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Assembly system of direct modified superparamagnetic iron oxide nanoparticles for target-specific MRI contrast agents

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ABSTRACT

We report the direct modification of SPIOs with a biomolecule and the target-specific assembly system of these modified SPIOs for using MRI contrast agents. The transverse relaxation rate of the aqueous solutions containing the modified SPIOs was altered by the dispersion state.

Since magnetic resonance imaging (MRI) provides noninvasive, three-dimensional examination of biological events in living organisms, it is one of powerful diagnostic tools in modern clinical medicine. Recent researches have been paid attentions to the development of MRI contrast agents not only for image enhancement but also for monitoring bioreactions or environmental changes in vivo.[1] Superparamagnetic iron oxide nanoparticles (SPIOs) are attractive materials for the stimuli-responsive MRI contrast agents, because their magnetism can be modulated by the dispersion state, and significant studies including gene expression, cancer imaging, and cellular trafficking have been performed.[2]

Direct modification with small molecules on the naked SPIOs has several inherent merits. The thin surface layer can provide a greater response to the applied magnetic field with SPIOs and increase the cell permeability.[3] These properties are crucially beneficial for improving the sensitivity and the selectivity of SPIOs-based MRI contrast agents. Previous works have reported the assembling system of SPIOs using biotin–avidin, antigen–antibody, and aptamer–small molecule interactions, however, very few were reported that the small molecules linked to SPIOs can work as the mediator for the formation of the cluster.[4] In addition, for enhancing the ability of SPIOs as a molecular imaging probe, it has been still required to sophisticate the surface modification of SPIOs without loss of the affinity and the specificity.

Herein, we describe the direct modification of SPIOs with a biomolecule and the target-selective assembly systems of the modified SPIOs for the application to the highly sensitive MRI biosensors. Biotin, hemin, and phosphorylated DNA can be immobilized onto the SPIOs via the hydrogen bonding with carboxylic acid or phosphorylic acid groups, and the modified SPIOs can form clusters in the presence of the small amount of the target. In addition, the measurements of the proton transverse (T2) relaxation rate of the aqueous mixtures containing the modified SPIOs revealed that the assembly of the modified SPIOs had an ability to enhance the acceleration of the T2 relaxation of water tissue and to show a darker contrast in MR images. As a result, the sensitivity can be improved at least 10-fold higher than those of previous works.

We prepared the modified SPIOs via the ligand exchange method from citric acid because the small volume of the total shape could hardly disturb the biomolecule–target interaction for assembling.[5,6] The standard procedure was according to Ref. 5 Aqueous solution of the SPIOs (100 μg/mL) was sonicated in the presence of 1 μM biotin, hemin, and 5′-phosphorylated DNA (**pDNA**, 5′-pCCT GCG TGT TAG AAC CGT-3′) for 1 min at 25 °C. In the case of hemin and **pDNA** modification, from the UV absorption measurements of the supernatant of the mixture solution, it was confirmed that these molecules were quantitatively absorbed on the SPIOs (K_d = 0.12 and 1.19 nM, respectively). The maximum number of hemin or **pDNA** on the single particle was evaluated as 1.0×10^4 and 1.0×10^2, respectively.[7] Mono-dispersed SPIOs were obtained

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after washing, and it was confirmed from the measurements of dynamic light scattering (DLS) that the modified SPIOs before and after ligand exchanging could maintain dispersed states at least for 7 days in water.

Scheme 1 outlines the assembly system of the modified SPIOs in this study. Initially, we executed the cluster formation with biotin–avidin interaction potential for a protein sensor. Figure 1a shows the result.[8] The average number of biotin molecules on the SPIOs was calculated from stoichiometry. The precipitation was formed immediately after adding avidin to the solution containing the biotin-tethered SPIOs at 25 °C. From the DLS measurements, the diameter of the biotin-tethered SPIOs (19.5 ± 5.2 nm) was increased to be 51.2 ± 22.4 nm by the addition of the 2000-times diluted avidin solution. Consequently, this system represents the highly sensitive protein sensor by the naked eyes with the minimum detection limit of 10 fmol of avidin with 5 mg/mL of the biotin-tethered SPIOs.

The aptamer DNA (5′-GTG GGT CAT TGT GGG TGG GTG TGG AAA AAT ACA ATA TAA CGC TAG GC-3′) consisted of three parts. The 24 bases from the 5′-end showed high affinity with hemin (K_d = 0.15 μM) in 50 mM sodium acetate buffer (pH 6.0) containing 10 mM KCl was connected to the 18 bases from the 3′-end as the complementary of the target sequence with the five bases of A runs as the linker.[9] The complex of hemin-tethered SPIOs with the aptamer DNA can be expected to form the cluster mediated by the target DNA (5′-GCC TAG CGT TAT ATT GTA AAA AAA GCC TAG CGT TAT ATT GTA AAA AAA GCC TAG CGT TAT ATT GTA AAA AAA GCC TAG CGT TAT ATT GTA-3′) possessed four tandem 18 bases of a specific sequence. Figure 1b is the summary of the result. The hemin-tethered SPIOs (5 mg/mL) were dispersed and added to 200 nM the aptamer DNA in 50 mM sodium acetate buffer (pH 6.0) containing 10 mM KCl at 25 °C. The cluster of the SPIOs was obtained only from the sample containing both the aptamer DNA and the target sequence after 15 min standing. On the other hand, slight precipitation was obtained from the negative controls. These data indicate that the cluster of the SPIOs should be formed via the hemin–aptamer interaction without non-specific absorption of phosphodiester backbone in DNA onto the SPIOs.

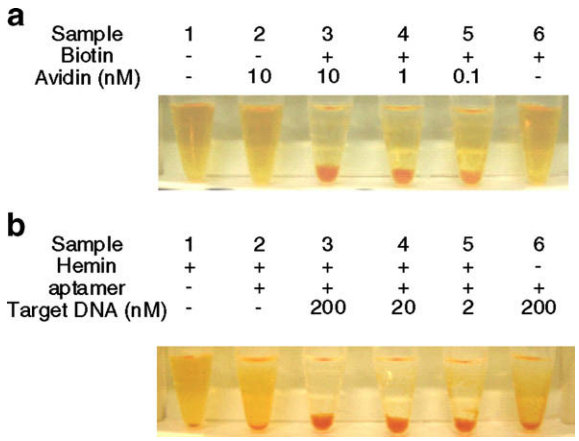
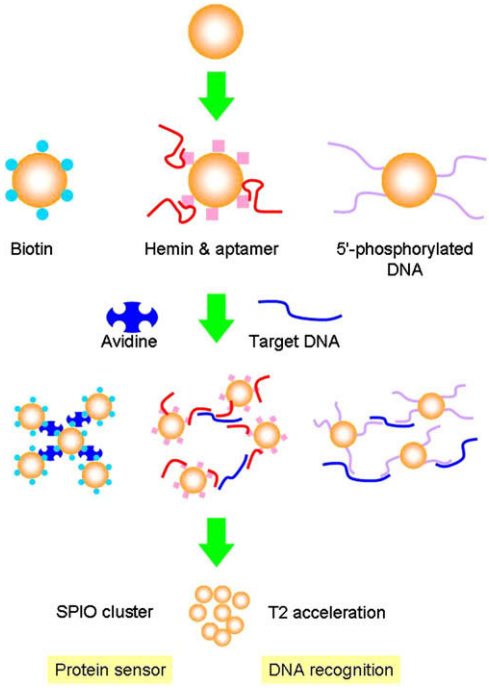


Figure 1. The cluster formation of (a) the biotin-tethered and (b) the hemin–aptamer DNA complex-modified SPIOs by the addition of the target molecules in 50 mM sodium acetate buffer (pH 6.0) containing 10 mM KCl at 25 °C. All images were taken after 15 min standing.

From the measurements of the dissociation constants of 5′-hydroxy terminal, 5′-carboxyl terminal, and **pDNA** to the SPIOs, it was revealed that the phosphate monoester group at the 5′-end has higher affinity to the surface of the SPIOs.[10] We immobilized **pDNA** onto the SPIOs with the same manner for the sequence-specific cluster formation and the application to the NMR probes. Figure 2a shows the cluster formation of the **pDNA**-tethered SPIOs in the presence of the target consisting of the tandem repeat of the complementary sequence. Since the precipitation was significantly less observed even in the sample containing not-complementary tandem sequence, the interaction between the SPIOs and the phoshodiester backbone in DNA could be ignored. In addition, the redispersion was accomplished with the enzymatic digestion of alkaline phosphatase.[11] These results represent that the responsiveness to bioreactions could be attached to the SPIOs.



Scheme 1.

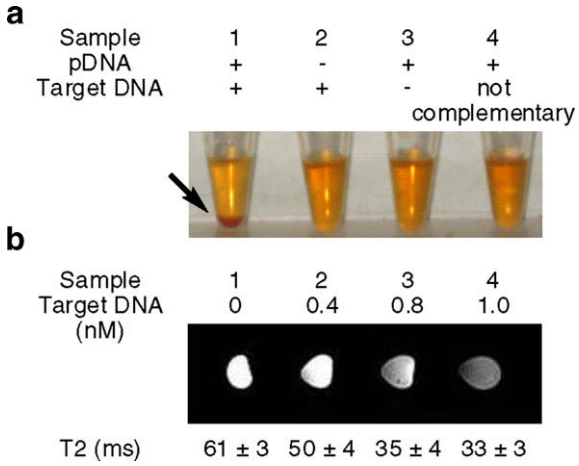


Figure 2. The cluster formation of the **pDNA**-tethered SPIOs in 50 mM sodium acetate buffer (pH 6.0) containing 10 mM KCl at 25 °C. (a) Images were taken after 15 min standing. (b) All samples contained 1.1 μg/mL of the modified SPIOs were added to the target DNA and sealed into 5-mm glass tubes. *T*2-weighted coronal MRI image (7 T, spin–echo sequence: repetition time TR = 3000 ms, echo time TE = 100 ms) of the various concentrated samples and their relaxation times. The *T*2 values were calculated from the average of three sets of independent experiments, and error values represent standard deviation.

The magnetic properties of dispersed SPIOs are significantly different from those in clusters and can be detected by MRI.[4,12] As compared to disperse SPIOs, the clusters are more effective in decreasing the *T*2 relaxation time, and therefore give rise to a darker *T*2-weighted MR image. Figure 2b shows the *T*2-weighted MR image of the samples containing 1.1 μg/mL the **pDNA**-tethered SPIOs at 7 T at 25 °C. We measured the *T*2 rate of each solution for evaluating the detection limit for the target DNA. The significant decrement of the *T*2 value appeared from the sample containing 0.4 nM the target DNA (40 fmol). The MR image showed the darker effect at the modified SPIOs-containing samples, corresponding to the decrement of the *T*2 values. These data suggest that the sequence-specific NMR probe has been realized, and the sensitivity (0.1 μg/mL) can be improved approximately 10-fold higher than that of previous system reported as a highly sensitive MRI sensor.[13]

In conclusion, we report the direct modification of SPIOs with a biomolecule and the target-selective assembly systems using the modified SPIOs. The direct modified SPIOs can acquire significant higher sensitivity as the protein sensor, or the target-specific MRI contrast agents. Thus, we can show here that the direct modification should be effective strategy to sophisticating the ability of the SPIOs. Our findings can be expected to be potential applications for cell cycle monitoring or in vivo imaging using MRI.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.035.

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