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Silica stationary phase functionalized by 4-carboxy-benzoboroxole with enhanced boronate affinity nature for selective capture and separation of *cis*-diol compounds

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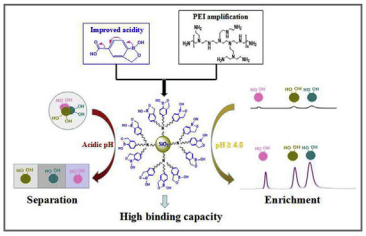
HIGHLIGHTS

- 4-Carboxy-benzoboroxole (CBB)-functionalized polyethyleneimine (PEI)-grafted silica stationary phase was prepared.
- The SiO_2@PEI-CBB showed excellent selectivity, the lowest binding pH and high binding capacities towards *cis*-diols.
- The secondary separation capability was pH-dependent, inspiring pH-gradient method for *cis*-diols separation.
- Enrichment of urinary nucleosides with excellent selectivity and efficiency was demonstrated.
- The stationary phase greatly expanded the application scope of boronate affinity materials.

GRAPHICAL ABSTRACT



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ABSTRACT

4-Carboxy-benzoboroxole was designed and synthesized. It was then combined with the modification effect of polyethyleneimine (PEI) for the preparation of boronate affinity silica stationary phase. The stationary phase showed improved binding strength with dissociation constant (K_d) towards xanthosine as low as 2.48×10^{-4} M. The column showed excellent selectivity, high binding capacities (88.3 μmol adenosine g^{-1}, pH 7.0) and the lowest binding pH (4.0 for cytidine and as low as 2.24 for xanthosine). These binding properties were superior to the existing boronate affinity materials, facilitating the selective extraction of trace *cis*-diol compounds in complex samples and greatly expanding the application scope of boronate affinity chromatography. In addition, the column showed secondary separation capability under acidic conditions and this secondary separation capability was investigated thoroughly. It was found that the separation was pH-dependent and mainly determined by binding strength with the possibility of involvement of other interaction, providing alternative strategy for the separation of *cis*-diol compounds. The feasibility and practicability were demonstrated through the selective enrichment of

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nucleosides in urine samples and the results indicated the excellent performance and great potential for the extraction of trace *cis*-diol compounds in complex samples.

1. Introduction

Cis-diol biomolecules, such as catechols, nucleotides, nucleosides, carbohydrates and glycoproteins, are of great biological significance and many of them are model analytes of proteomics, metabolomics or glycomics. However, *cis*-diol compounds in biosamples usually exist in very low abundance along with abundant interfering components. It is usually a necessary prerequisite to selectively isolate and enrich the target *cis*-diol compounds before their analysis. Boronate affinity technique has been proved to be a facile and efficient way for selective capture and enrichment of *cis*-diol compounds. The principle bases on the pH-dependent formation (usually alkaline conditions) and dissociation (acidic conditions) of five or six-membered cyclic esters between boronate ligand and *cis*-diol molecules. Boronate affinity materials based on various supports, such as monolithic column [1–12], nanoparticles [13–23], silica [24–27], fibrous cotton [28], polymers [29–31] and graphene [32], have been developed and applied to the selective capture and enrichment of *cis*-diol compounds. However, there are still challenges in lowering the binding pH and improving the binding strength and binding capacity of boronate affinity materials.

Conventional boronic acids usually require a basic pH for strong binding, which gives rise to the risk of degradation of labile molecules [33,34]. The strategies which employ phenylboronic acid with electron-withdrawing groups, such as sulfonyl [3] and fluoro [11], Wulff-type phenylboronic acid [10,35] (with intramolecular B-N coordination), benzoboroxole (also called improved Wulff-type phenylboronic acid with intramolecular five membered cyclic boronate) [8] and pyridinylboronic acid [36] as well as a strategy called "teamed boronate affinity" [2,7,14] have reduced the binding pH to neutral or medium acidic conditions. The pyridinylboronic acid functionalized monolithic capillary showed binding pH as low as 4.5 [36]. So, it is a great challenge to further lower the binding pH. Boronate affinity ligands with superior binding nature can effectively lower the binding pH of boronate affinity materials [3,8,10]. Additionally, it has been demonstrated that the adoption of boronate ligands with superior *cis*-diol binding properties was a direct way to improve binding strength [8,10,11]. Recently, a novel strategy, which combined boronate affinity with molecular imprinting, was able to dramatically enhance the binding strength towards *cis*-diol compounds with multiple *cis*-diol moieties, such as glycoproteins [37–41]. As to the improvement of binding capacity, the adoption of improved boronate ligand [8] and amplification of the number of boronic acid ligand through polymers, such as polyethyleneimine (PEI) [11,22,28], dendrimers [16,26] and chain polymer brushes through surface-initiated polymerization [24,25,29], have been proved to be efficient ways. So, it is a promising way to combine the superior boronate affinity ligand with amplification strategy for lowering binding pH and improving binding strength and binding capacity of boronate affinity materials.

Benzoboroxoles are a unique class of boronic acids which show excellent water solubility and improved sugar binding capacity in neutral water conditions [42–44]. The unique intramolecular five membered cyclic boronate structures were stable in acidic condition, bringing about extraordinary binding nature towards *cis*-diol compounds. They have been used as binding ligands for the detection of saccharide [45] and oligosaccharide [46]. 3-Carboxybenzoboroxole has been synthesized and used to prepare boronate affinity monolithic column and the column exhibited excellent selectivity and affinity with binding pH as low as 5.0 [8]. The monolithic column was off-line hyphenated with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the efficient analysis of glycoproteins/glycopeptides [47]. Besides, the monolithic column showed secondary separation capability towards *cis*-diol compounds in acidic conditions. The interaction responsible for the separation was not deeply understood yet. The secondary separation capability was also observed in other boronate affinity columns [3,36] and hydrogen bonding and anion exchange were considered in charge of the separation ability respectively. Deep insight into the interaction between benzoboroxole and *cis*-diol compounds is necessary for a better understanding and applications of this kind of boronate ligand.

In this study, to explore in depth the properties of benzoboroxole functionalized materials and further lower the binding pH as well as improve the binding strength and binding capacity of boronate affinity materials, 4-carboxy-benzoboroxole (CBB) was designed and synthesized. It was then combined with PEI modification strategy for the preparation of boronate affinity silica stationary phase. The properties, including binding selectivity, secondary separation ability, binding strength, binding pH and binding capacity, were thoroughly investigated. The K_d values towards six *cis*-diol nucleosides were determined to deeply understand the interaction between benzoboroxole and *cis*-diol compounds, facilitating the target-oriented design of boronate affinity materials. The practicability of the stationary phase was demonstrated through the selective extraction of trace nucleosides in urine samples.

2. Experimental

2.1. Materials and reagents

Methyl 4-bromo-3-methylbenzoate, Polyethyleneimine (PEI, MW ~10,000 Da), (γ-glycidoxypropyl)trimethoxysilane (GPTS), *N,N*-diisopropylethylamine (DIPEA), o-Benzotriazol-1-yl-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxy-7-azabenzotriazole (HOAt) were obtained from J&K Scientific Ltd. (Beijing, China). SiO_2 (with particle size of 5 μm, pore size of 100 Å and specific surface area of 290 m^2/g) was provided by Dalian Replete Scientific Instruments Co. Ltd. (Dalian, China). [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium (II) ($Pd(dppf)_2Cl_2$), *N*-bromosuccinimide (NBS), bis(pinacolato)diborane, triethylamine (TEA), cytidine (C), adenosine (A), uridine (U), inosine (I), guanosine (G), xanthosine (X), deoxyadenosine (DA), thymidine (T) were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). HPLC grade acetonitrile (ACN), acetic acid (HOAc), formic acid 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.