Cross relaxation and spin diffusion in the proton NMR of hydrated collagen

PROTON NMR of water is being used extensively to probe the molecular dynamics of water molecules in biological systems such as protein solutions, hydrated macromolecules, cells and tissue. The nuclear magnetic relaxation rates R_1 (spin–lattice) and R_2 (spin–spin) can be analysed in terms of the rotational motions of the water molecules[1–3]. A crucial assumption in this analysis is that the proton relaxation of the water proceeds independently of that of the macromolecules. Kimmich and Noack[4–6] claim that this assumption may be incorrect for proton spin–lattice relaxation because of spin diffusion, but clear evidence of spin diffusion in hydrated biological samples has not been reported. We show here that the proton spin–lattice relaxation behaviour in hydrated collagen is dominated by cross relaxation between the water protons and the macromolecular protons as a result of spin diffusion; the macromolecular spin–lattice relaxation contributes significantly to the water proton R_1.

The proton spin–lattice relaxation of ribbons of reconstituted collagen[7], hydrated with H_2O or D_2O, was studied by pulsed NMR. The spin–lattice relaxation was determined from a comparison of the free induction decay (FID) signal after a $(180° - t - 90°)$ pulse pair with that after a single 90° pulse. The pulse pair yields the partially relaxed magnetisation $M_z(t)$ whereas the single 90° pulse yields the equilibrium magnetisation M_0. The spin lattice relaxation rate was determined from a logarithmic plot of the reduced magnetisation $m(t) = -(M_z(t) - M_0)/2M_0$ against t.

The FID of hydrated collagen consists of two components (see Fig. 1). Protons in the rigid collagen matrix constitute a

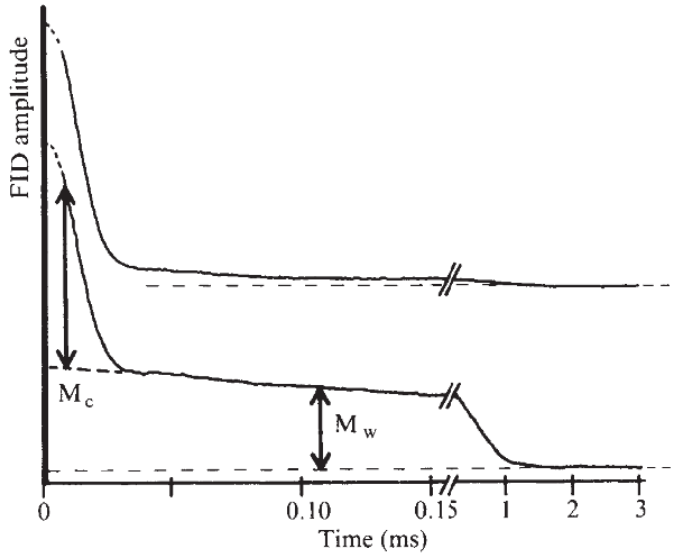

Fig. 1 Proton FID signals in reconstituted collagen, hydrated with 28 g water per 100 g collagen. Lower curve: hydrated with H_2O. Upper curve: hydrated with 95% D_2O. The collagen signal intensity $M_c(t)$ was measured immediately after receiver recovery from the 90° pulse, 10 μs after the beginning of the pulse. The water signal intensity $M_w(t)$ was measured 60, 110 and/or 400 μs after the pulse; the spin–lattice relaxation decay at these times is the same. The collagen fibres were oriented perpendicular to the magnetic field. Measurements were performed at room temperature with a Bruker pulse spectrometer operating at 30 MHz; a Varian V-2708 magnet was used, equipped with a Varian V-3508 flux stabiliser. The 90° pulse length was 1.5 μs. The FID signals were averaged with a combination of a Biomation 802 signal digitiser and a Nicolet 7002 were averaged together.

fast relaxing part ($R_2^* = 60{,}000\ s^{-1}$), and the water protons relax much more slowly ($R_2^* \lesssim 5{,}000\ s^{-1}$). This identification of the two signal components is supported by the observations that the intensity of the water fraction changes in direct proportion to the amount of hydration water and to the isotopic dilution with D_2O, and that the relative intensities of the two fractions correspond to the calculated ratio of collagen protons to water protons (three to seven, respectively, for the sample shown in Fig. 1). No static dipolar splitting of the water signal was apparent, in agreement with other studies of natural collagen under similar conditions[8,9].

The two components of the FID can easily be separated (see Fig. 1) so that we could study the spin–lattice relaxation of the water and of the macromolecular protons separately (Fig. 2). Measurements at different water contents all showed the following characteristics (see Fig. 2): (1) The relaxation rate R_1 of both water and collagen protons is the same if the initial 10 ms of the relaxation decays is ignored. (2) The beginning of the water relaxation decay is clearly concave (curve *c*) and that of collagen is convex (curve *b*). Both relaxation curves are described by a sum of two exponentials. (3) When collagen is hydrated with D_2O, then the relaxation rate of the collagen protons is single-exponential and proceeds at a rate which is much slower than in the presence of H_2O (curve *a*).

Proton NMR relaxation is brought about by magnetic dipolar interactions. Substitution of D_2O for H_2O changes the nuclear dipolar interactions between collagen protons and water nuclear spins. Assuming that the dynamics of hydration are similar for H_2O and D_2O, then the dipolar interaction between a collagen proton and a water proton is of the order of $(\gamma_H/\gamma_D)^2\ (I_H/I_D)\times(I_H+1)/(I_D+1) \simeq 16$ times more effective at inducing nuclear spin transitions than the interaction between a collagen proton and a water deuteron[10]. The large change in R_1 of the collagen protons on substitution of D_2O for H_2O clearly indicates that the macromolecular protons 'feel' the presence of water protons. Consequently, dipolar coupling between water and macromolecular protons must be an important factor in the proton relaxation mechanism in hydrated collagen.

The non-exponentiality of the water relaxation might be ascribed to the presence of two slowly exchanging water fractions. This seems unwarranted, however, as it does not explain the convex curvature in the collagen relaxation, or account for the similar relaxation rates of the two components after the initial 10 ms of decay. We can explain all of our observations by realising that the dipolar coupling between water and collagen protons provides a way for cross relaxation between the water and the collagen protons by the spin diffusion mechanism[10,11].

We will assume that the protons in the water phase as well as in the collagen phase have, at any time, a uniform spin temperature, that is, the longitudinal magnetisation $M_z(t)$ in each phase is uniform. Within the collagen phase spin diffusion among the collagen protons will rapidly establish a common spin temperature, whereas in the water phase this is accomplished by rapid chemical exchange. The water phase may also include exchangeable collagen protons. Let p_w and p_c be the fraction of protons in the water phase and in the collagen phase respectively, with the proton relaxation rates in the absence of cross relaxation denoted by R_{1w} and R_{1c}. It should be noted that R_{1w} comprises the contributions to the water proton relaxation rate which are normally included in theoretical treatments, for example, effects from bound and free fractions of water[1–3]. Whenever a proton of the water phase resides near a collagen proton, the two protons are coupled by the magnetic dipolar interaction and a mutual spin flip can occur[10]. Such a spin flip exchanges spin energy between the two proton phases,

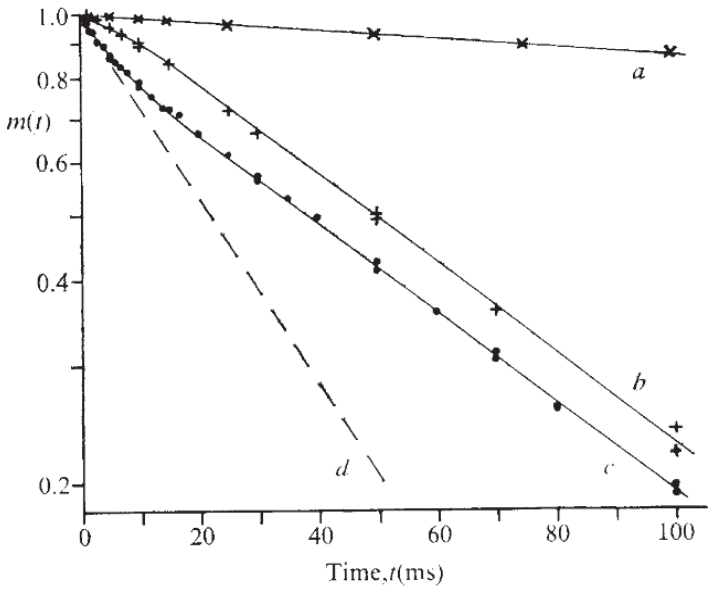

Fig. 2 Proton spin–lattice relaxation decays for the reduced magnetisation $m(t)$ in hydrated reconstituted collagen for the same samples as in Fig. 1. Measurements of $M_z(t)$ and M_0 were alternated and the FID signals were averaged into two halves of the memory of the signal averaging system. This alternation ensures very accurate R_1 measurements since slow changes in time of signal gain and/or of field drift affect $M_z(t)$ and M_0 in the same way and are compensated. Curve *a*, $m_c(t)$ for the collagen proton signal, hydrated with 95% D_2O (×). Curve *b*, $m_c(t)$ for the collagen proton signal, hydrated with H_2O (+). Curve *c*, $m_w(t)$ for the water proton signal of H_2O (●). A least squares fit of equation (2) to $m_w(t)$ yields the estimates, $R_1 = 15\pm1\ s^{-1}$; $c_w^- = 0.89\pm0.01$; $R_1^+ = 160\pm15\ s^{-1}$; $c_w^+ = 0.11\pm0.01$. Curve *a* gives $R_{1c} = 3\pm0.5\ s^{-1}$. With $p_w = 0.3$, equation (3) gives $R_{1w} = 31\pm4\ s^{-1}$; $k_c = 40\pm10\ s^{-1}$; $k_w = 100\pm25\ s^{-1}$; $c_c^- = 1.08\pm0.01$; $c_c^+ = -0.08\pm0.01$. Solid lines represent the theoretical decays obtained with these values of the parameters. Curve *d* represents the water relaxation decay with a relaxation rate $R_{1w} = 31\ s^{-1}$ as it would occur in the absence of cross relaxation.

leading to cross relaxation between the two phases. Spin diffusion may occur through protons on water molecules that are temporarily bound to the collagen, or through rapidly exchanging collagen protons. Spin diffusion will be effective in particular, if such bound nuclei exchange at a rate which is slow compared to ω_o, the NMR measuring frequency[10,11].

We can introduce a cross relaxation rate k_c, the rate at which magnetisation diffuses from the collagen to the water; k_w is the reverse rate. We can then write a set of modified Bloch equations, similar to those well-known for the case of chemical exchange:

$$dm_w(t)/dt = -R_{1w}m_w(t) - k_w m_w(t) + k_w m_c(t) \quad (1a)$$

$$dm_c(t)/dt = -R_{1c}m_c(t) - k_c m_c(t) + k_c m_w(t) \quad (1b)$$

Unlike the case of chemical exchange, (1) applies only to the z-component of the magnetisation; spin diffusion does not lead to cross relaxation in the transverse magnetisation.

The solution of the set of coupled differential equations (1) predicts that the relaxation in either phase is described by two apparent relaxation rates R_1^+ and R_1^- which are the same for both phases. The complete solution[12] is (the subscript i refers to either of the phases)

$$m_i(t) = c_i^+ \exp(-R_1^+ t) + c_i^- \exp(-R_1^- t) \quad (2)$$

where

$$R_1^\pm = \tfrac{1}{2}(R_{1c} + R_{1w} + k_c + k_w) \pm \tfrac{1}{2}\sqrt{[(R_{1c} - R_{1w} + k_c - k_w)^2 + 4k_c k_w]} \quad (3a)$$

$$c_i^\pm = \pm (R_1^\mp - R_{1i})/(R_1^- - R_1^+) \quad (3b)$$

$$k_w = (p_c/p_w)k_c \quad (3c)$$

From (3b) follows

$$R_{1i} = c_i^+ R_1^+ + c_i^- R_1^- \quad (3d)$$

With these expressions all the characteristics of the spin-lattice relaxation can be explained quantitatively.

The two fractions in the relaxation curve of the water signal are clearly resolved, permitting the determination of all the parameters in equation (2). A fit to the data points (see Fig. 2) yields R_1^+, R_1^-, c_w^+ and c_w^-; using these values, R_{1w} is calculated, from equation (3d), to be $31 \pm 4\ s^{-1}$. A similar calculation for the collagen proton signal decay is less accurate, but gives $R_{1c} \approx 3\ s^{-1}$, which is similar to R_{1c} measured in D_2O. p_c and p_w can be determined from the relative signal intensities in the FID, extrapolated to zero time (Fig. 1), or from the known amounts of protons in the two phases. Within experimental error the two methods give the same values for p_c and p_w.

The parameters that describe the complete relaxation behaviour consistent with equations (3) are given in the legend to Fig. 2. For the cross relaxation rate k_c we obtain an estimate of $40 \pm 10\ s^{-1}$ for a hydration of 28 g H_2O per 100 g collagen. We believe that this is the first time that a figure has been given for the proton spin diffusion rate from a protein to the hydration water. Our measurements show that the water phase constitutes the relaxation 'sink' for the collagen proton phase. This result differs with the model of Kimmich and Noack[4-6], who proposed that the water relaxation proceeds via relaxation 'sinks' located with the macromolecular phase.

Usually, water relaxation rates in hydrated biological systems are obtained from plots similar to Fig. 2. It is common practice, however, to ignore, or avoid measurements (because of associated experimental difficulties) during the initial 10 ms of the relaxation decay. It is clear from our analysis that this practice would lead to a measurement of an apparent relaxation rate of water (R_1^- in the present case), which is not necessarily the same as R_{1w}. We find for the hydrated collagen sample in Fig. 2 that $R_{1w} \approx 2R_1^-$. Clearly, cross relaxation can significantly affect water proton spin–lattice relaxation in biological samples. Its importance must be more carefully evaluated before applying molecular dynamical interpretations to apparent water relaxation rates. We are currently measuring cross relaxation effects in other hydrated biological systems and evaluating its consequences for a published analysis[9] of the relaxation behaviour in hydrated collagen.

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