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Fluorescent boronic acid terminated polymer grafted silica particles synthesized *via* click chemistry for affinity separation of saccharides

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ABSTRACT

Boronic acids are important for effective separation of biological active *cis*-diols. For the purpose of constructing a new type of saccharide-sensitive material which can not only provide convenient separation but also improve the access of boronic acid to guest molecules, the fluorogenic boronic acid terminated, thermo-sensitive polymers (BA-polyNIPAm) were grafted to an alkyne modified silica gel through the exploitation of click chemistry. The BA-polyNIPAm grafted silica gel (BA-polyNIPAm-SG) was characterized by FT-IR, fluorescence spectra, fluorescence microscopy, elemental analysis (EA), thermal gravimetric analysis (TGA), scanning electron microscope (SEM) and so on. BA-polyNIPAm-SG displayed affinity binding ability for saccharides under physiological pH value and allowed saccharides to be conveniently separated from solution. The maximum binding capacities for fructose and glucose are 83.2 μmol/g and 70.4 μmol/g polymer, respectively. The intensity of fluorescence emission of BA-polyNIPAm-SG increased with the increasing of fructose concentration. The present study provides a new kind of composite material which contains moveable and flexible grippers for recognizing and binding guest molecules.

1. Introduction

Carbohydrate widely exists in nature, which is treated as one of the three substantial units as protein and nucleic acid due to their roles as structural materials, sources of energy, biological functions and environmental analytes. For biomedical applications, it is highly desirable that saccharide-sensitive systems respond at physiological pH in aqueous media [1–6]. Boronic acid derivatives can bind to *cis*-diols tightly through ester formation. This binding property allows boronic acid to be a competitive chelator for diol appended molecules such as saccharides in water. Therefore, boronic acid derivatives have been widely exploited for the design of functional materials for detection and affinity separation of saccharides over the past decade [7–22]. In practical application, challenges such as the recovery and recycling of boronic acid, improving access to the target molecules, *etc.* still remain to be addressed. Currently, there are two main approaches used to address these concerns, namely immobilization of boronic acid ligands onto a suitable supporter [23–25] and synthesis of boronic acid containing polymers [26–32].

Various boronic acid ligands have been developed in the past. The common feature of these ligands is that they contain one boronic acid moiety able to bind *cis*-diols, and one functional group (*e.g.* amino, thiol, or polymerizable vinyl group) that can be used for immobilization on solid support [23]. In a previous work, Uddin et al. [24] developed a fluorescent clickable boronic acids derivative, *i.e.*, 3-(2-azido-acetylamino) phenylboronic acid (APBA). More interestingly, when this boronic acid was conjugated to an alkyne-functionalized material *via* click reaction (which changed the terminal azide into a triazole ring), the immobilized boronic acid remained the favorable fluorescence response. In another work of Xu et al. [25], APBA was conjugated to a thermo-responsive polymer, poly(N-isopropylacrylamide) (polyNIPAm) by click reaction. This boronic acid terminated, thermo-sensitive poymer (BA-polyNIPAm) has been used for separation of saccharides from water. In order to separate BA-polyNIPAm from aqueous solution by filtration, the temperature must be kept above its lower critical solution temperature (LCST). This is sometimes an inconvenience in practical application.

For constructing a new type of saccharide-sensitive material, we intend to anchor the thermo-responsive polymer (BA-polyNIPAm) on a suitable supporter. By doing this, the boronic acid "gripper" is linked to the supporter with a flexible arm. Therefore, the "grippers" (*i.e.* binding sites) of the material are moveable and flexible, which should lead to improved accessibility and binding capacity. On the other hand, immobilization on a solid support can provide convenient separation

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(*e.g.* a simple centrifugation step or a filtration step) and thus, allowing boronic acid to be easily recovered for repeated use.

Due to the feature of high surface area, convenient to be modified, proper physical properties and cost-effective, silica materials have been used as a variety of supporters matrices in separation, solid phase extraction, catalysis, binding assays, *etc.* The copper-catalyzed azide-alkyne cycloaddition (CuAAC) is a prototypical example of click reaction that has been recognized as a facile and versatile chemistry for bioconjugation and functionalization of polymeric architectures [33–38]. Therefore, we intend to anchor BA-polyNIPAm on the surface of silica gel through the exploitation of click chemistry.

In this work, the bromine of BA-polyNIPAm has been transformed into azide group by means of nucleophilic displacement reaction to give the clickable polymer (BA-polyNIPAm-N_3). Then BA-polyNIPAm-N_3 was grafted to an alkyne modified silica *via* click reaction. The saccharides affinity binding and the corresponding fluorescence response of the prepared material were achieved in aqueous media under physiological pH conditions.

2. Experimental section

2.1. Materials and characterization

Aminopropyl silica gel (particle size 15–35 μm, Fluka Chemika, 09297), 3-Aminophenylboronic acid hemisulfate, bromoacetyl bromide, $CuSO_4$, CuBr (98%), sodium ascorbate, sodium azide, Tris(2-dimethylaminoethyl)amine (Me_6TREN), propargylamine, propargyl chlorofomate, 2-bromoisobutyryl bromide, D-fructose, D-glucose dimethylsulfoxide-d_6 (99.9 at.% D) and chloroform-d (≥99.8 at.% D) were purchased from Sigma-Aldrich. CuBr was stirred overnight in acetic acid, filtered, washed with acetone and dried in vacuo before use. N-isopropylacrylamide (NIPAm) was purchased from Acros and recrystallized from toluene/hexane (2:1, v/v). 2-propanol, tetrahydrofuran (THF) and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich and used without further purification. Ultrapure water (18.2 MΩ cm) obtained from an ELGA LabWater System (Vivendi Water Systems Ltd.) was used throughout the experiments. All other solvents purchased from commercial resources were of analytical grade.

Attenuated total reflection (ATR) infrared spectra were recorded using a Perkin-Elmer FTIR instrument (Perkin-Elmer Instruments). UV–vis absorption spectra were recorded with a Beckman Coulter DU 800 UV/Vis Spectrophotometer. Fluorescence emission was measured using a QuantaMaster C-60/2000 spectrofluorometer (Photon Technology International, Lawrenceville, NJ, USA). ^{1}H NMR spectra were recorded on a 400 MHz Superconducting Magnet NMR Spectrometer (Bruker B-ACS60). The elemental analysis was measured on a Vario EL CHNS Elemental Analyzer (Elementar, Germany). The surface morphologies of the particles were observed with a scanning electron microscope (SEM; JEOL JSM-6610LV). The thermal gravimetric analysis (TGA) was performed using a TG 209F3 (Netzsch, Germany) at heating rate 10 °C/min under N_2 atmosphere. The samples were heated from 40 °C to 800 °C.

MALDI-TOF Mass Spectra were acquired using a 4700 Proteomics Analyzer (Applied Biosystems/MDS SCIEX, USA) in the positive reflector mode. The samples were dissolved in THF and the concentration was 0.2 mg/ml. The matrix solution consisted of 50% (v/v) acetonitrile in water, 5 mg/ml α-cyano-4-hydroxy cinnamic acid and 0.1% (v/v) phosphoric acid. The matrix solution was mixed with sample on a stainless target plate. Typically, 0.5 μl of sample was mixed with 0.5 μl matrix solution spiked with two internal standard peptides (m/z = 904.468 and m/z = 2465.199). The two internal standards allowed accurate mass calibration with a mass deviation less than 20 ppm.

For the fluorescence microscopy measurement, the synthesized materials were deposited on a glass slide and observed under a Nikon Eclipse E400 epifluorescence microscope equipped with a CCD camera. The conditions of measurement were: exposure time, 0.2 s; readout rate, 1 MHz at 16 bit; preamplifier gain, 5×; output amplifier, conventional.

An Alltech HPLC system (USA) equipped with an ELSD 2000 detector and an Alltech prevail carbohydrate analytic column (250 mm × 4.6 mm) were used for determining the concentrations of fructose and glucose. The mobile phase was prepared with methanol and water (80:20, v/v). The flow rate of mobile phase was 1.0 ml/min. The column temperature was set at 85 °C and sample injection volume was 20 μl.

BA-polyNIPAm was synthesized according to the reported procedures [25]. The synthetic route, the FT-IR, ^{1}H NMR spectra and UV/Vis of BA-polyNIPAm and the reaction intermediates can be seen in the Supporting Information.

The molecular weight of the synthesized polyNIPAm was determined by MALDI-TOF mass spectrometry (Supporting Information). Based on the result of MALDI-TOF measurement, the average degree of polymerization of polyNIPAm is calculated to be about 15. Direct determination of the molecular weight of BA-polyNIPAm was not successful, as this polymer gave a rather poor ionization results under the experimental condition.

2.2. Synthesis of BA-polyNIPAm-N_3

BA-polyNIPAm (0.18 g), NaN_3 (32.5 mg, 0.5 mmol) and DMF (10 ml) were added to a 100 ml round-bottom flask. The reaction mixture was allowed to stir at 50 °C for 48 h. After the reaction, most of the DMF was removed. The residue was diluted with THF, and then passed through a neutral alumina column to remove residual sodium salts. The solid product was then precipitated from diethyl ether and dried by vacuum.

2.3. Synthesis of alkyne immobilized silica

To a solution of triethylamine (0.50 ml) in THF (12 ml) at ice-water temperature, aminopropyl silica gel (0.50 g) and propargyl chlorofomate (0.21 ml, 2.0 mmol) were sequentially added. The reaction mixture was warmed to room temperature and stirred overnight. The silica particles were isolated by centrifugation, thoroughly washed with water and methanol. The particles were then dried by vacuum.

2.4. Linking BA-polyNIPAm-N_3 to the alkyne-silica

BA-polyNIPAm-N_3 (0.15 g), alkyne-silica(0.20 g), CuBr (0.0144 g, 0.10 mmol) and DMF (15.0 ml) were added to a 100 ml dried flask. The mixture was deoxygenated by bubbling with nitrogen for 40 min. Then Me_6TREN (0.0276 g, 0.12 mmol) was added. The mixture was heated to 85 °C and magnetically stirred under nitrogen atmosphere for 48 h. After the reaction, the particles were collected by centrifugation and washed thoroughly with water and methanol until no fluorescence could be observed from the supernatant. Then, the particles were dried by vacuum.

2.5. Etching silica gel from BA-polyNIPAm-SG by hydrofluoric acid

To remove the silica gel from BA-polyNIPAm-SG, 0.200 g of BA-polyNIPAm-SG was transferred to a plastic tube and stirred in 12.0 ml of diluted hydrofluoric acid at room temperature for 12 h. After the reaction, water was removed by bubbling with nitrogen. The residue was treated by THF and the insoluble residue was removed by filtration. The solid polymers were precipitated from diethyl ether, collected by centrifugation, washed with pure methanol and then dried in a vacuum chamber.

2.6. Measurement of fluorescence response to addition of saccharides

To a set of 15 ml calibrated test tubes, 4.0 mg of BA-polyNIPAm-SG, 0.5 ml of 0.20 M phosphate buffer solution (PBS) (pH 7.4), and a given