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Selective external surface functionalization of large-pore silica materials capable of protein loading

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ABSTRACT

Differentiating the chemical properties of the external and pore surfaces of sol-gel derived mesoporous materials by selective functionalization is important to advancing their application as platforms for biological catalysis, sensing and drug delivery. Prior selective functionalization approaches to concentrating functional groups at the external surface of particles have been limited to small pores (≤5.5 nm diameter) incapable of loading large biological molecules. This work investigates the selective exterior surface functionalization by amines of larger-pored (>7 nm diameter) mesoporous silica particles, which are synthesized by dual surfactant templating and hydrothermal aging. Previously developed selective functionalization techniques rely on choice of functionalization precursor, functionalization reaction time, or pore blocking (by leaving pore templates in as-synthesized materials). The effectiveness of these strategies are compared for larger-pored materials using the precursors (3-aminopropyl)tris(methoxyethoxyethoxy)silane (APTMEES) and (3-aminopropyl)triethoxysilane (APTES). The extent of amine functionalization is determined as a function of precursor reaction time (10 or 20 min) in both as-synthesized and template-extracted materials by confocal laser scanning microscopy of the ~10 μm diameter particles tagged with fluorescein isothiocyanate. Reaction time, regardless of pore template presence, is demonstrated to be the controlling variable for achieving selective exterior functionalization in these larger pored mesoporous materials. Under the conditions used, 10 min of functionalization with APTMEES localizes amine groups at the exterior of the particles, while 20 min functionalizes both the exterior and the interior pore surfaces. Protein accessibility within pores, before and after selective and full functionalization, is visually confirmed by confocal fluorescence imaging of Rhodamine B tagged lysozyme loaded particles.

1. Introduction

Complementary advances in the synthesis of mesoporous silica materials (MSMs) and the uses of proteins in biotechnology present new opportunities for enzyme catalysis, protein delivery, and protein-based sensing using silica platforms [1–7]. The synthesis of templated mesoporous silica materials with tunable ordered pores of size appropriately large for protein loading (5 nm–12 nm) has become routine [8,9]. These materials can be synthesized in a variety of morphologies such as thin films and particles [10,11]. The uses of enzyme loaded mesoporous silica materials range from biosensors to biocatalysts for the detection of a variety of compounds such as carbohydrates, aromatics and aquatic toxins [12–17].

The benefits of using mesoporous silica materials for the entrapment of proteins stem from the tunable properties of the materials. SBA type materials, synthesized with the tri-block copolymers such as Pluronic P-123 ((ethylene oxide)$_{20}$(propylene oxide)$_{70}$(ethylene oxide)$_{20}$), have transformed the synthesis of ordered large-pored metal oxides with a variety of tunable morphologies such as thin films and particles with different ordered mesostructures [10,11,18]. The addition of the ionic surfactant cetyltrimethylammonium bromide (CTAB) permits the synthesis of SBA-15 particles with spherical morphology and diameters in the micron range [10]. Hydrothermal aging of particles has proven to be an effective means of tuning pore diameters from 3.5 nm to 11.6 nm using temperatures between 60 °C and 130 °C [19–21]. Tunable pore sizes within mesoporous silica particles can be used for

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protein protection, protein separations and to sustain protein activity [21,22].

Protein loading within the pores of mesoporous silica is frequently established by bulk measurements of protein concentration or activity assays before and after exposure to particles [15,23]. However, activity measurements can be difficult to interpret clearly due to limited accessibility of the substrate to the enzyme within pores. In addition, neither method can directly determine the location of proteins within the materials. Confocal laser scanning microscopy (CLSM), on the other hand, has the benefit of using fluorescent imaging to visualize the location of proteins on the surface or within the particles [22,24]. For example, the diffusional resistance of enhanced green fluorescent proteins has been visualized within particles with pore sizes between 2.9 nm and 5.5 nm in both rod shaped and spherical particles [25]. Size selective protein separations have been visually confirmed using fluorescently tagged lysozyme and bovine serum albumin within 12 nm diameter pores of SBA-15 silica [22]. Recently, our group has demonstrated the use of spherical MSMs for pore-size dependent protection of proteins within SBA-15 spherical particles in the presence of hydrolyzing proteases [21]. Hydrothermal aging was used to tune pore sizes between 5.4 nm and 11.6 nm in diameter where an optimum diameter of 7.3 nm permitted access of a smaller fluorescent protein, while protecting it from a larger protease. CLSM provided both confirmation of fluorescent activity and location of the protein within the particles.

The synthesis of separate organic functional domains, either on the exterior particle surface or in the pores, has the potential to further enhance the application of large-pored organic – inorganic porous structures for protein applications. Selective functionalization could regulate particle interactions with their environment, covalently anchor proteins either within pores or on the exterior surface of particles, screen molecules entering and exiting pores or be used in particle targeting with antibodies. Functionalization of mesoporous silica with amines, in particular, has been shown to stabilize enzymes in mesopores [26], to modulate interactions of peptides and proteins with silica [27–31] and to control particle uptake and cytotoxicity [32–35]. Due to the versatile chemistry of primary amines on the surface of silica, amine functionalization allows for direct tagging with fluorophores or use as a building block for more advanced functionalization [24,36,37]. Precursors typically used to modify mesoporous silica include aminopropyl alkoxysilanes (e.g. 3-aminopropyltriethoxysilane (APTES) and 3-aminopropyltris(methoxyethoxyethoxy)silane (APTMEES)).

Selective interior or exterior functionalization of ordered mesoporous silica has been demonstrated by using pore blocking in mesoporous silica (where an organic functionality is grafted while the pore template still resides in the pores) [38–42], by functionalizing non-selectively followed by diffusion-limited deprotection [43], by adding combinations of precursors at different times to generate layers with different functionality [44,45], by intentionally functionalizing with bulky functional groups, sterically hindered from pore accessibility [46] and by passivating the external surface with an inert organic group followed by adding a second reactive silane to functionalize the internal pore surface [38]. Specifically for amines, selective external functionalization of silica with small mesopores (less than 5.5 nm) has been demonstrated through the selection of an amino silane precursor of suitable size and reactivity, and via pore blocking by the templating surfactant [45,47–49]. For example, Gartmann and Brühwiler demonstrated exterior functionalization using the larger and less reactive aminosilane APTMEES for particles with 2.9 nm pores [24]. By functionalizing mesoporous materials in the presence of the unextracted P123/CTAB template, exterior surface functionalization was demonstrated for pore sizes up to 5.5 nm using APTMEES as the precursor. By varying the solvent, Gartmann and Brühwiler concluded that slow diffusion of (perhaps partially hydrolyzed) APTMEES from a nonpolar medium was the most likely cause for external functionalization, although partitioning and diffusion effects were not considered separately in their work [24]. Selective exterior aminosilane functionalization has been interpreted from FTIR and NMR data [47,48], and visual confirmation of functional groups location via fluorescent tagging has been demonstrated with CLSM [24,37,43]. However, the techniques used to functionalize small pored materials (pore blocking, choice of aminosilanes, and reaction time for functionalization) have not been translated to selective functionalization of large pore porous materials (pore diameter > 5.5 nm) [24,45,47]. Pores for protein loading and protection from environmental destabilization are required to be large in diameter (in excess of 5 nm) to accommodate protein dimensions, so a need exists to test and develop methods for selective functionalization of large pored materials [21,50].

The goal of this work is to demonstrate strategies for the selective exterior functionalization of large-pore (>7 nm diameter) mesoporous silica particles with aminosilanes. SBA-15 sphere (SBAS) materials are employed in this study, which are micron-scale particles with tunable large mesopores obtained through dual-surfactant templating and hydrothermal aging. Synthesis conditions have been chosen (90 °C hydrothermal aging temperature) to obtain 5 μm–15 μm particles, appropriate for imaging by CLSM, with 7.4 nm diameter pores. The aminosilanes, APTES and APTMEES, are selected for their differing reactivity and size. The density of amine functional groups on the silica particles as a function of reaction time with the precursor (10 or 20 min) is examined for both as-synthesized (materials with the pore template left in the pores) and template extracted materials using FTIR and by a fluorescamine assay following dissolution of the particles. Functional group location and protein accessibility in the functionalized material is confirmed via imaging using confocal microscopy after fluorescent tagging.

2. Experimental

2.1. Materials

Hydrochloric acid (12.1 M, ACS certified) and hexanes were purchased from Fisher Scientific. Tetraethyl orthosilicate (TEOS, ≥98%) and Fluorescamine (Synthetic, 100% pure) were purchased from Acros Organics. Cetyltrimethylammonium bromide (CTAB, 98%) was purchased from Research Organics. Ethanol (200 proof) was purchased from Decon Labs. Rhodamine B isothiocyanate (TRITC), fluorescein isothiocyanate (FITC, 90%), 3-aminopropyltriethoxy silane (APTES, 99%) and Pluronic P123 triblock copolymer ($(EO)_{20}(PO)_{70}(EO)_{20}$ where EO is an ethylene oxide unit and PO is a propylene oxide unit, $MW_{avg} = 5800$) were purchased from Sigma Aldrich. 3-aminopropyltris(methoxyethoxyethoxy) silane (APTMEES, 95%) was purchased from Gelest Inc. All amino-silanes were stored under nitrogen after initial use. Handling was performed under nitrogen in a glove bag. Lyophilized lysozyme (LYS, ≥20,000 units/mg) was purchased from MP Biomedicals. Proteins were purified after tagging with TRITC using Thermo Scientific Pierce polyacrylamide desalting spin columns with 6K MW cutoffs from Fisher Scientific. All dilutions were made using 18.2 MΩ DIUF water purchased from Fisher Scientific.

2.2. Materials synthesis

Spherical SBA-15 particles were prepared using synthesis procedures adapted from Gartmann and Brühwiler, as modified from

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