Sensors and Actuators B 181 (2013) 29–37

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A solid phase-based nanopore sensing system for biomolecules using lipid-loaded mesoporous silica MCM-41 and a fluorescent dye

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ARTICLE INFO

Article history:
Received 14 November 2012
Received in revised form 15 January 2013
Accepted 18 January 2013
Available online 28 January 2013

Keywords:
Mesoporous silica material
Solid phase fluorometry
Fluorescent dyes
Lipid-loaded nanopores
BSA
Anti-BSA antibody

ABSTRACT

We describe a nanopore sensing system for biomolecules by using the nanopores of lipid-loaded, receptor-modified mesoporous silica MCM-41 and a fluorescence dye. The pores of MCM-41 are loaded with a lipid mixture, *i.e.*, DOPC($C_{18:1}$):DOPE($C_{18:1}$) = 9:1 in a molar ratio. BSA as a receptor is modified *via* the amine moiety of DOPE($C_{18:1}$). Upon binding to anti-BSA antibody in a solution in the presence of Rhodamine B (RB) as a fluorescent marker, the amount of RB going into the lipid-loaded nanopores is decreased. The solid phase-based fluorometric measurement of the sediment of the bovine serum albumin (BSA)-modified MCM-41 could determine anti-BSA antibody in the concentration range from 1.0×10^{-10} to 1.0×10^{-7} g mL^{-1} with detection limit of 1.0×10^{-11} g mL^{-1} (S/N = 3). The high sensitivity of the nanopore sensing system was ascribed to the amplified feature of an ion-flux and the enhancement of fluorescence of RB in the pores.

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1. Introduction

The nanopores of well controlled geometry have attracted attention for the design of sensing systems for molecules in solution. Mesoporous silica MCM-41 possesses a regular array of uniform unidimensional mesopores with narrow pore size distribution, whose size is tunable in the range from approximately 2 nm to 10 nm [1–3]. The application of MCM-41 and its analogs to the development of molecular sensing systems is based on several characteristic features such as large surface area, large volume, chemical stability and hydrophilic nature [4–6]. The pore dimensions of MCM-41 offer the possibility of accommodating ions and molecules within the pores [7,8]. Enzyme-functionalized mesoporous silica for bioanalysis has been discussed in a recent review [9].

Not only the ability of enzyme accommodation [10–14] but also the ability of controlling the penetration of molecules (analytes) in and out of nanopores has been exploited for designing sensing systems [15,16]. The penetration of an analyte itself into the nanopores causes the modulation of optical properties of fluorescent receptor or a probe in the pores. On the other hand, the amplified feature of an ion-flux through nanosize pores, due to channel-like behavior that transports ions rather than an analyte itself, is useful for the design of highly sensitive molecular sensing systems [17]. Synthetic nanopore membranes prepared by using solid supports have been developed for the detection of biomolecules such as protein [18–21] and DNA [22–25]. The molecular sizes of protein and the nanotube mouth are compatible and hence, analyte binding plugs the nanotube, which is detected as a blockage of the ion current. The chemical modification of peptide nucleic acid (PNA) on the inner wall of gold nanotubes allows discriminating complementary DNA sequences, which can be detected as a decrease in the flux of an optical marker [24]. We reported the channel-like transport of ions under the application of an electrochemical potential gradient through biotinylated MCM-41 in planar bilayer lipid membranes [26] and lipid-loaded MCM-41 [27]. Anion sensing based on the spontaneous release of an optical marker from the pores of mesoporous silica has also been reported by modifying a polyamine derivative as a molecular gate, which selectively recognizes ions such as ATP^{4-} and blocks the release of an optical marker [28,29].

Another potential approach for designing a highly sensitive optical method, taking an advantage of the above-mentioned ion-flux method, is the combination of the channel-like transport of a fluorescent marker into nanopores and its irreversible accommodation into the pores for the enrichment of the marker. Such sensing systems are expected to be highly sensitive and practical, because fluorescence from solid phase can be directly measured. In addition, the confined nanospace has the unique chemical and optical properties [30], which is different from a bulk solution, because of restricted freedom of dye movement [31], a shift in acid–base equilibrium [32] and suppressed dimerization of dyes [33–35].

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http://dx.doi.org/10.1016/j.snb.2013.01.039

In the present paper, we describe a solid-phase fluorometric method using lipid-loaded MCM-41 and a fluorescent dye, in which the transport of the dye into the nanopores is regulated by a receptor introduced *via* the head group of lipid in the pores. The emission of fluorescence from the dye in the pores is measured based on solid-phase fluorometry. The lipid molecules in the pores offer hydrophobic environment, which is effective for enhancing the fluorescence of the accommodated dye, and also provide binding sites for the introduction of a receptor, which can be activated by the conventional maleimide-thiol coupling method. Upon binding to an analyte, the receptor gate allows suppressing the amount of the fluorescent dye going into the hydrophobic pores. Since MCM-41 spontaneously precipitates in a solution, capturing fluorescence images of its sediment enables us to quantify the analyte. The working principle of the nanopore sensing system is discussed using BSA as a protein receptor and anti-BSA antibody (anti-BSA) as an analyte.

2. Experimental

2.1. Materials

Rhodamine 6G (R6G) and sulfo-rhodamine B (Sulfo-RB) were obtained from Tokyo Kasei Co. (Tokyo, Japan). Rhodamine B (RB) and fluorescein were obtained from Wako Pure Chemicals Co. (Osaka, Japan). 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC($C_{18:1}$), 50 mg mL^{-1} chloroform solution), 1-2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine (DPPC($C_{16:1}$), 10 mg mL^{-1} chloroform solution), 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (DMPC($C_{14:1}$), 10 mg mL^{-1} chloroform solution), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE($C_{18:1}$), 10 mg mL^{-1} chloroform solution) and L-α-phosphatidylethanolamine (PE, purity >99%, 10 mg mL^{-1} chloroform solution) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Albumin from bovine serum (BSA, >97%,) was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO) and dithiothreitol (DTT) was from Nacalai Tesque Co. (Kyoto, Japan). Rabbit polyclonal anti-BSA was obtained from Bethyl Lab. (TX, USA). *N*-Succinimidyl 3-(2-pyridyldithio) propionate (SPDP) and fluorescein isothiocyanate (FITC) were obtained from Thermo Scientific (Rockford, IL). Silica particles (Admafine silica SO-C5, regular type, particle diameter 1.4 μm, specific area 4.3 m^2 g^{-1}) was obtained from Admatechs Co. (Miyoshi, Japan). Rabbit anti-substance P antibody (polyclonal) was obtained from Funakoshi Co. (Tokyo, Japan). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic (HEPES) acid and *N*-(4-maleimidobutyryloxy)-sulfosuccinimide, sodium salt (sulfo-GMBS) were obtained from Dojindo Laboratories (Kumamoto, Japan). Uric acid, dimethyl sulfoxide (DMSO, for biochemical) and chloroform were obtained from Wako Pure Chemicals. 96-Well plates (flat and round bottoms) were obtained from Nunc A/S (Roskilde, Denmark). Human serum (type AB, male, H4522-20ML), albumin from human serum (lyophilized powder, 97–99%) and γ-globulins from human blood (>99%) were obtained from Sigma Chemical (St. Louis, MO, USA). Milli-Q water (Millipore reagent water system, Bedford, MA) was used throughout the experiments.

2.2. Preparation of lipid-loaded MCM-41 samples

Mesoporous silica MCM-41 was synthesized according to the reported procedure [36]. The pore diameter was calculated to be 2.8 nm, using a d_{100} value of 3.26 nm. Before use, MCM-41 was sonicated in 2.8 mL chloroform. Then, MCM-41 (30 mg) was incubated with a chloroform solution (0.22 mL) containing 10 mg mL^{-1} L-α-phosphatidylethanolamine (PE) at −20 °C for 60 min. The precipitates were washed three times with chloroform and dried overnight under reduced pressure at room temperature. The amount of loaded PE, determined by the procedure described below, was 57 nmol mg^{-1} MCM-41. The loading of other lipids, *i.e*., DOPC($C_{18:1}$), DPPC($C_{16:1}$), DMPC($C_{14:1}$) and a mixture of DOPC($C_{18:1}$) and DOPE($C_{18:1}$) (9:1 in a molar ratio), was performed in the same manner as above. The PE-loaded MCM-41 and MCM-41 loaded with a mixture of DOPC($C_{18:1}$) and DOPE($C_{18:1}$) (molar ratio of 9:1) hereafter are called PE-MCM-41 and DOPE($C_{18:1}$)-MCM-41, respectively.

The amount (mol) of PE and DOPE($C_{18:1}$) loaded on MCM-41 was quantified by converting the lipid to pyridyldithio moiety-linked ones with a heterofunctional crosslinker SPDP [37]. Lipid-loaded MCM-41 (1 mg) was suspended in 0.10 mL of 10 mM HEPES/NaOH buffer (pH 7.4) (abbreviated as a HEPES solution) and incubated with 1 mL of 1 mM SPDP in a HEPES solution containing 3% DMSO. After washing with a HEPES solution, the MCM-41 sample was treated with 1 mL of 0.10 M DTT in a HEPES solution at 20 °C for 60 min, in order to release pyridine-2-thione (molar absorption coefficient ε = 8080 at 343 nm) from the MCM-41 material into the solution [38]. The amount of lipid molecules was determined by measuring absorbance of the solution. The leak of PE from the pores was negligible even after three times washing with a HEPES solution (Fig. S1).

When the dye-loaded MCM-41 materials were repeatedly washed with a HEPES, the slight leak of RB from the materials occurred. However, the leak was suppressed when PE was present in the pores (Fig. S2). On the other hand, R6G remained on PE-MCM-41, and also on MCM-41, even after the 3rd washing.

2.3. Preparation of BSA with sulfhydryl groups

First, bovine serum albumin (BSA) with pyridyldithio groups was prepared by adding 12.5 μL of 20 mM SPDP in dimethyl sulfoxide (DMSO) to 0.50 mL of 4 mg mL^{-1} BSA in a 10 mM NaH_2PO_4 buffer containing 0.15 M NaCl and 10 mM EDTA (pH 7.4) (abbreviated as a PBS–EDTA buffer). After incubation for 60 min at room temperature, the pyridyldithio moiety-modified BSA was isolated by gel filtration with Zeba Desalt Spin Columns (Thermo Scientific). The concentration of eluted pyridyldithio moiety-modified BSA (BSA-SPDP) was determined by measuring absorbance at 280 nm. This solution can be stored for at least one week. Then, BSA with sulfhydryl groups (BSA-SH) was prepared by reducing BSA-SPDP (50 μL) with 50 μL of 0.15 M DTT in 0.10 M CH_3COONa containing 0.10 M NaCl (pH 4.5) (an acetate buffer). The BSA-SH was isolated by gel filtration with Centri•Spin-10 (Princeton Separation, Adelphia, NJ) and finally diluted to 50 μL with an acetate buffer. BSA-SH was prepared immediately before use.

2.4. Modification of lipid-loaded MCM-41 with BSA-SH

Introduction of BSA-SH on DOPE($C_{18:1}$)-MCM-41 was carried out as follows. A suspension of 0.1 mg lipid-loaded MCM-41 in 0.1 mL of a 10 mM NaH_2PO_4 containing 0.15 M NaCl (pH 7.4) (a phosphate buffer) was mixed with 0.10 mL of 1 mM sulfo-GMBS in a phosphate buffer. After removal of excess sulfo-GMBS by centrifugation, a 0.10-mL portion of the suspension in a phosphate buffer was mixed with 15 μL of BSA-SH (20 μg mL^{-1}) and 35 μL of a phosphate buffer, followed by incubation under a dark place for 1 h. After washing the mixture with a phosphate buffer by centrifugation, a 0.6 mg mL^{-1} suspension of BSA-modified MCM-41 in a phosphate buffer was prepared and used for fluorometric assay of anti-BSA.

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