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

N. Takaya¹, H. Watanabe¹, and F. Mitsumori¹
¹National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan

Introduction

T₂ contrast in human brain is utilized for disease diagnosis and elucidation of brain functions. We recently reported that the apparent transverse relaxation rate ($R₂^\dagger = 1/T₂^\dagger$) 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 ($f_M = 1 – \text{water fraction}$)[1,2]. Thus, $R₂^\dagger$ is assumed to be expressed as a linear combination of [Fe] and $f_M$ ($R₂^\dagger = \alpha [\text{Fe}] + \beta f_M + \gamma$) [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 combination 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

Figure 1 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 $\alpha = 0.248\pm0.002, \beta = 9.37\pm0.07$ with a regression coefficient of 0.99, when $\gamma$ 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 $\alpha, \beta$ and $\gamma$. It should be noted that the coefficient $\alpha$ 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

We thank a support from Grant-in Aid for Scientific Research Japan (19390327). We also thank to Y. Yoshikawa, J. Hayashi and K. Kushida for their help in $T_2$ measurements.

References