Notes

Synthesis of Ordered Biosilica Materials

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Biogenic silica with amazing diversity of nanostructure shells, fibers and granules in diatoms and sponges is mediated by proteins and polysaccharides and forms at ambient pressure and temperatures. Chemical synthetic methods, in contrast, have to rely on extreme pH and/or surfactants to induce the condensation of silica precursors into specific patterns. One kind of benign synthesis method through plant cell wall template-directed ordered biosilica materials under ambient conditions is intriguing in this context. Organized silica materials in intercellular spaces of epidermal cells of tall fescue leaves were synthesized through molecular recognition between Si-OH and polysaccharide-OH or glycoprotein-OH of main components of plant cell walls and cellular processing as well when $Si(OEt)_4$ was supplied rather than monosilicic acid. The biosynthesis of structural silica in tall fescue plant was correlated with the Si species applied, reflecting the slower condensation from tetraethoxysilane (TEOS) and thus providing greater opportunities for structural control by the underlying matrix of cell walls. The composition was estimated by energy dispersive X-ray (EDX) spectra on a scanning electron microscope. All organized structures showed carbon, oxygen and silicon peaks, indicating that their formations differ from natural siliceous process.

Keywords ordered biosilica material, template synthesis, biomineralization, cell wall, tall fescue plant

Introduction

The precise control of the polymerization of silica precursors determines the structures and properties of a wide range of siloxane-based materials. In contrast to anthropogenic and geological syntheses of these materials that require extremes of temperature, pressure, or pH, living systems produce a remarkable diversity of nanostructured silica at ambient temperatures and pressures and at near-neutral pH.[1] In biological systems such as diatom and sponge, the formation of structural silica with precisely controlled morphology is directed by proteins and polysaccharides.[2,3] Cha *et al*. showed that silicatein—a protein found in the silica spicules of the sponge—can hydrolyse and condense the precursor molecule tetraethoxysilane (TEOS) to form silica structures with controlled shapes under ambient conditions.[4,5] Moreover, Kröger *et al*. isolated a set of polycationic peptides (called silaffins) from diatom cell walls of lower plants that induce the formation of silica nanospheres at neutral pH *in vitro*.[6,7]

The capacity of plants for soluble silica absorption varies considerably according to genotype and environments. After absorption of silica precursors into plant body, the biomineralization processes begin to form

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amorphous silica that encompasses an almost infinite variety of structural forms from ordered aggregates to extended gel-like materials.[8] The sequence of biomineralization events appears to be linked to changes in organic cell wall template deposition.[9] The role of organic templates or membrane substrates may be essential to nanofabrications of biosilica since they provide organized surfaces for silica decoration.

A clear solution of tetraethoxysilane (TEOS) and Na_2SiO_3 was added as Si sources of plant absorption, respectively, in culture medium to cultivate zoysiagrass (*Zoysia japonica L.*)[10,11] and aloe (*Aloe vera L.*)[12] because they are silica-accumulating plants. The elaborate mesostructured silica in intercellular spaces of epidermal cells of zoysiagrass leaves and leaf spicules of aloe plant was synthesized when $Si(OEt)_4$ was applied rather than monosilicic acid molecular species. It was found that different cell walls did affect significantly the formation of the ordered SiO_2 that are controlled by amphiphillic properties and stereochemical nature of active cell walls composed mainly of cellulose microfibrils and matrix polysaccharides.

In this paper, to examine whether other plants with different cell walls of leaf epidermal apoplast and Si chemical forms affect the patterns and the cooperative synthesis of ordered mesostructured biosilica materials, here we describe the generation of the highly ordered silica mediated by cell wall template of tall fescue (*Festuca arundinacea*) plant.

Experimental

Seedling cultures

To examine the depositions of silica in the leaves, germinated boxes were used to control growth conditions in the incubator chamber. TEOS and sodium silicate (Na_2SiO_3) were chosen and dropped directly into the sterilized seed surfaces in the germinated boxes. Meanwhile, the growth humidity was kept with distilled water. The leaves of tall fescue were cut in thin sections and deposited onto Formvar-coated Cu grids for transmission electron microscope 10 days after germination.

TEM *and* SEM *characterization*

Leaves of tall fescue were prefixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.4, 2 h), fixed with 1% OsO_4 in the same buffer (2 h), dehydrated in a series of acetone and embedded in spur resin. Thin sections of glutaraldehyde fixed and unstained leaves were collected on Cu-grids and viewed with a transmission electron microscope (JEOL-200 cx) and scanning electron microscope (JEOL-6301F).

Result and discussion

TEM images illustrating the biosilica of the intercellular spaces of tall fescue showed ordered non-continuous superlattice arrays that are rough in outline and virtually of constant size, the major axes averaging 0.5 μm. Elaborate mesoscopic silica structures in intercellular spaces of epidermal cells of tall fescue leaves were constructed when $Si(OEt)_4$ was supplied rather than monosilicic acid (Fig. 1a—c). It is found that the Si species used did affect significantly the formation of the ordered silica materials comparable to the random arrays that form with Na_2SiO_3 as a control (Fig. 2a and 2b). All organized structures showed carbon, oxygen and silicon peaks (Fig. 3), indicating that their formation differs from natural siliceous process. High-resolution images of individual siliceous domains revealed irregular incoherent fringes in all selected microareas (images not shown).

The deposition mechanism can be explained by the negative surface charge of silanol SiOH groups of the silica precursor which inhibits association with the high hydroxyl group -OH of polysaccharides and glycoproteins existing in the cell wall. Cell wall is fundamental to the structural organization of biogenic silica and acts as spatial restraints by imparting scalar and vectorial control on siliceous structures.

The hydrophilic properties of the cell walls are clearly important in apoplastic molecular recognition interaction between Si sources and the cell wall matrix. Hydrophilic silicic acid hydrogel droplets or clusters spread out on the walls, resulting in a smaller contact angle, whereas a liquid droplet of TEOS solution spreads onto the walls with a relatively large contact angle.[13] This may reflect the slower condensation from TEOS, thus providing greater opportunities for structural control by the underlying matrix of cell walls. When silica is considered to grow by alternative attachment of monosilicic acid and TEOS aggregates to cell wall templates, the

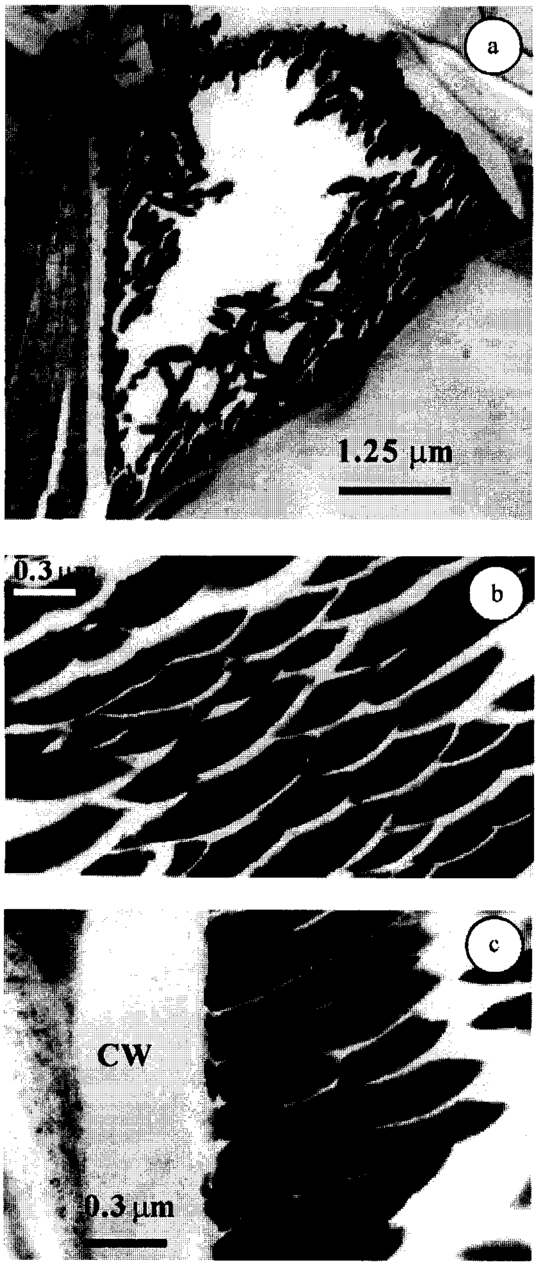

Fig. 1 a, TEM image of biosilica deposited onto the intercellular spaces of leaf epidermal cell of tall fescue plant by applying TEOS. b, ordered micrometer sized spheroidal rod-like silica. c, silica rod self-assembled on the cell wall template (CW) (a, scale bar, 1.25 μm; b and c, scale bars, 0.3 μm).

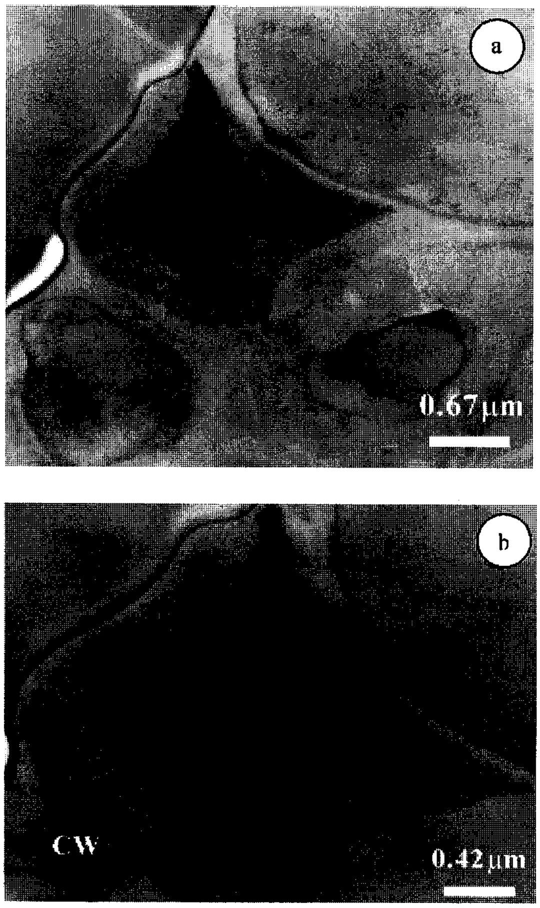

Fig. 2 Disordered silica deposited onto the leaf epidermal cell wall of tall fescue by applying Na_2SiO_3 (a, scale bar, 0.67 μm; b, scale bar, 0.42 μm).

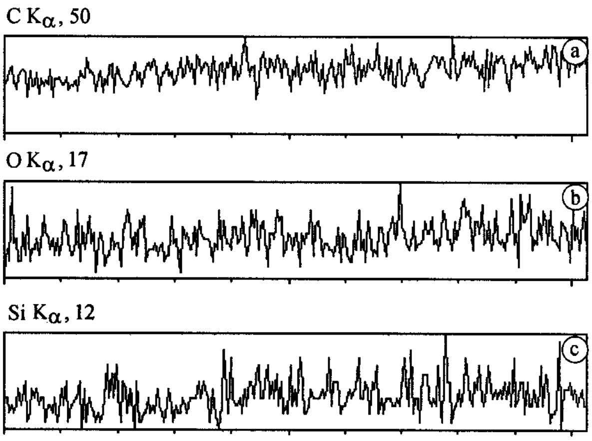

Fig. 3 The concentration variations of the carbon (a), oxygen (b) and silicon (c) elements with scanning one silica rod on a scanning electron microscope (Scanning length, 1.0 μm).

differences of contact angles play an essential role in the initial phases of silica formation.

The biomineralization process as biological strategies offers valuable insights into the scope and nature of biomimetic materials chemistry at the inorganic-organic template interfaces.[14-16] Further work is necessary to determine the sizes and shapes of biosilica controlled by biochemical composition and amphiphilic properties of cell wall templates of plants between organic templates and inorganic precursors as well.

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