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Enzyme Responsive Nanocontainers with Cyclodextrin Gatekeepers and Synergistic Effects in Release of Guests

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Stimuli-responsive silica nanoparticles (Si-MPs) with porous reservoirs have great potential for useful applications in biotechnology and medicine due to their unique responsiveness and high stability.[1,2] In particular, these nanocontainers equipped with gatekeepers are enormously appealing not only as sensory machines but also as delivery vehicles.[1,2] A variety of stimuli-responsive gatekeepers have been introduced onto the surface of Si-MPs to control the release of guest molecules in response to external stimuli, such as pH, light, and redox potential.[3,4] Although these systems have been investigated as controlled release systems or potential drug delivery vehicles, we still need to optimize their performances such as stability and enhanced sensitivity to external stimuli to expand their potentials in a variety of areas.[5]

Here, we report on the enzyme-responsive release of guest molecules from Si-MPs with cyclodextrin (CD) gatekeepers in response to α-amylase and lipase. Because the abnormal increase of these enzymes is intimately associated with acute pancreatitis, their early detection or utilization of these enzymes as stimuli for nanocarriers is of clinical significance.[6]

As an enzyme-responsive nanocontainer, we employed the functional Si-MPs that are composed of the surface CD gatekeepers, functional stalks, and fluorescence probes within porous channels (Figure 1). The torus shaped CD was initially chosen to block the porous channel of Si-MP. The CD moiety of the gatekeeper can be hydrolyzed by α-amylase, and the stalk part was designed to be degraded by lipase to exhibit enzyme-responsive characteristics in the release of guests.

For this purpose, we prepared Si-MP-0 with an ~60 nm diameter (average pore diameter = 2.5 nm).[5] The surface of Si-MP-0 was functionalized with amine groups by treatment with 3-aminopropyltriethoxysilane to obtain Si-MP-NH_2, which was then allowed to react with propargyl bromide to provide Si-MP-alkyne. To obtain Si-MP-CD, the β-CD gatekeeper was tethered onto the surface of Si-MP-alkyne by click coupling of mono-6-azido-β-CD.[7] For preparation of Si-MP-NBE-CD which contains the ester moiety in the stalk part, a reference procedure was employed.[5] The pore of Si-MPs was loaded with a fluorescent dye, calcein.

The weight percentages of CDs on Si-MP-CD and Si-MP-NBE-CD measured by an amylase assay kit were 1.30% and 0.87%, respectively.[7] These systems enabled us to control the release of calcein molecules from the porous channel when enzymatic stimuli were applied to degrade the CD or stalk moieties of the gatekeeper, as shown in Figure 1D. As shown in Figure 2, when α-amylase (10 U/mL) was added to the phosphate buffered saline (PBS, pH 7.4) solution of Si-MP-NBE-CD and Si-MP-CD, we observed a significant increase in the fluorescence intensity of calcein, which indicates that calcein molecules were released from the pore of Si-MPs due to degradation of CD gatekeepers by α-amylase. On the other hand, in the absence of α-amylase, the fluorescence intensity of calcein was not increased. As an additional control experiment, addition of denatured α-amylase into the solutions of

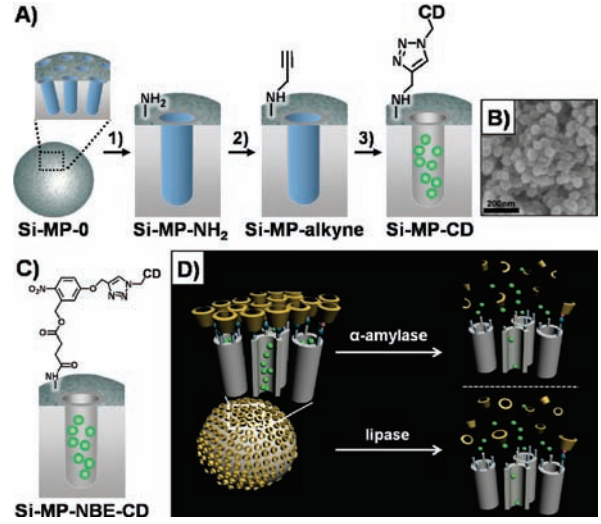

Figure 1. (A) Synthetic route to Si-MP-CD: (1) 3-aminopropyltriethoxysilane; (2) succinic anhydride and triethylamine; (3) removal of CTAB, calcein, $CuSO_4$, sodium ascorbate, and mono-6-azido-β-CD. (B) FE-SEM image of Si-MP-CD. (C) The structure of surface functional motifs on Si-MP-NBE-CD. (D) Schematic illustration for enzyme-triggered release of guest molecules from the pore of CD-covered nanocontainers.

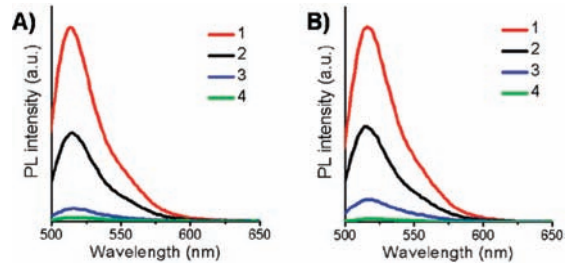

Figure 2. Fluorescence spectra of calcein from the PBS solution of (A) Si-MP-NBE-CD and (B) Si-MP-CD, respectively. In the presence of (1) α-amylase (10 U/mL), (2) α-amylase (10 U/mL) preincubated with the inhibitor protein for 90 min, (3) α-amylase (10 U/mL) preincubated with acarbose, and (4) in the absence of α-amylase.

Si-MP-NBE-CD and Si-MP-CD did not increase the fluorescence of calcein (Figure 3D).[7]

These results indicate that the guest molecules are kept in the pore without an enzymatic stimulus which can degrade the CD gatekeeper. In addition, the activity of α-amylase in degrading CD to open the gate of the pore of both Si-MP-NBE-CD and Si-MP-CD was suppressed after addition of α-amylase preincubated with the inhibitor protein. As shown in Figure 2, when Si-MP-NBE-CD and Si-MP-CD were treated with α-amylase preincubated with the inhibitor protein for 90 min in PBS, the fluorescence intensity of the Si-MP solutions became respectively 46% and 49% of the maximum fluorescence intensity in the presence of α-amylase,

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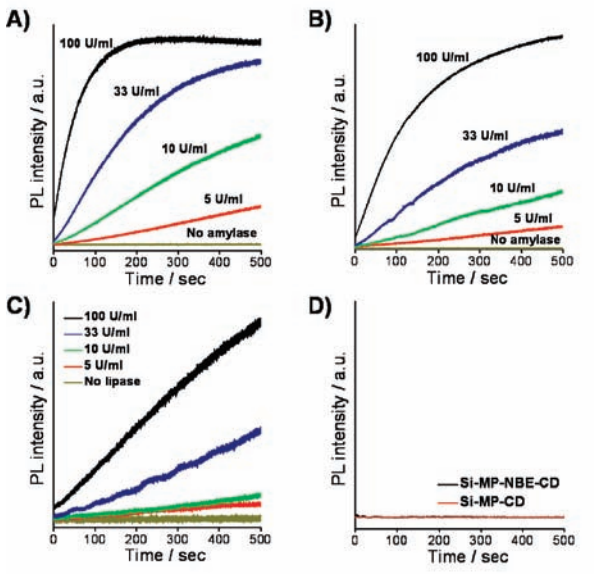

Figure 3. Time courses of fluorescence intensity (at $\lambda_{em} = 514$ nm) of calcein from (A) Si-MP-NBE-CD with α-amylase, (B) Si-MP-CD with α-amylase, (C) Si-MP-NBE-CD with lipase, and (D) Si-MPs with denatured α-amylase.

which is similar to the reported value of the suppressed activity (~50%) of α-amylase by the inhibitor protein.[8,9] These results indicate that the controlled release properties of our nanocontainer systems with the CD gatekeeper are sensitive to the enzymatic activity of α-amylase. To trace the enzymatic activity of α-amylase, we added different amounts of α-amylase into the solutions of Si-MPs (Figure 3A and 3B). While no appreciable changes were observed in the absence of α-amylase, the fluorescence intensity of calcein from Si-MP-NBE-CD or Si-MP-CD increased with increasing α-amylase concentration, which suggests that the CD-covered nanocontainers can discriminate the enzymatic activity of α-amylase (Figure 3A and 3B). In addition, since the stalk part of the gatekeeper in Si-MP-NBE-CD contains an ester moiety which can be hydrolyzed by enzymes such as lipase and esterase, Si-MP-NBE-CD was treated with lipase to investigate the susceptibility of the release property of Si-MP-NBE-CD to lipase. In this experiment, we observed an increase of the fluorescence intensity of calcein in Si-MP-NBE-CD through time-course fluorescence analysis (Figure 3C), suggesting that the ester moiety can be degraded by lipase to remove CD gatekeepers.

We investigated a synergistic effect by employing multiple stimuli for the release of guest molecules from Si-MP-NBE-CD which consists of the enzyme- and photoresponsive surface functionality. For Si-MP-NBE-CD, β-CD was introduced on the surface of Si-MP through an *o*-nitrobenzyl ester moiety as a photocleavable linker.[5] As shown in Figure 4A, when both stimuli were simultaneously applied to the solution of Si-MP-NBE-CD, the increase in the fluorescence intensity of calcein was more accelerated, indicating that the release of calcein molecules from Si-MP-NBE-CD was synergistically affected by the photostimulus and enzymatic activity of α-amylase.

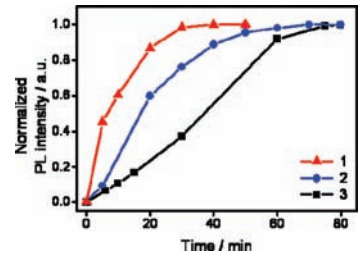

Figure 4. Time courses of normalized fluorescence intensity (at $\lambda_{em} = 514$ nm) of calcein from Si-MP-NBE-CD: (1) α-amylase (33 U/mL) + UV, (2) α-amylase (33 U/mL), (3) UV.

In summary, we have demonstrated that the incorporation of CD gatekeepers on the surface of Si-MPs provided an enzyme responsive character for α-amylase. In addition, the lipase-induced release of guests was investigated as well based on the hydrolysis of the ester moiety in the stalk part of the gatekeeper. The synergistic responsiveness in the controlled release of guest molecules by dual stimuli of α-amylase and UV irradiation was demonstrated. Our system would provide diverse applications in diverse areas such as diagnostics, imaging, and drug delivery.

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Supporting Information Available: Detailed synthetic procedures and spectral characterization for Si-MP-CD. This material is available free of charge via the Internet at http://pubs.acs.org.

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