

Boronate-containing thermoresponsive polymers as sugar sensitive materials

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Introduction

Reversible complex formation of borate and phenylboronate ions with mono- and oligosaccharides in aqueous solution is well known. These interactions have been employed in separation and detection of compounds containing sugar molecules and synthesis of sugar-responsive materials. Equilibrium constants of the complex formation (K_{ass}) between borate group and particular diols in the saccharide molecules vary from 6 to 2000 M^{-1} for different sugars, suggesting high selectivity of borate interaction with sugars. Recently, some efforts were made to synthesize water-soluble copolymers containing phenylboronic acid, and to study their properties from the viewpoint of application for sugar sensing and responding materials and lectin-like materials. In this study, interaction of thermoresponsive boronate-containing copolymers with some carbohydrates and polyols have been studied by a shift of its phase transition temperature (T_p), which might be caused by the binding of the molecules to the copolymer [1, 2]. Furthermore, we investigated to analyze the interaction of thermoresponsive boronate-containing copolymers with some carbohydrates using UV spectral measurement.

Results and discussion

Thermoresponsive boronate-containing copolymers of N-acryloyl-3-aminophenylboronic acid (AAPBA) and N-isopropylacrylamide (NIPAAm) were prepared by free radical polymerization [1]. The molecular structure of the copolymer is shown in Fig. 1. The content of phenylboronic acid in the (NIPAAm-AAPBA)-copolymer was 18 mol% which was determined by $^1\text{H-NMR}$ spectrum of the copolymer. The number- and weight-average molecular mass (M_n and M_w) of the (NIPAAm-AAPBA)-copolymer were 47,000 g/mol and 183,000 g/mol, respectively, and M_w/M_n was 3.9, as determined from the SEC chromatogram. The molecular mass at peak-top of SEC (M_p) was 224,000 g/mol.

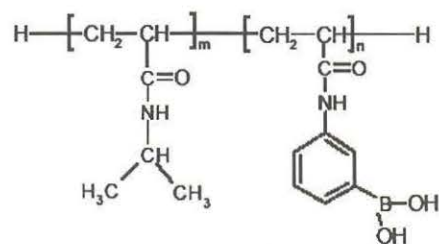


Fig. 1 Structure of (NIPAAm-AAPBA)-copolymer

Thermal precipitation property of (NIPAAM-AAPBA)-copolymer

The (NIPAAM-AAPBA)-copolymer exhibits a typical thermal precipitation behavior (Fig. 2). The optical transmittance of the copolymer solution sharply decreased at a specific temperature, which increased with an increase in pH. The increase would be due to the ionization of phenylboronate group from an uncharged trigonal form, which is hydrophobic, to a charged boronate anion, which is a hydrophilic. The thermal precipitation of poly(NIPAAM-AAPBA) at pH 9.7, where almost all phenylboronate groups would be in a form of charged boronic anion, was almost the same as that of poly-NIPAAM. This precipitation behavior of poly(NIPAAM-AAPBA) is considered as follows; the introduction of non-charged hydrophobic phenylboronate group into the polymer chain of poly-NIPAAM causes the decrease of the phase transition temperature; the ionization of phenylboronic group by increasing pH counterweights hydrophobicity, hence charged poly(NIPAAM-AAPBA) precipitated at nearly the same temperature as that of poly-NIPAAM.

Effect of sugars on the thermal precipitation of poly(NIPAAM-AAPBA)

The thermal precipitation of poly(NIPAAM-AAPBA) was measured in the presence of glucose. The thermal precipitation at pH 6.4-6.5, where almost all of boronic acid groups would be in the uncharged form, was unaffected by glucose. This indicates that the uncharged form of boronic acid does not interact with glucose. On the other hand, the thermal precipitation of the copolymer at pH 9.3-9.5, where boronic acid group would be in the charged form, was shifted to higher temperatures by the addition of extremely small amount of glucose, 5.6 μ M (1.0×10^{-3} mg/ml) (Fig. 3). The thermal precipitation temperature increased with an increase in the glucose concentration. The increase in T_p is caused by an increase in hydrophilicity of the

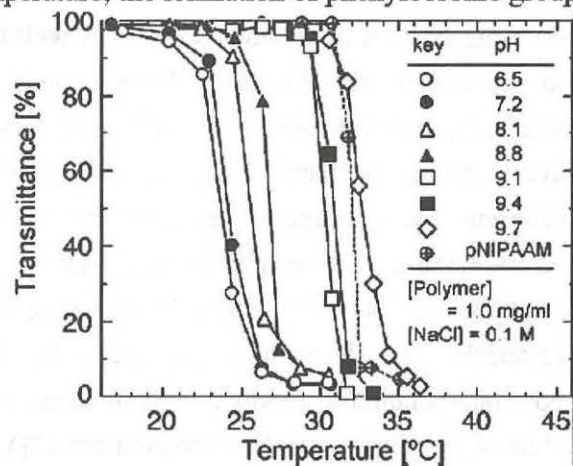


Fig. 2 Temperature dependence of optical transmittance of poly(NIPAAM-AAPBA) and poly-NIPAAM at various pH values.

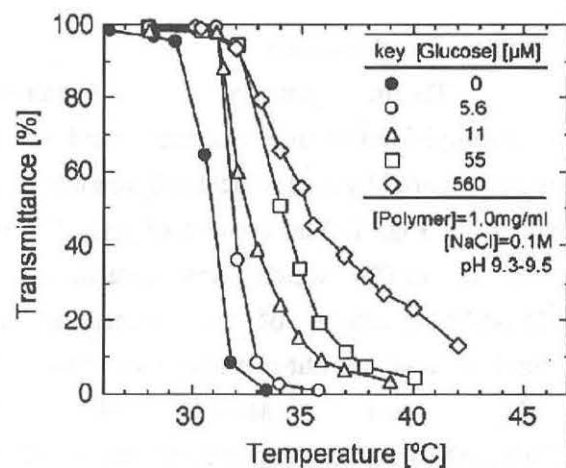


Fig. 3 Temperature dependence of optical transmittance of poly(NIPAAM-AAPBA) in the absence and presence of glucose at pH 9.3-9.5

copolymer due to the formation of the complex between phenylboronic acid group of the copolymer and glucose (Fig. 4).

The effect of the addition of sugars and polyol compounds on T_P of poly(NIPAAm-AAPBA) was examined (Fig. 5). The increase in T_P caused by the addition of fructose was about 7-10 °C at pH higher than 8. The effect of mannitol on T_P was almost the same as that of glucose. In the presence of galactose, though the small increase in T_P , 1-3 °C, was observed at pH 7.5-8.8. The order of the increase in T_P is the following: fructose > glucose ≈ mannitol > galactose. This order except glucose seems to reflect the association constant for a complex between monomeric phenylboronic acid and sugar (fructose > mannitol > galactose > glucose) [3]. Glucose has an ability to form a 1:2 complex with phenylboronic acid. Therefore, glucose might show a specific high affinity with phenylboronates in poly(NIPAAm-AAPBA) due to multi-point interaction like chelating of glucose with two pendant phenylboronates in the polymer chain as mentioned above.

A small increase in T_P was observed in the presence of glycerol and oligosaccharides at pH 7.5-8.7 while no effect was observed at pH > 9 (Fig. 5b). The affinity of these additives towards phenylboronate groups would be relatively low compared with monosaccharides used.

From the above results, the effect of sugars and polyols on the increase in T_P of poly(NIPAAm-AAPBA) is concluded as follows: fructose > glucose ≈ mannitol > pentaerythritol > galactose > Tris > glycerol.

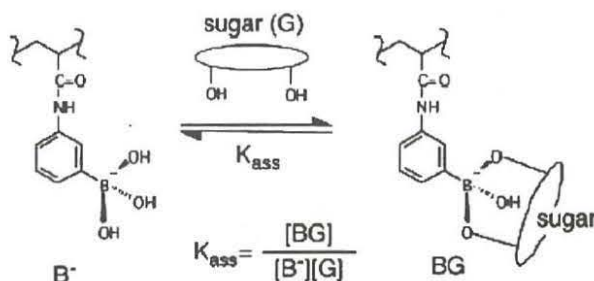


Fig. 4 Equilibrium of between a charged phenylboronate anion and sugar in aqueous media.

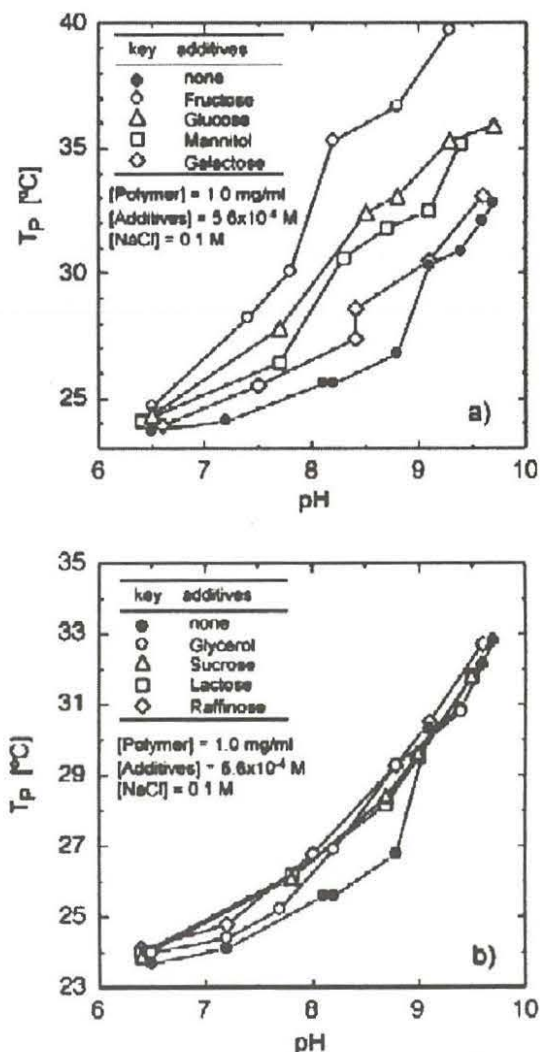


Fig. 5 Effect of pH on T_P of poly(NIPAAm-AAPBA) in the presence of various sugars and polyol compounds. a) monosaccharides, and b) glycerol and oligosaccharides.

Analysis of interaction between of poly(NIPAAM-AAPBA) and sugars

In order to analyse the interaction between of poly(NIPAAM-AAPBA) and sugars, UV spectrum of the copolymer dissolved in water at 297 K was measured at various pH values using a photo-probe type spectrophotometer (USB2000, Ocean Optics, Inc., USA). A Small change of the spectra by the pH change observed, however the difference was relatively small. So, the difference UV spectra for the copolymer at various pH values were taken from the spectrum observed at the lowest pH values as a reference (Fig. 6). This spectral change would be caused by the ionization of the phenylboronate group of the copolymer from an uncharged trigonal form (B) to a charged boronate anion (B⁻) [1]. Almost all of the boronate acid groups would be in the uncharged form at pH lower than 6, hence the spectral change is considered to be caused by the increase in the concentration of the charged boronate anion and the decrease of the uncharged one. The change rates of the absorbance at 287 nm for the copolymer solution, and also for the copolymer solution containing fructose at various concentrations, were plotted against their pH values in Fig. 7. The absorbance at 287 nm decreased sharply with increase in pH value. This absorbance change at 287 nm can be attributed to the decrease of the uncharged form. The curve of the absorbance change was shifted to lower pH region by an increase in the fructose concentration. Fructose can be interact with the charged boronate anion. Hence, this shift shows the consumption of the uncharged form by the formation of the complex between the charged boronate anion and fructose and the equilibrium between the uncharged form and the boronate anion.

References

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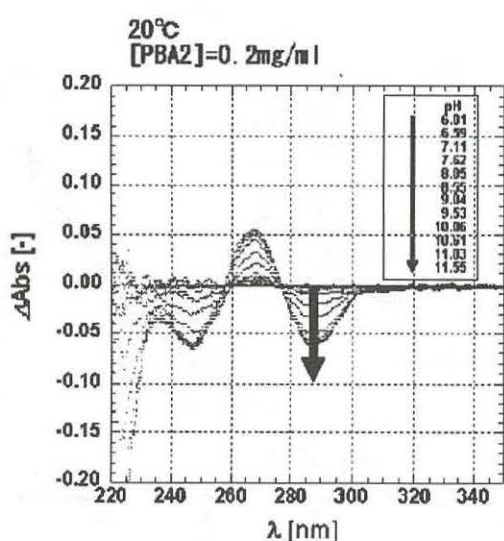


Fig. 6 Difference adsorption spectra between unionized and ionized forms of the copolymer

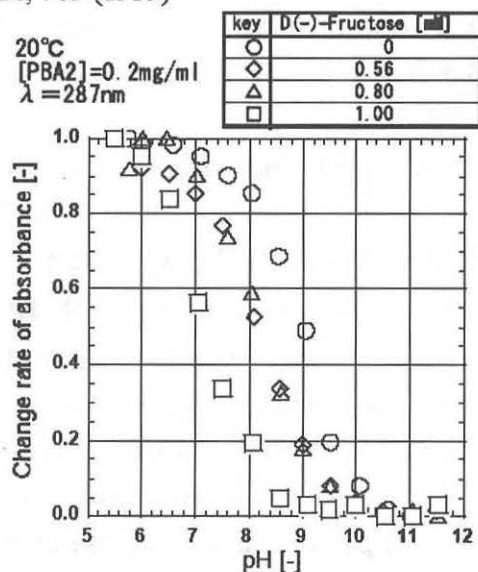


Fig. 7 Change rates of absorbance of the boronate copolymer solution containing fructose.