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Lectin Recognition Characteristics of Carbohydrate-Polyrotaxane Beads

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We prepared maltose-polyrotaxane (Mal-PRX)-immobilized beads by 4 steps: (1) making polypseudorotaxane using amine-terminated poly(ethylene glycol)-immobilized polystyrene (PEG-PS) beads and α-cyclodextrins, (2) capping the amine terminal of PEG in the polypseudorotaxane beads, (3) succination and (4) conjugating with maltose. The Mal-PRX-immobilized beads were mixed with biotinylated concanavalin A (Con A) to check the multivalent interaction with lectin. Quantum dot-streptavidin conjugate (QD-SV) was further introduced to enhance the fluorescence for the fluorescence microscope observation. The surface of Mal-PRX-immobilized beads became bright red by the emission 655 nm of QD-SV.

Keywords Beads; concanavaline A; lectin; maltose; polyrotaxane; quantam dot

Introduction

Cell membrane surface of, e.g., E. coli and related bacteria has receptor proteins to recognize carbohydrates [1–2], so that the detection of carbohydrate ligands with rapid and high sensitivity was expected as an ideal biological screening tool. In contrast to the low affinity and specificity monovalent analogs [3–5], the protein-carbohydrate interactions were dramatically enhanced by "cluster-glycoside effect" when multivalent ligand structures were introduced in such as linear polymers, glycopolymers, liposomes, dendrimers, beads and nanoparticles. However, the increase of local density and the number of carbohydrates often causes mismatching in the lectin recognition, and hence the reduction of mismatching by the design of biomaterials is required to improve the multivalent feature. In order to enhance the multivalent interaction, Yui's group has focused on mechanically locked structure of polyrotaxanes, in which many α-cyclodextrins (α-CDs) are threaded onto a poly(ethylene glycol) (PEG) chain capped with bulky end-groups. Maltosyl groups were successfully introduced to the polyrotaxane (PRX) with the different number of α-CDs for multivalent interaction with concanavalin A (Con A) [6]. Recently, they also reported the maltose-polyrotaxane conjugate (Mal-PRX) in which the maltose

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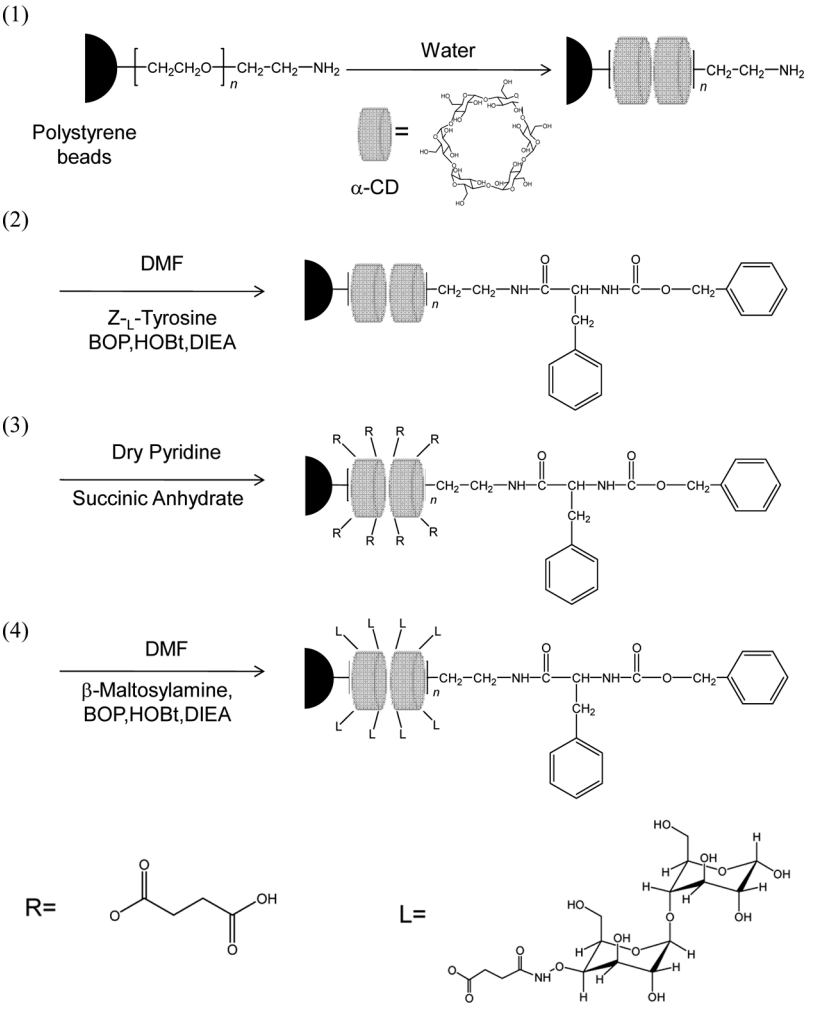
as a ligand to hydroxyl group of cyclodextrin with highly mobile nature contributes to the multivalent interactions [7]. Therefore, it is expected that the immobilization of Mal-PRX as a multivalent ligand onto microbeads (i.e., polymeric microspheres of diameters of 1–50 μm) enables a wide variety of biotechnological applications including biochemical synthesis, drug discovery, genomics, proteomics, biosensing, separations, and drug delivery.

In this study, Mal-PRX was immobilized on the surface of polystyrene (PS) beads, and the recognition of Con A was evaluated by using quantum dot technologies.

Experimental

Preparation and Characterization of Mal-PRX-Immobilized PS Beads

Mal-PRX-immobilized beads were prepared by 4 steps as shown in Scheme 1: (1) Preparation of polypseudorotaxane using amine-terminated PEG-immobilized PS



Scheme 1. Synthesis of ligand-polyrotaxane immobilized polystyrene beads.

beads (NovaSyn TG amino resin, PEG Mw: 3000 ~ 4000, 130 μm) and α-CDs, (2) capping the amine terminal of PEG in the polypseudorotaxane beads, (3) succination and (4) conjugating with maltose. Polyseudorotaxane formation on the surface of NovaSyn TG amino resin was carried out by mixing the beads (0.5 g) with saturated aqueous solution of α-CDs. Capping the amine terminal of PEG in the polypseudorotaxane beads with Z-$_L$-Tyrosine (Z-Tyr) was carried out by mixing the polyseudorotaxane beads (0.93 g) and Z-Tyr (1.37 g, 4.4 mM) in dry DMF (10 mL), together with a condensing agent (benzotriazol-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate: BOP, 1.95 g, 4.4 mM) and catalysts (1-hydroxybenzotriazole (HOBt, 0.59 g, 4.4 mM) and *N*, *N*-diisopropylethylamine (DIEA, 0.57 g, 4.4 mM)). After that, succinic anhydride was introduced to react with hydroxyl groups of α-CDs in the PRX-immobilized beads to prepare carboxyethylester polyrotaxane (CEE-PRX)-immobilized beads. The Z-Tyr-terminated polyrotaxane beads (0.36 g) and succucinic anhydride (4.1 g) were dissolved in dry pyridine (20 mL) and stirred at room temperature. Finally, β-maltosylamine was conjugated with the CEE-PRX-immobilized beads (0.24 g) in the presence of BOP (0.82 g, 2.1 mM), HOBt (0.29 g, 2.1 mM) and DIEA (0.27 g, 2.1 mM). The immobilization of polypseudorotaxane, PRX, CEE-PRX and Mal-PRX was analyzed by ^{1}H-NMR measurements (JNM-ECP400, JEOL Ltd.) in 1M NaOD, the condition of which induced the degradation of polyrotaxane structure, so that the amounts of α-CDs, CEE and maltose could be determined.

Digital Microscope Imaging of Polyrotaxane-Immobilized Beads

We used a digital microscope (VH-8000, KEYENCE Co.) to check the surface state of polystyrene beads with poly(ethtylene glycol) (PEG) chains and/or α-CD introduced polyrotaxane chains.

Analysis of Con A Recognition on Mal-PRX-Immobilized Surface

Mal-PRX-immobilized beads were mixed with Dulbecco's Phosphate Buffered Saline (DPBS, containing 137 mM NaCl) containing 2% bovine serum albumin (BSA), and incubated for 4 hours at 4°C. And then the Mal-PRX beads were mixed with biotinylated Con A, a kind of lectin. Nanocrystal-streptavidin conjugate (Qdot^{TM} 655 streptavidin conjugate (QD-SV), Invitrogen Co.) was further introduced into the Mal-PRX/biotinylated Con A solution. The fluorescence from the final solutions was investigated by the fluorescence microscopy in order to check the Con A recognition on the Mal-PRX beads surface (excitation wavelength: 400~440 nm).

Results and Disucussion

Figure 1 shows a digital microscope image of PEG-immobilized PS beads after adding to water (Fig. 1A) and to α-CD saturated aqueous solution (Fig. 1B). Though the beads surfaces seem to be sharp in water (Fig. 1(A)), they became unclear in α-CD saturated solution. These results indicate that α-CD molecules are threaded onto the PEG chain located on the beads surface. After the capping reaction of the terminal of PEG in the polypseudorotaxane-immobilized beads, the PRX

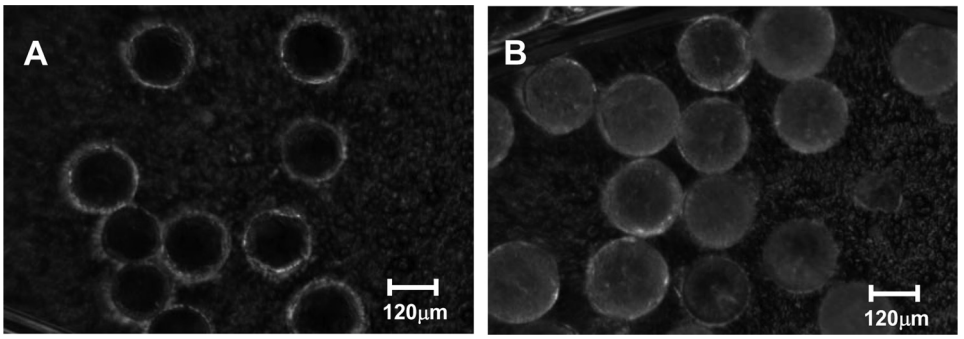


Figure 1. Digital microscope images of PEG-immobilized polystyrene beads: (A) before adding α-CD and (B) after adding α-CD. (Scale bar is 120 μm.)

structure was firstly confirmed by the microscope observation in water and in DMSO. As shown in Figure 2A, the surface in water was unclear. On the other hand, the surface became transparent in DMSO (Fig. 2B). These results suggest that the hydrogen bonds between α-CD molecules exist in water even after the capping reaction, but they were suppressed in the hydrophobic DMSO. This phenomenon is typical in the PRX structure, and hence the PRXs are suggested to exist on the surface. The results of FT-IR imaging of PRX beads surface supported the real immobilization of PRX (data not shown). Z-$_L$-Tyrosine groups of Mal-PRX were removed by cleaving the C−N bond in 1M NaOD to determine the number of α-CDs on the beads surface. Figure 3 shows the ^{1}H-NMR spectra of Mal-PRX beads in 1 M NaOD. It was suggested that the number of introduced maltose per α-CD molecule was five.

The interaction between the Mal-PRX beads with Con A was analyzed by fluorescence microscope observation after adding biotinylated Con A and QD-SV. The interactions among beads surface, maltose-immobilized polyrotaxane, biotinylated Con A (lectin) and streptavidin conjugated QD are schematically illustrated in Figure 4. Figure 5 shows fluorescence microscope images of PRX (Fig. 5A) and Mal-PRX beads (Fig. 5B). The yellow-colored emission of Mal-PRX-immobilized beads was observed, which is due to autofluorescence of Mal-PRX beads (Fig. 5B).

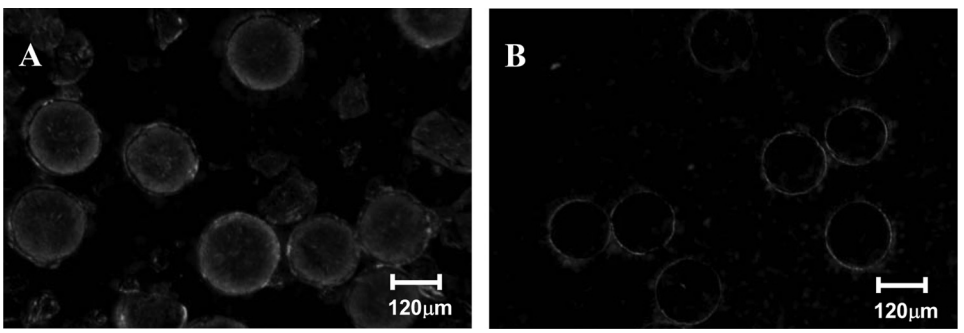


Figure 2. Digital microscope images of polyrotaxane beads: (A) in water and (B) in DMSO. (Scale bar is 120 μm.)

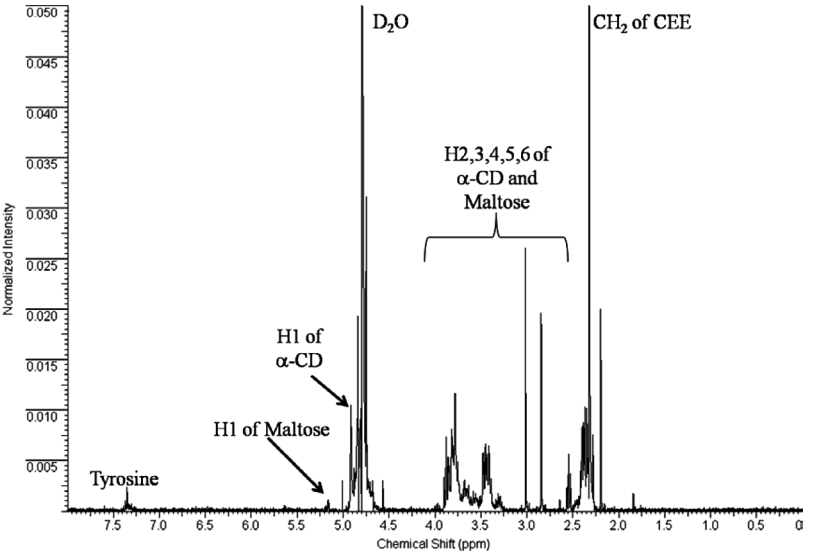


Figure 3. ^{1}H-NMR spectra of maltose-polyrotaxane immobilized beads in 1M NaOD.

PRX beads also showed the autofluorescence, but the intensity was weaker than that of Mal-PRX beads. Figure 6 shows fluorescence microscope image of PRX (Fig. 6A) and Mal-PRX (Fig. 6B) beads after adding biotinylated Con A and QD-SV. The surface of Mal-PRX-immobilized beads became bright red. On the other hand, the red color on PRX surface was hardly observed. These results suggest that maltose groups in Mal-PRX recognized biotinylated Con A followed by QD-SV interaction. It should be noted that no red color was observed without adding biotinylated Con A in the Mal-PRX-immobilized beads suspension due to the lack of molecular recognition (data not shown).

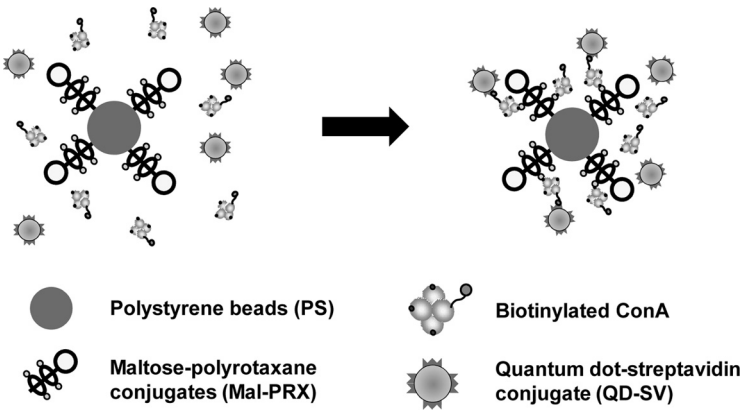


Figure 4. Schematic representation of the interactions among beads surface, maltose-immobilized polyrotaxane (Mal-PRX), biotinylated Con A (lectin) and quantum dot-streptavidin conjugate (QD-SV) The interaction is analyzed by fluorescence microscope observation after adding biotinated Con A and QD-SV.

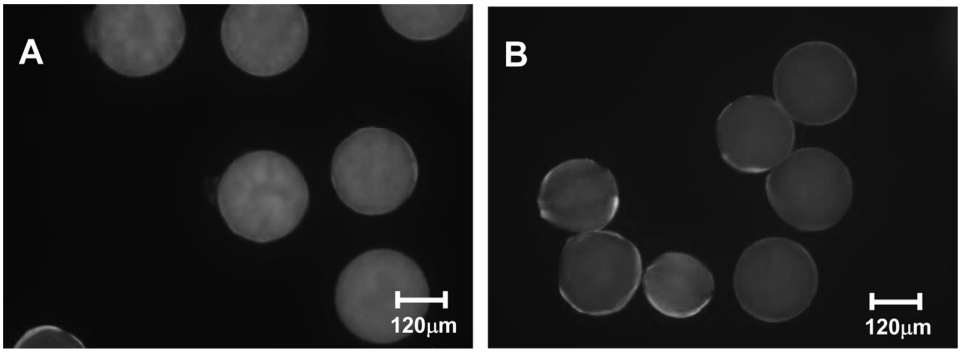


Figure 5. Fluorescence microscope images before adding Con A and QD-SV: (A) PRX beads and (B) Mal-PRX beads. (Scale bar is 120 μm.)

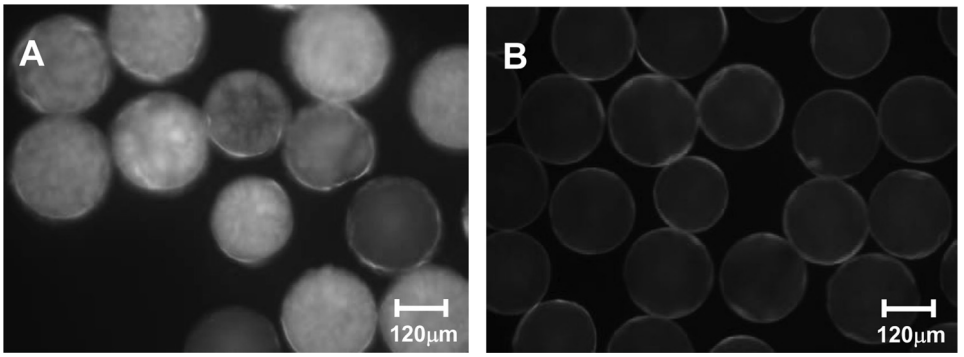


Figure 6. Fluorescence microscope images after adding Con A and QD-SV: (A) PRX beads and (B) Mal-PRX beads. (Scale bar is 120 μm.)

Conclusion

In summary, we prepared polyrotaxane modified by maltose as carbohydrate ligands on PS beads by 4 steps: (1) Preparation of polypseudorotaxane using PEG-PS beads and α-CD, (2) capping the amine terminal of PEG in the polypseudorotaxane beads, (3) succination and (4) conjugating with maltose. From a ^{1}H NMR spectrum of Mal-PRX beads in 1M NaOD, the number of introductions of the maltose per α-CD molecule was estimated five. The Mal-PRX-immobilized beads were mixed with biotinylated Con A and QD-SV, and the Con A recognition by the beads surface was evaluated by the fluorescence microscope observation. The surface of Mal-PRX beads became bright red due to the emission 655 nm of QD-SV. These results suggest that maltose groups in Mal-PRX recognized biotynated Con A followed by the specific interaction with QD-SV.

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