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Solvent Suppression Using a Spin Lock in 2D and 3D NMR Spectroscopy with H_2O Solutions

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The ability to observe all polypeptide backbone amide protons in ^1H NMR spectra is a preliminary condition for the use of NMR to determine the structure of proteins in solution (*1*). The acquisition of homonuclear 2D ^1H NMR spectra of proteins in H_2O solution (*2*) was therefore a turning point in the methodological development which made it possible to obtain complete ^1H NMR assignments of proteins (*3, 4*), and on this basis to determine three-dimensional protein structures (*5*). For proteins at slightly acidic pH, where the amide proton exchange rate is sufficiently slow, the most widely used solvent suppression method is by selective presaturation of the H_2O line (*1, 2, 6*). However, under certain conditions of pH and temperature, amide protons may exchange sufficiently fast with the bulk water for their signal intensity to be significantly reduced on presaturation of H_2O. In addition, bleaching of spectral regions near the water line and concomitant loss of information in the two-dimensional spectra may also result from presaturation of the water resonance (*2*). As an alternative to solvent presaturation, one of many selective excitation methods (*7–10*) may be used to suppress the water signal. The main disadvantages of selective excitation techniques lie in the facts that they bring about phase distortions in 2D NMR spectra and that they cannot be combined with uniform spectral excitation across the entire spectrum. Today heteronuclear NMR experiments are increasingly used in protein structure determinations to provide supporting information for the resonance assignments and to facilitate the collection of the ^1H NMR data needed as input for the structure determination (*11–13*). In this paper we present a technique whereby the water signal can be suppressed in heteronuclear NMR experiments with proton detection, without the need for either water presaturation or selective excitation methods.

It has been previously shown that suppression of undesired proton magnetization in heteronuclear spectra can be greatly enhanced by the use of spin-lock pulses (*14*). Here, spin-lock purge pulses are used during the application of heteronuclear pulse sequences to suppress the magnetization due to water protons. Figures 1A and 1B show, respectively, the experimental schemes for 2D [^{15}N, ^1H]-COSY (*15*) and 3D NOESY-[^{15}N, ^1H]-COSY (*12*) supplemented by spin-lock purge pulses for solvent ssion.

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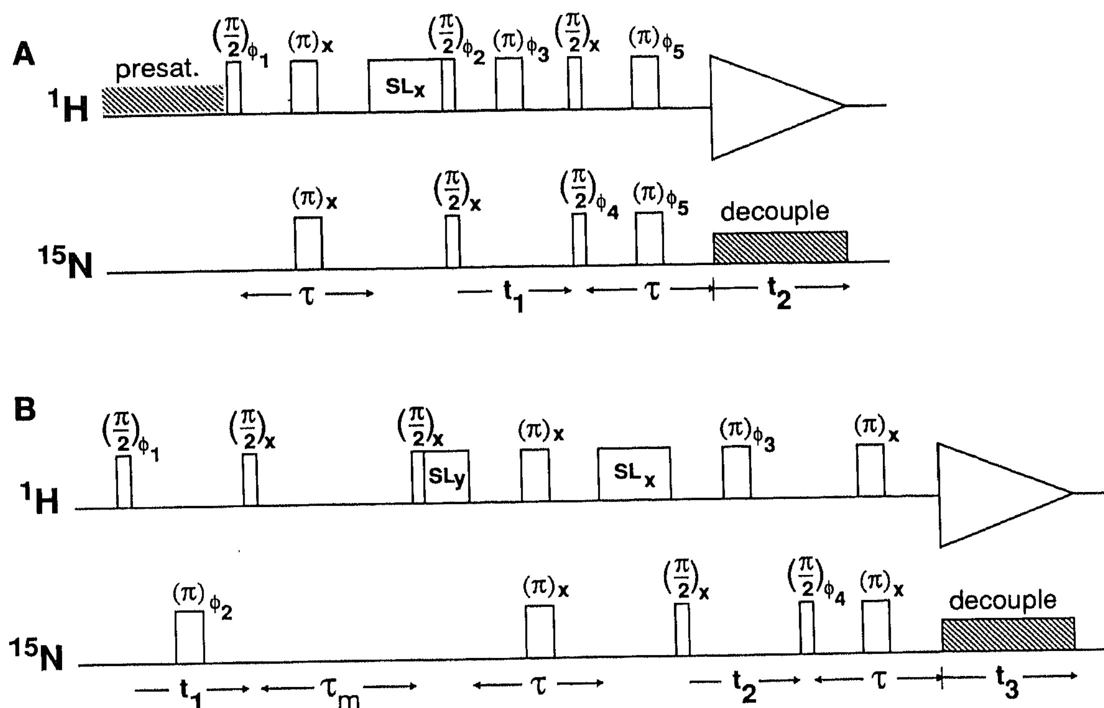

FIG. 1. (A) Experimental scheme for [^{15}N, ^{1}H]-COSY (*15*) extended by a spin-lock pulse (*14*) for solvent suppression. This scheme was used with and without presaturation of H_2O (indicated by dashed lines during the preparation period). The phases ϕ_1, ϕ_3, ϕ_4, and ϕ_5 are independently alternated between x and $-x$, and the phase ϕ_2 between y and $-y$, resulting in a 32-step phase cycle. The receiver phase is alternated together with ϕ_1, ϕ_2, and ϕ_4. Quadrature detection in ω_1 is achieved by applying TPPI (*18*) to the first two ^{15}N pulses. τ equals $1/[2\,^1J(^1\text{H}, ^{15}\text{N})]$. SL denotes the spin-lock pulse. (B) Three-dimensional NOESY-[^{15}N, ^{1}H]-COSY (*12*) with water suppression using spin-lock pulses. The phases ϕ_1 to ϕ_4 are independently alternated between x and $-x$, resulting in a 16-step phase cycle. The receiver phase is alternated together with ϕ_1 and ϕ_4. Quadrature detection in ω_1 is achieved by subjecting ϕ_1 to TPPI, and in ω_2 by applying TPPI to the first three ^{15}N pulses.

The purging effect of the spin-lock pulse is best explained using the product operator notation of Sørensen *et al.* (*16*), where I and S denote proton spins and ^{15}N spins, respectively. Considering the scheme of Fig. 1A we start with equilibrium z magnetization I_z. After the sequence $[(\pi/2)_x(^1\text{H})-\tau/2-(\pi)_x(^1\text{H}, ^{15}\text{N})-\tau/2]$ with $\tau = 1/[2\,^1J(^{15}\text{N}, ^1\text{H})]$, we have heteronuclear antiphase magnetization, $2I_xS_z$, aligned along the x axis for all ^{15}N-bound protons. The protons of the water resonance do not couple to other protons and are represented by in-phase magnetization I_y. The following spin-lock pulse along the x axis retains the magnetization of the ^{15}N-bound protons and randomizes the water magnetization by its radiofrequency field inhomogeneity. Efficient water suppression is achieved with spin-lock pulses of 2 ms duration and the same RF amplitude as the other proton pulses. This suppression scheme is quite insensitive to pulse imperfections of the refocusing $(\pi)_x(^1\text{H})$ pulse if the carrier frequency is set at the frequency of the water resonance. The same principles can be used for water suppression in NOESY and TOCSY with ^{15}N- or ^{13}C-labeled compounds. Figure 1B shows an example of the use of this suppression scheme with 3D NOESY-[^{15}N, ^{1}H]-COSY. The pulse sequence selects for z magnetization I_z during the mixing time τ_m. The following $(\pi/2)_x(^1\text{H})$ pulse converts I_z into transverse mag-

netization I_y. Then it is spin-locked by the spin-lock pulse SL_y, which randomizes all magnetization that was not aligned along the z axis during the mixing period. After this "trim pulse" the sequence $[\tau/2-\pi(^1H, ^{15}N)-\tau/2]$ converts the magnetization I_y from ^{15}N-bound protons into antiphase magnetization $2I_xS_z$, while the water magnetization remains aligned along the y axis. The following spin-lock purge pulse SL_x randomizes the water magnetization and retains the desired proton magnetization $2I_xS_z$. In order to avoid refocusing effects from the use of more than one spin lock, the two spin-lock pulses should be of different duration (*17*). Compared to the 3D NOESY-[^{15}N, ^1H]-COSY pulse sequence devised by Fesik and Zuiderweg (*12*), the use of the spin-lock pulses for water suppression requires additional refocusing $(\pi)_x(^1H, ^{15}N)$ pulses in the middle of each delay τ to prevent the evolution of proton magnetization under the chemical-shift Hamiltonian.

The water suppression schemes of Fig. 1 were applied with cyclophilin. This protein cannot be studied at pH values below 6.0, so that numerous rapidly exchanging amide protons could not be observed when the water line was presaturated. Cyclophilin contains 165 amino acids and has a molecular weight of about 17,900. The cDNA of the human protein was expressed in *Escherichia coli*, and 99% labeling with ^{15}N was achieved by growing the bacterial cells on a minimal medium containing ^{15}N ammonium sulfate as the sole nitrogen source. For the NMR experiments in Figs. 2 and 3 a 2 mM solution of cyclophilin in a mixed solvent of 90% H_2O/10% D_2O at pH 6.0 was used. All measurements were made at a temperature of 26°C on a Bruker AM 600 instrument.

As an initial test of the proposed water suppression with a spin lock, [^{15}N, ^1H]-COSY spectra of cyclophilin were recorded with and without presaturation of the water resonance, and otherwise identical conditions (Fig. 2). In Fig. 2A those resonances are identified for which the signal intensity is clearly reduced by the water presaturation. Although the majority of the resonances were not or only slightly affected, certain resonances lost a significant amount of intensity in spectrum B. In a different presentation, the comparison of corresponding cross sections from spectra A and B clearly shows that the water presaturation caused a reduction of the peak intensities II and III of more than 50% relative to peak I (Figs. 2C and 2D). These observations thus demonstrate that the experiment of Fig. 1A can yield clean and complete [^{15}N, ^1H]-COSY fingerprints of a protein in H_2O solution under conditions where water preirradiation causes significant loss of signal intensity. The latter is probably due in part to saturation transfer by chemical exchange of amide protons, and in part to spin diffusion starting from water protons or α protons located at the water proton chemical shift (*1*).

The spin-lock suppression technique was also applied in a 3D NOESY-[^{15}N, ^1H]-COSY experiment (Fig. 1B) with ^{15}N-labeled cyclophilin. For comparison the corresponding region of a conventional, homonuclear 2D ^1H NOESY spectrum is also shown (Fig. 3A). The extensive overlap of resonances in Fig. 3A makes clear that ^{15}N-resolved 3D NMR was indicated as a means to facilitate the spectral analysis. The 2D ^1H-NOESY experiment was acquired with selective water presaturation, and the band of bleached resonances along ω_2 at $\omega_1 \approx 4.75$–4.85 ppm is readily apparent in Fig. 3A. In contrast, the two cross sections taken from the corresponding 3D spectrum acquired without water presaturation show several resonances at and very near

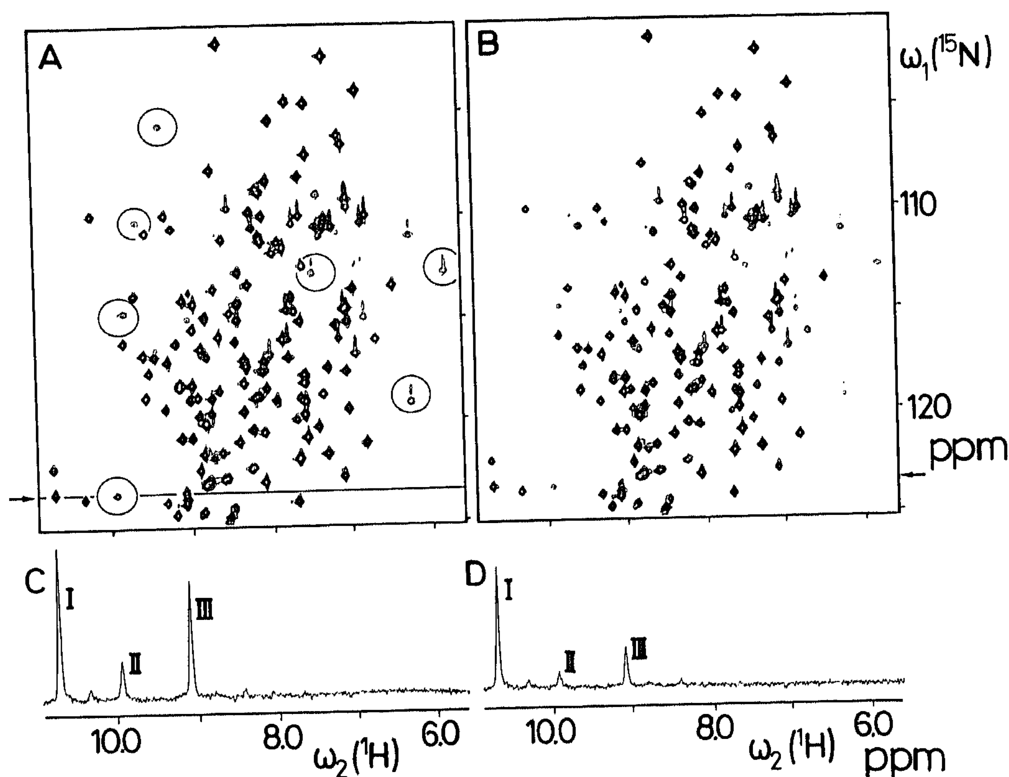

FIG. 2. Part of two phase-sensitive [^{15}N, ^1H]-COSY spectra of ^{15}N-labeled cyclophilin (2 m*M* protein in a mixed solvent of 90% H_2O/10% D_2O, with 10 m*M* [$^2H_3$]sodium acetate, 10 m*M* disodium phosphate, and 2 m*M* sodium dithionite, pH 6.0, 26°C, recorded at 600 MHz proton frequency on a Bruker AM600 spectrometer). Spectra (A) and (B) were acquired using the pulse sequence in Fig. 1A, without and with presaturation of the water resonance for 1 s during the preparation period, respectively. τ = 5.6 ms, t_{1max} = 134 ms, and t_{2max} = 127 ms; ^{15}N decoupling during acquisition using WALTZ with suppression of cycling sidebands (*19*). (C) and (D) Cross sections taken from (A) and (B), respectively, at the position indicated by the arrows. In spectrum (A) some of the resonances with clearly decreased intensity in (B) are circled. ^{15}N chemical shifts were referenced to liquid NH_3 (*20*).

to the chemical shift of water in ω_1 (Figs. 3B and 3C). These are mainly due to NOE interactions of amide protons with α protons that have chemical shifts near that of the water. Additional resonances at the water chemical shift may arise either from chemical exchange of NH protons with the bulk water, or from NOEs between H_2O and protons in the protein (*21*). Comparison of Fig. 3A with Figs. 3B and 3C also presents a nice illustration of the improved resolution achieved with the heteronuclear 3D NMR experiment (*12*).

In conclusion, the experimental data and their interpretation presented in this Communication clearly demonstrate the advantages of obtaining spectra of proteins in water using nonselective ^1H pulses without the need for presaturation of the water signal. Using the experimental schemes of Fig. 1, uniform excitation of the entire spectrum is achieved and a complete spectrum of the amide protons can be observed under conditions of pH and temperature, where water presaturation would cause the disappearance of the resonance lines of solvent-exposed labile protons and α protons with chemical shifts near that of the water (*1*). The 3D NMR experiment of Fig. 1B could further facilitate investigations of protein hydration in aqueous solu-

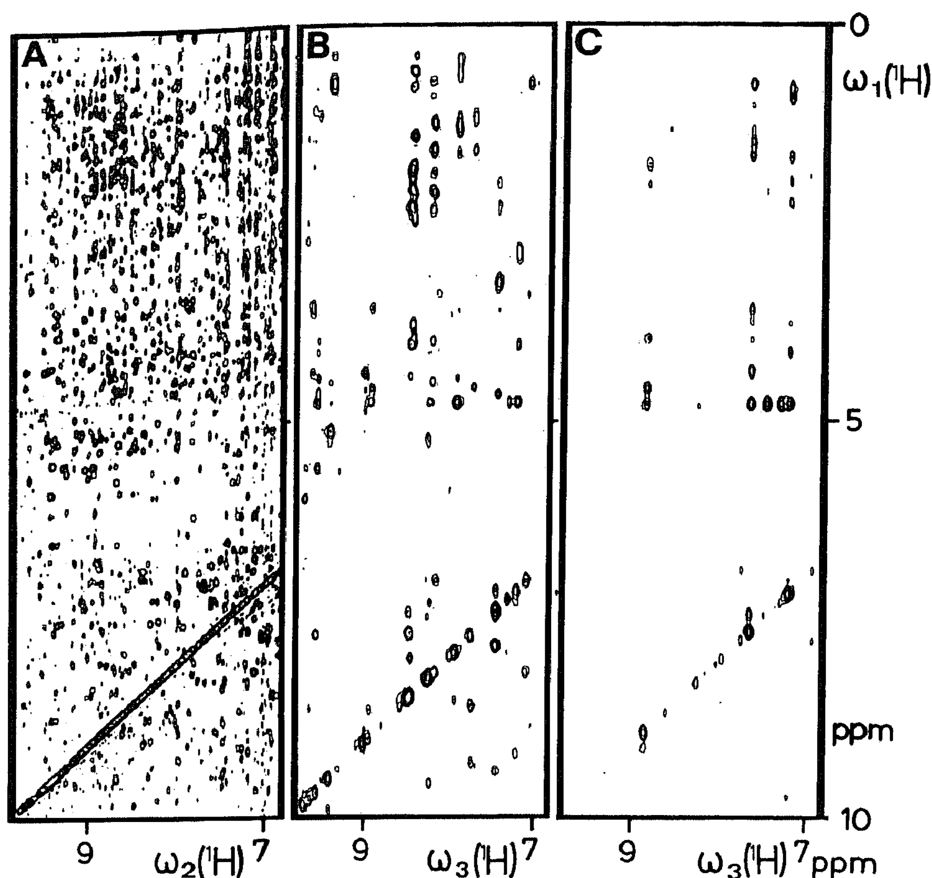

FIG. 3. (A) Two-dimensional ^{1}H NOESY spectrum of unlabeled cyclophilin (same sample conditions as those in Fig. 2, mixing time = 120 ms, total experimental time about 44 hours). Presaturation of the water resonance for 1 s was used during the preparation period, and also for the whole duration of the mixing time. (B) and (C) Cross sections from a three-dimensional NOESY-[^{15}N, ^{1}H]-COSY spectrum of ^{15}N-labeled cyclophilin (same sample conditions as those in Fig. 2). The pulse sequence of Fig. 1B was used, with spin-lock pulses of 0.5 ms (SL_y) and 2 ms (SL_x) duration. The cross sections were taken perpendicular to the ω_2(^{15}N) axis; (B) plane 28 and (C) plane 42 from a total of 64 planes. The total experimental time was 5 days, τ_m = 100 ms, τ = 5.6 ms, t_{1max} = 13.4 ms, t_{2max} = 16.9 ms, and t_{3max} = 63.5 ms, and ^{15}N decoupling during acquisition was achieved using WALTZ (*19*). The sweep width used in ω_1 and ω_3 was 8064 Hz, and in ω_2 it was 2840 Hz; 214 points were acquired in t_1, 76 points were acquired in t_2, and 1024 points were acquired in t_3. Prior to Fourier transformation, zero filling to 1024 points in ω_1, to 128 points in ω_2, and to 2048 points in ω_3 was used. Sine-bell weighting functions were applied, with phase shifts of $\pi/3$ in ω_3 and $\pi/2$ in both ω_1 and ω_2. Polynomial baseline correction was applied along ω_3 following the initial 2D Fourier transformation of the data from (t_1, t_2, t_3) to (t_1, ω_2, ω_3).

tion, following up on the recently described procedures using homonuclear 2D ^{1}H NMR (*21*).

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REFERENCES

1. K. WÜTHRICH, "NMR of Proteins and Nucleic Acids," Wiley, New York, 1986.
2. ANIL-KUMAR, G. WAGNER, R. R. ERNST, AND K. WÜTHRICH, *Biochem. Biophys. Res. Commun.* **96,** 1156 (1980).
3. G. WAGNER AND K. WÜTHRICH, *J. Mol. Biol.* **155,** 347 (1982).
4. G. WIDER, K. H. LEE, AND K. WÜTHRICH, *J. Mol. Biol.* **155,** 367 (1982).
5. K. WÜTHRICH, G. WIDER, G. WAGNER, AND W. BRAUN, *J. Mol. Biol.* **155,** 311 (1982).
6. G. WIDER, R. V. HOSUR, AND K. WÜTHRICH, *J. Magn. Reson.* **52,** 130 (1983).
7. V. SKLENÁŘ, R. TSCHUDIN, AND A. BAX, *J. Magn. Reson.* **75,** 352 (1987).
8. A. REDFIELD AND R. K. GUPTA, *J. Chem. Phys.* **54,** 1418 (1971).
9. P. J. HORE, *J. Magn. Reson.* **55,** 283 (1983).
10. P. PLATEAU AND M. GUÉRON, *J. Am. Chem. Soc.* **104,** 7310 (1982).
11. R. H. GRIFFEY AND A. G. REDFIELD, *Q. Rev. Biophys.* **19,** 51 (1987).
12. S. W. FESIK AND E. R. P. ZUIDERWEG, *J. Magn. Reson.* **78,** 588 (1988).
13. H. SENN, G. OTTING, AND K. WÜTHRICH, *J. Am. Chem. Soc.* **109,** 1090 (1987).
14. G. OTTING AND K. WÜTHRICH, *J. Magn. Reson.* **76,** 569 (1988).
15. G. BODENHAUSEN AND D. RUBEN, *Chem. Phys. Lett.* **69,** 185 (1980).
16. O. W. SØRENSEN, G. W. EICH, M. H. LEVITT, G. BODENHAUSEN, AND R. R. ERNST, *Prog. NMR Spectrosc.* **16,** 163 (1983).
17. C. J. R. COUNSELL, M. H. LEVITT, AND R. R. ERNST, *J. Magn. Reson.* **64,** 470 (1985).
18. D. MARION AND K. WÜTHRICH, *Biochem. Biophys. Res. Commun.* **113,** 967 (1983).
19. A. J. SHAKA, P. B. BARKER, C. J. BAUER, AND R. FREEMAN, *J. Magn. Reson.* **67,** 396 (1986).
20. D. H. LIVE, D. G. DAVIS, W. C. AGOSTA, AND D. COWBURN, *J. Am. Chem. Soc.* **106,** 1939 (1984).
21. G. OTTING AND K. WÜTHRICH, *J. Am. Chem. Soc.* **111,** 1871 (1989).