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Sugar-binding property of magnetite particles modified with dihydroxyborylphenyl groups via graft polymerization of acrylic acid

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Abstract

In order to combine sugar-binding property and magnetism, dihydroxyborylphenyl (DHBP) groups were attached to magnetite particles via graft polymerization of acrylic acid. The graft polymerization was carried out in a redox system consisting of mercapto groups introduced onto the surfaces of magnetite particles and ceric ions. DHBP groups were attached 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 sugars and compared with that of the free phenylboronic acid. The attached DHBP groups gave a large value of binding constant K for the complexation with adenosine having a pair of *cis*-OH groups, whereas the K values for the DHBP groups with adenosine phosphates were extremely small. With respect to the complexation with 2′-deoxyadenosine, cooperative interaction of neighboring DHBP groups was suggested. Although the value of acidity index pK_a of the attached DHBP was larger than that of free phenylboronic acid, the pK_a value was decreased by coexistent basic groups.

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

1. Introduction

Arylboronic acids are well known to form cyclic boronates with sugars at the sites of vicinal 1,2-diols and 1,3-diols involving an exocyclic CH_2OH moiety [1–5]. These complexations have been applied to separation [6,7], transport [8,9] and detection [10,11] of sugars. In addition, the complexations of arylboronic acids with sugars have played important roles in single-step glycosidation of sugars [12,13]. For practical application, it is of great interest to immobilize arylboronic acids on insoluble supports because of such advantages as easy separation and reusability of the immobilized arylboronic acids. If magnetizable particles are modified by attaching arylboronic acids, magnetic separation and/or transport of sugars can be achieved by using the modified particles.

The authors are interested in surface modification of magnetizable particles with functional substances. In previous studies, enzyme molecules were immobilized covalently on magnetite particles [14–16]. The immobilization process included graft polymerization of acrylic acid from the surfaces of magnetite particles, which was initiated by redox reaction between mercapto groups introduced onto the surfaces of the particles and ceric ions. Enzyme molecules were immobilized by the condensation reaction with carboxyl groups of the poly(acrylic acid) grafted onto the surfaces. The immobilized enzymes could be handled magnetically in reactor application.

The surface modification technique with graft polymerization is a useful tool to immobilize such functional organic compounds as arylboronic acids on inorganic substances. In the present study, phenylboronic acid was attached to magnetite particles via graft polymerization of acrylic acid: poly(acrylic acid) chains were grafted onto the surfaces of magnetite particles by the redox polymerization, and dihydroxyborylphenyl (DHBP) groups were attached through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). Sugar-binding property of the magnetite particles modified thus with DHBP groups was

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examined under various conditions and compared with that of free phenylboronic acid.

2. Experimental

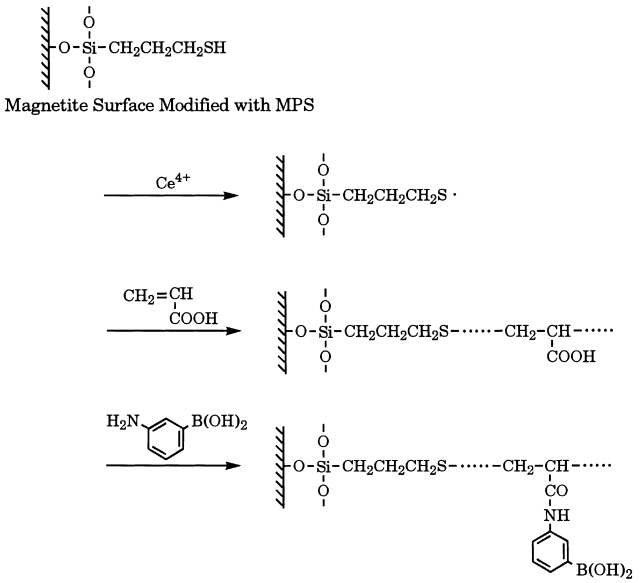
2.1. Materials

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 from Tokyo Kasei Kogyo Co., 3-mercaptopropyltrimethoxysilane (MPS) from Kanto Chemical Co. and ceric diammonium nitrate from Wako Pure Chemical Ind. 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 [17]: 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.

Graft polymerization of acrylic acid was carried out by following Scheme 1. 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. Although the magnetite particles can be separated with a magnet, centrifugal separation is favorable for experimental convenience and reproducibility. The amount of grafted poly(acrylic acid) was determined from the weight increase of the magnetite.



Scheme 1. Graft polymerization of acrylic acid from magnetite surface and attaching of DHBP groups.

2.3. Attaching of DHBP groups to magnetite particles

DHBP groups were attached to the poly(acrylic acid)-grafted magnetite (PAA-magnetite), as shown in Scheme 1, 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.

2.4. Determination of attached DHBP groups

The amount of attached DHBP groups was determined gravimetrically from the weight increase of PAA-magnetite accompanying the DHBP-attaching reaction. In order to confirm reliability of the gravimetric determination, several samples of DHBP-magnetite were treated with sodium hydroxide solution to hydrolyze amide linkage between DHBP groups and PAA-magnetite, and the amount of

liberated 3-aminophenylboronic acid was determined by means of the ^{11}B NMR spectroscopy. The results of gravimetry and NMR measurement were in good agreement for the determination of attached DHBP groups.

2.5. Complexation of attached DHBP groups with sugars

Complexation of the DHBP groups attached to PAA-magnetite was examined with the following sugars shown in Fig. 1: adenosine (**1**), 2′-deoxyadenosine (**2**), adenosine 5′-monophosphate (**3**), adenosine 5′-diphosphate (**4**), adenosine 5′-triphosphate (**5**), D-glucose (**6**), *p*-nitrophenyl-β-D-glucopyranoside (**7**) and *p*-nitrophenyl-β-D-galactopyranoside (**8**). All these sugars but D-glucose are characterized by UV absorption due to adenyl or *p*-nitrophenyl groups. They were chosen for analytical convenience.

The complexation was carried out by mixing DHBP-magnetite with each sugar in the following buffers of various pH values: KH_2PO_4–NaH_2PO_4 buffer (pH 5.0–8.0), Na_2CO_3–$NaHCO_3$ buffer (pH 9.0–10.0) and NaH_2PO_4–NaOH buffer (pH 11.0). The mixture was stirred for 1.0 h at room temperature, and then the DHBP-magnetite in the mixture was precipitated with a magnet, separated from supernatant and washed with the same buffer as used for the complexation. The bound sugars were liberated by dispersing the DHBP-magnetite in CH_3-COONa–HCl buffer (pH 2.0). It was confirmed by ^1H NMR that the sugars in Fig. 1 were stable in the solution of pH 2.0. The amounts of sugars **1**, **2**, **3**, **4** and **5** were determined by UV photometry from absorbance at 260 nm, **7** and **8** from absorbance at 300 nm, and **6** by Somogyi–Nelson method [18,19].

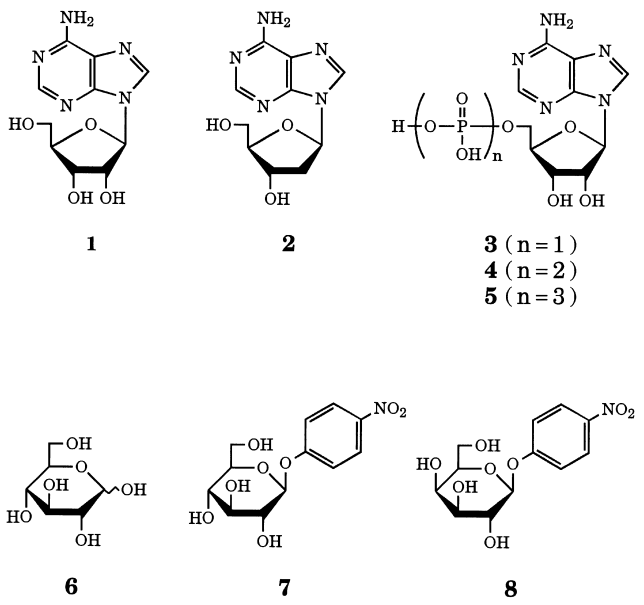


Fig. 1. Sugars employed for complexation with DHBP groups.

3. Results and discussion

3.1. Graft polymerization of acrylic acid and attaching of DHBP groups

Results of the graft polymerization of acrylic acid from the surfaces of magnetite particles have been reported in detail elsewhere [14]. In the present study, PAA-magnetite samples with various contents of grafted poly(acrylic acid) were prepared by the polymerizations for different periods of time: 10–64 mg of poly(acrylic acid) was grafted onto 1.0 g of magnetite.

DHBP groups were attached to these PAA-magnetite samples through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). In Fig. 2, the amount of attached DHBP groups is plotted against that of the carboxyl groups. It appears that the 3-aminophenylboronic acid reacts stoichiometrically with the carboxyl groups to attach DHBP groups to PAA-magnetite.

3.2. Effect of pH on complexation of attached DHBP groups with adenosine

Sugars are bound to DHBP groups by the complexation with anionized form of boronic acid moiety as shown in Scheme 2, where the acidity constant K_a and the binding constant K are defined as follows:

$$K_a = [BA^-][H^+]/[BA] \quad (1)$$

$$K = [\text{Complex}]/([BA^-][\text{Sugar}]). \quad (2)$$

The degree of anionization defined as $[BA^-]/([BA] + [BA^-])$ is derived from Eq. (1) and is given by

$$[BA^-]/([BA] + [BA^-]) = 1/(1 + [H^+]/K_a) \quad (3)$$

which means that the $[BA^-]$ increases with decreasing $[H^+]$

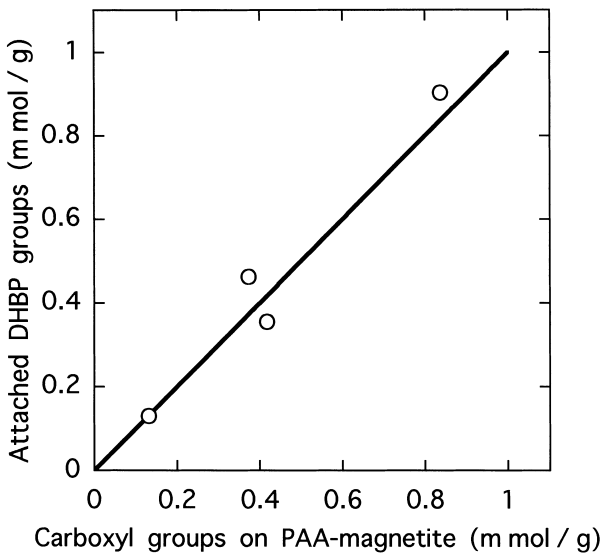
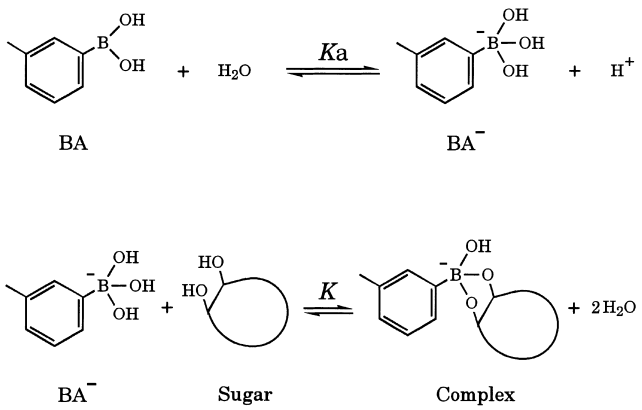


Fig. 2. Amount of DHBP groups attached to PAA-magnetite.



Scheme 2. Complexation of arylboronic acids with sugars.

and, therefore, the complexation occurs preferably in the case that pH is larger than the acidity index pK_a of DHBP groups. In the present study, the pK_a of the DHBP groups on magnetite particles was determined by the complexation with adenosine. DHBP-magnetite and adenosine were mixed in buffers of various pH values, and the complexation was monitored by measuring UV absorbance at 260 nm of the supernatant in each mixture. The results are shown in Fig. 3, where the ratio of the absorbance at each pH to that at pH 11.0 is presented as relative absorbance. Since the absorbance changes in proportion to a change in the concentration of sugar depending proportionally on a change in the degree of anionization, the data can be fitted to a curve given by

$$\text{Relative absorbance} = a - b/(1 + [H^+]/K_a) \quad (4)$$

where a and b are constants. In Fig. 3, the regression curve given by

$$\text{Relative absorbance} = 1.66 - 0.73/(1 + [H^+]/10^{-9.2}) \quad (5)$$

is shown, and the pK_a value determined from Eq. (5) is 9.2. This value is larger than the pK_a value (8.7–8.9) of phenylboronic acid in a homogeneous system [5,20]. The difference in pK_a between free phenylboronic acid and the DHBP groups on magnetite particles will be discussed later.

3.3. Complexation of attached DHBP groups with various sugars

Complexation of the attached DHBP groups was examined for furanosides (**1**–**5**), D-glucose (**6**) and pyranosides (**7**, **8**) at pH 10. Adenosine derivatives (**3**–**5**) were adopted as charge-carrying sugars. They have negative charge due to their phosphate groups under basic condition.

First of all, the effect of sugar concentration on the complexation was investigated with adenosine. In Fig. 4, the concentration of adenosine bound to the DHBP groups on magnetite particles is plotted against that of free adenosine. It is reasonable to consider that the data can be fitted to a curve given by the following equation, derived from Eqs. (1) and (2):

$$[\text{Complex}] = [\text{BA}]_0 \text{A}[\text{Sugar}]/(1 + \text{A}[\text{Sugar}]) \quad (6)$$

where [Complex] and [Sugar] are equal to concentrations of bound and free sugars, respectively, $[BA]_0$ is an initial concentration of the DHBP groups on magnetite, and the A is given by $K/(1 + [H^+]/K_a)$. The regression curve based on Eq. (6) is shown in Fig. 4 and the determined value of binding constant K is 630 M^{-1}.

In the same way as for adenosine, the binding constant K was determined for the other sugars. Table 1 shows the values of K for the complexes of the DHBP groups on magnetite with the sugars (**1**–**8**) together with those for the complexes of free phenylboronic acid. The data for free

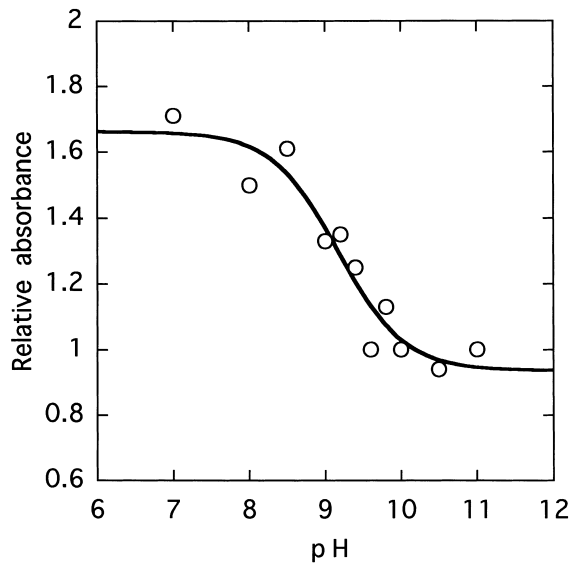


Fig. 3. Effect of pH on complexation of attached DHBP groups with adenosine.

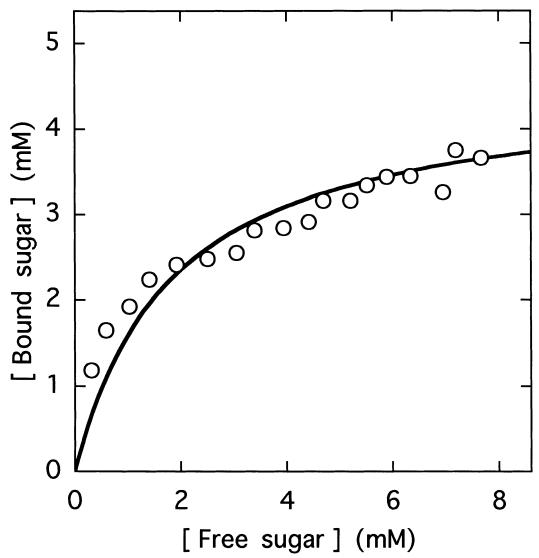


Fig. 4. Dependence of complexation of attached DHBP groups with adenosine on concentration of free adenosine at pH 10.

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

Sugar		Binding constant, K (M^{-1})	
		DHBP groups on magnetite particles	Phenylboronic acid
Adenosine	(**1**)	630	1470
2′-Deoxyadenosine	(**2**)	20	0
Adenosine 5′-monophosphate	(**3**)	30	370
Adenosine 5′-diphosphate	(**4**)	0	360
Adenosine 5′-triphosphate	(**5**)	0	230
D-Glucose	(**6**)	20	100
p-Nitrophenyl-β-D-glucopyranoside	(**7**)	<10	<10
p-Nitrophenyl-β-D-galactopyranoside	(**8**)	10	20

phenylboronic acid were obtained by the means of ^{11}B NMR spectroscopy [5].

It has been reported that the free phenylboronic acid forms a stable complex with a pair of *cis*-OH groups on a furanose ring [5,21]. Therefore, the binding site of adenosine is considered to be the pair of *cis*-2′,3′-OH groups on its ribose ring. This is supported by the result that a large value of K was observed for adenosine, whereas no complexation was observed for the adenosine without 2′-OH group (2′-deoxyadenosine). Although the binding sites of adenosine 5′-monophosphate, diphosphate and triphosphate were also considered to be the pair of *cis*-2′,3′-OH groups, the values of K for these adenosine derivatives were smaller than that for the adenosine, probably due to the limited complexation caused by the charge interaction between trihydroxoborate ($-B(OH)_3^-$) and phosphate ($-PO_3^{2-}$, $-PO_3^-$)groups. The results for D-glucose, *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-β-D-galactopyranoside are consistent with a previous report [5].

The DHBP groups on magnetite gave smaller values of K than free phenylboronic acid for the complexation with all the sugars but 2′-deoxyadenosine. These results suggest that the surface of magnetite was so crowded with the attached DHBP groups that steric hindrance occurred against the complexation with the sugars. It is interesting to note the results for the adenosine derivatives. The values of K for adenosine 5′-monophosphate, diphosphate and triphosphate were extremely small compared with the case of free phenylboronic acid. In case of the DHBP groups, which are concentrated on the surface of magnetite, it seems that the charge interaction with phosphate groups of the adenosine derivatives is enhanced to inhibit the complexation. On the other hand, the complexation with 2′-deoxyadenosine was observed for the DHBP groups on magnetite whereas free phenylboronic acid did not form the complex. This result may be attributed to the cooperative interaction of neighboring DHBP groups with 3′- and 5′-OH of 2′-deoxyadenosine.

3.4. Control of acidity index of DHBP groups on magnetite particles

As described in Section 3.1, the DHBP groups attached to magnetite gave a larger value of acidity index pK_a than the free phenylboronic acid. This result is similar to the phenomena observed for the self-assembled monolayers carrying carboxyl groups [22–24] and can be interpreted as the effect of densely immobilized DHBP groups. On the surface of DHBP-magnetite, the concentration of boronic acid moiety is much higher than in the homogeneous solution of phenylboronic acid, and it follows that the repulsive interaction [24] between emerging trihydroxoborate is considered to suppress further anionization of DHBP groups on the surface.

Such a large pK_a value is unfavorable for practical separation and/or transport of sugars with DHBP-magnetite. In order to lower the value of pK_a, DHBP groups were attached to magnetite particles with 2-dimethylaminoethyl (DMAE) groups through amide linkages by the condensation of PAA-magnetite with equivalents of 3-aminophenylboronic acid and *N*,*N*-dimethylethylenediamine. The magnetite modified with DHBP and DMAE groups (DHBP/DMAE-magnetite) was obtained in the same manner as described in the experimental Section 2.3. On the basis of the weight increase of PAA-magnetite and the amount of 3-aminophenylboronic acid liberated by hydrolysis with sodium hydroxide (determined by ^{11}B NMR analysis), the amounts of DHBP and DMAE groups on 1.0 g of DHBP/DMAE-magnetite were determined to be 0.12 and 0.08 mmol, respectively.

DHBP/DMAE-magnetite and adenosine were mixed in buffers of various pH values, and the complexation was monitored in the same manner as for DHBP-magnetite. The results are shown in Fig. 5, where the regression curve given by

$$\text{Relative absorbance} = 1.55 - 0.55/(1 + [H^+]/10^{-8.3}) \quad (7)$$

is presented, and the pK_a value determined from Eq. (7) is 8.3. It should be noted that this pK_a value is smaller than that of free phenylboronic acid. The result suggests that

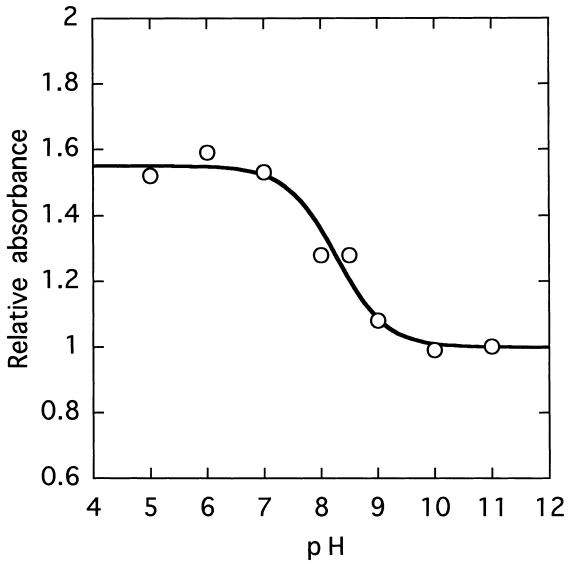


Fig. 5. Effect of pH on complexation of attached DHBP groups (coexistent with DMAE groups) with adenosine.

anionization of the DHBP groups on magnetite particles was promoted by coexistent basic DMAE groups. However, the determined value of K for DHBP/DMAE-magnetite ($290\ M^{-1}$) was smaller than that for DHBP-magnetite, showing that affinity of the DHBP groups to the sugar was decreased by the introduction of DMAE groups.

4. Conclusions

Poly(acrylic acid) was grafted onto the surface of magnetite particles by the redox polymerization of acrylic acid initiated from the surface. Succeedingly, DHBP groups were attached through amide linkages by the condensation reaction of 3-aminophenylboronic acid with carboxyl groups of the grafted poly(acrylic acid). It was found that 3-aminophenylboronic acid reacted stoichiometrically with the carboxyl groups to attach DHBP groups to magnetite particles. 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 sugars and compared with that of free phenylboronic acid.

The attached DHBP groups, as well as free phenylboronic acid, gave a large value of binding constant K for the complexation with adenosine having a pair of *cis*-OH groups on its ribose ring. The K values for the complexation of the DHBP groups with such charge-carrying sugars as adenosine phosphates were extremely small compared with those of free phenylboronic acid. The complexation with 2′-deoxyadenosine was observed for the DHBP groups, unlike for free phenylboronic acid, probably due to cooperative interaction of neighboring DHBP groups with 3′- and 5′-OH of 2′-deoxyadenosine. On the other hand, the DHBP groups gave a larger value of acidity index pK_a than free phenylboronic acid. However, when the DHBP groups were attached together with DMAE groups, the pK_a value was observed to become smaller than that of free phenylboronic acid. Taking into account the importance of pK_a in practical application, it is a point of interest that the anionization of DHBP groups on magnetite particles can be controlled by coexistent basic groups.

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