Transverse relaxation of water in ferritin gel: relative contributions of iron and gel

N. Takaya[1], H. Watanabe[1], and F. Mitsumori[1]

[1]National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan

Introduction

T_2 contrast in human brain is utilized for disease diagnosis and elucidation of brain functions. We recently reported that the apparent transverse relaxation rate ($R_2^\dagger = 1/T_2^\dagger$) of tissue water in human brain is well explained by contributions from regional non-hemin iron concentration ([Fe], mostly ferritin) and from macromolecular mass fraction (f_M = 1 – water fraction)[1,2]. Thus, $R_2^\dagger$ is assumed to be expressed as a linear combination of [Fe] and f_M ($R_2^\dagger = \alpha\,[Fe] + \beta\, f_M + \gamma$) [eq.1]. In the ferritin solution it is well known that T_2 of water proton linearly decreases with ferritin iron concentration, and the effect is again linearly dependent on the observe field strength (B_0) [3, 4]. Enormous amount of studies has been conducted on the relaxation of macromolecules. However, there is no report whether the transverse relaxation of water proton in ferritin solution is expressed as a linear conbination of iron and macromolecules when macromolecules coexist in the solution. In the present work, we examined the behavior of T_2 of water proton in solution and gel of ferritin with varying concentrations of ferritin and agarose concentrations.

Materials and Methods

Sample preparation: Horse spleen ferritin with an iron loading factor of ~1000 Fe atoms/molecule purchased from Calbiochem was diluted to 50mM NaCl solution. Iron concentration in the solution were varied from 0 to 60mg/100g. Various amounts of agarose were added to the ferritin solution to give the final concentrations of 0, 0.5, 1.0 or 1.5%. These solutions were placed in 5mm NMR tubes and warmed to 80°C in a water bath for 5min with vigorous mixing, then cooled down to be gel.

T_2 measurements: 1.9T (Varian), 4.7T (Varian), 9.4T (Varian), 11.7T (JEOL), 14.1T (Varian), and 18.8T (JEOL) MRI or NMR spectrometers were used for T_2 measurements. T_2 measurements were performed using a Carr-Purcell-Meiboom-Gill (CPMG) method with a fixed echo spacing of 2ms, and rectangular 90° (270μs), and 180° (540μs) pulses. Variable numbers of echo were collected dependent on the sample T_2 values. Intensity of water signal in echo train was fitted with a single exponential curve and T_2 value was obtained.

Result and Discussions

Figure1 shows R_2 values of water at 4.7T as a function of ferritin iron concentration ([Fe]) in ferritin solution and agarose gels (0.5 ~1.5%). R_2 linearly increases with iron concentration over the range of 0 ~60mg/100g in each agarose concentration. Multiple regression analysis of the observed R_2 using equation 1 gave a result of $\alpha = 0.248 \pm 0.002$, $\beta = 9.37 \pm 0.07$ with a regression coefficient of 0.99, when γ was fixed to 0.43 obtained with 50mM NaCl solution. This result demonstrated that transverse relaxation of ferritin solution and gel is expressed as a linear combination of contributions from iron and from gels as in the case of human brain. When the measurement was performed at various magnetic field (B_0) from 1.9 ~18.8T, the same analysis was possible with varying coefficients α, β and γ. It should be noted that the coefficient α due to iron contribution linearly increased with B_0 as shown in Fig.2. This result indicated that the multiple regression analysis successfully discriminates relaxations due to iron and macromolecules in the ferritin gel sample, and R_2 due to iron in ferritin gel shows the same B_0 dependence as in the solution.

Acknowledgements

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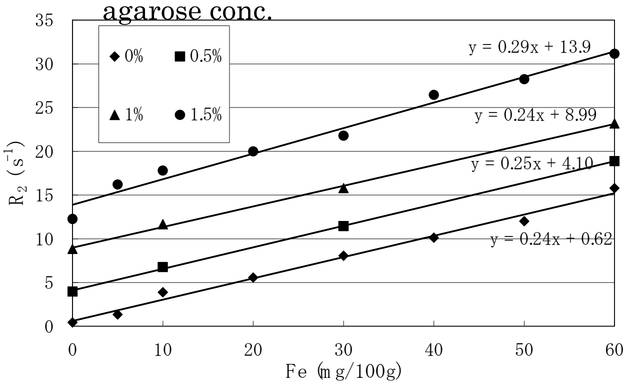

Fig.1. R_2 values of water at 4.7T as a function of the ferritin iron concentration ([Fe]) in ferritin solution (◆) and agarose gels (0.5 %(■), 1 %(▲), and 1.5 %(●))

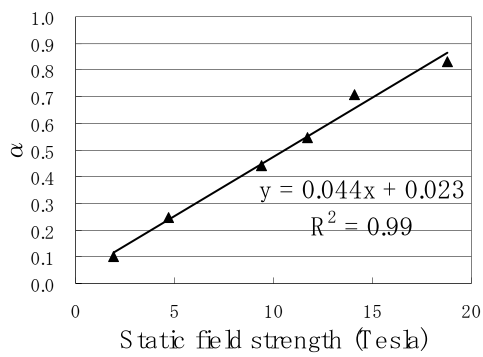

Fig.2 Linear increase in α dependent on B_0

References

[1] Mitsumori F, Watanabe H, Takaya N, Garwood M: Magn. Reson. Med., 58, 1054 (2007). [2] Mitsumori F, Watanabe H, Takaya N: Magn. Reson. Med., in press (2009). [3] Vymazal J, et al.: Magn. Reson. Med., 27, 368 (1992).
[4] Gossuin Y, et al.: Magn. Reson. Med., 48, 959 (2002).