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Notes

Evaluation of Interactions between Monosaccharides and a Stationary Phase Modified with Alkylboronic Acid by Means of a Liquid-Chromatographic Method

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Introduction

It is a well-known fact that saccharides play important roles in a variety of biological processes. Their crucial roles in cell-to-cell interactions and biological recognition are now becoming clear and, as a result, interest in saccharide chemistry is increasing. Therefore, the need for an analytical method to evaluate the recognition properties of saccharides is clear.

Boronic acid derivatives are known to form covalent bonds with compounds having a *cis*-diol moiety, resulting the formation of cyclic esters.[1,2] Since many saccharides contain a *cis*-diol moiety, various approaches have been developed for the detection and separation of saccharides by taking advantage of this unique property. Weith *et al*. reported on the synthesis of cellulose derivatives containing a dihydroxyboryl group and their use in the separation of saccharides and nucleic acid components.[3] Wulff *et al*. prepared a macroporous polymer modified with a boronic acid derivative by a molecular imprinting procedure, and was able to separate racemic saccharides using the prepared polymer.[4] Czarnik *et al*. synthesized a boronic acid-introduced anthracence derivative, and applied to the fluorometric sensing of saccharides.[5] Shinkai *et al*. designed and synthesized several compounds containing a boronic acid moiety, and used them as a fluorescent sensor based on a photoinduced electron-transfer effect.[6] Shinkai *et al*. also designed a boronic acid derivative, which contains two boronic acid moieties at suitable positions, and achieved monosaccharide sensing in circular-dichroism measurements.[7] Although many applications of boronic acid derivatives have been reported for the detection and separation of saccharides, a systematic evaluation between the boronic acid moiety and saccharides has not been investigated to date.

In this report, in order to clarify the interaction between monosaccharides and a boronic acid moiety, the immobilization of an alkylboronic acid with a support suitable for use in reverse-phase liquid chromatography was attempted *via* a hydrophobic interaction among alkyl groups. The interaction between saccharides with this modified support was investigated by measuring the retention time using liquid chromatography.

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Experimental

Reagents and apparatus

Reagents were obtained from Wako Pure Chemicals (Osaka, Japan) and Kishida Chemical Co., Ltd. (Osaka, Japan). 1-Decylboronic acid was synthesized by the reaction of 1-bromodecane with trimethyl borate in THF, and was then characterized by ^{1}H-NMR and elemental analysis.

The HPLC system was composed of a reverse-phase liquid-chromatographic column (Octadecyl-2PW), an injector (7125, Rheodyne), an optical rotatory detector (OR-990, JASCO), and a recorder (TYPE 3066, Yokogawa Electric Corporation).

Procedures

The procedure used for immobilization was as follows. 1-Decylboronic acid (0.6 g) was dissolved in 20 mL of EtOH/H_2O (1:1, v/v), and the resulting solution was circulated through a column packed with a polymer support at a flow rate of 0.5 mL min^{-1} for 5 h. Water was then introduced into the column at a flow rate of 0.5 mL min^{-1} for 5 h to remove any unimmobilized 1-decylboronic acid from the column.

To evaluate interactions between monosaccharides and alkylboronic acid immobilized on the support, 20 μL of a 5 mM monosaccharide solution of six different monosaccharides (glucose, mannose, galactose, arabinose, sorbose, and fructose) was injected into the column separately. Solutions of 20 mM phosphate buffer adjusted to pH 8, and 20 mM carbonate buffer adjusted to pH 9, 9.5, 10, 10.5, 11, were used as eluents, followed by a measurement of the retention time.

Results and Discussion

A schematic illustration of the immobilization of 1-decylboronic acid on the column support is shown in Fig. 1. Since the polymer support (particle size, 5 μm, total weight, 0.65 g) contained a C_{18} alkyl chain on the surface, 1-decylboronic acid could be immobilized on the support through hydrophobic interactions with the octadecyl group bound to the support. In order to measure the amount of 1-decylboronic acid immobilized on the support, the modified column was purged with organic solvents (ethanol and dichloromethane). The eluent was then dried *in vacuo*, and 0.47 g of 1-decylboronic

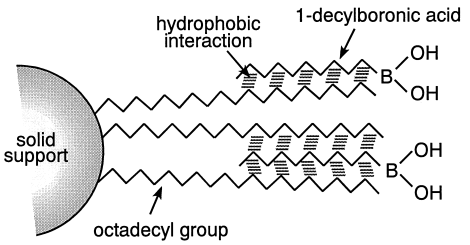

Fig. 1 Immobilization of an alkylboronic acid on the support surface *via* hydrophobic interactions.

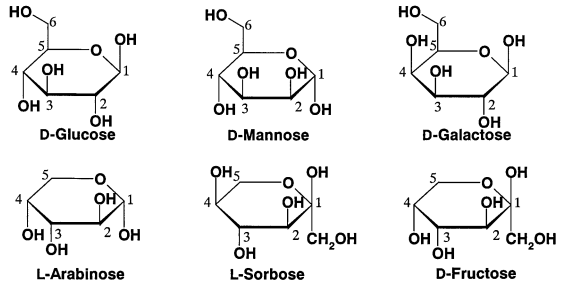

Fig. 2 Chemical structures of saccharides used in this study. For sorbose and fructose, the notation of the position number in Fig. 2 does not correspond to the IUPAC recommendation. This was done to make it easier to demonstrate the interaction between each monosaccharide and the alkylboronic acid. Position 1 of sorbose and fructose in Fig. 2 corresponds position 2 in the IUPAC recommendation.

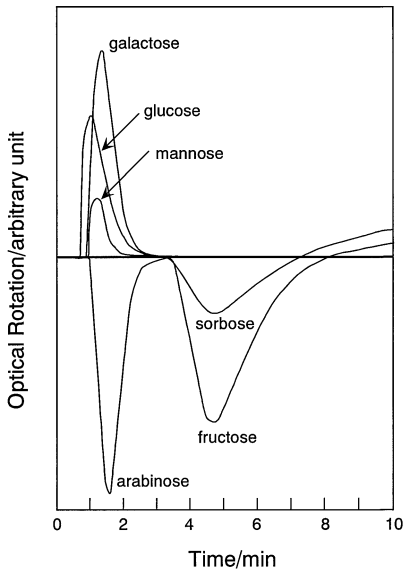

Fig. 3 Elution diagrams of monosaccharides. Glucose, mannose, galactose, arabinose, sorbose, and fructose were used as samples. Conditions: eluent, carbonate buffer at pH 10.5; flow rate of eluent, 0.2 mL min^{-1}; sample volume and concentration, 20 μL and 0.5 mM.

acid was found to have been eluted from the column. Therefore, the average capacity of the decylboronic acid-immobilized polymer support was calculated to be 1.1 mmol/g-resin.

Glucose, mannose, galactose, arabinose, sorbose and fructose were used as samples for evaluating the interaction between alkylboronic acid and saccharides (Fig. 2). Sucrose was used as a reference, since it is thought to have no interaction with boronic acid derivatives.[8] The elution diagrams for each monosaccharide are shown in Fig. 3; a pH 10.5 carbonate buffer solution was used as an eluent. The capacity factors for the monosaccharides were calculated from the retention time in Fig. 3 and the retention time of sucrose. Table 1 gives the capacity factors for monosaccharides obtained by different pH buffer solutions. As can be seen from Fig. 3, the peak of each monosaccharide was broadened. This may be due to a strong interaction of monosaccharides to the stationary phase. The mixed monosaccharides could not be completely separated. However, many interesting findings about the interaction between the boronic acid and the monosaccharides were obtained from the capacity factors for the monosaccharides. The capacity factors for monosaccharides are larger in the order glucose < mannose < galactose < arabinose < sorbose < fructose for an eluent at pH 10.5. This sequence suggests that the degree of the interaction between the immobilized boronic acid and the monosaccharides is closely dependent on the number and orientation of the hydroxyl groups in each monosaccharide. The number of *cis*-diol moieties in the monosaccharides is 0 for glucose and 1 for mannose, galactose, arabinose, sorbose, and 2 for fructose. Mannose has a *cis*-diol moiety at the 2,3-positions, sorbose at the 1,2-positions, and galactose and arabionose at the 3,4-positions. Fructose has two *cis*-diol moieties at the 3,4- and 1,2-positions. Since the sequence of the capacity factor is in the order of glucose < mannose < galactose < arabinose < sorbose < fructose, and the capacity factor corresponds to the extent of the interaction between the boronic acid and the monosaccharide, the sequence of the capacity factor observed is in agreement with the number of *cis*-diol moieties in the monosaccharide (glucose < mannose, galactose, arabinose, sorbose < fructose). In addition, for saccharides having one *cis*-diol moiety, *i.e.*, mannose, galactose, arabinose, and sorbose, since the sequence of the capacity factors is larger in the order mannose < galactose < arabinose < sorbose, the degree of the interaction between the boronic acid and the monosaccharides appears to be stronger in the order 2,3-*cis*-diol < 3,4-*cis*-diol < 1,2-*cis*-diol. The capacity factor of galactose is smaller than that of arabinose. Thus, the interaction of boronic acid with galactose is weaker than that with arabinose, although both galactose and arabinose contain a 3,4-*cis*-diol moiety. This seems to be due to the fact that the bulky 6-CH_2OH group in the galactose molecule sterically hinders complex formation between the boronic acid and the 3,4-*cis*-diol moiety, compared with arabinose.

The sequence of the capacity factors for monosaccharides obtained by our column were larger in the order glucose < mannose < galactose < arabinose < sorbose < fructose, as described above. On the other hand, the sequence of the capacity factors for monosaccharides obtained by a conventional anion-exchange column using a borate buffer solution as an eluent was quite different from our results. For example, the capacity factors obtained by an anion-exchange column were larger in the order mannose < galactose < glucose < fructose < sorbose.[9] The difference in sequence of the capacity factors obtained by both columns indicates that the separation mechanisms are basically different between them. Namely, the capacity factor for our column depends on the degree of the interaction between the immobilized boronic acid and the monosaccharides; on the other hand, the capacity factor for the anion-exchange column depends on the degree of the distribution property of the borate-monosaccharide complex anion formed by a reaction with the eluent. Shinkai *et al.*

Table 1 Capacity factors of saccharides on a stationary phase modified with 1-decylboronic acid

Sample	pH of eluent					
	8.0	9.0	9.5	10.0	10.5	11.0
Glucose	0	0	0	0	0.13	0.54
Mannose	0	0.04	0.04	0.04	0.17	0.38
Galactose	0	0.04	0.04	0.20	0.33	0.71
Arabinose	0	0.04	0.04	0.8	0.46	0.83
Sorbose	0.08	0.15	0.16	2.1	3.1	—
Fructose	0.08	0.21	0.32	2.1	3.2	5.9

Conditions: flow rate of eluent, 0.2 mL min^{-1}; sample volume and concentration, 20 μL and 0.5 μM. —: the sample could not be detected. Sucrose was used for a marker of non-retained solute on the stationary phase. The capacity factor (k') of a monosaccharide was calculated from the following equation: $k' = (t_R - t_0)/t_0$; t_R, the retention time of each monosaccharide; t_0, the retention time of the reference sucrose.

reported that the stability constants between the monosaccharides and the boronic acid derivative synthesized by them were larger in the order glucose < galactose < fructose.[6] Their report also supports the sequence of the capacity factor for monosaccharide obtained by our column.

As can be seen from Table 1, the pH of the eluent clearly affects the nature of the capacity factors of monosaccharides. The capacity factors are relatively small when the pH of the eluent is below 9.5. The capacity factors tend to be larger with increasing pH of the eluent. This result is in good agreement with the fact that complex formation between boronic acid and the *cis*-diol moiety proceeds more rapidly in basic media. This suggests that appropriate control of the pH of the eluent would enhance the effective separation of a mixed solution of saccharides. No significant retention of saccharides was observed on a bare octadecyl column, which was not treated with 1-decylboronic acid (data not shown).

In conclusion, the degree of interactions between boronic acid and saccharides appears to be largely dependent on the number and position of the *cis*-diol moiety contained by a saccharide. The method which we describe here would be one of the simple and effective techniques for investigating the interaction between the saccharides and boronic acid. We continue to evaluate the retention behavior of more complex saccharides, such as oligosaccharides, using the boronic acid-modified column developed here.

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