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ScienceDirect
Acta Biomaterialia 4 (2008) 725–732
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

A novel method for preparing a protein-encapsulated bioaerogel: Using a red fluorescent protein as a model

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Received 23 June 2007; received in revised form 5 October 2007; accepted 11 October 2007
Available online 26 October 2007

Abstract

A recombinant red fluorescent protein, DsRed, was chosen as a model protein to prepare a protein-encapsulated bioaerogel, DsRed-SAG. It was prepared using sol–gel polymerization of tetraethyl orthosilicate (TEOS) with an ionic liquid as the solvent and pore-forming agent. The DsRed-SAG bioaerogel was characterized by Fourier transformation infrared, scanning electron microscopy and Brunauer–Emmett-Teller measurements. It was found that the as-prepared bioaerogel had high porosity, and the silica network exhibited little shrinkage during the drying process. The stability of the bioaerogel was monitored by fluorescence spectroscopy and confirmed by confocal laser scanning microscopy. In addition, the protection of the encapsulated proteins by the silica network was further investigated using the degradation test by a protease. The results indicated that the as-prepared protein was quite stable during formation of the protein-containing wet gel and extraction of the ionic liquid, demonstrating that the new method can be extended to prepare other protein-encapsulated bioaerogels.

Keywords: Bioaerogel; DsRed; Ionic liquid; Protein-encapsulation

1. Introduction

Since Avnir et al. reported a key paper describing the entrapment of proteins into alkoxysilane-derived silicate glasses via the sol–gel method in 1990 [1], many sol–gel-derived composite materials, entrapped with a wide variety of biological species, including enzymes, antibodies, regulatory proteins, membrane-bound proteins, nucleic acids and even whole cells, have been reported [2–8]. Applications of biocomposite materials studied include the development of biosensors, stationary phases for affinity chromatography, immuno adsorbent and solid-phase microextraction coatings, controlled release agents and solid-phase biocatalysts. The entrapment of biological species into sol–gel materials and their applications have been reviewed by Livage [5], Brennan et al. [9,10], Gill [11] and Pierre [12] Those studies showed that the requirements for useful bioimmobilizates include a high density of immobilized biomolecules, high activity, long-term stability under potentially adverse reaction conditions, good accessibility to analytes, rapid response times, and resistance to leaching and/or desorption [9].

As is known, due to the cross-linking of the matrix, the removal of alcohol and water produced and the drying process, the pores of the immobilized biomolecules prepared

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doi:10.1016/j.actbio.2007.10.007

by the traditional sol–gel polymerization method usually shrink to the range of 2–20 nm while the volume shrinks up to 85% of its initial volume. Therefore, these immobilized biomaterials belong to the dense xerogel type and are difficult to use both in the realm of analysis due to inaccessibility of external analytes to the entrapped proteins and as a biosensor due to slow response times [9].

On the other hand, silica aerogels are materials that usually comprise more than 96% porosity, while the remaining 4% is a network structure of silicon dioxide. Due to their unique characteristics, such as high porosity, large surface area, low density and low thermal conductivity, silica aerogels have been used as advance materials in the applications of thermal insulation, electrical batteries, nuclear waste storage, catalysis, acoustic insulation, adsorbents, etc. [13]. In addition, due to their chemically and mechanically robust and biocompatible properties, silica aerogels also hold promise as biocompatible scaffolds for the immobilization of biological materials in a variety of applications [14] and as matrices in the design of biosensors [14–17]. Nevertheless, to date, these bioaerogels have been prepared either by absorbing the biomaterials after the formation of silica aerogels or by encapsulating the biomaterials in the wet gel followed by a supercritical drying process to avoid the collapse of the structure [15–21]. Since the protein to be encapsulated in the aerogel scaffold is easily damaged in the harsh supercritical drying process, a special method has been reported in which the self-organized cytochrome *c*-containing metal superstructures are encapsulated into a mesoporous silica-based nanoarchitecture [15–17,21]. However, massive amounts of proteins are required for the formation of the metal superstructures by this method.

An easily detected protein can facilitate monitoring of the protein-encapsulation process. Because of their intrinsic bright and visible fluorescence, fluorescent proteins, such as the green fluorescent protein (GFP) and its derivative mutants, have been widely used as reporters in molecular biology studies of gene expression, protein dynamics and localization in various biological systems [22,23]. Recently, a red fluorescent protein (DsRed) gene was cloned from the reef coral, *Discosoma* sp. [24]. The coral-derived red fluorescent protein, with its significantly red-shifted excitation (558 nm) and emission (583 nm) maxima, high stability, quantum yield and relative brightness, has attracted increasing interest for its good complementation to the GFP, with excitation and emission maxima at 488 and 507 nm, respectively.

The conventional supercritical extraction was used for removing the pore fluid in preparation of the aerogel. This is because the method keeps away from the collapse of the gel structure due to avoiding the large capillary forces exerted on the gel structure at the liquid–gas interfaces. In this study, DsRed, a stable, intrinsic bright and visible red fluorescent protein, was chosen as a model to prepare the protein-encapsulated bioaerogel, DsRed-SAG, via the sol–gel polymerization of tetraethyl orthosilicate (TEOS) without using the harsh supercritical drying process. During the preparation of the pure SAG and DsRed-SAG, an ionic liquid (IL) was used as the solvent and the pore-forming agent to maintain the gel structure during long time hydrolysis and condensation. Besides, after extraction out of the IL with ethanol by the Soxhlet extraction, ethanol was frozen and removed by the freeze-drying method to keep their network structures. This is because in the drying process, the frozen solvent was sublimed from solid to vapor; therefore, the large capillary forces exerted on the gel structure at the liquid–gas interfaces were also avoided as in the supercritical method. The as-prepared bioaerogel was characterized by Fourier transformation infrared (FTIR), scanning electron microscopic (SEM) and Brunauer–Emmett–Teller (BET) measurements. Besides, due to the fluorescent characteristic of DsRed, this stable protein was able to be examined by eye and its stability could be monitored by fluorescence spectrometry during the sol–gel polymerization process and after the extraction of IL. Furthermore, the dispersion of the protein was investigated by confocal laser scanning microscopy (CLSM).

2. Experimental procedures

2.1. Materials

The precursor, TEOS, was obtained from Acros Company. Methanol, ethanol, 1-chlorobutane, acetonitrile, sodium tetrafluoroborate and 1-methylimidazol were purchased from Acros. All chemicals were used as received.

2.2. Preparation of 1-n-butyl-3-methylimidazolium tetrafluoroborate (BMIBF) [25,26]

To a solution of 1-methylimidazole (82.0 g, 1 mol) in acetonitrile (50 g) at room temperature 1-chlorobutane (370 g, 4 mol) was added dropwise with stirring overnight. After distilling off the acetonitrile, the remaining salt, 1-butyl-3-methylimidazolium chloride (BMIM) was dried at −50 °C through freeze-drying. Then 218 g (1 mol) of BMIM was added to a solution of sodium tetrafluoroborate (132 g, 1.2 mol) in acetonitrile (100 g). The solution was mechanically stirred for two days at room temperature. The reaction mixture was filtered, and the volatiles were removed under reduced pressure. The resulting ionic liquid product, 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIM^+BF_4^-$, abbreviated as BMIBF), was finally obtained by filtration and dried at −50 °C through freeze-drying.

2.3. Preparation of the silica aerogel

The silica aerogel was prepared by the sol–gel process using the IL, BMIBF, as the pore-forming agent and solvent. In a typical run, 5.8 g (28 mmol) of TEOS, 1.9 g (58 mmol) of methanol, 3.0 g (12 mmol) of BMIBF and 3.4 g (189 mmol) of water were mixed in a bottle. A mono-

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