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Preparation of a boronate affinity silica stationary phase with enhanced binding properties towards *cis*-diol compounds

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ABSTRACT

In this study, a boronate affinity silica stationary phase with enhanced binding properties towards *cis*-diol compounds was prepared through the combination of surface-initiated atom transfer radical polymerization (SI-ATRP) with a Wulff-type boronate as affinity ligand. The stationary phase showed good hydrophilicity and improved binding strength toward adenosine, with binding constant to be as low as 2.38×10^{-4} M. The column exhibited excellent binding specificity, low binding pH (≥5.5) and high binding capacities (80.1 μmol adenosine g^{-1} at pH 7.0 and 45.2 μmol adenosine g^{-1} at pH 5.5, respectively). The stationary phase was applied as adsorbent for the selective extraction of nucleosides in human urine with excellent specificity and high enrichment efficiency. These results demonstrated that this stationary phase could be favorably applied for selective capture and enrichment of *cis*-diol compounds in complex samples.

1. Introduction

Cis-diol compounds are of great biological significance. For example, modified nucleosides in urine have been considered as potential tumor markers for many diseases [1], such as leukemia [2], small cell carcinoma of the lung [3] and breast cancer [4]. Most of the *cis*-diol compounds in real samples usually exist in very low abundance, such as glycoproteins and glycopeptides, with the presence of abundant interfering components. So, selective enrichment of the target *cis*-diol molecules and meanwhile removal of the interferring species is a key issue for the separation and analysis of *cis*-diol compounds in complex samples.

Boronate affinity based technique has been proved to be an efficient means for selectively capture and enrichment of *cis*-diol compounds. The unique selectivity comes from the formation of reversible and pH-dependent five of six-membered cyclic esters between boronic acid ligand and *cis*-diol compounds. The cycle esters form in an alkaline aqueous solution and dissociate when the medium is changed to acidic pH. Various boronate affinity materials based on different type of supports, such as monolithic column [5–17], nanoparticles [18–28], silica [29–32], fibrous cotton [33], polymers [34–36] and graphene [37], have been developed and applied to the selective extraction or enrichment of *cis*-diol compounds. Recently, much attention has been attracted to improve the binding strength and binding capacity of boronate affinity materials. As to the improvement of binding capacity, it is an efficient and straightforward way to amplify the number of boronic acid ligand on the support materials. Amplification through the modificaiton the support with polymers, such as polyethyleneimine (PEI) [17,25,33], dendrimers [23,31] and chain polymer brushes through surface-initiated polymerizetion [29,30,34], had been proved to be efficient way to substantially increase the binding capacity of boronate affinity materials. As for the improvement of binding strength, the adoption of boronate ligands with superior *cis*-diol binding properties was a direct way [9,14,17]. Besides, the combination of boronate affinity with molecular imprinting technique had also been proved to be an ingenious strategy, which was able to dramatically enhance the binding strength (with dissociation constants, K_d, values within 10^{-8}–10^{-10} M) and specificity toward target glycoproteins [38–42]. So, the combination of amplification strategy with superior boronate affinity ligand is a promising way to improve binding capacity and binding strength of boronate affinity materials.

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The typical methods for the modification of surface support with polymers generally can be catagorized into "grafting to" and "grafting from". In the "grafting to" method, polymers, such as PEI [17] and dendrimer [23], were covalently grafted to solid supports. This may result in a limited grafting density due to the significant steric hindrance of large molecular polymers and limit the further improvement of adsorption capacity. In ref [17], the PEI 70,000 modified boronate affinity monolithic column showed lower binding capacity (18.9 μmol mL^{-1}) than that modified with PEI 10,000 (28.6 μmol mL^{-1}). This might be due to the large steric hindrance of PEI 70,000, which led to a lower grafting density, resulting the decrease of the number of active sites for the attachment of boronate affinty ligand. Compared with "grafting to" method, high grafting density of polymer chain on a solid support can be achieved with "grafting from" method due to the diffusion of monomers into the active sites [43,44]. Surface-initiated atom transfer radical polymerization (SI-ATRP) was an efficient "grafting from" method to modify solid surface with polymer brushes with high grafting density and the polymer chains adopted a unique stretched conformation due to the strong exclusion forces [45,46]. This approach has been successfully used to produce boronate affinity materials [30,47] and other type materials [48,49] with high binding capacity towards target analyte. Besides, the relationship between the binding capacity and the polymer brushes has been investigated in detail [30]. However, an alkaline binding pH is usually acquired, due to the weak acidity of most of the boronate affinity ligands, which may lead to degradation of labile compounds, especially bimolecular in biosamples. To reduce the binding pH of boronate affinity related technique, strategies, including adoption of boronate affinity ligand with specific molecular structure [8,9,14,15] and the teamed boronate affinity methods [5,13,20] were developed and effectively reduce the binding pH to neutral or moderate acidic.

Wulff-type phenylboronic acid featured intramolecular B-N coordination [9,50] and demonstrated binding capacity to *cis*-diol compounds at neutral and even moderate acidic pH, extending the application scope of boronate affinity materials to acidic biological samples, such as urine, tears and saliva. A Wulff-type boronate, (3-(dimethylaminomethyl)aniline-4-pinacol boronate, termed as Wulff-PBA here) had been synthesized and applied for the preparation of boronate affinity monolithic capillary for the selective capture of *cis*-diol compounds at pH as low as 5.5 [9]. The monolithic capillary showed excellent binding properties towards *cis*-diol compounds. However, the binding capacity was relative low, which might be due to the relative small number of boronate affinity ligand attached the capillary.

Herein, we described the preparation of a boronate affinity silica stationary phase with enhanced binding properties towards *cis*-diol compounds. The silica was first grafted with poly(glycidyl methacrylate) (PGMA) brushes through SI-ATRP, dramatically amplifying the number of active sites. Then, Wulff-PBA was covalently anchored through ring-opening reaction. Its binding strength towards *cis*-diol compounds was investigated. The properties of the column based on the stationary phase, including hydrophilicity-hydrophobicity, boronate affinity selectivity, binding pH and binding capacity, were investigated in detail. Furthermore, the stationary phase was applied in the selective extraction of nucleosides from real urine samples.

2. Experimental

2.1. Chemicals and reagents

Aminopropyltrimethoxysilane (APTES) and 2-bromoisobutyryl bromide (BiBB) were obtained from J&K Scientific Ltd. (Beijing, China). Silica gel (with particle size of 5 μm, pore size of 100 Å and specific surface area of 290 $m^2\ g^{-1}$) was provided by Dalian Replete Scientific Instruments Co. Ltd. (Dalian, China). The Wulff-type PBA (3-(dimethylaminomethyl)aniline-4-pinacol boronate) was synthesized according to a previously reported route [9]. Glycidyl methacrylate (GMA), 2,2′-bipyridine (Bipy), CuBr, $CuBr_2$, triethylamine (TEA), thymidine (T), deoxyadenosine (DA), adenosine (A), uridine (U) and cytidine (C) were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Toluene, acrylamide and thiourea were purchased from Alfa-Aesar (Tianjin, China). HPLC grade acetonitrile (ACN), acetic acid (HOAc) and methanol were used for the HPLC analysis. Ultrapure water was obtained from MilliQ gradient ultrapure water system (Millipore Inc., MA, USA). All the other chemicals were of analytical grade without further treatment.

2.2. Synthesis of the silica stationary phase

The overall synthesis procedure is shown in Fig. 1A. Prior to use, SiO_2 was activated by hydrochloric acid/H_2O (1/1, v/v) solution with stirring for 24 h, then washed with deionized water to neutral and dried under vacuum at 120 °C.

Aminopropyl functionalized silica was prepared according to the method described in previous literature [51] with some modification. Silica (11.3 g) and APTES (8.0 mL) were added into 100 mL anhydrous toluene under nitrogen atmosphere and the mixture was refluxed for 12 h under nitrogen with continuous stirring. After cooling, the product was separated by centrifuge, and then washed successively with toluene and ethanol for three times respectively, followed by drying under vacuum at 40 °C. The resulting product was referred as SiO_2-NH_2. Then, SiO_2-NH_2 (6.0 g) and TEA (3.0 mL) were added into dichloromethane (80 mL) in an ice bath with magnetic stirring. A solution containing BiBB (4.0 mL), DMAP (38 mg) and dichloromethane (80 mL) was added dropwise. The mixture was kept at 0 °C for 1 h and then at room temperature for 24 h. The obtained ATRP initiator SiO_2@Br was washed with dichloromethane, ethanol and ultrapure water, and then dried under vacuum.

The obtained SiO_2@Br (2.0 g) was used as an initiator and dispersed in methanol/water solution (30 mL, 5/1, v/v). Then, GMA (2.6 mL), CuBr (143 mg), $CuBr_2$ (44.6 mg) and Bipy (312 mg) were successively added under nitrogen. The mixture was vacuumed and bubbled with nitrogen six times. The polymerization proceeded at 60 °C for 24 h under continuous magnetic stirring. The final product SiO_2@PGMA were collected by centrifuge and washed with methanol and ultrapure water three times respectively, followed by drying under vacuum at 40 °C. Subsequently, SiO_2@PGMA (3.0 g) was mixed with acetonitrile (70 mL), Wulff-PBA (0.35 g) and TEA (0.4 mL). The mixture was heated at 60 °C for 12 h with stirring to undergo the ring opening process, resulting in SiO_2@PGMA-Wulff. Afterward, the SiO_2@PGMA-Wulff was isolated by centrifuge, washed with acetonitrile and deionized water successively for several times, and dried under vacuum at 40 °C.

Before boronate affinity analysis, the SiO_2@PGMA-Wulff column was washed with100 mM HOAc at a flow rate of 0.25 mL min^{-1} for 30 min, in order to dissociate the pinacol ester, and equilibrated with the loading buffer until a stable baseline was achieved. The procedure of reversible capture/release of the *cis*-diol compounds along with corresponding experimental conditions is shown in Fig. 1B.

2.3. Characterization of the prepared stationary phase

The carbon, hydrogen and nitrogen contents of activated Silica, SiO_2-NH_2, SiO_2@Br, SiO_2@PGMA and SiO_2@PGMA-Wulff were determined by elemental analysis on an Elementar Vario EL cube (Hanau, Germany). FT-IR spectra of the samples were obtained on a