Amplification of radiation damping in a 600-MHz NMR spectrometer: Application to the study of water–protein interactions

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Summary

A new application of a recently developed electronic radiation-damping (RD) control system is presented. It is possible to amplify radiation damping so as to make the water magnetization return back to its equilibrium direction in a time shorter than the characteristic RD time. Certain types of experiments involving radiation damping as a selective inversion pulse can be significantly improved by this new method. Moreover, amplification of RD is shown to improve water suppression and consequently the dynamics of 2D NOESY experiments on proteins.

Introduction

Radiation damping (RD) (Bloembergen and Pound, 1954; Bloom, 1957) has become an everyday concern over the past few years in high-field NMR of biomolecules, and has stimulated a great number of spectroscopists to find efficient methods to eliminate it. Most of these methods rely on the use of gradient pulses, which wash out the water transverse magnetization and consequently prevent RD to occur, and are obviously restricted to evolution periods. However, recently, several new methods have been proposed in the literature, which aim at eliminating the causes of RD rather than its consequences (Abergel et al., 1995; Anklin et al., 1995; Broekaert and Jeener, 1995; Louis-Joseph et al., 1995). The strategy developed in our group is to eliminate the RD field by using a simple electronic device. This system is able to detect the phase and amplitude of the RD field by analysing the water signal and to create a magnetization-dependent field in the sample, which compensates the RD field at all times. It is thus possible to cancel RD during evolution or acquisition periods of any 1D or multidimensional NMR experiment. In this article, we wish to present an original application of this RD control system, in which not only suppression but also amplification of RD at 600 MHz are used in the study of protons involved in magnetization exchange with water.

We briefly recall here the basic ideas of the method (Fig. 1), since further details can be found elsewhere (Abergel et al., 1995; Louis-Joseph et al., 1995). It is well-known that RD is caused by the interaction of solvent magnetization (the water) with the detecting network, resulting in an induced magnetic field which is perpendicular and proportional to the transverse water magnetization. The method used here is original in that a compensating field which points in the direction opposite to that of the RD field is generated from the water signal by an electronic system (Fig. 1a). In this sense the approach can be viewed as essentially relying on the manipulation of the water nuclear spins via a radiofrequency (rf) magnetic field. Moreover, since both the phase and the intensity of the correcting field are controlled, reverse use of this system can be made as well, leading to the amplification of RD. Indeed, one can think of generating an rf field, the phase of which is identical to that of the RD field, and which intensity can be made arbitrarily large (Fig. 1b). Obviously, this contribution adds to the RD field and therefore increases its effect.

Radiation damping accelerates the return of the water magnetization to equilibrium, and gives rise to what could be called an apparent spin-lattice relaxation time T_1^*, which is conveniently adapted by the RD control system. In order to avoid confusion, we shall stress that in this context, T_1^* by no means suggests that RD is an actual

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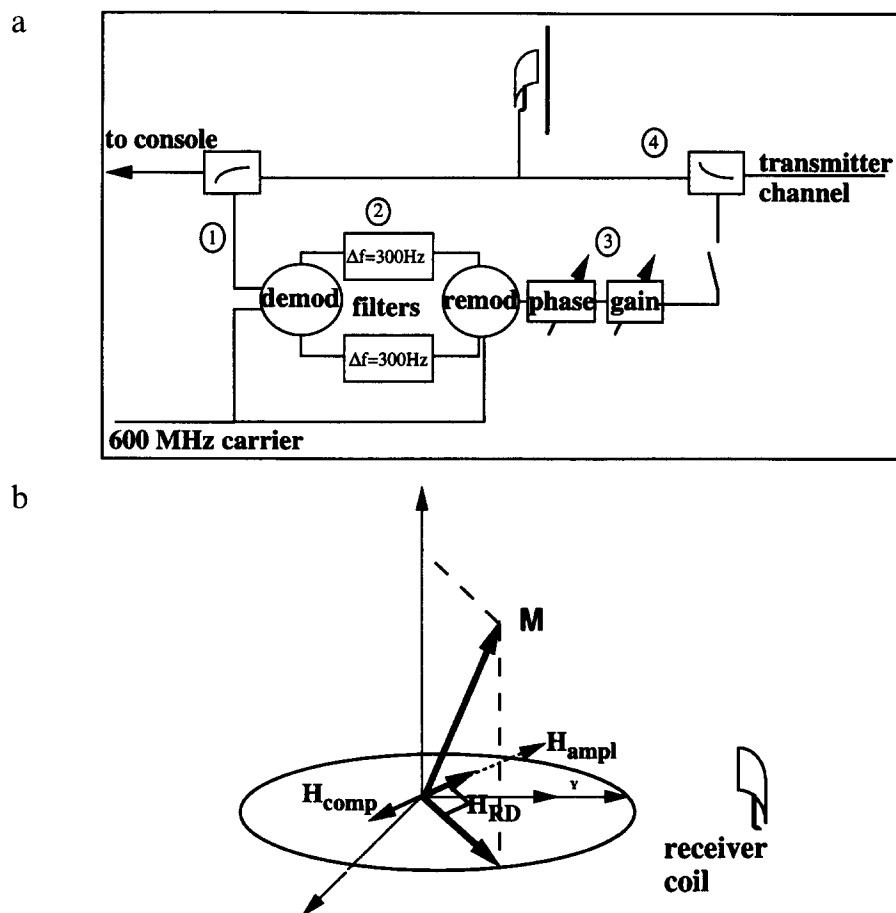*To whom correspondence should be addressed.

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Fig. 1. (a) Scheme of the radiation-damping (RD) control unit. The RD control system functions as follows: the rf signal is picked up at the output of the ^{1}H pre-amplifier receiver by a directional coupler and fed into a phase-sensitive detector in which demodulation at 600 MHz takes place. Next, the obtained real and imaginary components of the water signal are selectively retained by low-pass filters (active second-order Bessel filters with a bandwidth of 300 Hz were used for this purpose). The low-frequency water signal is then transformed back to 600 MHz. Then, after phase and gain adjustment, the signal is fed back into the probe via a directional coupler. Note that a coherent modulation is needed, and that the carrier used for modulation–demodulation must be locked to the ^{1}H carrier of the spectrometer. Phase- and amplitude-tuning facilities of the output signal are available for correction adjustment. Phase inversion of this signal, as well as activation of the system, may be controlled by the spectrometer pulse programmer. (b) The RD field lies in the xy plane and is proportional and perpendicular to the transverse water magnetization. The RD control unit is capable of generating in the sample a compensating field H_{comp}, which cancels the RD field. It can also create an amplifying field H_{ampl}, so as to accelerate the return of the water magnetization to the equilibrium direction.

relaxation mechanism. When RD is completely suppressed, T_1^* equals the actual spin-lattice relaxation time T_1. Alternatively, T_1^* can be conveniently made shorter by both inverting the phase of the compensating field and increasing its intensity.

Recently, a new kind of experiment was proposed, which aims at observing magnetization-transfer processes between water protons and protein protons (Otting and Liepinsh, 1995). Otting suggested that RD could be used in this purpose as a means of achieving 'selective inversion' of the water magnetization. A hard inversion pulse is followed by a fixed mixing delay τ_m, during which RD brings the water magnetization towards equilibrium while leaving other spins unaffected, owing to the weakness of the RD field. The return of the water magnetization to equilibrium under RD is expected to be faster than the one achieved by T_1 relaxation of the magnetization of the

protein protons of interest. Simultaneously, NOE and/or exchange between water protons and labile protons of the protein take place. A read-pulse cluster is then applied for signal detection. The resulting spectrum contains lines originating from magnetization transfer between water protons and protein protons. On the other hand, in the absence of RD, the water magnetization remains aligned along –z, and the experiment merely produces a reference spectrum. Subtraction of signals yielded by both spectra retains only a signal from those spins which undergo magnetization transfer with water (either NOE or exchange); see Figs. 2a and b.

However, it should be pointed out that this experiment suffers two limitations. Firstly, for the sake of efficiency, the delay τ_m has to be larger than the duration of complete return to equilibrium of the water magnetization. Secondly, the RD field has only weak intensity and may
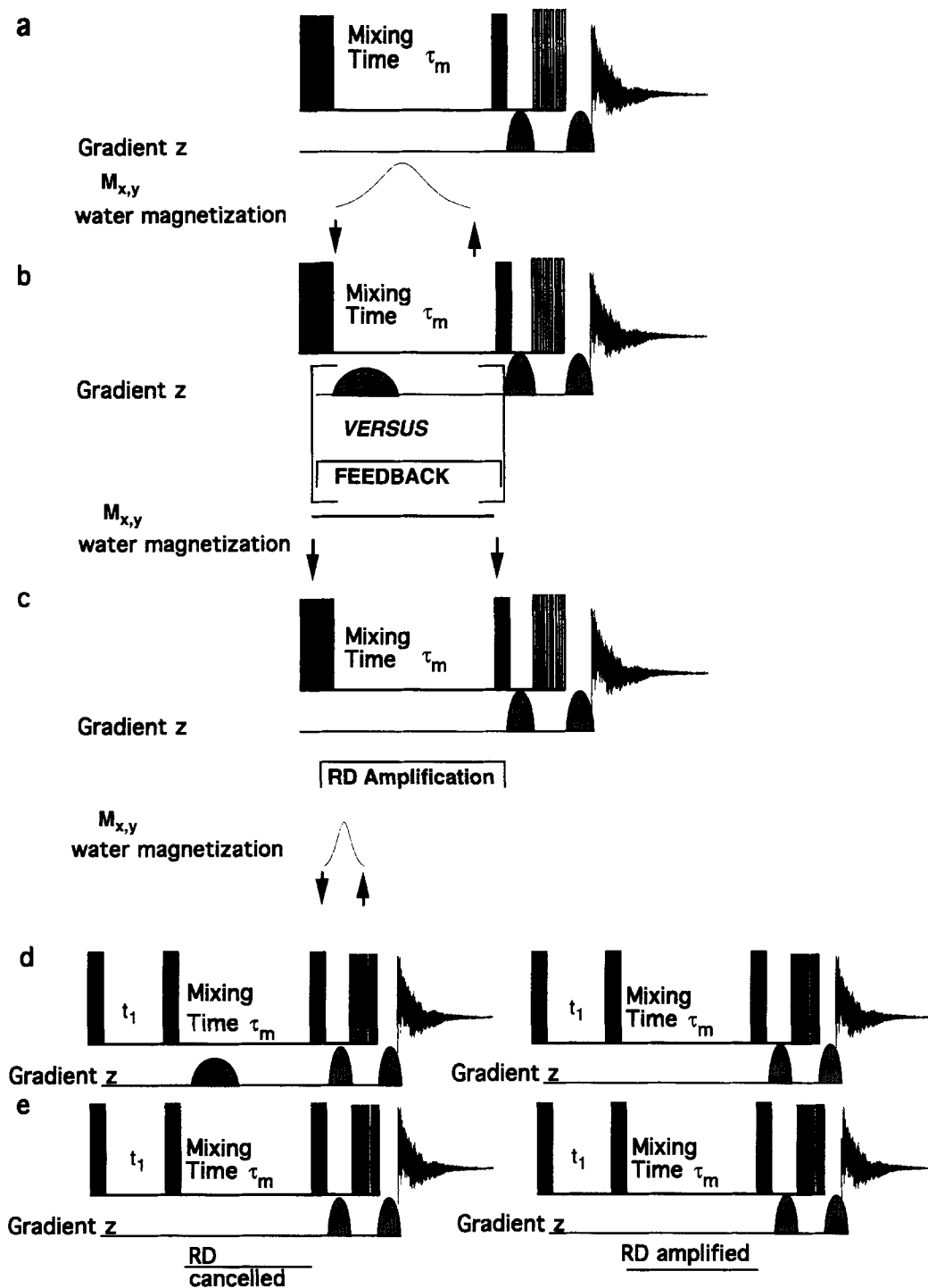
Fig. 2. Pulse sequences used in 1D and 2D experiments. Narrow bars denote 90° pulses and wider bars represent 180° pulses. In these experiments, a WaterGate read-pulse cluster, which comprises a composite selective pulse sandwiched between two gradient pulses, was used. In (a), the basic experiment using radiation damping (RD) as an inversion pulse for the water-selective NOE experiment is depicted. After a 180° hard pulse, inversion of the water magnetization with respect to the magnetization of the spins of interest is achieved under the action of the RD field during the mixing time τ_m. In (b), RD is eliminated during the mixing time. This constitutes the reference experiment without RD. In the Otting-and-Liepinsh version of the experiment, which served as a comparison of both techniques, RD was conveniently eliminated by a single 8-ms gradient pulse at the beginning of the mixing time, owing to the relatively long recovery time of the water magnetization (of the order of 150 ms). On the other hand, in our version, RD was cancelled by the feedback method. In (c), RD amplification yields faster water-selective inversion, which in turn enhances the efficiency of the experiment (see text for details). In (d) and (e), the 2D generalisation is shown of the enhanced difference experiments provided by (a), (b), and (c). In (d), RD is eliminated by a gradient pulse at the beginning of the mixing time, while selective inversion of the water magnetization is accomplished through RD. This constitutes a reference set of experiments for the 2D NOESY difference experiment. In (e), the set of experiments which yield improved difference experiment includes pulse sequences in which RD is eliminated or amplified by selective neutralisation or amplification of the RD field, respectively.
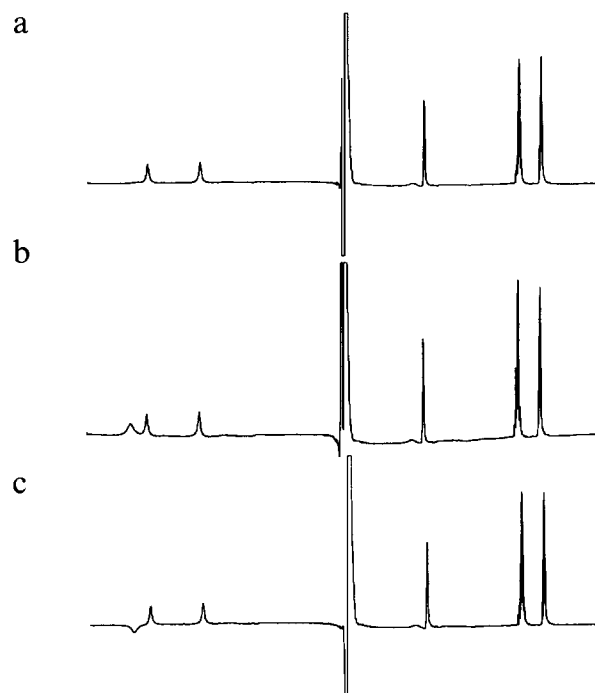
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Fig. 3. The experiments described in Figs. 2a–c are performed on a sample of 60-mM glutamine at 600 MHz (mixing time = 51 ms). The resonance on the left-hand side of the spectrum corresponds to the NH_4^+ group. The spectrum obtained in presence, in absence, and amplification of radiation damping (RD) are represented in (a), (b) and (c), respectively. Comparison of (a) and (c) shows that the use of RD amplification allows the detection of the leftmost resonance. This demonstrates the increase in the net magnetization transfer obtained by a faster return of the water magnetization to equilibrium.
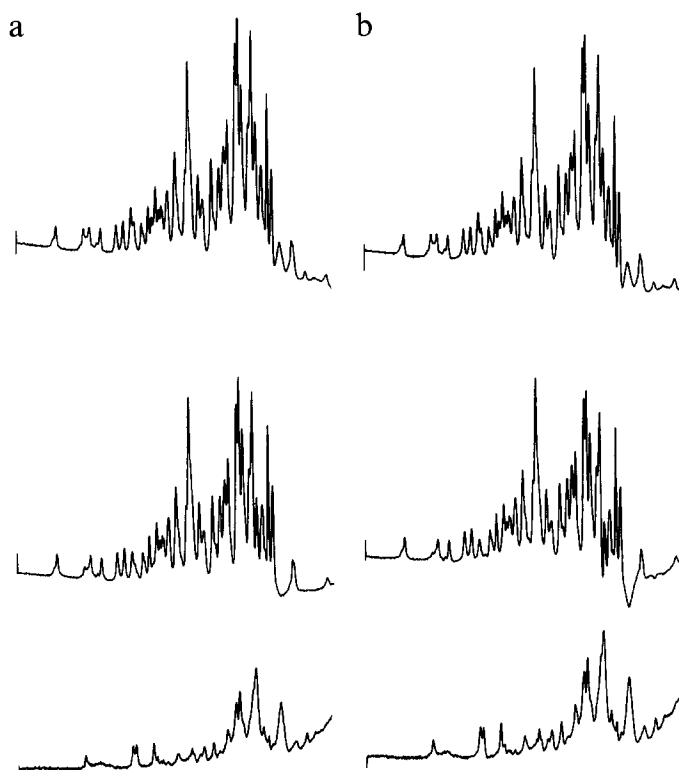

Fig. 4. Selective exchange experiments performed on 3-mM BPTI at 279 K (mixing time = 90 ms). In the spectra on the left (a) the low-field region of the reference spectra with radiation damping (RD) eliminated by a gradient pulse, and in the presence of RD (top and middle traces, respectively) are presented. The lower trace shows the resulting difference spectrum, which selectively exhibits protons exchanging magnetization with water. In the spectra on the right (b), similar experiments are shown, with RD eliminated and amplified by the RD control unit, respectively. Enhancement of magnetization transfer in (b) yields a gain in peak intensity by a factor of approximately 1.5.
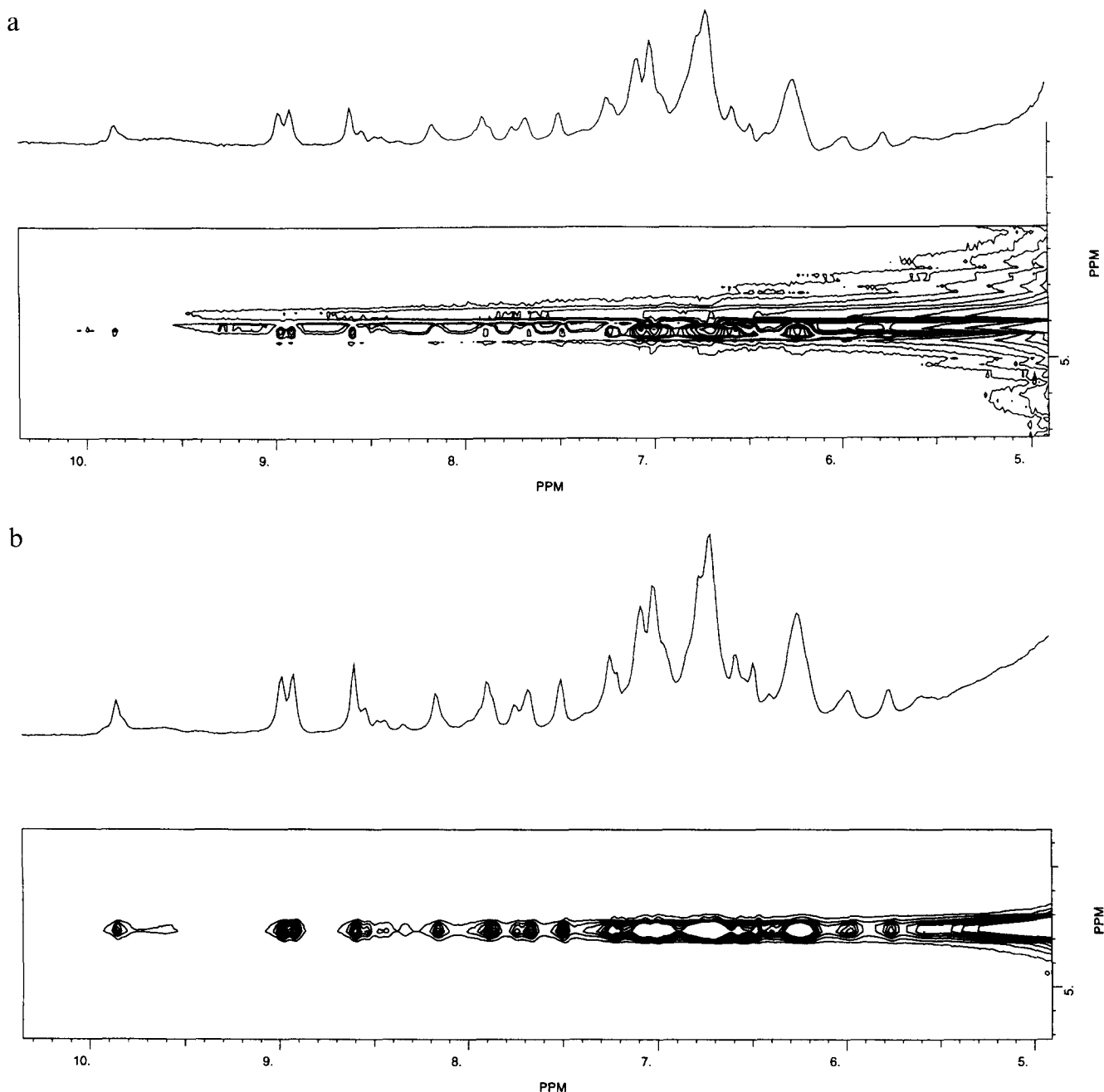
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Fig. 5. Plots of the water-exchanging-protons region of the 2D NOESY difference experiments (mixing time = 90 ms). The associated spectra in (a) and (b) correspond to the water line of the 2D experiment: in (a) without amplification of radiation damping; in (b) the amplification of radiation damping yields important signal enhancement.

yield complete water inversion in a time comparable with the T_1 relaxation time of the spins of interest, thus altering selective inversion of the water with respect to the spins of the protein (complete recovery time which was of the order of 150 ms on the pulsed field-gradient probe of our 600 MHz spectrometer). This could be stated in the following manner. In order to have the best-achievable NOE enhancement factor in a conventional 1D transient NOE experiment, it is necessary that complete inversion of the irradiated spin occurs. Transposed in the context of the experiment described above, it means that the time neces-

sary for the water magnetization to return to equilibrium (via RD) must be short enough compared to the recovery time of the spins that belong to the molecule under study (via T_1 relaxation), which is not always the case. This will be exemplified below for the case of glutamine (Fig. 3).

Methods

The RD control system which was developed in our laboratory provides a convenient way to overcome these problems, and indeed, it appeared to be a very efficient
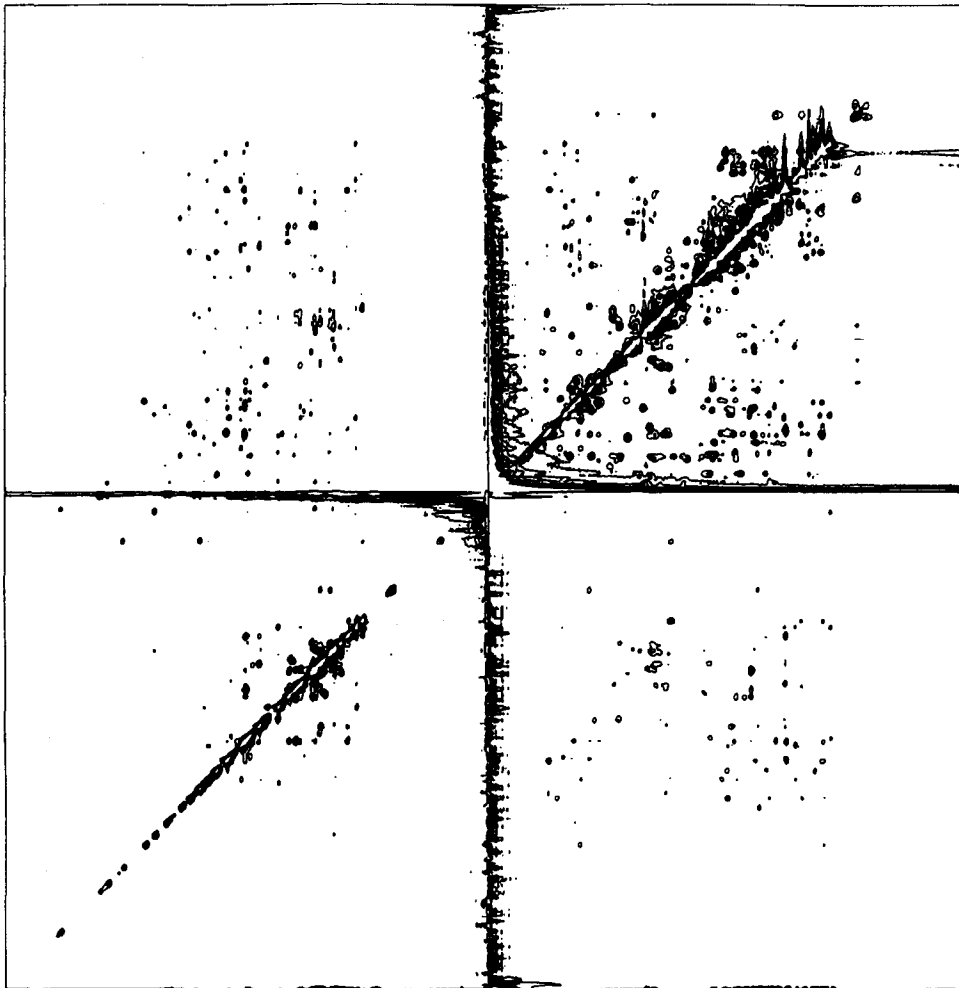
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Fig 6. Two-dimensional NOESY experiment (same conditions as in Fig. 5) with amplification of radiation damping used as a water 'flip-back' pulse. Figure (b) shows better water solvent suppression than in conventional experiment (a), due to complete alignment of the water magnetization along +z at the end of the detection period.

way of performing specific return of the water magnetization to equilibrium. As stated above, the solvent-magnetization-dependent field generated in the sample is both gain- and phase-controlled. Consequently, making this field equal and opposite to the RD field (RD eliminated) yields the reference spectrum in the NOE difference experiment, corresponding to a long T_1^*. Alternatively, when this correcting field is made parallel to the actual RD field and with sufficiently high gain (RD amplification), complete water-magnetization inversion is achieved in less than 40 ms.

It may be noted here that it should be indeed possible to make use of a selective pulse to help the RD field achieve the return of the water magnetization to its equilibrium direction. A pulse scheme of this kind has already been reported (Otting and Liepinsh, 1995) in which a selective pulse initiating the RD and conferring the water magnetization to the xy plane is followed by a 90° hard pulse. This experimental scheme was found to be less sensitive than the mere use of RD, supposedly because

'not all the water magnetization arrives in the transverse plane simultaneously and with exactly the same phase' (Otting and Liepinsh, 1995). This fact is not surprising, since the initial phase of the recovering magnetization is unpredictable, and is likely to vary from one experiment to another. In this context, our approach has several advantages over conventional selective pulses. Indeed, absolutely no knowledge about the phase of the water magnetization is required, since the feedback signal is slaved to that of the water signal. In particular, all the water magnetization is taken back to the z-axis, which ensures maximum sensibility. Moreover, the applied correction is autocalibrated with respect to its duration, since the RD field goes to zero as soon as the water magnetization is parallel to the z-axis. It compensates for the non-perfect calibration of the first 180° inversion pulse, and consequently there is no need for a subsequent homospoil pulse (as in Otting and Liepinsh, 1995). Full advantage is thus taken of the cooperative RD phenomenon. Finally, implementation is very easy, since calibration of the dur-
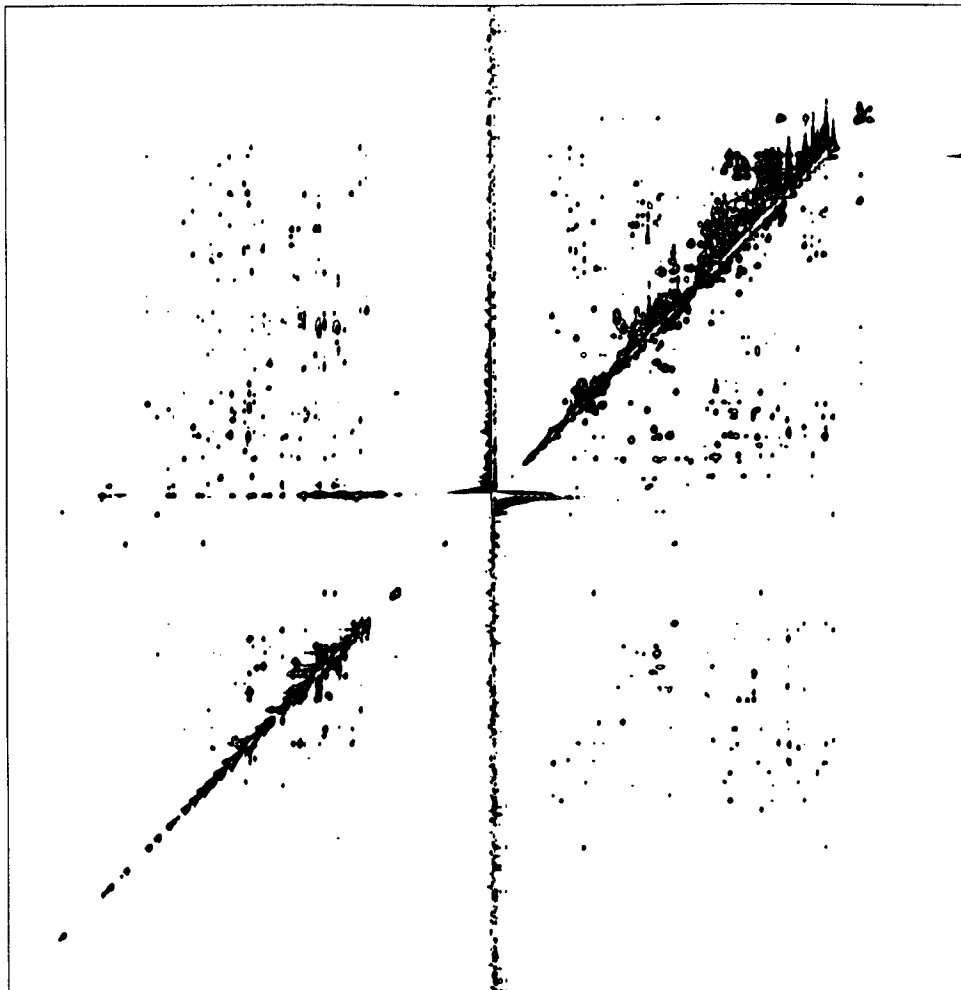
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Fig 6. (continued).

ation is determined by directly observing the water FID return to zero.

There are some advantages in using the approach to force the water magnetization to its equilibrium direction, in several respects. Firstly, it should be noted that amplification of RD as performed in the experiments presented in this article allows quasi-complete recovery of the water magnetization within about 40 ms. From this, the maximum of the correcting-field intensity can be estimated to be less than about 40 Hz, which ensures a good enough selectivity. Secondly, when used in multidimensional experiments in order to make the water magnetization return to +z, our method of amplification of RD by detecting the phase and amplitude of the water magnetization provides a new means of achieving complete and rapid return of the water magnetization to its equilibrium direction, irrespective of its evolution during nonconstant delays. Thirdly, selective control of RD, including both elimination and amplification without perturbing the evolution of magnetization or coherences of the spins of interest can only be obtained by an approach of this kind.

Results and Discussion

A first test experiment was performed on a 60-mM glutamine sample at 600 MHz (Fig. 3). The total mixing time was 51 ms. Elimination of RD was achieved by amplification of the RD-compensating field during 50 ms, followed by a 1-ms purging gradient pulse. In the case of RD amplification, the pulse was switched off after 40 ms, which ensured complete return of the water magnetization to equilibrium. The equivalent protons of the NH_3^+ group are in exchange with water protons. They give rise to a resonance located on the left-hand side of the spectrum, attesting for a slow-exchange regime (Wüthrich, 1986). This peak is conserved in the experiment in which the RD is cancelled during the mixing time (Fig. 3b). On the other hand, when RD is accelerated, the water magnetization is completely inverted with respect to the NH_3^+ protons, and a negative peak is detected, accounting for water–proton exchange (Fig. 3c). Note that in the case in which RD is present, though not accelerated, the sign and intensity of the NH_3^+ peak vary according to the amount of water magnetization which has

returned to the equilibrium direction (Fig. 3a). Comparison of Figs. 3a and 3c clearly shows that the outcome of the difference experiment is significantly improved when RD is amplified, and illustrates the efficiency of our RD control system.

A comparison was made between this improved method on the one hand, and a more conventional approach, which uses gradients to eliminate RD in a reference experiment (as in Fig. 2b), and in which RD is used without amplification, on the other hand (as in Fig. 2a). As expected, both reference experiments (with a 8-ms gradient pulse at the beginning of the mixing time versus RD-compensating field) yielded identical results. In contrast, experiments in which RD is present (as in Fig. 2a) or amplified (as in Fig. 2c), respectively, yield spectra that exhibit differences in some of the peak intensities. Results of this comparison are shown in Fig. 4. Experiments were performed on a 3-mM sample of BPTI ($\tau_m = 90$ ms; duration of the RD-accelerating pulse $= 40$ ms; temperature $= 6$ °C). It is noteworthy to stress that no purging pulse at the end of the mixing time was used. In all of the above experiments, we used a composite-pulse version (Sklenář et al., 1993) of the WaterGate (Piotto et al., 1992) read-pulse cluster. Difference spectra clearly show that a gain in intensity by a factor of approximately 1.5 is achieved (bottom traces of Fig. 4).

As mentioned above, this method should reveal to be useful in multidimensional experiments, in which uncontrolled behaviour of the water magnetization is expected during variable delays. In order to make a clear evaluation of the method, two sets of NOESY difference experiments were performed and spectra compared, as depicted in Figs. 2d and 2e. Again, elimination of RD was performed by a gradient pulse or an RD-correcting field, respectively. Return of the water magnetization towards equilibrium was achieved by RD alone or by its amplification. In the latter case, return of the water magnetization to equilibrium was achieved within less than 40 ms. In either case, no gradient purging pulse was used at the end of the mixing time. Similarly, 2D NOESY difference spectra can be obtained, which exhibit signals only at the water chemical shift, so that the experiments selectively detect protons that are in exchange (NOE/chemical exchange) with water. As in the 1D case, comparison of both sets of experiments shows a significant improvement of the peak intensities (Fig. 5). This demonstrates the feasability of the method in multidimensional experiments. Moreover, the use of our RD-controlling unit provides better water suppression (as displayed in Fig. 6). This is interesting, because for relatively short mixing times (< 100 ms) a nonnegligible amount of water remains in the xy plane, which alters both the solvent suppression and the dynamics of the experiment. In this case, the RD amplification acts as a flip-back pulse.

Conclusion

In conclusion, we have demonstrated that RD can be controlled by a simple electronic device and it proves to be of special interest in NMR spectroscopy. Indeed, the water apparent longitudinal relaxation time T_1^* can be conveniently controlled at will, so as to accelerate or slow down the water magnetization returning to equilibrium. This in turn was successfully applied to the study of water–protein interactions. The concept of water longitudinal relaxation-time control also suggests further application to signal-to-noise improvement in multipulse NMR spectroscopy.

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