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Temperature-controlled release by changes to the secondary structure of peptides anchored on mesoporous silica supports

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Changes in the conformation of a peptide anchored onto the external surface of silica mesoporous nanoparticles have been used to design novel temperature-controlled delivery systems.

In the last few years, anchoring organic or biological molecules on certain inorganic supports has resulted in the design of hybrid materials showing advanced cooperative functional behaviors.1 One appealing concept in this area is related with the design of gated solids for advanced delivery applications.2 These new materials contain switchable molecular-based entities which control the on-command release of previously entrapped guests. In this context, silica mesoporous supports have been widely used as scaffolds given their distinctive characteristics, such as inertness, robustness, thermal stability, high homogeneous porosity, tunable pore sizes and high loading capacity.3 Moreover, by decorating the mesoporous material with a wide collection of organic moieties, linkers and capping agents, researchers have prepared systems that can be triggered with target stimuli, such as light,4 changes in pH5 or redox potential,6 temperature,7 and the presence of certain ions, molecules or biomolecules.8 In particular, the development of gated mesoporous silica nanoparticles using bio-molecules is highly appealing and, for instance, aptamers,9 antibodies,10 DNA fragments11 and peptides12 have been used as caps in the preparation of advanced gated nanodevices.

When dealing with the stimuli available for uncapping protocols, changes in temperature are an attractive trigger that can be used by simply selecting global or local temperature changes.

In previously reported examples, delivery at a certain temperature has been achieved using the thermosensitive poly(N-isopropylacrylamide) (PNIPAAm) polymer,13 paraffins14 or supramolecules such as rotaxanes15 as caps. Yet despite these interesting abiotic examples, the use of bio-molecules for the preparation of nanoscopic gated materials that are able to release an entrapped cargo upon changes in temperature is rare. In this context, unique reported examples deal with the use of temperature-induced ds-DNA melting processes.16 Moreover, the use of small peptide sequences to prepare temperature-driven-controlled delivery nanodevices has been described very recently.17

Scheme 1. Schematic representation of gated material S1-P. The release of the loaded safranine dye was achieved by a progressive α-helix-to-disordered transformation when temperature increased.

In this context, we envisioned a new approach to design gated materials in which peptides could act as caps and in which uncapping process would be triggered by changes in temperature. The underlying idea was to use the well-known temperature-controlled α-helix-to-disordered transformation that occurs in certain peptides in order to design new gated supports. With this application in mind, we have prepared a series of mesoporous silica nanoparticles using a short, disordered peptide sequence, aromatic tryptophan, as caps. Upon increases in temperature, the peptide undergoes a conformational change that results in loss of its secondary structure, subsequently releasing the entrapped guest. Herein, we report the synthesis and characterization of mesoporous silica nanoparticles functionalized with short peptide sequences, as well as the use of these materials for temperature-triggered delivery of encapsulated molecules.
aim in mind, a self-aggregating 17-mer peptide, designed to adopt a high level of alpha-helical conformation, was used as a biocompatible gate. Folding in α-helical bundles was expected to inhibit cargo delivery, whereas transformation to a disordered conformation would reduce the steric crowding around the pore outlets with the subsequent cargo release.

The designed capped support is depicted in Scheme 1. It is based on the use of mesoporous silica nanoparticles loaded with a suitable dye (i.e., safranine O) and containing the 17-mer peptide anchored on the external surface. The capping peptide sequence was based on the previously well-characterized peptide scaffold Ac-SAAEAXAXXAEAXAKG-NH₂.18 Fixed and variable positions on the scaffold were designed to preserve the tendency of the peptide to fold into an α-helix conformation, and to minimize alternative secondary structures while allowing sequence diversity. Residues Ser-1 and Gly-17 have N- and C-terminal α-helical, end-capping properties.19 Two charged residues Glu (E; positions 4 and 12) and Lys (K; positions 8 and 16), were incorporated to favor both aqueous solubility and the formation of salt bridges, which stabilize the helical-bundle conformation.20 Alanine residues were chosen for their intrinsic alpha-helix-stabilizing properties.21 The final sequence selected was H-SAAEAYKRIAELAKG-OH (P).

The starting MCM-41 mesoporous support was prepared by using tetraethyl orthosilicate (TEOS) as a hydrolytic inorganic precursor and the surfactant hexadecyltrimethylammonium bromide (CTABr) as a porogen species.22 After removing the surfactant by calcination, the MCM-41 solid was obtained. The MCM-41 structure of the starting material was confirmed by X-ray diffraction and transmission electron microscopy (TEM, see Figure 1). The N₂ adsorption-desorption isotherms of the prepared phase show a typical type IV curve with a specific surface area of 1096.5 m² g⁻¹, a narrow pore size distribution and an average pore diameter of 3.09 nm. The inorganic support was then loaded with safranine O as a suitable reporter and the outer surface was functionalized with the 3-(azidopropyl)triethoxysilane groups (solid S1). In another step, the final capped nanoparticles S1-P were prepared by grafting the corresponding 4-pentenoic-P derivatives onto the surface of S1 by using the copper(I)-catalyzed Huisgen azide/alkyne 1,3-dipolar cycloaddition “click” reaction.23

Solid S1 was characterized by using standard procedures (see Supporting Information). The X-ray diffraction pattern of S1 (see Figure 1) indicates that the loading process with the dye and the further functionalization with azido groups did not modify the structure of the mesoporous scaffold. This can be concluded from the presence of the (100) diffraction peak characteristic of the MCM-41-type mesoporous materials. Furthermore, the presence of the mesoporous structure in the final functionalized solid S1-P was also confirmed by TEM analysis (see Figure 1). The final S1-P material was obtained as spherical particles with diameters of approximately 80-100 nm. The N₂ adsorption-desorption isotherm of S1 (see Supporting Information) was typical of mesoporous systems with filled mesopores, and a significant decrease in the N₂ volume adsorbed was observed (a specific surface area of 90.7 m² g⁻¹ for S1 was determined) when compared with the starting MCM-41 material. The organic content in S1 and S1-P was determined by thermogravimetric and elemental analyses. In particular, solid S1 contained 0.544 mmol of safranine O/g SiO₂ and 0.246 mmol of azide/g SiO₂, whereas the amount of organic matter in S1-P was 0.250 mmol of safranine O/g SiO₂ and 0.041 mmol of P/g SiO₂. Taking into account an external surface area of S1 (ca. 80 m² g⁻¹) and the amount of azide and peptide in S1 and S1-P solids the surface coverage was estimated to be 1.56 azide/nm² and 0.26 peptide/nm² with average distances of 8 and 19.6 Å for azide and peptide respectively.

Figure 1. Powder X-ray patterns of a) as-synthesized MCM41, b) calcined MCM-41, c) solid S1 containing safranine dye and 3-azidopropyltriethoxysilane and d) final solid S1-P. TEM images of e) solid S1-P showing the typical hexagonal porosity of the MCM-41 mesoporous matrix.

To evaluate the structural changes of peptide P in solution, circular dichroism (CD) spectroscopy studies were performed in 10 mM phosphate buffer at pH 7 with 25 mM NaCl, and the thermal denaturation curves were recorded at different temperatures to study the thermal stability of the peptide. The CD spectra of P at low temperatures exhibited two strong negative bands at 222 nm (assigned to the amide n→π* transition) and 208 nm (amide π→π* and a strong positive band at 190 nm (amide π→π*), which are characteristic of the peptides adopting a helicoidal conformation that changes to a random coil disposition upon heating (see Figure 2). Moreover, the CD spectrum evidences that the partial helicity loss (or denaturation) is reversible upon cooling to the original temperature.

In another step, the gating properties of the solid were studied. In a typical experiment, S1-P was suspended in phosphate buffer (pH 7) and the suspension was stirred at the same temperatures as those used for the CD measurements. At a certain time (3 h), the suspension was centrifuged to remove the solid. Dye delivery into the solution was then measured by safranine O fluorescence at 585 nm (λ ex 520 nm). The delivery profile of the dye at different temperatures is displayed in Figure 2. Solid S1-P was tightly capped up to a temperature of ca. 40°C and then the amount delivered increased with temperature. Whereas a simple temperature-dependent diffusion-controlled process would result in a continuous cargo delivery, Figure 2 shows that no payload release was observed from S1-P over a wide temperature range (from 4°C to ca. 40°C), which
This result suggests that mesoporous nanoparticles functionalized with certain peptides can be used to design capped materials in which the cargo delivery can be triggered by temperature changes. After bearing in mind that the transformation from α-helix to a random coil is reversible (vide ante), it also occurred to us that SI-P could be reloaded and reused. In order to test this appealing possibility, solid SI-P was suspended in PBS at 90°C until safranine O was completely released. Then the solid was filtered and dried under vacuum. Afterward, the empty solid was suspended in a PBS solution of safranine O at 90°C. Then the solid was suddenly introduced into an ice bath (in order to assure the rapid transformation of peptides into their α-helix conformation), filtered and dried. By means of UV-vis studies, the safranine O content in the reloaded material was determined as 0.147 g dye/gSiO₂, a value which comes close to that found in the starting SI-P. Furthermore, the re-loaded solid was suspended in PBS at 4°C for 12 h and no delivery was observed (< 4% of loaded dye). Moreover, the studies of the cargo release at different temperatures displayed a similar profile to that shown in Figure 2.

In summary, herein we report for the first time a mesoporous hybrid material capped with a peptide sequence capable of releasing an entrapped dye (safranine O) by changes in temperature. Cargo delivery correlated well with the change of the peptide conformation from α-helix to a random coil. Moreover, the peptide-functionalized support can be reloaded and reused by taking advantage of the fact that the α-helix to the random coil conformation transformation is a reversible process. The possibility of using different mesoporous supports and a large variety of peptide sequences makes this approach appealing for the design of new temperature-responsive reusable gated materials for different applications.

Notes and references


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