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Stimuli-Responsive Conformational Conversion of Peptide Gatekeepers for Controlled Release of Guests from Mesoporous Silica Nanocontainers

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Supporting Information

ABSTRACT: The use of peptides as gatekeepers for payloads of mesoporous silica nanoparticles would allow triggering the release of guests by various biological stimuli. We investigated the effect of peptide conformation on their gatekeeping capability by employing two model peptides with a turn or a random structure. The conformation-dependent gatekeeping properties provided an opportunity to utilize the conformational conversion of peptides as a valuable motif for stimuli-responsive gatekeepers. Based on that investigation, we demonstrated that Fmoc-CGGC-SS-Si, which exhibited a zero-release property without any stimuli due to a turn-like conformation induced by the intramolecular disulfide bond, can be triggered to release guests by converting its conformation to a random structure, induced by reduction of the disulfide bond upon addition of glutathione. We further demonstrated that the conformational conversion of Fmoc-CGGC by Zn(II) ion can also be utilized as a triggering motif.

Mesoporous silica nanoparticles (MSNs) have been of great interest as nanocarrier platforms for efficient drug delivery due to their facile surface functionalization, high biocompatibility, low cytotoxicity, chemical stability, and sufficient encapsulation of guest molecules in the nanochannel.[1−4] There are two options for drug encapsulation into the MSNs: physical trapping in the mesopore, and cleavable chemical bond formation between MSNs and drug molecules.[5−9] Drug encapsulation by physical trapping is preferred due to its advantages in avoiding chemical bond formation, such as high loading percent, no unnecessary chemical modification of the drug molecule, and ease of application for various drugs. Upon physical trapping of a drug in the pore, it becomes essential to prevent its release during circulation in the bloodstream before reaching the target site. This zero-release property can be achieved by using a stimuli-responsive gatekeeper, thereby reducing side effects of the drug at the non-target sites.[10,11] Recently, various gatekeepers at the surface of MSNs have been reported, including cyclodextrin,[12−14] cucurbituril,[15] rotaxanes,[14,16−19] dendrimers,[20] polymers,[16,21,22] inorganic nanoparticles,[23−25] and peptides.[26,27] These gatekeepers were designed to release guest molecules when triggered by external stimuli such as pH, light, redox potential, and enzymes.[1,2] Among these various gatekeepers, peptides are one of the most attractive types (see Figure 1) due to their bioresponsiveness, biological activity, and biocompatibility for triggered release of guests in the nanochannel.

The introduction of peptides as gatekeepers on the surface would provide MSNs that allow triggering the release of guest molecules by a variety of biological stimuli, including specific enzymes, at specific target sites.[1,2,26,27] Recently, Heise et al. reported MSNs with a short oligopeptide (DAAR) as a gatekeeper.[26] This system showed a controlled release of 4 kDa FITC-dextran from the MSNs triggered by protease. MSNs with cleavable gatekeepers using enzyme-responsive long peptides (18-mer) were also reported, in which the release of blocked small guest molecules was triggered by peptidase.[27] However, more extensive research is needed for successful utilization of peptides as effective gatekeepers with stimuli-responsive triggering capability in specific biological environments. For example, the relationship between the conformations of peptides and their gatekeeping capability needs to be comprehensively understood for better design of peptide gatekeeper motifs that undergo conformational transformations triggered by external stimuli.

Toward that objective, we investigated the gatekeeping capability of two selected model peptides, Fmoc-CPGC and Fmoc-CGGC, which would have different secondary structures due to the ProGly sequence in the former inducing a turn structures.[28−30] Furthermore, on the basis of that investigation, we reasoned that a peptide capable of conformational conversion between a turn and a random structure would provide a stimuli-responsive gatekeeping property. Therefore, we also investigated the stimuli-responsiveness of peptide gatekeepers with conformational conversion which can be triggered by biological stimuli (i.e., glutathione (GSH) or Zn(II)).

In order to investigate the gatekeeping properties of the two model peptides, Fmoc-CPGC and Fmoc-CGGC, which would have different secondary structures, we prepared MCM-41-type MSNs (Si-NP) with 2.5 nm pore size according to procedure reported in ref 12. Transmission electron microscopy (TEM) images showed the hexagonally ordered porous nature and spherical shape of Si-NPs with about 80 nm diameter (Figure S1). The surface of the Si-NPs was modified using 3-aminopropyltriethoxysilane to introduce an amino group (Si-NH_2).[14] The amino group on the surface of Si-NH_2 was confirmed using FT-IR spectrum, which showed a N−H bend

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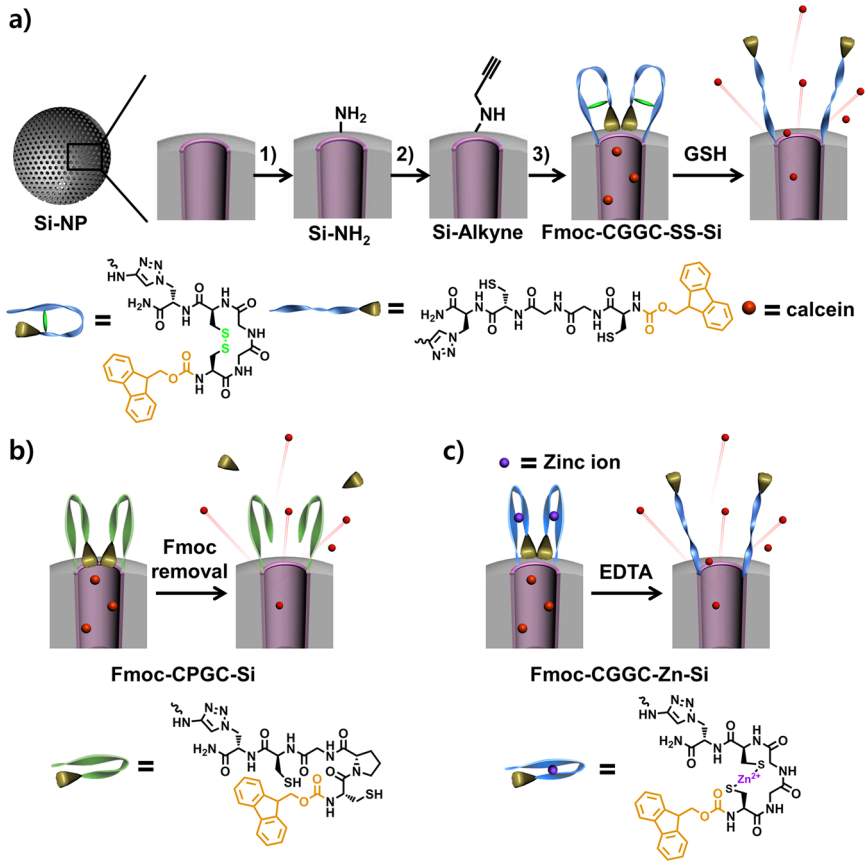

Figure 1. (a) Schematic representation of MSNs with peptide gatekeepers. Conditions: (1) aminopropyltriethoxysilane; (2) propargyl bromide; (3) CTAB removal, calcein loading, Fmoc-CGGC-SS-Azide, copper(II) sulfate, and sodium ascorbate. Below, schematic representation of Fmoc-CPGC-Si (b) and Fmoc-CGGC-Zn-Si (c). Amino acid abbreviations: C, cysteine; G, glycine; P, proline.

absorption peak at 1508 cm^{-1} (Figure S2). Furthermore, the zeta potential value changed from −8.65 mV (Si-NP) to +32.09 mV (Si-NH_2) after treatment with 3-aminopropyltriethoxysilane due to the presence of amino groups on the surface. Next, Si-NH_2 was modified using propargyl bromide to introduce an alkyne unit (Si-Alkyne). The introduction of an alkyne group also was confirmed by observation of the alkyne stretching band at 2133 cm^{-1} in the FT-IR spectrum (Figure S2). The zeta potential value of Si-Alkyne was +32.51 mV. The surfactant template, cetyltrimethylammonium bromide (CTAB), was eliminated from Si-Alkyne by using ammonium nitrate in ethanol. The guest molecules (calcein) were loaded into the pores of surfactant-removed Si-Alkyne by soaking in a dimethylformamide solution of calcein for 1 day. The peptides, Fmoc-CPGC-Azide and Fmoc-CGGC-Azide, were then conjugated onto the surface of Si-Alkyne, respectively, using a click reaction with microwave irradiation.[31,32] Fmoc-CPGC-Azide and Fmoc-CGGC-Azide were prepared by solid-phase peptide synthesis using Fmoc-chemistry (see Supporting Information). The presence of a S−CH_2 bend absorption peak at 1450 cm^{-1} and amide II near 1580 cm^{-1} in the FT-IR spectra of MSNs with surface peptide gatekeepers Fmoc-CPGC-Si and Fmoc-CGGC-Si indicated successful introduction of the peptide gatekeeper on the surface of MSNs (Figure S2). The substitution yields of Fmoc-CPGC-Si and Fmoc-CGGC-Si on the NP surface were 0.127 and 0.112 mmol/g, respectively, estimated by the Fmoc titration (see Supporting Information). TEM images of Fmoc-CPGC-Si and Fmoc-CGGC-Si in Figure 2 indicate that the mesoporous nature and diameter of the NPs were maintained after sequential surface modification.

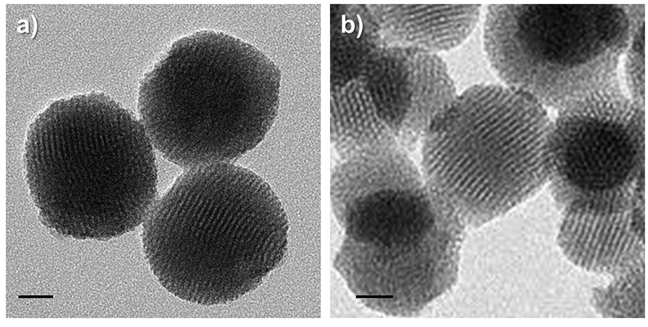

Figure 2. TEM images of Fmoc-CPGC-Si (a) and Fmoc-CGGC-Si (b). The scale bar is 20 nm.

The secondary structure of the peptides was investigated by circular dichroism (CD) spectroscopy (Figure 3, see Supporting Information for details). Fmoc-CPGC-Azide exhibited a considerable negative band at 225 nm, which indicated that Fmoc-CPGC-Azide would adopt a turn structure induced by the ProGly sequence.[28−30] On the other hand, Fmoc-CGGC-Si showed a weak negative band below 210 nm, which indicated that the major conformation of the peptide would be a random structure.

The gatekeeping properties of the peptides with turn (Fmoc-CPGC) and random structures (Fmoc-CGGC) were inves-

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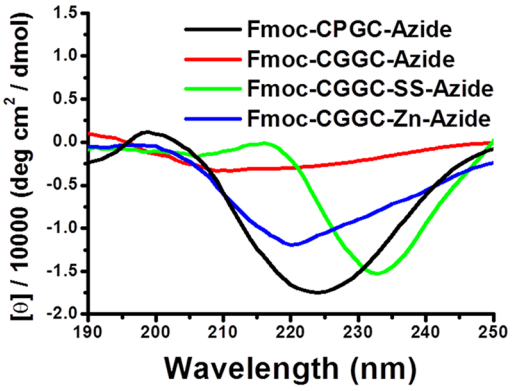

Figure 3. CD spectra of Fmoc-CPGC-Azide, Fmoc-CGGC-Azide, Fmoc-CGGC-SS-Azide, and Fmoc-CGGC-Zn-Azide.

tigated by monitoring the release of calcein loaded in MSNs in phosphate-buffered saline (PBS) buffer (pH 7.4). As shown in Figure 4a, the calcein molecules in Fmoc-CPGC-Si were not

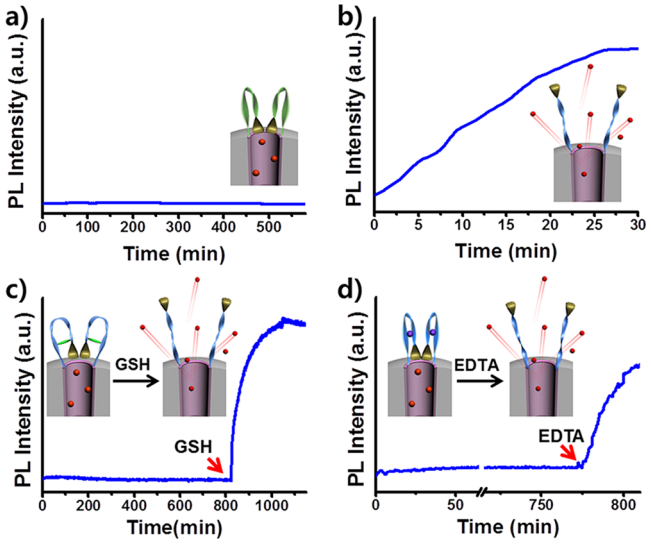

Figure 4. Release profile of guest molecules from Fmoc-CPGC-Si (a), Fmoc-CGGC-Si (b), Fmoc-CGGC-SS-Si (c), and Fmoc-CGGC-Zn-Si (d) in PBS buffer (pH 7.4).

released over 500 min without external stimulus. On the other hand, as shown in Figure 4b, the guest molecules in the nanochannel of Fmoc-CGGC-Si were released over time. These results indicated that Fmoc-CPGC with a turn structure inhibited the release of the guest molecules in MSNs, while Fmoc-CGGC with a random structure could not inhibit the release of the entrapped guest molecules. Upon removal of the Fmoc unit at the N-terminal, the entrapped guest molecules in Fmoc-CPGC-Si were released over time, as shown in Figures 1b and S3. Therefore, these results indicated that both the turn structure of CPGC and the Fmoc moiety played collaborative roles in the peptide gatekeeper to keep the entrapped guest molecules in the nanochannel of MSNs.

On the basis of these results, we reasoned that a peptide capable of conformational conversion between a turn and a random structure could be utilized as a stimulus-responsive gatekeeper. As a proof of concept, we prepared Fmoc-CGGC-SS-Si, in which two cysteine units were linked via a disulfide bond to provide a gatekeeping capability with a turn-like structure, which could be converted to Fmoc-CGGC-Si with a random structure to open the orifice of the nanochannel by reducing the intramolecular disulfide bond. We prepared Fmoc-CGGC-SS-Si by click coupling of calcein-loaded Si-Alkyne with Fmoc-CGGC-SS-Azide, which was synthesized from Fmoc-CGGC-Azide by oxidation in acetonitrile/water (80:20, v/v) (see Supporting Information). The CD spectrum of Fmoc-CGGC-SS-Azide, with an intramolecular disulfide bond, exhibited a strong negative band around 230 nm, as shown in Figure 3, suggesting that the intramolecular disulfide bond of Fmoc-CGGC induced the peptide to adopt a turn-like structure.[30,33] The release profile of calcein-loaded Fmoc-CGGC-SS-Si (substitution yield of Fmoc-CGGC-SS = 0.121 mmol/g) in Figure 4c showed that the guest molecules were not released over 800 min in PBS buffer (pH 7.4). Upon addition of GSH (1 mM) as a biological stimulus—which is expressed in numerous cancer cells with high levels[34−36]—the guest molecules in the nanochannel of Fmoc-CGGC-SS-Si were released, as shown in Figure 4c. The loading percentage of calcein in the channel of Fmoc-CGGC-SS-Si was 6.5 wt%, estimated from UV/vis absorbance of calcein in the supernatant after sufficient release of calcein triggered by GSH.

In addition, we further investigated the gatekeeping capability of the peptide with conformational conversion induced by a Zn(II) ion. Having peptide sequence common with that of the zinc finger domain (CXXC),[37,38] Fmoc-CGGC might adopt a turn structure upon addition of Zn(II) ion, which plays a critical role in several cellular functions, including regulation of gene expression, cofactors of metalloenzymes, and signaling at neural synapses.[39−41] Upon addition of Zn(II) ion into calcein-loaded Fmoc-CGGC-Si before release, the two thiol groups of CGGC would chelate a Zn(II) ion to convert the peptide to a turn structure (Fmoc-CGGC-Zn-Si). As shown in Figure 3, the CD spectrum of Fmoc-CGGC-Zn-Azide (Fmoc-CGGC-Azide in the presence of Zn(II) ion) exhibited a considerable negative band at 220 nm,[30,37] which revealed that Fmoc-CGGC adopted a turn structure in the presence of Zn(II) ions due to the chelation. The release profile of calcein-loaded Fmoc-CGGC-Zn-Si in PBS buffer (pH 7.4) in Figure 4d showed that the guest molecules were not released without any stimuli over 750 min. Upon addition of EDTA to remove Zn(II) ion from Fmoc-CGGC-Zn and to induce conformational change from turn to random structure (Fmoc-CGGC), the guest molecules were released, as shown in Figure 4d. These results corroborated that the conformational conversion of the peptides triggered by biological stimuli (i.e., GSH or Zn(II)) could be utilized as a motif for effective gatekeepers of MSNs with an on-demand release property.

In conclusion, we demonstrated that the gatekeeping capability of two peptides, Fmoc-CPGC and Fmoc-CGGC, was highly dependent on the secondary structure. This result led us to utilize the conformational conversion of peptides as a valuable motif for stimuli-responsive peptide gatekeepers for MSNs. As a model system, we prepared Fmoc-CGGC-SS-Si, which exhibited a zero-release property without any stimuli in physiological conditions due to a turn-like conformation induced by the intramolecular disulfide bond. Upon addition of GSH, the guest molecules in the MSN were triggered to be released by the conformational conversion from a turn-like to a random structure, induced by reduction of the disulfide bond. We further demonstrated that the conversion of the secondary structure of Fmoc-CGGC by Zn(II) ion can also be utilized as a useful trigger for stimulus-responsive gatekeeping. These results

provide valuable information for the optimized design of stimuli-responsive multifunctional peptide gatekeepers which would be useful delivery vehicles with on-demand release characteristics.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, FT-IR spectra, and TEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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