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New ligands for boronate affinity chromatography

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Abstract

A new type of boronate affinity ligand was synthesized which contained an internal coordinate bond between a carbonyl oxygen and a boron atom. This coordination makes the boron atom tetrahedral, which is favorable for boronate esterification with cis-diols. The ligand formed a catechol ester in 2-(N-cyclohexylamino)ethanesulfonic acid, Tris and phosphate buffers, respectively, at 0.5, 0.6 and 1.1 pH units lower than did phenylboronic acid. When the ligand was coupled to an agarose gel, the new matrix esterified with catechol at pH 7.5. When the ligand was coupled to a cellulose gel, the new matrix esterified with catechol at pH 7.0. In comparison, the commercial *m*-aminophenylboronic acid–agarose does not form an ester with catechol below pH 8.0.

1. Introduction

Boronate affinity chromatography was first used to separate nucleic acid components and carbohydrates by Weith et al. in 1970 [1]. Since then, the specificity of boronate has been used to separate *cis*-diol-containing compounds, including catechols, nucleic acids, glycoproteins and carbohydrates [2]. In recent years it has been applied to separate γ-glutamyltransferase in patients with hepatocellular carcinoma [3]; fast boronate affinity chromatography of glycosylated hemoglobin [4]; purify human platelet glycocalicin [5]; determine glycosylated albumin concentration [6]; determine 5-S-cysteinyldopa in human urine [7]; isolate 3,4-dihydroxyphenylalanine-containing proteins [8]; measure glycosylated hemoglobin in dried blood [9]; and isolate 2-hydroxycarboxylic acids [10]. Boronate affinity chromatography has also been used in clinical studies. For example, immobilized phenylboronate has been used to measure the level of glycosylated hemoglobin in the diagnosis of diabetes [11–13] and glycemia [14]. Measuring levels of hypoxanthine, uridine and inosine using boronate chromatography has been useful in the diagnosis of several pathological disorders [15,16]. Thus boronate affinity chromatography has been employed for a wide variety of applications in both basic biochemistry and clinical chemistry.

The earliest, and most widely used, boronate ligand is 3-aminophenylboronic acid (3aPBA or mPBA). It can be coupled to a solid support through the anilino group, and the *m*-amino substitution lowers its pK_a [17]. In all applications of 3aPBA, the pH must be basic, i.e. pH > 8. The pK_a of 3aPBA is 8.8 [18], so the pH should be as high as reasonably possible for optimum binding. However, in many cases the analytes lose their biological activities at such high pH values, which is the major limitation to

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expanded use of boronate affinity chromatography [19]. Attempts to lower the pK_a of boronate ligands have involved ligands such as *p*-bromophenyl boronate [17,20], *p*-(ω-aminoethyl) phenylboronate [21], and *p*-vinylbenzene boronate [22] and, more recently, the introduction of strong electron-withdrawing groups on the phenyl ring. For example, Soundararajan et al. [19] put a (N-methyl) carboxyamido group on the phenyl ring, while Singhal et al. [23] put a nitro group on the phenylboronate. Aliphatic boronate ligands with a five-membered stable complex formation have also been employed [24], and proposed [25]. In spite of these efforts, the goal of obtaining a ligand which can form a complex with *cis*-diols at neutral pH has not been attained [26].

The pH is a crucial factor in the reaction between boronates and *cis*-diols. Phenylboronic acid does not complex with D-glucose below pH 6, but is fully complexed above pH 9 [27]. It is not known if other factors determine the pH at which boronates react with *cis*-diols. The pK_a of the boronate may be the critical factor, but the optimal pH for esterification is not the same as the pK_a of the ligand. For example, the maximal esterification of adenosine occurred at pH 7.8, 7.3 and 8.2 during incubation with phenylboronic acid (PBA, $pK_a = 8.9$), *p*-nitrophenylboronic acid (4nPBA, $pK_a = 7.0$), and *p*-methylphenylboronic acid (4mPBA, $pK_a \approx 9$), respectively [28]. Maximal esterification with PBA occurs at 1.1 pH unit below its pK_a, whereas maximal esterification with 4nPBA occurs at 0.3 pH unit above its pK_a. This is similar to esterification between β-methylribofuranoside (mRib) and 3aPBA ($pK_a = 8.8$) and 4nPBA [23]. Esterification occurs 1.8 pH units below the pK_a of 3aPBA and 0.4 pH unit below the pK_a of 4nPBA. Thus the pK_a of a ligand is not consistent with the pH at which esterification occurs, which indicates that lowering the pK_a of a ligand may not be the essential factor needed to lower the optimal pH for esterification.

A tetrahedral boronate may be the favorable configuration for forming esters with *cis*-diol compounds, possibly because a tetrahedral boronate is less strained. We propose that the concentration of tetrahedral conformation, rather than pK_a, is the key factor for esterification. This is supported by the observation that in the esterification of ethylene glycol with 3-acetamidophenylboronic acid (trigonal boronate) and 3,6-diacetamidophenylboronic acid (tetrahedral boronate) in dimethyl sulfoxide (DMSO), the formation constant of the latter is 115 times higher than the former [29]. Further evidence comes from the observation by Wulff et al. [30], who showed that the rate of esterification of PBA with α-propylene glycol was several orders greater in the presence of piperidine, probably because the piperidine nitrogen donated a pair of electrons to the boron atom to form a coordinate nitrogen–boron bond. The resulting boronate was tetrahedral, as shown by ^{11}B NMR. In contrast, although triethylamine has a p*K* similar to that of piperidine, it did not accelerate the rate of esterification. Therefore, basicity is not the key here. One possible explanation is that steric hindrance associated with triethylamine prevents the formation of a tetrahedral boronate. If so, a boronate affinity adsorbent with the structure shown in Fig. 1 would be a better chromatography matrix at lower pH values than conventional boronate affinity adsorbents.

To determine this, we synthesized and crystallized the model compounds catechol [2-(diethylamino)carbonyl,4-methyl]phenylboronate (**I**) and catechol [2-(diisopropylamino)carbonyl]-phenylboronate (**II**) (Fig. 2). X-Ray crystallography demonstrated that there was indeed an internal coordination bond between carbonyl oxygen and boron, and that the boron was tetrahedral, which was also supported by ^{11}B NMR [31]. Subsequently, we synthesized the boronate affinity ligand, catechol [2-(diethyla-

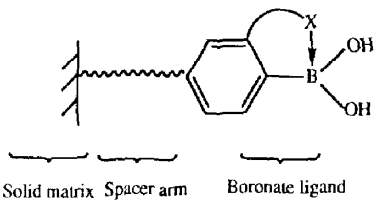

Fig. 1. The proposed internally coordinated boronate affinity matrix.

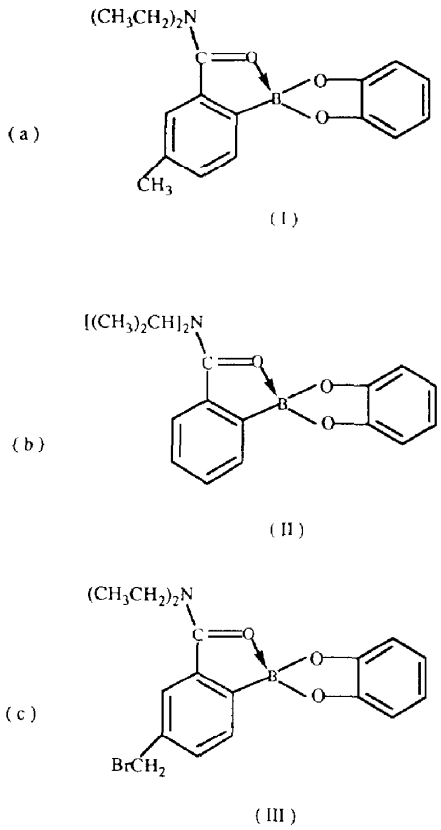

Fig. 2. The boronate affinity ligands synthesized: (a) catechol [2-(diethylamino)carbonyl,4-methyl]phenylboronate (**I**); (b) catechol [2-(diisopropylamino)carbonyl]phenylboronate (**II**); (c) catechol [2-(diethylamino)carbonyl,4-bromomethyl]phenylboronate (**III**).

mino)carbonyl, 4-bromomethyl]phenyl boronate (**III**) (Fig. 2), which was coupled to agarose and cellulose and used in boronate affinity chromatography.

2. Experimental

2.1. Materials

sec.-Butyllithium (^{sec}BuLi), N,N,N′,N′-tetramethylethylenediamine (TMEDA), tetrahydrofuran (THF), dimethylformamide (DMF), 2,5-dimethoxybenzyl alcohol, trimethyl borate, carbon tetrachloride, bromine, PBA and catechol were purchased from Aldrich (Milwaukee, WI, USA). N,N-Diethyl-*m*-tolumide and N,N-diisopropylbenzamide were purchased from Lancaster (Windham, NH, USA). Cysteamine agarose, sulfhydryl cellulose, *m*-aminophenylboronic acid–agarose (3aPBA-agarose), dithiothreitol (DTT), sorbitol, 2-(N-cyclohexylamino)ethanesulfonic acid (CHES), Tris and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma (St. Louis, MO, USA). Azomethine H and 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), were products of Pierce (Rockford, IL, USA). All other reagents were of analytical grade. NMR was performed on a Varian XL300 NMR spectrometer; elemental analysis was done by Atlantic Microlab (Norcross, GA, USA); mass spectrometry was performed by Mass Spectrometry Services, Montana State University; chromatography columns were from Sepracor (Marlborough, MA, USA); liquid chromatography was performed on a Bio-Rad Econo System (Hercules, CA, USA).

2.2. Methods

Synthesis of ligands (Fig. 3)

Synthesis of **I** and **II**

A modification of the method described by Beak and Brown [32] was used to synthesize **I** and **II**. All glassware and syringes were oven-dried. The reactions were carried out under purified nitrogen. THF was freshly distilled from potassium metal under nitrogen. TMEDA was freshly distilled from calcium hydride under nitrogen. ^{sec}BuLi was standardized prior to use by titration using 2,5-dimethoxybenzyl alcohol as an indicator [33]. The procedure used to synthesize **I** is described below.

TMEDA (2 ml, 13.0 mmol) in 30 ml dry THF at −78°C was added dropwise to 12 ml ^{sec}BuLi (13.0 mmol). After 10 min, 2.1 g of N,N-diethyl-*m*-tolumide (11.0 mmol) in 20 ml dry THF were added dropwise. The mixture was then stirred for 1 h at −78°C. Trimethyl borate, 8.0 ml (70.4 mmol), was added rapidly and the mixture was then stirred for an additional 20 h during which it was allowed to warm to room temperature. The

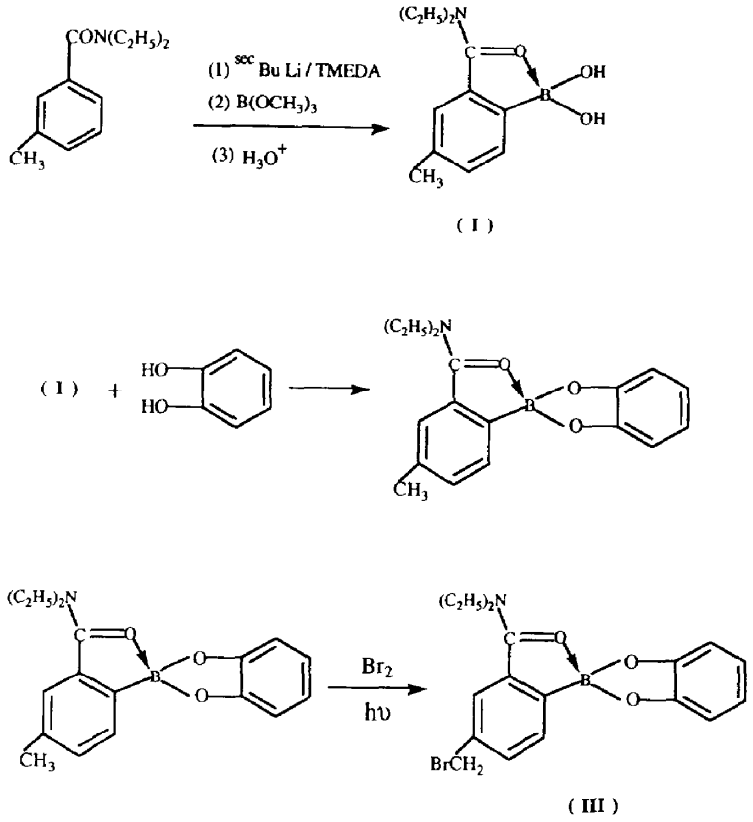

Fig. 3. Synthesis of **III**.

reaction mixture was then poured into a separating funnel containing 30 ml saturated aqueous NH_4Cl (pH 5.7) and 30 ml diethyl ether. The water phase was extracted twice and the combined ether phase was dried over $MgSO_4$. Evaporation of solvent in vacuo gave 2.46 g (10.5 mmol, 95.4%) [2-(diethylamino)carbonyl,4-methyl]phenylboronic acid.

Using a Dean–Stark water separator, the azeotrope of water and benzene was distilled from a solution of 2.1 g (8.9 mmol) of [2-(diethylamino)carbonyl,4-methyl]phenylboronic acid in 100 ml benzene. The reaction mixture cooled to 70°C and 1.0 g of catechol (9.1 mmol) was added. Distillation then continued for 3 h. Evaporation of the solvent in vacuo yielded 2.91 g (89.1%) of crude product, **I**. Recrystallization from toluene gave white needles. m.p. 173–175°C. ^1H NMR (300 MHz, C^2HCl_3) δ (ppm) 7.66 (d, 1H), 7.50 (s, 1H), 7.44 (d, 1H), 6.88 (m, 2H), 6.78 (m, 2H), 3.95 (q, 2H), 3.70 (q, 2H), 2.45 (s, 3H), 1.50 (t, 3H), 1.30 (t, 3H). ^{11}B NMR δ (ppm) 13.5 ppm, single peak. IR (KBr), ν 1620 cm^{-1}, 806 cm^{-1}, 740 cm^{-1}, 701 cm^{-1}. Mass spectrum (m/z, ion): 295, 223, 195, 167, 105. Analysis: calculated for $C_{18}H_{20}O_3BN \cdot 0.5C_7H_8$: C, 72.69; H, 6.81; N, 3.94; found: C, 72.16; H, 6.68; N, 3.91.

II was synthesized according to the same procedure, using N,N-diisopropylbenzamide in place of N,N-diethyl-*m*-tolumide m.p. 179–180°C. ^1H NMR (300 MHz, C^2HCl_3) δ (ppm) 7.78 (d, 1H), 7.68 (d, 1H), 7.60 (t, 1H), 7.42 (t, 1H), 6.80 (m, 4H), 5.05 (m, 1H), 3.80 (m, 1H), 1.46 (q, 12H). ^{11}B NMR δ (ppm) 13.5 ppm, single peak. IR (KBr), ν 1620 cm^{-1}, 746 cm^{-1}. Mass spectrum (m/z, ion): 323, 280, 223, 195, 167, 136, 105. Analysis: calculated for $C_{19}H_{22}O_3BN \cdot C_6H_6O_2$: C, 69.30; H, 6.51; N, 3.23; found: C, 69.21; H, 6.57; N, 3.13.

Synthesis of ligand **III** [34].

II (0.828 g, 2.68 mmol) in 100 ml dry carbon tetrachloride in a 200-ml flask was equipped with a Claisen head, a condenser and a receiver. Carbon tetrachloride (20 ml) was distilled from the mixture to insure the absence of water in the solvent. The distillation apparatus was replaced quickly by a dropping funnel containing 4.30 g of bromine (26.8 mmol) in 15 ml of dry carbon tetrachloride. Bromine was added dropwise with illumination from two 150-W tungsten bulbs held 3 in. (1 in. = 2.54 cm) from the flask. The mixture was stirred vigorously and maintained at a gentle reflux until the color due to the presence of bromine in reaction flask disappeared. Addition of bromine ceased when the color no longer disappeared after 5 min. Evaporation of the solvent yielded 0.94 g of a gray cream powder, which was used for coupling without further purification. ^{1}H NMR showed that the benzylmethyl group was brominated. ^{11}B NMR showed a peak at 13.5 ppm.

Interaction with catechol in solution

The reaction of catechol [2-(diisopropylamino)carbonyl]phenylboronic acid (DICP) and PBA with catechol (1:2 ratio) in CHES, Tris and phosphate buffers at various pH values was studied by ^{11}B NMR on a Varian XL300 NMR spectrometer. Samples contained 10% $^{2}H_2O$ to provide an NMR locking signal.

^{11}B NMR spectroscopy

^{11}B NMR spectra were recorded at 25°C on a Varian XL300 NMR spectrometer at 96.25 MHz, using boron trifluoride diethyl etherate as the external standard. Boronate derivatives and catechol were dissolved in various buffers containing 10% $^{2}H_2O$ at different pH values. ^{11}B NMR chemical shifts were measured above the boron background present in the NMR tubes, and the ^{11}B NMR peaks appeared as sharp peaks above the bell-shaped background.

Coupling of the boronate ligand [35,36] (Fig. 4)

Coupling of the boronate ligand on agarose

Cysteamine agarose (5 ml) was washed with excess anhydrous DMF to remove water, then transferred to a 100-ml flask containing 0.1 g **III**, 0.05 g anhydrous K_2CO_3 and 50 ml DMF. The mixture was stirred for 4 h at room temperature

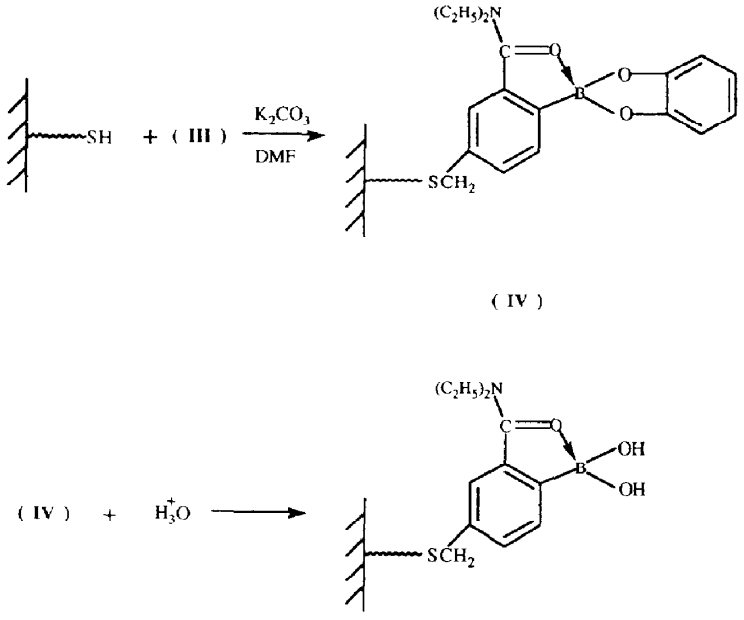

Fig. 4. Immobilization of the boronate affinity ligand **III** on a solid matrix.

under nitrogen, after which the gel was washed with 50 ml DMF, then 50 ml 50 m*M* HEPES, pH 8.5.

Coupling of the boronate ligand on cellulose

Sulfhydryl cellulose (1 g) was added to a 100-ml flask containing 15 ml anhydrous DMF and 0.415 g DTT. The mixture was stirred for 1 h at room temperature under nitrogen. The gel was washed thoroughly using DMF, and then transferred to a 50-ml flask containing 0.55 g **III** and 0.05 g anhydrous K_2CO_3. The mixture was stirred for 4 h at room temperature under nitrogen. After reaction, the gel was washed with DMF, then 50 m*M* HEPES, pH 8.5.

During the coupling reactions, the sulfhydryl concentration was monitored by Ellman's assay [37], and the boron concentration was monitored by the azomethine H method [38].

Affinity chromatography

A 1-ml column (Sepracor) was packed with 1 ml of gel. The column was connected to an automated liquid chromatographic system (Bio-Rad). It was first washed with 1% acetic acid to hydrolyze catechol boronate to boronic acid, after which it was equilibrated with 50 m*M* HEPES. Catechol samples were dissolved in the equilibration buffer. After application of the sample, the column was washed with equilibration buffer until the absorbency returned to the baseline. Elution was accomplished using 1% acetic acid or 0.2 *M* sorbitol in the equilibration buffer. In the case of elution using sorbitol, the column was regenerated by treatment with 1% acetic acid, followed by re-equilibration with the washing buffer.

3. Results and discussion

3.1. DICP and PBA interact with catechol in solution

Although DICP can not be coupled to a solid matrix, we used it as a model to study boronate interaction with *cis*-diols in solution. The results were in good agreement with those obtained using immobilized boronate affinity columns, as demonstrated by Singhal et al. [23]. Phenylboronic acid was used as the standard since its structure is closely related to that of commercial boronate affinity matrices.

In CHES buffers (Fig. 5), catechol boronate ester between phenylboronic acid and catechol formed at pH 9.0, as shown by ^{11}B NMR, whereas catechol boronate ester between [2-(diisopropylamino)carbonyl]phenylboronic acid and catechol occurred at pH 7.9. Free phenylboronic acid has a chemical shift of 29.6 ppm on ^{11}B NMR, which indicates a trigonal boron. The chemical shift of catechol phenylboronates were approximately 10 ppm (9.6 ppm for DICP ester and 10.4 ppm for PBA ester in this case) on ^{11}B NMR, which indicates a tetrahedral boron. Free

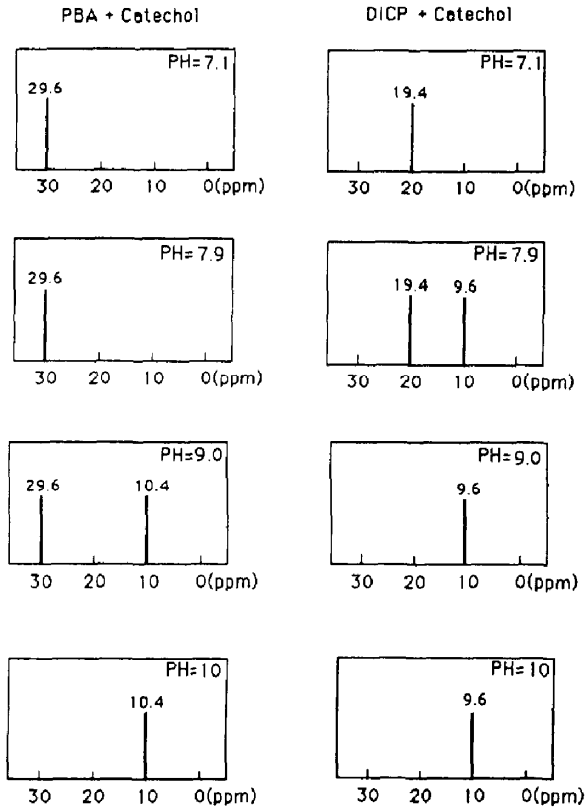

Fig. 5. Interaction between boronate affinity ligands and catechol. The reaction of PBA and DICP with catechol (1:2 ratio) in CHES buffer at various pH values. Numbers shown are chemical shift on ^{11}B NMR. Samples contained 10% ^2H_2O to provide an NMR locking signal.

[2-(diisopropylamino)carbonyl]phenylboronic acid had a chemical shift of 19.4 ppm on ^{11}B NMR, suggesting that the boron was partially tetrahedral. At pH values higher than the "critical pH" (where the catechol esters start to form), all boronate groups eventually became esterified with catechol, and demonstrated only one ^{11}B NMR peak at about 10 ppm. At pH values lower than the "critical pH", no esterification occurred, and only a peak for the free boronate ligand (29.6 ppm for PBA and 19.4 ppm for DICP) was seen. As shown in Fig. 5, [2-(diisopropylamino)carbonyl]phenylboronic acid esterified with catechol at 1.1 pH unit lower than did phenylboronic acid.

In Tris buffers (Fig. 6), DICP began to form an ester bond with catechol at pH 7.0 compared to 7.6 for PBA, which is 0.6 pH unit lower. In phosphate buffers (Fig. 7), esterification between [2-(diisopropylamino)carbonyl]phenyl boronic acid and catechol occurred at pH 6.6, while esterification between phenylboronic acid and catechol formed at pH 7.1, which is 0.5 pH unit lower than for PBA. It is known that buffers have various effects on boronate affinity chromatography [39].

3.2. *Affinity chromatography on [2-(diethylamino)carbonyl,4-bromomethyl]phenylboronate–agarose (DECBP-agarose)*

The chromatography of catechol on DECBP-agarose gel is shown in Fig. 8a and b. The

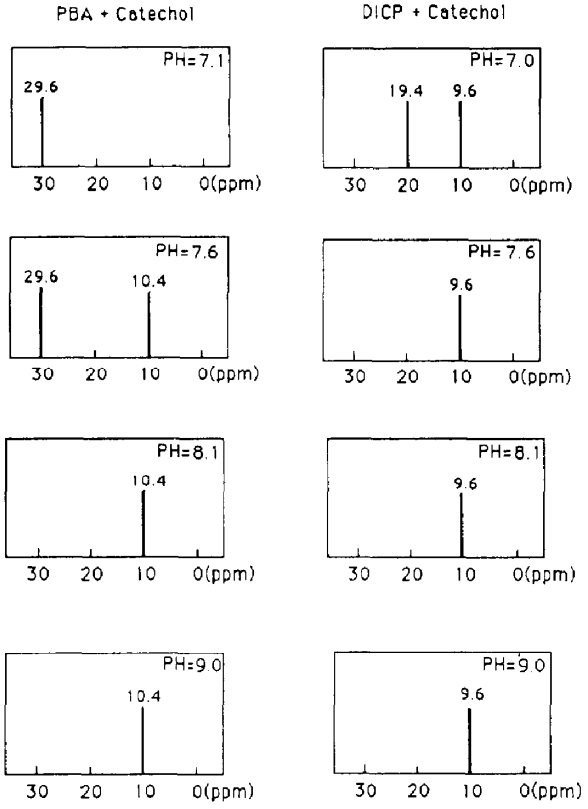

Fig. 6. Interaction between boronate affinity ligands and catechol. PBA and DICP were reacted with catechol (1:2 ratio) in Tris buffers at various pH values. Numbers shown are chemical shift on ^{11}B NMR. Samples contained 10% $^{2}H_2O$ to provide NMR locking signal.

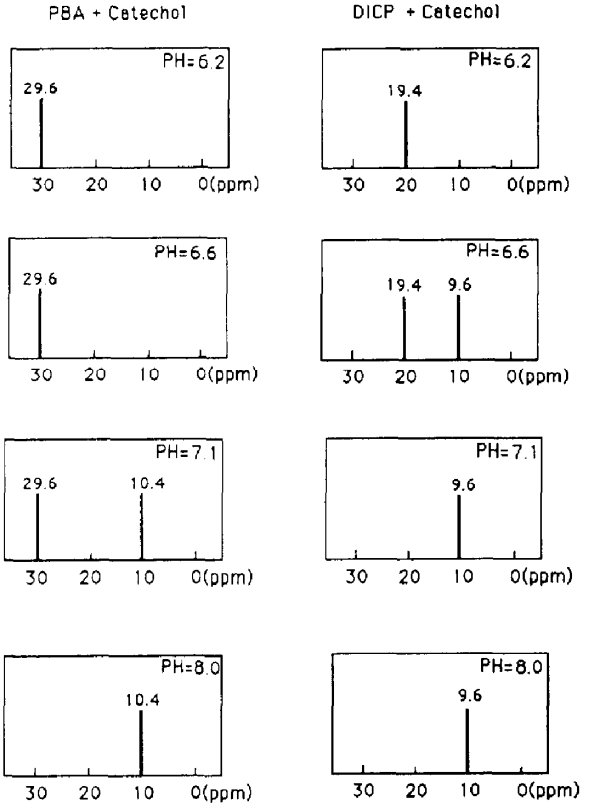

Fig. 7. Interaction between boronate affinity ligands and catechol. PBA and DICP were reacted with catechol (1:2 ratio) in phosphate buffers at various pH values. Numbers shown are chemical shift on ^{11}B NMR. Samples contained 10% $^{2}H_2O$ to provide NMR locking signal.

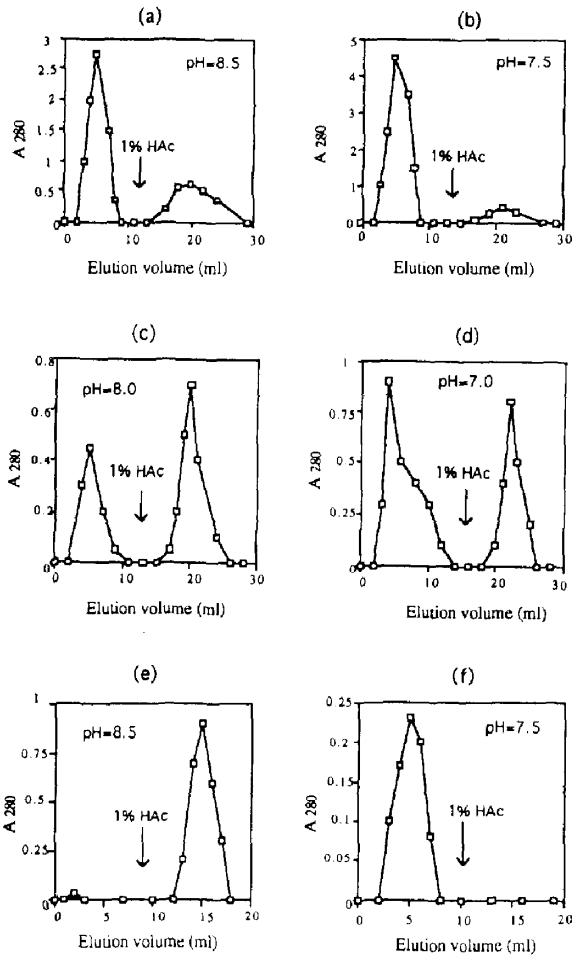

Fig. 8. Chromatography of catechol on DECBP-agarose gel, DECBP-cellulose gel and mPBA-agarose gel. (a) Column: 1 ml DECBP-agarose gel (0.14 μmol boronate); washing buffer: 50 m*M* HEPES, pH 8.5; sample: 0.25 μmol catechol in washing buffer; elution: 1% acetic acid; flow-rate: 2.0 ml/min. (b) Column: 1 ml DECBP-agarose gel (0.14 μmol boronate); washing buffer: 50 m*M* HEPES, pH 7.5; sample: 0.33 μmol catechol in washing buffer; elution: 1% acetic acid; flow-rate: 2.0 ml/min. (c) Column: 1 ml DECBP-cellulose gel (0.66 μmol boronate); washing buffer: 50 m*M* HEPES, pH 8.0; sample: 1.0 μmol catechol in washing buffer; elution: 1% acetic acid; flow-rate: 1.0 ml/min. (d) Column: 1 ml DECBP-cellulose gel (0.66 μmol boronate); washing buffer: 50 m*M* HEPES, pH 7.0; sample: 1.0 μmol catechol in washing buffer; elution: 1% acetic acid; flow-rate: 1.0 ml/min. (e) Column: 1 ml mPBA-agarose gel (14 μmol boronate); washing buffer: 50 m*M* HEPES, pH 8.5; sample: 2.5 μmol catechol in washing buffer; elution: 1% acetic acid; flow-rate: 2.0 ml/min. (f) Column: 1 ml mPBA-agarose gel (14 μmol boronate); washing buffer: 50 m*M* HEPES, pH 7.5; sample: 0.6 μmol catechol in washing buffer; elution: 1% acetic acid; flow-rate: 2.0 ml/min.

column was overloaded with an excess of catechol. After unbound catechol was eluted with washing buffer, 1% acetic acid was used to elute catechol bound to the gel. At pH 8.5, catechol was easily bound to the gel, and subsequently eluted with 1% acetic acid. At pH 7.5, catechol binding also occurred although the elution peak was somewhat smaller than that at pH 8.5.

3.3. Affinity chromatography on [2-(diethylamino)carbonyl,4-bromomethyl]phenylboronate–cellulose (DECBP-cellulose)

The chromatography of catechol on DECBP-cellulose gel, as shown in Fig. 8c and d, followed the same procedures as for DECBP-agarose. At pH 8.0 and 7.0, catechol bound to the gel, and gave a sharp peak upon elution by 1% acetic acid. Elution using 0.2 *M* sorbitol in the washing buffer gave similar results. Chromatography at pH 6.0 showed that some binding occurred, although the amount of binding was considerably less than at pH 7.0.

3.4. Affinity chromatography on m-aminophenylboronic acid–agarose (mPBA-agarose)

These experiments were performed as a comparison to commercial mPBA-agarose gel. As shown in Fig. 8e and f, at pH 8.5, 50 m*M* HEPES, catechol was bound to the gel, and gave a sharp elution peak. However, at pH 7.5, as was expected, no catechol was bound to the gel. Results from additional chromatography showed no binding occurred until the pH reached 8.0.

These investigations demonstrated that the new internally coordinated boronate ligands can esterify with catechol at neutral pH values, both in solution and when bound to the boronate affinity columns. In solution, DICP esterified with catechol at 0.5, 0.6 and 1.1 pH unit lower than PBA did in CHES, Tris and phosphate buffers, respectively. When **III** was coupled to agarose gel, the new matrix esterified with catechol at pH 7.5. When **III** was coupled to cellulose gel, the new matrix esterifies with catechol

at pH 7.1. In comparison, the commercial mPBA-agarose did not esterify with catechol below pH 8.0. In addition, the adsorbent might also reduce secondary ionic and charge transfer interactions in boronate affinity chromatography [39], since no boron anion is formed and the boron no longer has an empty orbital for charge transfer. Therefore, utilizing this type of ligand might increase the overall application of boronate affinity chromatography.

Acknowledgements

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References

[1] H.L. Weith, J.L. Wiebers and P.T. Gilham, *Biochemistry*, 9 (1970) 4396.
[2] W.H. Scouten, *Solid Phase Biochemistry*, Wiley, New York, (1983) p.149.
[3] T. Yamamoto, Y. Amuro, Y. Matsuda, H. Nakaoka, S. Shimomura, T. Hada and K. Higashino, *Am. J. Gastroenterol.*, 86 (1991) 495.
[4] S. Hjertén and J. Li, *J. Chromatogr.*, 500 (1990) 543.
[5] R. DeCristofaro, R. Landolfi, B. Bizzi and M. Castagnola, *J. Chromatogr.*, 426 (1988) 376.
[6] R.N. Jahnson and J.R. Baker, *Clin. Chem. (Winston-Salem, N.C.)*, 34 (1988) 1456.
[7] C. Hansson, B. Kaagedal and M. Kaellgerg, *J. Chromatogr.*, 420 (1987) 146.
[8] C.J. Hawkins, M.F. Lavin, D.L. Parry and I.L. Ross, *Anal. Biochem.*, 159 (1986) 187.
[9] J. Eross, D. Kreutzmann, C. Crowell and M. Silink, *Clin. Chem. (Winston-Salem, N.C.)*, 32 (1986) 2222.
[10] S. Higa and S. Kishimoto, *Anal. Biochem.*, 154 (1986) 71.
[11] D.C. Klenk, G.T. Hermanson, R.I. Krohn, E.K. Fujimoto, A.K. Malia, P.K. Smith, J.D. England, H.M. Wiedmeyer, R.R. Little and D.E. Goldstein, *Clin. Chem. (Winston-Salem, N.C.)*, 28 (1982) 2088.
[12] B.J. Gould, P.M. Hall and G.H. Cook, *Clin. Chim. Acta*, 125 (1982) 41.
[13] R. Kluckiger, T. Woodtli and W. Berger, *Diabetes*, 33 (1984) 73.
[14] H.F. Bunn, *Am. J. Med.*, 70 (1981) 325.
[15] K. Nakano, K. Shindo, K. Yaraka and H. Yamamoto, *J. Chromatogr.*, 332 (1985) 21.
[16] K. Nakano, K. Shindo, K. Yaraka and H. Yamamoto, *J. Chromatogr.*, 332 (1985) 127.
[17] A. Yurkevich, I. Kolodkina, E. Ivanova and E. Pichuzhkina, *Carbohydr. Res.*, 43 (1975) 215.
[18] H.-G. Boit, *Beilstein*, 16(3), Springer, Berlin, 1974, p. 1284.
[19] S. Soundararajan, M. Badawi, C.M. Kohlrust and J. Hageman, *Anal. Biochem.*, 178 (1989) 125.
[20] E. Ivanova, S. Panchenko, I. Kolodkina and A. Yurkevich, *Z. Obshcheikhimii*, 45 (1975) 208.
[21] V. Akparov and V. Stepanov, *J. Chromatogr.*, 155 (1978) 329.
[22] C. Elliger, B. Chan and W. Stanley, *J. Chromatogr.*, 104 (1975) 57.
[23] R.P. Singhal, B. Ramamurthy, N. Govindraj and Y. Sarwar, *J. Chromatogr.*, 543 (1991) 17.
[24] V. Adamek, X. Liu, Y. Zhang, K. Adamkova and W.H. Scouten, *J. Chromatogr.*, 625 (1992) 91.
[25] R.P. Singhal and S.S.M. DeSilva, *Adv. Chromatogr.*, 31 (1992) 307.
[26] J.R. Mazzeo and I.S. Krull, *Biochromatography*, 4 (1989) 124.
[27] S.A. Barker, A.K. Chopra, B.W. Hatt and P.J. Somers, *Carbohydr. Res.*, 26 (1973) 33.
[28] R.J. Ferrier, *Adv. Carbohydr. Chem. Biochem.*, 35 (1978) 31.
[29] S.X. Cai and J.F.W. Keana, *Bioconjugate Chem.*, 2 (1991) 317.
[30] G. Wulff, R. Dederichs, R. Grotstollen and C. Jupe, in T.C.J. Gribnau, J. Visser and R.J.F. Nivard (Editors), *Affinity Chromatography and Related Techniques —Theoretical Aspects/Industrial and Biomedical Applications (Proceedings of the 4th International Symposium, Veldhoven, 22–26 June 1981)*, Elsevier, Amsterdam, 1982, p. 207.
[31] X. Liu, J.L. Hubbard and W.H. Scouten, *J. Organomet. Chem.*, (1994) submitted for publication.
[32] P. Beak and R.A. Brown, *J. Org. Chem.*, 47 (1982) 34.
[33] M.R. Winkle, J.M. Lansinger and R.C. Ronald, *Chem. Commun.*, (1980) 87.
[34] P. Tschampel and H.R. Snyder, *J. Org. Chem.*, 29 (1964) 2168.
[35] J.M. Khurana and P.K. Sahoo, *Synthetic Commun.*, (1992) 1691.
[36] Y.S. Or, R.F. Clark and J.R. Luly, *J. Org. Chem.*, 56 (1991) 3146.
[37] G.L. Ellman, *Arch. Biochem. Biophys.*, 82 (1959) 70.
[38] T.P. Gaines and G.A. Mitchell, *Commun. Soil Sci. Plant Anal.*, 10 (1979) 1099
[39] S. Fulton, *Boronate Ligands in Biochemical Separations*, Amicon Corp., Danvers, MA, 1981, p. 6.