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Characterization of a magnetic carrier encapsulating europium and ferrite nanoparticles for biomolecular recognition and imaging

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ABSTRACT

In this study, FG beads (ferrite nanoparticles in the core covered with poly-(styrene-*co*-glycidyl methacrylate)) were made into fluorescent magnetic carriers (FMCs) containing the fluorescent substance, europium ion (Eu^{3+}) complex. The developed FMCs showed several notable features such as high fluorescence intensity and high dispersibility in water. More importantly, FMCs did not leak Eu^{3+} complex. It is expected that the FMCs will be a useful tool for biomolecular recognition and imaging and contribute to advancement of a wide range of research fields, including cell biology and molecular imaging.

1. Introduction

Since its emergence in 1955, cell imaging technology which can visualize localized target molecules with fluorescence has been performed widely [1]. This technology has made it possible to visualize and identify localized target molecules in living cells. Notably, fusion proteins with green fluorescent protein (GFP) have been utilized frequently for visualization of the cell [2]. In biological studies, it is essential to analyze the function of biomolecules and to clarify their precise mechanisms of action [3–7]. However, it takes time to elucidate biological phenomena, because separate analyses of functions are performed and target molecules are identified independently. Thus, it is necessary to develop new technologies to make it possible to separate and analyze the target molecules at the same time.

We have successfully developed functional magnetic carriers (called FG beads, which includes ferrite nanoparticles in the core covered with poly-(styrene-*co*-glycidyl methacrylate)) for affinity purification, which show a practical response to magnetic fields [8,9]. In preparation of FG beads, we could achieve encapsulation of several 40 nm ferrite particles into the polymer matrix despite their strong tendency for magnetic self-aggregation. The hydrodynamic diameter of FG beads was about 200 nm and they were stable and dispersible in a wide range of organic solvents without aggregation and deconstruction [9]. These remarkable features enabled us to immobilize water-insoluble compounds onto FG beads under non-aqueous condition. Notably, FG beads showed extremely high performance in affinity purification such that the targets toward drugs without non-specific binding of proteins could be isolated with high recovery and purity, compared to commercially available carriers [9]. Therefore, we expected that FG beads would be suitable as fluorescently labeled magnetic carriers for the visualization of target molecules.

In this study, we developed novel poly(styrene-*co*-glycidyl methacrylate) magnetic carriers encapsulating Eu^{3+} complex for biomolecular recognition and imaging. We selected europium iron (Eu^{3+}) complex for a fluorescent substance which have maximal excitation wavelength at ultraviolet region and maximal fluorescence wavelength at 615 nm, because FG beads had absorbance region at 400–600 nm of wavelength derived from ferrite nanoparticles and absorbance region of the FG beads did not crossover the excitation and fluorescent regions of the Eu^{3+} complex. Various organic solvents having reversible swelling effects on the polymer matrix were studied in order to evaluate the encapsulation efficiency of Eu^{3+} complexes into FG beads.

2. Experimental procedures

2.1. Materials

Acetone, *N*,*N*-dimethylformamide (DMF), 1,4-dioxane, ethanol, and dimethylsulfoxide (DMSO) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Europium acetate was purchased from

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SIGMA-Aldrich Corporation (St. Louis, USA). Thenoyltrifluoroacetone (TTA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and trioctyl phosphine oxide (TOPO) was purchased from DOJINDO LABORATORIES (Kumamoto, Japan).

2.2. Preparation of Eu^{3+} complexes ($Eu(TTA)_3(TOPO)_3$)

Eu^{3+} complexes were prepared following a previously reported procedure [10]. A total of 0.2 g of europium acetate and 0.758 g of TTA were dissolved in 50 mL of distilled water. The solution was adjusted to pH 5.5 by the addition of 1.0 M aqueous sodium hydroxide (NaOH) and stirred at 50 °C for 24 h in the absence of light. The obtained product, $Eu(TTA)_3$, was filtrated and dried at 70 °C. $Eu(TTA)_3(TOPO)_3$ was obtained by mixing with 77.3 mg of TOPO and 87.0 mg of $Eu(TTA)^3$ in 41 ml of acetone.

2.3. Preparation and characterization of fluorescent magnetic carriers (FMCs)

Functional magnetic carriers (FG beads) were prepared through admicellar polymerization [9]. Fluorescent magnetic carriers were prepared by encapsulating Eu^{3+} complexes into FG beads. The solvent used to prepare the FG beads which was exchanged from distilled water to organic solvents of acetone, DMF, 1,4-dioxane, ethanol, and DMSO by repeating collection and washing of beads and integrating into organic solvents. The diameter of FG beads in various organic solvents was determined by the dynamic light scattering/photon correlation spectroscopy (PCS), using FPAR-1000 (Otsuka Electronics Co. Ltd.). In the resulting swollen state, FG beads with the maximal diameter were selected for the encapsulation of Eu^{3+} complexes. The obtained FMC's were observed by the transmission electron microscopy (TEM) on the H-7500 (Hitachi High Technology Corp.) with a beam voltage of 100 kV. The fluorescent intensity was measured by LS-55 (Perkin Elmer Inc.). The supernatant of FMCs' suspension in water was obtained by magnetic separation and centrifugation.

2.4. Preparation of aminated FMCs

Aminated FMCs were prepared as described previously [9]. FMCs were suspended in 3.0 M NH_4OH, incubated at 70 °C for 24 h and dialyzed against distilled water. Aminated FMCs served as the positive control for the cationic carrier which exhibited a large amount of non-specific protein binding [11].

2.5. Verification of non-specific protein binding to FMCs

A total of 0.25 mg of FG beads, FMCs, and aminated FMCs were suspended in a binding medium (20 mM HEPES–NaOH [pH 7.9], 0.1% NP-40, 10% glycerol, 1.0 M (KCl), 0.2 mM EDTA, 1.0 mM DTT, and 0.2 mM PMSF). Each carrier was incubated with 500 µl of crude cellular extract (1.0 mg/ml of protein) that was obtained from the HeLa cytoplasmic fraction and equilibrated to the binding medium [12] at 4 °C for 4 h with occasional agitation. After washing with 200 µl of binding medium three times, the carriers were treated with 30 µl of high-salinity medium (20 mM HEPES–NaOH [pH 7.9], 0.1% NP-40, 10% glycerol, 1.0 M KCl, 0.2 mM EDTA, 1.0 mM DTT, 0.2 mM PMSF). Bound proteins were eluted with SDS-PAGE loading dye (50 mM Tris–HCl [pH 6.8], 125 mM 2-mercaptoethanol, 2% SDS, and 0.025% bromophenol blue, 10% glycerol) and subjected to SDS-PAGE, followed by silver staining.

3. Results and discussion

If fluorescence is immobilized on the surface of carriers, non-specifically protein adsorption and aggregation of the beads would occur. Therefore, the encapsulation of a fluorescent substance into FG beads was attempted. In order to encapsulate the fluorescent substance into FG beads efficiently, the swelling properties of the beads from the addition of various organic solvents were considered. First, the size of FG beads in organic solvents was examined. Fig. 1 shows diameters of FG beads in acetone, DMF, 1,4-dioxane, ethanol, and DMSO and FG beads after a replacement of buffer to distilled water again from each of the organic solvents. Only in acetone were FG beads swollen (D_w = 231.9 nm), followed by shrinkage in water (D_w = 203.8 nm). The result indicates that acetone possesses high affinity for poly (styrene-*co*-glycidyl methacrylate) of FG beads due to the similarities in their solubility parameters (SP) ($SP_{poly\ (styrene-co-glycidyl\ methacrylate}$ = 8.6–10.7, $S_{Pacetone}$ = 9.8). Therefore, acetone was selected as the organic solvent for the encapsulation of Eu^{3+} complex into FG beads.

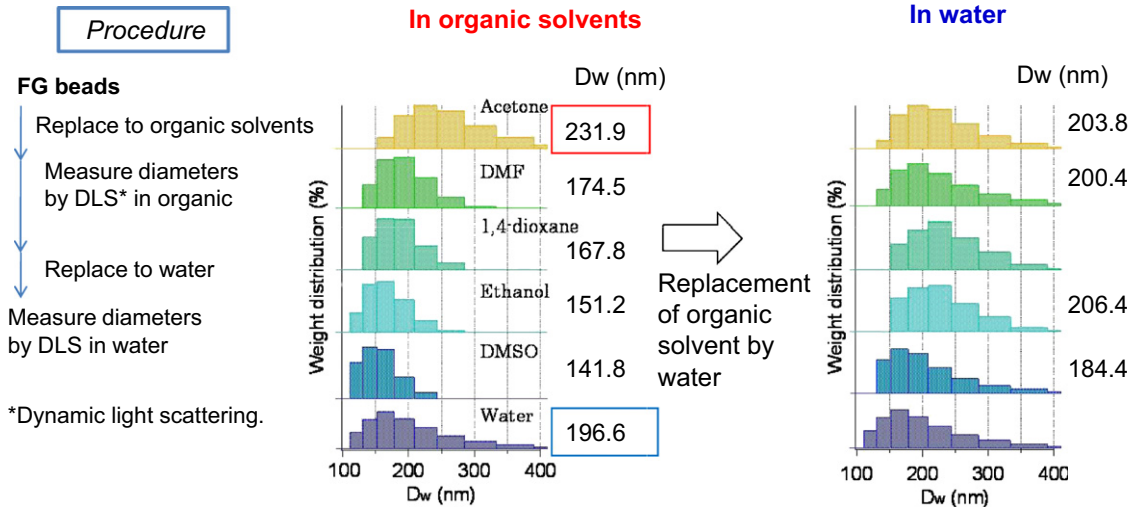

Fig. 1. Size distribution of FG beads in several organic solvents (acetone, DMF, 1,4-dioxane, ethanol, and DMSO) and distilled water. Right histogram displays size distribution of FG beads suspended in several organic solvents and distilled water. Left histogram displays size distribution of FG beads in distilled water after suspension buffer was replaced from organic solvents to distilled water again.