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Characterization of silica particles prepared via urease-catalyzed urea hydrolysis and activity of urease in sol–gel silica matrix

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ABSTRACT

Urease templated precipitation of silica synthesized by sol–gel chemistry produces a composite material allowing high urease activity. This study investigates the structural properties of the composite material that allow for the retention of the urease hydrolysis activity. Scanning (SEM) and transmission (TEM) electron microscopy reveal that the composite has a mesoporous structure composed of closely packed spherical structures ~20–50 nm in diameter. Brunauer–Emmett–Teller (BET) analysis revealed that the surface area and pore volume of the composite prepared under the conditions of 50 mM urea and 25 °C is relatively high (324 m^2/g and 1.0 cm^3/g). These values are equivalent to those of usual mesoporous silica materials synthesized from the self-assembly of triblock copolymers as organic templates. In addition, after encapsulating in a sol–gel silica matrix, urease retained high activity (~90% of the activity compared with native urease). Our results suggest a new method for synthesizing mesoporous silica materials with highly tunable pore sizes and shapes under mild conditions.

1. Introduction

At present, there is great interest in the production of naturally inspired or biomimetic materials and functionalized nanomaterials for use in many areas of research from biology to physics and engineering. Many research areas and industries use lengthy and complex processes that employ harsh chemicals and conditions to fabricate the desired products. Therefore, the field of biomimetic and functionalized materials needs to show improvements in the fabrication and scope of application to reduce production times and hazardous waste while maintaining the optimum conditions for biological materials and reactions [1–4].

Silica particles find varied applications in chromatography, bio-separation, imaging, and the synthesis of multifunctional nanocomposites [5–8]. However, it is important to characterize the particles. In particular, it is important to consider the particle size and morphology because the physical, electrical, and optical properties of silica particles vary with size [9–12]. In addition, since the discovery of ordered mesoporous silica (MPS), great efforts have been extended to advance the morphological/textural control of such ordered mesoporous materials. Among these, the construction of nanoscale MPS has long been one of the most attractive targets for various groups aiming to improve the efficiency of mesopore usage and rapid mass transfer. This is of great importance for application in catalysis, adsorption, and separation as well as in inclusion chemistry [13–23]. It is advantageous if the silica particles can be prepared under neutral conditions using more environmentally friendly chemicals by a biomineralization method utilizing biomolecules such as peptides, enzymes, and microbes [24–27].

Biomimetic synthesis of highly ordered silica from synthetic precursors such as tetraethoxysilane (TEOS) using biomolecules as templates has been extensively studied. This process offers precise control over the nanostructures of the obtained inorganic materials. The most widely explored biomolecules were probably the silaffins isolated from diatom cell walls [28,29] and the silicateins isolated from marine sponges [30]. On the other hand, synthetic polyamines like polyethyleneimines and polyallylamine [24] and cationic polypeptides like poly(L-lysine) and poly(L-arginine) were able to catalyze silicic acid deposition [31–33]. For the former, it was determined that a microphase separation between polyamines and anions was necessary to induce formation of silica nanospheres. For the latter, the inherent secondary structure of polypeptides offered additional parameters to tune the resulting silica structures.

Encapsulation technologies have attracted considerable attention because of an increased interest in fields such as biotechnology, medicine, pharmaceutics, catalysis, ecology, and nutrition [34–36]. Encapsulation can concentrate and shield the biomolecules in order to protect them in a defined volume and to create a single compartment that represents a microenvironment separated from the outer environment. There are several strategies for encapsulating

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biomolecules in sol–gel silica materials [37–40]. However, most of the strategies are based on the usual process, in which alkoxysilanes are mixed with alcohol and polymerized in the presence of acidic or basic conditions. Although this method has been used successfully for many years, it involves heating the particles to temperatures well above ambient as well as the use of highly basic pH; both conditions are highly damaging to proteins and other biomolecules.

Urease (EC 3.5.1.5; urea amidohydrolase) is a nickel-dependent enzyme that catalyzes the hydrolysis of urea to form ammonia and carbon dioxide [41]. Urease immobilized on polymeric membranes, inorganic supports, and microcapsules opened the way for constructing urea sensors, heavy metal sensors, and membrane bioreactors for urea determination and urea removal applications [42–44]. Recently, inorganic materials like hydroxyapatite, calcium carbonate, zinc oxide, and yttrium oxide have been obtained in solutions by enzyme catalyzed decomposition of urea by urease. These enzymatically assisted routes offer the possibility of synthesizing a number of inorganic materials with excellent control over the structural organization [45–47]. However, urease immobilization and encapsulation on silica particles prepared via urease-catalyzed urea hydrolysis reactions are rarely reported. This is especially true for the characteristics of silica particles and the activity of urease inside sol–gel silica networks.

The study presented here used urease to mimic the biosilication process and to determine how silica particle production can be controlled in a systematic way. We used the hydrolytic enzyme and urease combined with the hydrolysis of urea and a hydrolysis and polycondensation of tetraethoxysilane, to form silica particles via nature inspired conditions. Silica particles produced using these enzymatic processes have been characterized by nitrogen adsorption–desorption experiments, thermogravimetric analysis (TGA), and solid-state ^{29}Si- and ^{13}C-nuclear magnetic resonance (NMR). Stability data on enzymes encapsulated during the silication process, which indicates that the enzyme activity is not inhibited as is the case of free enzyme in solution, is also demonstrated for the first time.

2. Experimental

2.1. Materials

Urease from jack bean (molecular weight, 48 kDa; isoelectric point, 5.0; optimal pH 6.0; optimal temperature, 60 °C) was purchased from Wako Chemicals Co., Japan. An urease activity kit (BUN Kinos) was purchased from Kinos Laboratories Inc, Japan. TEOS was purchased from Shin-Etsu Chemical Co., Japan. All materials were of analytical grade and used as received without further purification.

2.2. Preparation of silica particles by urease-catalyzed reactions

Silica synthesis was carried out using urease in a phosphate buffer containing urea as an enzyme substrate. Urease (10 mg) was added to a mixture of 5, 20, or 50 mM urea in a 1 mM phosphate buffer of pH 6.0 (1.5 ml). TEOS (1.5 ml) was added and the mixture was stirred for 3 days at 25 °C. On gel formation, the solid material was crushed in distilled water and the suspension was centrifuged to collect the silica–urease composites. The silica gel was freeze-dried and the solid obtained was refrigerated before use in activity measurements and material characterization.

2.3. Characterization of samples

Particle morphology of silica–urease composite materials was determined by scanning electron microscopy (SEM) using an Hitachi S-3000 field emission (FE) SEM system with 10 kV accelerating voltage for imaging. For transmission electron microscopy (TEM) studies, a small aliquot was taken from a suspension of methanol and placed in a lacey carbon-coated TEM grid that was pulled through suspension and allowed to dry in air. The resulting sample was examined with a JEOL JEM 2010 operated at 200 kV. The surface area and pore diameter were measured using nitrogen (N_2) adsorption/desorption measurements in a Shimadzu TriStar 3000 system. The data were evaluated using the Brunauer–Emmett–Teller (BET) and Barrett–Joyer–Halenda (BJH) models to calculate the surface area and pore volumes/pore size distributions, respectively. Samples were prepared by degassing at 80 °C for 1 h and then at 110 °C for 3 h. Thermogravimetry and differential thermal analysis (TG/DTA) was measured with a Seiko Instruments Japan TG–DTA 320 using a heating rate of 10 °C/min in the range room temperature −1000 °C. N_2 gas flowed in during TG–DTA after the TG–DTA furnace had been evacuated; the amount of powder used for each measurement was approximately 15 mg. To characterize protein stability in sol–gel silica matrix, a differential scanning calorimeter (DSC, Seiko Instruments 120U, Japan) was used and the sample temperature was increased from 30 to 120 °C at a scanning rate of 1 °C/min in a nitrogen atmosphere using Al_2O_3 as a reference. Solid-state NMR experiments were performed on Varian Unity Inova 300 NMR spectrometer equipped with a Varian 7 mM probe. Larmor frequencies for ^{29}Si and ^{13}C nuclei are 59.587 and 75.429 MHz, respectively. ^{13}C cross-polarization (CP) magic-angle spinning (MAS) NMR spectra were obtained using the regular ^{1}H–^{13}C CP/MAS sequence at 2 ms contact time with an H decoupling radio frequency as the continuous wave. The CP/MAS spectra were measured with 15,000 scans at the magic angle at 4 kHz with 5 s recycled time. ^{29}Si dipolar decoupling (DD) MAS NMR spectra were obtained using ^{29}Si single pulse excitation with H decoupling radio frequency as a continuous wave. The DD/MAS spectra were measured with 864 scans at the magic angle at 5 kHz and 100 s recycled time. Deconvolution of the ^{29}Si MAS NMR spectra was performed with PEAKFIT 4.12 software. The ^{29}Si and ^{13}C chemical shifts were externally referenced to trimethylsilane (TMS) at 0.0 ppm. Zeta potentials of silica particles and urease were determined in a 10 mM phosphate buffer solution at pH 7.0 using a zeta potential and particle size analyzer (ELSZ-2, Otsuka Electronics Co., Japan).

Table 1
Specific surface areas and pore volumes of the prepared silica particle.

Urea conc. (mM)/ reaction temp. (°C)[a]	Total dry mass of silica (mg)	Surface area[b] (m^2/g)	Pore volume[c] (cm^3/g)
5/25	13	15.4	0.04
20/25	26	93.5	0.23
50/25	110	234.3	0.98
50/4	168	324.5	1.00
50/60	365	151.5	0.54

[a] Reaction conditions are detailed in the experimental section.
[b] Specific surface area is determined according to BET.
[c] Obtained according to BJH analysis for an adsorption branch of diameters ranging from 1.7 to 300 nm.

2.4. Activity assay of urease encapsulated in sol–gel silica particles

Catalytic activity of silica–urease composites and free solution enzyme were assayed using a urease activity kit (a colorimetric assay) following the procedure from the supplier. Free urease (1 mg) or silica–urease composite (one-tenth of total composite yield, Table 1) was tested for enzyme activity. The catalytic activity of free enzyme solution before encapsulation was assumed to be 100%.