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Note

Synthesis and application of a boronic acid-substituted silica for high-performance liquid affinity chromatography

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Borate is known to form complexes with polyols under alkaline conditions[1,2], and boronic acid linked to polymer matrices (agarose, cellulose, polyacrylamide) was used as an affinity ligand for *cis*-diol compounds of biological interest such as carbohydrates, ribonucleosides and ribonucleotides, nucleic acids and glycosylated polymers[3–7]. Recently, boronic acid-substituted silica has been synthesized and applied to the high-performance liquid affinity chromatography (HPLAC) of nucleosides, nucleotides and carbohydrates under isocratic conditions[8].

We attempted to apply a two-step pH-dependent elution under low pressure, introduced originally for the selective clean-up (prefractionation) of nucleosides in urine and serum[3], to HPLAC using a support synthesized according to ref. 8. This application should avoid the pH-dependent shrinkage and swelling of the polymer matrix and should reduce analysis time. However, the procedure failed, due to the elution of UV-absorbing material of the modified silica under acidic conditions. We, therefore, prepared a new boronic acid-substituted silica suitable for pH-shift-dependent prefractionation of polyols.

EXPERIMENTAL

Chemicals

LiChrosorb Si 100, 5 μm, was obtained from E. Merck (Darmstadt, F.R.G.), *m*-aminobenzeneboronic acid hemisulphate and nucleosides from Sigma (München, F.R.G.). γ-Chloropropyltrimethoxysilane was a gift from Dynamit Nobel (Troisdorf, F.R.G.).

Synthesis of γ-chloropropylsilane-substituted silica (Scheme 1, reaction I)

To 1.5 g LiChrosorb Si 100, 5 μm, dried at 160°C overnight and slurried in 60 ml dried toluene, 5 ml γ-chloropropyltrimethoxysilane were added under argon. The stirred mixture was refluxed under argon for 24 h. The substituted silica was filtered

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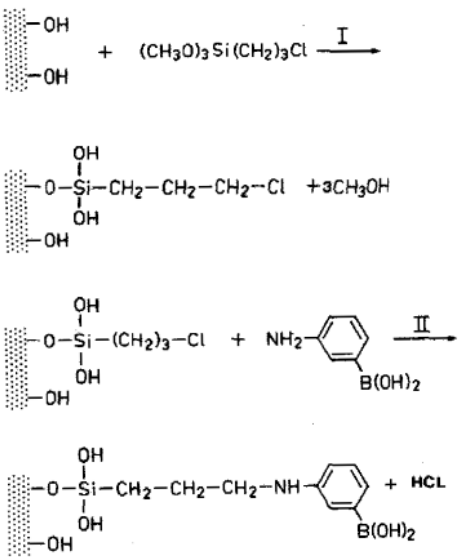
off, washed on a glass filter-funnel with dichloromethane, methanol, dichloromethane and diethyl ether (50 ml of each) and finally sucked dry.

m-Aminobenzeneboronic acid (200 mg) was added to 40 ml of water and sonicated (10 min). The pH was then adjusted to 7.5 using sodium hydroxide. For synthesis control, 50 μl of this solution were diluted in 2 ml of 0.1 *M* ammonium dihydrogenphosphate buffer, pH 7.0, followed by the determination of the UV absorption at 293 nm (ε of boronic acid: 1.61 cm^2 μmol^{-1}).



Scheme 1.

Synthesis of boronic acid silica (Scheme 1, reaction II)

One gram of γ-chloropropylsilane-substituted silica was added to the aqueous boronic acid solution and sonicated for 10 min. For synthesis control, a 200-μl aliquot was centrifuged at 2000 *g* and the UV absorption determined at 293 nm. This procedure allows the calculation of the amount of non-specifically bound boronic acid (< 5%). The reaction mixture was then stirred for 24 h at 90°C. The extent of boronic acid substitution was determined spectrophotometrically in the supernatant to be 145 μmol per g γ-chloropropylsilane-substituted silica. Elementary analysis of the boronic acid silica yielded a C/B ratio of 11.4 (calculated 9.8).

Chromatography

The chromatographic system used was as described previously[9]. Boronic acid-substituted silica was packed in a stainless-steel column (30 × 4 mm I.D.) with an ascending slurry packing technique.

RESULTS AND DISCUSSION

Synthesis of boronic acid-substituted silica

A modification of the method described by Glad *et al.*[8] for the preparation of boronic acid-substituted silica via γ-glycidoxypropyltrimethoxysilane was necessary,

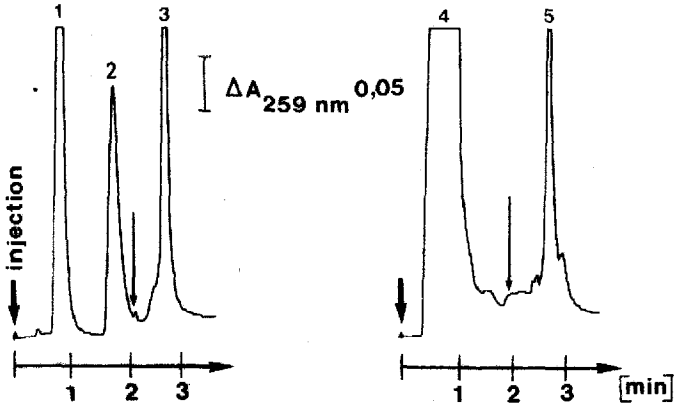


Fig. 1. Left: fractional separation of a synthetic mixture of deoxythymidine (1), deoxyadenosine (2), pseudouridine (3), and adenosine with boronic acid silica under pH-step gradient conditions. Elution sequence: 2 min 0.1 *M* ammonium dihydrogenphosphate pH 8.3; 0.15 *M* ammonium formate pH 3.5 (indicated by an arrow); flow-rate 1 ml/min. Detection: UV, 259 nm. Right: clean-up and group-specific separation of *cis*-diol compounds from 50 µl membrane-filtered urine; 4 = urinary UV-absorbing compounds; 5 = urinary *cis*-diol compounds. Elution sequence as before.

as application of the functionalized material in the pH-step gradient was found to produce UV-absorbing substances. In addition, these compounds coeluted with *cis*-diols and interfered in the subsequent reversed-phase HPLC analysis of nucleosides. Therefore, by analogy to ref. 10, we attempted to couple *m*-aminobenzeneboronic acid (ABBA) to γ-chloropropyltrimethoxysilane prior to substitution of silica. As ABBA is only slightly soluble in most common solvents, the reaction was performed in dimethylformamide at 100°C. Reversed-phase HPLC control (conditions as in Fig. 2) of the reaction mixture revealed, however, that ABBA degrades under these conditions yielding at least three different UV-absorbing side products. These materials could not be removed from the affinity support even by extensive washing.

It was then decided to use the traditional method, *i.e.*, to substitute silica first with γ-chloropropyltrimethoxysilane. Subsequently, the ABBA was coupled to the γ-chloropropyl-substituted silica in water at 90°C. This procedure eliminated any degradation of ABBA.

Separation of cis-diols

Fig. 1 (left) demonstrates that the prepared material is an effective affinity support for the pH-shift-dependent separation of a synthetic mixture of ribo- and deoxyribonucleosides. A control run, *i.e.*, a simple acid–base shift, revealed no elution of UV-absorbing ABBA-related products (not shown). The functionality of the affinity support, *i.e.*, the group-specific retardation and pH-dependent elution, was further demonstrated for urinary UV-absorbing *cis*-diol compounds (Fig. 1, right).

Fig. 2 shows the reversed-phase HPLC elution profiles of aliquots of the acidic fraction of a synthetic mixture of nucleosides (top) and of a native urine sample (bottom). The urinary ribonucleosides were characterized by UV and ^{1}H NMR spectroscopy and substance-specific chemical reactions[6,11].

In conclusion, the prepared boronic acid silica affinity column can be used

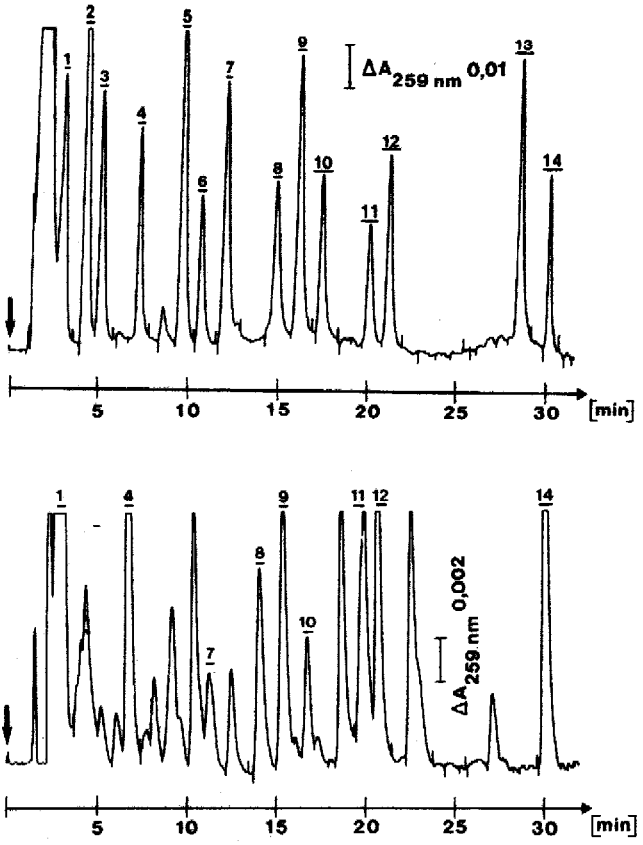


Fig. 2. Reversed-phase separation of the acidic fraction of nucleosides from a synthetic mixture (top) and urinary UV-absorbing *cis*-diols (bottom) obtained by HPLAC with pH-step gradient elution (Fig. 1). Peaks: 1 = pseudouridine (t_R 3.0 min); 2 = uridine (4.2); 3 = cytidine (5.1); 4 = N-1-methyladenosine (7.3); 5 = inosine (9.7); 6 = guanosine (10.8); 7 = adenosine (12.1); 8 = N-1-methylinosine (15.0); 9 = N-1-methylguanosine (16.3); 10 = N-2-methylguanosine (17.5); 11 = N-6-methyladenosine (20.3); 12 = N-2-dimethylguanosine (21.3); 13 = N-6-dimethyladenosine (28.7); 14 = N-6-(carbamoylthreonyl)adenosine (30.4). Chromatographic conditions: LiChrosorb RP-18,7 μm, 250 × 4 mm I.D. Elution: 0.15 *M* ammonium formate pH 3.5; after 2 min a linear gradient up to 8% methanol in 10 min followed by a linear gradient up to 30% methanol in 20 min; flow-rate 1.8 ml/min. Detection: UV, 259 nm.

under high pressure as well as pH-dependent conditions for the group-selective separation and enrichment of *cis*-diols from biological fluids.

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