Polym. Bull. (2010) 65:807–814
DOI 10.1007/s00289-010-0278-x

ORIGINAL PAPER

Sugar-dependent solubility and fluorescence property of copolymers consisting of phenylboronic acid and 2-hydroxyethyl methacrylate moieties

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Received: 9 February 2010 / Revised: 9 April 2010 / Accepted: 17 April 2010 /
Published online: 7 May 2010

Abstract Copolymers consisting of *N*-3-acrylamidophenylboronic acid (APBA) and 2-hydroxyethyl methacrylate moieties (HEMA) were synthesized and their solubility and fluorescence properties were evaluated in the presence of sugar. The APBA–HEMA copolymer composed of 25 mol% of APBA moiety was found to be poorly soluble in water at pH 7.4. However, the water solubility of APBA–HEMA was improved in the presence of fructose in solution. The solubility of APBA–HEMA was influenced by fructose in a concentration-dependent manner, due to the formation of boronate ester of APBA moiety with fructose added. In addition, APBA–HEMA was modified with fluorescein isothiocyanate (FITC) for the fluorometric detection of sugars. The fluorescence intensity of FITC-modified APBA–HEMA was dependent on the type and concentration of sugars in solution. The fluorescence intensity of FITC-modified APBA–HEMA was highly enhanced by the addition of fructose, while the fluorescent response was negligibly small when other sugars were added. Thus, usefulness of FITC-modified APBA–HEMA for the selective determination of fructose was demonstrated.

Keywords Phenylboronic acid · Boronate ester · Fructose · Optical density · Fluorescence

Introduction

The development of sugar-sensitive materials has been currently a focal subject in biomaterials science and technology [1–3]. Phenylboronic acid (PBA) is known to

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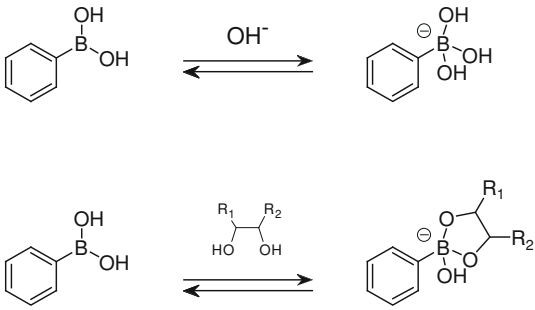

Fig. 1 Acid–base and diol binding equilibria of phenylboronic acid

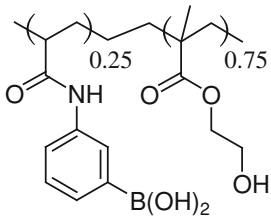

Fig. 2 Chemical structure of APBA–HEMA

bind with high affinity to compounds containing diol moieties through reversible ester formation (Fig. 1) [4, 5]. PBA-based sugar-responsive functional materials in microparticle and microcapsule forms have been developed to use for drug delivery systems (DDS) [6–8]. In particular, there have been many reports on the sugar sensor for which PBA derivatives were used as the recognition portion [9, 10], in which UV–visible spectra [11–13], fluorescence emission spectra [14, 15], and electrochemical property [16, 17] were changed upon binding sugar.

The principle of sugar detection in these reports relied on characteristic spectrophotometric or electrochemical changes of PBA. This study reports the sugar-sensitive solubility and fluorescence property of copolymers that include the PBA moiety. We have synthesized a copolymer from *N*-3-acrylamidophenylboronic acid (APBA) and 2-hydroxyethyl methacrylate (HEMA; Fig. 2). The water solubility of the APBA–HEMA copolymer was limited when the content of APBA unit in the copolymer was 25% or higher. However, the solubility of APBA–HEMA was improved upon addition of fructose, due to the formation of a phenylboronate ester accompanied by the addition of OH^- ion to the boron atom and contribution of hydroxyl groups from the sugar (Fig. 1). In other words, the solubility of APBA–HEMA depended on the kind and concentration of the sugar added in the solution.

In addition, we have modified APBA–HEMA with fluorescein isothiocyanate (FITC-modified APBA–HEMA) for studying the effects of sugar on the fluorometric property. It has been known that fluorescence of FITC derivatives is quite sensitive to the physical and chemical parameters of the environment where the dye is located. For example, the FITC fluorescence is quenched upon being confined on the surface of proteins [18, 19] and/or due to the close proximity of the FITC residues [20, 21]. This property of FITC fluorescence was successfully utilized for the determination of binding sites of avidin using FITC–biotin conjugates, whose

fluorescence was significantly quenched upon binding to avidin [19]. In fact, we have found that fluorescence intensities of FITC-modified APBA–HEMA increased upon addition of fructose.

Experimental section

Materials

3-Aminophenylboronic acid hemisulfate, acryloyl chloride, 2-hydroxyethyl methacrylate, and 2,2′-azodiisobutyronitrile (AIBN) were obtained from Tokyo Kasei Co. (Tokyo, Japan). Other reagents were of the highest grade available and used without further purification.

N-3-Acrylamidophenylboronic acid was synthesized according to the reported procedure [10]. Briefly, 3-aminophenylboronic acid hemisulfate (1.0 g) was dissolved in NaOH solution (2 mol L^{-1}, 11 mL) at 0 °C. Chilled acryloyl chloride (1.0 g) was added dropwise to the 3-aminophenylboronic acid solution under vigorous stirring over 15 min. HCl solution (1 mol L^{-1}) was slowly added to the reaction mixture until the pH reached 1.0. White solids precipitated, which were filtered and washed with cold water.

APBA–HEMA was synthesized by free radical polymerization as follows. 2-Hydroxyethyl methacrylate (412 mg), *N*-3-acrylamidophenylboronic acid (188 mg), and AIBN (6 mg) were dissolved in 10 mL of dimethylsulfoxide. The mixture was bubbled with N_2 gas for 30 min, and heated at 70 °C for 24 h. After cooling to room temperature, the reaction mixture was dialyzed against excess amount of DI water for 24 h. The content of APBA moiety in the resulting copolymer was calculated to be about 25% from the ratio of nitrogen and carbon contents in elemental analysis. Calculated values for the copolymer composed of 25% APBA residue, C: 55.86%, H: 6.89%, N: 2.41%. Found, C: 52.37%, H: 7.06%, N: 2.26%. FITC-modified APBA–HEMA was synthesized by modifying copolymer prepared in a similar manner adding 1% of allylamine with FITC.

Apparatus

Optical density (OD) was recorded on a Shimadzu 3100PC spectrophotometer (Kyoto, Japan) using quartz cell with 10 mm light pass length. Fluorescence spectra were measured using a Shimadzu RF-5300PC spectrofluorophotometer (Kyoto, Japan).

Results and discussion

A preliminary examination suggested a limited solubility of APBA–HEMA in water at pH 7.0. Therefore, we have measured OD of aqueous APBA–HEMA solutions (100 μg mL^{-1}) at pH 5.0–10.0. Figure 3 shows the OD at 600 nm of APBA–HEMA solutions measured using a quartz cuvette with 10 mm light pass length. The

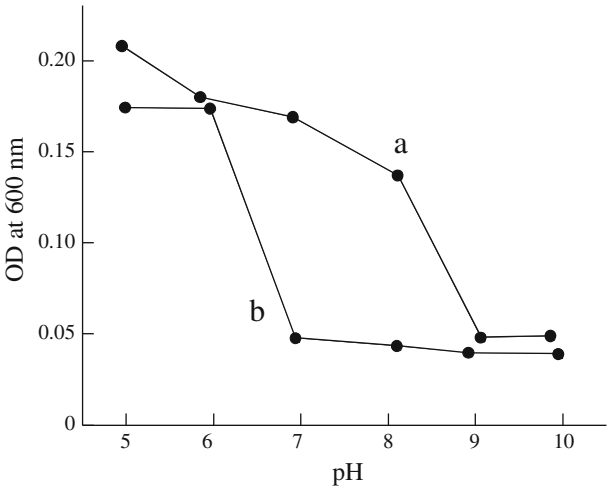

Fig. 3 OD of APBA–HEMA (100 μg mL^{-1}) in different pH solutions in the absence (*a*) and presence (*b*) of 100 mmol L^{-1} fructose, measured in HEPES buffer solution

results show that APBA–HEMA did not completely dissolve in water in the pH range tested. The OD of the solution was ca 0.2 in weakly acidic pH (<pH 7) in the absence of fructose, while the OD decreased in the solutions at pH 8 or higher. This is probably because an OH^- ion coordinated to the boron atom of PBA in the copolymer (see Fig. 1, pK_a of PBA is 8.8 [11]). On the other hand, in the presence of 100 mmol L^{-1} fructose, the OD was significantly reduced at pH 7.0 and 8.0 as compared to those without fructose. Since the pK_a of PBA is 8.8, it exists in molecular form at low pH, while at high pH an OH^- ion coordinates to the molecule (Fig. 1). It is known that the pK_a of PBA shifts to the acidic side upon binding diol compounds [11]. In this case, the pK_a of PBA in APBA–HEMA may be shifted by addition of fructose, and the coordination of OH^- ion was accelerated at pH 7.0 and 8.0. Thus, PBA moiety in the polymer existed in negatively charged form, resulting in the enhanced solubility in the presence of fructose.

The OD response characteristics of APBA–HEMA to different concentrations of fructose at pH 7.0 were investigated over time (Fig. 4). Fructose was added to 100 μg mL^{-1} APBA–HEMA solution to the final concentrations of 0, 10, 50, 75, and 100 mmol L^{-1}. A significant decrease in the OD_t/OD_0 value was observed when 75 and 100 mmol L^{-1} fructose was added. The OD decreased down to 62 and 69% after 60 min in the presence of 75 and 100 mmol L^{-1} fructose, respectively. However, the OD of the solution exhibited lower response to fructose at the concentrations of <50 mmol L^{-1}. The OD was not changed by the addition of other kinds of sugars including 100 mmol L^{-1} glucose, mannose, galactose, and maltose (data not shown). Thus, the response was found to be selective to fructose. The binding constants of these saccharides to PBA are: fructose, 160 M^{-1}; galactose, 15 M^{-1}; mannose, 13 M^{-1}; glucose, 4.6 M^{-1}; and maltose, 2.5 M^{-1} in pH 7.4 phosphate buffer solution [15]. These saccharides may interact with PBA moiety in

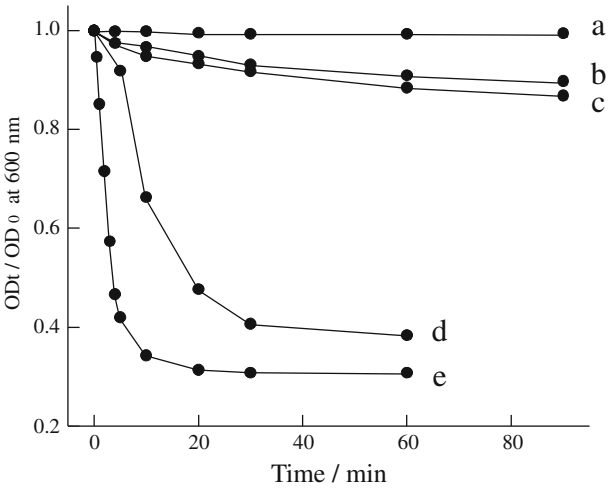

Fig. 4 Time-course of changes in OD_t/OD_0 of 100 μg mL^{-1} APBA–HEMA upon addition of 0 (*a*), 10 (*b*), 50 (*c*), 75 (*d*), and 100 mmol L^{-1} fructose (*e*), measured in pH 7.0 HEPES buffer solution

APBA–HEMA depending on the binding constant, but a high affinity such as that of fructose is necessary to decrease OD.

The effect of APBA content in APBA–HEMA copolymer on the solubility with and without fructose was also studied. For this purpose, copolymers with 15 and 35% APBA residues were synthesized in a similar manner by changing the ratio of APBA and HEMA monomers. The copolymer with 15% APBA residues nearly completely dissolved in pH 7.0 solution. The OD value of the solution of 100 μg mL^{-1} copolymer with 35% APBA residues at pH 7.0 was 0.3 as compared to 0.2 for the copolymer with 25% APBA residues. Thus, the solubility of the copolymer with 35% APBA residues was slightly lower than that of the copolymer with 25% APBA residues in the absence of fructose. The OD decreased with fructose addition in a similar manner as for 25% APBA copolymer (data not shown).

The sugar-sensitive property of APBA–HEMA copolymer may be applicable to the determination of sugars. Therefore, we have modified 25% APBA copolymer with FITC and its fluorescence was studied in the presence of sugars. FITC derivatives are known to be quite sensitive to the physical and chemical parameters of the environment in which the dye is located. It is thought that FITC residues in the modified APBA–HEMA are in a hydrophobic environment in solution because the copolymer is not fully soluble. In contrast, FITC residues may be exposed to hydrophilic environment in the presence of sugar. Consequently, the fluorescence intensity of the copolymer may change upon sugar binding. Figure 5 shows the fluorescence emission spectra of 1 μg mL^{-1} FITC-modified copolymer upon addition of fructose in the solution at pH 7.4. Figure 5 clearly shows that the fluorescence intensities of FITC in the copolymer increased with the addition of fructose as expected. This is probably originating from the fact that the copolymer is not fully soluble at pH 7.4, and the fluorescence was quenched in the dispersed state. On the other hand, the fluorescence was not quenched when the solubility of the

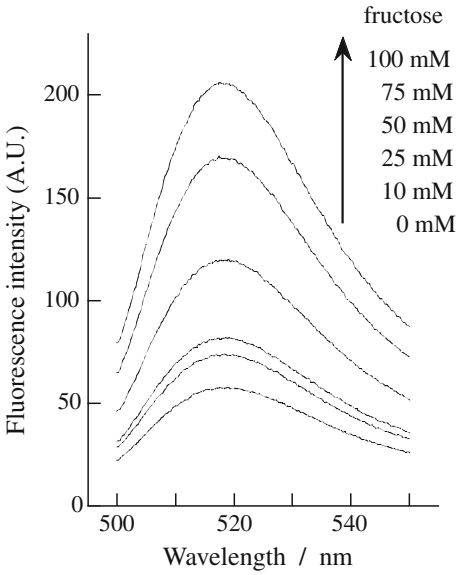

Fig. 5 Effects of fructose on the fluorescence spectra of 1 μg mL^{-1} FITC-modified APBA–HEMA. The fluorescence spectra were recorded 30 min after addition of fructose. The wavelength of excitation light used was 488 nm, and the spectra were measured in pH 7.4 HEPES buffer solution

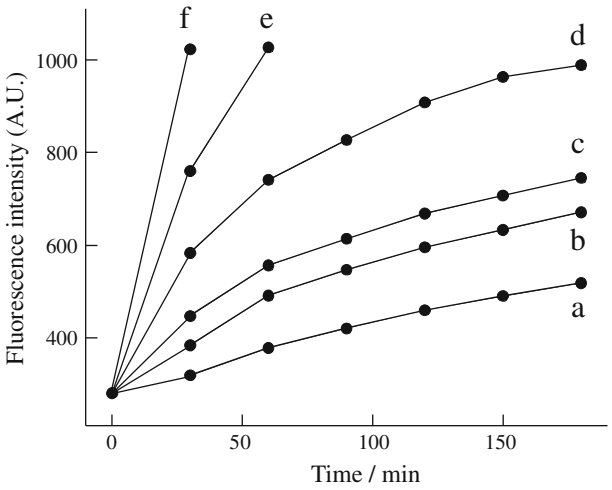

Fig. 6 Time-course of changes in fluorescence intensity at 520 nm of 1 μg mL^{-1} FITC-modified APBA–HEMA upon addition of 0 (*a*), 10 (*b*), 25 (*c*), 50 (*d*), 75 (*e*), and 100 mmol L^{-1} fructose (**f**). The wavelength of excitation light used was 488 nm, and the spectra were measured in pH 7.4 HEPES buffer solution

copolymer increased as a result of fructose binding. In fact, we were able to detect <10 mmol L^{-1} fructose using fluorimetry of the FITC-modified copolymer.

Figure 6 shows the fluorescence response of FITC-modified copolymer to the different concentration of fructose. The copolymer showed the fluorescence response to fructose of 10–100 mmol L^{-1} ranges. The fluorescence intensity of the copolymer also increased without fructose probably because the copolymer

dissolved gradually over time at the low concentrations. The fluorescence intensity of the copolymer slightly increased in the presence of fructose <10 mmol L^{-1}, but baseline drift disturbed the accurate measurements. The effects of other kinds of saccharides (glucose, mannose, galactose, and maltose) on the fluorescence intensity were very small. Thus, the fluorescence response was selective to fructose.

Conclusions

The solubility (or OD) of copolymer APBA–HEMA was sensitive to pH and fructose in aqueous solution. The solubility was improved in the presence of fructose due to the binding of fructose with APBA residue in the copolymer associated with the addition of OH^- ion to boron atom. Furthermore, it has been found that the fluorescence intensity of FITC-modified APBA–HEMA copolymer was enhanced in the presence of 10–100 mM fructose. Thus, APBA–HEMA copolymer may be useful for developing sugar-sensitive materials.

Acknowledgment This study was supported, in part, by a Grant-in-Aid for Young Scientists (B) (21790031) from Japan Society for Promotion of Sciences (JSPS).

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