The ADC of Water Limits Magnetization Transfer Process under Physiological Conditions

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Introduction

Magnetization Transfer (MT) between water molecules and the exchangeable hydrogens of biomolecules (BM) is a powerful source of endogenous contrast in Magnetic Resonance Imaging[1]. The mechanisms underlying this phenomenon remain however, incompletely understood, making MT images difficult to interpret. Chemical exchange and dipolar cross-relaxation between water molecules and the BM [2], have been proposed as the main processes underlying MT. Both may be affected by changes in the microviscosity surrounding the BM and the ADC's of the interacting molecules, but few studies have investigated this aspect in detail[3, 4]. In this study we address the influence of microviscosity and water ADC's of water on the MT process using a combination of DOSY NMR spectroscopy and Molecular Interaction Maps, a computational chemistry resource providing energies of solvation over different regions of the BM [5].

Materials and Methods

As model system we used solutions of 1M glutathione (oxidized) containing or not 50% glycerol. We obtained 1D-spectra, determined water T_1, water ADC's and Z-spectra representing the ratio of intensities Ms/Mo of the water resonance (M_o; without presaturation, M_s; with 1s of presaturation in the range -2000 $< \nu_{H2O} < 8000$ Hz) using a Bruker AVANCE spectrometer interfaced with and 11.7 Tesla WB Magnet operating at 500.13 MHz for [1]H (Figure 1A and 1B). For translational diffusion measurements of the water, we implemented a 2D DOSY sequence using stimulated echoes and one spoil gradient (Pulse Field Gradient Spin Echo Method, PFGSE). ADC's for water molecules were calculated from the Stejskal-Tanner equation (Δ: 100 ms-200ms, δ: 1-4 ms, G: 4.258 e+03 Hz/G). T_1's were obtained using inversion-recovery sequence. To evaluate the activation energy E_{ac} of water molecules in contact with glutathione we used the GRID program. Knowledge of the activation energy in every region of the BM, allows the calculation of Molecular Interaction Field Maps providing information on local solvation of individual molecular groups. Negative values of E_{ac} (Kcal.mol^{-1}) indicate the preferred distribution of water molecules as compared to positive E_{ac} values.

Results

Figure 1A depicts representative Z spectra of the glutathione solution with two overlapping z-peaks ca. 4.8 ppm and 8.0 ppm, respectively. The first corresponds to MT between water protons while the second reflects a combined MT between NH groups of glutathione and water (NH $_2$ γ-Glu; 7.6 ppm, NH Cys; 8.4 ppm and NH Gly; 8.2 ppm). Figure 1B shows that the percentage of MT decreased in the presence of 50% glycerol, from 73% (1A) to 17% (1B), at physiological pH=7.0 and temperature 37[0]C. The presence of glycerol in the glutathione solution, decreased the diffusion coefficient of water from $1.245 \cdot 10^{-09} \pm 0.015 \cdot 10^{-09}$ to $0.260 \cdot 10^{-9} \pm 0.015 \cdot 10^{-09}$ m$^2 \cdot$s^{-1} and the values of water T_1 from 1.338 ± 0.002 to 0.647 ± 0.001s. Molecular Interaction Field Maps showed that the most favorable interaction between water and glutathione occurs near the –OH group of γ-glutamic with an energy of activation: -19.5 Kcal·mol^{-1}. Concerning the solvation of NH groups, Molecular Interaction Field Maps showed that even though the E_{ac} is more favorable for the interaction of water with the NH group of glycine than with the NH group of cysteine, the population of water molecules is arranged spatially more favorably around the NH groups of cysteine (Figure 1C).
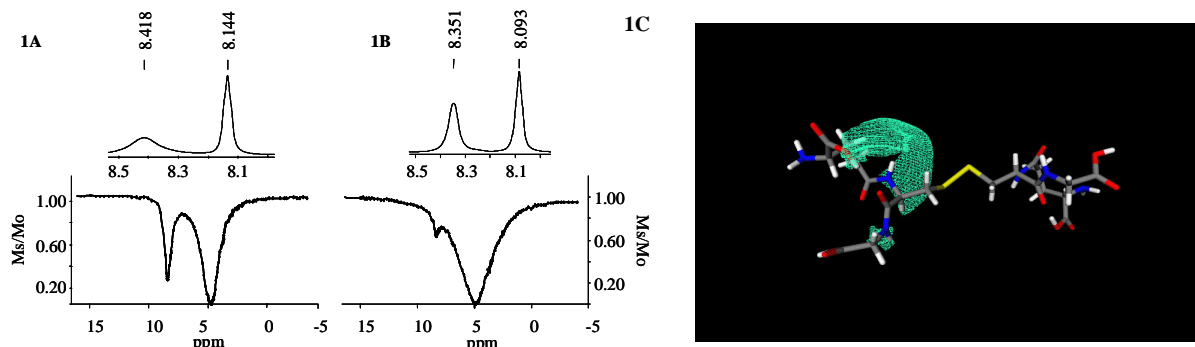
Figure 1.A: Z-spectra of an aqueous solution of 1M L-glutathione oxidized (pH 7.0, 310K). The peak centered at 4.7 ppm corresponds to magnetization transfers between water molecules, while the peak centered at 8 ppm reflects magnetization transfers between water molecules and NH groups of glutathione. The percentage of MT between NH groups of Glutathione and water, corrected with the symmetric peak, gave 73% MT. **1B**: same as in **A** but in a 1M L-glutathione solution containing 50% (w/v) glycerol. In this case, NH MT decreased to 17% only. Insets: 1D spectra showing the intensities of both NH peaks with (1B) or without (1A) glycerol: 8.4 ppm-cysteine, 8.1 ppm-glycine. **1C:** Molecular Interaction Field (MIF) Map, revealing the distribution of water molecules solvating the NH groups of glutathione. The population of water molecules interacting with the NH's cysteine region is larger than NH's glycine region.

Discussion and Conclusion

Our results suggest that the apparent translational diffusion coefficient of water influences appreciably MT transfers with biomolecules at physiological pH and temperature. Moreover, our data reveal that this effect is heterogeneous within the biomolecule, being more pronounced in those groups with better solvation. In this respect it is interesting to note how the presence of 50% glycerol, does not affect the GlyNH resonance, while increases significantly the CysNH resonance (insets Fig.1). The present study also reveals an important role for Molecular Interaction Field Maps as determined with the GRID program, providing clues for the interpretation of hydration profiles. Further refinements of this approach will include Molecular Dynamic calculations. Finally, the results of the present study suggest that MT contrast observed in MR images may contain an important contribution from the local water ADC's. Correlation of MT and ADC weighed MRI images may help to further understand the quantitative relationships between these variables in vivo.

References

1. Ward and Balaban, Mag. Res. Med 2000, 44, 799-802
2. Liepinsh, E., Otting, G. Mag Res Med. 1996, 35, 30-42.
3. Cabrita J. E., Berger S. Magn. Reson. Chem. 2002, 40:S122-127
4. Moonen CTH, Van Gelderen P., Vuister GW, Van Zjil PCM. J. Magn Reson. 1992; 97:419
5. Jager R, Kast SM. J Mol Graph Model. 2001; 20(2):123-31.