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Dextran-magnetite complex: temperature dependence of its NMR relaxivity

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The NMR relaxivity of proton by dextran-magnetite (DM) complex was examined at temperatures from 20 to 80 °C. The decrease in T_1 relaxivity with increasing temperature is due to the thermal activation of motion of water molecules in and/or surrounding DM particle. The decrease in magnetization of magnetite core is the secondary effect. The temperature dependence of T_1 relaxivity was found to depend on molecular weight of dextran. The motion of dextran chains binding to core may affect the thermal activation of "heavy water" interacted with dextran. The Arrhenius plots of T_1 relaxivity showed different slopes at 20–40 °C and 50–80 °C, while, no change in slope was observed for $Fe(NO_3)_3$, $CuSO_4$ and Gd-DTPA solutions.

1. Introduction

The nuclear magnetic resonance imaging (MRI) contrast agent is a biocompatible pharmaceutical in addition to a good probe of the nuclear magnetic resonance (NMR) relaxation. The main conditions necessary to the contrast agent are (i) high relaxivity, (ii) specific *in vivo* distribution, (iii) *in vivo* stability, (iv) *in vivo* excretability and (v) lack of toxicity [1].

The dextran-magnetite (DM) complex was recently developed as a new biocompatible magnetic fluid [2]. The complex is composed of a magnetite core and some dextrans directly binding to core [3, 4]. The complex has a super-paramagnetic property [5] and the NMR relaxivity is much larger than that of conventional MRI contrast agent of paramagnetic ion [4]. Since the dextran chains closely cover the surface of magnetite core, DM complex is safe for living body and applicable to medical field such as MRI and hyperthermia [2]. The uptake and metabolic behaviors of DM complex in rat organs suggest that DM complex is suitable for a MRI contrast agent of tumors of reticuloendothelial system, particularly of liver [6–9].

The physical and chemical properties of DM solution, the stability of DM solution etc. depend significantly on molecular weight of dextran chain (chain length) [4, 9]. These molecular weight dependences seem to be associated with the conformation of dextran chains covering core. For example, the conformation is close to a fully-extended state for a short chain (molecular weight less than 2000) and takes a random-coiled state for a long chain (greater than 20 000) [3, 4].

However, the interaction between water molecules and dextran chains covering core has not yet been studied. The molecular weight dependence may not be explained without knowledge about the water-dextran interaction. The behavior of DM particles in living body may also be closely related to the water-dextran interaction. Thus, in this study, we have attempted to reveal the water-dextran interaction through the temperature dependence of NMR relaxivity of DM solution.

2. Materials and methods

Three kinds of DM complexes were prepared using alkali-treated dextrans with number average molecular weight of 2000, 4200 and 9600. The weight average molecular weights of the dextrans are 4000, 7000 and 20 000, respectively. As shown in Table I, six samples from No. 1 to No. 6 were prepared by "one step method" reported previously [4]. No. 1 and No. 2 are DM samples prepared using dextran with molecular weight of 2000 but the core sizes are different. No. 3 and No. 4 are a

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TABLE I Core size and NMR relaxivity of dextran-magnetite (DM) complexes prepared in this study

Sample	M	D_c(nm)	R_1(mM^{-1}s^{-1})	R_2(mM^{-1}s^{-1})
No. 1	2000	8.1	37	150
No. 2	2000	7.8	23	100
No. 3	4200	5.7	25	150
No. 4	4200	7.7	43	200
No. 5	9600	7.1	26	100
No. 6	9600	8.2	38	150

M, number average molecular weight of dextran. D_c, average size of magnetite core in diameter. R_1, T_1 relaxivity of DM solution at 38 °C (body temperature of rat), where T_1 is the spin-lattice relaxation time of proton (^{1}H). The concentration of DM complex (mM) is expressed in Fe base. R_2, T_2 relaxivity of DM solution at 38 °C, where T_2 is the spin-spin relaxation time.

group and also No. 5 and No. 6 are a group. The respective groups are called as DM(2000), DM(4200) and DM(9600) in this paper, where the numbers in parentheses represent the molecular weight of dextran.

Using X-ray diffraction pattern of the powdered DM sample, the core size was determined from the most intense (3 1 1) peak in the same manner reported previously [3]. The NMR relaxivities (R_1 and R_2) of DM sample were determined from the following equations [1].

$$1/T_1 - 1/T_1(w) = R_1 c \qquad (1)$$

$$1/T_2 - 1/T_2(w) = R_2 c \qquad (2)$$

Here, T_1 is the spin-lattice relaxation time of proton (^{1}H) for DM solution. T_2 is the spin-spin relaxation time for DM solution. $T_1(w)$ and $T_1(w)$ represent the data for distilled water. The parameter c represents the concentration of DM complex which is expressed in Fe base. All the T_1 and T_2 data were measured using a pulsed NMR instrument (Bruker, minispec PC-120; 0.47 T, 20 MHz). Table I shows R_1 and R_2 at 38 °C (body temperature of rat) for all the DM samples. Both R_1 and R_2 of the large core samples are greater than those of the small core samples.

The NMR experiments were carried out at 20, 30, 40, 50, 60, 70 and 80 °C. The sample temperatures were controlled with an accuracy of ± 0.05 °C, using a temperature controller (Lauda, RM6). The T_1 data were obtained at all the temperatures, while, the T_2 data were not obtained at 60–80 °C because of large error. It is known that DM solution becomes unstable at temperature higher than 80 °C (aggregation and/or precipitation of DM particles occur within several days) [4]. However, in the present study, no change was detected for all the samples.

The NMR experiments at 20–80 °C were also carried out for $Fe(NO_3)_3$, Gd-DTPA, $CuSO_4$ and dextran solutions, where DTPA is diethylene-triamine-penta-acetic acid (Gd-DTPA is a conventional MRI contrast agent [1]). The high concentration solutions were used for Gd-DTPA and $CuSO_4$, since their R_1 are much smaller than R_1 of DM sample. Table II shows the concentrations of all the samples used in NMR experiment.

The magnetization of a dense DM solution (1 M in Fe base) was measured up to $H = 1 \times 10^4$ Oe using a vibrating sample magnetometer (Toei-Kogyo, VSM-5-18 Auto). The measurement was carried out at 20, 60 and 80 °C.

TABLE II Concentrations of samples used in NMR experiment

Sample	Concentrations
DM complex	0, 0.05, 0.1, 0.2 mM (in Fe base)
$Fe(NO_3)_3$	0, 0.1, 0.2, 0.4 mM
Gd-DTPA	0, 1, 2, 4 mM
$CuSO_4$	0, 1, 2, 4 mM
Dextran	0.1 mM

3. Results

Fig. 1 shows a typical temperature dependence of T_1 for DM sample. The data with the same accuracy were also obtained for other DM samples. The straight lines indicate that the T_1 relaxation by DM complex satisfies Equation 1 at various temperatures. The slope of the straight line decreases with increasing temperature. Since the slope represents R_1, these data suggest that R_1 of DM solution decreases at higher temperature. On the other hand, the data points on the ordinate indicate that $1/T_1(w)$ decreases with increasing temperature, i.e., $T_1(w)$ increases with temperature. The increase in $T_1(w)$ is due to the thermal activation of motion of water molecules. Thus, two reasons are considered for the decrease in R_1 at higher temperature: (i) decrease in

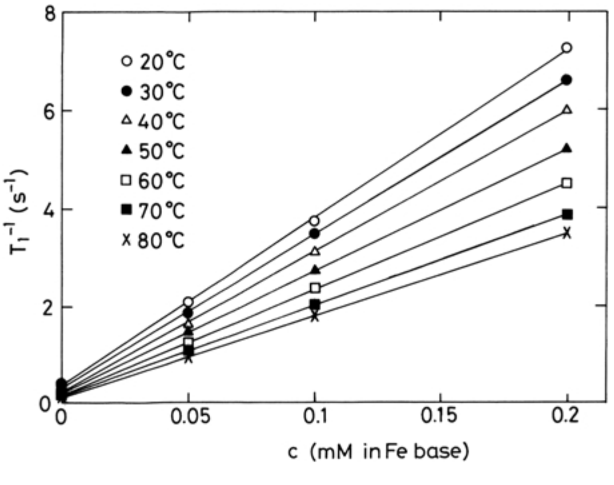

Figure 1 $1/T_1$ versus c at temperatures from 20 to 80 °C. Here, T_1 is the spin-lattice relaxation time of proton (^{1}H) and c is the concentration of dextran-magnetite (DM) complex which is expressed in Fe base. These data were obtained using No. 6 sample listed in Table I.

magnetic strength by magnetite core and (ii) increase in the motion of water molecules.

Fig. 2 shows typical magnetization (M-H) curves for a dense DM solution at 20, 60 and 80 °C. The magnetization rises sharply with external field and increases gradually over H = $\sim 3 \times 10^3$ Oe. This result agrees with that DM solution has a super-paramagnetic property [2]. The decrease in magnetization from 20 to 80 °C is about 8% at around H = 5×10^3 Oe. While, for the same DM sample, the decrease in R_1 from 20 to 80 °C is about 31%. This difference suggests that the decrease in R_1 is mainly due to the thermal activation of water molecules and the change in magnetization is the secondary effect.

We plotted $\ln R_1$ against $10^3/T$, assuming that the temperature dependence of R_1 is an Arrhenius type. Here, $\ln R_1$ is the natural logarithm of R_1 and T is the absolute temperature. Fig. 3 shows the relationship between $\ln R_1$ and $10^3/T$ for all the DM samples. The plots can be fitted with the two straight lines which cover temperature ranges of 20–40 °C and 50–80 °C. The arrow indicates the inflection point where lie between 40 and 50 °C.

Since the magnetization of magnetite core increases with the core size, R_1 of DM sample with large core is greater than with small core. Thus, the value of R_1 itself is not important in the present study. On the other hand, since the thermal activation of water molecule is not related to magnetization of core, the slope (temperature dependence of R_1) does not depend on core size. Note that the slope of No. 1 is almost the same as that of No. 2. Table III summarizes the slopes at the low and high temperature regions. The slope (k_1) at the low temperature region is about a half of the slope (k_2) at the high temperature region. Both k_1 and k_2 decrease with an increase in molecular weight of dextran but the ratio (k_2/k_1) inversely increases.

Fig. 4 shows the plots of $\ln(1/T_1)$ versus $10^3/T$ for pure water and two dextran solutions. The concentration (0.1 mM) of the dextran samples is approximately equal to the concentration of dextran in 0.2 mM DM solution. All the data points can be fitted with a straight line over the temperature range of 20–80 °C. The data points of dextran samples lie at almost the same positions as for the pure water, suggesting that the dextran molecule itself has no effect on T_1 of water. This slope (k) is listed in Table III. The value of k is 3–5 times as large as k_1 of DM sample and 1.5–2 times as large as k_2 of DM sample. The thermal activation energy for the motion of pure water can be estimated from k to be 4.1 kcal/mol. The activation energy is consistent with the value reported by other group (4.7 kcal/mol) [10].

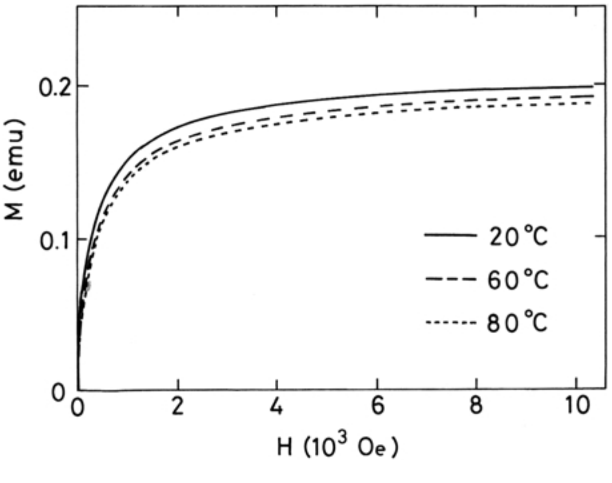

Figure 2 Magnetization (M-H) curves of DM solution at 20, 60 and 80 °C. These data were obtained using No. 6 sample.

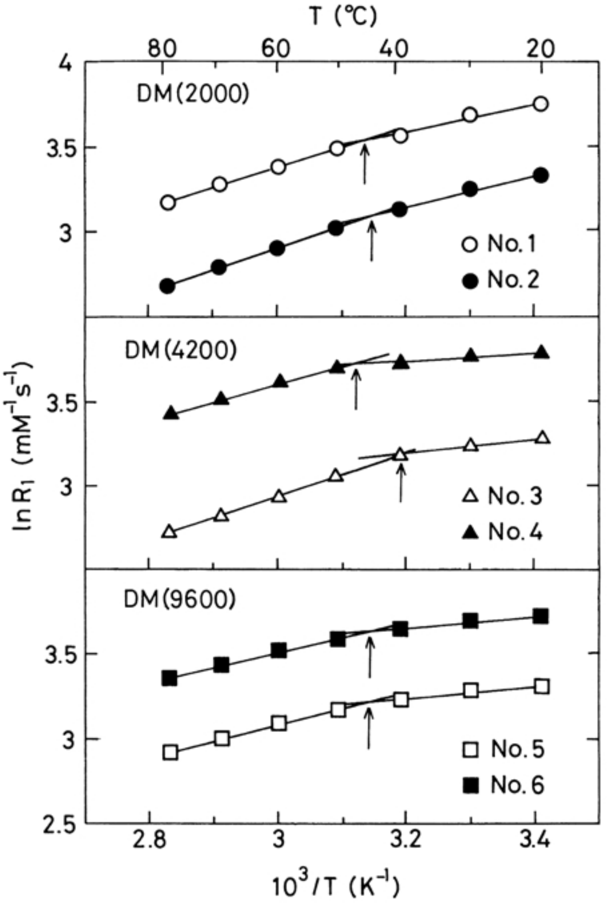

Figure 3 $\ln R_1$ versus $10^3/T$ for DM samples. Here, $\ln R_1$ is the natural logarithm of R_1 and T is the absolute temperature. The notation of DM(2000) represents the DM samples (No. 1 and No. 2) which were prepared using dextran with molecular weight of 2000. The intersection of two straight lines is marked by an arrow. The symbol size represents data accuracy.

Fig. 5 shows the plots of $\ln R_1$ versus $10^3/T$ for $Fe(NO_3)_3$, Gd-DTPA and $CuSO_4$ solutions. The $Fe(NO_3)_3$ data at temperatures higher than 50 °C are omitted in this figure, since the sample became muddy above 50 °C. The respective plots can be fitted with a straight line and the slopes of three samples are similar. These slopes (k) are also listed in Table III. The values of k are about a half of k of pure water and close to k_2 of DM sample rather than k_1.

4. Discussion

The strength H of the magnetic field generated by a magnetite core is written as $H \infty M/r^2$, where M is the magnetization of core and r is the radial distance from the center of core [1]. Thus, the magnetic field near core is effective to the relaxation of proton but the field away from core is not effective.

TABLE III Slope of Arrhenius plots of T_1 relaxivity

Sample	k	k_1	k_2	k_2/k_1
DM(2000)	—	0.87	1.3	1.5
DM(4200)	—	0.59	1.0	1.7
DM(9600)	—	0.37	0.92	2.5
water	2.1	—	—	—
dextran(2000)	2.1	—	—	—
dextran(9600)	2.1	—	—	—
$Fe(NO_3)_3$	1.1	—	—	—
Gd-DTPA	1.1	—	—	—
$CuSO_4$	1.3	—	—	—

k, slope of straight line at 20–80 °C (see Figs 4 and 5). k_1, slope of straight line at 20–40 °C (see Fig. 3). k_2, slope of straight line at 50–80 °C. The values of k, k_1 and k_2 are expressed in arbitrary units. DM(2000), DM sample prepared using dextran with molecular weight of 2000. Dextran(2000), dextran solution of molecular weight of 2000. DTPA represents diethylene-triamine-pentaacetic acid (Gd-DTPA is a conventional MRI contrast agent [1]).

The water molecules in and/or surrounding DM particle are mainly concerned in the T_1 relaxivity of proton, since the relaxation effect by core is limited to the local area outside core. These water molecules are interacted with dextran chains binding to core and motion of the water molecules is restricted by the interaction. Thus, the thermal activation of ''heavy water'' (not D_2O) interacted with dextran may be less than that of free water. This idea agrees with the result that k_1 and k_2 of DM samples are less than k of free water. The same idea is applicable to the paramagnetic ions and Gd-DTPA, since k of these samples are less than k of free water.

Both k_1 and k_2 of DM samples decrease with an increase in molecular weight of dextran. A large interaction is responsible for a large suppression of the motion of water molecules. However, degree of the water-dextran interaction may be almost constant irrespective of molecular weight of dextran. The decrease in both k_1 and k_2 suggest that the motion of ''heavy water'' is influenced by the motion of dextran chain itself. The motion of dextran chain may depend on not only the chain length but also the conformation. The conformation of dextrans covering core changes by the chain length, as shown in Fig. 6. According to the previous study [4], the conformation is close to a fully-extended state for DM(2000) and to a random-coiled state for DM(9600). The conformation for DM(4200) is considered to lie between DM(2000) and DM(9600).

In the case of the fully-extended conformation, the motion of dextrans may be more active in comparison with the random-coiled conformation, since no tangled chain is observed for the fully-extended conformation. Thus, the motion of ''heavy water'' also becomes more active for the fully-extended conformation. This idea agrees with the result that both k_1 and k_2 decrease with increasing molecular weight of dextran. In summary, the thermal activation of ''heavy water'' decreases from the fully-extended conformation to the random-coiled conformation.

There lies an inflection point between 40 and 50 °C for all the DM samples. It seems to be characteristic of DM complex, since such thermal transition is not observed for $Fe(NO_3)_3$, $CuSO_4$ and Gd-DTPA samples. However, Yoshino, Yoshida and Takahashi reported that a similar transition concerning the motion of D_2O was observed at around 30 °C in the temperature dependence of T_1 of enflurane-solubilized D_2O system [11]. According to

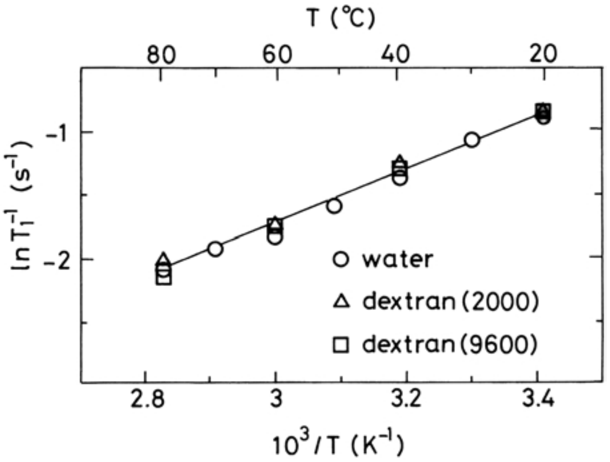

Figure 4 $\ln(1/T_1)$ versus $10^3/T$ for pure water and two dextran solutions. The notation of dextran (2000) represents dextran sample of molecular weight of 2000. The symbol size represents data accuracy.

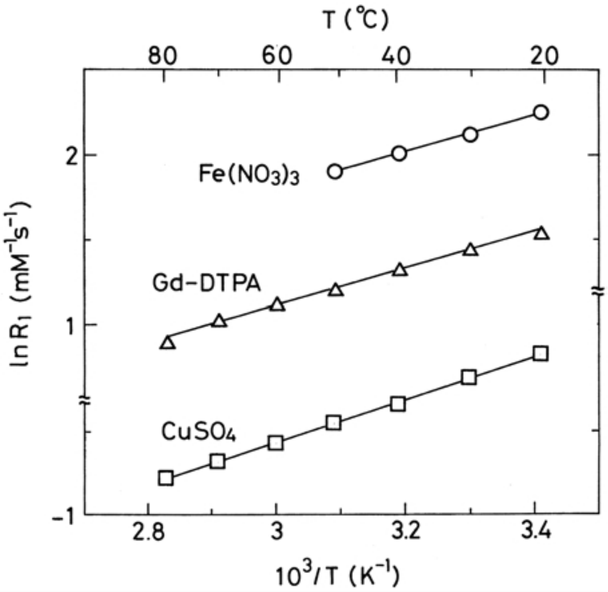

Figure 5 $\ln R_1$ versus $10^3/T$ for $Fe(NO_3)_3$, Gd-DTPA and $CuSO_4$ solutions. Gd-DTPA is a conventional MRI contrast agent. The symbol size represents data accuracy.

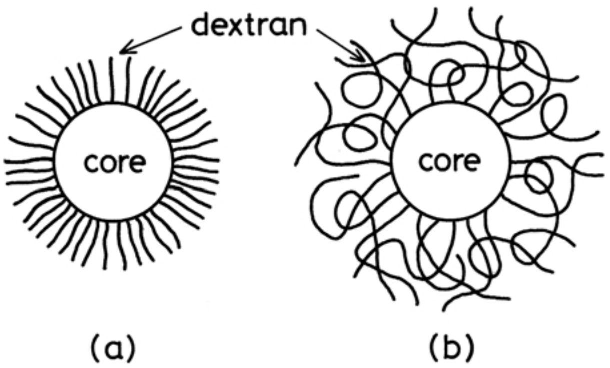

Figure 6 Structural model of DM complex in water: (a) DM(2000); (b) DM(9600). The core size is exaggerated. Note the conformation of dextran chains covering core.

their idea, this transition is due to change in the interaction between D_2O and enflurane in the system [11].

In the case of DM complex, the thermal transition occurs at almost the same temperature, irrespective of molecular weight of dextran. Thus, the transition may be associated with some change in the local environment of ''heavy water'' rather than change in the conformation of dextran chains. The transition seems to be due to some change in the water-dextran interaction, in the same way as for the enflurane-solubilized D_2O system.

The relation of $k_1 < k_2$ is seen for all the DM samples. This relation indicates that the motion of ''heavy water'' becomes active at higher temperature above the inflection point. If the water-dextran interaction becomes weak above the transition temperature in the same way as for the D_2O-enflurane interaction [11], the above relation may be reasonably explained by the change in water-dextran interaction by temperature. On the other hand, the ratio of k_2 and k_1 (k_2/k_1) increases with increasing molecular weight of dextran. This is due to the large decrease in k_1 with molecular weight of dextran, because k_2 also decreases. The change in k_2/k_1 seems to be associated with the conformation of dextrans but the reason is not clear at present.

5. Conclusions

Three kinds of dextran-magnetite (DM) complexes were prepared using alkali-treated dextrans with molecular weight of 2000, 4200 and 9600. The T_1 relaxivity of proton by DM complex was examined at temperatures from 20 to 80 °C. The results are summarized as follows.

1. The T_1 relaxivity decreases with increasing temperature. The decrease is due to the thermal activation of motion of water molecules in and/or surrounding DM particle. The decrease in magnetization of magnetite core is the secondary effect.
2. The thermal activation of the water molecules decreases with an increase in molecular weight of dextran. The reason can be explained from the motion of dextran chains binding to core which depends on the conformation of dextrans.
3. The slope of Arrhenius plots of the T_1 relaxivity is different at 20–40 °C and 50–80 °C for all the DM samples. The thermal transition may be associated with some change in the water-dextran interaction, since such transition is not observed for $Fe(NO_3)_3$, $CuSO_4$ and Gd-DTPA solutions.

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