2-spin-order. (B) Delayed heteronuclear COSY. The observed difference spectrum corresponds to A–B. The vertical bars represent radiofrequency pulses, where the different pulse lengths ($\pi/2$, π, spin lock pulse SL) are distinguished by the widths of the bars. The phases of the pulses are indicated above the pulse symbols. The phases φ_1 and φ_2 were independently alternated between y and $-$y, and the phases φ_3 and φ_4 between x and $-$x. The delay τ_1 was tuned to $1/[2^1J(S,H)]$. The phases of the first two pulses on S are subjected to time-proportional phase incrementation (TPPI; Marion and Wüthrich, 1983). Heteronuclear decoupling is applied during the evolution and detection periods.

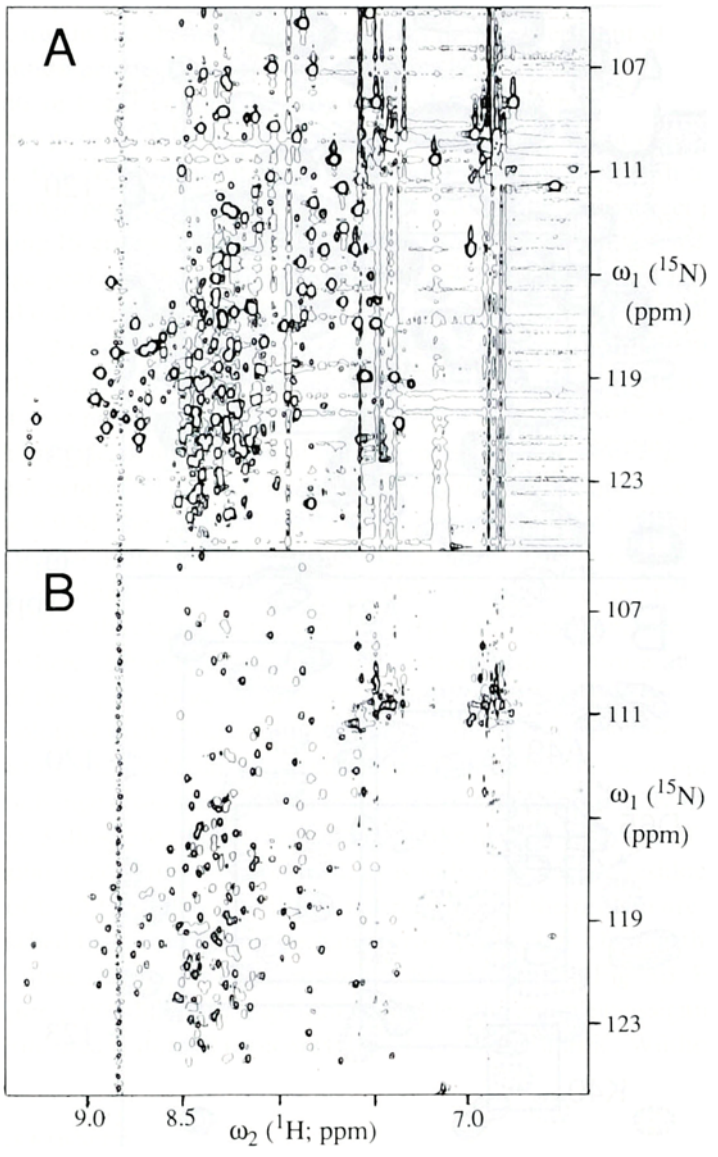

Fig. 2. (A) 2D exchange-relayed [^{15}N,^{1}H]-COSY spectrum recorded using the experimental scheme of Fig. 1A with uniformly ^{15}N-labeled 434 repressor(1–69) (protein concentration 4 mM; solvent 10% D_2O/90% H_2O; 4.2 M urea; 20 mM $NaClO_4$; pH 4.8; T = 18 C). A Bruker AM-600 instrument was used and 150 t_1-values were collected, with t_{1max} = 54 ms and t_{2max} = 131 ms. The measuring time was 18 h. Before Fourier transformation, the time-domain data were multiplied with sine-bell windows (DeMarco and Wüthrich, 1976) along both t_1 and t_2; with phase shifts of $\pi/5$ and $\pi/9$, respectively. (B) 2D difference spectrum obtained by subtraction of a data set obtained with the pulse sequence of Fig. 1B from the data set of Fig. 2A. The two spectra were recorded in the interleaved mode with identical experimental parameters, and plotted at the same ground contour level. Negative signals are plotted with a single contour level.

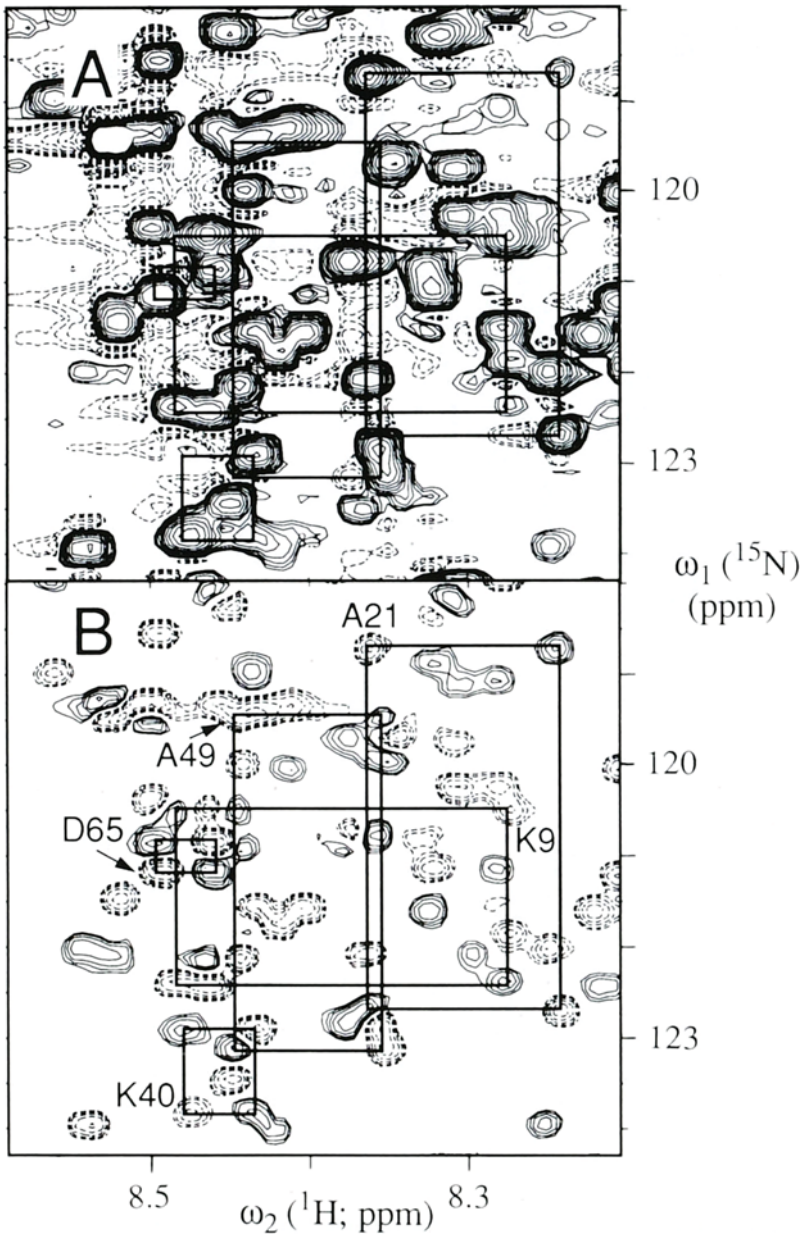

Fig. 3. Region ($\omega_1 = 118.0$–124.3 ppm, $\omega_2 = 8.2$–8.6 ppm) of (A) spectrum 2A and (B) spectrum 2B on an expanded scale. Solid lines represent positive contour levels corresponding to exchange peaks, and the broken lines stand for negative levels corresponding to direct correlation peaks. The rectangular patterns illustrate how corresponding peaks in the urea-unfolded form and the native protein (identified by the one-letter amino acid code and sequence number) can be related by simple rectangular patterns.

The second experiment needed to obtain the desired difference spectrum deviates from the scheme of Fig. 1A, in that the order of the mixing time τ_m and the evolution time t_1 is interchanged (Fig. 1B). As a consequence, the longitudinal 2-spin-order I_zS_z is not frequency-labeled, and the intensity that is located in the chemical exchange peaks in the experiment of Fig. 1A remains part of the direct correlation peaks. Therefore, the difference between the two data sets resulting from the two experiments in Fig. 1 contains greatly reduced direct correlation peaks: the residual intensity of each direct peak is equal to but opposite in sign to the sum of the intensities of the exchange cross peaks that derive from it (in Figs. 2 and 3 we show data with a two-state system, where a single exchange peak derives from each direct peak), and hence direct peaks corresponding to nonexchanging IS groups are completely suppressed. The absolute sensitivity for observation of the exchange peaks in the difference spectrum is 0.5 times that in Fig. 1A, because these peaks are measured only during half of the total accumulation time and the random noise is effectively increased by $\sqrt{2}$ when the difference is computed. However, this reduction of the absolute sensitivity is largely compensated by the suppression of unwanted artefacts arising from the strong direct correlation peaks in the experiment of Fig. 1A.

Figures 2 and 3 illustrate an application of the difference experiment of Fig. 1 for studies of partially denatured, uniformly ^{15}N-labeled 434 repressor(1–69). In the presence of urea concentrations from 3.0 M to 6.0 M, the ^1H NMR spectrum of this protein consists of two sets of resonances corresponding, respectively, to the spectrum of the native protein recorded in the absence of urea, and to a urea-unfolded form of the protein. These two forms of the protein are in slow exchange. Figure 2A shows the exchange-relayed [^{15}N,^1H]-COSY spectrum recorded with the experiment of Fig. 1A, where strong direct ^{15}N–^1H correlation peaks, t_1-noise and baseline artifacts cover a large part of the ω_1–ω_2 plane. In the corresponding difference spectrum (Fig. 2B; note that negative peaks are plotted with a single contour line) the intensity of the direct peaks, the t_1-noise and the baseline distortions are drastically reduced (the t_1-noise band at 8.88 ppm is an instrumental artifact). The exchange cross peaks are much better resolved and readily identifiable. The expanded plots in Fig. 3 illustrate how corresponding peaks of the two forms of the protein can be related. A rectangular peak pattern is obtained, as is indicated for five residues. Since the direct correlation peaks of both the native and the denatured form of the protein are negative, they can readily be distinguished from the positive exchange cross peaks. It is obvious that it is much more difficult to identify these relations in the spectrum of Fig. 3A than in Fig. 3B. For the 434 repressor(1–69) in 4.2 M urea, this difference experiment enabled the complete assignment of the ^1H and ^{15}N resonances of the urea-unfolded protein (D. Neri, G. Wider and K. Wüthrich, to be published).

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