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Nanoparticle-Target Interactions Parallel Antibody-Protein Interactions

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Abstract

Magnetic particles can act as magnetic relaxation switches (MRSw's) when they bind to target analytes, and switch between their dispersed and aggregated states resulting in changes in the spin-spin relaxation time (T_2) of their surrounding water protons. Both nanoparticles (NPs, 10-100 nm) and micron-sized particles (MPs) have been employed as MRSw's, to sense drugs, metabolites, oligonucleotides, proteins, bacteria and mammalian cells. To better understand how NPs or MPs interact with targets, we employed as a molecular recognition system the reaction between the Tag peptide of the influenza virus hemagglutinin and a monoclonal antibody to that peptide (anti-Tag). To obtain targets of different size and valency, we attached the Tag peptide to BSA (Mw= 65000 Daltons, diameter = 8 nm) and to Latex spheres (diameter = 900 nm). To obtain magnetic probes of very different sizes, anti-Tag was conjugated to 40 nm NPs and 1 μ m MPs. MP and NP probes reacted with Tag peptide targets in a manner similar to antibody/antigen reactions in solution, exhibiting so-called prozone effects. MPs detected all types of targets with higher sensitivity than NPs with targets of higher valency being better detected than those of lower valency. The Tag/anti-tag recognition system can be used to synthesize combinations of molecular targets and magnetic probes, to more fully understand the aggregation reaction that occurs when probes bind targets in solution and the ensuing changes in water relaxation times that result.

INTRODUCTION

Magnetic nanoparticles in the size range of 10 to 100 nm (NPs) and micron-sized magnetic particles (MPs) act as magnetic relaxation switches (MRSw's) when they bind to molecular targets and switch between their dispersed and aggregated states with changes in the spin-spin relaxation time (T_2) of water protons. Although both NPs and MPs can be used as MRSw's and induce changes in T_2 upon aggregation, those changes are in opposite directions. With NP based MRSw assays, target induced NP aggregation causes a T_2 decrease (type I MRSw assay) while with MP based assays MP aggregation causes a T_2 increase (type II MRSw assay). The physical basis for this different behavior of NPs and MPs upon aggregation has been explained.¹ Briefly, magnetic spheres of increasing size (increasing magnetic moments) produce larger magnetic field inhomogeneities that are more effective at dephasing the spins of water protons which diffuse through them. Hence T_2 decreases as magnetic NPs aggregate. However,

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SUPPORTING INFORMATION AVAILABLE

Tables of MRSw type I and type II sensitivities reported in the literature and TEM images of aggregates of anti Tag-NP with a multivalent particle target, Latex-(Tag)_{5,300}. This material is available free of charge via the Internet at <http://pubs.acs.org>.

eventually magnetic spheres become so large, and so few in number at a given iron concentration, that many water protons fail to “experience” a magnetic field inhomogeneity. In this diffusion-limited case, T_2 increases as the size of NP aggregates increases. This diffusion-limited case applies when MPs are induced to aggregate. Precipitation was not observed in our experiments, as evidenced by the highly reproducible T_2 values we obtained throughout these studies. See also References 2 and 9. MRSw based assays can detect widely different types of target analytes, ranging from small analytes such as calcium ions³, oligonucleotides⁴ and antibodies⁵ to large analytes such as viruses⁶ and bacteria^{7, 8}. However, interpreting the MRSw literature is complicated by the facts that there are several types of MRSw assays, two of which are discussed here, and many different molecular recognition systems. Many reports use a specific antibody/antigen molecular recognition system, a specific magnetic particle probe, and detect a specific analyte, making it difficult to ascertain the general features of reactions between magnetic probes and target analytes from literature studies.

Here we report the behavior of NP-based type I and MP-based type II MRSw assay systems when they bind to synthesized molecular targets of different valency and size. To obtain targets of different size and valency, while maintaining the same molecular recognition system, we attached the Tag peptide from hemagglutinin of influenza virus to two substrates, BSA (diameter = 8 nm) and Latex beads (diameter = 900 nm). Tag peptide was attached to BSA at two levels or valencies, giving a total of three types of targets. We also attached the anti-Tag IgG to NPs and MPs to obtain magnetic probes of different sizes, whose physical properties have been described in detail elsewhere.⁹ By synthesizing molecular targets, we were able to study the interaction of two magnetic probes with three types of targets, all employing the same Tag/anti-Tag molecular recognition system.

EXPERIMENTAL METHODS

General Information

Particle size distribution was determined by dynamic light scattering (DLS) using Zetasizer (Malvern, Southborough, MA). T_2 was measured by relaxometry (0.47 T Minispec mq20; Bruker, Billerica, MA). BSA was purchased from Sigma and 0.9 μ m aminated Latex from Bangs Laboratories. Streptavidin coated MP, MyOne-SAv, was purchased from Invitrogen and LC-(+)-Biotin hydrazide from Molecular Biosciences. Zeba Spin Columns and sodium periodate were obtained from Pierce and from Sigma respectively. TEM images were collected on a JEOL JEM-2011 electron microscope operated at an accelerating voltage of 200 kV.

Target Analyte Design

Activation of carriers was conducted by reacting BSA with sulfo-succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) or aminated Latex with *N*-succinimidyl 3-(2-pyridyldithio)-propionate (SPDP). The Tag peptide (YPYDVDPYAK(FI)GGC) was conjugated to activated BSA or to Latex beads as described previously.⁹ Attached peptides were quantitated by absorbance.¹⁰

Magnetic Probe Synthesis

Anti Tag IgG (Millipore, MA) was conjugated to amino cross-linked dextran caged iron oxide nanoparticles (amino NPs, amino-CLIO) by the reaction between sulfhydryl group-induced IgG and sulfo-SMCC activated amino NPs as reported previously.¹¹ After purification through size exclusion chromatography (Sephacryl 300 column), the average number of attached IgG per NP was quantitated by iron concentration assay and by BCA protein assay (Pierce).¹¹

To attach anti Tag IgG onto the MP (MyOne-SAv), anti Tag IgG was first biotinylated.¹² Anti Tag IgG was oxidized with 10 mM NaIO₄ solution in sodium acetate buffer (0.1 M, pH 5.5)

and purified by using a Zeba spin column. The oxidized anti Tag IgG was added with 0.1 volume of 10× PBS and LC-biotin-hydrazide in DMSO (room temperature, 2 h). Biotinylated anti Tag IgG was purified with Zeba spin columns and stored at 4 °C. After washing twice with PBS following magnetic separation and removal of the supernatant, MyOne-SAv in 100 μL were suspended in 400 μL PBS and mixed with the biotinylated anti Tag IgG (room temperature, 4–6 h). Following magnetic separation and washing in PBS with 0.1 % Tween 20, unreacted SAv binding sites on the surface of MyOne-SAv was blocked with 20 μM biotin-EG3-NH₂ (3 h). The final product was washed and stored in PBS-BT (PBS with 0.1% BSA and 0.1% Tween, pH 7.4) until further usage.

MRSw Assays

Anti Tag-NP probes were incubated with BSA-(Tag)_n (n=2 or 6), or Latex-(Tag)_{5,300} (~2 h, room temperature) prior to T₂ measurement. A magnetic field enhancement strategy was applied to anti Tag-MP containing samples.⁹ Anti Tag-MP probes were incubated with BSA-(Tag)_n (n=2 or 6) or Latex-(Tag)_{5,300} (20 min, room temperature), then magnetically aggregated in the magnetic field of 0.47 T in the relaxometer (10 min, 40°C), and removed from the relaxometer and allowed to disaggregate (10 min, 40°C), and placed again in the relaxometer for 30 sec to obtain a T₂ measurement. A ΔT₂ was obtained as the difference in T₂ between samples with and without an appropriate molecular target. The transient exposure to a magnetic field amplifies molecular target induced binding between MPs as we⁹ and others¹³ have observed.

RESULTS AND DISCUSSION

Figure 1A summarizes the sizes and valences of the three target analytes and two magnetic probes, NPs and MPs, we employed. Figures 1B and 1C illustrate the interactions between different magnetic probes and different target analytes.

We first examined the response of NP-based type I MRSw to increasing concentrations of target analytes of different valencies or sizes as shown in Figure 2A. The changes in T₂ values as a function of target concentration were obtained and fitted to a four-parameter equation, with EC₅₀ values and projected sensitivity concentrations (PSC) provided in Table 1. PSC was defined as the target concentration with 5 ms of T₂ difference from the control T₂ value and derived from the curve parameters.⁹ The divalent target, BSA-(Tag)₂, provided EC₅₀ value of 9.1 nM, while the hexavalent target BSA-(Tag)₆ showed a five fold decrease in the EC₅₀ value (1.7 nM). However, when the target was the larger and more multivalent Latex-(Tag)_{5,300}, EC₅₀ and PSC were reduced by at least three orders of magnitude. (Table 1) Thus in using a single molecular recognition system (variable Tag target analytes, but constant anti Tag NP probes), the sensitivity of NP-based MRSw type I assays was highly dependent on target size and valency. These results are consistent with various literature reports where target size and valency were varied along with molecular recognition systems. These results indicated that type I MRSw assays were able to detect viruses and bacteria with high sensitivities^{5,7,8} but have lower sensitivities with other types of targets^{2, 4, 5, 11}. A more extensive review of the literature on this topic is provided in Table S-1.

We then examined the response of MP-based type II MRSw assays to increasing concentrations of the same target analytes (Figure 2B), using the exposure to 0.47 T magnetic field of the relaxometer to enhance sensitivity of MP based probes as described.⁹ Again EC₅₀'s and PSC's are provided in Table 1. Type II assays had lower EC₅₀'s and lower PSC's with all target analytes, with the difference between Type I and Type II assays being greater with small, low valency targets.

We next examined the reaction of Type I NPs with BSA-(Tag)₆ over a broad concentration range, both as the T₂ obtained and size of particles present using dynamic light scattering (Figure 3). As the target concentration increased, T₂ first decreased and then increased, which can be explained by the so-called prozone effect¹¹ where the excessive presence of target analytes saturates the binding sites of the NP probes and hampers aggregation of the NPs. As it is done with antibody-protein reactions, we term the point of the greatest T₂ decrease and size increase as the equivalence point¹⁴⁻¹⁶. Size measurements indicated that particle size increased as T₂ decreased with the maximum size occurring at the T₂ minimum. Similar behavior was obtained when Latex-(Tag)_{5,300} replaced BSA-(Tag)₆ (Figure 4). However, as shown in Figure 4, aggregate size continually increases as target concentration increases, which did not occur in Figure 3. The explanation for the resulting biphasic change in T₂ seen with Figure 4 lies with biphasic effect of the size of magnetic particles on T₂ explained in the Introduction. As the target size increased from BSA-(Tag)₆ to Latex-(Tag)_{5,300}, the ratio of target analytes to NP probes at the equivalence point decreased from 0.54 (= 9.1 nM BSA-(Tag)₆) to 0.00005 (= 0.98 pM Latex-(Tag)_{5,300}). The molar concentration of target analytes is defined as that of BSA or Latex, not of the Tag peptide attached to them.

We then repeated the experiments of Figures 3 and 4 with Type II MPs as shown in Figures 5 and 6. When Tag-MPs reacted with the increasing concentration of targets, a maximum T₂ was obtained with the largest size of aggregates. Indeed around the equivalence points the size of particle aggregates was too large to be determined by DLS. As in the case with Type I NPs, the ratio of target analytes to MP probes at the equivalence point was decreased from 5,500 (= 0.30 nM BSA-(Tag)₆) to 5.5 (= 0.42 pM Latex-(Tag)_{5,300}) when the size of the target increased from BSA-(Tag)₆ to Latex-(Tag)_{5,300}. Saturation of binding sites led to decreased T₂ values and smaller aggregates at the region of target excess compared with those at equivalence.

With the combinations of magnetic probes and targets employed (Figures, 3-6), increasing concentrations of targets produced a maximum T₂ change, or equivalence point, which decreased with further increases in target concentration. The equivalence point corresponded to the maximal size aggregates formed by reaction between probes and targets. A comparison of the detection of molecular targets by anti Tag MP and anti Tag NP probes (Table 1) indicated both probes detected more highly multivalent target analytes far more effectively than low valency analytes. In addition, target detection with MPs was superior to NPs with all types of molecular targets.

Here we compared two magnetic probes, the anti Tag NP and the anti Tag MP, that had similar Fe based R₂'s and but very different numbers of Fe atoms per particle. The NP had an R₂ of 50 mM⁻¹sec⁻¹, with 8000 Fe's per particle, while the MP had an R₂ of 43 mM⁻¹sec⁻¹, with 2.8 × 10⁹ Fe's per particle.¹ The effects of many variables in magnetic particle design on their performance as probes with MRSw's, including magnetization per mole metal and relaxivity per mole metal, were not varied with the current study and will be the subject of future publications.

CONCLUSIONS

The reactions between magnetic particles and targets were similar to the reactions between antibodies and multivalent proteins, with points of maximum complex formation or equivalence points obtained as the concentration of targets was increased. Type II MP based assays were more sensitive than Type I NP based assays with all types of targets. Both types of MRSw assays showed enhanced sensitivities as the valency or the target analyte was increased. The Tag/anti Tag recognition system was used to synthesize both antibody based magnetic probes and peptide antigen based molecular targets. This enables the study of their

aggregation reaction between different types of probes and targets in solution and determination of the associated changes in proton relaxation times.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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(A)

	target analytes			magnetic probes	
	BSA-(Tag) ₂	BSA-(Tag) ₆	Latex-(Tag) _{5,300}	anti Tag-NP	anti Tag-MP
size (nm)	8	8	900	70	1,000
valency	1.8	5.6	5,300	2.6	> 1,000

(B) NP Based Type I

(C) MP Based Type II

Figure 1. Design of materials and reaction schemes of type I NP based and type II MP based MRSw's
 (A) Design of target analytes and magnetic probes. Three target analytes were prepared by attaching the Tag peptide to bovine serum albumin (BSA) or Latex. Magnetic probes were synthesized by attachment of the anti Tag antibody to NPs or MPs. (B) Scheme of NP-based MRSw type I assays. Anti Tag-NPs were reacted with three different types of target analytes to form NP aggregates that led to T_2 decreases. (C) Scheme of MP-based MRSw type II assays. Anti Tag-MPs were reacted with three different types of target analytes respectively to form MP aggregates that caused T_2 increases.

(A) NP Based Type I

(B) MP Based Type II

Figure 2. Results of NP-based type I and MP-based type II MRSw assays
Normalized T₂ difference (%ΔT₂) was shown as a function of the concentration of target analytes. □: BSA-(Tag)₂, △: BSA-(Tag)₆, ○: Latex-(Tag)_{5,300}

Figure 3. Equivalence principle for NP based Type I MRSw assays reacting with a multivalent protein target BSA-(Tag)₆ at different target to probe ratios

(A) T_2 values decreased as the number of targets per NP probe increased with the NP probe concentration constant. By analogy to standard antibody-antigen reactions, three types of regions of reaction were obtained. These were probe excess, equivalence, and target excess. The dotted line is the T_2 without target present. (B) Size of particles present by DLS at each of the three regions in (A). (C) Schematic diagram of each of the three regions in (A). T_2 decrease was highest at equivalence where the aggregate size was maximal. Saturation of NP binding sites with excessive amount of targets led to smaller aggregates, which shows so-called the prozone effect.

Figure 4. Equivalence principle of NP based type I MRSw assays for a multivalent particle target, Latex-(Tag)_{5,300}, at different target to probe ratios

(A) T_2 decreased as the number of the targets per NP probe increased with the NP probe concentration constant. Three regions of reaction were obtained which were probe excess, equivalence, and target excess. The dotted line is the T_2 without target present. (B) Size distribution of particles present by DLS at each of the three regions in (A). (C) Schematic diagram of each of the three regions in (A). See Figure S-1 for TEM images. T_2 values in the target excess region showed MP based Type II MRSw-like behavior and increased above the control T_2 as the ratio of target to probe was increased, because NP bound Latex likely acted as pseudo-MP.

Figure 5. Equivalence principle of MP based Type II MRSw assays for a multivalent protein target BSA-(Tag)₆ at different target to probe ratios

(A) T_2 increased as the number of the targets per MP probe increased with the MP probe concentration constant. Three types of regions of reaction were obtained which were probe excess, equivalence, and target excess. The dotted line is the T_2 without target present. (B) Size distribution of particles present by DLS at each of the three regions in (A). (C) Schematic diagram of each of the three regions in (A). Excessive presence of targets showed the prozone effect where saturated MP binding sites hampered aggregation of MP and led to decreased T_2 compared with that at equivalence.

Figure 6. MP based type II MRSw assays reacting with the large multivalent particle target, Latex-(Tag)_{5,300}, at different target to probe ratios

(A) T_2 values increased as the number of the targets per MP probe increased with the MP probe concentration constant. Three regions of reaction were obtained which were probe excess, equivalence, and target excess. The dotted line is the T_2 without target present. (B) Size distribution of particles present by DLS at each of the three regions in (A). (C) Schematic diagram of each of the three regions in (A). The excessive amount of Latex targets in the target excess region led to smaller aggregates and decreased T_2 compared with those at equivalence. Increased amount of unbound Latex in the target excess region is shown at the size range below 1 μ m by DLS.

Table 1
EC50 and sensitivities of type I and type II MRSw as a function of size and valency of target analytes.

MRSw	Target	Target size (nm)	Target valency	EC50	PSC*
Type I (NP)	BSA-Tag ₂	8	2	9.1 nM	35 nM
	BSA-Tag ₆	8	6	1.7 nM	1.2 nM
	Latex-Tag _{5,300}	900	5,300	0.26 pM	87 fM
Type II (MP)	BSA-Tag ₂	8	2	0.83 nM	56 pM
	BSA-Tag ₆	8	6	17 pM	4 pM
	Latex-Tag _{5,300}	900	5,300	84 fM	1.2 fM

* PSC: projected sensitivity concentration