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Magnetic Resonance Imaging 22 (2004) 683–688

MAGNETIC RESONANCE IMAGING

Proton T_1 and T_2 relaxivities of serum proteins

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Received 1 November 2003; accepted 29 January 2004

Abstract

In the present study, T_1 and T_2 of phantoms containing serum sets with varying amounts of proteins, serum samples with certain amounts of proteins, serum diluted by distilled water, and serum treated with iron were measured. In addition, T_1 and T_2 of phantoms containing normal serum, diluted serum, and albumin-doped serum were also measured. Relaxation rates were plotted versus protein concentrations. The slope of relation was taken as relaxivity. The T_1 relaxivities of proteins were ranged from 0.035 to 0.080 $s^{-1}(g/dl)^{-1}$, whereas T_2 relaxivities were ranged from 0.24 to 0.68 $s^{-1}(g/dl)^{-1}$. The T_1 and T_2 relaxivities of transferrin iron were 2.40 and 2.60 $mM^{-1}s^{-1}$, respectively. The contributions of diamagnetic proteins and transferrin iron to the relaxation rate of serum were also calculated for each of diluted serum, normal and albumin-doped serum. The contributions and the average TP relaxivities(calculated by using individual relaxivities and the ratios of protein fractions in TP) were used for TP calculations. The agreement between the calculated TP and TP by autoanalyzer and also the agreement between average TP relaxivities and the TP relaxivities determined from dilution experiments show that the data of relaxivities are reliable. The results suggest that individual protein relaxivities explain the influence of serum TP composition on T_1 and T_2 relaxation times. © 2004 Elsevier Inc. All rights reserved.

Keywords: MRI; T_1; T_2; Relaxivity; Proteins

1. Introduction

Serum is known to be composed of water (90–92%), serum proteins (7–8%) and inorganic substances (1–2%). Serum proteins make up total protein of serum (TP). An increase or decrease in the concentrations of individual proteins, caused by malignant tumors and other diseases, influences serum total protein composition [1,2]. Such an influence may also alter total protein content. The changes in the TP or TP composition will affect both T_1 and T_2 because individual proteins contribute to the relaxation rates [2–7].

The contribution of serum proteins to the relaxation rates in serum (CSP for diamagnetic proteins and CTI for transferrin iron) can be expressed as relaxivity, which is the increase in relaxation rate normalized to the concentration of proteins or to that of iron. The T_1 and T_2 relaxivities of some TP constituents such as albumin and γ-globulins were determined from solution experiments [4,5,8], and T_1 relaxivity of transferrin iron is known [6,7,9]. However, the protein solutions are not identical to serum for relaxation studies [10], and previous studies do not include the relaxivities of all major TP components and the T_2 relaxivity of transferrin iron. On the other hand, over the past three decades, the relaxation times in serum from healthy and sick individuals have been compared for many groups [11–21], and these types of studies are still of scientific interest [22–25]. The normalization of each individual contribution makes possible the calculation of the relaxation rates in various serum groups. Such normalization may explain overlappings and differences in relaxation rates of healthy and diseased serum. The relaxivity mesurements should therefore be done in serum and they also should be extended to all major proteins making up serum.

Dilution experiments indicate that the relaxation rates in serum are linearly proportional to TP content [4,5]. This implies that the TP in serum can be calculated by using the CSP and the TP relaxivities. The TP calculation may lead to an equation that explains the effect of TP composition on the relaxation rates in various serum groups, as well as the confirmation of the relaxivities.

In this work, proton relaxivities of major serum proteins, transferrin iron, and the CSP in normal serum, diluted se-

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0730-725X/04/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.mri.2004.02.001

rum, and albumin-doped serum were determined. Relaxivity of fibrinogen, which is a plasma protein, was measured. The results were used for TP calculation, and for analysis of the relaxation change in various serum groups.

2. Materials and methods

2.1. Material

Certain volume of a pooled serum was collected from healthy volunteers and used for determinations of relaxivity, CSP and CTI. Serum proteins (albumin, γ-globulins, α-globulins, $(\alpha+\beta)$-globulins, $(\gamma+\beta)$-globulins, fibrinogen and lysozyme) were purchased from Sigma (St. Louis, MO, USA).

2.2. Phantoms

Except $(\gamma+\beta)$-globulins and fibrinogen, the relaxivity measurements were carried out on a set of samples for each type of protein. Protein concentration in each set ranged from 0 (pure serum) to 21 g/dl. Due to precipitation, $(\gamma+\beta)$-globulins were measured at single concentration (3 g/dl) and fibrinogen was measured at two concentrations. Fibrinogen (0.75g/dl) was well-dissolved in serum, but a partial aggregation occured with 2.5 g/dl of fibrinogen. In addition, a set of samples from pooled serum treated with ferrous iron was used for the determination of transferrin relaxivities. In this case, the treatment of serum with iron and the removal of excess iron by magnesium carbonate were made as in a previous study [26]. Furthermore, the pooled serum was diluted by distilled water in the ratios of ⅓ and ⅔. The samples obtained were used for determination of TP relaxivities. Finally, three sets of samples prepared from a different pooled serum were used for CSP determinations. The first set was normal serum, the second set was composed of samples diluted by distilled water in the ratio of ⅓, and the third set was composed of serum samples doped with 3 g/dl of albumin.

Samples were transferred into cylindrical glass tubes, which were already placed in a plastic phantom. The samples used for relaxivity determinations are shown in the rows of the upper images in Fig. 1, whereas those for CSP determinations are shown in the rows of the lower images in Fig. 1. To check reproducibility, the relaxivity measurements were repeated three times by using different pooled serums (different phantoms), whereas the CSP and TP determinations were repeated twice. Reproducible results were obtained.

2.3. Determinations by conventional methods

The total protein, serum iron, and total iron binding capacity of serum (TIBC) in the pooled serum were determined by autoanalyzer (Abbott, Toshiba-Aeroset, Otawara/

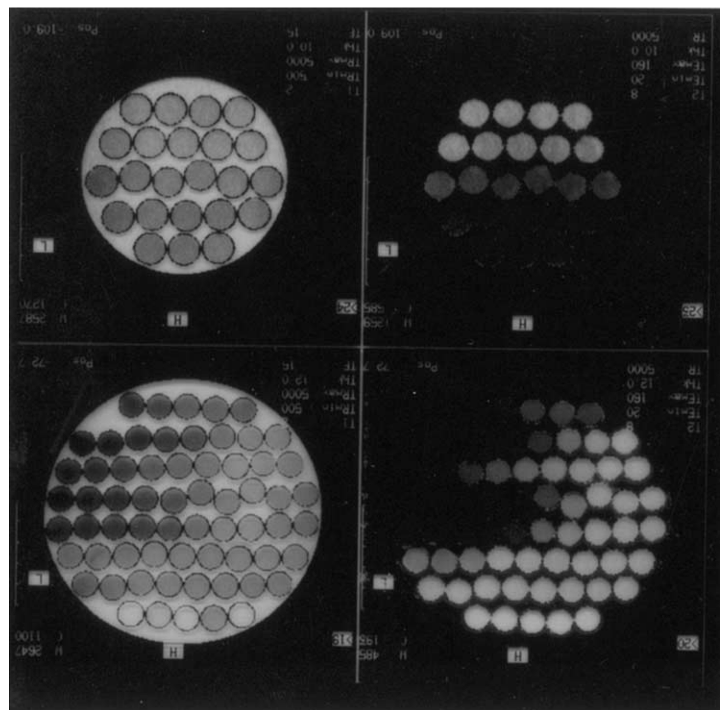

Fig. 1. (a) T_2(left upper) and T_1(right upper) maps used for relaxivity measurements. Each row represents a set. Up to down: Diluted serum, serum treated with iron, the set at one and two concentrations (fibrinogen and $\gamma+\beta$-globulins), lysozyme, γ-globulins, albumin, $(\alpha+\beta)$-globulins and α-globulins. (b) T_2 (left lower) and T_1 (right lower) maps used for CSP. Upper two rows show serum doped with albumin, whereas lower two rows present serum diluted with water. The medium row denotes normal serum.

SHI, Tochigi-Ken, Japan), whereas protein fractions were determined by electrophoresis (Interlab, Microtech 6481SO, Rome, Italy). Serum iron and TIBC were found to be 130 and 420 μg/dl, respectively.

2.4. Relaxation measurements

Relaxation measurements were carried out at 1T by using a Siemens Magnetom SP 10 MR scanner. A Carr-Purcell pulse sequence with multi-echoes(SE8P) was used for T_2 measurements. TR was set at 5000 ms. Echo delays were changed from 20 to 160 ms. A SE single echo sequence (SE_15B130) was used for T_1 measurements. In this case TRmin and TRmax were chosen as 500 ms and 5000 ms, respectively. Field-of-view (FOV) and slice thickness were 160 mm × 160 mm and 12 mm, respectively. T_1 and T_2 were obtained from T1 and T2 maps.

2.5. Determination of proton relaxivities

The relaxation rates in the sets were plotted versus concentrations of the proteins added. The relaxivities were determined from the slope of linear part of the relation between the relaxation rates ($1/T_1$ and $1/T_2$) and protein concentrations [4,5,7–10]. Relaxivities of both fibrinogen and ($\gamma+\beta$)-globulins were found by dividing the increase in relaxation rate to the corresponding concentrations, whereas relaxivities of transferrin iron were determined by using the increase in relaxation rate and iron, which saturates the unsaturating iron binding capacity of serum transferrin (TIBC minus serum iron).

Electrophoretic analysis showed that the pooled serum is consist of albumin (63%), γ-globulins (17%) and ($\alpha+\beta$)-globulins (20%). Proton relaxivities of the diamagnetic TP can therefore be averaged as follows:

$$R_{itp}=0.63R_{ialb}+0.17\ R_{i\gamma}+0.20\ R_{i(\alpha+\beta)}\quad i{:}1,2 \tag{1}$$

For comparative purposes, the R_{itp} was also determined by dilution of the pooled serum used for relaxivity determinations.

2.6. Contribution of serum proteins (CSP)

Phantoms containing diluted serum, normal serum, and albumin-doped serum were reconstructed for measurements of T_1 and T_2 relaxation rates (lower images in Fig. 1). The measured relaxation rates in serum can be written as [3–7]

$$1/T_i = 1/T_{iw} + 1/T_{itp} + 1/T_{itr} \tag{2}$$

where subscribes w, tp, and tr denote water, serum total protein, and transferrin iron, respectively. From Eq. (2), $1/T_{itp}$ can be solved as follows:

$$1/T_{itp} = (CSP)_i = 1/T_i - (1/T_{iw} + 1/T_{itr}) \tag{3}$$

Because the $1/T_i$, $1/T_{iw}$, and$1/T_{itr}$ are known, the contribution of diamagnetic proteins can be calculated.

2.7. Calculation of TP by MR

The proton T_1 and T_2 relaxivities (R_1 and R_2) of TP can be written as [2,5,8]

$$R_{iTP} = \frac{1/T_{itp}}{C} = \frac{1/T_i - (1/T_{iw} + 1/T_{itr})}{C} \tag{4}$$

where C is concentration of total protein. The C can be solved from Eq. (3) as

$$C(\text{g/dl}) = \frac{1/T_{ip}}{R_{itp}} = \frac{1/T_i\ -\ (1/T_{iw}\ +\ 1/T_{itr})}{R_{itp}} \tag{5}$$

Equation (5) gives the TP in serum. For comparative purposes, the TP was also determined by conventional methods.

3. Results and discussion

The relaxation rates versus concentrations of various proteins are given in Figs. 2 and 3, which correspond to 0–15 g/dl of protein addition. The increases in relaxation rates due to ($\gamma+\beta$)-globulins were 0.9 s^{-1} for T_2 and 0.24 s^{-1} for T_1, whereas the enhancements caused by well-dissolved fibrinogen were 0.38 s^{-1} for T_2 and 0.023 s^{-1} for T_1. The increase in relaxation rate due to added iron, which saturates unbounding capacity of transferrin, was 0.12 s^{-1} for T_1 and 0.13 s^{-1} for T_2.

The relaxivities determined by the slopes and those measured at certain concentrations of ($\gamma+\beta$)-globulins, fibrinogen and transferrin iron are shown in Table 1. As can be seen, the proton T_2 relaxivities are greater than those of T_1 by a factor of 3.8 to 16.7. The relaxivities of partially

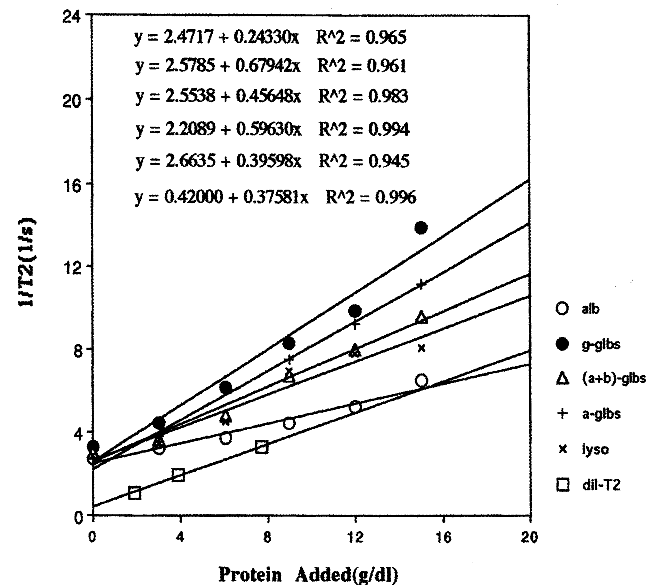

Fig. 2. The $1/T_2$ relaxation rate versus concentrations of proteins added. The order of formulas is the same as that of symbols.

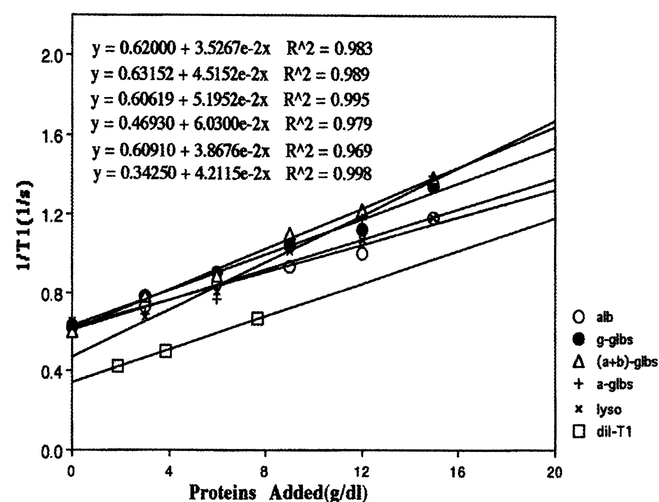

Fig. 3. The $1/T_1$ relaxation rate versus concentrations of proteins added. The order of formulas is the same as that of symbols.

aggregated fibrinogen were longer than those in well-dissolved fibrinogen. This indicates that either higher concentrations of fibrinogen or those of $(\gamma+\beta)$-globulins cause an error in the relaxivity determinations. T_1 and T_2 relaxivities of transferrin were almost equal. The results for albumin, γ-globulins, and T_1 relaxivity of transferrin iron are consistent with previous findings [4,5,8,9,26].

Relaxations are caused by local magnetic fields, which are induced by random molecular motions of widely varying frequency. The components of the local magnetic fields with Larmor frequency can induce spin-lattice relaxation rate, whereas the spin-spin relaxation rate may have contribution from any local field, including the zero frequency one. For this reason, the $1/T_2$ rate is generally larger than the $1/T_1$ [27]. In the case of protein solutions, both $1/T_1$ and $1/T_2$ arise from the same dipolar relaxation mechanisms at the low fields. The dipolar contributions to the $1/T_2$ and $1/T_1$ disperse towards 0.3 of their low field values with increasing fields [27]. However, there is an additional contribution to $1/T_2$ that increases quadratically at high fields. This field-dependent contribution arises from acid- and base-catalyzed proton exchange, and dominates the $1/T_2$ [28]. The T_{2R}/T_{1R} ratios given in Table 1 for diamagnetic proteins may be explained by the field-dependent contribution. On the other hand, under certain circumstances, such as $w_s^2\tau^2 << 1, w_I^2\tau^2 << 1$ and neglection of the spin exchange term, the $1/T_1$ and $1/T_2$ in coordination sphere of an ion are equal. The equality of the transferrin relaxivities implies that these circumstances are valid at the present frequency used for T_1 and T_2 measurements.

Table 2
Contribution of transferrin iron and serum proteins to 1/T1 and 1/T2 in serum, expressed as s^{-1} and TP determined by MRI and autoanalyzer

Serum	CTI to T_1 and T_2	CSP to T_1	TP by T_1	CSP to T_2	TP by T_2	TP-auto
Diluted	0.04	0.20	5	1.65	4.6	4.8
Normal	0.06	0.28	7	2.251	6.3	6.8
Doped	0.06	0.40	10	3.47	9.6	10

The present dilution experiments, in agreement with previous ones [4,5], show a linear dependence of relaxation rates on serum TP. This implies that the data in Table 1 can be utilized for TP determinations, which in turn will serve to confirm the relaxivities. TP determination by MRI requires the relaxivities of TP and also the CTI and CSP in serum. The CSP and CTI are presented in Table 2. The CTI and CSP in diluted samples are lower than those in native serum, whereas CSP of albumin-doped serum increases. The results are consistent with the dilution and addition of albumin.

Inserting the relaxivities in Table 1 into Eq. (1) gives 0.04 $(g/dl)^{-1}s^{-1}$ for T_1 relaxivity of TP and 0.36 $(g/dl)^{-1}s^{-1}$ for the T_2 relaxivity. These are in good agreement with the TP relaxivities obtained by dilution, which are about 0.38 $(g/dl)^{-1}s^{-1}$ for T_2 relaxivity and 0.042 $(g/dl)^{-1}s^{-1}$ for T_1 relaxivity of TP. Then serum TP can be calculated from Eq. (5) by using R_{iTP} and $(CSP)_i$. TP values by MRI and by autoanalyzer are also presented in Table 2. The calculated values are consistent with those by autoanalyzer. The similarity between the average TP relaxivities and the TP relaxivities from serum dilution, and also the similarity between TP values by MRI and those by conventional methods imply that the relaxivities in Table 1 are reliable. By using Eq. (5), the relaxation rate in healthy serum can therefore be aproximated as

$$1/T_{ih} = 1/T_{iw} + C^h_{alb}\,R_{alb} + C^h_{\gamma\text{-}glbs}\,R_{\gamma\text{-}glbs} + C^h_{(\alpha+\beta)\text{-}glbs}\,R_{(\alpha+\beta)\text{-}glbs} + C^h_{tr}\,R_{tr} \quad (6)$$

where h denotes healthy, and C and R denote concentrations and relaxivities, respectively. The terms on the right side of Eq. (6) show the contributions of free water, albumin, γ-globulins, $(\alpha+\beta)$-globulins, and transferrin iron, respectively. Because albumin, γ-globulins, $(\alpha+\beta)$-globulins

Table 1
Proton relaxivities of some serum proteins and serum diluted by distilled water, expressed as $(g/dl)^{-1}\ s^{-1}$

Relaxivity	Alb.	γ-glbs	$(\alpha+\beta)$-glbs	α-glbs	Lyso	$(\gamma+\beta)$-glbs	Fib	Trf	Dil-ser
R_{T2}	0.240	0.680	0.46	0.6	0.400	0.30	0.50	2.60	0.38
R_{T1}	0.035	0.045	0.052	0.06	0.039	0.08	0.03	2.40	0.042
R_{T2}/R_{T1}	6.9	15	8.9	10	10	3.8	16.7	1.1	9

make up almost TP, the contribution of other globulins were neglected.

Equation (6) can qualitatively be expressed in the following way: Serum contains water, proteins, inorganic salts, and various organic compounds. Serum water is found in the free and bound states. Exchange of water between the two environments is so sufficiently rapid that relaxation rates in serum can be expressed in terms of a fast exchange of protons between free and bound state [4,5,8,27,28]. The percentage of bound water is altered by changes in the concentration of individual proteins making up TP [27,28]. Both T_1 and T_2 will therefore be dependent on TP composition.

Because TP in diluted serum and TP in albumin-doped serum [obtained from Eq. (5) by using the relaxivities] are similar to those obtained by autoanalyzer, there are probably no differences between relaxivities of individiual proteins in healthy and diseased serum. In fact, the composition of serum from patients with hypoprotein resembles diluted samples to some extent, whereas that with hyperprotein resembles albumin-doped serum. The relaxation rates in diseased serum should therefore be considered as

$$1/T_{id} = 1/T_{iw} + C^d_{alb}\, R_{alb} + C^d_{\gamma\text{-}glb}\, R_{\gamma\text{-}glbs} + C^d_{\alpha+\beta-glbs}\, R_{\alpha+\beta-glbs} + C^d_{tr}\, R_{tr} \quad (7)$$

where d denotes diseased serum. The difference of relaxation rates in healthy and diseased serum will be as follows

$$1/T_{id} - 1/T_{ih} = \Delta C_{alb}\, R_{alb} + \Delta C_{\gamma\text{-}glbs}\, R_{\gamma\text{-}glbs} + \Delta C_{\alpha+\beta\text{-}glbs}\, R_{\alpha+\beta\text{-}glbs} + \Delta C_{tr}\, R_{tr} \quad (8)$$

where

$$\Delta C_{alb} = C^d_{alb} - C^h_{alb}$$

$$\Delta C_{\gamma\text{-}glb} = C^d_{\gamma\text{-}glbs} - C^h_{\gamma\text{-}glb}$$

$$\Delta C_{\alpha+\beta\text{-}glbs} = C^d_{\alpha+\beta\text{-}glbs} - C^h_{\alpha+\beta\text{-}glbs}$$

$$\text{and } \Delta C_{tr} = C^d_{tr} - C^h_{tr}$$

Equation (8) can explain the effect of TP composition on serum relaxation rates. The effect of TP and TP composition may occur in several ways: (a) Both TP and TP composition may change by diseases. If all proteins increase, Eq. (8) will take the following form:

$$1/T_{id} = 1/T_{ih} + A \quad (9)$$

where A is equal to the right side of Eq. (8). As a result of positive contribution, T_{id} decreases. If all proteins decrease, Eq. (8) will be

$$1/T_{id} = 1/T_{ih} - B \quad (10)$$

where B is equal to the right side of Eq. (8). As a result of negative contribution, T_{id} increases. (b) If some proteins increase while others decrease, TP composition is changed by diseases. However, TP content can change or not change. In this case, the sign and magnitudes of the contributions are dependent on fractional change of each protein and the corresponding relaxivity. Accordingly, the contributions can be positive, negative, or they can be zero, as given in Eq. (11)

$$1/T_{id} = 1/T_{ih} + C,\ 1/T_{id} = 1/T_{ih} - D,\ 1/T_{id} = 1/T_{ih} \quad (11)$$

where C and D are each the sum of positive and negative contributions.

Earlier data has reported a wide range of relaxation times for each of the normal and diseased serum groups [2,11–14]. In these studies, it was found that the relaxation times in diseased serum are lower and higher than those in normal serum, and also, relaxation times in normal serum overlap with those in diseased serum. Because earlier data have been conducted at lower frequencies and not included the percentage of protein fractions, reproductions of T_1 and T_2 in earlier data by using the present relaxivities are impossible. Nevertheless, the distributions in each group should be explained by Eqs. (9–11).

When TP in the diseased group remains in the range of that in normal serum, the sum of protein increase may be equal to the sum of protein decrease. Nevertheless, the relaxation rates are changed in this case because individual proteins have different relaxivities. For example, in a previous work [2], the concentration of albumin in lung carcinoma and non-tumor diseases decreased nearly 0.7 g/dl relative to control, but concentration of γ-globulins increased nearly 0.5 g/dl. TP was the same for all groups. Inserting these individual protein changes and the relaxivities in Table 1 into Eq. (8) gives $(-0.17)\ s^{-1}$ for the contribution of albumin to $1/T_2$ and $(+0.31)\ s^{-1}$ for the contribution of γ-globulins to $1/T_2$. This corresponds to a net increase of $(+0.14)\ s^{-1}$ in $1/T_2$. Despite equality of TP in all groups, T_2 in diseased groups was shortened relative to normal serum. This is consistent with the relaxation changes in diseased groups. Equation (8) and related analysis indicate that only changes in serum TP composition are a main source of the relaxation changes in various serum groups.

The studies on the comparison of relaxation times in healthy and diseased biological fluids, including blood and serum, are still of scientific interest [22–25]. These studies compare various diseased groups [20–23,25] and reveal the effect of solution composition on relaxation times. However, in none of them were the results interpreted in terms of relaxivities and protein contents. Equation (8) and related discussion may therefore be helpful for selection of patient groups in relaxation studies and useful for analysis of such studies. On the other hand, conventional TP determination methods are based on the use of several chemicals, and are time consuming. For this reason, the search for a new method to determine TP in serum is important [29–32]. The data based on the determinations of CSP and the relaxivities of proteins in serum imply that serum total protein may be

determined by MRI. The data may contribute to the efforts on finding a new method for TP determination.

4. Conclusions

In conclusion, present results suggest that T_2 relaxivities of individual serum proteins and the contributions of serum proteins to the $1/T_2$ are significantly greater than those of T_1. The results also suggest that relaxation changes in various serum groups can be explained by the contents and relaxivities of individual serum proteins.

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