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Journal of Magnetism and Magnetic Materials 293 (2005) 252–258
Journal of magnetism and magnetic materials
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Investigation of interactions between dendrimer-coated magnetite nanoparticles and bovine serum albumin

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Available online 5 March 2005

Abstract

We investigated the interactions between dendrimer-coated magnetite nanoparticles (MNPs) and the protein serum albumin. The investigation was based on the fluorescence quenching of tryptophan residue of serum albumin after binding with the dendrimer-coated magnetite nanoparticles. The extent of the interactions between bovine serum albumin and dendrimer-coated MNPs strongly depends on their surface groups and pH value.
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PACS: 32.50.+d; 33.50.Dq; 87.68.+z

Keywords: Poly(amidoamine) dendrimer; Magnetite nanoparticles (MNPs); Bovine serum albumin (BSA); Dendrimer; PAMAM; Protein interaction; Surface dependence; pH Dependence

1. Introduction

Biocompatible magnetic fluids (BMFs) are composed of magnetic nanoparticles (MNPs) surface-coated with organic molecules and stabilized as aqueous colloids at neutral pH and salinity of 0.9% NaCl (physiological condition). These colloids have been introduced as a promising material basis for biological, biomedical, and biotechnology applications [1], with emphasis in the treatment of neoplasic diseases. The efficiency of any treatment based on BMFs depends upon the ability of the tumor tissue to accumulate and selectively retain the magnetic-based material. The binding of BMFs to various blood components influences their distribution among normal and tumor tissues, as well as their partition among the various compartments of tumor tissues.

Dendrimers are a new class of polymeric materials. They are globular, highly branched, monodisperse macromolecules. Due to their structure, dendrimers promise to be new, effective biomedical materials as oligonucleotide transfection agents and drug carriers. More information about biological properties of dendrimers is crucial

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doi:10.1016/j.jmmm.2005.02.018

for further investigation of dendrimers in therapeutic applications [2].

As a result of the recent findings, it seems to be crucial to investigate the interaction of dendrimer-coated MNPs with live blood cells and plasma proteins, as well as the immune response of the organism after BMF intravenous administration. In the present work, we investigated whether polyamidoamine dendrimer-coated MNPs might interact with bovine serum albumin (BSA) and change its conformation. Serum albumins are the most abundant proteins in plasma. As the major soluble protein constituents of circulatory system, they have many physiological functions and play a key role in the transport of many endogenous and exogenous ligands. For many drugs binding to serum albumin is a critical determinant of their distribution and pharmacokinetics [3]. BSA possesses a high helical content (about 67%) and molecular weight of 66.4 kDa (calculated from the amino acid composition). BSA consists of 583 amino acids in a single polypeptide chain. It is built from three structurally homologous domains (I, II and III). Each domain is the product of two subdomains (IA, IB, etc.). Serum albumin is postulated to have a heart-shaped structure with dimensions of $8.0 \times 8.0 \times 3.0$ nm [4].

Dendrimer-coated MNPs binding to BSA and other biological macromolecules should be investigated using different spectroscopy techniques. The fluorimetric assays have been chosen due to the high sensitivity of the technique in probing the intrinsic fluorescence of the tryptophan residue from BSA, which is quenched by the binding of dendrimer-coated MNPs to specific sites. This provides a strategy to investigate the interaction between dendrimer-coated MNPs and BSA through the evaluation of specific parameters that clearly describe the binding process, such as the quenching constant, the binding constant and the stoichiometry of the complex [5,6].

2. Materials and methods

2.1. Materials

Essentially fatty-acid-free (fraction V) BSA and 3-aminopropyltrimethoxysilane ($NH_2(CH_2)_3$–Si–$(OCH_3)_3$, APTS) was purchased from Sigma (USA). BSA was used without further purification. PAMAM dendrimers (generation 3.5 and 4.0) were obtained from Aldrich (UK). All other chemicals were of analytical grade. Water used to prepare solutions was double distilled. Chemical condensation reaction in alkaline media has been routinely used to obtain MNPs based on spinel ferrites [7].

2.2. Preparation of magnetite nanoparticles coated with amino-silane

25 ml MNP aqueous suspension (5 wt%) prepared above was diluted to 150 ml by water. The suspension was treated by ultrasonic wave for 30 min. Ten milliliter of APTS was added into it with stirring for 7 h. The result solution was washed with water for 5 times. MNP-APTS represents the magnetite nanoparticle modified only with APTS.

2.3. Preparation of magnetite nanoparticle coated with PAMAM dendrimer

Deionized water (1 ml) was added to 1 ml of the aqueous suspension of magnetite nanoparticle described above (pH 6.5). Fifty microliter of the 2 mg/ml PAMAM dendrimer (generations 3.5) aqueous solution was added. The mixture was immersed in a sonicating water bath at room temperature for 7 h and then washed with water for 5 times by magnetic separation. The dendrimer-coated MNP possesses surface ester groups (MNP-G3.5). After washing, 200 μl ethylenediamine was added to the suspension with stirring for 3 h. The magnetite suspension was then washed for 5 times with water to get dendrimer-coated MNP with amino surface groups (MNP-G4.0).

2.4. Fluorescence measurement of BSA binding to dendrimer-coated MNPs

BSA was dissolved in phosphate-buffered saline (PBS: 150 mmol/l NaCl, 1.9 mmol/l NaH_2PO_4, 8.1 mmol/l Na_2HPO_4, pH 7.4) at a concentration of 5 μmol/l. Fluorescence spectra were taken with a Perkin-Elmer LS-55 spectrofluorometer.

Samples were thermostatted at 25 °C. Excitation wavelength of 295 nm was used to avoid the contribution from tyrosine residues. The emission spectra were recorded from 300 to 440 nm. The excitation and emission slit widths were set to 10 and 3.4 nm, respectively. Samples were contained in 1-cm path length quartz cuvettes and were continuously stirred. Next, increasing concentrations of dendrimer-coated MNP, ranging from 0.25 to 1000 μmol/l, were added to BSA from a stock solution in PBS (0.9 mmol/l) and fluorescence of tryptophan residues was measured. Phosphate buffers with different pH were used to investigate pH-effect on BSA fluorescence in the presence of dendrimer-coated MNPs.

2.5. Instrumentation

TEM pictures were taken with a JEM 100-CXII microscope (JEOL, Japan) at 100 kV. The Zeta potential of dendrimer-coated MNP aqueous suspension was measured by using MALVERN ZETAZIZER 2000 instrument (UK).

3. Results and discussion

3.1. Size and dispersivity study of dendrimer-coated MNP particles by using transmission electron microscopy (TEM)

Fig. 1 shows a detail of the TEM pictures of the MNPs coated with PAMAM dendrimer, from where the particle size polydispersity profile was taken. Fig. 1(d) shows the particle size histogram obtained from the TEM data. A mean particle diameter of $D_m = 8.0 \pm 0.1$ nm and a standard deviation of $\sigma = 0.35 \pm 0.01$ were obtained from Eq. (1) [8]. MNP-APTS particles are seen as dense aggregates, MNP-G3.5 and MNP-G4.0 MNPs show excellent dispersivity and they assembled into clusters with dimension of about 50 nm as shown in Fig. 1(b), (c). The clusters were

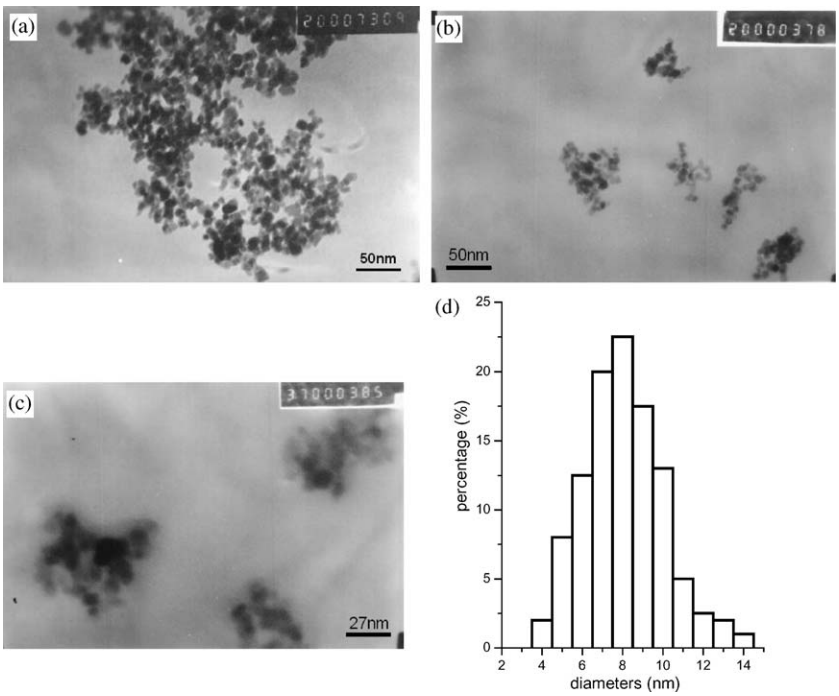

Fig. 1. TEM images of dendrimer-coated MNPs in water. (a) MNP-ATPS; (b) MNP-G3.5; (c) MNP-G4.0; (d) Particle size histogram obtained from TEM data.

surrounded by an outer layer of dendrimer with thickness of about 4 nm as shown in Fig. 1(c), agreeing very well with the G4.0 PAMAM dendrimer diameter of 4.2 nm [2].

$$P(D) = \frac{\exp(-2\sigma^2)}{D_m\sigma(2\pi)^{1/2}} \exp\{-\ln^2(D/D_m)/2\sigma^2\}. \quad (1)$$

3.2. Surface charge investigation of dendrimer-coated MNPs with different surface groups

The negative charge developed in a magnetite nanoparticle is assumed to be due to partially bonded oxygen atoms (M–O–) at the nanoparticle surface [9]. The primary amino groups on the surface of PAMAM dendrimers have a pK of 9.5 [10]. The extent of ionization of amino groups of PAMAM dendrimers was estimated using the Henderson–Hasselbach equation [10]:

$$\mathrm{pH} = \mathrm{pK} + \log\frac{[\mathrm{NH_2}]}{[\mathrm{NH_3^+}]}. \quad (2)$$

At studied pH range (from 5.7 to 8.0) almost all (97–100%) amino groups were positively charged [10]. As demonstrated in Fig. 2, at pH 7.4 and salinity 0.9% NaCl (physiological condition), MNP-G4.0 and MNP-ATPS were positively charged due to their amino surface groups, only MNP-G3.5 bear little charge on their surface.

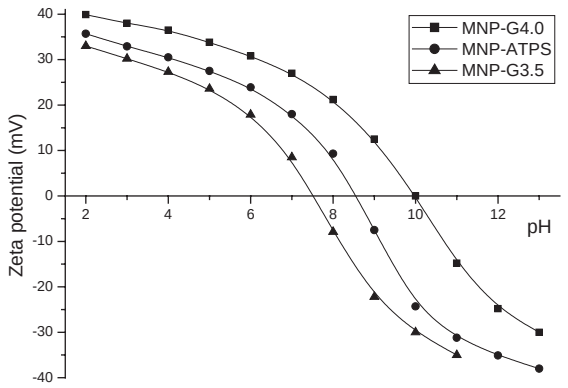

Fig. 2. Zeta potential curves of MNPs coated with ATPS, G3.5 PAMAM dendrimer and G4.0 PAMAM dendrimer in water.

3.3. Interaction of dendrimer-coated MNPs and BSA

The decrease in the fluorescence intensity was the most marked change in the fluorescence spectrum observed upon addition of dendrimers and dendrimer-coated MNPs (Fig. 3). The effect was the strongest for MNP-G4.0, less pronounced for G4.0 PAMAM dendrimer and MNP-G3.5, and the weakest for G3.5 PAMAM dendrimers.

The wavelength of the fluorescence maximum for BSA was at 350 nm, which indicated that the tryptophan residue (Trp134) is in contact with bound water molecules [10]. Commonly used method to study the environment of tryptophans is measuring the shift in the wavelength of emission maximum. The shift in the position of emission maximum corresponds to the changes of the polarity around chromophore molecule. The red shift indicates that tryptophans are, on average, more exposed to the solvent, whereas the blue shift is a consequence of transferring tryptophan residues into a more hydrophobic environment [10]. After adding dendrimer-coated MNPs, the position of emission maximum changed slightly (variations did not exceed 10 nm,

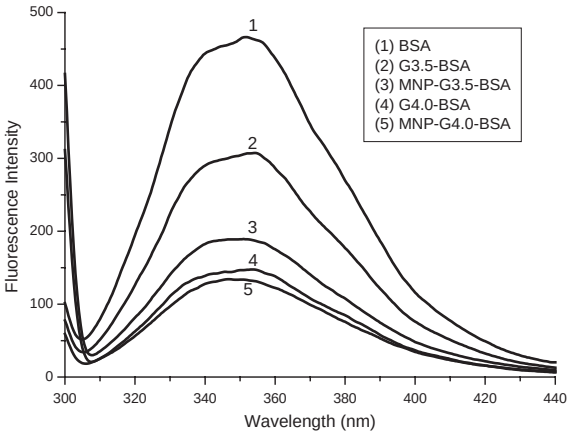

Fig. 3. BSA fluorescence quenching caused by binding with dendrimers and dendrimer-coated MNPs. (1) BSA solution (5 μmol/l), (2) after binding with G3.5 PAMAM dendrimer at a concentration of 20 μmol/l, (3) after binding with MNP-G3.5 at a concentration of 20 μmol/l, (4) after binding with 20 μmol/l G4.0 PAMAM dendrimer, and (5) after binding with 20 μmol/l MNP-G4.0.

Fig. 3, curves 3 and 5). Very little blue shift upon addition of PAMAM dendrimers of generation 4.0 and 3.5 was observed (Fig. 3, curves 2 and 4). Dendrimer-coated MNP alter the position of emission maximum stronger than dendrimers. This shift indicates that the tryptophan residue is placed in a less polar environment [10].

For MNP-G4.0, the increasing concentrations caused a linear reduction in the fluorescence of tryptophan residues (Fig. 4). Analysis of the fluorescence data were performed using the method of Macaroff [5] and Tedesco [6,11]. According to that approach the tryptophan residue fluorescence intensity scales with the dendrimer-coated MNP concentration ([M]) through the following Eq. (3):

$$\frac{F_0 - F}{F - F_s} = \left(\frac{[M]}{K_{diss}}\right)^n. \quad (3)$$

The binding constant K_b is obtained by plotting $\log[(F_0 - F)/(F - F_s)]$ versus $\log[M]$, where F_0 and F_s are the relative fluorescence intensities of the protein alone and the protein saturated with the dendrimer-coated MNPs, respectively. The slope of the double-logarithm plot obtained from the experimental data is the number of equivalent binding sites (n), whereas the value of $\log[M]$ at $\log[(F_0 - F)/(F - F_s)] = 0$ equals to the logarithm of the dissociation constant K_{diss}. The reciprocal of

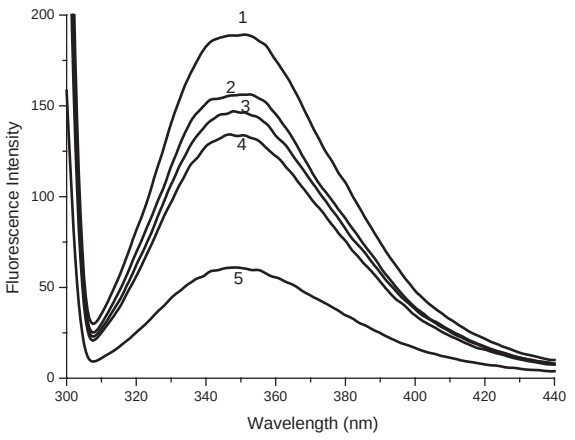

Fig. 4. Effect of MNP-G4.0 concentration on the fluorescence spectra of BSA. The concentration of MNP-G4.0 increases from top to bottom: (1) 5 μmol/l, (2) 22 μmol/l, (3) 50 μmol/l, (4) 84 μmol/l, and (5) 1000 μmol/l.

K_{diss} is the binding constant K_b. The fluorescence intensity values (F) were obtained from the area under the fluorescence spectra, in the range of our investigation (300 to 440 nm).

Fig. 5 represents the plot of $\log[(F_0 - F)/(F - F_s)]$ versus $\log[M]$ for MNP-G4.0, MNP-APTS, and MNP-G3.5. Values of K_b and n obtained from Fig. 5 are shown in Table 1.

The data shown in Table 1 indicates that the MNP-G4.0 and MNP-APTS are more strongly bound to the BSA than MNP-G3.5. MNP-G4.0 is about six times more specific than the MNP-G3.5. It means that more protein molecules per MNP particle are carried out if MNPs are surface-coated with G4.0 PAMAM dendrimer.

Alternatively, the data were analysed by Stern–Volmer equation [10]:

$$F_0/F = 1 + K_{SV}[M], \quad (4)$$

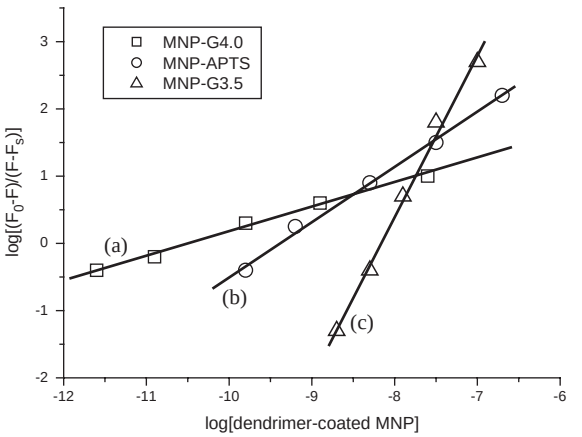

Fig. 5. Double-logarithmic plot of the quenching of BSA fluorescence by dendrimer-coated MNPs: (a) MNP-G4.0, (b) MNP-APTS, and (c) MNP-G3.5.

Table 1
Binding constant (K_b) and number of binding sites (n) of different dendrimer-coated MNP incubated with BSA

Sample	K_b (10^8 M^{-1})	n
MNP-APTS	21.1	0.81
MNP-G3.5	1.36	2.01
MNP-G4.0	483.5	0.31

where F_0 and F are, respectively, BSA fluorescence intensities in the absence and presence of dendrimer-coated MNP, K_{SV} is the Stern–Volmer dynamic quenching constant and $[M]$ is the concentration of the dendrimer-coated MNPs. The equation assumes a linear plot of F_0/F versus $[M]$ and the slope equals to K_{SV} as shown in Fig. 6. The Stern–Volmer constants express chromophore accessibility to the dendrimer-coated MNP [10]. The Stern–Volmer constants for the quenching of tryptophan fluorescence by different dendrimer-coated MNPs are presented in Table 2. K_{SV} were calculated from plots shown in Fig. 6. The strongest quenching of BSA fluorescence was observed for MNP-G4.0 ($K_{SV} = 22.5$).

For MNP-G4.0 the pH dependence was also investigated. The K_{SV} value sharply increased for pH 9.0 (Fig. 7). It can be a result of two facts. First, BSA is an acidic protein. The isoionic point

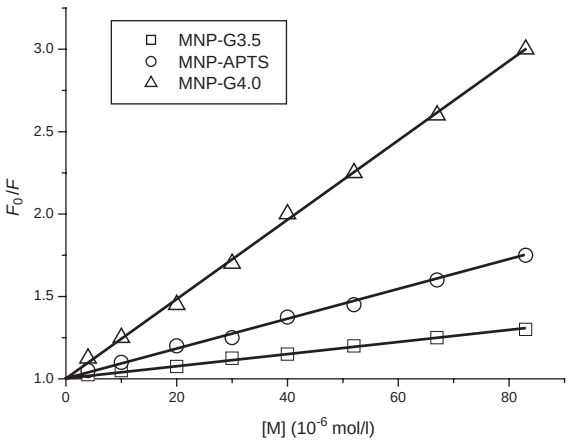

Fig. 6. Stern–Volmer plots for tryptophan fluorescence quenching by dendrimer-coated MNP with different concentration.

Table 2
Stern–Volmer constants of the quenching of tryptophan by dendrimer-coated MNPs

Sample	Stern–Volmer constant K_{SV} [m M^{-1}]
MNP-ATPS	7.51
MNP-G3.5	3.12
MNP-G4.0	22.5

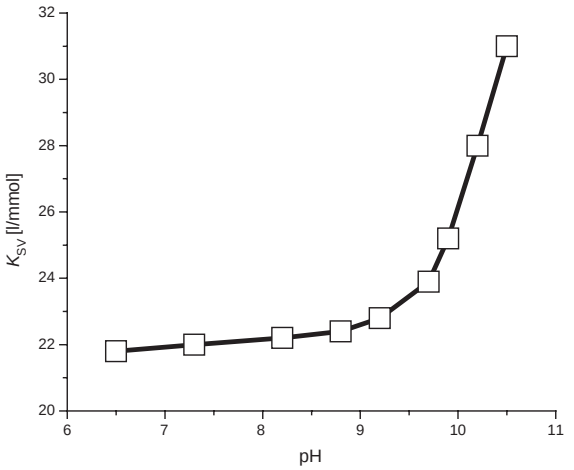

Fig. 7. The effect of pH on the Stern–Volmer dynamic quenching constant by MNP-G4.0.

is about pH 5.2. At this pH, all amino groups are protonated. At pH 9.0, the net charge becomes negative (Fig. 2), thus the electrostatic interactions between dendrimers' amino groups and the protein molecule are enhanced. Second, BSA undergoes expansion above pH 8.0 and at pH 9.0; it changes conformation to the basic form. It is a result of breaking salt bridges between domain I and domain III. It causes stronger ligand binding to subdomain IIB [10]. Therefore, better availability of Trp213 residue to the dendrimer may be involved in enhancing of quenching effect.

The interactions between dendrimer-coated MNPs and BSA are probably of electrostatic nature. That is why they are the weakest for neutral ester-terminated dendrimer coated MNP (MNP-G3.5). At pH 7.4 (the pH of blood), BSA has the negative net charge (−17); therefore, MNP-G4.0 particles with amino-terminated cationic surface have the biggest impact on the protein.

4. Conclusions

In summary, determination of both the binding constant (K_b) and the binding stoichiometry (n) of MNP-ATPS, MNP-G3.5 and MNP-G4.0 to BSA, using the intrinsic fluorescence of the tryptophan residue, has been successfully performed. The

binding constant of MNP-G4.0 is much higher than that of MNP-ATPS and MNP-G3.5. Likewise, the binding stoichiometry of MNP-G4.0 is about six times the values found from MNP-APTS.

Stern–Volmer dynamic quenching constant (K_{SV}) also showed that there are strong interactions between dendrimer-coated MNP and BSA, and the extent of the impact depends strongly on their surface groups. The strength of interactions between protein and dendrimer-coated MNPs depends on the type of dendrimer surface and can be ordered as MNP-G4.0 > MNP-APTS > MNP-G3.5.

We also know that neoplastic tissue are easily associate with proteins and lipoproteins, based on the over expression of surface receptor, allowing us to use this strategy as a way to increase the uptake of specific proteins complex drug against this neoplastic tissue. In conclusion, from the three samples investigated, the dendrimer-coated MNP with amino surface groups would be the most efficient in localizing the serum albumin protein complex with or without drug associated to them in a specific site as far as the external magnetic field effect is concerned.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 30471599) and Shanghai Development Foundation of Science and Technology (No. 03ZR14057).

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