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Band-selective editing of exchange-relay in protein-water NOE experiments

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Abstract

A pulse sequence is proposed which uses a train of band-selective pulses for the editing of slow chemical exchange-relay effects in experiments designed to study water-macromolecule interactions. Compared to previous methods, this experiment does not require knowledge of the exact chemical shift of the relaying labile protons and needs only the recording of a single experiment to edit the relay through different exchanging groups resonating at different frequencies. The pulse sequence has been implemented using Gaussian cascades and was applied to the study of the hydration of HEW lysozyme.

The structure and dynamics of biomolecules are significantly affected by the aqueous environment (Otting et al., 1991; Gerothanassis, 1994). Hydrogen bonds, electrostatic screening and hydrophobic effects (Billeter, 1995) are profoundly influenced by water. In addition, proteins (Brooks et al., 1988) and DNA (Berman, 1994) often contain hydration water molecules as an integral part of their structure. The structure and kinetics of water molecules in the proximity of macromolecule protons can be investigated through the measurement of water-solute NOEs (Pitner et al., 1974; Glickson et al., 1976; Otting and Wüthrich, 1989; Otting et al., 1991; Otting, 1997).

The interpretation of water-selective NOE experiments (Otting, 1997) is often hampered by the presence of intense chemical exchange-relayed NOEs arising from the two step magnetization transfer $H_2O \rightarrow XH \rightarrow H_m$, where XH denotes a nitrogen or oxygen bound labile proton and H_m any proton belonging to the macromolecular solute (Glickson et al., 1976; Johnston and Redfield, 1977; Stoez and Redfield, 1978; Boelens et al., 1985; Van de Ven et al., 1988; Otting and Wüthrich, 1989; Otting et al., 1991; Otting, 1997). Similarly to spin-diffusion effects, the exchange-relayed NOEs are formally a second order effect; however their intensity is often comparable or higher than that of direct NOEs between water and macromolecule protons because the rates of proton exchange with water can significantly exceed those of dipolar cross-relaxation (Liepinsh and Otting, 1996).

At present there are two independent methods to rule out exchange-relayed contributions to the observed NOEs. One approach is structure-based and treats as ambiguous all NOEs to solute protons within a radius of 4.5 Å from labile groups XH in chemical exchange with water (Otting et al., 1991; Otting and Liepinsh, 1995). The other method does not rely on the three-dimensional structure and uses continuous wave irradiation or selective inversion of the relaying XH proton to quench undesired two-step transfers (Olejniczak et al., 1986; Massefski and Redfield, 1988; Fejzo et al., 1991; Dalvit, 1998; Melacini et al., 1998). The latter approach has the advantage of providing insight into hydration in the proximity of labile groups, but it presupposes exact knowledge of the chemical shift of the relaying XH labile proton. This requirement cannot be always fulfilled if the XH resonance is in a crowded spectral region. In addition, multiple experiments are required to suppress chemical-exchange relay through XH groups resonat-

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ing at different chemical shifts. This communication shows how these limitations can be overcome using a train of band-selective pulses for the simultaneous inversion of all relaying labile XH groups resonating in a well-defined frequency band.

Hyperbolic secant inversion pulses have been previously employed to suppress spin diffusion by splitting the cross-relaxation network of a macromolecule into two non-interacting subsets (Macura et al., 1992; Hoogstraten et al., 1993). Alternatively, spin diffusion can be quenched using an audiomodulated band-selective pulse which performs a simultaneous double selective inversion of a selected spin pair (Zwahlen et al., 1994; Vincent et al., 1996). When band-selective pulses are used to edit relay through chemical exchange the delay between subsequent inversions must be shorter than the shortest characteristic lifetime of the relaying XH spins. This condition ensures a linear regime for magnetization exchange and an efficient decoupling of the inverted protons from the rest of the relaxation network (Fejzo et al., 1991; Zwahlen et al., 1996). Therefore chemical exchange-relay is best edited by band-selective pulses characterized by small inversion bandwidth-pulse duration products, such as the Gaussian cascades G^3 or Q^3 (Emsley and Bodenhausen, 1990, 1992).

Gaussian cascades inversion pulses have narrow transition regions between the effective inversion frequency band and the two flanking non-inverted regions of the spectrum (Emsley and Bodenhausen, 1990, 1992). This property is especially relevant in the context of water-selective NOE experiments because at the fields commonly used for high resolution NMR the relaying Thr or Ser hydroxyls in slow chemical exchange and the water protons resonate within a few hundred Hz from each other (Liepinsh et al., 1992). However, even though the Gaussian cascade shape ensures that spins resonating in the non-inverted bands are brought to their original $+z$ or $-z$ orientation at the end of the pulse, during the band-selective pulse itself magnetization is still subject to excursions about the longitudinal axis (Figure 1).

Spin evolution during the shaped pulses is especially significant when a train of multiple inversion pulses is used during the NOE mixing period. Specifically, three major effects need to be considered: a) Losses of magnetization caused by longitudinal and transverse cross-relaxation during the band-selective pulses (Zwahlen et al., 1994); b) Projection averaging of longitudinal and transverse cross-relaxation rate constants; c) If difference spectroscopy is used,

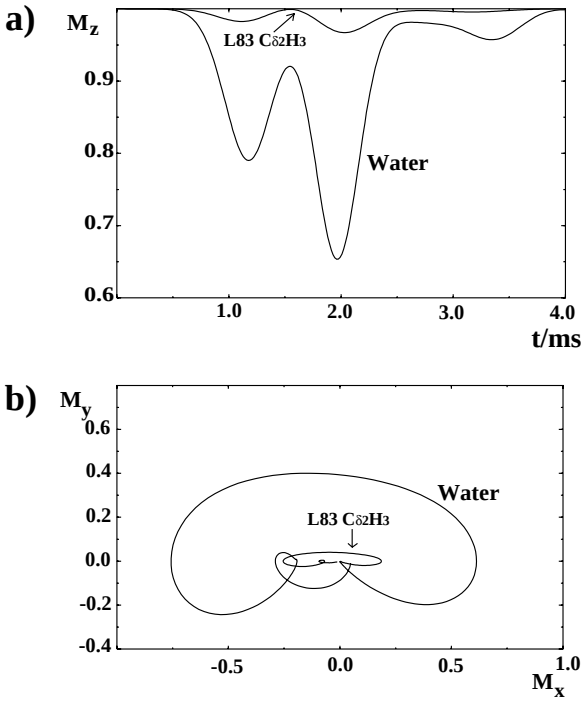

Figure 1. Evolution of the magnetization of a single spin during a 4 ms inversion Gaussian cascade G^3 pulse with a maximum peak amplitude of 772 Hz acting on an unit vector initially aligned with the $+z$ axis. The pulse shape was generated with the program 'Shape' (Bruker, Inc.) and the trajectories were computed using the program 'Pulsetool' with two representative offsets: -1060 Hz (water resonance) and -3026 Hz (L83 $C_{\delta 2}H_3$ resonance). a) Evolution of the M_z component. b) Evolution of the transverse component. Here time is used as a parameter.

transient or residual transverse components of the water magnetization can trigger radiation damping during the NOE mixing time. Effects a) and b) can be compensated for by calibration using complementary experiments in which two consecutive band-selective pulses are applied during the mixing time. Two consecutive inversions are to first approximation a unity operation; therefore the comparison of this calibration spectrum with a reference NOE experiment run without band-selective pulses allows to estimate the correction factor *k* associated with each couple of band-selective pulses. If *n* couples of band-selective pulses are present in the NOE mixing time, the total correction factor is then estimated to be k^n (Zwahlen et al., 1994). The effect of radiation damping (point c)) can be prevented by inserting G_z gradients between the band-selective pulses. Alternatively, gradients can be used for coherence selection (Dalvit, 1995) so that water magnetization is dephased during the NOE mix-

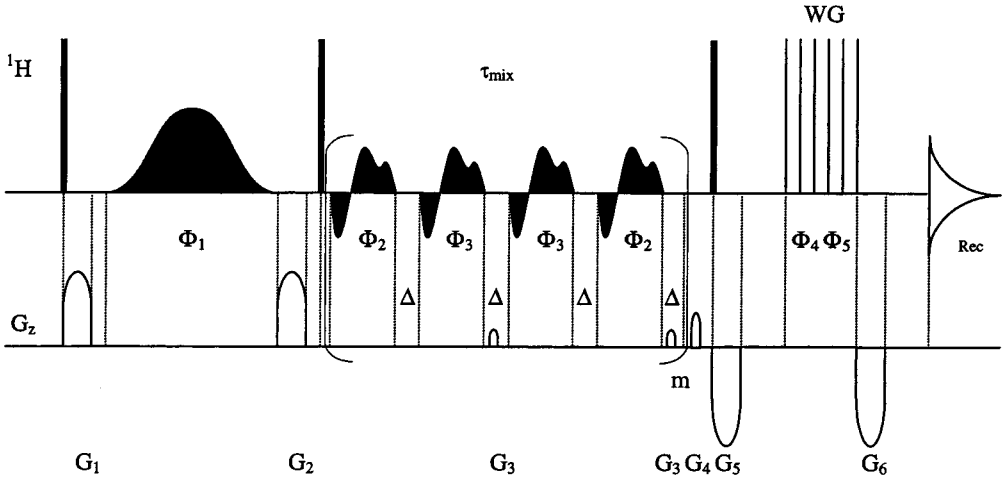

Figure 2. Pulse sequence for the slow chemical exchange-relay edited water-selective NOE experiment. Thick vertical bars indicate 90° hard pulses. Between the first two 90° pulses water is selectively inverted by a 50 ms Gaussian pulse (Dalvit, 1995). During the mixing period a train of band selective G^3 Gaussian cascades repetitively inverts labile protons in slow chemical exchange with water. Gradients denoted as G_3 and G_4 dephase unwanted transverse magnetization and prevent radiation damping during the mixing period. Water is suppressed before acquisition using the Watergate block implemented with the 3–9–19 pulse train (Sklenar et al., 1993). All gradients have a sinus amplitude profile. G_1, G_2, G_5 and G_6 last 2.4 ms, while G_3 and G_4 have a duration of 0.4 ms. The strengths of G_1-G_6 are: 4.3 G/cm, 4.3 G/cm, 1.1 G/cm, 2.2 G/cm, −5.8 G/cm and −5.8 G/cm, respectively. Every gradient is followed by a delay for gradient recovery before pulses are applied; for G_1-G_4 this is 0.4 ms, for G_5 and G_6 it is 0.8 ms. The delay Δ between the band-selective pulses must therefore be longer than (0.4 + 0.4) ms. Phase cycling is used to select the desired coherences according to the phases: Φ_1 = x, y, −x, −y; Φ_2 = y; Φ_3 = −y; Φ_4 = x; Φ_5 = −x; Φ_{Rec} = x, −x; all other phases are x.

ing time and radiation damping is eliminated at the expense of recording only half of the signal acquired with difference spectroscopy.

The principles discussed above have led to the design of the pulse sequence shown in Figure 2 employing NOE difference spectroscopy. The water magnetization is initially selected through a spin pinging block with a 50 ms selective Gaussian pulse flanked by gradients to eliminate radiation damping during inversion (Dalvit, 1996; Dalvit and Hommel, 1995). A train of G^3 Gaussian cascades is then applied during the NOE mixing time by repeating the block (G^3_y G^3_{-y} G^3_{-y} G^3_y) including two gradients. Before the hard 90° pulse at the end of the mixing time residual transverse magnetization is dephased by a stronger gradient. The intense water signal is then suppressed by the Watergate scheme (Sklenar et al., 1993) prior to acquisition.

The pulse sequence of Figure 2 has been applied to HEW lysozyme (HEWL). Using this sequence without band-selective pulses, a water-selective NOE spectrum of HEWL at pH 4.1 and 36 °C was acquired as a reference (Figure 3, top). As shown by NOESY/ROESY comparisons the peaks containing the largest chemical exchange contributions resonate between 5.9 ppm and 7.7 ppm (Otting et al., 1997) (see horizontal bar in Figure 3, bottom). In a 500 MHz spectrometer, the relaying resonances in this region can be effectively inverted by 4 ms long G^3 pulses with a maximum peak amplitude of 772 Hz and centered at the center of this frequency band. These Gaussian cascades do not invert water magnetization (Figure 1a) and generate only a small residual transverse water component (Figure 1b). The sequence of Figure 2 was therefore run using the above mentioned G^3 pulses and resulted in the spectrum shown in Figure 3, bottom. This spectrum was calibrated according to the method discussed above, leading to a correcting factor of ∼1.17 for well-resolved representative resonances in the amide and aliphatic region of the spectrum.

The efficiency of the band-selective train of pulses is supported by the observed quenching of the previously characterized (Otting et al., 1997; Melacini et al., 1998) NOE contributions relayed by different XH groups: the NOEs exchange-relayed by the hydroxyl groups resonating at the high field extreme of the XH band are effectively edited as shown by the arrows in Figure 4 at 0.97 ppm, 1.14 ppm and 1.65 ppm; the peak at 1.65 ppm is decreased only by 35% as expected on the basis of overlapping residual signals including direct NOEs with water (Melacini et al., 1998). Similarly, the 37% NOE contribution

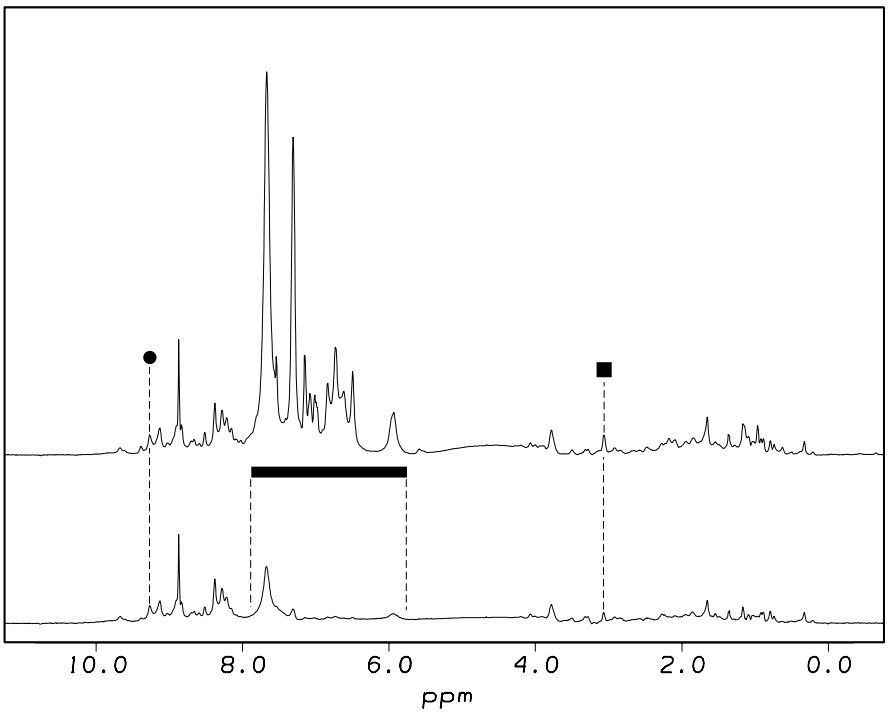

Figure 3. (Top) Reference 1D water-selective NOE experiment of HEWL in the same experimental conditions as Otting et al. (1997) but at pH 4.1. The spectrum was acquired on a Bruker AMX500 spectrometer using the sequence of Figure 1a without the band-selective pulses during the 41 ms mixing time (Dalvit, 1996). The number of scans was 5K in order to observe also weak NOEs with water molecules. The relaxation delay after acquisition was 5 s. The residual water peak was removed through convolution filtering. The spectral region indicated by the horizontal bar from 5.9 ppm to 7.7 ppm contains resonances for which the chemical exchange with water prevails over dipolar cross-relaxation, as indicated by the conserved positive sign in the corresponding ROESY experiment (Otting et al., 1997). The dot (●) indicates a representative direct NOE between water and a protein proton, I55 NH (Otting et al., 1997). The filled square (■) denotes a representative chemical exchange-relayed NOE at 3.07 ppm. A more detailed picture of the NOEs in the aliphatic region can be found in the expansions shown in Figure 4. (Bottom) 1D water-selective NOE experiment of HEWL acquired and processed as spectrum (a) but with a train of 4 ms G^3 pulses separated by 1 ms delays (pulse sequence of Figure 2). The G^3 pulses repetitively invert magnetization resonating in the 5.9–7.7 ppm band (see horizontal bar).

at 3.07 ppm exchange-relayed by the nitrogen-bound labile proton resonating at 7.67 ppm (Melacini et al., 1998) in the low field extreme of the XH band is quenched in the spectrum of Figure 3, bottom. These observations show that the band-selective pulses act effectively on the whole region of relaying XH groups. This conclusion is further confirmed by the editing of the peak at 0.65 ppm (arrow in Figure 4) which is exchange-relayed by the labile protons resonating at 7.30 ppm as indicated by 2D-e-PHOGSY-NOE-NOESY experiments.

Finally, Figure 4b shows that the train of band-selective inversions does not effectively quench NOEs exchange-relayed via labile protons resonating at the water frequency (i.e. the NOEs to the methyl protons of T47 at 1.37 ppm and of T51 at 0.34 ppm relayed by their hydroxyls in fast chemical exchange with water). In these cases either a structure-based approach is used or the sample conditions are modified in order to slow down the chemical exchange. For instance, if the temperature is lowered to 4–5 °C and the pH is brought to 6–7, the relaying Ser and Thr hydroxyls are observed as well-resolved resonances at approximately 5.4–6.2 ppm in the absence of exchange catalysts (Liepinsh et al., 1992). Under these conditions it would then be possible to edit the chemical-exchange relay processes experimentally. Work in this direction is now ongoing in our laboratory.

In conclusion, this communication shows how band-selective Gaussian cascades can be used in water-selective NOE experiments for the efficient editing of chemical-exchange relayed NOE contributions. Unlike previous experiments (Dalvit, 1998; Melacini et al., 1998), no exact knowledge of the relaying proton chemical-shift is required. In addition, no multiple experiments are needed to suppress relay through different XH groups since all relaying groups in a

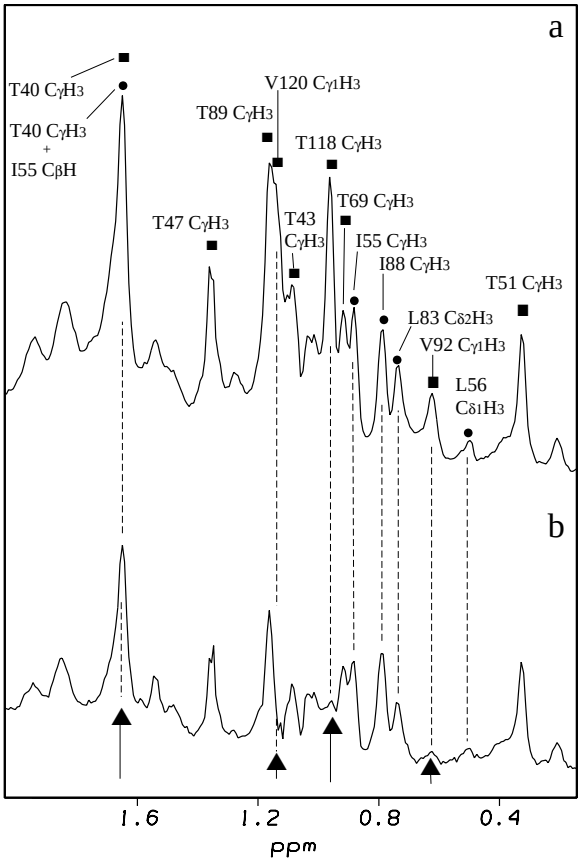

Figure 4. Expanded regions of the spectra shown in the upper and lower parts of Figure 3, respectively. Dots (●) indicate contributions to the observed peaks arising from direct intermolecular NOEs with water, while filled squares (■) denote contributions from exchange-relayed NOEs corresponding to the two-step transfer $H_2O \rightarrow XH \rightarrow$ protein proton, where X = N or O (Otting et al., 1997). Arrows indicate the exchange-relayed NOEs which are edited by the train of bandselective G^3 pulses. The same nomenclature as in Otting et al. (1997) is used for the assignments.

region of interest are simultaneously inverted by the band-selective pulses.

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