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Information from the Water Stripe in TOCSY Experiments on Systems with Exchangeable Protons[1]

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The water "stripes" of the TOCSY maps of aqueous solutions of sucrose, of a 15-aminoacid peptide, and of several of the constituent aminoacids are shown to contain correlations at the resonance frequencies of protons which are scalar coupled to OH or NH protons which exchange with the solvent. Theoretical analysis of chemical exchange during the spin-lock period in TOCSY elucidates the origin of these correlations, and shows that their intensities vary with the duration of the spin-lock period and with the exchange rate.

In NMR studies of proteins and peptides (*1*), conformational information is obtained via ^{1}H TOCSY, ROESY, and NOESY experiments which probe the scalar and dipolar interactions between the N–H and C–H groups along the backbone. Most studies are done in water solutions near neutral pH where the rate of exchange between the solvent accessible backbone N–H protons and water protons is slow (rate constant $\approx$ 1–2 s^{-1}). On the other hand, the rate of proton exchange between water and the hydroxyl groups in sugars is very fast, so that the resonances of the hydroxyl and water protons are coalesced into a single line. No information about the scalar coupling between hydroxyl protons and other protons in the sugar molecule can be obtained under these conditions, so most NMR studies of saccharides and oligosaccharides are performed in D_2O rather than in H_2O (*2*). A problem with NMR spectra obtained in H_2O is the presence of the extremely intense signal from the water protons—the water ''stripes'' in the f_1 and f_2 dimensions of the 2D maps. Several effective methods are available to minimize the intensity of the solvent resonance in 2D spectroscopy (*3*). In these suppression methods, the water proton magnetization is saturated so that there is essentially no water proton magnetization during the t_1 labeling period. Magnetization which appears at the water proton frequency in f_2 can arise in two ways—recovery of water proton magnetization as the system relaxes back toward equilibrium (f_1 will appear at the center of the f_1 window since this magnetization was not present during t_1), or magnetization transferred from some other proton(s) in the molecule via chemical exchange with the water protons (f_1 will reflect the source of the transferred magnetization by indicating its identity during t_1). In this report, we suggest that it may be advantageous to perform experiments on H_2O solutions since examination of features contained in the water stripes of 2D NMR contour maps may be of assistance in the assignment of molecular fragments containing protons which exchange rapidly with the water protons (e.g., OH, SH, NH protons). All spectra reported here were obtained on a Varian Unity 500 spectrometer using a TOCSY pulse sequence (*4*) with a spin-lock sequence consisting of a series of MLEV-16 (*5*) plus a 60° pulse element, preceded by saturation of the water resonance with the transmitter for 1.5 s at power level $\gamma B_1 = 110$ Hz.

Some preliminary experiments on simple model compounds were undertaken. The 500-MHz ^{1}H TOCSY map of sucrose in water solution is shown in Fig. 1. Of particular interest is the water stripe at $f_2 = 4.8$ ppm. Since the water suppression was very effective, an examination of this water stripe provides reliable identification of the magnetizations which can be transferred to the water protons during the spin-lock period in TOCSY. Correlations to all protons in the sucrose molecule except G1 are present. There is too much overlap of the resonances of the G5, F6, and G6 protons (δ 3.85 ppm) to claim unequivocally that all of these protons have correlations in the water stripe, but a weak correlation to F5 does appear in the water stripe. In a TOCSY experiment on l-butanol in water done under conditions identical to those for the sucrose experiment, the water stripe contained only a single correlation to the methylene protons α to the hydroxyl group. We suggest, therefore, that the strongest correlations which appear in the water stripe are to protons in C$\mathbf{H}_n$OH fragments—that is, to protons which are scalar coupled to the OH protons which exchange with the solvent protons. The anomalous appearance in the water stripe of the very weak correlation to the sucrose F5 proton, which is not directly scalar coupled to an OH proton, is undoubtedly due to reasonably large scalar F5–F4 and F5–F6 proton–proton couplings which provide indirect TOCSY connections to the OH protons on the F4 and F6 centers (and hence to the OH protons at these centers) during the spin-lock period.

In order to delineate the correlations which may be con-

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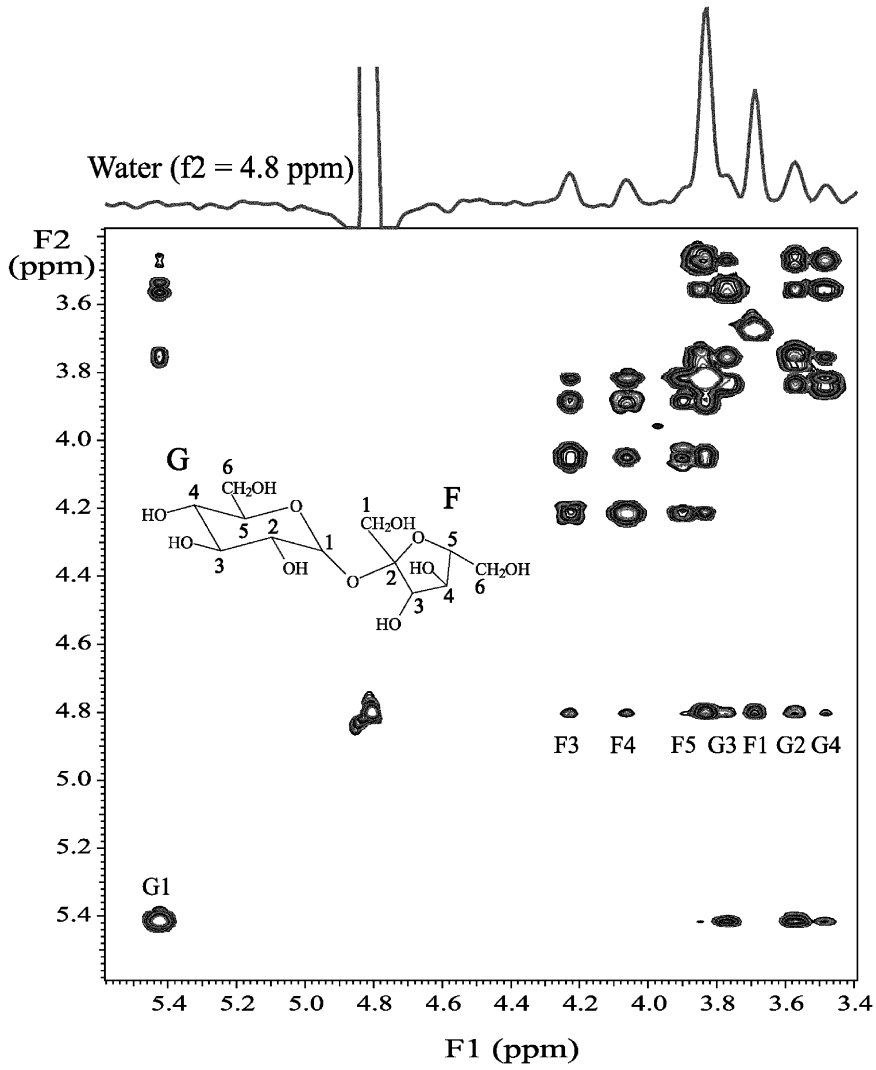

FIG. 1. Part of the 500-MHz TOCSY spectrum of 67 mM sucrose in 80% H_2O/20% D_2O. The water stripe is plotted above the contour map. Water suppression was performed by transmitter presaturation and the spin lock period was 0.05 s. An acquisition time of 0.25 s was used to define 2000 Hz. Eight scans were acquired with a recycle delay of 1.3 s for each of the 512 t_1 increments.

tained in the water stripe in peptides and proteins, we have performed TOCSY experiments on a peptide (**I**) containing 15 amino acid residues in the sequence KEGYLVDKNTGCKYE with a C-terminal amide. Peptide **I** contains residues which have exchangeable protons in side chains: one cysteine (C) with an SH proton, one threonine (T) and two tyrosines (Y) with OH protons, and three lysine (K) residues which have exchangeable NH protons. Sections of the TOCSY maps of **I** obtained in experiments at high (6.7) and low (4.0) pH are given in Figs. 2 and 3, respectively. At both pHs, exchange between the water protons and the amide protons in the peptide backbone is sufficiently slow that strong $C_\alpha\mathbf{H}$–N**H** correlations were observed. At high pH (Fig. 2), the water stripe shown just above the contour map contains a weak and broad correlation for the backbone N**H** protons (7~9 ppm), a weak correlation to the $C_\beta\mathbf{H}$ proton of threonine at 4.35 ppm, and a weak correlation near 2.9 ppm which is probably the shoulder of the strong asparagine $C_\alpha\mathbf{H}$–$C_\beta\mathbf{H}$ correlation which lies just above the ''water stripe.'' There was no evidence of any correlation to the aromatic protons in the tyrosine or lysine residues in the water stripe, nor were there any signals attributable to the terminal NH protons in the lysine residues, or to the SH protons in cysteine.

At low pH (4.0), exchange between the water protons and the backbone NH protons of the peptide is expected to be slower than at high pH (*6*). The most striking feature of the TOCSY map of **I** at low pH (Fig. 3) is the appearance of a series of correlations between the NH_3^+ protons (7.6 ppm) and other protons in the lysine residues: Strong correlations to $C_\epsilon\mathbf{H}_2$ (3.01 ppm) and $C_\delta\mathbf{H}_2$ (1.70 ppm) and a medium correlation to $C_\gamma\mathbf{H}_2$ (1.45 ppm) in the lysines are observed (the resonances for the individual lysines are not resolved). In the

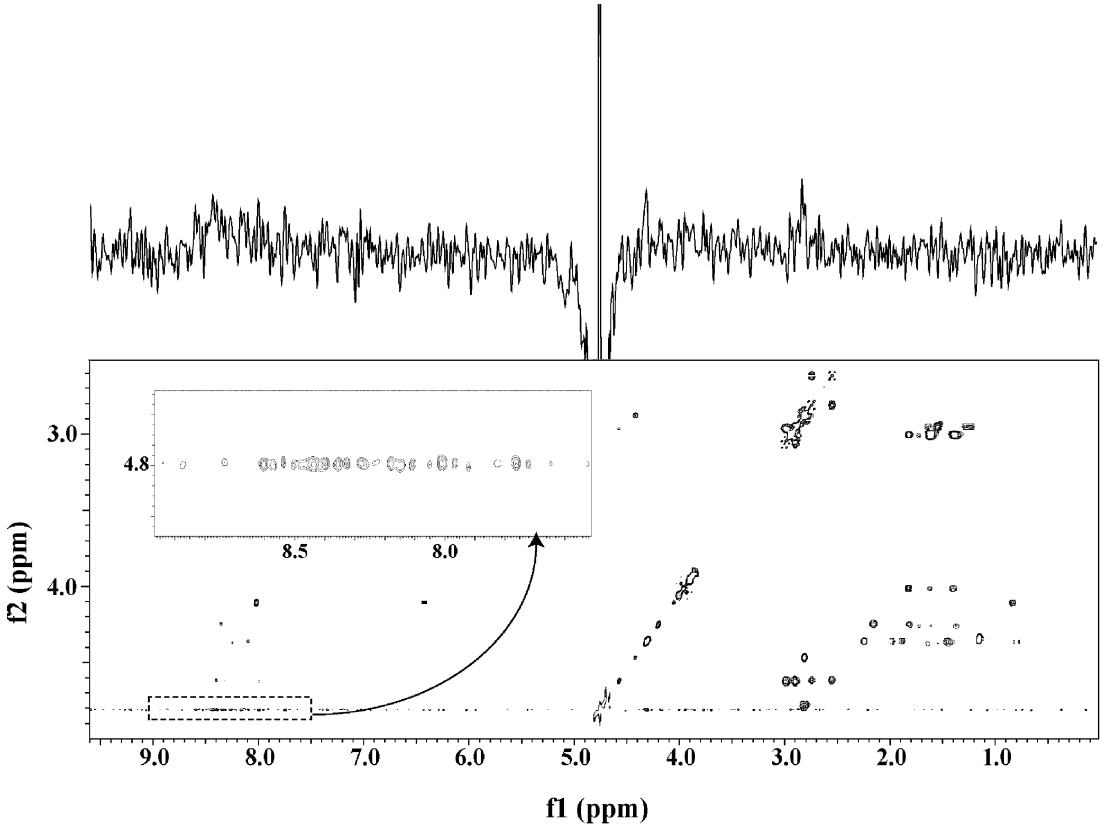

FIG. 2. Part of the TOCSY spectrum of peptide **I** (12 mM in 80% H_2O/20% D_2O) at pH 6.7. The water stripe is plotted above the contour map and an expansion of the 7.5–9.0 ppm region is shown in the inset. Experiment parameters as in Fig. 1, except a 4800-Hz sweep width and 32 scans/t_1 were used.

water stripe shown above the contour map, we find a number of correlations, but only to protons in the lysines: a strong correlation to $C_\epsilon\mathbf{H}_2$, a weak correlation to $C_\delta\mathbf{H}_2$, and a very weak correlation to $N\mathbf{H}_3^+$.

In order to confirm our interpretations of the correlations present in the TOCSY maps of peptide **I**, we have investigated 1D and TOCSY spectra of the aminoacids cysteine, lysine, threonine, and tyrosine in water solutions and summarize our findings here. For lysine at pH 5.7, there were no detectable signals from the ϵ-$N\mathbf{H}_3^+$ protons, but the water stripe in the TOCSY map contained a strong correlation to the $C_\epsilon\mathbf{H}_2$ protons and a weak one to the $C_\delta\mathbf{H}_2$ protons. At pH 3.9, lysine had resolved, but broad, ϵ- and α-NH_3^+ resonances; the ϵ-NH_3^+ showed strong TOCSY correlations to the $C_\epsilon\mathbf{H}_2$ and $C_\delta\mathbf{H}_2$ protons, and progressively weaker ones to the $C_\gamma\mathbf{H}_2$ and $C_\beta\mathbf{H}_2$ protons; and the water stripe contained strong correlations to $C_\epsilon\mathbf{H}_2$, medium correlations to $C_\alpha\mathbf{H}_2$, and weaker ones to $C_\delta\mathbf{H}_2$ and $C_\beta\mathbf{H}_2$. The correlations to $C_\alpha\mathbf{H}_2$ and $C_\beta\mathbf{H}_2$ undoubtedly arise via scalar coupling to the α-NH_3^+ protons which exchange with water. No ϵ- and α-NH_3^+ correlations were detectable in the water stripe of the lysine TOCSY map at pH 3.9 due to the large widths and low amplitudes of the ϵ- and α-NH_3^+ resonances. TOCSY experiments on threonine at pH 5.6 gave strong $C_\beta\mathbf{H}$ and $C_\gamma\mathbf{H}_3$ correlations and a weaker one for $C_\alpha\mathbf{H}$ in the water stripe. No correlations appeared in the water stripes of the TOCSY maps for tyrosine at pH 0.9 or for cysteine at pH 6.7. All of the relevant spectral features observed in these experiments on the individual amino acids are consistent with the data on peptide **I**.

The experimental observations of TOCSY correlations in the water stripe from $C\mathbf{H}_n$ protons which belong to scalar coupling networks containing NH or OH protons imply that magnetization from $C\mathbf{H}_n$ is transferred to water via chemical exchange. This magnetization transfer can be described quantitatively for the CH–XH$\cdots$$H_{water}$ spin triad in which we designate product operators for the C**H** proton with symbol **A**, operators for the X**H** proton with symbol **X**, and operators for the water proton as **W**. During the spin-lock period of the TOCSY experiment, the spin Hamiltonian for the CH–XH fragment is (*7*)

$$\mathcal{H} = 2\pi J\mathbf{A}\cdot\mathbf{X}, \qquad [1]$$

where J is the C**H**–X**H** scalar coupling constant. In the absence of exchange, the **A** and **X** magnetizations interconvert in a cyclic fashion with period $1/J$ so that the t_1-labeled $\mathbf{A}_x$ magnetization present at the beginning of the spin-lock

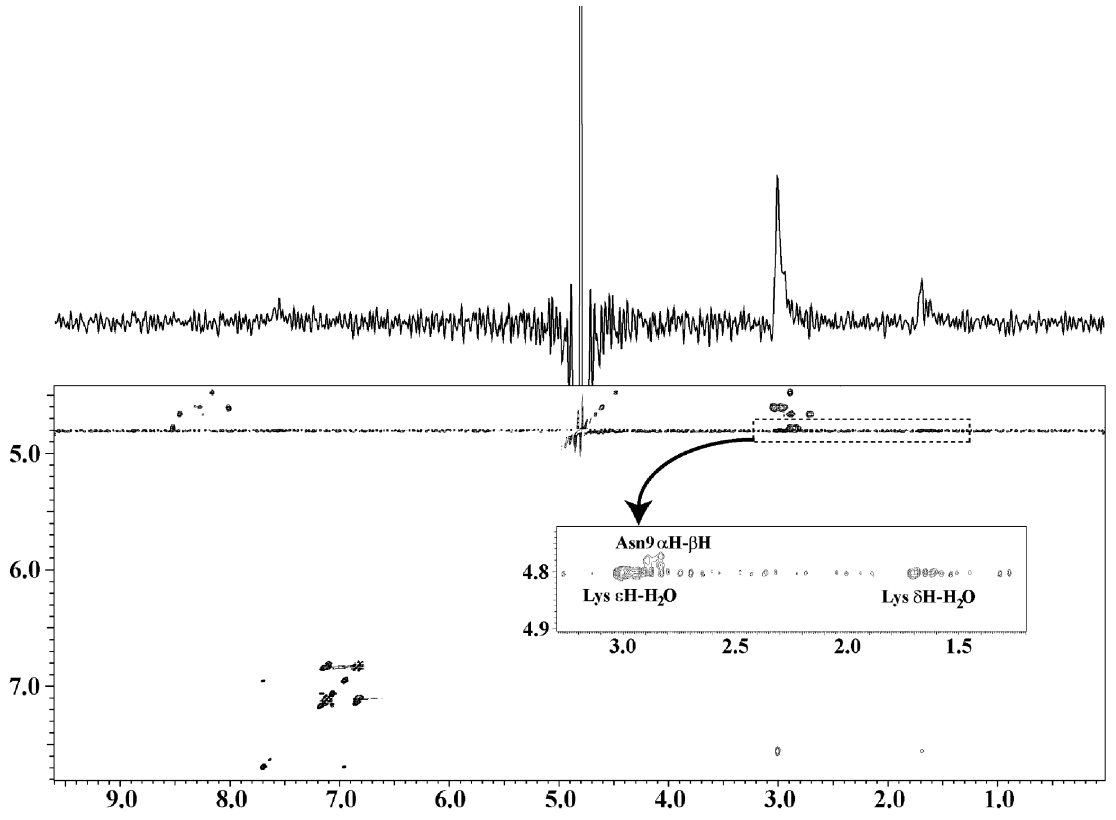

FIG. 3. Part of the TOCSY spectrum of peptide **I** (12 mM in 80% H_2O/20% D_2O) at pH 4.0. The water stripe is plotted above the contour map and an expansion of the 1.2–3.3 ppm region is shown in the inset together with assignments of the strongest peaks. Experiment parameters as in Fig. 2.

period generates t_1-labeled components of **X** magnetization ($\mathbf{X}_x$ and $2\mathbf{X}_y\mathbf{A}_z$), whose amplitudes oscillate at frequency J as they are interchanged with **A** magnetization ($\mathbf{A}_x$ and $2\mathbf{A}_y\mathbf{X}_z$) by the scalar interaction (*7*). When exchange is present, however, all of the components of the **X** and **W** magnetizations are interconnected by the exchange events, which implies that the t_1-labeled $\mathbf{A}_x$ magnetization present at the beginning of the spin-lock period will generate **W** magnetization ($\mathbf{W}_x$) during the spin-lock period. A full product operator calculation of the behavior of the coherences formed during the spin-lock period from the **A**, **X**, and **W** spin operators in the -1 coherence level was carried out using the procedures of Ref. (*8*) and confirms this qualitative description. Shown in Fig. 4 are the calculated magnetization components $\mathbf{A}_x$, $\mathbf{X}_x$, and $\mathbf{W}_x$ which originate from t_1-labeled $\mathbf{A}_x$ magnetization and develop during the spin-lock period of TOCSY for two values of the exchange rate constant $k = 1.0\ s^{-1}$ and $k = 10\ s^{-1}$, for a system in which $J = 7.5$ Hz and equal solute and solute concentrations. For $k = 1.0\ s^{-1}$ (Fig. 4b), the dominant feature is the slowly damped periodic interchange of $\mathbf{A}_x$ and $\mathbf{X}_x$ magnetizations, with the amplitude of the $\mathbf{W}_x$ magnetization exhibiting slow exponential growth with rate constant $1.5\ s^{-1}$ throughout the spin-lock period and only small amplitude oscillations. At the higher rate of exchange (Fig. 4a), the $\mathbf{W}_x$ magnetization grows much more rapidly and has oscillations of larger amplitude, whereas the amplitude of the periodic interchange of $\mathbf{A}_x$ and $\mathbf{X}_x$ magnetizations is more rapidly damped than at the slower exchange rate. These calculated

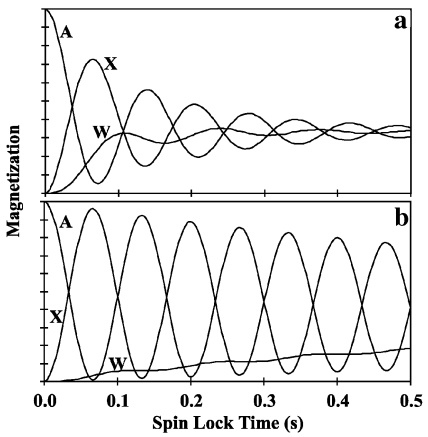

FIG. 4. Calculated amplitudes of magnetization transferred from CH-proton (**A**) to XH-proton (**X**) and water proton (**W**) during a spin-lock period for exchange rates $10.0\ s^{-1}$ (a) and $1.0\ s^{-1}$ (b) for a fragment CH–XH with $J =$ 7.5 Hz.

curves indicate that correlations to C**H** protons in CH–XH fragments should be observed in the water stripe of a TOCSY map if the XH proton exchanges with water at a suitable rate.

In this report, we have shown that, even in cases of rapid chemical exchange where the resonances of solvent-exchangeable protons cannot be distinguished from the solvent resonance, TOCSY experiments may be used to identify the chemical shifts of protons which are scalar coupled to these solvent-exchangeable protons provided effective solvent suppression is attainable. As one of the reviewers has pointed out, one may obtain increased sensitivity by studying the water resonance along f_2 at $f_1 = 4.8$ ppm in TOCSY experiments without presaturation (*9*). Earlier methods of obtaining such information relied on temperature reduction and/or solvent modification as means of decreasing the exchange rate so the resonances of solvent-exchangeable protons were resolved. The observations reported here indicate that information contained in the water stripe of a TOCSY map may be used in establishing the resonance assignments of peptides, proteins, saccharides, and nucleotides.

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