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Polyethyleneimine-grafted boronate affinity materials for selective enrichment of *cis*-diol-containing compounds

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ABSTRACT

Polyethyleneimine (PEI)-grafted and 3-acrylamidophenylboronic acid (AAPBA)-functionalized SiO_2 boronate affinity materials were synthesized for the selective enrichment of *cis*-diol-containing compounds. Characterization results of scanning electron microscopy, Fourier transform infrared spectroscopy, elemental analysis, zeta potential, and X-ray photoelectron spectroscopy indicated the successful fabrication of SiO_2@PEI–AAPBA materials. Chromatographic separation of test mixtures reveals that SiO_2@PEI–AAPBA has high selective enrichment ability for *cis*-diol-containing compounds. The binding pH between SiO_2@PEI–AAPBA and catechol was found to be as low as pH 4.5, while that between SiO_2@PEI–AAPBA and adenosine was only ~7.5. This difference might be attributed to the strong electrostatic repulsion between the solid phase and analytes at a low pH. Furthermore, a diphasic separation column was fabricated based on boronate affinity chromatography, C18-reversed-phase chromatography and applied in pressurized capillary electrochromatography (pCEC). Results showed that four polar nucleosides could be well captured by the boronate affinity chromatography (BAC) section and separated by reversed phase pCEC. Finally, SiO_2@PEI_{600}–AAPBA-based solid-phase extraction technology was applied to the purification of ribonucleosides in real urine samples, and results of UHPLC–MS/MS revealed that the intensities of the extracted ions (a neutral mass loss of *m*/*z* 132.04 Da) of the ribonucleosides were significantly enhanced after the enrichment.

1. Introduction

cis-Diol-containing biomolecules such as glycoproteins, glycolipids, nucleosides, carbohydrates, and catechols play a crucial role in many fields of biotechnology and medicine. For example, glycosylation is one of the most common post-translational modifications, and glycoproteins play fundamental roles in many biological processes [1]. Numerous studies have shown that many potential biomarkers and clinical therapeutic targets are *cis*-diol-containing biomolecules [2–7]. Therefore, their selective detection in biological samples will facilitate the discovery of potential biomarkers. Unfortunately, most *cis*-diol-containing biomolecules in real samples are of low abundance and difficult to analyze quantitatively because of the matrix effect in mass spectrometric detection [8]. Therefore, effective enrichment and purification of *cis*-diol-containing biomolecules are essential.

Boronate affinity chromatography (BAC) is a powerful tool for selective separation and enrichment of *cis*-diol-containing compounds. The fundamental principle of BAC is based on the reversible covalent complexation between boronic acids and *cis*-diols in an alkaline aqueous solution (pH ≥ 8.5) to afford the corresponding cyclic boronate esters that dissociate in acidic environment. However, conventional boronic acids, taking phenylboronic acid for example, cannot strongly bind to *cis*-diol-containing compounds in near-neutral or weakly acidic solutions because of the requirement of an alkaline pH for significant binding; furthermore, conventional boronic acids exhibit limited binding affinity for trace amounts of compounds. In general, the properties of BAC are determined by both the boronic acid ligands and support materials [9], that is to say, materials with strong boronate affinity binding ability at lower pH condition and high-density boronate affinity groups are needed. Therefore, based on these requirements, several strategies have been developed for synthesizing appropriate support materials and aromatic boronic acids or their derivatives with a lower pKa value [10–15].

Polyethyleneimine (PEI) polymers have been widely used as coating materials for inorganic nanoparticles (NPs) and polymeric carriers for gene delivery because of their strong electrostatic affinity for polynucleic acids [16–18]. Due to a high density of

Abbreviations: PEI, polyethyleneimine; AAPBA, 3-acrylamidophenylboronic acid; BAC, boronate affinity chromatography; pCEC, pressurized capillary electrochromatography; SPE, solid-phase extraction

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amino groups, PEI polymers can supply a large number of active binding sites for grafting, which can greatly increase the amount of functional ligands in the chemical modification of a polymer surface [19], known as brushes or tentacles. Moreover, PEI significantly improved the hydrophilicity of bonded materials facilitating applications [20] in the separation and detection of biological samples, particularly reducing nonspecific adsorption significantly. Thus, these unique features of PEI make it an ideal support material for improving the binding density of interesting functional monomers.

Based on the excellent features of both PEI and boronic acids, Peng et al. [21] reported a phenylboronic acid-modified PEI, which significantly improved gene transfection efficiency compared to unmodified PEI. The efficiency of PEI was attributed to a high charge density at physiological media and a high buffer capacity at weakly acidic media. Li et al. [22] reported a new type of magnetic NPs, namely, Fe_3O_4@SiO_2@PEI–FPBA (FPAB=4-formylphenylboronic acid), which was found to show a much higher binding capacity for *cis*-diol-containing compounds.

The objective of this study is to combine the features of PEI and BAC to synthesize a novel boronate affinity material with strong binding capacity for isolating and purifying *cis*-diol-containing biomolecules, particularly those at a low concentration. Thus, a novel SiO_2@PEI–AAPBA affinity material was prepared, and 3-acrylamidophenylboronic acid (AAPBA) was selected as the boronate affinity ligand to grow on PEI, which was covalently immobilized on a SiO_2 material, by the Michael addition reaction between the amino groups of PEI and the α,β-unsaturated carbonyl groups of AAPBA. SiO_2@PEI–AAPBA was characterized and applied for chromatographic separation to confirm its utility and specificity. Further, the separation mechanism of this material was investigated, and the effect of the molecular weight of PEI on the binding ability of the material was elucidated for the first time. Moreover, a single column packed serially by boronate affinity materials and C18-reversed-phase was utilized in pressurized capillary electrochromatography (pCEC) for the separation of complex mixtures containing *cis*-diols. Finally, a solid-phase extraction–high-performance liquid chromatography–mass spectrometry (SPE–HPLC–MS) platform based on SiO_2@PEI_{600}–AAPBA was applied to the enrichment and separation of urinary ribonucleosides in real urine samples.

2. Materials and methods

2.1. Materials

3-Aminophenylboronic acid (APBA) monohydrate was purchased from Beijing Zhongsheng Huateng Technology Co., Ltd. Acryloyl chloride, stabilized with 400 ppm phenothiazine, as well as the polyethyleneimine molecules with four different molecular weights (PEI, MW~600 Da, ~1800 Da, 10,000 Da, 99%, and ~70,000 Da, 50%, marked as PEI_{600}, PEI_{1800}, $PEI_{10,000}$, and $PEI_{70,000}$, respectively) was bought from J&K Chemical Ltd. and used directly without further purification. Tetraethylorthosilicate (TEOS), γ-(2,3-epoxypropoxy) propytrimethoxysilane (KH-560), methyl alcohol, ethyl alcohol, formic acid, ammonium formate, anhydrous ether, and toluene were purchased from the Sinopharm Chemical Reagent Co., Ltd. The toluene must be used after the dehydration treatment. Adenosine, deoxyadenosine, catechol, resorcinol, hydroquinol, uridine, guanosine, and uracil were purchased from the Aladdin Industrial Inc. Ultrapure water was purified using a Molelement water purification system (Molecular, Chongqing, China). Other reagents utilized were of analytical grade or better. Silica spheres (particle size ~3 μm and ~20 μm, pore diameter ~12 nm) and C18–SiO_2 spheres (particle size ~3 μm, pore diameter ~12 nm) were achieved from the Global Chromatography (Suzhou, China). Fused-silica capillary tubes (100 μm ID) were obtained from the Yongnian Fiber Plant (Hebei, China).

2.2. Instruments

Scanning electron microscopy (SEM) of the morphology for sub-2 μm SiO_2 was carried out on a Hitachi S-4800 SEM instrument. The samples were prepared by dispensing drops of aqueous suspension onto aluminum foil fragments which were adhered onto the objective table by the conductive tapes. A Nano ZS Zetasizer (Mlavern Instruments Ltd.) was used to determine the zeta potential of the materials using the aqueous suspension at pH=7. Fourier-transform infrared spectrometric (FT-IR) spectra were recorded on a Nicolet 6700 (Thermo Fisher, USA) FT-IR spectrometer using KBr pellet, which were used to further prove the coating of the functional groups. X-ray photoelectron spectroscope (XPS, AXIS UltraDLD, Kratos, Japan) with Al Kα X-ray (energy=1486.3 eV) as excitation source was used to probe the surface element. The elemental analysis was performed with a Vario EL Cube (Elementar, Germany) type apparatus. The capillary chromatographic operations were completed on a Trisep-2100 pCEC (Unimicro Technologies, USA) instrument with a UV absorbance detector. The SPE part was performed on a CNW 12 Position Vacuum Manifold Set. And the UHPLC–MS/MS system consisted of an ACQUITY ultra performance liquid chromatographic system (Waters, Milford, MA, USA) coupled with an API 5500 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with a Turbo Ion Source (ESI) operating with positive mode was used.

2.3. Synthesis of 3-acrylaminophenylboronic acid

The functional ligand AAPBA was synthesized by reacting APBA with acryloyl chloride in an aqueous solution containing sodium hydroxide according to the method previously reported [23]. First, APBA (1.14 g) was dissolved in aqueous sodium hydroxide (1.2 M, 25 mL), and the resulting solution was cooled in the ice-bath. Then, acryloyl chloride (1.2 mL) was added dropwise over a period of ~2 min under vigorous stirring. After 1 h, the mixture was warmed to room temperature, and the pH of the mixture was adjusted to ~1 by a hydrochloric acid solution (1 M). The resulting beige precipitates were filtered and washed several times with cold water (~5 mL). Then the precipitates were dissolved in ultrapure water (20 mL) at 85 °C, and the impurities were filtered off. The filtrate was left to stand overnight at room temperature, and the resulting light needle-like crystals of the product were filtered and dried in a desiccator. ^{1}H NMR spectrum of the product was consistent with previously published data. ^{1}H NMR (400 Hz): δ ppm 9.21 (s, 1H, NH), 8.01 (s, 2H, B–OH), 7.90 (d, 1H, Ar–H), 7.57 (d, 1H,Ar–H), 7.29 (t, 1H, Ar–H), 7.13 (s, 1H, Ar–H), 6.46 (dd, 1H, CH), 6.33 (dd, 1H, C=CH_2), 5.68 (dd, 1H, C=CH_2).

2.4. Preparation of SiO_2@PEI–AAPBA microspheres

2.4.1. Procedure for preparing sub-2 μm SiO_2@PEI–AAPBA microspheres with four different molecular weights

Procedure for preparing sub-2 μm SiO_2@PEI–AAPBA microspheres with four different molecular weights: (i) nonporous sub-2 μm silica (SiO_2) microspheres were synthesized by the modified two-phase sol–gel method [24]. The obtained SiO_2 microspheres were activated in a hydrochloric acid solution (12 wt%) for 24 h, washed thoroughly with ultrapure water until neutral pH, and dried under vacuum at 45 °C after filtration. (ii) To modify the SiO_2 surface with epoxy groups, the activated SiO_2 microspheres (1 g) were refluxed with KH-560 (4 mL) in anhydrous toluene (60 mL) for 24 h under nitrogen. The resulting epoxy-modified SiO_2 microspheres (denoted as SiO_2–epoxy) were isolated and washed in sequence with toluene, acetone, and