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Preparation and characterization of fluorophenylboronic acid-functionalized monolithic columns for high affinity capture of *cis*-diol containing compounds

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ABSTRACT

Boronic acids are important ligands for the selective recognition and capture of *cis*-diol containing compounds, such as nucleosides and glycoproteins. In a recent study, it was found that 2,4-difluoro-3-formyl-phenylboronic acid (DFFPBA) exhibited an ultrahigh boronate affinity for binding with monosaccharides. Herein three DFFPBA-functionalized monolithic columns with varying spacer arms were synthesized and characterized. Different *cis*-diol containing compounds were used for the evaluation of the boronate affinity of the DFFPBA-functionalized monoliths. The DFFPBA-functionalized monoliths exhibited advantageous characteristics. These monoliths exhibited an ultrahigh boronate affinity toward *cis*-diol containing compounds. Moreover, the monolith with appropriate spacer arm exhibited a low binding pH (6.0) for *cis*-diols of small molecular weight. These advantages made DFFPBA-functionalized monoliths suitable for the enrichment of trace *cis*-diol containing compounds in neutral and weak acidic real samples. In addition, it was interesting that the length of spacer arms strongly influenced the boronate affinity: increasing the spacer arm resulted in apparently reduced boronate affinity, and inappropriate spacer arm length even eliminated the boronate affinity toward glycoprotein. To explain such a phenomenon, a possible mechanism was proposed. Finally, the potential of DFFPBA-functionalized monoliths for real applications was demonstrated with the selective enrichment of modified nucleosides from human urine.

1. Introduction

Boronate affinity is a unique means for the selective recognition and enrichment of *cis*-diol containing biomolecules, such as glycoproteins, glycopeptides, nucleosides and saccharides. The principle is based on reversible covalent complex formation/dissociation between boronic acids and *cis*-diol containing compounds in an alkaline/acidic aqueous solution. Such a pH switchable property has made boronic acids excellent ligands in many areas such as molecular recognition, proteomics and metabolomics [1–12]. Monolithic columns, as compared with conventional chromatographic columns, exhibit several attractive features, including easy fabrication, low back pressure and fast convective mass transfer [13,14]. Due to the advantageous characteristics of the two aspects, boronate affinity monolithic columns have gained rapid development in recent years [15]. However, there are still several obstacles to wider applications. First, conventional boronic acids require a basic pH for substantial binding with *cis*-diols, and thereby they fail to strongly bind with *cis*-diol containing compounds in near-neutral or weak acidic samples such as serum and urine. Second, regular boronic acids such as phenylboronic acid exhibit limited affinity and thereby fail to bind *cis*-diol containing compounds at trace concentration.

Many efforts have been made to enhance boronate affinity in neutral and weak acidic conditions through reducing the binding pH. Four types of boronic acids with lowered binding pH have been reported: (1) electron-withdrawing type, with an electron-withdrawing group (e.g., nitro or sulfonyl) in the phenyl ring [16–20]; (2) Wulff-type, with a nitrogen atom adjacent to the boron atom to form an intramolecular B–N coordination [21,22]; (3) improved Wulff-type, with an oxygen atom adjacent to the boron atom to form an intramolecular B–O coordination [23–27]; (4) teamed boronate affinity, with two individual molecules, an amine and a boronic acid, forming a molecular team through intermolecular B–N corrdination [28,29]. So far, the lowest binding pH was reported to be 5.0, provided by an improved Wulff-type boronic acid (3-carboxybenzoboroxole) [27]. However, in a recent comparative study on the binding strength between representative monosaccharides and a variety of typical boronic acids [30], it was found that 3-carboxybenzoboroxole exhibited much lower

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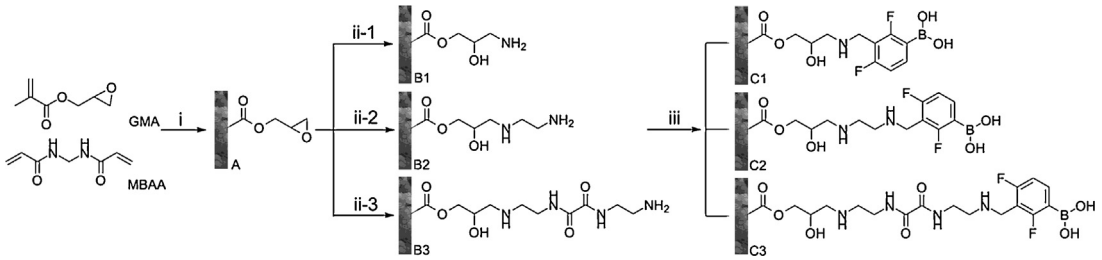

Fig. 1. Schematic of the preparation of DFFPBA-functionalized monolithic columns with different spacer arms.

affinity at pH 7.4 and 6.0, as compared with other boronic acids. Rather, 2,4-difluoro-3-formyl-phenylboronic acid (DFFPBA) exhibited ultrahigh affinity at pH 7.4 and 6.0, with affinity toward fructose 8.2–9.5 times higher than that of 3-carboxybenzoboroxole. Besides, only a few literatures have studied on fluorophenylboronic acids, which were immobilized on hydrogel matrix for the design of fluorescent biosensors [31–33]. To the best of our knowledge, there has been no report on exploring the characteristics of fluorophenylboronic acid-functionalized monolithic materials to date.

In this study, we report the preparation and characteristics of three DFFPBA-functionalized monolithic columns with varying spacer arms. DFFPBA was chosen as the ligand, because not only it exhibited ultrahigh boronate affinity but also it exhibited a relatively lower pK_a value (6.5). As expected, the prepared DFFPBA-functionalized monolithic columns exhibited an untrahigh boronate affinity toward *cis*-diol containing compounds. Meanwhile, the DFFPBA-functionalized monolith with appropriate spacer arm can capture *cis*-diol containing compounds at pH as low as 6.0. More interestingly, it was observed that the length of spacer arms strongly influenced the affinity toward *cis*-diol containing compounds; with the spacer arm increasing the boronate affinity reduced. Extremely, boronate affinity toward glycoprotein was even eliminated with inappropriate spacer arm length. A possible mechanism was proposed to explain such a phenomenon. Finally, a DFFPBA-functionalized monolithic column was applied for the selective enrichment of modified nucleosides from human urine.

2. Experimental

2.1. Materials

DFFPBA, glycidyl methacrylate (GMA), horseradish peroxidase (HRP), ribonuclease A (RNase A) and HPLC grade acetonitrile (ACN) were purchased from Sigma (St. Louis, MO, USA). 3-Formylphenylboronic acid (FOPBA), *N*,*N*′-methylenebisacrylamide (MBAA), dodecanol, adenosine, deoxyadenosine and catechol were purchased from Alfa Aesar (Ward Hill, MA, USA). Dimethyl sulfoxide (DMSO) and 2,2-azobisisobutyronitrile (AIBN, recrystallized in methanol before use) was purchased from Shanghai Fourth Reagent & H.V. Chemicals (Shanghai, China). *N*,*N*′-bis(2-aminoethyl)oxamide (BAEO) was synthesized according to the reference [34]. Deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, MA, USA). Other chemical reagents were of analytical grade. Fused-silica capillaries with 150 μm I.D. and 375 μm O.D. were purchased from Yongnian Optic Fiber Plant (Hebei, China).

2.2. Instrumentation

All capillary liquid chromatography (CLC) were performed on an UltiMate 3000 high pressure liquid chromatography system (Dionex, Sunnyvale, CA) equipped with an LPG-3 × 00 micropump, an FLM-3100 microflow manager (1:100 split ratio) which guarantees a constant flow rate, an VWD-3400 variable-wavelength UV–vis absorbance detector with a 3 nL flow cell for on-column detection and an WPS-3000 automatic sampler. Chromeleon software from Dionex was used for system operation and data acquisition and processing. The flow rate was set at 1 μL/min and the column temperature was set at 25 °C in all experiments. The effective lengths of monolithic columns used in all CLC experiments were 25 cm unless other specifications.

Scanning electron microscopy (SEM) analyses were performed on a Hitachi FE-SEM S-4800 (Tokyo, Japan). Nitrogen adsorption-desorption measurements were conducted at 77 K on an ASAP2020 instrument (Micromeritics, Norcross, GA, USA). The FT-IR spectrum was acquired on a Thermo Nicolet iS10 FT-IR spectrometer (Waltham, MA, USA). A Synergy Mx microplate reader (BioTek, USA) was used for the UV-absorption experiments. Micellar electrokinetic chromatographic (MEKC) analyses were carried out on a Beckman Coulter P/ACE MDQ system (Beckman Instruments, Fullerton, CA, USA). The separation conditions were set according to the reference [8]. The running buffer contained 25 mM borate, 25 mM phosphate buffer and 25 mM cetyltrimethylammonium bromide (CTAB) at pH 9.50. The separation voltage was −15 kV with UV detection wavelength at 254 nm. Sample was injected at 3.4 kPa for 10 s.

2.3. Preparation of the monolithic columns

The synthesis route of base monolithic column is illustrated in Fig. 1 (reaction i). Prior to the preparation of the monolith, the capillary was pretreated with acid, alkali and γ-MAPS according to the procedure reported previously [22]. The base monolithic column was synthesized by thermo-initiated free radical copolymerization. Briefly, a mixture containing GMA (47 mg), MBAA (43 mg), DMSO (140 mg), dodecanol (125 mg) and AIBN (1 mg) was vortexed for 5 min and sonicated for 30 min to obtain a homogeneous solution. The vinylized capillary was filled with the polymerization mixture, sealed with rubber at both ends and then submerged into a water bath at 75 °C for 12 h. After the polymerization reaction, the resulting monolithic column was washed with methanol and acetonitrile successively to remove non-reacted residues. This capillary monolithic column is designated as A in the following text.

The obtained base monolithic column was then reacted with different amines and the synthesis routes are illustrated in Fig. 1(reaction ii). Column B1 was obtained by post-modification of column A with a solution of $NH_3{\cdot}H_2O$/ACN (1:1, v/v) pumped through at 60 °C for 12 h (Fig. 1, ii-1). Columns B2 and B3 were obtained post-modification of column A with ethylenediamine/ACN (1:1, v/v) (Fig. 1, ii-2) and BAEO/Et_3N/DMSO (1/5/50, w/w/w) (Fig. 1, ii-3), respectively.

The preparation procedure for DFFPBA-functionalized monolithic columns is illustrated in Fig. 1(reaction iii). Columns B1, B2 and B3 were treated with DFFPBA/ACN (14 mg/200 μL) and