Molecular Gates

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Enzyme-Responsive Controlled Release Using Mesoporous Silica Supports Capped with Lactose**

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The development of gated stimuli-responsive materials is a new research field that brings molecular and supramolecular concepts to the frontiers of the nanoscience.[1] Most of these systems are inspired by biochannels and biogates, and in biotools in general, that utilize movable mechanisms triggered by specific stimuli. One appealing application of such stimuli-sensitive systems is their use as containers that are able to deliver cargo under controlled conditions using external triggers.[2] Traditional delivery systems are based on polymers that are usually not stimuli-responsive but release the cargo by diffusion-controlled processes or through degradation of the polymer container.[3] As a promising alternative, silica mesoporous supports (SMPSs) provide unique features, such as stability, biocompatibility, large load capacity, and the possibility of including gate-like scaffoldings on the external surface for the design of nanodevices for on-command delivery applications.[4] To date, several SMPS-based systems with control release properties have been reported that contain different gated structures, which in most of cases use pH, redox changes, and light as stimuli for uncapping the pores.[5] Despite these reported examples, the approach of using gated SMPSs for the development of real delivery systems is still in its infancy. For instance, some of the reported solids have gating features in non-aqueous solvents, or employ external stimuli that can not be used under physiological conditions. Additionally, and in particular, there is an almost complete lack of SMPS-based systems with selective delivery in the presence of target biomolecules. To our knowledge, only two recent examples that involve the use of enzyme-mediated hydrolysis for the controlled opening of silica nanocontainers have been described.[6,7] In a first proof-of-concept, Zink et al. loaded a mesoporous support with rhodamine B, and functionalized the external surface with a [2]rotaxane (formed by the inclusion of an α-cyclodextrin onto a polyethyleneglycol fragment) that was capped with an ester-linked adamantyl stopper.[6] The functionalized SMPS device showed "zero release" until the addition of porcine liver esterase (PLE) that induced dethreading of the [2]rotaxane owing to hydrolysis of the adamantyl ester. In a second example, Bein et al. attached avidin caps on biotinylated SMPSs. The avidin–biotin complex formation resulted in a tight closure of the pores. Addition of the protease trypsin resulted in the hydrolysis of the attached protein avidin and release of the entrapped guest (fluorescein).[7] However, and despite the appeal of this attractive stimuli-responsible strategy on SMPSs, no more examples of enzymatic triggers have been reported.

Herein, we describe the synthesis of a lactose-capped SMPS that is selectively uncapped using β-D-galactosidase by the rupture of a glycosidic bond. Enzyme β-D-galactosidase is a glycoside hydrolase involved in the hydrolysis of the disaccharide lactose into the monosaccharides galactose and glucose. In humans, β-D-galactosidase is present predominantly along the brush border membrane of the differentiated enterocytes lining the vili of the small intestine. The prepared gated material S1 is depicted in Scheme 1.

The SMPS (a calcined MCM41-like material) was prepared following well-known procedures.[8] The MCM-41 structure of the starting material was confirmed by X-ray diffraction (Figure 1) and TEM. The N_2 adsorption–desorption isotherms showed a typical type IV curve with a specific surface area of 1200 $m^2 g^{-1}$, a narrow pore distribution, and average pore diameter of 2.4 nm (Figure 2, Table 1). This SMPS was first loaded with $[Ru(bipy)_3]Cl_2$, which was used as dye for monitoring the enzyme-triggered process. The SMPS was then reacted with the capping molecule **3**, which is a lactose derivative that consists of β-D-galactose and β-D-glucose monosaccharides linked through a β1→4 glycosidic bond. Molecule **3** was prepared by reaction of lactose **1** with 3-aminopropyltriethoxysilane (**2**) in ethanol (see the Support-

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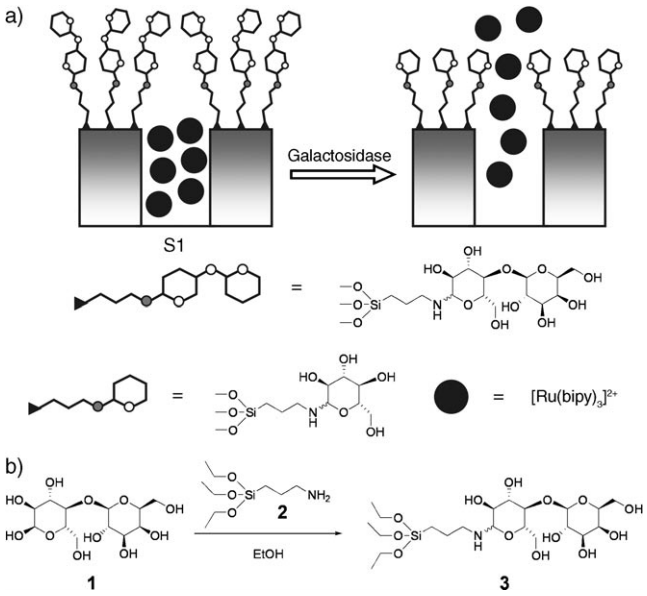

Scheme 1. a) Representation of the gated material S1 capped with trialkoxysilane lactose derivative **3**. b) Synthesis of **3**.

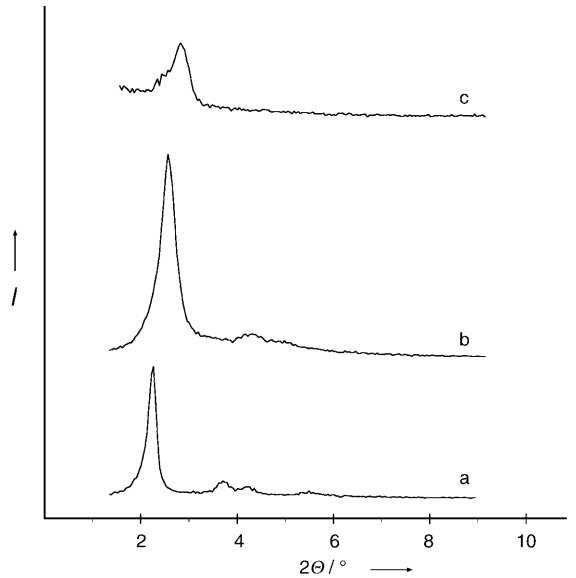

Figure 1. X-ray pattern of a) MCM-41 as synthesized, b) MCM-41 calcined, and c) the S1 hybrid material.

ing Information).[9] The anchoring of **3** is expected to inhibit cargo delivery owing to the formation of a network of disaccharides linked by hydrogen bonding interactions around the pore outlets. Addition of β-D-galactosidase would then uncap the mesopores by hydrolysis of the 1→4 glycosidic bonds, allowing the release of the guest.

Standard techniques were used for the characterization of S1. Figure 1c shows the powder X-ray pattern; S1 has the expected features of the MCM-41 phase with a reduction of the low-angle peak intensity (related to a reduction in contrast because the pore voids are filled with the ruthenium(II) complex). Additionally, the MCM-41 mesoporous

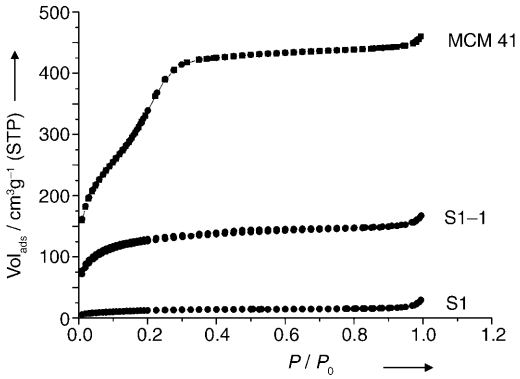

Figure 2. Nitrogen sorption isotherms of the samples MCM-41, S1, and S1–1.

Table 1: BET specific surface values, pore volumes, and pore sizes calculated from the N_2 adsorption–desorption isotherms for selected materials.

	S_{BET} [$m^2 g^{-1}$]	Pore volume [$cm^3 g^{-1}$]	Pore size [nm]
MCM-41	1200	0.47	2.40
S1	45	0.04	–
S1–1	450	0.15	–

type structure of S1 is clearly observed in the TEM image (see the Supporting Information). From thermogravimetric and elemental analysis, contents of S1 were calculated to be 0.283 lactose groups/Si ($mmol\,g^{-1}$) and 0.194 dye molecules/Si ($mmol\,g^{-1}$).

The N_2 adsorption–desorption isotherms of S1 are typical for mesoporous systems with filled mesopores (Figure 2). This solid shows flat curves when compared to those of the MCM-41 parent material at the same scale, thus indicating significant pore blocking and the subsequent absence of appreciable porosity. This result is expected bearing in mind that the solid has a high content of dye filling the pores. This lack of porosity together with the typical mesoporous-like X-ray powder diffraction profile and the TEM images for S1 (see the Supporting Information) provide direct evidence of the high efficiency of the dye loading.

To study the enzyme-responsive controlled release in detail using the SMPS S1 capped with lactose, several experiments were carried out. The response of S1 at pH 7.5 in the absence of β-D-galactosidase was studied; this pH value was selected as it is the pH in the intestine at which enzymatic saccharide degradation reactions typically occur. In a typical experiment, 10 mg of S1 were suspended in 25 mL of water, the suspension was stirred, and the delivery of the dye was monitored by the fluorescence band of the $[Ru(bipy)_3]^{2+}$ dye at $\lambda_{max} = 610$ nm in the aqueous phase ($\lambda_{ex} = 454$ nm).[10] A negligible release of the cargo was observed (Figure 3). It was also found (not shown) that release of the cargo was negligible at acidic pH (simulating conditions in the stomach).

In contrast, the presence of the enzyme induces cargo release from S1 (Figure 3). As stated above, this behavior is ascribed to the enzymatic hydrolysis of the glycosidic bond in the anchored lactose that induces a release of the galactose

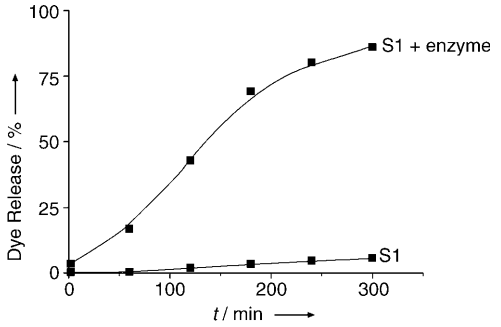

Figure 3. Release profiles of $[Ru(bipy)_3]^{2+}$ dye from water suspensions of gated solid S1 in the absence and in the presence of enzyme at pH 7.5.

fragment, leaving only a glucose derivative anchored to the surface. This hydrolysis results in a decrease in the size of the appended group, allowing delivery of the entrapped dye. The lactose modification by the action of the enzyme was confirmed by the detection of galactose, obtained through the galactosidase-induced rupture of the 1→4 glycosidic bond, in the aqueous solution using ^{1}H NMR spectroscopy and HPLC experiments. The release profile of S1 in the presence of β-D-galactosidase also shows a constant release of the cargo over at least five hours, which is an especially interesting behavior that avoids peaks in the rate of delivery.

To further study the effect that the enzyme attack has on the pore accessibility, the solid S1–1 was prepared and characterized. S1–1 was obtained from S1 after the uncapping process using β-D-galactosidase and further release of most of the entrapped dye. S1–1 still contains residual color, which is most likely due to strongly adsorbed dye that could not be delivered under the experimental conditions. The N_2 adsorption–desorption curve of S1–1 is somewhat similar in shape to that of S1 (Figure 2). However, both the Brunauer–Emmett–Teller (BET) surface area and the Barrett–Joyner–Halenda (BJH) pore volume (Table 1) show a large increase after the dye release, indicating that a significant level of porosity is recovered.

From the above results, it can be deduced that relatively small changes in the pore-blocking structure (the transformation of a disaccharide into a monosaccharide) results in remarkable changes in the delivery behavior. To further demonstrate this point, we prepared solid S2, which is similar to S1, but contains the commercially available monosaccharide *N*-(3-*t*riethoxysilylpropyl)gluconamide on the surface (see the Supporting Information). Whereas the disaccharide-functionalized material S1 shows negligible release without enzyme present (Figure 3), the monosaccharide-containing material S2 shows a significant $[Ru(bipy)_3]^{2+}$ delivery under similar experimental conditions (see the Supporting Information, Figure S4). These results thus show that the induced delivery in the presence of galactosidase is due to the enzymatic-mediated rupture of the glycosidic bond and size reduction of the saccharide chain at the surface.

Further control experiments were carried out. A hybrid material containing the $[Ru(bipy)_3]^{2+}$ dye (S3), but lacking the anchored disaccharide **3**, was synthesized to assess the effect of the lactose grafted in the outer of the MCM-41 pores. Aqueous suspensions of S3 alone at pH 7.5 showed a fast dye release; however, dye release was completely inhibited from S3 in the presence of β-D-galactosidase, which is most likely due to an unselective adsorption of the enzyme on the surface through interactions with the silanol groups. This behavior draws attention to the crucial role played by the lactose moieties in the gating mechanism; that is, the grafting of the lactose onto the surface SMPS prevents the unspecific adsorption of the β-D-glacatosidase enzyme but allows the enzymatic rupture of the lactose pore-blocking caps and cargo release.

To further demonstrate that β-D-galactosidase is responsible for dye release, two additional experiments were carried out. In one experiment, the enzyme was denatured by heating enzyme solutions (pH 7.5) at 60 °C for 60 min before addition of S1. In the other, solid S1 was treated with the digestive protease pepsin. In both experiments, no release of the dye was observed, which again points to the selective β-D-glacatosidase enzymatic hydrolysis as the mechanism responsible of the opening of the mesopores.

In conclusion, we have demonstrated that the attachment of a lactose derivative as gatekeeper on the surface of SMPSs supports provides a suitable method for the design of mesoporous systems that are able to deliver entrapped guests by a biocontrolled uncapping using β-D-galactosidase. We believe that gated systems based on SMPSs containing gate functions triggered by target biomolecules, such as enzymes, is a very promising route for the development of smart controlled-delivery nanodevices for potential biomedical applications, and a number of new advances in this area are anticipated.

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