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Research Article

Glucose-Responsive Gel from Phenylborate Polymer and Poly(Vinyl Alcohol): Prompt Response at Physiological pH Through the Interaction of Borate with Amino Group in the Gel

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Purpose. To design glucose-responsive gels based on the complexation between polymers having phenylboronic acid groups and poly (vinyl alcohol). Specifically, high-glucose sensitivity at physiological pH was achieved through the interaction of phenylborate with amino groups.

Method. Terpolymers of *m*-acrylamidophenylboronic acid, N,N-dimethylaminopropylacrylamide (DMAPAA), and N,N-dimethylacrylamide were prepared. DMAPAA was introduced in the terpolymer to stabilize phenylborate-polyol complex at physiological pH. The effect of amino groups on complex stabilization was estimated from viscosity as well as UV spectrum measurements.

Results. A good correlation was observed between complexation rate and fraction of phenylborate as well as DMAPAA in the terpolymers. In line with this increased complexation rate, UV difference spectra measurement revealed that ionization of phenylboronic acid was facilitated in the terpolymer due to the interaction with DMAPAA. Further, sensitive change in the complexation rate was demonstrated with a variation in glucose concentration, which is in sharp contrast with the poor glucose-sensitivity of the polymer without DMAPAA.

Conclusions. The introduction of an amino group into phenylborate polymers was quite effective for increasing the complexation ability and the glucose responsivity at physiological pH. These results suggest the feasibility of this complex-gel system in designing a self-regulated insulin-releasing device.

KEY WORDS: boronic acid; poly(vinyl alcohol); amino group; glucose gel.

INTRODUCTION

For the treatment of diabetes, self-regulated releasing systems of insulin responding to the blood glucose concentration have been developed by many research groups. In these systems, biological components such as enzyme (glucose oxidase) (1) and lectin (concanavalin A) are used as glucose sensing units to trigger a change in the properties of the matrix (2,3). However there is always the problem of stability, toxicity, and antigenicity of these biological components. To overcome these problems, we have focused on the development of a self-regulated releasing system of insulin composed of totally synthetic matrices.

Phenylboronic acid group is used in our study as the glucose sensing moiety. A polymer having phenylboronic acid group as a side chain can form a complex gel with a polyol polymer such as poly(vinyl alcohol) (PVA) through the covalent complex formation between the pendant phenylborate and hydroxyl groups (4). When glucose is added to this complex gel, the gel swells due to a decrease in the cross-linking density caused by the substitution reaction of glucose with the pendant hydroxyl groups of the polymer toward borate groups. This leads to an accelerated release of loaded compounds, in this case insulin, from the complex gel. This exchange reaction is reversible so as to reduce insulin release through the reformation of borate-polyol crosslinking in the complex gel with a decreased concentration of glucose.

However, the glucose sensitivity of the complex gel prepared to date was observed only under alkaline conditions (pH 9.0 ~) (5). Phenylboronic acid is in an equilibrium of two

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ABBREVIATIONS: AAPBA, *m*-acrylamidophenylboronic acid; DMAPAA, N, N-dimethylaminopropylacrylamide; DMAA, N,N-dimethyacrylamide; PVA, poly(vinyl alcohol); NVP, N-vinyl-2-pyrrolidone; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; V-65, 2,2′-azobis(2,4-dimethylvaleronitrile); DMSO-d6, deuterated dimethyl sulfoxide; DBA, poly (*m*-acrylamidophenylboronic acid-co-N,N-dimethylaminopropylacrylamide-co-N,N-dimethyacrylamide).

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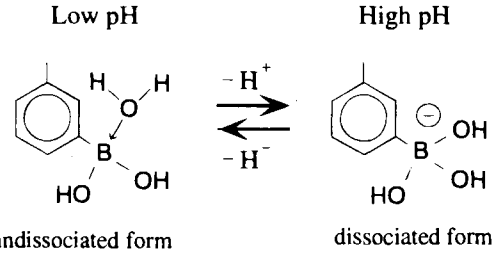


Scheme 1.

forms (undissociated and dissociated forms) in aqueous media as shown in Scheme 1.

The complex of undissociated boronic acid with glucose is unstable in water due to the high susceptibility to hydrolysis, whereas ionized phenylborate forms a stable complex with glucose even in aqueous solution. As the pKa of *m*-alkylamidophenylboronic acid is approximately 8.6 (6), a fraction of the phenylborate anion is not adequate to give the complex gel enough sensitivity to glucose under physiological conditions. To overcome this problem, incorporation of flanking amino groups into the phenylborate polymer was done to achieve the interaction of the amino group with the phenylboronic acid group, thus protecting the borate-polyol complex from nucleophilic attack by a water molecule (7) (Fig. 1).

In this paper, we studied the interaction of an amino group with the phenylboronic acid group in the side chain of the polymer using UV difference spectrum measurements. Furthermore, the effect of pH as well as of the amino group content in the copolymer on the formation of polymer complex was studied in detail to determine the optimum composition in order to achieve a prompt response toward glucose.

EXPERIMENTAL

Materials

m-Aminophenylboronic acid monohydrate (Sigma), acrylic acid (Wako Pure Chemical Industries Co., Ltd., Japan), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC-HCl) (Peptide Institute Inc., Japan) were used as received. N,N-Dimethylaminopropylacrylamide (DMAPAA) and N,N-dimethylacrylamide (DMAA) were purchased from Wako Pure Chemical Industries Co., Ltd. (Japan), and were distilled under reduced pressure (b.p. for DMAPAA; 91°C/1 mmHg, b.p. for DMAA; 51°C/1 mmHg) before use. 2,2′-Azobis(2,4-dimethylvaleronitrile), V-65, was purchased from Wako Pure Chemical Industries Co., Ltd. and recrystallized from water before use. Poly(vinyl alcohol) was purchased from Wako Pure Chemical Industries Co., Ltd. (Japan) and used without further purification. Ethanol was distilled over sodium, and stored under an argon atmosphere.

Methods

Synthesis of m-*acrylamidophenylboronic Acid (AAPBA) (4)*

75 mmol of EDC·HCl was added to 75 mmoles of *m*-aminophenylboronic acid monohydrate and 75 mmoles of acrylic acid in 100 ml of water, adjusted to pH 4.8 with 3 mol/l NaOH and cooled to 4°C. The mixture was stirred for 1 h after the addition was completed. The solution was then extracted with four 100 ml portions of diethylether in order to collect AAPBA in the diethylether layer. After removal of the diethylether by evaporation, the residue was recrystallized from water to obtain needle-shaped crystals. The ^{1}H-NMR (JEOL Co., Ltd., EX-400) spectrum of the compound in DMSO-d6 was consistent with the proposed structure.

Copolymerization of AAPBA, DMAPAA, and DMAA

The radical copolymerization of AAPBA, DMAPAA, and DMAA was carried out in a sealed glass ampoule *in vacuo* using V-65 as an initiator in ethanol at 45°C with varying feed ratios to prepare terpolymers with different composition (Table 1). After a 30 min reaction, the ampoule was opened, and the contents were poured into a large excess of diethylether. The precipitated polymer was filtered and dried under vacuum.

Characterization of Terpolymer, Poly(AAPBA-DMAPAA-DMAA) (DBA)

The copolymer composition was calculated from the ^{1}H-NMR (JEOL EX-400) spectrum in DMSO-d6 measured at 80°C. The composition of some copolymers was also determined from the combined analysis of atomic absorption spectroscopy (Shimadzu AA-680) of the boron atom and acid-base titration, and the results were compared with that obtained from ^{1}H-NMR. In this study, the DBAx/y abbreviation was used to express the copolymer composition, where x stands for the mol% of AAPBA, and y stands for the mol% of DMAPAA in DBA. A static light scattering measurement (Otsuka Electronics DLS-700) was carried out in distilled ethanol to determine the molecular weight of the copolymer.

UV Difference Spectrum Measurement

Acidic and basic DBA solutions were prepared, respectively, from a 5 ml aliquot of DBA aqueous solution (boronic acid concentration was adjusted to 1 mmol/l) by adding the same volume (5 ml) of 0.01 N HCl or 0.01 N NaOH. A 172 mg NaCl sample was added to both solutions to adjust the ionic

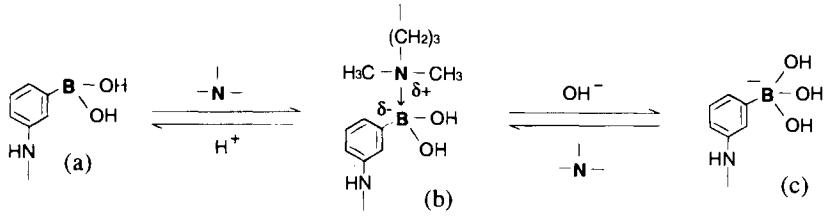


Fig. 1. Equilibria of phenylboronic acid in aqueous media in the presence of amino groups.

Table 1. The Composition of Poly(AAPBA-co-DMAPAA-co-DMAA)

Code name	Feed composition			Yield (%)	Copolymer composition			$\overline{Mw}$
	AAPBA	DMAPAA	DMAA		AAPBA	DMAPAA	DMAA	
DBA11/ 0	10.0	0.0	90.0	49.5	11.0	0.0	89.0	—
DBA13/ 0	10.0	0.0	90.0	34.7	12.5	0.0	87.5	39000
DBA10/ 3	10.0	5.0	85.0	31.0	9.5	3.2	87.3	—
DBA13/ 6	8.5	8.5	83.0	29.9	12.7	6.4	80.9	37000
DBA11/10	7.5	31.5	61.0	22.1	10.7	10.4	78.9	34000
DBA13/12	10.0	10.0	80.0	23.0	13.0	11.6	75.4	—
DBA11/11	10.0	15.0	75.0	41.0	11.2	10.8	78.0	—
DBA11/16	10.0	15.0	75.0	35.6	10.6	16.1	73.3	—

Note: total monomer conc.: 0.1 g/ml; reaction time: 30 min.; reaction temp.: 45°C; initiator: V-65(0.01 mol/l); solvent: ethanol.
The compositions were determined by ^{1}H-NMR. The abbreviation DBA x/y was used to express the composition. The x stands for the mol% composition of AAPBA, and y stands for the mol% of DMAPAA in DBA, respectively. $\overline{Mw}$ was measured by static light scattering using ethanol as solvent.

strength. The solutions were then diluted to 20 ml using distilled water. DBA solutions with a given pH were prepared by mixing these acidic and basic DBA solutions in an appropriate ratio. UV difference spectra of these DBA solutions were measured using a Shimadzu UV-3000 spectrophotometer with DBA solution at pH 5.0 as the reference.

Complexation Time Measurement

Complex formation was estimated from the viscosity change in the complex system. The time-dependent change in the viscosity of the mixed solution of DBA and poly(vinyl alcohol) was measured using a coagulation analyzer (SALSTEDT BIOMATIC2000), in which a vibrating rod is used as a sensor of solution viscosity. The relative viscosity at time t (V_t) is given by the following equation (1):

$$V_t = \frac{I_t - I_0}{I_{max} - I_0} \quad (1)$$

where I_t is an output at time t, I_{max} is an output given at the condition where the vibrating rod is fixed by clamping, and I_0 is an output of the control using 0.4 ml of solvent (in this case, buffer solution). In this study, the time required for the solution to reach half of the plateaued value of its V_t is defined as the complexation time. V_t value can be converted into the viscosity value measured by a rotational viscometer using a calibration curve as reported elsewhere (4). The concentration of polymer solutions (DBA, PVA) used in this measurement was 1 wt%, and the measurement starts when both DBA and PVA solutions are mixed together. Buffers used in this measurement were acetic acid/NaOH/NaCl buffer solution (<pH 5.5), phosphate buffer solution (6.5 < pH < 8.0), sodium *p*-phenosulfonate/NaOH buffer solution (8.0 < pH < 9.0), and $NaHCO_3$/NaOH/NaCl buffer solution (> pH 9.0). The ionic strength of all the buffer solutions was kept at 0.15 by adding an appropriate amount of NaCl. Complexation time measurements were carried out both in the presence and absence of a certain amount of glucose.

RESULTS AND DISCUSSION

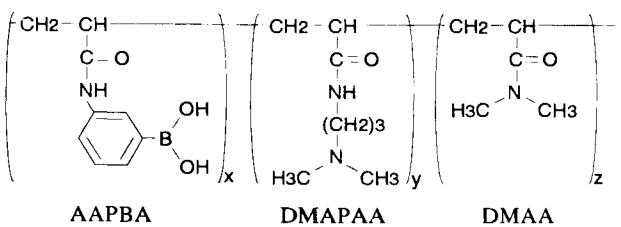
Synthesis of Linear Terpolymer (DBAx/y) Composed of AAPBA, DMAPAA, and DMAA.

The composition of the DBA terpolymer shown in Scheme 2 was determined by ^{1}H-NMR.

The peak intensities of the methyl and methylene protons of DMAPAA, the phenyl protons of AAPBA, and the methylene protons of the main chain were used for the calculation. The computer assisted peak separation software (JEOL Co., Ltd., MacAlice) was used to determine the integrated intensities. The compositions of the DBA terpolymer with varying ratios of AAPBA, DMAPAA, and DMAA are summarized in Table 1. The compositions determined from the combined analysis of the atomic absorption spectroscopy of the boron atom and acid-base titration were consistent with those obtained by ^{1}H-NMR. The weight-averaged molecular weights ($\overline{Mw}$) of the DBA samples with different composition were determined by static light-scattering in ethanol, and are also given in Table 1. The $\overline{Mw}$ ranged between 30,000 and 40,000 for all of the measured samples.

UV Difference Spectrum Measurement

In the phenylboronic acid moiety, the borate group directly binds to the phenyl ring, which has a crucial effect on its absorption bands. Benzene and its derivatives are known to have E-, K- and B- absorption bands in the UV region. The position and strength of these bands are very sensitive to the kind and number of substituents. Thus, ionization of the boronic acid moiety should have a crucial effect on the UV spectrum of the phenylboronic acid derivatives. Soundararajan *et. al.* applied the method of the difference absorption spectrum to determine the pKa's of arylboronic acid and its derivatives (8). Here, we utilized their method to estimate the interaction of the amino group of the DMAPAA unit in DBA with the phenylboronic acid moieties. In the UV absorption spectrum of DBA in buffer, the absorbance around 285 nm decreased with an increase in pH, reflecting the



AAPBA DMAPAA DMAA

Scheme 2.

increased ratio of borate anion to unionized boronic acid. The absorption difference spectra were then measured at each pH using pH 5.0 solution as a reference for DBA13/0 and pH 4.0 solution as a reference for DBA11/11. As the pKa of *m*-propionamidophenylboronic acid, a model compound for the phenylboronic acid moiety in DBA polymer, is 8.63, all of the boronic acid groups in the DBA polymer should be in the unionized form at pH 5.0. Thus, the difference in absorbance at 285 nm directly corresponds to the ratio of the unionized and ionized phenylboronic acid groups in DBA. The fraction of borate anion ($B_{ion}(\%)$) can be calculated from the results of the difference spectra based on the following equation (2):

$$B_{ion}(\%) = \frac{Abs. - Abs_{BH}}{Abs._{B^-} - Abs._{BH}} \times 100 \qquad (2)$$

where $Abs._B$ is the absorbance at pH 11 (100% ionized borate), $Abs._{BH}$ is the absorbance at pH 4 or 5 (100% unionized boronic acid), and *Abs.* is the absorbance at a given pH. The results for DBA13/0 and DBA11/11 are shown in Fig. 2.

$B_{ion}(\%)$ increased with a pH increase for both samples, yet in the lower pH region (<pH 8), a higher $B_{ion}(\%)$ was achieved for DBA11/11 compared to DBA13/0, suggesting the interaction of the amino group with the boronic acid group to form a complex as schematically shown in Fig. 1. At pH 7.4, DBA11/11 has twice the amount of ionized borate ((b)+(c) in Fig. 1) than DBA13/0.

Complexation Time Measurement

As the formation of the interpolymer complex leads to an increase in the viscosity of the solution, a kinetic viscosity measurement using a blood-coagulometer was carried out to estimate the formation of the polymer complex. As shown in Fig. 3, increases in both the amino group (DMAPAA) and phenylboronic acid (AAPBA) contents in DBA led to a significant decrease in the complexation time, indicating an increased efficiency to form the polymer complex. Particularly, a slight change in AAPBA contents in the region of 11 to 13% crucially affects the complexation time, which might reflect a change in the property of the polymer chain, probably conformation, in this region.

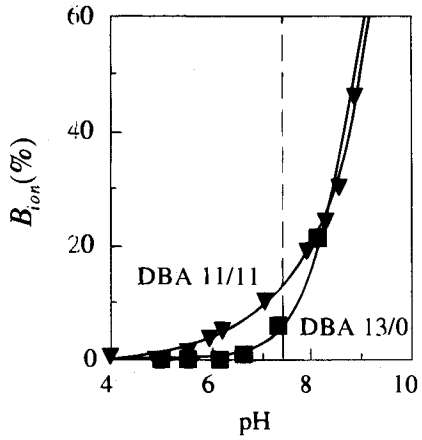


Fig. 2. Change in the fraction of borate anion ($B_{ion}(\%)$) with pH for phenylborate polymer with or without amino groups.

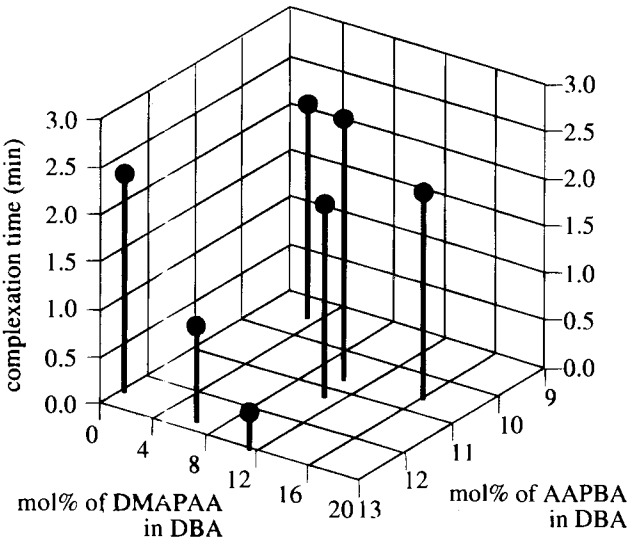


Fig. 3. Effect of DBA composition on the complexation time in DBA-PVA system.

The effect of pH on the complexation time was then compared to the polymer with (DBA13/12) or without (DBA11/0) the amino group (Fig. 4). At pH 8.6, the complexation times for the DBA11/0-PVA system and the DBA13/12-PVA system have very similar values of 4 sec and 5 sec, respectively, because most of the boronic acid groups are in the ionized form under this condition. With decreasing pH, a difference in the complexation time then becomes significant between these two systems. At pH 7.4, the complexation time of DBA11/0-PVA increased to 40 sec, whereas the complexation time of DBA13/12-PVA remained as short as 7 sec. This significant difference in the complexation time in the region of moderate pH between the two systems is consistent with the UV measurement results, suggesting an interaction of amino groups with the boronic acid group to increase the fraction of boronic acid in the ionized (or complex) form. With a further decrease in pH, DBA13/12 then showed a dramatic increase in the complexation time at

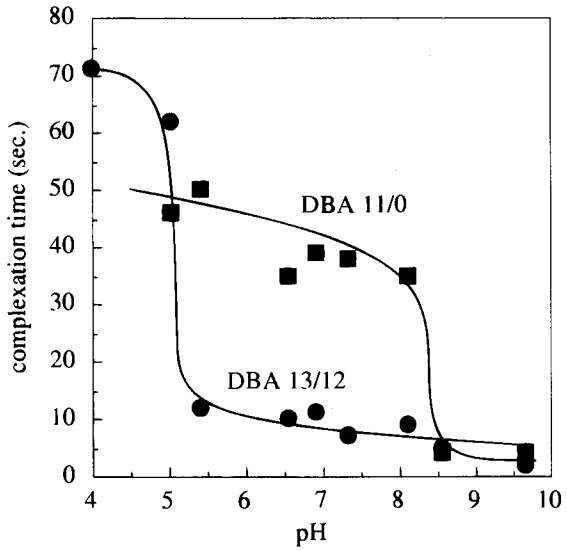


Fig. 4. Effect of pH on the complexation of DBA-PVA system.

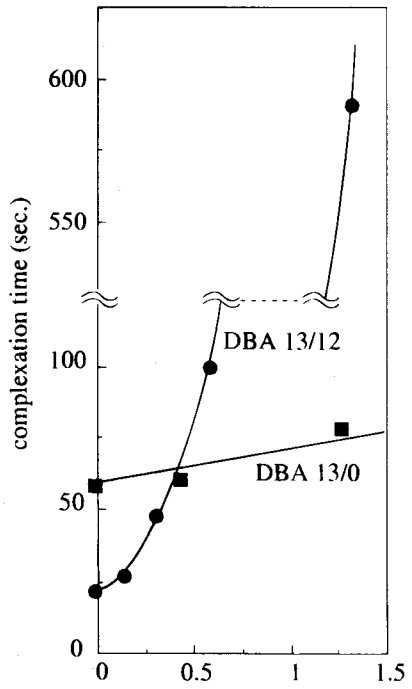


Fig. 5. Effect of pre-added glucose on the complexation of DBA-PVA system.

pH 5.0. It is noted that all the borate should be neutralized at this pH. Thus, no stable complex with polyol was formed below this pH.

It is of interest to measure the complexation of DBA with PVA in the presence of varying amounts of glucose from the standpoint of estimating the substitution ability of PVA with glucose toward the phenylborate group. Indeed, reversible complexation responding to a change in glucose concentration should be essential for the future application of this system in the glucose-responsive insulin-releasing device.

Fig. 5 shows the effect of pre-added glucose on the complexation between DBA and PVA. An increase in the molar ratio of the pre-added glucose to the monomer units of PVA leads to a significant increase in the complexation time for the DBA13/12-PVA system. No obvious increase in viscosity was observed within the measuring time-period (40 min) when the [glucose]/[-CH_2-CH(OH)] ratio was more than 1.5. On the other hand, a prompt response was achieved in the region with a low concentration of glucose. This result clearly indicates the reversible nature of the complexation between two polymers responding to a glucose concentration at physiological pH, and is quite promising for constructing the gel-system used in the self-regulated insulin releasing system for diabetes treatment. Indeed, reversible swelling and de-swelling of the complexed gel prepared from DBA and PVA was demonstrated under physiological pH by varying glucose concentration (the manuscript in preparation).

In a sharp contrast to the DBA13/12-PVA system, the complexation time was scarcely changed with glucose concentration for the DBA13/0-PVA system. Namely, this system has a poor response to glucose. As the complexation of DBA13/0-PVA is rather slow even at the glucose-free condition and has no retardation by coexisting glucose, this process may not involve the covalent complex formation between borate and the polyol groups. It should be noted that at pH 7.4, most of the borate groups in the copolymer exist in an undissociated form which cannot form a stable covalent complex with polyol compounds in aqueous media. Presumably, polymer association through hydrogen bonding may occur in the DBA13/0-PVA system at pH 7.4, because the boronic acid group is highly polar, and offers several sites for hydrogen bonding (9). By shifting the pH to an alkali condition (pH 9), even the DBA13/0-PVA system showed a good response to glucose (data not shown), which is in line with an increase in the borate anion.

In conclusion, the terpolymer of AAPBA, DMAPAA, and DMAA (DBA) forms a polymer complex with PVA under physiological pH. The complex formation is quite sensitive to glucose concentration in the medium, and the rate of the complexation can be controlled over a wide range by varying the glucose concentration. This is in a sharp contrast to the borate polymer without an amine moiety, where the complexation with PVA is slow and unresponsive to glucose. Because of the high-sensitivity to glucose at physiological pH, DBA-PVA gel system is feasible to use in a glucose-responsive self-regulated insulin delivery system by packaging into the biocompatible semipermeable membrane.

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