Supramolecular Chemistry

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Controlled Release of Guest Molecules from Mesoporous Silica Particles Based on a pH-Responsive Polypseudorotaxane Motif**

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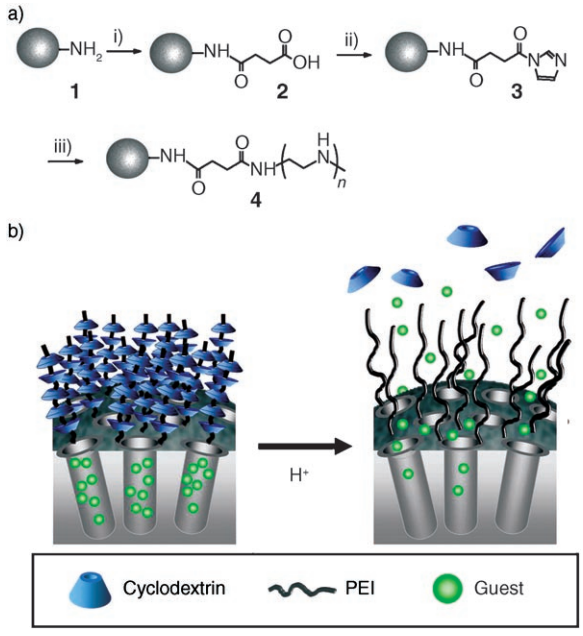
Cyclodextrins (CDs) have been a target of intensive research owing to unique structural, physical, and chemical properties.[1] In particular, their capability to form inclusion complexes with guest molecules would be the most interesting characteristics. Furthermore, inclusion complexes with various types of guest molecules could be reversibly dissociated by external stimuli.[1] These interesting features have provided a unique route to construct nanomachines by using CDs.[1] Several groups have reported on functional rotaxane systems based on cyclodextrin as a host. Recently, pH-responsive polypseudorotaxanes consisting of polyethyleneimine (PEI) and CDs were reported.[2–4] Furthermore, Lee et al. reported on the pH-dependent polypseudorotaxane formation of α-CD with the triblock copolymer (PEI–*b*-PEG–*b*-PEI; PEG = poly(ethylene glycol)) where CDs undergo unique reversible complexation and decomplexation selectively with the PEI block through pH variation.[4] Therefore, we reasoned that the incorporation of this type of reversible pseudorotaxane onto the surface of mesoporous materials can create pH-responsive nanocarrier systems in which CDs play the role of a pH-responsive valve for pores of mesoporous materials.

Controlled-release systems have attracted great attention owing to their applicability in the area of drug delivery.[5] To date, a diverse class of organic carriers, such as micelles, liposomes, and polymeric nanoparticles, have been investigated for their utility as delivery systems.[5] However, organic carriers such as micelles suffer from poor stability owing to biochemical attack. Recently, amorphous mesoporous silica particles were suggested as useful carriers because of their stability, controllable pore diameter, and biocompatibility.[6] Several groups reported on controlled release systems using mesoporous silica. Fujiwara and co-workers described the photoinduced release of drug molecules from coumarin-functionalized mesoporous materials.[7] The thermoresponsive release system was demonstrated by using a mesoporous material modified with poly(*N*-isopropyl acrylamide).[8] The pH-responsive release systems based on mesoporous silica were reported by using a polyelectrolyte motif.[9] Stoddart and co-workers reported a redox-controlled nanovalve based on mesoporous silica materials with a pseudorotaxane.[10] More recently, a base-triggered controlled-release system was demonstrated.[11] However, CD–rotaxane-based pH responsive systems on silica materials have not been reported.

Herein, we report on the pH-controlled release of guest molecules entrapped in the pore of a mesoporous silica particle (Si-MP) that is blocked by the surface-grafted pH-responsive PEI/CD polypseudorotaxane. The working principle of this system is schematically described in Scheme 1 and Figure 1. This system consists of biocompatible components such as silica particles, PEI, and CD. Low-molecular-weight linear PEI, which is known as a biocompatible polycationic polymer,[11] was used as the guest polymer for CD hosts. CDs present several advantages as drug-delivery agents with the ability to protect the drug from physical, chemical, and enzymatic degradation and to enhance cell-membrane permeability.[12] As a reservoir for guest molecules, surface-functionalized Si-MP was selected. The guest molecules in the



Scheme 1. a) Synthetic route to PEI-functionalized silica particles. b) Schematic of pH-responsive release of guest molecules from the pore of Si-MP. i) succinic anhydride, triethylamine; ii) 1,1′-carbonyldiimidazole; iii) PEI–NH_2.

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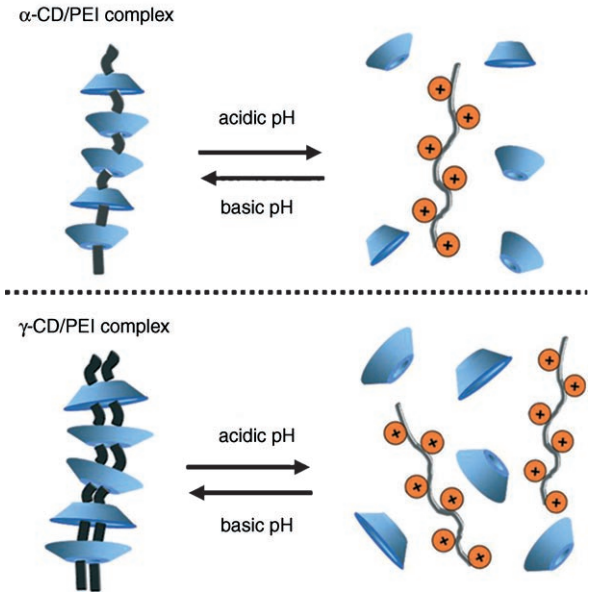

Figure 1. Schematic illustration of the pH dependence of polypseudorotaxane formation from PEI and CDs.

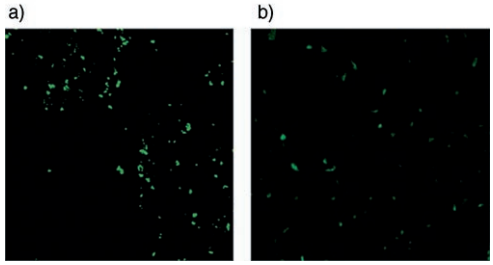

Figure 2. Confocal laser microscopy images: a) calcein-loaded α-CD/**4** and b) calcein-loaded γ-CD/**4**.

pore can be released through pH-triggered dethreading of PEI/CD polypseudorotaxane to open the pore of Si-MP.

PEI ($M_n = 1100$) with a terminal amine unit (PEI–NH_2) and spherical Si-MP were prepared according to published procedures.[13,14] The structure of Si-MP was confirmed by powder X-ray diffraction (PXRD), transmission electron microscopy (TEM), and environmental scanning electron microscopy (E-SEM). The mean diameter of the silica particles estimated by E-SEM was about 425 nm.[15] The lattice spacing of ordered pores with hexagonal structures was 3.6 nm as measured by PXRD. The average diameter of the pore measured by BET analysis was 1.8 nm. The surface of Si-MP was functionalized with amine groups by treatment with 3-aminopropyltriethoxysilane to yield silica particle **1** (Figure 1).[10,16] The surface functionalization of Si-MP was monitored by FTIR spectroscopy.[15] The silica particle **2** functionalized with a carboxylic group was obtained by allowing particle **1** to react with succinic anhydride in the presence of triethylamine. The resultant mixture was purified by centrifugation and washed with ethanol. The FTIR spectrum of particle **2** exhibits new absorption bands in the amide I (1641 cm^{-1}) and amide II (1569 cm^{-1}) regions and at 1725 cm^{-1} owing to the carbonyl-group stretching of the carboxy group. The carboxy unit on the surface of silica particle **2** was activated by 1,1′-carbonyldiimidazole to yield particle **3**, which was then reacted with PEI–NH_2 in THF to prepare particle **4**. The FTIR spectrum of particle **4** showed a sharp band at 3270 cm^{-1} from the crystalline phase of linear PEI.[17]

Calcein was loaded as a guest molecule into the pore of a silica particle by soaking particle **4** in a phosphate-buffered saline (PBS) solution (pH 7.4) of calcein. Then, α-CD was added into the mixture to provide calcein-loaded α-CD/**4** in which the pore was blocked by PEI/α-CD polypseudorotaxane. The pH value of the solution was adjusted to 11. The excess amount of calcein was removed by centrifugation and washing with water. The disappearance of the IR band at 3270 cm^{-1} is attributed to inclusion of the surface PEI segments in α-CD.[15]

After purification, the resulting particles were resuspended at pH 11. The emission at 520 nm from the solution ($\lambda_{ex} = 490$ nm) indicates that calcein molecules reside in the pore of the mesoporous silica particle. The loading of calcein in the silica particle with the surface α-CD/PEI (α-CD/**4**) was visualized by confocal laser scanning microscopy (CLSM; Figure 2). The self-quenching induced by calcein aggregation within the pore of α-CD/**4** results in weak fluorescence of calcein.[15] The calcein-loaded particle with γ-CD/**4** was also prepared by the same procedure. The encapsulation of calcein in the pore of γ-CD/**4** was also confirmed by the fluorescence spectra and CLSM (Figure 2 and the Supporting Information). However, in the case of β-CD, the guest molecules could not be effectively blocked in the pore because PEI and β-CD did not form a stable polyseudorotaxane.

The threading and dethreading process of α- and γ-CD onto the PEI block can be controlled through pH variation.[3,4] In particular, α- and γ-CD are threaded onto PEI with a maximum yield of the polyseudorotaxane complex at pH 11.[3] Furthermore, CDs can be dethreaded from the PEI block at pH values below 8.0 owing to the weak interaction of the protonated PEI chain with the hydrophobic interior of CDs.[3,4] Therefore, to clearly demonstrate the pH-responsive release property of our system, the pH value of the solutions of calcein-loaded α-CD/**4** and γ-CD/**4** was adjusted from 11 to 5.5. In both cases, the release of calcein guest molecules from the pore owing to dethreading of CDs from the PEI chain was monitored by fluorescence measurement.[18] The fluorescence intensity of the solution of calcein-loaded CD/**4** was monitored at 520 nm over time (Figure 3). At pH 11, weak fluorescence intensity was observed owing to self-quenching of calcein molecules in the pore. On the other hand, for example, after adjusting to pH 5.5, an immediate increase in fluorescence intensity was observed. This indicated that calcein molecules were released from the pore through pH-induced dethreading of CDs from the PEI chains that blocked the pore (Figure 3).

In summary, we have demonstrated controlled release of guest molecules from mesoporous silica particles by using a pH-sensitive CD/PEI polypseudorotaxane motif. The pore of particle **4** was filled with guest molecules and then blocked by threading of CDs onto the surface-grafted PEI chains at pH 11. At pH 5.5, the guest molecules were released from the

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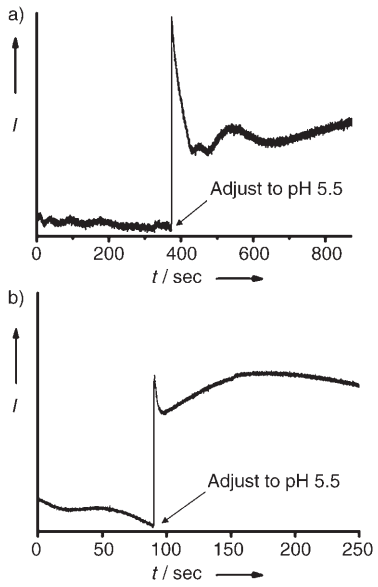

Figure 3. Variation of fluorescence intensity ($\lambda_{em} = 520$ nm) with respect to time and pH for a) calcein-loaded α-CD/**4** and b) γ-CD/**4**.

pore of CD/**4** by the reversible dethreading of CDs from the PEI chain. This system deserves attention as one of expanded applications of CD-based polypseudorotaxanes that can act as a molecular gate. This approach would provide routes to various useful systems including molecular machines, stimuli-responsive nanocarriers, and sensors.[1,7,19,20]

Experimental Section

Loading, capping, and release experiments: The filling of the pores with calcein was carried out by soaking particle **4** (0.5 mg) in a PBS solution (10 mL, pH 7.4) of calcein (0.6 mg). α-Cyclodextrin was added into the mixture of silica particle **4** and calcein, and then the pH of the solution was adjusted to 11. The excess amount of calcein was removed by centrifugation (8000 rpm, 30 min) and washing with water. The emission at 520 nm ($\lambda_{ex} = 490$ nm) from the solution indicates that calcein molecules are inside CD/**4**. Calcein-loaded CD/**4** particles were visualized by CLSM. To investigate the pH-responsive release properties of our system, the solution of calcein-loaded CD/**4** was adjusted to different pH values.

Fluorescence measurements: All the fluorescence measurements were performed by using a Shimadzu RF-5301PC spectrofluorophotometer. The emission and excitation slit widths were set at 1.5 nm with $\lambda_{ex} = 490$ nm.

TEM analysis: TEM was performed by using a Philips CM 200, operated at an acceleration voltage of 120 kV. For the preparation of dispersed samples in water, a drop of sample solution (100 mg L^{-1}) was placed onto a 300-mesh copper grid coated with carbon. About 2 min after deposition, the grid was tapped with filter paper to remove surface water. The samples were air-dried before measurement.

SEM analysis: The E-SEM image was obtained on an FEI XL-30 field emission gun E-SEM instrument (accelerating voltage: 10–15 kV; pressure range: 0.8–0.9 Torr). E-SEM samples were prepared by transferring a drop of sample solution onto a 200-mesh carbon-coated copper grid. About 5 minutes after transfer, excess water was removed with filter paper. The samples were air-dried before measurement.

CLSM analysis: The CLSM image was obtained on a BioRad MRC-1024. The CLSM samples were prepared by transferring a drop of sample solution onto a cover slip. The cover slip was then sandwiched with another cover slip.

PXRD experiment: PXRD patterns were recorded at room temperature on a Rigaku model RINT-2000 counter diffractometer with a $Cu_{K\alpha}$ radiation source (operated at 40 kV, 40 mA).

BET: The pore size was measured at 77 K on a Quantachrome instrument.

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