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SP1 based self-assembled selective molecular nanochannels

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ABSTRACT

Controlling the permeability and porosity of an inorganic layer using biomolecule building blocks has raised interest for nanotechnological applications. The challenge lies mostly in the fabrication, usually a long, expensive and tedious process, involving many steps. Using biomaterials for this purpose is highly appealing; due to both ease of fabrication and the final output, that contains a bioelement. The biomolecule, specifically, stable protein 1 (SP1), serving as the scaffold for our pattern, is of great stability and durability, and presents size, charge and structural selectivity towards electroactive species. Here, we demonstrate the ability of SP1 to form a rigid template within a sol-gel matrix, allowing selective electron transfer to the gold electrode. Specifically, a thiolated SP1 was first adsorbed on a gold surface followed by filling the non-occupied areas by sol-gel. The latter was electrochemically deposited. The various steps were carefully characterized. Finally, we studied the electrochemistry of numerous redox couple at the Au/SP1/sol-gel interface and found that the nanochannel array shows charge and structural selectivity, which is based on the interactions between the redox species and the functionalities of SP1. The resulted surface shows promise towards electrochemical sensing applications.

1. Introduction

The ability to direct nanostructures through self-assembly of organic and inorganic building blocks has both scientific and technological importance. The utilization of nanoporous substrates in applications such as selective ion transport, biomolecule separation, seeded templating, and catalysis, necessitates an efficient control over the porosity and permeability properties of the surface [1,2].

Fabrication of such nanoscale arrays is one of the main challenges in nanotechnology. The common approach to produce nanoarrays relies very often on lithography, which consists of long, complex and expensive multistep processes. An alternative method involves the use of biological materials, such as proteins, to form ordered two-dimensional arrays [3,4]. Proteins are attractive candidates for this task due to their well-defined structure and ability to add structural modifications and functionality using genetic engineering. Such manipulations enable both optimization of the self-assembly and the ability to attach to various inorganic surfaces through affinity tags. However, one of the drawbacks of proteins is their sensitivity to different environmental conditions such as temperature, pH, proteases, etc. Hence, a protein that is both durable and can direct different functional groups is of great interest and could be employed as a building block in designing thin films and functionalized coatings.

Stable Protein 1 (SP1) is a ring like protein complex, self-assembled from 12 identical monomers. The oligomeric form of SP1 reveals an exceptionally stable structure, demonstrated by its stability in high temperatures, protease resistance, wide pH range tolerance and stability in detergents and organic solvents [5,6]. The crystal structure of SP1 reveals a ring diameter of 11 nm, an inner pore of 2–3 nm, and a width of 4–5 nm [7]. This unique conformation of the protein, which is not prone to conformational changes in its structure, allows it to assume an interesting role as a channel, appealing to various applications in nanotechnology. Recently, SP1 and its derivatives demonstrated their ability to generate hydrophilic nanochannels in the plasma membrane of living cells [8]. This feature was further exploited to implement a progressive growth of single metal nanoparticles (NPs) on a membrane self-assembled SP1 template, on indium-tin oxide (ITO) surfaces [9].

Herein, we present an electrode composed of SP1 as template on gold surfaces, confined in a sol-gel matrix. A variant of SP1, *namely*, C81-SP1, was used, which contains cysteine residues on the protein rim and facilitates the protein adherence to gold surfaces [10]. Specifically, the C81-SP1 was initially adsorbed onto an Au surface followed by the electrochemical deposition of a sol-gel matrix using 3-mercpatopropyltrimethoxysilane (MPTMS) to improve adhesion. This resulted in a

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blocking thin layer interwoven with SP1 nanochannels serving as an array of nanoelectrodes. The various steps in assembling this nanostructure were carefully characterized and optimized. Finally, we found by studying a large variety of organic electroactive molecules that the SP1 nanochannel array exhibits charge selectivity as well as it inhibits the permeation of catechol derivatives. Yet, no size selectivity was found for organic or inorganic small molecules.

2. Experimental

2.1. Materials

3-Mercaptopropyltrimethoxysilane (MPTMS, 95%), ferrocenemethanol (97%), ferrocenecarboxylic acid (97.0%), catechol (99 + %), 3,4-dihydroxyphenylacetic acid (dopac, 98%), 9,10-anthraquinone-2,6-disulfonic acid disodium salt (≥ 98%), Potassium hexacyanoferate, (~ 99%), dopamine (98%), Hexaammineruthenium(III) chloride (98%), silver nitrate (99.0%) and pyrrole (98%) were purchased from Sigma-Aldrich; ethanol (absolute), hydrochloric acid (37%) and potassium nitrate (99.0%) from Merck; sodium chloride (AR) and sodium hydroxide (AR) from Bio-Lab ltd.; hydroquinone from Acros Organics; Bacto™ tryptone and Bacto™ yeast extract from BD-Becton, Dickinson and company. All materials were used as received without further treatment. Solutions were prepared from deionized water (Barnstead Easypure, UV system).

2.2. Instrumentation

Electrochemical experiments were carried out using a PC controlled CHI-750B electrochemical workstation bipotensiostat (CH Instruments Inc., USA). Extra-high-resolution scanning electron microscopy (XHR-SEM) images were acquired using Magellan XHR 400 L (FEI Co. Ltd.). Energy-dispersive X-ray mapping (EDX) was carried out with an accelerating voltage of 10 kV. FTIR spectra were collected at a resolution of 2 cm^{-1} using an Equinox 55 (Bruker) spectrometer, equipped with nitrogen cooled mercury cadmium telluride (MCT) detector. For each sample, 2000 scans were collected. Attenuated total reflection (ATR) measurements were collected using the same instrument coupled with an ATR device, for each spectrum, 2500 scans were collected with a resolution of 4 cm^{-1}.

3. Methods

Expression and purification of C81-SP1 variant was performed as described previously [10]. Briefly, the C81-SP1 vector was constructed using site-directed mutagenesis on the ΔNSP1 template previously described in the work by Wang et al. [6]. *E. coli* BL21 (DE3) bacteria were used for expression. Bacteria were grown on Luria–Bertani (LB) media at 37 °C and induced with 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG) added to the media at 0.8 O.D. Cell pellets were resuspended in lysis buffer (50 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$ pH 8), and sonicated. The insoluble pellets were separated by centrifugation at 14000*g* for 15 min. Inclusion bodies of C81-SP1 were washed with IB washing buffer (20 mM Tris-HCl, 2 M urea, pH 8) and then centrifuged at 14000*g* for 15 min. The pellets were resuspended in denaturation buffer (20 mM Tris-HCl pH 8, 6 M urea, 10 mM dithiothreitol (DTT)) and diluted to a protein concentration of 5 mg mL^{-1}. Denatured proteins were then refolded by dialysis against 20 mM Tris-HCl (pH 7) and 1 mM DTT for 4 days. A Hitrap Q Sepharose ion-exchange FPLC column (5 mL, Amersham Biosciences, Piscataway, NJ USA) was used to purify the protein. Proteins were loaded on the column using 20 mM Tris pH 7 buffer at a flow rate of 3 mL min^{-1}. The purified protein was eluted in a stepwise isocratic manner at 400 mM NaCl.

The substrates were thermally evaporated Au (200 nm thick films, 99.999%, with a thin Cr underlayer) onto glass plates (Berliner Glass, 1.1 mm thickness). The Au substrates (1.3 × 0.7 cm^2) were cleaned by consecutive baths of deionized water and ethanol, dried under nitrogen flow and thermally annealed [11]. The gold plates were further cleaned using UV ozone cleaner (UVO cleaner, Jelight Company Inc., CA) for 20 min followed by flame annealing. For the SP1 modified plates, 40 μL of SP1 solution (1.5 mg mL^{-1}) was placed on the gold plate to cover 2/3 of the surface for 30 s, gently rinsed with deionized water and dried with nitrogen flow.

Modification of the gold plates (surface area in solution was always 0.7 cm^2) was carried out by electrodeposition from a pre-hydrolyzed solution of 0.2 M MPTMS. The hydrolysis step was optimized to the following conditions: a 1:1 mixture of ethanol:water was used as a solvent (ethanol was not used as a sole solvent to avoid denaturation of the protein adsorbed to the gold surface), hydrochloric acid was used to adjust to pH 2, and the hydrolysis was carried out at room temperature for 90 min. Following the hydrolysis step, the sol was treated by a concentrated NaOH solution to achieve pH 4.5, to favor the electrochemically assisted sol-gel condensation. Electrodeposition was carried out by applying a constant potential of − 1.2 V vs. Ag/AgBr quasi reference electrode (QRE) for 10 min. We found, based on our previous work, that this quasi reference electrode is more stable than the conventional Ag/AgCl. It is important to note, that since a QRE was used, prior to each experiment, a CV was carried out to calibrate the QRE. Finally, the modified electrodes were cured for 1 h in 60 °C, to drive the condensation to completion. The working electrodes used for the deposition were evaporated gold on glass, modified with SP1 or bare.

Cyclic voltammetry (CV) was performed using a three-electrode system using the gold substrate as the working electrode, a Pt wire as a counter electrode and Ag/AgBr as quasi reference electrode. First, cyclic voltammetry was carried out on a bare gold electrode in a 0.1 M of potassium nitrate solution containing 1 mM of silver nitrate. According to the resulted voltammogram, a deposition potential was chosen. Then, each of the following gold electrodes; bare, SP1 modified, coated with MPTMS and SP1 modified coated with MPTMS were subjected to the same procedure: a short pulse of 1, 5 or 10 s of the chosen potential was applied to each gold electrode.

Different solutions containing various redox couples at a concentration of 0.5 mM were used for this set of experiments. CV was carried out with the treated gold electrodes (bare, modified with MPTMS and modified with SP1 and MPTMS) under conditions (potential window and pH, i.e., buffered solution) that varied in accordance with the redox couple, as is described in detail in Table 1. In these experiments, the reference electrode used was a commercial Ag/AgCl (KCl 1 M) electrode (CH Instruments Inc., USA).

The critical cross-section diameter is the diameter (in Å) of the smallest cylindrical pore that the molecule can enter without distortion [12]. This was estimated using ChemDraw software based on space-filling models.

4. Results and discussion

Understanding the adsorption of the protein onto the gold surface is a key element in this work. Gold surfaces were subjected to the C81-SP1 mutant, containing cysteine amino acids, which bear thiol residues on the protein rim. Since SP1 is a dodecamer, each complex contains 12 thiol groups, six on each face of the protein ring [10].

The process of the gold electrode preparation, C81-SP1 adsorption and the sol-gel matrix electrodeposition are schematically described in Fig. 1. Protein deposition conditions used for electrode coverage were derived from previous studies [10]. It should be noted that SP1 denaturation must be avoided upon adsorption, because the denatured protein loses its unique barrel-type structure, which is essential for controlling the accessibility of electroactive species to the electrode surface, vide infra. The protein presence on gold was characterized by FTIR spectroscopy (detailed in the SI, Fig. S1) prior to the silanization step. Briefly, the indicative peaks typical to the SP1 were detected using FTIR transmittance measurements, and then compared to the ATR