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Adsorption and desorption characteristics of DNA onto the surface of amino functional mesoporous silica with various particle morphologies

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ABSTRACT

Recently, deoxyribonucleic acid (DNA) adsorption on solid materials has been reported for applications such as genetic diagnosis of diseases, gene delivery, and biosensors. Mesoporous silica (MPS) is an excellent carrier because of its high surface area and large pore volume. Functionalization of the MPS surface can be controlled by silane coupling reagents, and the MPS particle morphology can be easily changed by the synthetic conditions. In this study, to evaluate the ability of DNA adsorption on MPS, the MPS surface was functionalized using four reagents, 3-aminopropyltriethoxysilane ($-NH_2$), *N*-(2-aminoethyl)-3-aminopropyltriethoxysilane ($-2ENH_2$), *N*-(6-aminohexyl)aminopropyltrimethoxysilane ($-2HNH_2$), and (3-trimethoxysilylpropyl)diethylenetriamine ($-3NH_2$), each having a different number of amino groups and alkyl chain lengths. Moreover, we prepared three types of MPSs with different particle morphologies: sheet-type structure (MPS sheet), spherical MPS (MCM-41s), and nonporous spherical silica. A high adsorption capacity was observed in MPS sheets with $-2HNH_2$ (sheet-$2HNH_2$) and $-3NH_2$ (sheet-$3NH_2$), as well as MCM-41s with $-3NH_2$ (41s-$3NH_2$). The adsorption and desorption rates of DNA on these three MPSs were then examined and the best results were obtained with 41s-$3NH_2$. These results demonstrate that the amino functionalized MPS materials are useful DNA adsorbents.

1. Introduction

Deoxyribonucleic acid (DNA) is a biological polymer with two helical chains. Each chain is composed of a sugar phosphate backbone consisting of four primary nucleobases (adenine, guanine, thymine, and cytosine) [1,2]. The sequence of these bases directly influences protein synthesis and gene characteristics [3,4]. Therefore, DNA is applied for the genetic diagnosis of diseases [5] and in gene delivery [6] and biosensors [7]. DNA adsorbed on solid materials is often used for these applications.

For DNA adsorption on solid materials, various solid particles have been investigated, such as mesoporous silica (MPS) [8,9], zirconia [10], indium tin oxide (ITO) [11], and gold nanoparticles [12]. The driving forces of DNA adsorption on these materials are suggested to be covalent bonding and electrostatic interaction between DNA and the particle surface [13]. Chemical adsorption occurs by covalent bonding, in which DNA and the surface ligands share electrons. On the other hand, electrostatic interaction is a type of physical adsorption, which occurs by the difference in charge between DNA and the particles.

MPS, which is SiO_2 particles having nanosized pores of 2–50 nm, is widely used as an adsorbent and catalyst support [14]. This material has several advantages, including its high surface area, large pore volume, and its facile pore size change and surface functionalization capabilities. The mesopores of MPS are formed using surfactant templates, and the pore size distribution and pore structure are controlled by the type of surfactants and their organic chain lengths. In addition, the SiO_2 surfaces possess SiOH groups; therefore, it is possible for the surface functional groups to change into various organic groups, including amino (NH_2) [15], carboxyl (COOH) [16], and methyl (CH_3) [17] groups, using the silane coupling method.

In our previous studies, enzymes and proteins were immobilized on MPSs having different pore size distributions and particle morphologies [18–20]. These immobilized proteins were protected from denaturation using organic solvents, acidic solutions, and chaotropic reagents [21]. It was suggested that MPS was useful as an adsorbent and immobilizing material for various biomolecules.

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Moreover, we were successful in developing sheet-like MPS (MPS sheet) using a dual-templating method, thereby improving the enzyme and metal ion adsorbent capabilities of the sheet material [19,22,23].

Some studies have reported that DNA adsorption on MPS proceeds by electrostatic interactions between the negative charge of the DNA phosphate groups and the positive charge of the MPS surface functionalized with silane coupling reagents [24]. In previous studies of these types of adsorbents, silica was generally functionalized using 3-aminopropyltriethoxysilane, which has a single amino group [9]. For example, the optimal amount of amino modification on MCM-41 for adsorption of plasmid DNA (pDNA) was investigated, and the results showed that a higher amount of pDNA was adsorbed owing to the presence of a higher number of amino groups. However, there is a possibility of significantly improving the adsorption ability by changing the structure of the coupling reagent. There are various types of amino functionalizing reagents having different chain lengths and different numbers of amino groups. Calleja et al. reported the adsorption of carbon dioxide (CO_2) on amino-functionalized MPS by grafting the surface with aminopropyl, ethylene-diamine, and diethylenetriamine organosilanes. A high amount of adsorption was achieved by increasing the number of amino groups [25]. Kanann et al. described a large difference in the coordination of metal ions (zinc and copper) to immobilized chelating ligands having two or three amino groups [26]. It was presumed that the formation of hydrogen bonds between the silanol and the amino groups of the surface ligand led to a decrease in the adsorbable amino groups.

In this study, we attempted to increase the amount of DNA adsorption on MPS by replacing the general coupling reagent, 3-aminopropyltriethoxysilane ($-NH_2$), with other reagents having different numbers of amino groups and chain lengths, such as (2-aminoethyl)-3-aminopropyltriethoxysilane ($-2ENH_2$), *N*-(6-aminohexyl) aminopropyltrimethoxysilane ($-2HNH_2$), and (3-trimethoxysilylpropyl) diethylenetriamine ($-3NH_2$). Furthermore, we focused on the particle morphologies of three types of MPS: MPS with a sheet-type structure (MPS sheet), conventional spherical MPS (MCM-41s), and nonporous spherical silica (silica). To evaluate the DNA adsorption characteristics, adsorption isotherms using Langmuir and Freundlich models, adsorption and desorption rates, and the degradation ability of adsorbed DNA by DNase I were also investigated.

2. Experimental

2.1. Materials

Nonporous silica (NanoTeK® silicon oxide) was purchased from C. I. KASEI Co., Japan. Cetyltrimethylammonium chloride (CTAC), L-alanine, and palmitoyl chloride were purchased from Wako Pure Chemical Industries, Japan. Tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane ($-NH_2$), and tetramethoxysilane (TMOS) were purchased from Shin-Etsu Chemical Co., Japan. *N*-(2-Aminoethyl)-3-aminopropyltriethoxysilane ($-2ENH_2$), *N*-(6-aminohexyl) aminopropyltrimethoxysilane ($-2HNH_2$), and (3-trimethoxysilylpropyl) diethylenetriamine ($-3NH_2$) were obtained from AZmax Co., Japan. Triblock copolymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$), DNA sodium salt from salmon testes, deoxyribonuclease I from bovine pancreas (DNase I), albumin from bovine serum (BSA), and immunoglobulin G (IgG) from human serum were purchased from Sigma–Aldrich (St. Louis, MO). Lambda DNA (λDNA) was obtained from Life Technologies Co., (Carlsbad, CA). PrimeGel™ Agarose and SYBR® Green I Nucleic acid Gel Stain were purchased from TaKaRa Bio Inc. (Japan). Nucleic acid sample buffer was purchased from BIO-RAD (Hercules, CA). All materials and chemicals used were of reagent grade.

2.2. Synthesis of MPS

2.2.1. MPS sheet

The MPS sheet was prepared according to our previous report [19]. The chiral organic template, *N*-palmitoyl-L-alanine (C_{16}-L-Ala), was prepared from palmitoyl chloride and L-alanine. To synthesize MPS sheet, 0.31 g of C_{16}-L-Ala was dissolved in deionized water (25.5 mL), and then 10 mL of 0.1 M NaOH was added. After the addition of Pluronic P123 (0.3 g), the mixture was stirred for 1 h. The mixture was cooled in an ice bath, and then 3.5 mL of 0.1 M HCl was added to achieve a pH of 9–10. The silica precursor reagents containing TEOS (1.46 g) and (3-aminopropyl) triethoxysilane (APTES) (0.28 g) were dropped into the mixture for 5 min under vigorous stirring, with continued stirring for 3 h. The suspension was maintained under static conditions for 24 h at room temperature. The particles were collected by centrifugation, and then hydrothermal treatment was carried out at 100 °C for 24 h. The solid was collected with deionized water and was centrifuged. The solid product was washed with ethanol and acetone. Finally, the sample was calcined at 500 °C for 4 h.

2.2.2. MCM-41s

MCM-41s, which has a spherical morphology with a two-dimensional (2D) hexagonal pore structure, was synthesized by a similar method as described elsewhere [27]. Sodium hydroxide (1 M, 2.28 mL) was added to a solution of methanol (360 g) and deionized water (440 g), and then CTAC (3.52 g) was added to the solution. After stirring for 1 h, TMOS (1.32 g) was slowly dropped into the mixture and the resulting solution was stirred at room temperature. The mixture was maintained under static conditions overnight. The solid was collected by centrifugation at 6000 rpm and washed with deionized water, ethanol, and acetone. Finally, the particles were calcined at 500 °C to remove the organic template.

2.3. Preparation of amino-functionalized MPS

To adsorb DNA onto the MPS, the MPS surface was functionalized by amino-containing organosilanes. MPS (50 mg) was mixed in 10 mL of toluene, and then 1.0 mL of an aminosilane reagent, 3-aminopropyltriethoxysilane ($-NH_2$), *N*-(2-aminoethyl)-3-aminopropyltriethoxysilane ($-2ENH_2$), *N*-(6-aminohexyl)aminopropyltrimethoxysilane ($-2HNH_2$), or (3-trimethoxysilylpropyl) diethylenetriamine ($-3NH_2$), was added to the mixture. After the mixture was refluxed for 6 h, the composite was separated by centrifugation. The precipitate was washed with ethanol and acetone. The solid was dried overnight at room temperature (Scheme 1).

2.4. Materials characterization

To analyze the morphology of the synthesized MPS materials, field emission scanning electron microscopy (FE-SEM) was carried out using an S4300 (Hitachi Co., Japan) at an acceleration voltage of 10 kV. Transmission electron microscopy (TEM; JEM 2010, JELO, Japan) was performed at an operating voltage of 200 kV. The surface areas, pore volumes, and pore size distributions of the samples were calculated by the Brunauer–Emmett–Teller (BET) and the Barret–Joyner–Halenda (BJH) methods using a TriStar 3000 Micromeritics analyzer (Shimadzu Co., Japan). Small-angle X-ray diffractometry (XRD; Rint 2100V/PC, Rigaku Co., Japan) patterns were recorded over a scanning range of 0.6–12° (2θ) for investigation of the mesopore characteristics. For confirmation of surface functionalization, Fourier transform-infrared (FT-IR) spectra were