Protein Hydration Viewed by High-Resolution NMR Spectroscopy: Implications for Magnetic Resonance Image Contrast

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Recent high-resolution nuclear magnetic resonance (NMR) experiments indicate that the residence times of hydration water molecules on protein surfaces are in the subnanosecond time scale.[1] This result bears important consequences for the interpretation of image contrast in magnetic resonance (MR) imaging experiments, where the contrast is based on magnetization transfer between tissue and water. This Account presents and discusses the respective NMR experiments leading to these conclusions. The short lifetimes of the association between polypeptide chains and their hydration water compare well with average values deduced from relaxation measurements of the water signal in protein solutions.[2] The high-resolution experiments discussed here measure the residence times near individual protein protons, thus affording a much more detailed view of protein hydration. The same experiments detect the proton exchange from OH and NH groups with the solvent.

Magnetization transfer between macromolecules and water generates contrast in MR imaging, since it provides a mechanism by which the detected water signal "senses" the properties of different proteins, lipids, etc. in its environment. To date, it is usually believed that the exchange of hydration water with bulk water is the major molecular mechanism for magnetization transfer between tissue and bulk water.[2–4] Consequently, rather unusual hydration models have been postulated to explain the observed magnetization transfer.[2,5] However, the combined results from the hydration studies discussed here and proton exchange rate measurements suggest that proton exchange beween the macromolecular phase and the water cannot be neglected and is at least in some cases more important for image contrast than the exchange between hydration water and bulk water.

Nuclear Overhauser Effects for the Detection of Hydration Water Molecules

NMR spectroscopy is one of the most powerful and universally applicable techniques to study chemical or conformational equilibria in solution.[6] Thereby, the subnanosecond time range can be addressed by relaxation studies of proton (^{1}H) magnetization which is sensitive to processes occurring at rates comparable to $2\pi\nu$, where ν is the precession frequency of the nuclear spins in the magnetic field (Larmor frequency). Of all relaxation phenomena, the nuclear Overhauser effect (NOE) stands out for its key role in the determination of the three-dimensional structure of proteins and DNA.[7] The estimation of the residence times of hydration water molecules on protein surfaces from the signs of the NOEs observed between the water and the macromolecule is the focus of the present Account.

A NOE is equivalent to dipolar cross-relaxation between two nuclear spins which are sufficiently close in space that the interaction between the nuclear magnetic dipole moments induces measurable magnetization transfer between the nuclei. The strength of this dipole–dipole interaction decays with $1/r^6$ with increasing distance r between the two spins. In practice, NOEs are observed only for protons which are closer than 5 Å.[7] Therefore, NOEs observed in aqueous protein solutions between protein protons and water protons are always interactions with the water molecules of the innermost hydration layer. This is fortunate for the study of protein hydration, because only a single, averaged signal is observed for all water protons in the NMR spectrum due to the exchange of the water molecules or their protons between the different chemical environments with rates larger than 1000 s^{-1}.[8] The short-range nature of the water–protein NOEs ensures that all NOEs observed with the single, averaged water signal are in fact with hydration water, even though hydration water contributes only little to the overall water signal, which is dominated by the free, unbound bulk water.

NOEs are most readily observed by two-dimensional NOE spectroscopy (NOESY).[6] There, the NOE-medi-

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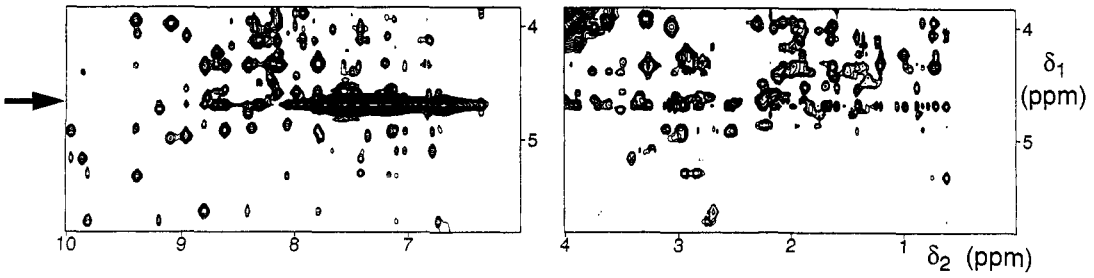

Figure 1. Selected spectral regions of a two-dimensional NOESY spectrum recorded with a solution of bovine pancreatic trypsin inhibitor. The intermolecular water–protein cross peaks are aligned along the horizontal chemical shift axis at the chemical shift of the water signal (δ_1 = 4.8 ppm, indicated by the arrow). The central part of the spectrum at δ_2 = 4.8 ppm is not shown, because it is obscured by the dominant water signal. Protein concentration: 20 mM in 90% H_2O/10% D_2O, pH 6.9, 36 °C. The relaxation agent GdDTPA was added at a concentration of 750 μM to enhance the relaxation of the water resonance. The spectrum was recorded at 400 MHz with a mixing time of 50 ms. Positive and negative levels were plotted without distinction.

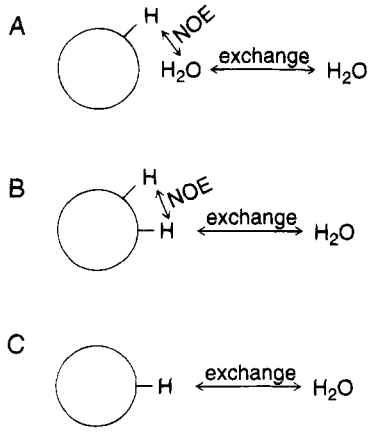

Figure 2. Three different mechanisms leading to intermolecular water–protein cross peaks in NOESY and ROESY spectra. (A) The magnetization is transferred by magnetic dipole–dipole interaction (NOE) between nonexchangeable protein protons and bound hydration water which is in chemical exchange with the free, unbound bulk water. Because of the rapid exchange, bound and free water appear at the same chemical shift in the NMR spectrum. (B) The magnetization is transferred between nonexchangeable protein protons and the bulk water via a labile OH or NH proton of the protein which exchanges with the solvent. Because of exchange rates >1000 s^{-1} between the labile protein protons and the water, the OH and NH protons appear in the NMR spectrum at the chemical shift of the bulk water. (C) Chemical exchange of labile protein protons with the bulk water with rates smaller than 1000 s^{-1} so that the exchangeable protein protons are observed as individual resonances separated from the water signal.

ated magnetization transfers are manifested as cross peaks at the frequencies of the interacting protons. Figure 1 shows as an example spectral regions of a NOESY spectrum recorded with an aqueous solution of bovine pancreatic trypsin inhibitor (BPTI). Besides cross peaks from NOEs between different proton resonances of the protein, there are a number of water–protein cross peaks aligned along the δ_2 chemical shift axis at the δ_1 chemical shift of the water resonance. These cross peaks can arise from three different magnetization transfer pathways (Figure 2): (i) intermolecular NOE between a protein proton and the protons of hydration water molecules which exchange with the bulk water, (ii) intramolecular NOE between a protein proton and a labile protein proton which is in rapid exchange with the bulk water and therefore appears at the water chemical shift, and (iii) chemical exchange of a labile protein proton with water protons. The first two can be distinguished from the last mechanism by comparison with a NOE spectrum in the rotating frame (ROESY).[9] While longitudinal magnetization is exchanged between different protons in a NOESY experiment, the ROESY experiment detects the exchange of transverse magnetization. One of the consequences is that chemical exchange peaks and cross peaks involving a single NOE transfer step have different signs in ROESY. The distinction between mechanisms i and ii is possible if the involvement of an exchangeable protein proton can be excluded from the knowledge of the three-dimensional structure of the protein, or if the NOEs with the exchangeable protein protons can be identified by different means, *e.g.*, by recording spectra at low temperature and suitable pH, where the proton exchange is sufficiently slow that the chemical shifts of the exchangeable protons are no longer averaged with the water chemical shift, and individual resonances and cross peaks can be observed for the labile protons.[10]

To date, no example is known in which different chemical shifts were observed for hydration water and bulk water, although the different chemical environments ought to induce chemical shift differences of up to several parts per million. To average this chemical shift difference requires that the lifetime of the hydration water molecules is shorter than about 1 ms.[8] Interestingly, this upper limit applies also to hydration water located in the interior of protein structures without any direct access to bulk water. Their short lifetimes are an example of the dynamic nature of protein structures which, for short times, makes the protein interior accessible to the bulk water and allows the exchange of water protons or entire water molecules.

NOE cross peaks between the water signal and the resonance of a protein identify hydration sites and convey information about the residence times of the hydration water molecules at these sites. An upper limit of 1 ms is indicated by the degeneracy of the water proton chemical shifts. As it turns out, information about the residence times in the subnanosecond time range is encoded by the size and sign of the NOEs.

NMR Hydration Studies of the Small Protein BPTI

NOE studies performed with the small globular protein BPTI show that by far the most prominent

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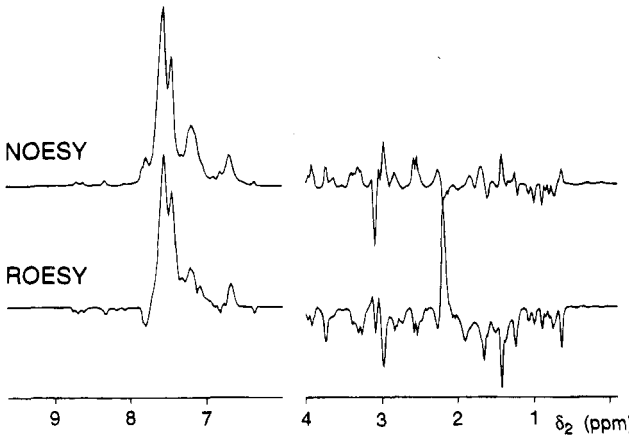

Figure 3. Water–protein cross peaks in NOESY and ROESY. Upper panel: cross section through the NOESY spectrum of Figure 1 taken at the position indicated by the arrow in Figure 1. Lower panel: corresponding cross section taken from a ROESY spectrum recorded under conditions identical with those of the NOESY experiment. For improved readability, the spectral regions containing the water–protein cross peaks between 6 and 9.5 ppm were scaled down by a factor of 8 and inverted to compensate for the sign inversion introduced by the excitation profile of the experimental scheme used.[12] The magnetization transfer by the mechanisms involving NOEs (Figure 2A,B) gives rise to negative peaks in the ROESY and positive or negative peaks in the NOESY spectra. Direct chemical exchange (Figure 2C) yields positive peaks in both spectra.

water–protein NOEs arise from internal hydration water molecules, whereas much weaker NOE intensities are observed for most of the surface hydration water. Figure 3 shows the cross sections through the NOESY and ROESY spectra of Figure 1 along the δ_2 chemical shift axis at the δ_1 chemical shift of the water line. The large signals between 6.5 and 7.8 ppm come from the chemical exchange of labile side chain protons with water.[11] They are readily identified as exchange peaks by the fact that they have the same, positive, sign in NOESY and ROESY. NOE cross peaks are identified by their negative sign in ROESY; they may be either positive or negative in NOESY (see below). The NOE cross peaks belong to one of three categories: (i) direct NOE with water; (ii) NOE with a rapidly exchanging protein proton, *e.g.*, the hydroxyl and amino protons of amino acid side chains; (iii) intraprotein NOE with nonexchangeable protons which accidentally appear at or near the water chemical shift. The signs and intensities of intraprotein cross peaks are sometimes affected by coherent magnetization transfer.[6,12] Comparison with spectra recorded in D_2O solution shows that there are only few peaks of type iii present in the cross section of Figure 3. Examples are the intense signals at $\delta_2 \approx 2.2$ and 3.1 ppm.

Because of extensive overlap between the protein resonances in a one-dimensional NMR spectrum, the total number of NOE peaks in the cross section of Figure 3 is difficult to assess. Further information can be obtained from three-dimensional NMR spectra, where the water–protein cross peaks are correlated via scalar coupling or NOE with further protein resonances in the third dimension, thus facilitating the assignment of the protons involved in NOEs with the water. In the case of BPTI, about 100 NOE cross peaks could be identified, from three-dimensional NMR spectra with the water resonance, that are also present but incompletely resolved in the cross sections of Figure 3. About 40 of those were shown to be intraprotein NOEs with hydroxyl protons from the side chains of threonine, serine, and tyrosine, whereas the other NOEs are with hydration water. The NOEs with hydration water are divided into two groups. The most intense NOEs involve the four internal water molecules which had been found earlier in single-crystal X-ray studies of BPTI.[1,11] In all three different crystal forms studied as well as in the solution structure, these water molecules are at conserved positions.[13–16] They are identified by about 20 positive water–protein NOESY cross peaks. A recent ^{17}O and ^{2}H relaxation study pinpointed their residence times to the range 10^{-4}–10^{-6} s^{-1} for one of them and 10^{-6}–10^{-8} s^{-1} for the other three.[17,18] The other group of NOEs with hydration water comprises some 40 relatively weak cross peaks with negative signs in NOESY. These cross peaks are exclusively observed with solvent-exposed protons on the surface of BPTI. In the NOESY cross section of Figure 3, the negative cross peaks are most clearly seen for the methyl groups in the chemical shift range between 0.8 and 1.1 ppm. Further negative NOESY cross peaks could be resolved in three-dimensional NMR data that are obscured in the cross section of Figure 3 by overlap with the more intense positive peaks.[12]

In BPTI, no positive NOESY cross peaks were observed for those hydration water molecules which are located on the protein surface in the single-crystal structures.[1] Experience gathered with different proteins and peptides shows that the sign and size of the water–protein NOE observed in NOESY is quite generally a criterion distinguishing ordered, internal water molecules from mobile surface hydration water.[19–25]

Residence Times of Hydration Water Molecules in the Subnanosecond Time Range

The sign of the intermolecular water–protein NOEs presents a qualitative criterion to determine whether the residence times of the corresponding hydration water molecules are short or long compared to the time range 0.1–1 ns. This conclusion results from model

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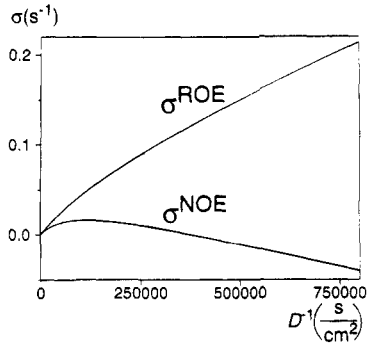

Figure 4. Cross-relaxation rates σ predicted for water–protein NOEs in NOESY and ROESY experiments as a function of the inverse of the diffusion constant D. The cross-relaxation rates translate directly into sign and intensity of the water–protein cross peaks, except that positive cross-relaxation rates give rise to negative cross peaks and *vice versa*. In the model used for the calculation,[26] protein and water molecules are represented by spheres of 12 and 2 Å radius, each with a proton spin lying 1 Å underneath the surface and rotational correlation times of 4 ns and 4 ps, respectively. The simulation is for a ^{1}H NMR frequency of 400 MHz.

calculations of the intermolecular water–protein NOEs. Quite generally, positive and negative NOEs are observed when the vector connecting two proton spins is modulated rapidly or slowly, respectively, on the time scale of the inverse Larmor frequency, *i.e.*, about 0.5 ns for an experiment at 400 MHz.[6] (Due to an old convention in NMR spectroscopy, *a positive NOE gives rise to negative cross peaks in NOESY and vice versa*. The following discussion will refer to the sign of the NOE rather than the sign of the corresponding cross peak.) Since biological macromolecules reorientate slowly on this time scale, the intraprotein NOEs are always negative. The observation of positive NOEs with water must therefore indicate that the vector connecting a protein proton with the proton of a nearby hydration water molecule is modulated by an additional motion which is faster than the reorientation of the protein. In principle, this additional motion could be a local reorientation of the water molecule at its hydration site or the exchange of water molecules in and out of the hydration site. However, explicit models describing the relaxation between two protons on the surface of the macromolecule do not predict positive NOEs unless one of the protons moves with respect to the other by amplitudes larger than the diameter of a water molecule, which is equivalent to the exchange of water molecules.[1]

The signs and intensities of the intermolecular water–protein NOEs seem to be best described by the diffusion model of Ayant *et al.*,[26] where water and protein molecules are represented by hard spheres of radius r_w and r_p, respectively, carrying a proton spin at some distance underneath the surface to account for the fact that the approach of the nuclei is limited by the van der Waals radii of the hydrogens. The spheres rotate with correlation times τ_w and τ_p and diffuse freely with respect to each other. The cross-relaxation rates σ^{NOE} and σ^{ROE} predicted for the NOESY and the ROESY experiment are shown in Figure 4 for this model. With the parameters indicated, the sign of the NOE in NOESY changes for a translational diffusion coefficient D of about 2.5×10^{-6} cm^2/s. This value is about one-tenth of the self-diffusion coefficient of pure water at 36 °C.[27] A recently proposed model, where the protein surface is assumed to be planar, predicts the change of sign of σ^{NOE} for a diffusion coefficient which is of the same order of magnitude as the value derived above for the relative diffusion of hard spheres.[28] The diffusion coefficient may be translated into a residence time of the water molecule at its hydration site by using the Einstein relation for three-dimensional diffusion,

$$\tau = \overline{x^2}/(6D)$$

where τ is the time it takes the water molecule to diffuse by an average displacement $(\overline{x^2})^{1/2}$. For $(\overline{x^2})^{1/2} = 4$ Å and $D = 2.5 \times 10^{-6}$ cm^2/s this time would be about 100 ps. Positive water–protein NOEs in NOESY would indicate shorter residence times of the hydration water molecules, and negative σ^{NOE} values would indicate longer residence times.

The time limit of 0.1 ns requires some qualifying comments. First of all, the force free interaction between ideal spheres is at best a poor description of protein hydration. Protein surfaces are not smooth, and hydrophilic and hydrophobic surfaces exert different forces onto hydration water molecules. A further problem is associated with the conversion of the diffusion coefficient into a residence time of the hydration water, since the surface topology of a protein can reduce the number of dimensions for hydration water diffusion. Assuming two-dimensional or one-dimensional diffusion, the residence time τ deduced from the diffusion coefficient would increase by a factor of 2 or 4, respectively.[29] (Note that in ref 1 the τ value was derived using $\tau = \overline{x^2}/D$.) Furthermore, the precise value of the diffusion coefficient for which the model predicts a change in sign for σ^{NOE} depends somewhat on the rotational correlation times and dimensions of the spheres used to represent protein and water molecules. For example, increasing the rotational correlation time of the sphere representing the water molecule to 300 ps increases the residence time estimate by about 20%. In summary, the sign change of σ^{NOE} may be expected to occur for residence times in the range 0.1–1 ns. These residence times are so short that they must apply to the entire hydration water molecules. Proton exchange between different water molecules is by orders of magnitude slower.[30]

Clearly, the use of a simple model to rationalize the sign of water–protein NOEs could be prone to over-interpretation. However, a sign change of σ^{NOE} for correlation times in the time range 0.1–1 ns is also predicted for other models, where the two protons of interest are linked to the surface of a sphere and the correlation times characterize the spatial reorientation of the vector between the two protons. The results are quite independent of whether the critical correlation time is given by the overall rotational tumbling of the molecule[1] or by some local motion of significant

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amplitude which is superimposed onto the slower reorientational tumbling of the sphere (*e.g.*, wobbling in a cone model[31,32]).

Independent confirmation for residence times in the subnanosecond time range comes from molecular dynamics (MD) simulations of hydrated proteins. These calculations use water models which are calibrated to correctly predict the microscopic properties of pure water, including the self-diffusion coefficient.[33,34] The diffusion coefficients of hydration water molecules on the protein surface, which are obtained from the MD simulations, are generally about 4 times smaller than in bulk water.[35,36] This result is in full agreement with the diffusion coefficients derived from the observation of positive water–protein NOEs using the diffusion model discussed above. The residence times of the hydration water molecules on the protein surface predicted by the MD simulations range from tens of picoseconds to a few hundred picoseconds,[35–37] which coincides perfectly with the time window accessible by NMR experiments measuring the sign and intensity of water–protein NOEs. The MD simulations predict similarly short residence times near polar and unpolar regions of the polypeptide, a result which could not easily be obtained from NOE data, since most polar groups either are devoid of hydrogen atoms or are involved in rapid proton exchange. Unfortunately, the time periods simulated to date are still much shorter than 10 ns, which is too short to calculate the NOE directly from MD simulations.

Finally, the conclusion that the residence times are shorter than 1 ns for most of the hydration water not only follows from the model calculations of the NOE and molecular dynamics simulations but also was deduced from the magnetic field dependence of the T_1 relaxation time of the water signal in aqueous protein solution.[2]

NMR and X-ray Detection of Hydration Water

Hydration water molecules at specific sites of a protein structure are quite routinely located in single crystals by X-ray crystallography as part of the structural refinement at a resolution of 2 Å or better. The evidence compiled for several proteins is that only a few of the water molecules detected by X-ray crystallography are also characterized by sizable negative NOEs in solution, *i.e.*, by residence times longer than 1 ns.[1,21–25] These are water molecules in the interior of a protein structure or solvent-exposed water molecules at defined hydration sites where they are also likely to play a structural role. Particularly nice examples for solvent-exposed, structural hydration water with prolonged residence times are the water molecules of the spine of hydration in the minor groove of A,T-rich B-DNA structures.[38–40] The majority of the X-ray detectable water, however, must diffuse almost unhindered in solution as evidenced by positive σ^{NOE} rates in NOESY and small or vanishing σ^{ROE} rates observed in ROESY (Figure 4). In other words, X-ray crystallography locates those hydration water molecules which are on the time average at defined locations with respect to the crystal lattice, irrespective of their residence times. Because of the rapid diffusion of the water molecules also in the protein crystal, water molecules are thus preferentially detected when steric constraints imposed by the crystal lattice reduce the degrees of freedom for diffusion. Consequently, many of the hydration water molecules observed by X-ray crystallography are found to be in contact with two or more neighboring macromolecules.[1,41] A more technical difficulty associated with the interpretation of X-ray crystallographic data is the fact that the number and location of water molecules included in the fit of the electron density map depend on the level of refinement, and different refinement algorithms may arrive at somewhat different results for the less well ordered hydration water molecules.[42,43]

The observation of hydration water by X-ray crystallography must not be mistaken as an indication that these molecules are structurally important or bound with relatively long residence times in solution. On the other hand, the few hydration water molecules with long residence times in solution, as judged by the criterion of negative σ^{NOE} values, are usually reliably detected by X-ray crystallography in the single crystals. Most of the hydration water, however, has subnanosecond residence times in solution. In fact, small flexible peptides without internal water or exchangeable side chain protons display positive σ^{NOE} values for all solvent accessible protons,[1] unless the mobility of the water is very much reduced by cooling to temperature below −20 °C.[20]

Magnetization Exchange between Proteins and Water for Contrast in MR Imaging

Most of the MR images recorded in clinical application rely, in the absence of any special paramagnetic relaxation agent, on proton density as well as different T_1 and T_2 relaxation times of the water magnetization in different types of tissue. However, in spite of a large body of experimental data, there is no general agreement about how, in the absence of paramagnetic ions, the relaxation parameters of the water protons "sense" the presence of the biological macromolecules.[2,18,44,45] Figure 5 illustrates two fundamentally different views. According to the most common belief, the relaxation of the water protons is primarily influenced by the exchange of water molecules between a pool of hydration water bound to the macromolecular surface and the bulk water (Figure 5, model

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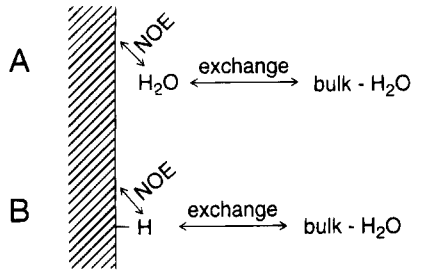

Figure 5. Two molecular models explaining image contrast in MR imaging obtained by magnetization transfer between macromolecules and water. (A) Magnetization relayed between the macromolecular phase and the free, unbound bulk water by intermolecular NOE and exchange of hydration water molecules (or hydration water protons). While the hydration water molecules are bound, their protons assume properties similar to those of the protons of the macromolecular phase, including rapid magnetization transfer by NOE. (B) Magnetization transfer effected by intramolecular NOE between nonexchangeable protons of the macromolecular phase and OH or NH groups carrying rapidly exchanging protons. No hydration water is directly involved in the magnetization transfer.

A).[5,46,47] While the water is bound to the macromolecular phase, proton magnetization is exchanged with the macromolecules by NOE. The alternative view assumes that the magnetization transfer is governed by proton exchange between hydroxyl or amino groups of the macromolecular phase and the water (Figure 5, model B).[45] The magnetization of the hydroxyl and amino protons is further coupled to the proton magnetization of the macromolecular phase by NOEs. The two views agree on the fact that the bulk water relaxation parameters reflect macromolecular properties because of magnetization exchange between the two phases, but they differ in the physical quantity encoded by the water proton relaxation parameters: in the first case, the conformation of the macromolecular surface is important for providing adequate hydration sites, where water molecules can join the macromolecular phase, whereas the second model identifies the covalently bound but exchangeable protons from the macromolecular phase as the determinant of water proton relaxation. In the following, we would like to argue for the importance of the magnetization transfer pathway of model B of Figure 5 (see also ref 18).

The situation is most clearly discussed for the recently proposed magnetization transfer experiment,[48] although magnetization transfer between the macromolecular and the aqueous phase is also crucial in T_1 and T_2 weighted MR images. In the magnetization transfer experiment, the image is obtained detecting the narrow water resonance after saturation of the broad background signal of the immobilized macromolecules by irradiation far away from the water resonance.[48] Because of magnetization exchange between the macromolecules and water, the water signal is decreased as well. Whereas proton exchange conserves the sign of the magnetization, the sign of the magnetization transferred by NOE can be of different sign (Figure 4). As shown above, most of the surface hydration water molecules diffuse so rapidly with respect to the protons of the macromolecule that σ^{NOE} is positive or very small. Positive σ^{NOE} values should lead to an *increase* of the water signal in the magnetization transfer experiment which is not observed. Therefore, the exchange of the few hydration water molecules which are bound with longer lifetimes and therefore interact with negative σ^{NOE} with the macromolecular phase (Figure 4) would have to explain the entire magnetization transfer according to model A of Figure 5.[46]

For globular proteins and glycoproteins in particular, the number of hydroxyl protons from threonine, serine, and tyrosine as well as sugar residues exceeds by far the number of structurally bound hydration water molecules. Furthermore, the side chain NH's of histidine, arginine, lysine, and some of the backbone amide groups exchange their protons quite rapidly with the water. These residues are hydrophilic and therefore mostly located at the protein surface exposed to the solvent. The proton exchange rates from the side chains of serine, threonine, tyrosine, arginine, and lysine were measured to be between 400 and 10 000 s^{-1} at 36 °C and neutral pH.[49] The proton exchange rate from the imidazole ring of histidine is in the same range,[50] and sugar hydroxyl protons exchange with rates similar to those of the hydroxyl protons of serine and threonine. The exchange from amino and hydroxyl groups is catalyzed by phosphate. Exchange catalysis is particularly pronounced for the hydroxyl groups of tyrosine, threonine, and serine, for which the proton exchange rates accelerate more than 10-fold in the presence of phosphate at intracellular concentrations (65 mM).[49] The proton exchange from hydroxyl groups is further catalyzed by amino and carboxyl groups. Thus, the exchange lifetimes of the labile hydroxyl and amino protons can be estimated to be the range between 1 μs and 1 ms under physiological conditions, which must make an important contribution to the overall magnetization transfer between the macromolecular phase and water.

Efficient magnetization transfer between the macromolecular phase and the bulk water according to model B of Figure 5 requires rapid exchange between hydroxyl or amino protons and water protons and fast magnetization transfer by NOE to the interior of the macromolecular phase. Although the proton exchange from hydroxyl and amino groups is generally slower than the exchange of hydration water molecules, the NOE transfer is still the rate-limiting step in globular proteins and most likely also in stationary tissue. In solids, the magnetization transfer rate by NOE may be as fast as 10 000 s^{-1}.[51,52] However, this rate is expected to be slower in the presence of local mobility.[53,54] Local mobility can be pronounced in particular for the long side chains of lysyl and arginyl residues, if they are solvent exposed.[32] On the other hand, the NOE is hardly affected by the proton exchange rates since residence times of more than 1 μs are longer than the correlation times governing the NOE between an

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exchangeable proton and the nearest nonexchangeable protons of the macromolecular phase. Consequently, the overall magnetization transfer rate by the mechanism of model B (Figure 5) increases with increasing density of exchangeable protons, with hydroxyl and amino groups being approximately equivalent for the overall magnetization transfer efficiency.

In small globular proteins and DNA, functional groups with exchangeable protons are far more abundant than water molecules bound with residence times longer than 1 ns. There is no reason why this should not be true for biological material in general. This strongly suggests that proton exchange rather than the hydration of biological tissue is the dominant mechanism effecting the magnetization transfer as measured either in a magnetization transfer experiment or as reflected in T_1 or T_2 weighted MR images.

Concluding Remarks

It has been noted earlier that the magnetization transfer between macromolecules and water can be consistently explained if it is attributed exclusively to proton exchange between the two phases.[44,45] There, it was assumed that hydration water does not couple to the magnetization of the macromolecule due to its short residence times. High-resolution NMR experiments have not shown conclusively that this assumption holds for proteins and DNA except for a few hydration water molecules which play a structural role. The importance of proton exchange for the ^1H relaxation of water is not contested by the finding that a few structural hydration water molecules can dominate the ^{17}O and ^2H relaxation of the bulk water in the absence of exchange catalysts.[17,18] Protons relax more slowly than deuterium, so that proton exchange is more effective in transferring magnetization than deuterium exchange. Under physiological conditions, where proton exchange is accelerated by phosphate, carboxyl, and amino groups, the proton exchange between the numerous OH and NH groups of biological tissue and the water clearly cannot be neglected as a contribution to the ^1H relaxation of water.

There is incidental support for the idea that the density of solvent accessible, exchangeable protons is the main determinant of magnetization transfer between water and biological material. For example, it was concluded from ^1H NMR experiments with lipid systems that "the presence of surface hydroxyl and/or amino groups on the macromolecule appears to be necessary for ... magnetization transfer".[5] Furthermore, it was observed that the abundance of cholesterol in white matter leads to increased T_1 relaxation rates compared to gray matter in MR images of brain.[2] Also this effect is readily explained by the proton exchange from the hydroxyl group present in cholesterol without invoking any unusual hydration model.[55,5] Hopefully, future experiments will assess quantitatively and conclusively the contributions from proton exchange and exchange of hydration water in different tissues.

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