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Radiation damping compensation of selective pulses in water–protein exchange spectroscopy

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Abstract

The observation of nuclear Overhauser effects (NOEs) between bound water and biological macromolecules such as proteins and nucleic acids can be improved by inverting the water resonance selectively while compensating for radiation damping effects. The efficiency of inversion, the offset profiles, and the appearance of 2D NOE-NOESY spectra can be improved in comparison with earlier methods.

Introduction

The observation of nuclear Overhauser effects (NOEs) between bound water and biological macromolecules (Otting et al., 1991; Dalvit and Hommel, 1995; Liepinsh and Otting, 1999) provides unique insight into the role of water in biological processes. In particular, interactions between a protein and internally bound water may provide insight into the stability and structure of the fold (Englander and Kallenbach, 1984; Westhof, 1987). Such experiments critically depend on the ability to efficiently invert the magnetization of the solvent in a highly selective manner while saturating the magnetization of the solute.

The initial period of these experiments involves the selective inversion of the water magnetization. In principle, one may consider a sort of 'difference spectroscopy', where the water magnetization is inverted in odd scans, and unperturbed in even scans. In practice, this is best achieved by a selective excitation ($M_z \rightarrow M_x$) followed by a non-selective $\pi/2$ pulse with a phase alternating between $+y$ and $-y$ ($M_x \rightarrow -M_z$ on odd scans, $M_x \rightarrow +M_z$ on even scans) as shown in Figure 1a. During a first mixing period τ_{m1}, the inverted water magnetization is transferred to the solute through chemical or magnetic exchange processes. Magnetization that has been transferred to the solute during τ_{m1} may be further transferred through cross-relaxation processes during a second mixing period τ_{m2} (Otting, 1997). Some of the sequences commonly used for this purpose are illustrated in Figure 1.

Experiments designed to monitor solvent–solute interactions may be subject to artefacts arising from the failure to achieve complete saturation of the solute magnetization. Residual solute magnetization at the beginning of τ_{m1}, which may be transferred through cross-relaxation during τ_{m1}, may be responsible for additional cross-relaxation processes during τ_{m2} in a mechanism similar to spin-diffusion. The resulting signals arising from solute–solute–solute interactions may be incorrectly interpreted as arising from solvent–solute–solute interactions.

Methods

These problems may be partly overcome by applying an excitation pulse of increased selectivity to the

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Supplementary material: The software for compensation of radiation damping during selective pulses will be released with the new version of Bruker XWIN-NMR. In addition, the C program can be obtained from the authors.

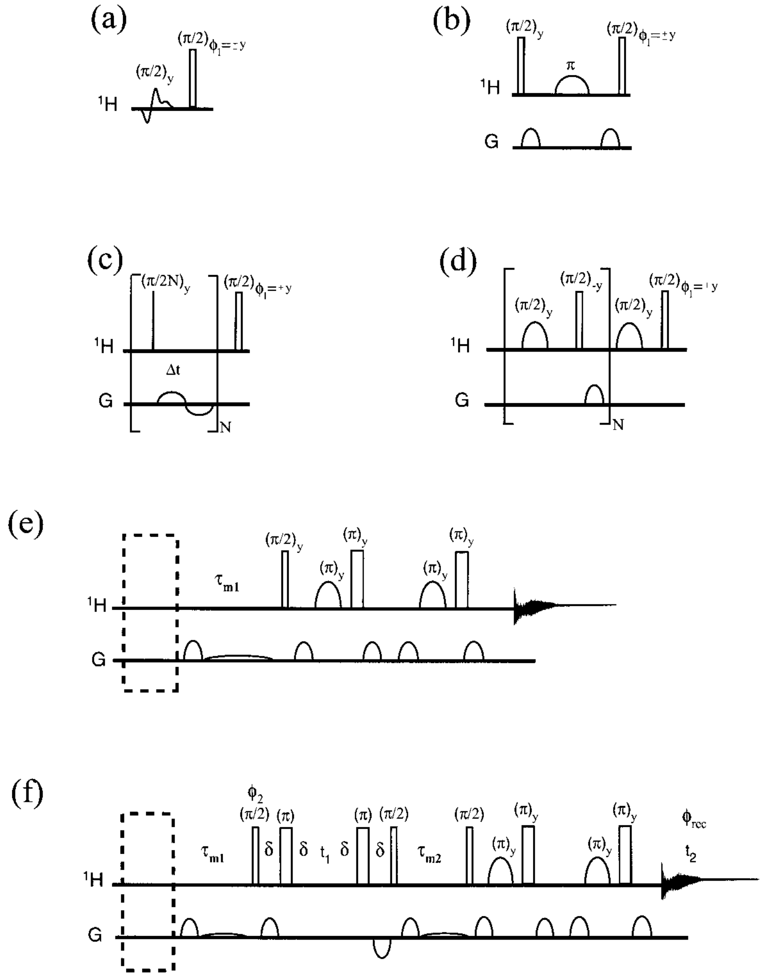

Figure 1. Pulse sequences used for the investigation of solvent–solute interactions. (a) New method with compensation of radiation damping. The solvent is inverted on alternate scans through the combination of a selective RD-compensated E-BURP-1 excitation pulse followed by a non-selective $(\pi/2)_{\pm y}$ pulse of alternating phase ($M_x \rightarrow -M_z$ or $M_x \rightarrow +M_z$ on odd or even scans, respectively). (b) Dephasing of solvent magnetization in e-PHOGSY with sine-shaped gradients and refocusing by a selective Gaussian π pulse. (c) The WANTED sequence uses a modification of DANTE, with a sine-shaped gradient of one period between successive pulses to prevent radiation damping. (d) Method proposed by Liepinsh and Otting (1999), using a series of semi-selective pulses without compensation of radiation damping repeated by an independent gradient to avoid accidental refocusing. Any of the sequences (a) to (d) can be inserted into the dashed boxes of sequences (e) and (f). Water suppression was achieved using the excitation sculpting sequence with 3 ms selective Gaussian pulses (Hwang and Shaka, 1995). (e) One-dimensional experiment for measuring exchange from solvent to solute used for Figure 3. (f) Two-dimensional version used in Figure 4. The following phases were cycled: $\phi_1 = \{y, -y, y, -y\}$, $\phi_2 = \{y, y, -y, -y\}$, with the receiver phase $\phi_{rec} = \{x, -x, -x, x\}$, all other phases being along x. The phases of the $(\pi/2)$ and (π) pulses preceding the evolution period t_1 were shifted in the manner of time-proportional phase increments (TPPI). Magnetization is gradient encoded before and after the evolution period t_1 by a pair of spin echoes with 1 ms bipolar sine-shaped gradients of ±10 G/cm to suppress radiation damping regardless of the duration of the evolution period, in a variation of a proposal by Sklenar (1995).

water signal. However, at high magnetic fields, the highly selective inversion of water magnetization is a non-trivial task because radiation damping (RD) tends to drive the magnetization back to its equilibrium position. Radiation damping arises from the radio frequency (RF) field induced in the coil by the intense precessing transverse magnetization of the solvent (Bloembergen and Pound, 1954). Once significant transverse magnetization has been created in an early part of the selective pulse, the radiation damping field is of sufficient magnitude to disturb the trajectory of the magnetization during the remainder of the selective pulse. For example, if a 50 ms pulse were used to manipulate water magnetization, this pulse would need to have an *average* RF field of 5 Hz to achieve excitation and 10 Hz for inversion. Assuming typical experimental conditions, a sample with 90% H_2O may have a radiation damping time constant (T_{RD}) of 7.5 ms for an inverse 5 mm probe in a 600 MHz spectrometer (Chen et al., 2000) leading to a maximum RF field due to radiation damping of 21 Hz. In the absence of suitable precautions, it is therefore not possible to use selective pulses that are significantly longer than T_{RD} (Mori et al., 1996) without sacrificing the quality of the offset profiles.

Several approaches have been proposed to circumvent radiation damping effects during selective excitation or inversion. The existing methods may be broadly divided into two classes. In several experiments gradients are applied in order to dephase the intense magnetization (Dalvit and Hommel, 1995; Dalvit, 1996; Mori et al., 1996; Böckmann and Guittet, 1997; Liepinsh and Otting, 1999). Among these methods, e-PHOGSY (Dalvit and Hommel, 1995; Figure 1b) is probably most widely used. Transverse magnetization is first excited by a hard 90° pulse and dephased by a strong gradient pulse before applying a selective refocusing pulse to the water magnetization, which leads to an echo after another strong gradient pulse. By applying a hard $(\pi/2)_{\pm y}$ pulse at the top of the gradient echo, the solvent magnetization is rotated alternatively to $-M_z$ and to $+M_z$. Radiation damping does not affect the refocusing pulse. One disadvantage of this method is that the efficiency of the inversion of water is attenuated by diffusion. In a variant of this idea known as WANTED (Böckmann and Guittet, 1997; Figure 1c) the $(\pi/2)$ pulse is split into N pulses, each with a nutation angle $(\pi/2N)$ in the manner of DANTE (Morris and Freeman, 1978), and oscillating gradients are applied in the intervals Δt, with a time-dependent gradient waveform so that the magnetization is refocused just before the next short pulse. Alternatively, it has been recently shown (Liepinsh and Otting, 1999; Figure 1d) that good selectivity may be obtained with a cascade of semi-selective pulses. This is achieved through an iterative process of applying a semi-selective $(\pi/2)_y$ pulse, a non-selective $(\pi/2)_{-y}$ pulse, and a gradient pulse to dephase residual transverse magnetization. The water magnetization is toggled back and forth by the series of N pulse pairs, first tipped away and then brought back to the north pole, while residual transverse magnetization (including that of the solvent) is dephased by the gradient pulses. This leaves only the water magnetization along the +z axis, so that it can be tipped into the transverse plane by a final semi-selective pulse. The magnetization is then tipped along the +z or −z axis by a non-selective pulse. This allows one to invert intense magnetization selectively, although the offset profile may not give a 'top hat' response with sharp transition regions as for pure-phase selective pulses (Emsley and Bodenhausen, 1989; Geen et al., 1989). Radiation damping may be ignored for a sufficiently short semi-selective pulse. It should be noted that a sufficient number of independent gradients must be used to prevent accidental refocusing. Alternatively, it is possible to reduce effects of radiation damping by switching the quality factor of the probe (Otting and Liepinsh, 1995). With the so-called Q-switched method, the shape of the selective pulse can be chopped up into a sequence of short pulses in the manner of a DANTE sequence while the Q factor of the probe is switched to a low value in the intervals between the short pulses.

It is also possible to manipulate intense magnetization in a selective manner without relying on gradient pulses (Abergel et al., 1995, 1996; Broekaert and Jeener, 1995; Bornet et al., 1999). If one measures the transverse magnetization which occurs during a selective pulse or any other interval in a pulse sequence, one may generate a secondary RF field with a magnitude that is proportional to the transverse magnetization through a feedback circuit. If the secondary RF field has a phase opposite to the radiation damping field, it is possible to eliminate radiation damping effects for any orientation of the intense magnetization by choosing a suitable proportionality constant.

We present a procedure which is similar in purpose to the feedback method, but which does not require any additional hardware. As previously described (Warren et al., 1989; Chen et al., 1999, 2000), it is possible to correct for the effects of radiation damping if the ideal trajectory of the magnetization during a selective pulse is known. This method, like the feed-back solution, cancels the RF field due to radiation damping by injecting a secondary field. In the feedback approach, this secondary field is produced through a separate RF channel. For the method illustrated here, the secondary RF field is superimposed directly on the waveform of the original selective pulse. In the feedback method, the magnitude of the secondary field is controlled by a proportionality constant chosen by the spectroscopist, whereas in our method, the amplitude of the secondary field relative to the original field is determined only by the value of T_{RD}. This can be determined by an inversion recovery experiment (Chen et al., 2000). If the pulses

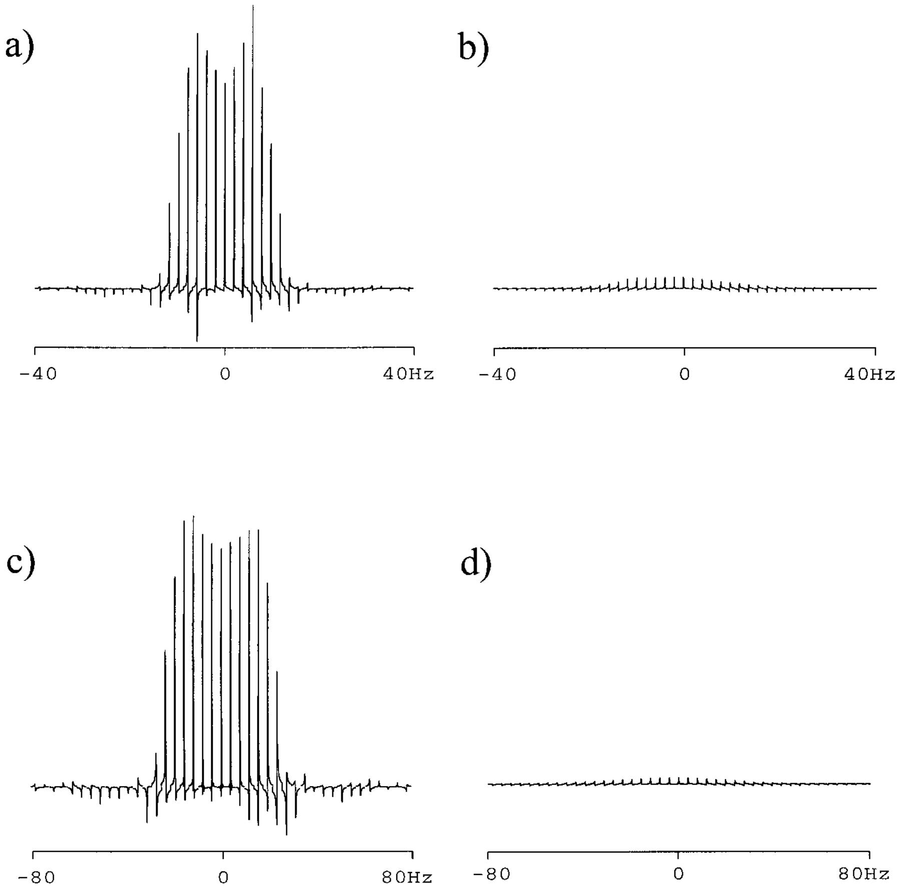

Figure 2. Offset profiles of a selective E-Burp-1 excitation pulse, measured in (a) and (b) using 200 ms pulses with a 600 MHz spectrometer equipped with a conventional probe, and in (c) and (d) using 100 ms pulses with a 500 MHz spectrometer with a cryoprobe. For the offset profiles (a) and (b), the frequency range spanned ±40 Hz from the water resonance in increments of 2 Hz, whereas for profiles (c) and (d) the range spanned ±80 Hz in increments of 4 Hz. The profiles shown in (a) and (c) were obtained using radiation damping compensated pulses as described herein. These are equivalent to the ideal behavior of a selective pulse in the absence of radiation damping. The time constant for radiation damping was determined by the inversion-recovery method (Chen et al., 2000), resulting in $T_{RD} = 7.5$ ms at 600 MHz and $T_{RD} = 2.6$ ms at 500 MHz with a cryoprobe. The compensation in (a) and (c) was performed without trial-and-error or iterative approaches. The profiles in (b) and (d) show the failure of uncompensated selective pulses in the presence of radiation damping. This is due to the weakness of the RF field compared to the RD field. These and all other figures were acquired using a solution of 2 mM lysozyme dissolved in $H_2O:D_2O = 9:1$ with 20 mM sodium acetate to maintain a pH of 4.5.

were repeated too rapidly, a steady-state would be established where the magnetization would be attenuated, which decreases the radiation damping rate R_{RD}. The efficiency of the RD compensated pulses is fairly insensitive to these variations.

Results and discussion

To achieve inversion of the water magnetization prior to the first mixing period, it is preferable to use a selective $90^\circ_{\pm y}$ pulse applied to water, followed by a non-selective $90^\circ_{\pm y}$ pulse to give either $-M_z$ or $+M_z$ magnetization and a gradient pulse to dephase residual transverse water magnetization and to dephase the magnetization of the solute. This method is preferable to a selective inversion of the water magnetization on odd scans because of artifacts associated with difference spectroscopy (Sobol et al., 1998). We were able to invert the water magnetization with an efficiency better than 92% even with highly selective pulses (Table 1). The importance of the compensation of radiation damping for a highly selective excitation pulse is illustrated in Figure 2b. Without RD compensation, a 200 ms selective E-Burp-1 excitation pulse (Geen et al., 1989) fails to excite significant transverse mag-

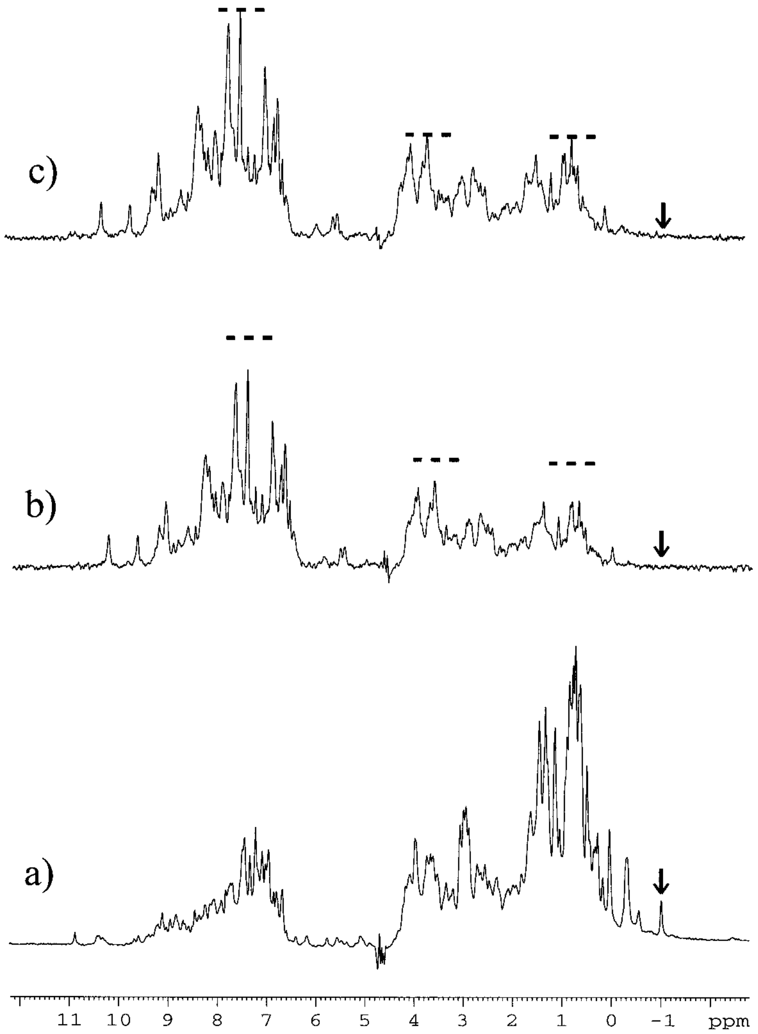

Figure 3. (a) Conventional one-dimensional proton spectrum of lysozyme at 303 K acquired with 4 scans with excitation sculpting preceded by a non-selective $\pi/2$ pulse. (b) Exchange from solvent to solute measured with the 1D experiment of Figure 1e in combination with the e-PHOGSY sequence of Figure 1b using a 40 ms selective Gaussian π pulse, sandwiched between two sine-shaped gradients of 1 ms and 6.5 G/cm. A stronger sine-shaped gradient of 1 ms and 30 G/cm was applied at the start of the mixing time $\tau_{m1} = 150$ ms, followed by a weaker sine-shaped gradient of 2 G/cm for the rest of τ_{m1}. After each pulsed field gradient, a 200 μs recovery time was inserted. A recovery delay of 2 s was used, and 32 scans were acquired. (c) Similar spectrum as in (b), using identical conditions except that the new sequence of Figure 1a was used to invert the water magnetization with a 40 ms selective E-BURP-1 excitation pulse compensated for radiation damping. Dashed lines are drawn over several peaks to aid in comparison between (b) and (c), which were both recorded with 32 scans and plotted with the same vertical scale. The gain in sensitivity in Figure 1c compared to Figure 1b is approximately 20%, a value consistent with the first row in Table 1. Note that a sharp peak in (a) at -1 ppm, which must belong to a buried methyl group, has been reduced to a level beneath the noise in (b) and (c) as shown by arrows, since there is no exchange from the solvent to a buried methyl group.

netization in a conventional probe with $T_{RD} = 7.5$ ms (Figure 2b). The problem is even more evident with a 100 ms E-Burp-1 pulse used with a cryoprobe with $T_{RD} = 2.6$ ms (Figure 2d).

The greater the efficiency of the inversion of the solvent magnetization, the more sensitive the measurement of chemical or magnetic exchange. The enhancement of the transfer of magnetization from the solvent to the solute is shown in Figure 3. The new RD compensated method is compared with the e-PHOGSY method, the latter experiment being acquired with parameters described by Dalvit and Hommel (1995) (see caption of Table 1). The RD compensated method remains efficient if the selectivity of solvent inversion is increased, in contrast to the e-PHOGSY experiment where the signal amplitudes are attenuated because of

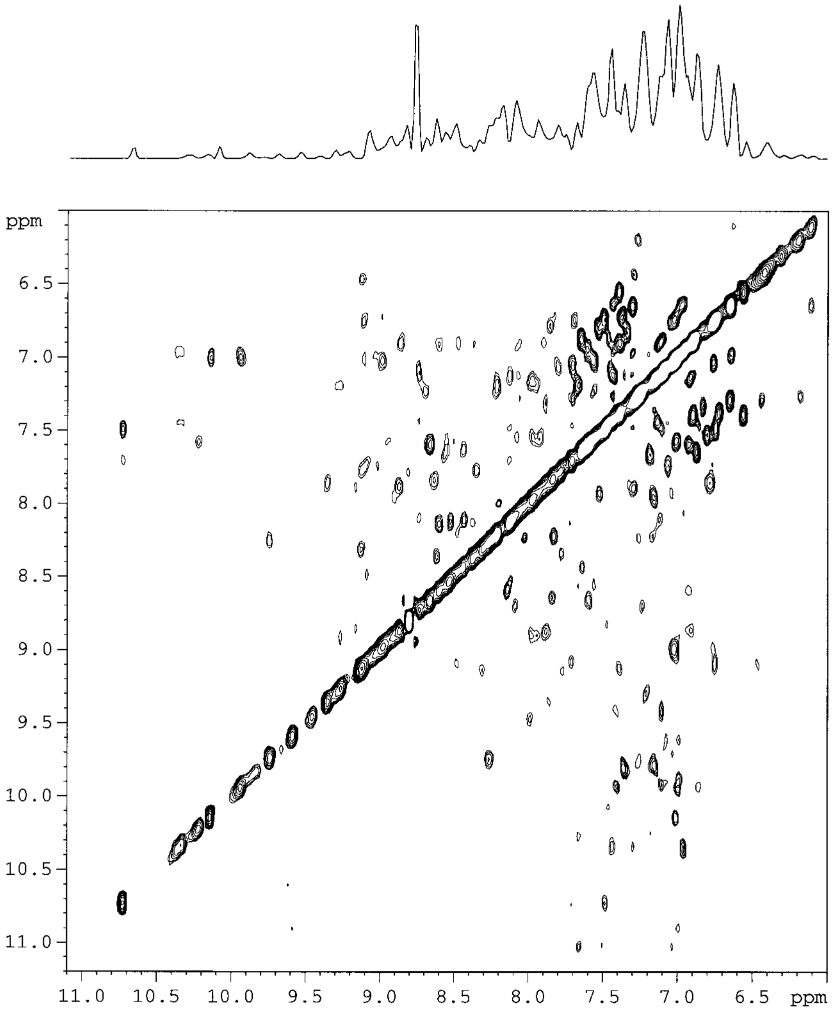

Figure 4. Low field part of a 2D exchange solvent–solute–solute experiment on lysozyme at 303 K using a 500 MHz spectrometer equipped with a cryoprobe, with the pulse sequence of Figure 1f, combined with Figure 1a. The experiment required approximately 8.5 h with 64 scans for each of 256 real data points in the t_1 domain, which spanned 15 ppm. The recycle delay was 1.5 s, the first mixing time $\tau_{m1} = 150$ ms, and the second $\tau_{m2} = 200$ ms. The spectrum was processed with States-TPPI using cosine window functions in both dimensions and zero-filling in the indirect dimension. The projection of the 2D spectrum is shown above.

diffusion of water (Stejskal and Tanner, 1965) if the duration of the refocusing pulse is increased and if the gradients are strong.

If some H^α protons in proteins are unwittingly excited by the selective pulse, they will give rise to misleading NOE peaks during the first mixing period. Attempts have been directed towards preventing complications due to cross relaxation from H^α protons and sophisticated methods have been developed (Melacini et al., 1999, and references therein). In fact, a long selective pulse may be sufficient to saturate the H^α magnetization. During the nutation caused by the rf field, the magnetization decays through a combination of T_1 and T_2 effects (Hajduk et al., 1993; Schwager and Bodenhausen, 1996). The transverse relaxation times of H^α protons in proteins are much shorter than those of water.

Compensation of radiation damping during selective pulses is possible even when radiation damping fields are strong, as demonstrated in Figure 2. The use of pulses of increasing length and hence increasing selectivity is analogous to increasing the radiation damping rate R_{RD} for a selective pulse of a given duration and selectivity. To substantiate this claim, the proposed method for observing solute–solvent interactions was performed as a two-dimensional experiment

Table 1. Efficiency of the excitation of water magnetization in the presence of radiation damping

Duration of selective pulse (ms)	Efficiency of water excitation (%)	
	e-PHOGSY with 180° Gaussian	RD-compensated E-Burp-1
40	80.7	97.8
80	69.8	95.9
120	59.5	94.7
160	50.3	93.9
200	43.8	92.8

The RD-compensated and e-PHOGSY methods are illustrated in Figure 1a and 1b, respectively. To measure transverse magnetization, the final $(\pi/2)_{\pm y}$ pulses were removed from both sequences. The percentages indicate the fraction of magnetization excited, using a selective pulse with a duration shown on the left, compared to a non-selective pulse. For the RD-compensated method, an E-BURP-1 excitation pulse was used, whereas a Gaussian refocusing pulse was used for the e-PHOGSY method. To minimise attenuation due to diffusion, 1 ms sine-shaped gradients with a maximum of 6.5 G/cm were used for the e-PHOGSY experiment, as described by Dalvit and Hommel (1995). This gradient strength is near the lower bound necessary to sufficiently defocus the solute magnetization. Stronger gradients lead to increased signal attenuation because of diffusion.

acquired with a prototype ‘cryoprobe’ operating at a temperature in the vicinity of 25 K. The lower temperature allows one to obtain a significant increase in the signal-to-noise ratio (Anderson et al., 1995), but radiation damping increases with the increased quality factor of the probe. At 500 MHz, this cryoprobe resulted in $T_{RD} = 2.6$ ms with a $H_2O:D_2O = 9:1$ sample. The same sample gave $T_{RD} = 7.5$ ms with a conventional probe at 600 MHz.

As observed in Figure 4, strong cross peaks are observed, representing magnetization that is transferred from the solvent to the solute in τ_{m1}, and is subsequently transferred in τ_{m2} through cross-relaxation within the protein. When the experiment was repeated using $\tau_{m1} = 3$ ms, both diagonal and cross peaks had vanishing amplitudes (less than 5% residual for the most intense peak).

Conclusions

We have presented an RD-compensated method to improve the sensitivity of experiments designed to monitor solvent–solute interactions. This is accomplished by modifying the shape of the selective pulse used for the initial excitation of the solvent magnetization. The implementation of the method implies a preliminary measurement of the time constant T_{RD} of radiation damping, as described elsewhere (Chen et al., 2000). The proposed method is expected to perform with equal success when applied at higher static fields. Even for very selective pulses with a duration that greatly exceeds the time constant T_{RD}, the RD-compensated method allows one to achieve a nearly quantitative inversion of the solvent magnetization.

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