Journal of Biomolecular NMR, 8 (1996) 87–92
ESCOM

J-Bio NMR 373

Suppression of radiation damping during selective excitation of the water signal: The WANTED sequence

Anja Böckmann and Eric Guittet*

Laboratoire de RMN, ICSN-CNRS, 1 Avenue de la Terrasse, F-91190 Gif-sur-Yvette, France

Received 26 April 1996
Accepted 14 June 1996

Keywords: Selective water excitation; Radiation damping; Water–protein interactions; BPTI; Fructose repressor

Summary

A new pulse sequence is presented allowing the use of long selective pulses at the water frequency using standard equipment. Radiation damping is suppressed during the pulse by the use of gradient echoes programmed between the single pulses of a DANTE train. This WANTED (*w*ater-selective D*ANTE* using gra*d*ients) sequence thus allows the observation of interactions with water without the use of special probe heads or filtering of undesired resonances. By combining the WANTED sequence with NOESY, ROESY and NOESY-HSQC experiments, we obtain selective 1D and 2D spectra fit to the observation of chemical exchange and dipolar interactions between water and protein protons.

Selective excitation of the solvent signal is an important tool in protein NMR to reduce the dimensionality of spectra acquired for the study of exchange processes between the labile protons and the water protons, or interactions between the protein and the solvent molecules (Grzesiek and Bax, 1993b; Kriwacki et al., 1993; Dalvit, 1995; Dalvit and Hommel, 1995; Otting and Liepinsh, 1995a,b; Mori et al., 1996). With the high magnetic fields and high-quality factor probe heads used in modern spectrometers, a severe problem is brought about by the phenomenon of radiation damping (RD). Radiation damping has its origin in the interactions between the precessing strong magnetization of the solvent protons and the radiofrequency (RF) coil (Bloembergen and Pound, 1954; Bloom, 1957). The magnetization induces an electromotive force in the coil, which in turn creates a current. This current is at the origin of a magnetic field which tends to bring the magnetization back to equilibrium. Thus, the apparent relaxation rate of the water protons decreases, causing line broadening of the water signal in both directly and indirectly detected dimensions. Another problem is that excitation of the water signal by long selective pulses can become impossible in the presence of radiation damping, due to the field created during the pulse (Otting and Liepinsh, 1995a). Several methods have been proposed very recently to suppress RD in the indirectly and directly detected dimensions, including the Q-switch (Otting and Liepinsh, 1995a), the feedback (Broekaert and Jeener, 1995; Louis-Joseph et al., 1995) and an overcoupled probe (Picard et al., 1995). The use of bipolar gradients during evolution periods (Sklenář, 1995; Zhang and Gorenstein, 1996) is one of the most popular methods, since it does not require hardware modifications for modern NMR spectrometers.

Suppression of RD during selective pulses has until now exclusively been realized using a Q-switched probe (Otting and Liepinsh, 1995a). For standard equipment, the use of short selective pulses (2–4 ms) overcoming the field of RD has been proposed. As they do not only excite water, but also other resonances close to the water frequency, as for example H^{α} protons in protein studies, filtering of the nondesired resonances becomes necessary. This can be achieved for ^{13}C-labelled proteins by using isotope-filtering methods (Grzesiek and Bax, 1993b); another approach for doubly labelled compounds is the MEXICO sequence proposed by Gemmecker et al. (1993), using excitation of all signals followed by a $^{15}N/^{13}C$ filter. For unlabelled proteins, a short selective pulse followed by a spin-echo filter, eliminating H^{α} resonating at the water frequency, can be applied (Mori et al., 1996). This

*To whom correspondence should be addressed.

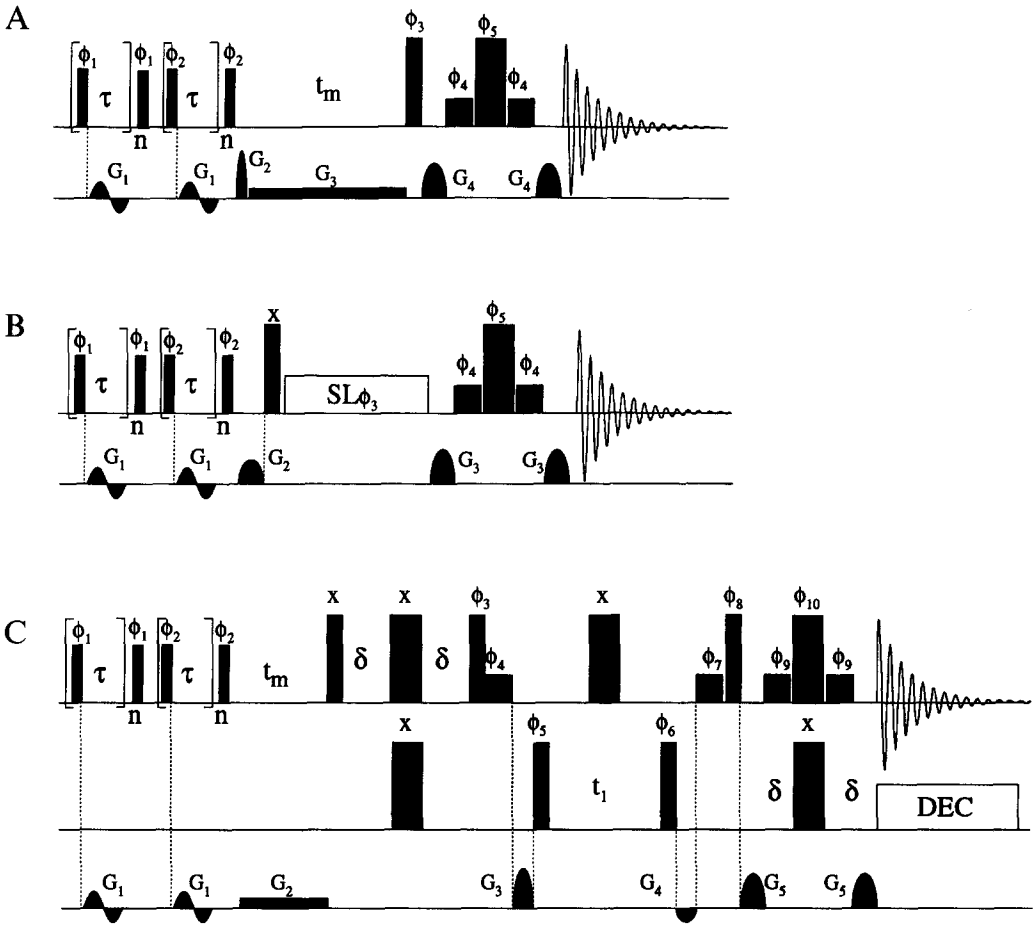

Fig. 1. Pulse schemes for the sequences described. Narrow and wide pulses correspond to 90° and 180° flip angles. The ^1H carrier was set at the H_2O frequency. (A) WANTED-NOESY sequence. The RF field strength is 1.6 kHz for the pulses of the DANTE pulse train (Morris and Freeman, 1978). Each train consists of 128 pulses. The delays τ are 200 μs. The bipolar gradients G_1 (Sklenář, 1995) have a sine-bell amplitude profile with a strength of 2.5 G/cm at maximal intensity. The gradient G_2 has a Gaussian amplitude profile, a duration of 1 ms and a strength of 5 G/cm. The gradient G_3 has a square amplitude profile, a duration of 49 ms and a strength of 25 mG/cm. The RF field strength for the low-power pulses of the WATERGATE (Piotto et al., 1992) sequence is 170 Hz. The gradients G_4 have a Gaussian amplitude profile and a strength of 20 G/cm at their center. The phases are the following: $\phi_1 = x$; $\phi_2 = x,-x$; $\phi_3 = 2x,2y,2(-x),2(-y)$; $\phi_4 = 2x,2y,2(-x),2(-y)$; $\phi_5 = 2(-x),2(-y),2x,2y$; $\phi_{acq} = x,-x,y,-y,-x,x,-y,y$. The interscan delays are 5 s. (B) WANTED-ROESY sequence. G_2 has a Gaussian amplitude profile and a strength of 1.5 G/cm. $\phi_1 = x$; $\phi_2 = x,-x$; $\phi_3 = 4x,4y,4(-x),4(-y)$; $\phi_4 = 2x,2(-x),2y,2(-y),2(-x),2x,2(-y),2y$; $\phi_5 = 4x,4y,4(-x),4(-y)$; $\phi_6 = 4(-x),4(-y),4x,4y$; $\phi_{acq} = 2(x,-x),2(y,-y),2(-x,x),2(-y,y)$. The spin-lock pulse has an RF field strength of 800 Hz. The other parameters are the same as for (A). (C) WANTED-NOESY-HSQC sequence. The parameters for the WANTED and NOESY parts are the same as for (A). The ^{15}N carrier frequency is set to 107.4 ppm. ^{15}N decoupling was achieved using the GARP sequence (Shaka et al., 1985). The duration of the delay δ is 2.7 ms. The gradients G_4 and G_5 are z-filter gradients of 5 G/cm and −1 G/cm and a duration of 2 ms and 800 μs, respectively. The RF field strength for the water-selective flip-back pulses (Grzesiek and Bax, 1993a,b) with phases ϕ_4 and ϕ_7 is 170 Hz. The water-selective pulses of the WATERGATE have the same characteristics. The interscan delay is 2 s. The phases are: $\phi_1 = x$; $\phi_2 = x,-x$; $\phi_3 = 2y,2(-y)$; $\phi_4 = -x,x$; $\phi_5 = 4x,4(-x)$; $\phi_6 = 16x,16(-x)$; $\phi_7 = 8(-x),8x$; $\phi_8 = 8x,8(-x)$; $\phi_9 = 8x,8(-x),8x,8(-x)$; $\phi_9 = 8(-x),8x,8(-x),8x$; $\phi_{acq} = x,2(-x),x,-x,2x,2(-x),2x,-x,x,2(-x),x,-x,2x,-x,x,2(-x),2x,2(-x),x,-x,2x,-x$.

sequence yet shows the drawback of signal losses due to diffusion, partial saturation of the water proton magnetization and T_2 relaxation of the water proton magnetization. Another possibility to realise long selective pulses is to dephase the water magnetization during the whole sequence (Dalvit, 1995) or during the selective pulse (Dalvit and Hommel, 1995), the latter version also allowing filtering of H^α. However, in both versions, signal losses similar to those in the experiment proposed by Mori et al. (1996) occur. Methods using radiation damping to selectively invert the solvent signal can suffer from ill-defined mixing times (Otting and Liepinsh, 1995b; Böckmann et al., 1996).

In this contribution, we introduce a new method allowing efficient and selective excitation of the water signal, based on the suppression of RD during the selective pulse using standard equipment. In this sequence, which we refer to as 'WANTED' (*w*ater selective D*ANTE* using gra*d*ients), radiation damping is suppressed by the use of gradient echoes during the delays of a DANTE (Morris and Freeman, 1978) pulse train. The suppression of RD during the delays of a pulse train has been proposed by Otting and Liepinsh (1995a) using a switchable probe; our approach is similar, but requires no other hardware than a z-gradient accessory. The selectivity of a DANTE pulse

train is determined by the total length of the train, and thus mainly by the length of the delays between the single pulses. During these delays, radiation damping takes place and counteracts the pulses. If the delays are long enough, the net effect of the pulses of the train can be totally cancelled by RD. In the WANTED sequence, radiation damping is suppressed during the delays by the use of bipolar gradients, as proposed by Sklenář (1995). The magnetization is dephased by the application of a first gradient after each pulse of the train. At half the delay, a second gradient of the same shape and duration, but of opposite sign is applied, which starts to refocus the magnetization. The refocusing is only complete just before the application of the next pulse. Thus, water magnetization is defocused during the entire delay between the pulses. By the resulting absence of radiation damping, the magnetization follows indeed the desired path prescribed by the pulse train. The WANTED sequence can be applied to obtain one or multidimensional spectra selective at the water frequency; it is easily adaptable to most existing pulse sequences. Since water magnetization is preserved, water flip-back pulses (Grzesiek and Bax, 1993a) can be included in the sequences. As a demonstration, we combined the WANTED sequence with three standard experiments important in the study of protein–water interactions and exchange processes: the NOESY, the ROESY and the NOESY-HSQC experiments. We thus obtain 1D and 2D spectra selective at the water frequency.

Figure 1A shows the WANTED-NOESY pulse sequence. The magnetization is brought into the xy plane by the first 90° DANTE train. The total length of the 90° pulse is 25 ms, showing very good selectivity and thus avoiding the excitation of most H^{α} protons. The gradients applied during the delays of the pulse train are sine-shaped and pass continuously from positive to negative in order to shorten switching delays. The second pulse train aligns for one scan the water magnetization along the –z axis, and for the second scan along the z axis, as determined by the phase ϕ_2. By incrementing the receiver phase by

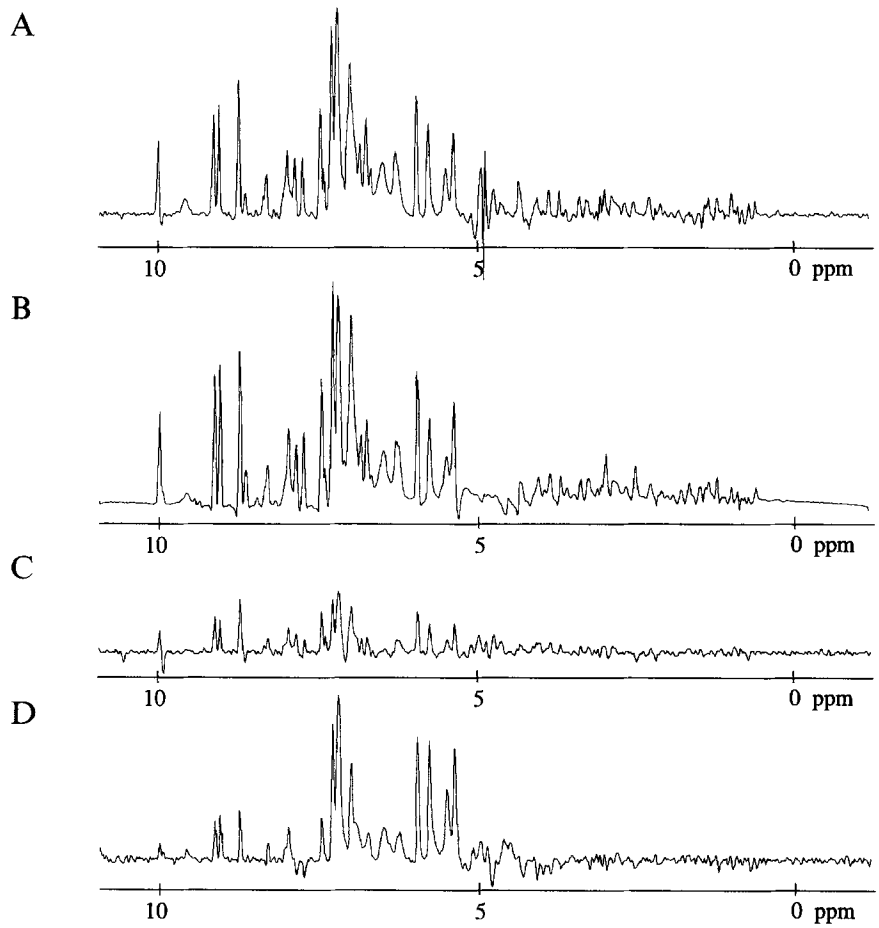

Fig. 2. Spectra of a 5.5-mM solution of BPTI at pH 6.5, 20 °C acquired on a Bruker AMX600 spectrometer using a triple-resonance HCN probehead with a self-shielded z-gradient coil. (A) WANTED-NOESY spectrum. The mixing time is 50 ms, the interscan delays are 5 s. The spectral width is 7353 Hz. The total recording time is about 45 min. (B) Cross section of a 2D NOESY spectrum. Radiation damping is suppressed during t_1 evolution and the mixing time (Sklenář, 1995). The mixing time is 50 ms, interscan delays are 5 s and the total recording time is 11 h. (C) Same pulse sequence and parameters as (A), but zero intensity of the pulsed field gradients during the WANTED selective pulse. (D) WANTED-ROESY spectrum. Exchange peaks are shown as positive signals, NOEs as negative signals. The mixing time is 25 ms, the interscan delays are 5 s, the total recording time is about 45 min.

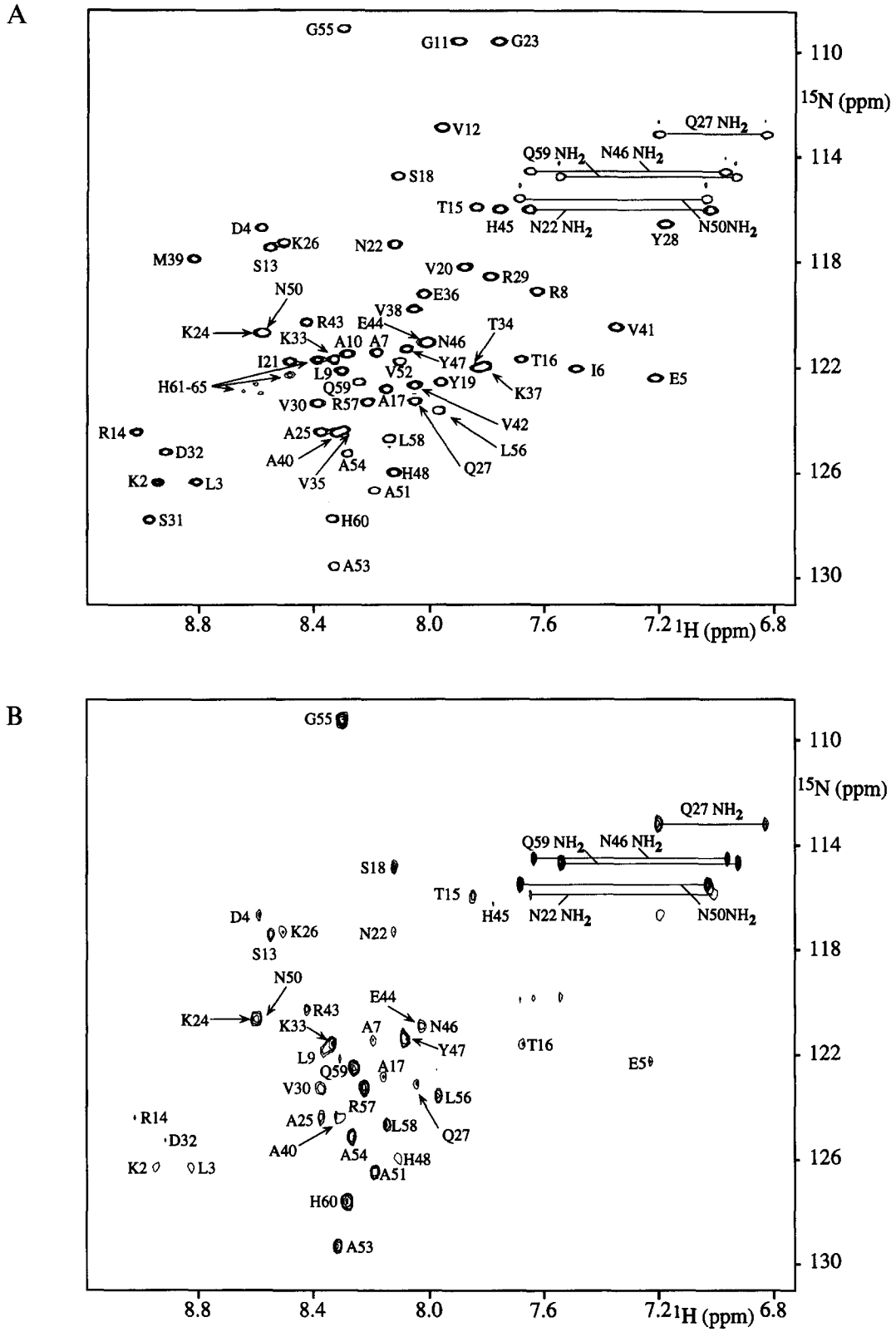

Fig. 3. Spectra of a 2 mM solution of FruR(1-57)* at pH 5.9, 25 °C, acquired on a Bruker AMX600 spectrometer using a triple-resonance HCN probehead with a self-shielded z-gradient coil. (A) Extract of a standard HSQC spectrum. (B) Same extract of the WANTED-NOESY-HSQC spectrum recorded with the pulse sequence shown in Fig. 1C. The signal intensities correspond directly to the exchange rates (Gemmecker et al., 1993; Böckmann et al., 1996). The mixing time is 50 ms. The spectral width is 7353 Hz and 4200 Hz for ^{1}H and ^{15}N, respectively. A total of 180×2048 increments were taken. Linear prediction up to 512 data points was applied in dimension 1 using the GIFA program (Delsuc, 1989) and data were zero-filled in t_1 up to 1024 points. The interscan delay is 2 s, the total recording time 13 h. Amide proton assignments were from Penin et al. (manuscript submitted for publication).

180° every second scan, one obtains a difference experiment retaining exclusively magnetization at the water frequency. During the mixing time, magnetization transfer by exchange and dipolar interactions takes place. A crusher gradient, programmed to destroy any residual transverse magnetization, is followed by a weak gradient in order to suppress radiation damping, whose presence would complicate the interpretation of the data obtained. No water flip-back pulse is applied in the one-dimensional spectra, thus avoiding alteration of the mixing time or signal losses due to T_2 relaxation. We use instead long (5 s) interscan delays, not a major problem in 1D spectra. Water suppression is achieved using the WATERGATE sequence (Piotto et al., 1992). The spectrum obtained with the gradients during the pulse, set to a value of 2.5 G/cm at its maximum point, is shown in Fig. 2A. The gradient strength is chosen to efficiently suppress radiation damping without loosing signal intensity due to incomplete field recovery. The chosen strength is obviously a compromise, since the short delays between the pulses do not allow complete suppression of radiation damping *and* complete field recovery. This also explains the observed amount of excited water magnetization of about 60% compared to a nonselective pulse. This ratio will probably increase with future developments in probe design. The application of rather weak gradients brings the advantage of minimizing signal losses due to diffusion of the water molecules during the delays. The course of the DANTE sequence is untouched by the gradients: as shown by Zhang and Gorenstein (1996), the application of a gradient echo does not influence the evolution of the magnetization under chemical shift or coupling.

The WANTED-NOESY spectrum of BPTI shown in Fig. 2A is very similar to the cross section through the 2D NOESY spectrum at the water frequency shown in Fig. 2B. Figure 2C shows a spectrum taken under the same conditions as the spectrum in Fig. 2A, but with the gradients during the WANTED pulse set to zero. The difference between the two spectra is clear-cut. Only the most intense resonances are observable, indicating residual excitation of the water frequency. The weakest resonances have completely disappeared, others are clearly reduced. Figure 2D shows the WANTED-ROESY spectrum taken with the sequence on Fig. 1B. The selective inversion by the WANTED pulse is followed by a crusher gradient destroying any residual transverse magnetization. A hard pulse brings the magnetization in the xy plane, where it is spin-locked during the mixing time. Water suppression is achieved by a WATERGATE sequence (Piotto et al., 1992). The spectrum shows the exchange signals with a positive sign and the dipolar cross-relaxation signals with a negative sign.

The WANTED sequence can also easily be adapted to obtain 2D spectra. For ^{15}N-labelled proteins, the extract at the water frequency of a NOESY-HSQC experiment is an important tool to study exchange processes or to observe NOEs between water protons and protein protons. The knowledge of amide proton exchange rates allows to obtain information about the protein dynamic structure; in the study of protein–DNA complexes, exchange rates are used to identify interfaces between the protein and the ligand and to assess changes in dynamics induced by the ligand. With the WANTED sequence, the 3D NOESY-HSQC experiments can be reduced to their two dimensional version selective at the water frequency, which can easily be recorded in less time and with a better resolution. Figure 1C shows the WANTED-NOESY-HSQC pulse sequence. The water magnetization is selectively inverted in the same manner as discussed for the WANTED-NOESY and -ROESY sequences. Radiation damping is suppressed during the 100-ms mixing time by a weak gradient. During the HSQC sequence, water magnetization is selectively flipped back prior to t_1 and to acquisition (Grzesiek and Bax, 1993a,b). Water suppression is achieved by the WATERGATE sequence (Piotto et al., 1992). Figure 3B shows the resulting spectra for FruR(1-57)*, the DNA-binding domain of the fructose-repressor protein (Scarabel et al., 1995; Penin et al., submitted). The amide protons of this protein have shown to exchange entirely against D_2O in less than 5 min. In contrast to the HSQC spectrum (Fig. 3A), one observes in the selective spectrum a variation of signal intensities for the different amide protons. Already by considering these intensities, one can classify the exchange rates of the amide protons of the protein (Gemmecker et al., 1993). The most intense signals indicating residence times on the ms–s time scale belong to residues 50–60 of the unstructured part of the protein. Signals no longer observable in the selective spectrum indicate slower exchange ($< s^{-1}$) and originate mostly from amide protons involved in hydrogen bonding (Böckmann et al., 1996).

The WANTED sequence represents the last element for the suppression of radiation damping during the entire pulse sequence with standard equipment and standard pulse sequences. It thus completes the work of Sklenář (1995) and of Zhang and Gorenstein (1996), comprising suppression of radiation damping by gradients during mixing, evolution and acquisition times. Its advantage over selective excitation in the presence of radiation damping (Kriwacki et al., 1993; Grzesiek and Bax, 1993b; Mori et al., 1996) is the free choice of the pulse length and thus of the selectivity; additional filtering of undesired resonances becomes unnecessary. The efficient approach here proposed should find applications for a number of studies of protein–water interactions in biomolecular systems.

Acknowledgements

We thank Dr. François Penin for providing samples of FruR(1-57)*. This work was supported by a fellowship

from the German Academic Exchange Service (A.B., DAAD Doktorandenstipendium aus Mitteln des zweiten Hochschulsonderprogramms).

References

Bloembergen, N. and Pound, R.V. (1954) *Phys. Rev.*, **95**, 8–12.

Bloom, S. (1957) *J. Appl. Phys.*, **28**, 800–805.

Böckmann, A., Penin, F. and Guittet, E. (1996) *FEBS Lett.*, **383**, 191–195.

Broekaert, P. and Jeener, J. (1995) *J. Magn. Reson.*, **A113**, 60–64.

Dalvit, C. (1995) *J. Magn. Reson.*, **A113**, 120–123.

Dalvit, C. and Hommel, U. (1995) *J. Magn. Reson.*, **B109**, 334–338.

Delsuc, M.A. (1989) In *Maximum Entropy and Bayesian Methods*, (Ed. Skilling, J.), Kluwer, Dordrecht, The Netherlands.

Gemmecker, G., Jahnke, W. and Kessler, H. (1993) *J. Am. Chem. Soc.*, **115**, 11620–11621.

Grzesiek, S. and Bax, A. (1993a) *J. Am. Chem. Soc.*, **115**, 12593–12594.

Grzesiek, S. and Bax, A. (1993b) *J. Biomol. NMR*, **3**, 627–638.

Kriwacki, R.W., Hill, R.B., Flanagan, J.M., Caradonna, J.P. and Prestegard, J.H. (1993) *J. Am. Chem. Soc.*, **115**, 8907–8911.

Louis-Joseph, A., Abergel, D. and Lallemand, J.-Y. (1995) *J. Biomol. NMR*, **5**, 212–216.

Mori, S., Berg, J.M. and Van Zijl, P.C.M. (1996) *J. Biomol. NMR*, **7**, 77–82.

Morris, G.A. and Freeman, R. (1978) *J. Magn. Reson.*, **29**, 433–462.

Otting, G. and Liepinsh, E. (1995a) *J. Magn. Reson.*, **B107**, 192–196.

Otting, G. and Liepinsh, E. (1995b) *J. Biomol. NMR*, **5**, 420–426.

Penin, F., Geourjon, C., Montserret, R., Yang, Y., Fillon, A., Bonod, C., Cortay, J.C., Nègre, D., Cozzone, A.J. and Deléage, G., manuscript submitted for publication.

Picard, L., v. Kienlin, M., Décorps, M. (1995) *J. Magn. Reson.*, **A117**, 262–266.

Piotto, M., Saudek, V. and Sklenář, V. (1992) *J. Biomol. NMR*, **2**, 661–665.

Scarabel, M., Penin, F., Bonod, C., Nègre, D., Cozzone, A.J. and Cortay, J.C. (1995) *Gene*, **153**, 9–15.

Shaka, A.J., Barker, P.B. and Freeman, R. (1985) *J. Magn. Reson.*, **64**, 547–552.

Sklenář, V. (1995) *J. Magn. Reson.*, **A114**, 132–135.

Zhang, S. and Gorenstein, D.G. (1996) *J. Magn. Reson.*, **A118**, 291–294.