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Complexation of sugars with dihydroxyborylphenyl groups attached to magnetite particles via graft polymerization of acrylic acid

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Abstract

For the purpose of magnetic handling of sugars, dihydroxyborylphenyl (DHBP) groups were attached to magnetite particles. In advance, the magnetite particles were modified by the graft polymerization of acrylic acid initiated in a redox system consisting of mercapto groups introduced onto their surfaces and ceric ions. Succeedingly, DHBP groups were attached to the magnetite particles through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). Complexation of the attached DHBP groups was examined with various monosaccharides, disaccharides and oligosaccharides, and compared with that of free phenylboronic acid. The attached DHBP groups gave larger values of binding constant K for the complexation with oligosaccharides than free phenylboronic acid, whereas the K values of the DHBP groups for the complexation with monosaccharides and disaccharides were smaller than those of free phenylboronic acid. It was suggested from the result that neighboring DHBP groups on the magnetite particles interacted cooperatively with vicinal OH pairs on both the reducing and nonreducing ends of oligosaccharides. With respect to the complexation with monosaccharides, both the DHBP groups and free phenylboronic acid gave the K values corresponding to the content of furanose-type isomer in aqueous solution of each saccharide.

Keywords: Poly(acrylic acid); Arylboronic acids; Magnetite particles

1. Introduction

Arylboronic acids have the properties to form complexes with sugars, which have been widely applied to separation [1,2], transport [3,4], detection [5,6] and single-step glycosidation of sugars [7,8]. It has been accepted that the complexation of arylboronic acids results from the formation of cyclic boronates with sugars at the sites of vicinal 1,2-diols and 1,3-diols involving an exocyclic methylol moiety [9–13]. The authors have been interested in the combination of the sugar-binding property and magnetism for magnetic handling of sugars in such fields as separation and transport. For this reason, dihydroxyborylphenyl (DHBP) groups were attached to magnetite particles via graft polymerization of acrylic acid in a previous study [14]. The graft polymerization was carried out in a redox system consisting of mercapto groups introduced onto the surfaces of magnetite particles and ceric ions [15–17], and DHBP groups were attached through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid).

The DHBP groups attached to magnetite particles gave a large value of binding constant K, as expected, for the complexation with adenosine having a pair of *cis*-hydroxyl groups on its ribose ring, whereas the K values for the complexation with adenosine phosphates were extremely small, probably due to enhanced charge interaction of phosphate groups with the trihydroxoborate groups concentrated on the surface of magnetite. Moreover, the complexation with 2′-deoxyadenosine was not observed for free phenylboronic acid, but observed for the DHBP groups attached to magnetite particles.

With respect to the complexation with 2′-deoxyadenosine, it was suggested that, on magnetite particle, neighboring DHBP

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groups interacted cooperatively with 3′- and 5′-hydroxyl groups of 2′-deoxyadenosine. Such interaction can be expected for the complexation with oligosaccharides, for it seems possible for some of them to have suitable conformations for fitting the steric arrangement of their hydroxyl groups to the cooperative action of neighboring DHBP groups. In the present study, the complexation of the DHBP groups on magnetic particles was examined with a variety of sugars (monosaccharides, disaccharides and oligosaccharides), and compared with the property of free phenylboronic acid on the basis of the *K* values for the complexation with these sugars.

2. Experimental

2.1. Materials

The magnetite used was MAT-305 obtained from Toda Kogyo Corp. in the form of spherical particles. It had an average particle size of 0.23 μm and a BET surface area of 7.2 m^2/g. Acrylic acid was purchased from Wako Pure Chemical Ind., and purified by distillation under reduced pressure. 3-Aminophenylboronic acid, 3-mercaptopropyltrimethoxysilane (MPS) and ceric diammonium nitrate were purchased from Tokyo Kasei Kogyo Co., Kanto Chemical Co. and Wako Pure Chemical Ind., respectively, which were used without further purification. 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CMC) from Aldrich Chemical Co. was used as a condensing agent. Other chemicals, solvents and sugars were of guaranteed-reagent or analytical grade and used without further purification.

2.2. Graft polymerization of acrylic acid from magnetite particles

Prior to graft polymerization of acrylic acid, the magnetite particles were treated with MPS in order to introduce mercapto groups onto their surfaces [18]: a mixture of 30 g of magnetite, 30 ml of MPS and 300 ml of dried toluene was refluxed under nitrogen for 20 h. The treated magnetite was filtered off, washed on a filter with dried toluene and then with methanol, and dried at 60 °C in vacuo.

The graft polymerization was carried out by following the scheme shown elsewhere [14]. Into a flask, 5.0 g of magnetite treated with MPS, 30 g of acrylic acid and 100 ml of distilled water were charged. After deaeration of the mixture, a solution of 2.0 mmol of ceric diammonium nitrate in 30 ml of 1 N nitric acid was added. The polymerization was carried out at 25 °C with stirring under nitrogen. After a given time, the polymerization was stopped by the addition of hydroquinone. The reaction mixture was diluted with distilled water and centrifuged at 10^5 m/s^2 until the magnetite particles were precipitated completely. The precipitated magnetite particles were dispersed in distilled water and centrifuged once more. This procedure was repeated several times and the precipitated particles were dried at a temperature below 60 °C in vacuo.

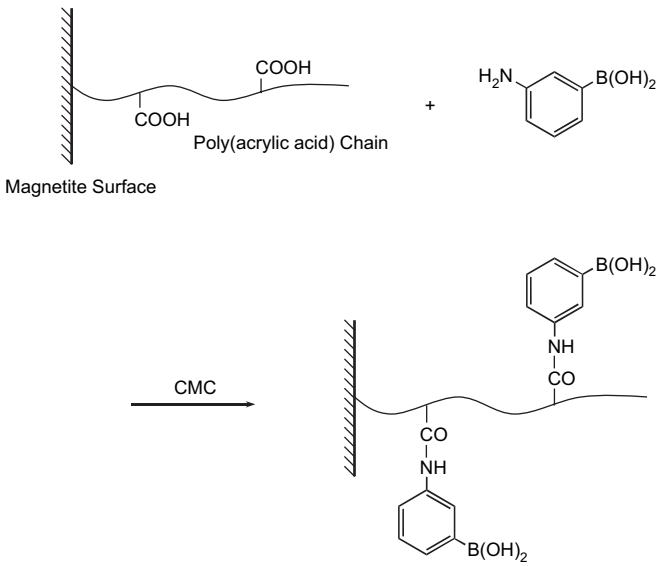
The amount of grafted poly(acrylic acid) was determined gravimetrically from the weight increase of the magnetite. In accordance with the result reported elsewhere [15], poly(acrylic acid) up to the amount of 75 mg was grafted on the surface of 1.0 g of magnetite particles by the graft polymerization within 1.0 h.

2.3. Attachment of DHBP groups to magnetite particles

As illustrated in Scheme 1, DHBP groups were attached to the poly(acrylic acid)-grafted magnetite (PAA-magnetite) through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). CMC was used as a water-soluble condensing agent.

A mixture of 4.0 g of PAA-magnetite, 1.5 equivalent of 3-aminophenylboronic acid per mole of carboxyl groups of the grafted poly(acrylic acid) and 100 ml of distilled water was placed into a flask and stirred for 10 min. Subsequently, the pH value of the mixture was adjusted to 6.0 with 1 N sodium hydroxide solution, and the same molar amount of CMC as 3-aminophenylboronic acid was added. Then the mixture was stirred at 25 °C, the pH value being maintained at 6.0 by the addition of 1 N hydrochloric acid. After 20 h of stirring, the reaction mixture was centrifuged at 10^5 m/s^2, and the magnetite particles were completely precipitated. The precipitated magnetite particles, i.e., the magnetite modified with DHBP groups (DHBP-magnetite), were dispersed in distilled water, filtered off, and washed on a filter with distilled water. This washing procedure was repeated several times, and the washed DHBP-magnetite was dried at a temperature below 60 °C in vacuo.

In order to determine the amount of attached DHBP groups, 0.1 g of the DHBP-magnetite was treated with 10 ml of 0.5 N sodium hydroxide solution at 90 °C for 120 h, and the amounts of boronic acid and 3-aminophenylboronic acid liberated by the treatment were measured by means of ^{11}B NMR spectroscopy. As shown in Fig. 1, ^{11}B NMR signals of boric acid and 3-aminophenylboronic acid are observed at



Scheme 1. Attachment of DHBP groups to poly(acrylic acid) grafted onto magnetite surface.

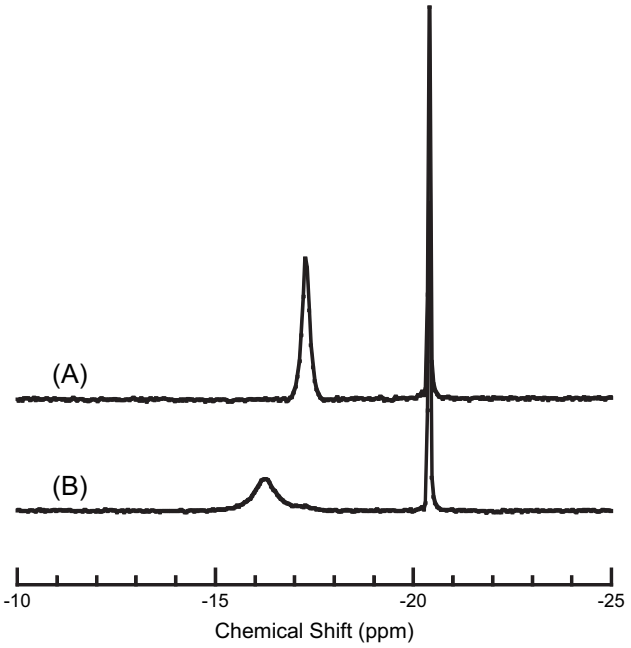


Fig. 1. ^{11}B NMR spectra of boric acid (A) and 3-aminophenylboronic acid (B) in alkaline D_2O solution. $NaBF_4$ was used as an internal standard (−20.4 ppm).

−17.3 ppm (A) and −16.3 ppm (B), respectively. In the present study, a sample containing 0.26 mmol/g of DHBP groups was used for the experiments of complexation with sugars.

2.4. Complexation of attached DHBP groups with sugars

Complexation of the DHBP groups attached to PAA-magnetite was examined with the following sugars: monosaccharides (glucose, fructose, galactose and mannose), disaccharides (maltose, cellobiose, lactose, melibiose and palatinose) and oligosaccharides (maltopentaose, maltohexaose and maltoheptaose). Their structural formulas are shown in Fig. 2.

Prior to the complexation, 0.3 g of DHBP-magnetite was dispersed in 40 ml of 0.1 M Na_2CO_3–$NaHCO_3$ buffer (pH 10.0) for 5.0 min at room temperature. Succeedingly, the complexation was carried out by mixing the dispersion with a given concentration of sugar solution. The mixture was stirred for 1.0 h at room temperature, and then the DHBP-magnetite in the mixture was precipitated with a magnet to be separated from supernatant. The amounts of bound sugars were determined from the concentration of free sugars in the supernatant measured by Somogyi–Nelson method [19,20].

3. Results and discussion

3.1. Complexation of free phenylboronic acid with various sugars

Prior to the investigation of the DHBP groups attached to magnetite particles, the complexation of free phenylboronic

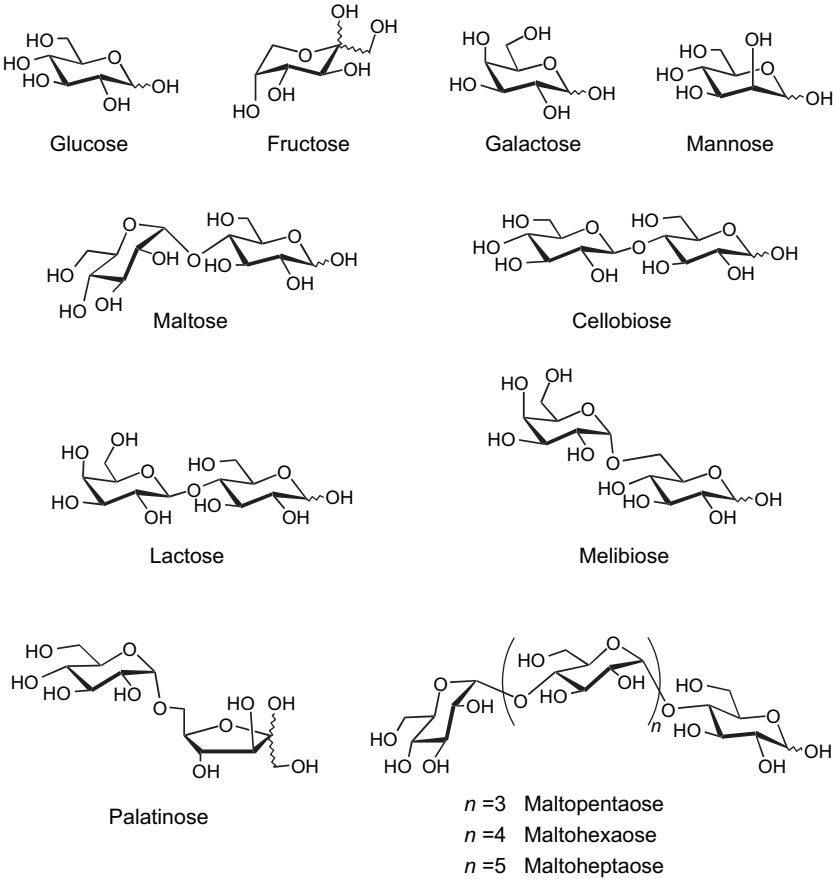


Fig. 2. Structural formulas of sugars employed for complexation with DHBP groups.

acid was examined with various sugars for the purpose of comparison with the property of the DHBP groups. As shown in Scheme 2, sugars are bound to arylboronic acids by the complexation with anionized form of a boronic acid moiety. The complexation was traced by means of UV spectroscopy.

With respect to the complexation of an arylboronic acid with sugars, the following equation can be derived [14]:

$$[\text{Complex}] = [\text{BA}]_0 A[\text{Sugar}]/(1 + A[\text{Sugar}]) \quad (1)$$

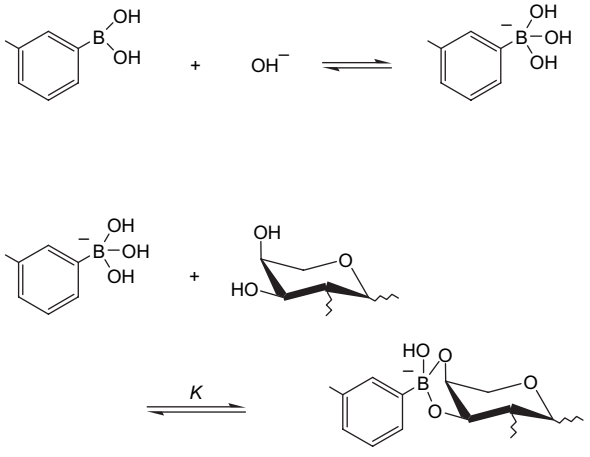
where [Complex] and [Sugar] are equal to concentrations of bound and free sugars, respectively, $[\text{BA}]_0$ is an initial concentration of the arylboronic acid, and A is given by $K/(1 + [\text{H}^+]/K_a)$. The parameters K and K_a represent the binding constant and the acidity constant of the arylboronic acid, respectively. In addition, it should be remarked that $1/(1 + [\text{H}^+]/K_a)$ is equal to the degree of anionization of arylboronic acids [14].

By addition of a sugar to phenylboronic acid solution, the UV absorbance due to free phenylboronic acid is decreased corresponding to the complex formation, and a difference in the UV absorbance (ΔI) can be measured. When the concentration of the sugar increases in the presence of a constant concentration of phenylboronic acid, ΔI increases until it becomes saturated to be a constant value (ΔI_{max}). Since ΔI and ΔI_{max} are proportional to [Complex] and $[\text{BA}]_0$, respectively, the relation between ΔI, ΔI_{max} and [Sugar] is derived from Eq. (1) and given by

$$1/\Delta I = 1/\Delta I_{max} + 1/(\Delta I_{max} A[\text{Sugar}]) \quad (2)$$

which means that plots of $1/\Delta I$ against 1/[Sugar] (Benesi–Hildebrand plots) give a straight line with the slope $1/(\Delta I_{max}\, A)$, i.e., $(1 + [\text{H}^+]/K_a)/(\Delta I_{max}\, K)$. Therefore, the value of binding constant K can be determined from the slope by considering $[\text{H}^+]$ of the solution and K_a of phenylboronic acid.

In the present study, Benesi–Hildebrand plots were attempted for the complexation of free phenylboronic acid with sugars, based on the UV absorbance at 267 nm. The plots for the complexation with monosaccharides and disaccharides gave straight lines according to Eq. (2). The K values at pH 10 for the complexation with those sugars were determined by taking account of the pK_a value (8.8) of phenylboronic acid in a homogeneous system [13,21]. However, the plots for oligosaccharides did not give straight lines and, therefore, the K values for the complexation with these sugars were determined by means of the ^{11}B NMR spectroscopy [13]. The K values determined for the sugars in Fig. 2 are listed in Table 1.



Scheme 2. Complexation of arylboronic acids with sugars.

In reference to the complexation of free phenylboronic acid with the monosaccharides, the determined values of K are in good accordance with the following values reported by Lorand and Edwards [22]: glucose, 110 M^{-1}; fructose, 4370 M^{-1}; galactose, 276 M^{-1}; mannose, 172 M^{-1}. Nagai et al. have studied the complexation of indolylboronic acid with a variety of sugars and reported the following values of K for the complexation with monosaccharides and disaccharides [23]: glucose, 71 M^{-1}; fructose, 6300 M^{-1}; maltose, 72 M^{-1}; cellobiose, 30 M^{-1}; lactose, 90 M^{-1}; melibiose, 580 M^{-1}; palatinose, 3400 M^{-1}. The results in Table 1 are consistent with these K values. Thus, the K values determined in the present study are considered to be reliable enough. However, for the complexation of indolylboronic acid with maltopentaose, the K value has been reported to be 930 M^{-1}, which is significantly larger than that for the complexation of phenylboronic acid. In Ref. [23], this result has been attributed to an extra interaction (so-called CH–π interaction) between the indole ring and the C–H moiety located apart from the binding sites on the oligosaccharide molecule.

3.2. Complexation of attached DHBP groups with various sugars

Complexation of the attached DHBP groups was examined with all the sugars shown in Fig. 2. By plotting the concentration of the sugars bound to the DHBP groups on magnetite particles against that of the corresponding free sugars, the effect of sugar concentration on the complexation was investigated quantitatively. Fig. 3 shows, as typical examples, the

Table 1
Binding constants for complexes of free phenylboronic acid and DHBP groups on magnetite particles with sugars at pH 10

Sugar	Binding constant K (M^{-1})	
	Phenylboronic acid	DHBP groups on magnetite particles
Monosaccharides		
Glucose	100	20
Fructose	5200	400
Galactose	370	180
Mannose	80	50
Disaccharides		
Maltose	60	0
Cellobiose	30	0
Lactose	110	0
Melibiose	500	70
Palatinose	1900	120
Oligosaccharides		
Maltopentaose	50	130
Maltohexaose	50	140
Maltoheptaose	40	210

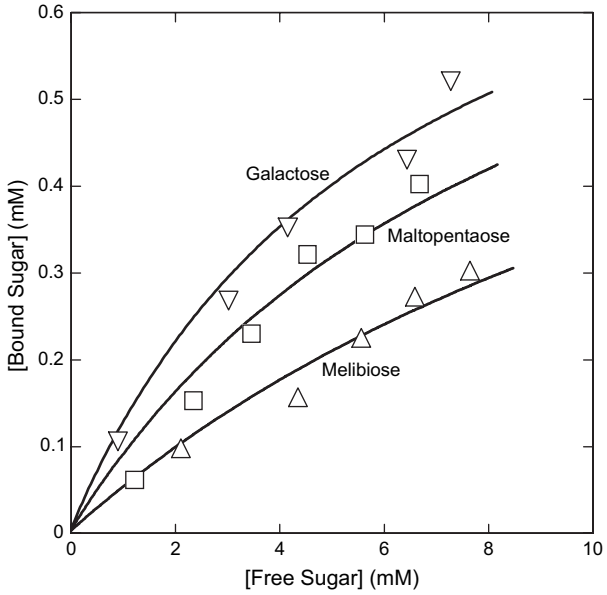


Fig. 3. Dependence of complexation of attached DHBP groups with galactose, melibiose and maltopentaose on concentration of each free sugar at pH 10. Initial concentration of the DHBP groups was 0.90 mM.

results obtained from the complexation with galactose, melibiose and maltopentaose, in which the regression curves based on Eq. (1) are fitted to the experimental data. From the regression curves, the values of binding constant K for the complexes with galactose, melibiose and maltopentaose were determined to be 180, 70 and 130 M^{-1}, respectively. In the same way as for these sugars, the K values were determined for the other sugars in Fig. 2. The determined values of K are listed in Table 1, together with those for the complexes of free phenylboronic acid.

For the complexation with monosaccharides and disaccharides, the DHBP groups attached to magnetite particles gave smaller values of K than free phenylboronic acid. As for the disaccharides, it was found that maltose, cellobiose and lactose were not bound to the DHBP groups at all. Thus, the DHBP groups on magnetite particles have less affinity to monosaccharides and disaccharides than phenylboronic acid in a homogeneous system, which is probably due to limited accessibility of the immobilized DHBP groups to these saccharides. The K values for the complexes of monosaccharides with both the DHBP groups on magnetite and phenylboronic acid were in the following order: fructose > galactose > glucose, mannose. In view of the fact that phenylboronic acid forms a stable complex with a pair of *cis*-OH groups on a furanose ring [13,24], it is a point of interest that the K values for the complexation of monosaccharides correspond to the content of furanose-type isomer in aqueous solution of each saccharide: fructose, 25% [25,26]; galactose, 7% [27]; mannose, 1% [28]; glucose, <1% [29]. On the other hand, it should be remarked that the K values for the complexes of the DHBP groups with oligosaccharides were larger than those of phenylboronic acid. This result suggests that neighboring DHBP groups on magnetite particles interact cooperatively with hydroxyl groups of the oligosaccharides. Such interaction was taken into account for the discussion on the result of the complexation with 2′-deoxyadenosine that the DHBP groups gave a considerable value of K for the complexation whereas free phenylboronic acid formed no complex [14].

3.3. Cooperative behavior of neighboring DHBP groups on magnetite particles

As seen in Table 1, the affinity of the oligosaccharides to the DHBP groups on magnetite particles seems to increase with increasing chain length of the sugars, whereas the affinity of them to free phenylboronic acid depends little on the chain length. In case of the DHBP groups concentrated on the surface of magnetite, it is likely that the complexation occurs with both the 1,2-OH pairs on the reducing ends and the 4,6-OH pairs on the nonreducing ends of the oligosaccharides. When the oligosaccharide chain is elongated, its conformation will become more suitable for fitting the steric arrangement of the vicinal OH pairs to such cooperative interactions. In addition, it is interesting to refer to the complexation of the DHBP groups with melibiose and lactose. Although these disaccharides consist of the identical monosaccharides (galactose and glucose), there appears a difference in the binding behavior. Melibiose is a flexible α-1,6-linked disaccharide and, therefore, its 1,2-OH pair on the reducing end and the 3,4- or 4,6-OH pair on the nonreducing end can interact cooperatively with the DHBP groups to give a considerable value of K. However, lactose, which is a less flexible β-1,4-linked disaccharide, forms no complex with the DHBP groups on magnetite particles.

In a practical application of DHBP-magnetite to magnetic handling of sugars, it is an important factor of the attached DHBP groups to have sufficient affinity to the sugar molecules. Since the DHBP groups, concentrated on the surfaces of magnetite particles, have considerable values of K for the complexation with oligosaccharides, DHBP-magnetite will be a promising tool for novel magnetic separation and/or transport of oligosaccharides. Apart from the magnetic separation, the surface modification technique with DHBP groups via the graft polymerization can be applied to the preparation of packing materials for chromatographic separation of sugars.

4. Conclusions

Magnetite particles were modified by the graft polymerization of acrylic acid initiated in a redox system consisting of mercapto groups introduced onto their surfaces and ceric ions. DHBP groups were attached to the magnetite particles through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). In order to apply the magnetite particles modified thus with DHBP groups to magnetic handling of sugars, complexation of the attached DHBP groups was examined with various monosaccharides, disaccharides and oligosaccharides, and compared with that of free phenylboronic acid.

The attached DHBP groups showed less affinity to monosaccharides and disaccharides, and gave smaller values of binding constant K for the complexation with these

saccharides than free phenylboronic acid. The less affinity was attributed to limited accessibility of the immobilized DHBP groups to monosaccharides and disaccharides. However, the *K* values for the complexes of the DHBP groups with oligosaccharides were larger than those of phenylboronic acid. It was suggested from this result that neighboring DHBP groups on the magnetite particles interacted cooperatively with vicinal OH pairs on both the reducing and nonreducing ends of oligosaccharides. In addition, with respect to the complexation with monosaccharides, both the DHBP groups and free phenylboronic acid gave the *K* values corresponding to the content of furanose-type isomer in aqueous solution of each saccharide.

The present study gave an interesting result that the DHBP groups attached onto the surfaces of magnetite particles have considerable values of *K* for the complexation with oligosaccharides. Therefore, DHBP-magnetite can be expected as a promising tool for novel magnetic handling, such as separation and transport, of oligosaccharides.

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