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Structure of dextran–magnetite complex: relation between conformation of dextran chains covering core and its molecular weight

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A homologous series of dextran–magnetite complex (DM) was synthesized using alkali-treated dextrans with various molecular weights from 1800 to 27 000. The structure of DM particles in water, particularly effect on molecular weight of dextran, was studied. The number of dextran chains binding to a core is possibly determined by the steric hindrance between dextran chains covering core. The conformation of dextran chains may change from a fully extended state to a random-coiled state with an increase in molecular weight.

1. Introduction

The dextran–magnetite complex (DM) is a biocompatible magnetic fluid which is different from conventional type with surfactant. DM was developed as a new medical agent applicable to magnetic resonance imaging and hyperthermia [1]. The physical properties of DM particle were studied by magnetization [2], nuclear magnetic resonance (NMR) [3, 4], electron microscopy [2], X-ray diffraction [2] and Mössbauer spectroscopy [5]. These data have been accounted for by the idea that DM particle is composed of a magnetite core and dextran chains binding directly to the core and exhibits a super-paramagnetic property. The uptake and metabolic behaviors of DM in rat organs were also studied from a view point of application in the medical field [6–13]. The results and the chemical properties of DM [2, 13] suggest that DM is a unique magnetic fluid with high stability and low toxicity for living body. However, little is known about the structure of DM particle in aqueous solution, although the structural information, particularly conformation of dextran chains covering core, is important for interpretation of the biological properties of DM.

In this study we prepared a homologous series of DM using magnetite ultrafine particles (8 nm in diameter) and six kinds of alkali-treated dextrans with various molecular weights (1800–27 000). The whole size of DM particle in water was measured by dynamic light scattering. The number of dextrans binding to a core was determined by chemical analysis. The conformation of dextrans covering core is discussed on the basis of these systematic data for molecular weight.

2. Materials and methods

Fig. 1(a) shows constitutional formula of the alkali-treated dextran chain which is made by heating dextran in NaOH solution. A carboxyl group is introduced at the reduction end by the heat treatment. The dextran chain binds to a magnetite core by linkage between the carboxyl group and an iron atom on the core surface (Fig. 1b) [14]. In this study we prepared the alkali-treated dextrans with number average molecular weights of 1800, 3600, 9000, 13 300, 17 900 and 27 000. The dispersion parameter $\langle M_w \rangle / \langle M_n \rangle$ for molecular weight was less than 1.5, where $\langle M_w \rangle$ and $\langle M_n \rangle$ are the weight and number average molecular weights, respectively.

Fig. 2 shows a flow chart of the synthetic procedure. The preparation of magnetite core is as follows. Anion-exchange resin slurry (Amberleit IRA-410, OH type, exchange capacity of 1.35 mol l^{-1}) of 3.24 l in volume was activated in advance by distilled water until 4.2 l in the total volume. $FeCl_3 \cdot 6H_2O$ (165.2 g) and $FeCl_2 \cdot 4H_2O$ (60.6 g) were dissolved in distilled water (530 ml) under N_2 atmosphere. The iron salts solution was added into the resin slurry with stirring and the reaction mixture was kept at pH 8–9. After several minutes, HCl was added into the mixture until pH 1.6 and then the resin was removed using a glass filter. The source magnetite sol of ultrafine particle was dialyzed through a cellophane film against running water until pH 2.5–3.0. The Fe yield of the sol thus obtained was about 70%.

For preparation of DM sample, the source magnetite sol was added into 20% (w/v) solution of alkali-treated dextran (weight of dextran was 4.5 times as great as that

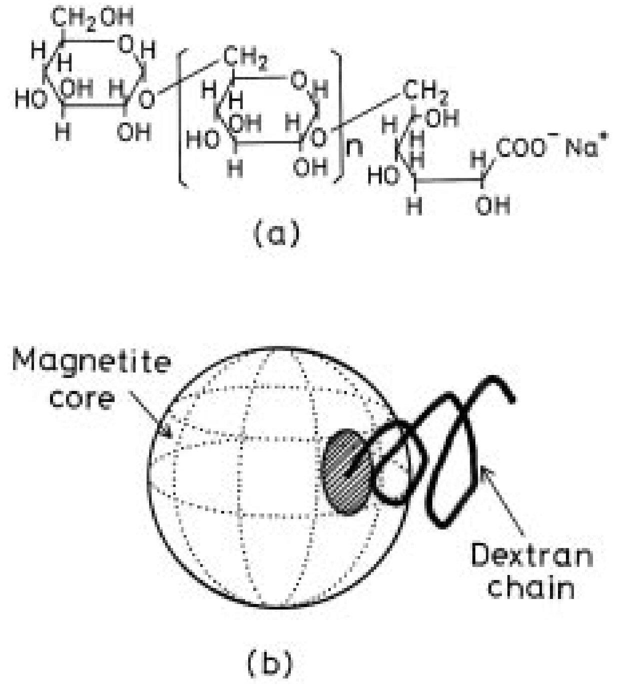

Figure 1 (a) Constitutional formula of alkali-treated dextran. (b) Illustration of a dextran chain binding to magnetite core. The core size is exaggerated. The hatched area represents the area of core surface occupied by a chain.

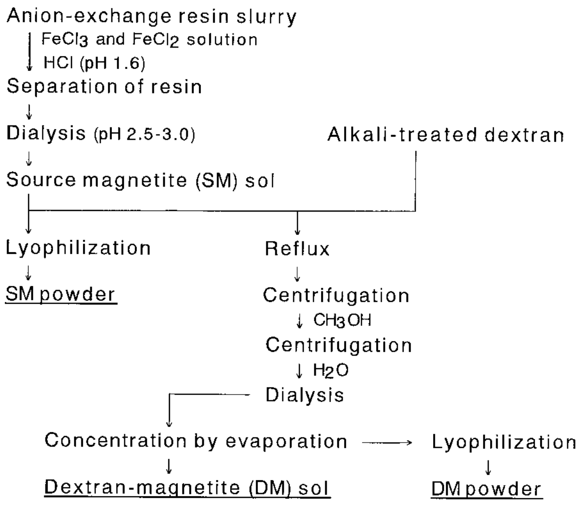

Figure 2 Flow chart of synthetic procedure of magnetite core and dextran–magnetite complex (DM).

of Fe). The mixture was refluxed for 1 h at 100 °C. Most of the dextran possibly bind to the magnetite particles in the reflux process. After cooling, CH_3OH was added up to 41–48% (w/v) to precipitated DM. After centrifugation (2000 ***g*** for 5 min), the precipitate was dialyzed against running water overnight. The DM solution was adjusted to pH 8 with a drop of NaOH solution and concentrated using an evaporator. The Fe yield of DM sol was 90–95%.

In this paper we refer to the DM samples obtained using the six kinds of dextrans as ‘‘DM-1, DM-2, DM-3, DM-4, DM-5 and DM-6’’ in the order of molecular weight of 1800, 3600, 9000, 13 300, 17 900 and 27 000, respectively. The powder samples of magnetite particle and DM were also prepared by lyophilization of the source magnetite sol and DM sol.

3. Results

3.1. Shape and size of magnetite core

X-ray diffraction of powder DM sample was measured using a conventional diffractometer (Fe-filtered CoK_α, $\lambda = 0.179$ nm) to check the crystal structure of core. The pattern was attributable to magnetite (Fe_3O_4), although all the diffraction peaks were broad because of ultrafine particle. The shape and size of core were examined using a transmission electron microscope (TEM). We prepared the TEM sample by dropping dilute DM solution on a microgrid coated with carbon film. The shape of core was approximately spherical and the size was 7.7 ± 1.0 nm in diameter. The core size was also estimated from the most intense (3 1 1) peak in the X-ray diffraction pattern using Scherrer's equation [15]. The size (8 ± 1.5 nm) is consistent with the result obtained by TEM observation, suggesting that most of the cores consist of a single domain of magnetite crystal.

3.2. Gel filtration

The gel chromatography of DM solution was carried out to remove unreacted dextran (free dextran). The gel (Toso Co., PW-5000) was equilibrated with 0.1 M phosphate buffer solution (pH 7.4). Fig. 3 shows the elution patterns of DM-1 and DM-6 solutions. The ordinate represents the dextran concentration which was measured by H_2SO_4-anthrone method. A large peak first appears and subsequently a small peak appears in both patterns. The fractions of the large peak contained iron and the iron concentration was proportional to the dextran concentration. Thus, the large peak corresponds to the elution of DM particle. On the other hand, the small peak corresponds to the elution of free dextran,

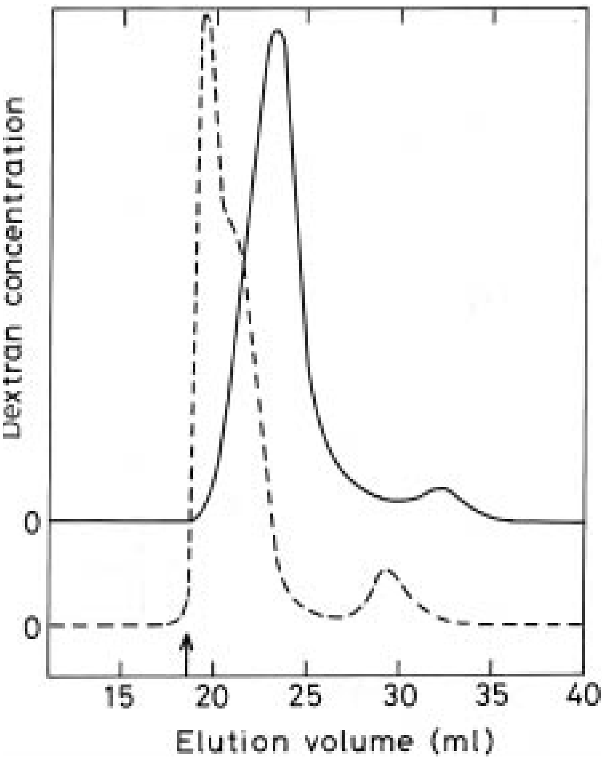

Figure 3 Typical elution pattern of gel chromatography for two DM solutions. The solid and dashed curves represent the patterns of DM synthesized using dextrans of 1800 and 27 000 in molecular weight, respectively. An arrow indicates the void volume of gel.

TABLE I Structural parameters of dextran–magnetite complexes synthesized in this study

Sample	M	N_d	R (nm)	V (nm^3)	L_d (nm)	V_d/N_d (nm^3)
DM-1	1800	81	14	11×10^3	10	1.4×10^2
DM-2	3600	75	19	30×10^3	15	3.8×10^2
DM-3	9000	70	26	72×10^3	22	10×10^2
DM-4	13 300	57	26	72×10^3	22	12×10^2
DM-5	17 900	50	26	72×10^3	22	14×10^2
DM-6	27 000	48	26	72×10^3	22	15×10^2

M, number average molecular weight of dextran. N_d, number of dextran chains binding to a magnetite core. R, radius of complex. V, volume of complex. L_d, thickness of dextran chain layer covering core. V_d, volume of the dextran layer. V_d/N_d, volume occupied by a dextran chain.

since no iron was detected in the fraction. These results suggest that DM sample without free dextran can be obtained by gel filtration technique.

In the dashed curve (DM-6), the large peak seems to consist of two components (we can see a shoulder in the peak). This is possibly due to the fact that the size of DM-6 is close to the void volume of gel (indicated by an arrow). It is suggested that the size of DM-6 is larger than that of DM-1, because large particles are eluted faster than small particles. Also, for free dextran, a difference in the molecular weights of two dextrans (1800 and 27 000) appears at the positions of small peaks.

3.3. Number of dextrans binding to a core

Fe concentration of the fractions containing DM was measured as follows. $NH_2OH \cdot HCl$ was added into the DM solution to convert all Fe^{3+} ions in core into Fe^{2+} ions. Then, the Fe^{2+} concentration was measured by *o*-phenanthroline method [16]. The ratio of dextran and Fe in weight estimated from both concentrations was 0.38, 0.68, 1.6, 1.9, 2.4 and 3.0 for DM-1 to DM-6, respectively. The ratio increases with an increase in molecular weight of dextran but the rate of increase decreases.

The number of Fe atoms in a magnetite core can be calculated with the core volume, density and Fe content. The volume (240 nm^3) is given by core size, assuming spherical shape. For the density and Fe content, we used the values (4.0 g cm^{-3}, 62% (w/w)) obtained from chemical analysis of the source magnetite core, although there are the data of magnetite crystal (5.2 g cm^{-3}, 72% (w/w)) [17].

Thus, the concentration of core in DM solution can be obtained from the number of Fe atoms in a core (1.0×10^4) and the Fe concentration. Because a DM particle contains a core, the concentration of core is equal to that of DM particle. Consequently, the number of dextran chains binding to a core (N_d), in other words, the number of dextrans in a DM particle is estimated from both concentrations of core and dextran for DM solution without free dextran. The value of N_d is listed in Table I together with some structural parameters of DM particle.

The area of core surface occupied by a dextran chain (S_d) (see the hatched area in Fig. 1b) is estimated from N_d and area of core surface (190 nm^2). Fig. 4 shows N_d and S_d plotted as a function of molecular weight of dextran. N_d decreases with an increase in molecular weight, while S_d increases. These data imply that the conformation of dextran chains depends on the molecular weight, i.e. chain length.

3.4. Whole size of DM particle

The dynamic (quasielastic) light scattering from DM particles was measured using a light scattering instrument with a He–Ne laser (Union Giken, SLS-600). The concentration of ~ 10 mM in Fe base was best for the sample. The scattering data were analyzed by the time-integral method [18]. We assumed that the shape of DM particles, as well as the core, is spherical, because dextran chains possibly bind uniformly to the core. The distribution of whole size (polydispersity) was similar for DM-1 to DM-6. The average radius (R) was 14 nm for DM-1, 19 nm for DM-2 and 26 nm for DM-3 to DM-6. The value of R is listed in Table I.

3.5. Nuclear magnetic resonance (NMR) relaxation power

The NMR relaxivity of DM solution for proton was measured using a pulsed NMR instrument (Bruker, PC-120 (20 MHz)) at 36 °C. The measurement was carried

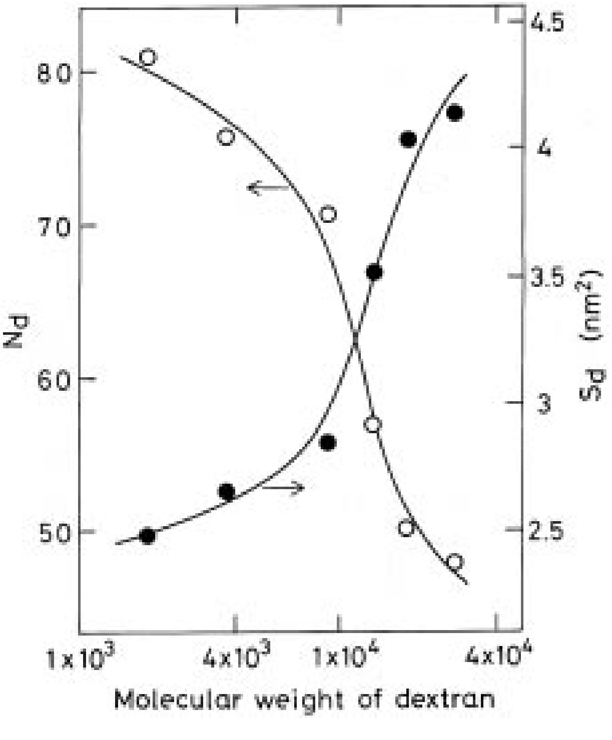

Figure 4 Number of dextran chains binding to a core (N_d) and area of core surface occupied by a chain (S_d) as a function of molecular weight of dextran.

out for four samples with different DM concentrations (0.1–1 mM in Fe base) and pure water. Two relaxivities corresponding to T_1 and T_2 were obtained from the concentration dependencies of $1/T_1$ and $1/T_2$ [3]. The relaxivity for T_1 was 48 (mM (Fe)$^{-1}$ s^{-1}) for DM-1, 44 for DM-2 and ~ 40 for DM-3 to DM-6. The relaxivity for T_2 was 240 (mM (Fe)$^{-1}$ s^{-1}) for DM-1, 220 for DM-2 and ~ 200 for DM-3 to DM-6. This tendency seems to be similar to that of the whole size of DM particle.

4. Discussion

The structure of the DM particle can be summarized as follows. (i) A DM particle is composed of a spherical core and several tens of dextran chains binding to the core. (ii) The dextran chains uniformly cover the core. (iii) The core locates at the center of the DM particle. (iv) The shape of the DM particle is spherical. Table I shows that the thickness of dextran layer (L_d) is constant for DM-3 to DM-6, although the volume occupied by a dextran chain (V_d/N_d) increases from 1000 (DM-3) to 1500 nm^3 (DM-6). This suggests that the conformation of dextran chains changes from DM-3 to DM-6.

Two extreme models can be proposed for the conformation of a dextran chain. One is a fully extended conformation. The chain length (L_{max}) is given by the number of glucose units in a dextran chain and the length of a glucose unit (~ 0.7 nm). Another is a fully folded conformation. The chain length (L_{min}) is obtained by the number of turns in a chain and the thickness of a turn (~ 1.4 nm). A turn consists of seven glucose units under the fully folded conformation. Table II shows L_{max} and L_{min} together with the number of glucose units in a dextran chain and the number of turns in a fully folded chain.

If $L_d > L_{max}$, the value of L_d is not reasonable. For DM-1, L_d is greater than L_{max} (for DM-2 to DM-6, $L_d \leq L_{max}$). The cause is possibly associated with the determination of whole size by dynamic light scattering technique. The following reasons are considered, because we could measure exact size of Latex particle in water. (i) The DM particle is not homogeneous in density. The density of bulk dextran (1.04 g cm^{-3}) is close to that of water [19]. Thus, the density of dextran layer is much smaller than that of magnetite core (4.0 g cm^{-3}). (ii) The boundary between the dextran layer and water is not so clear as that of latex/water system.

Senti *et al.* [20] suggested that the conformation of dextran with molecular weight greater than 50 000 is a random-coiled state and the shape of coil is spherical. If a dextran chain of 27 000 takes a random-coiled conformation, the volume of coil can be estimated to be ~ 1500 nm^3, from extrapolation of their data. This volume is consistent with V_d/N_d of DM-6 (see Table I). Thus, for long chains, the conformation of dextran binding to core may be similar to that of free dextran. Also, the hydration of dextran is possibly similar.

Fig. 5 shows the relationship among L_d, L_{max} and L_{min}. L_d of DM-1 and DM-2 are close to L_{max}. This suggests that the dextran chains take a fully extended conformation. In contrast, L_d of DM-6 is less than L_{min}. This is reasonable, if the dextran chain takes a random-coiled conformation. Fig. 6 shows two structural models

TABLE II Structural parameters of dextran chains used in this study

M	N_u	L_{max} (nm)	N_t	L_{min} (nm)
1800	11	8	1.5	2
3600	22	15	3	4
9000	56	40	7.5	10
13 300	82	60	11.5	15
17 900	110	80	15.5	20
27 000	170	120	23.5	30

M, number average molecular weight of dextran. N_u, number of glucose units in a dextran chain. L_{max}, length of a fully extended chain (maximum length). N_t, number of turns in a fully folded chain. L_{min}, length of the fully folded chain (minimum length).

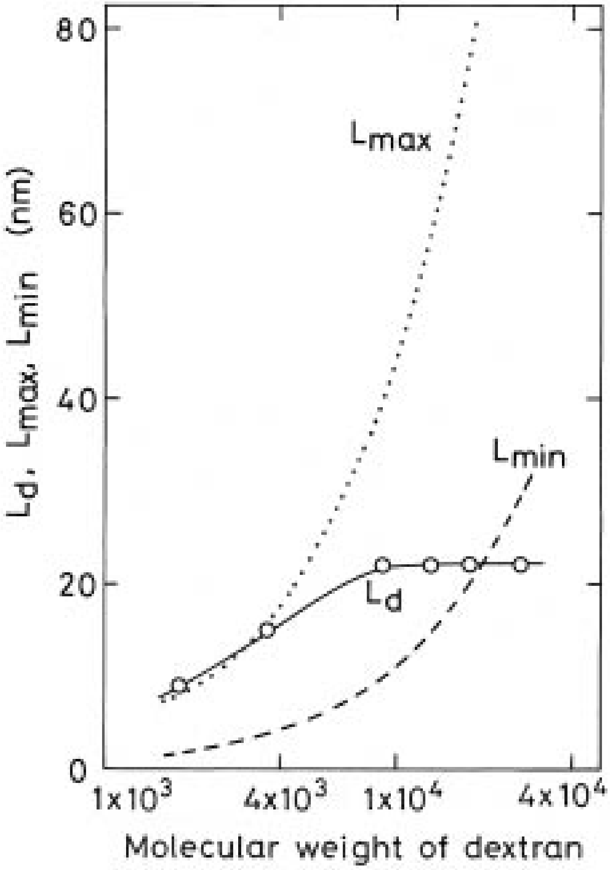

Figure 5 L_d, L_{max} and L_{min} versus molecular weight of dextran, where L_d is thickness of dextran chain layer covering core, L_{max} is length of a fully extended dextran chain and L_{min} is length of a fully folded dextran chain.

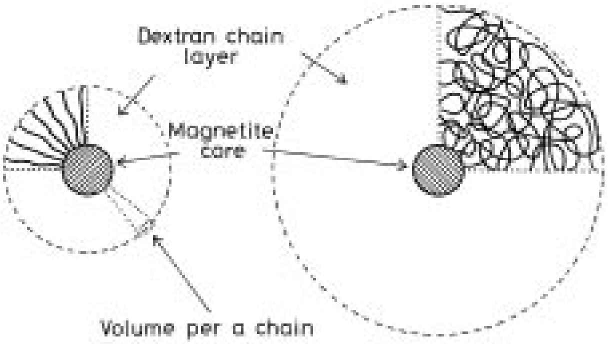

Figure 6 Structural model proposed for DM particle in water: (a) DM-1 (molecular weight of dextran chain is 1800); (b) DM-6 (27 000).

proposed for both conformations. L_d of the other samples lies between L_{min} and L_{max} and approaches L_{min} from DM-3 to DM-5. It is suggested that the conformation of dextran chains changes from the fully extended state to the random-coiled state with an increase in molecular weight.

The hydration of dextran chains covering the core may also depend on the conformation. The parameter V_d/N_d increases with an increase in molecular weight but the rate of increase decreases. This suggests that the number of hydration waters per a glucose unit possibly decreases by the change in conformation.

The number of Fe atoms on the surface of a core of 8 nm in diameter can be calculated as ~ 550 (~ 200 for A site and ~ 350 for B site in spinel structure). The number is 7–12 times as great as N_d. Thus, N_d is possibly determined by the steric hindrance between dextran chains covering core. In other words, N_d depends mainly on the volume of a hydrated dextran chain and its conformation. The core size is secondary for N_d, except for extremely small size.

The NMR relaxation power of DM particle depends on the strength of local magnetic field by core and the number of protons influenced by the magnetic field. The field strength is similar for all the samples, because the cores are the same. Thus, the relaxation power for DM-1 to DM-6 is associated with a change in the number of protons. If the relaxation power is related to the whole size of DM particle, it is suggested that the number of protons may decrease with an increase in whole size.

5. Conclusions

The structure of a homologous series of dextran–magnetite complex (DM) in water was studied to reveal the conformation of dextran chains of 1800 to 27 000 in molecular weight. In summary, the main results are as follows.

1. The number of dextran chains binding to a core decreases with an increase in molecular weight. This can be explained by the steric hindrance between dextran chains covering core.
2. The whole size of DM particle increases from 1800 to 9000 in molecular weight but is constant over 9000. This can be explained by the conformation of dextran chains changes from a fully extended state to a random-coiled state with an increase in molecular weight.

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