Electronic Supplementary Information

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Restricted Access Boronate Affinity Porous Monolith as a Protein A Mimetic for the Specific Capture of Immunoglobulin G

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Experimental section

Materials

Tris (2,3-epoxypropyl) isocyanurate (TEPIC), (3-aminopropyl) trimethoxysilane (ATMS) were obtained from Alfa Aesar (Ward Hill, MA, USA). Horseradish peroxidase (HRP), human α_1-acid glycoprotein (AGP), ribonuclease B (RNase B), ovalbumin (OVA), lactoferrin from bovine milk, adenosine, deoxyadenosine, sinapinic acid, *m*-aminophenylboronic acid (APBA), 4-mercaptophenyl-boronic acid (MPBA) and 1,6-hexamethylenediamine (HMDA) were all purchased from Sigma (St. Louis, MO, USA). α-Fetoprotein (AFP), trypsin inhibitor (TI), myoglobin, human serum albumin (HSA), bovine serum albumin (BSA), rat monoclonal antibody (mAb) against human AFP (anti-AFP mAb), rat mAb against human EPO (anti-EPO mAb), rat mAb against human carcinoembryonic antigen (anti-CEA mAb), rat mAb against human prostate-specific antigen (anti-PSA mAb), rat mAb against human ferrin (anti-FR mAb), and rabbit polyclonal antibody (pAb) against human anti-AFP (anti-AFP pAb), rabbit pAb against human anti-CEA (anti-CEA pAb), rabbit pAb against human ferrin (anti-FR pAb), rabbit pAb against human anti-PSA (anti-PSA pAb) were all purchased from Shuangliu Zhenglong Chemical and Biological Research Laboratory (Sichuan, China). Human recombinant erythropoietin (EPO) was purchased from the European Pharmacopoeia, which was pretreated prior to use according to the procedure described previously.[1] Human blood serum was kindly donated from Nanjing Railway Hospital, and it was diluted 10 times by 100 mM phosphate buffer (pH 8.5) before used. Polyethylene glycol 200 (PEG-200) and tetrahydrofuran (THF) were purchased from Sinapharm Chemicals (Shanghai, China). All other chemicals were of analytical or HPLC grade. Water used in all the experiments was purified by a Milli-Q Advantage A10 ultrapure water purification system (Millipore, Milford, MA, USA). Fused-silica capillaries of 150 μm i.d. and 375 μm o.d. were purchased from Yongnian Optic Plant (Hebei, China). *N*,*N*′-bis(2-aminoethyl) oxamide (BAEO) was synthesized according to the literature method.[2] Its NMR data was in agreement with literature. Relative ^{1}H NMR (500 MHz) data were: δ 7.82 (1H, s) δ 3.40 (2H, q, J = 6, 6.5, 6 Hz), δ 2.90 (2H, t, J = 6, 6.5 Hz) ppm.

Instrumentation

^{1}H NMR spectra were recorded on a Bruker Avance DMX 500 MHz instrument (Bruker Biospin GmbH, Rheinstetten, Germany) using solvent residual at peak as reference (7.27 ppm). Solid-state ^{11}B magic angle spinning nuclear magnetic resonance (MAS NMR) spectra were performed on Bruker Avance 400 spectrometer (Bruker Biospin

GmbH, Rheinstetten, Germany) operating at 128 MHz for the 11B nucleus using commercial trifle resonance 4 mm MAS NMR probe. The magic angle was adjusted by minimizing the line widths for the spinning sideband in the ^{23}Na MAS NMR. A HPLC pump was utilized to flush the monolithic columns. Scanning electron microscopy (SEM) characterization was performed on a FE-SEM S-4800 instrument (Hitachi, Tokyo, Japan). TriSep-2100 pCEC (Unimicro Technologies, Pleasanton, CA, USA) instrument with a UV-absorbance detector was used to carry out all chromatographic separations and enrichments. Capillary electrophoresis experiments were conducted on a P/ACE MDQ system (Beckman Coulter, Fullerton, CA, USA). The throughpore size of the monolith was determined using a PoreMaster-60 mercury intrusion porosimeter (Quantachrome, Boynton Beach, FL, USA). Specific surface areas and mesopore size were determined by the Brunauer-Emmett-Teller (BET) method with an ASAP 2020 physisorption analyzer (Micromeritics, Norcross, GA, USA).

Matrix-assisted laser desorption ionization-time of flight mass spectrometric (MALDI-TOF MS) analyses were implemented on an Autoflex mass spectrometer (Brucker Daltonics, Billerica, MA, USA) equipped with a delayed ion-extraction device and a pulsed nitrogen laser operated at 337 nm. The range of laser energy was adjusted to slightly above the threshold to obtain good resolution and signal-to-noise ratio. All mass spectra reported were obtained in the positive ion linear mode with delayed extraction for 500 ns and calibrated using an external calibration equation generated from the ion signal of apomyoblobin, aldolase and albumin. Spectra were acquired in the positive-ion mode at 25 Hz laser frequency, and each profile is composed of the accumulation of 500 laser shots. A 0.5 μL aliquot of elution was mixed on a polished steel target plate with 1 μL of matrix solution (10 mg/mL sinapinic acid in 50% acetonitrile, 0.1% trifluoroacetic acid) and allowed to dry at room temperature. Then it was washed by using ice cold water directly on the plate.

Preparation and characterization of the monolithic capillary columns

A capillary of 45 cm in length was rinsed sequentially with 1 M NaOH, water, 1 M HCl, and water for 30 min each. The capillary was then dried under a steam of nitrogen. A 1:1 (v/v) mixture of THF and ATMS were pumped through the capillary at 80 °C for 24 h. The modified capillary was then washed with methanol. TEPIC (80 mg), MPBA (23 mg), and BAEO (20 mg) were dissolved in PEG 200 (369 mg) by sonication at 40 °C. The remained homogeneous solution was injected into the modified capillary with a high-pressured pump. Both ends of capillary were sealed with rubber, and the capillary was subsequently submerged in a water bath at 80 °C for 12 h. The resulting monolithic

column was washed with methanol by a HPLC pump to flush out the unreacted monomers and porogens. The monolithic column was washed with 100 mM acetic acid (HAc) for 30 min before use.

The poly (TEPIC-co-(APBA-HMDA) monolithic capillary was prepared according to the previously reported method.[3]

Monolith for porous properties characterization

For characterization of the pore-size distribution and specific area, a monolith bed was synthesized in an empty regular stainless steel HPLC column. The monolithic column was washed with methanol by using a HPLC pump until the residual reagents were flushed out. Subsequently, the monolith was flushed out from the columns and cut into small pieces. The obtained stationary phase was dried under vacuum at 100 °C for 24 h.

Breakthrough capacity measurement

The breakthrough capacity of the boronate affinity monolithic column was measured by the frontal chromatography method. For small molecules, the measurement was carried out on the TriSep-2100 pCEC instrument. A monolithic column of 45 cm in length (effective length 40 cm) was used. First, the monolith was equilibrated with the loading buffer (100 mM phosphate buffer, pH 7.5) for 30 min. A test sample containing 1 mg/mL catechol and 0.01 mg/mL in the loading buffer was pumped through the column. Elution was carried out with 100 mM acetic acid solution. For antibody, the measurement was carried out on the P/ACE MDQ CE instrument, the effective length of the monolith was 5 cm, and the test sample was changed into a mixture of 0.05 mg/mL anti-AFP and 0.01 mg/mL deoxyadenosine in the loading buffer.

Chromatographic experiments

A monolithic of poly ((TEPIC-co-(MPBA-BAEO)) with a total length of 45 cm (effective length 40 cm) was used in all chromatographic experiments, and a detection window was created at the end of the monolithic column by heating with a thermal wire stripper. Samples were injected through an injection valve with an internal 600-nL sample loop. A three-port splitter was set before the injection valve to split the flow into a desirable and stable flow rate. The splitting ratio was set with 15:1. Mobile phase flow rate was set with 0.090 mL/ min, and column pressure was 16.5 MPa. The UV absorbance was monitored at 214 nm for quinol, catechol, proteins and antibodies; 260 nm for adenosine and deoxyadenosine.

Enrichment of EPO by the monolithic column for CZE and MS experiments

The procedure for the enrichment of antigen included five steps, including: 1) antibody immobilization, 2) washing, 3) antigen enrichment, 4) washing, and 5) elution. Phosphate buffer (100 mM, pH 7.4) was used as the loading buffer, while acetic acid solution (100 mM, pH 2.7) was used as the elution buffer. A monolithic column with length of 10 cm was first washed by loading buffer for 30 min. Then, excesses anti-EPO mAb (50 μL, 1 mg/mL) was added to a 0.5 mL micro-centrifuge tube which was placed in a tailor-made high-pressure bomb connected to a nitrogen gas cylinder, and the anti-EPO was introduced by applying 2 MPa of nitrogen until all the antibody was pushed through the column. After that, the sample was replaced by the loading buffer to remove the unreacted antibody for 10 times column volume. Then excess antigen EPO (20 μL, 1 mg/mL dissolved in 100 mM phosphate buffer, pH 7.5) was introduced into the monolithic column and incubated at 37 °C for 30 min. Then, it was washed by loading buffer to remove the unreacted antigen for 10 times column volume. The enriched EPO was then eluted by the elution buffer into tubes. Every 5 μL eluate was collected in one tube and five tubes were collected in total for CZE experiments and MS experiments.

CZE separation of EPO glycoforms

The experimental condition of CZE separation of EPO glycoforms was the same as the previous method.[4] In brief, a bare fused-silica capillary with 60 cm total length (50 cm effective length) and 50 μm i.d. was used for separation. The separation buffer was 10 mM sodium acetate containing 7 M urea, 10 mM Tricine, 3.9 mM putrescine, and 100 mM NaCl, pH 5.50. The separation voltage was 15 KV.

References

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2 H.J. Chang, O. Vogl, *J. Polymer Sci*. 1977, **15**, 311-322.

3 L.B. Ren, Z. Liu, Y.C. Liu, P. Dou, H.Y. Chen, *Angew. Chem., Int. Ed.* 2009, **48**, 6704–6707.

4 J. Liu, Z. Liu, M.C. Kang, S.C. Liu, H.Y. Chen, *J. Sep. Sci.* 2009, **32**, 422-429.

Supporting Figures.

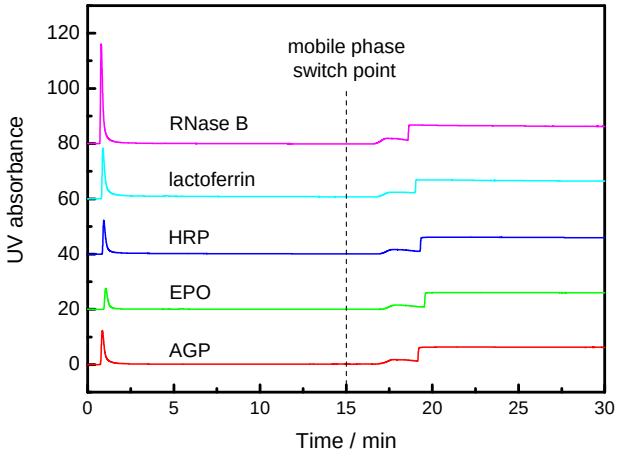

Fig. S1 Retention of glycoproteins on the poly (TEPIC-co-(APBA-HMDA)) monolith. Loading buffer, 100 mM phosphate buffer, pH 7.5; elution buffer, 100 mM acetic acid, pH 2.7.

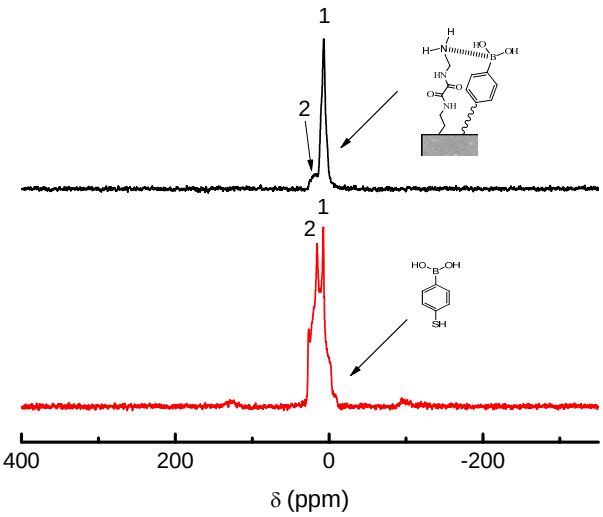

Fig. S2 ^{11}B MAS NMR spectrum for MPBA (bottom trace) and the poly(TEPIC-co-(MPBA-BAEO)) monolith (top trace). The boron atom of MPBA exhibited both sp^3 (represented by peak 1) and sp^2 (represented by peak 2) configuration while the boron atom of the monolith exhibited predominate sp^3 configuration. B atom of sp^3 configuration binds with cis-diol group with a much higher binding constant than B atom of sp^3 configuration does.

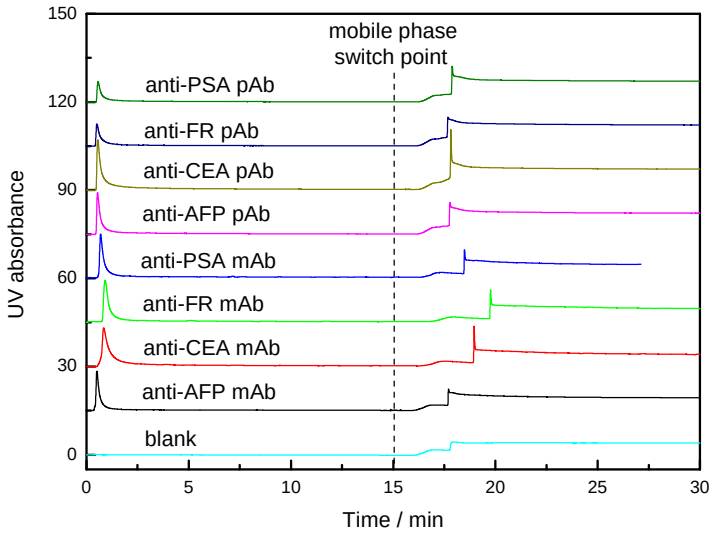

Fig. S3. Retention of antibodies on the poly (TEPIC-co-(APBA-HMDA)) monolith.