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Fructose-sensitive thermal transition behaviour of boronic ester-bearing telechelic poly(2-isopropyl-2-oxazoline)

Jiyoung Lee, [a] Jong Min Park[a] and Woo-Dong Jang*, [a]

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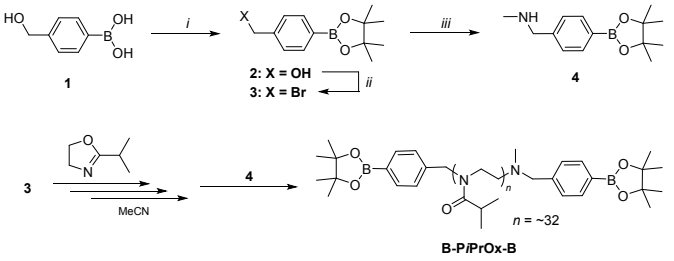
Boronic ester-bearing telechelic poly(2-isopropyl-2-oxazoline) (B-P*i*PrOx-B) exhibited a hydrophilic-hydrophobic phase transition near human-body temperature in aqueous media. The thermal transition temperature of B-P*i*PrOx-B changed notably upon the addition of fructose.

Stimuli-responsive polymers that change their physicochemical properties in response to external stimuli have been widely utilised in various fields.[1, 2, 3, 4, 5] In particular, thermoresponsive polymers are highly useful in bio-related applications such as sensors, bio-scaffolds, and smart biointerfaces because living systems are homeostatic to temperature.[6, 7, 8, 9] Especially, isothermally responsive polymers that shows hydrophilic-hydrophobic phase transition at specific temperature would be very useful and desirable for the bio-related applications.[10] Such isothermally responsive polymers can be obtained by combination of stimuli-responsive functional groups and thermoresponsive polymers. Poly(2-isopropyl-2-oxazoline) (**P*i*PrOx**) is a representative thermoresponsive polymer that exhibits hydrophilic-hydrophobic phase transition via a lower critical solution temperature (LCST).[11] **P*i*PrOx** has excellent biocompatibility and shows LCST behaviour near human-body temperature.[12, 13, 14] Therefore, the applications of **P*i*PrOx** in the biomedical field are being actively studied.[15, 16, 17] **P*i*PrOx** is synthesized by the cationic ring-opening polymerization (CROP) of an oxazoline monomer.[18, 19] Because the polymerization of **P*i*PrOx** proceeds via a living mechanism, the molecular weight and distribution of the polymer can be easily controlled.[20, 21, 22] More importantly, various functional groups can be introduced to the initiation and termination ends of **P*i*PrOx**.[23, 24, 25] By exploiting the merits of living polymerization, additional stimuli-responsive functional groups have been introduced to **P*i*PrOx** for the preparation of multi-modal stimuli-responsive polymers.[26, 27, 28] For example, hybrid systems of **P*i*PrOx** and azobenzene have been designed to obtain thermo- and photoresponsive functional materials.[29] Recently, we prepared uracil-bearing telechelic **P*i*PrOx** as a chemoresponsive **P*i*PrOx**, which exhibited a drastic change in thermal transition temperature in response to mercury ions.[30] In this study, we prepared a telechelic pinacol boronate-bearing **P*i*PrOx** (**B-P*i*PrOx-B**) as a new type of chemoresponsive polymer. Boronic esters are capable of undergoing exchange reactions with chemicals having diol groups[31, 32, 33, 34] and show particularly high reactivity to *cis*-diols. Therefore, the thermal transition temperature of **B-P*i*PrOx-B** can be varied by the addition of various saccharides through the boronic ester exchange reaction. Notably, fructose has the greatest effect on the thermal transition temperature increment. Because the continuous ingestion of the fructose is directly and indirectly related to the pathogenesis of obesity, type 2 diabetes and cardiovascular disease, fructose responsive polymers may provide potential applications.[35, 36, 37]



Scheme 1. Synthesis of **B-P*i*PrOx-B**. Reagents and conditions: i) pinacol, Na_2SO_4, THF, 25 °C; ii) CBr_4, PPh_3, THF, 0 °C; iii) methylamine, dimethoxymethane.

The synthesis **B-P*i*PrOx-B** is illustrated in Scheme 1. First, pinacol was allowed to react with 4-(hydroxymethyl)phenylboronic acid (**1**) to obtain **2**. The hydroxyl group of **2** was converted to bromide using CBr_4 and PPh_3 to yield **3**, which was used as the functional initiator for CROP. Methylamine was allowed to react with **3** to obtain **4**, which was used as the functional terminator in the CROP. For

[a]*Department of Chemistry, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea. E-mail: wdjang@yonsei.ac.kr (Prof. W.-D. Jang)*
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the CROP, the ratio of **3** and the monomer (2-isopropyl-2-oxazoline) was adjusted to 1:32, so that the theoretical number-average molecular weight (M_n) became approximately 4.0 kDa. When the monomer was fully consumed, an excess amount of **4** was added to the reaction mixture to obtain telechelic **B-P*i*PrOx-B**. Then, the remaining terminator and low-molecular-weight impurities were removed by recycling preparative size-exclusion chromatography (SEC). The products of each reaction were characterised by ^{1}H NMR, SEC, and MALDI-TOF-MS measurements. The ^{1}H NMR spectra of **B-P*i*PrOx-B** showed multiple peaks from 8.13 to 6.86 ppm, indicating the successful introduction of the phenyl groups into both the initiation and termination ends of the polymer (Fig. S4). The single peak at 1.31 ppm indicated the existence of pinacol moieties. The M_n and dispersity index ($Đ$) of **B-P*i*PrOx-B** were estimated from the results of SEC and MALDI-TOF-MS analyses. The result of analytical SEC revealed a single sharp peak at the retention time of 20.44 min. From the results of analytical SEC, the M_n and $Đ$ of **B-P*i*PrOx-B** were estimated to be 3.99 kDa and 1.12, respectively, using polystyrene standards. A similar molecular weight was deduced from MALDI-TOF-MS analysis. The MALDI-TOF-MS profile of **B-P*i*PrOx-B** exhibited sets of peaks at 113.08 Da intervals, corresponding to the molar mass of the monomeric 2-isopropyl-2-oxazoline (Fig. S5). The maximum peak appeared at 4.08 kDa, which coincided well with the results of SEC as well as the theoretically expected M_n value from the monomer to initiator ratio (Fig. S6). In addition to the molecular ion peaks, two sets of satellite peaks were observed, which were attributable to the fragment series formed by the removal of pinacol moieties under the MALDI-TOF-MS conditions.

Table 1. Time-dependent T_{CP} changes (°C) of **B-P*i*PrOx-B** (4.0 g/L in 10 mM PBS, pH 7.4) by addition of various saccharides (50 mM).

Time(h)	Maltose	Lactose	Glucose	Sucrose	Mannose	Galactose	Fructose
0*	0±0.11	0.1±0.08	0.5±0.11	0.1±0.07	1.3±0.10	1.7±0.10	5.7±0.11
1	0±0.10	0.1±0.09	0.6±0.12	0.3±0.11	1.6±0.14	1.7±0.09	5.7±0.10
3	0±0.09	0.3±0.10	0.6±0.10	0.4±0.09	1.7±0.13	1.7±0.11	5.7±0.08
5	0.2±0.07	0.4±0.07	0.8±0.11	0.8±0.07	1.7±0.12	1.8±0.11	5.7±0.11
24	0.3±0.10	0.4±0.09	0.8±0.09	1±0.11	1.7±0.10	1.8±0.10	5.7±0.11

*Immediately after addition.

The thermal transition temperatures of **B-P*i*PrOx-B** were determined by temperature-dependent transmittance measurements at 800 nm. For this experiment, **B-P*i*PrOx-B** was dissolved in physiological saline-containing 10 mM phosphate-buffered solution (PBS, 150 mM NaCl, pH 7.4), and the cloud point temperature (T_{CP}) was measured upon increasing the temperature a rate of 2 °C/min, where T_{CP} is defined as the temperature corresponding to a 50% decrease in the optical transmittance. Fig. 1a,b shows the temperature-dependent transmittance changes of **B-P*i*PrOx-B**. T_{CP} gradually decreased with increasing concentration of **B-P*i*PrOx-B** from 0.5 to 8.0 g/L, where the difference in T_{CP} was approximately 13 °C between 8.0 g/L and 0.5 g/L. These trends are similar to those observed for other types of thermoresponsive polymers.

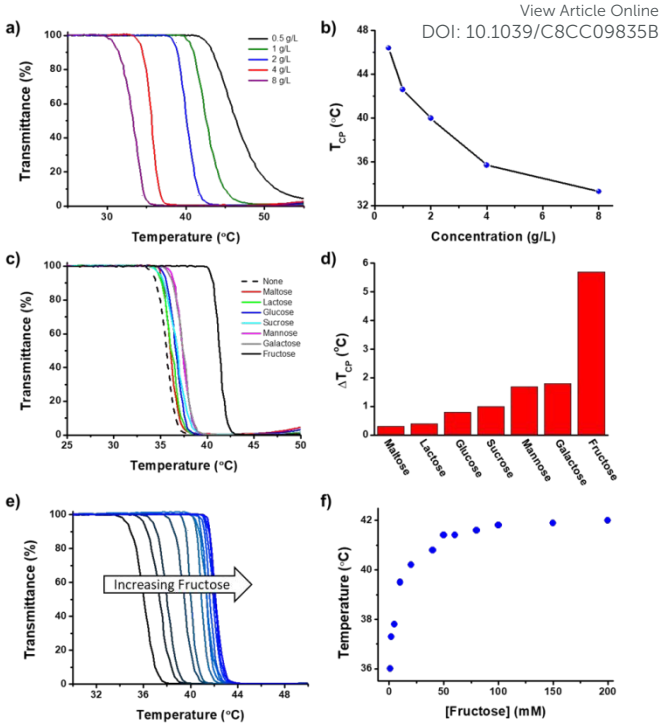

Figure 1. Thermoresponsiveness of **B-PiPrOx-B** in pH 7.4 PBS solution. a) Temperature-dependent transmittance changes of **B-P*i*PrOx-B** at several concentrations; b) concentration-dependent T_{CP} of **B-P*i*PrOx-B**; c) temperature-dependent transmittance changes of **B-P*i*PrOx-B** (4.0 g/L) with various saccharides (50 mM); d) T_{CP} changes of **B-P*i*PrOx-B** (4.0 g/L) by addition of various saccharides (50 mM); e) temperature-dependent transmittance changes of **B-P*i*PrOx-B** (4.0 g/L) with various concentrations of fructose (0, 1, 2, 5, 10, 20, 40, 50, 60, 80, 100, 150, and 200 mM); f) T_{CP} of **B-P*i*PrOx-B** (4.0 g/L) with various concentrations of fructose (0–200 mM).

Because boronic esters are capable of exchange reactions with chemicals having diol groups,[38] we investigated the changes in the thermal transition temperature of **B-P*i*PrOx-B** in the presence various saccharides such as maltose, lactose, glucose, sucrose, mannose, galactose, and fructose (Fig. 1c-d). To a solution of **B-P*i*PrOx-B** in 10 mM PBS (150 mM NaCl, pH 7.4), various saccharides were added, and the final concentration of **B-P*i*PrOx-B** and each saccharide was adjusted to 4.0 g/L and 50 mM, respectively. If the hydrophilic saccharides bond to the end of **B-P*i*PrOx-B** by an exchange reaction, the thermal transition temperature of the polymer might change because of the increasing hydrophilicity. As expected, T_{CP} of **B-P*i*PrOx-B** increased with the addition of various saccharides. However, the degree of T_{CP} change greatly depended on the nature of the saccharide (Fig. 1c,d, Table 1). For example, **B-P*i*PrOx-B** showed only a 0.3 °C increase in T_{CP} even after 24 h of maltose addition. In the case of galactose addition to **B-P*i*PrOx-B**, T_{CP} increased only by 1.8 °C after 5 h of galactose addition. Unlike case of the other saccharides, the addition of fructose greatly influenced the T_{CP} value of **B-P*i*PrOx-B**: T_{CP} increased by 5.7 °C with the addition of fructose. It is noteworthy that the T_{CP} change of **B-P*i*PrOx-B** was immediately saturated by the addition of fructose, indicating a high rate of reaction between **B-P*i*PrOx-B** and fructose. The fast response and large T_{CP} change of **B-P*i*PrOx-B** by fructose addition can be explained by the structural aspect of the diol moiety and the high stability of the

saccharide-conjugated boronic ester structure, respectively.[39, 40, 41] First, fructose has two syn-periplanar *cis*-diols, which would be suitable functional groups for the fast boronic ester exchange reaction.[42] Moreover, furanose would be more reactive than pyranose because of the large angle between the diol moieties, possibly reducing the angle strain in the five-membered pentagonal ring structure of the saccharide-conjugated boronic ester.[43] To confirm the influence of fructose on the thermal transition behaviour of **P*i*PrOx** segment, the temperature dependent transmittance changes of a propargyl-bearing telechelic **P*i*PrOx** (**Prop-P*i*PrOx-Prop**; Figure S7) with and without fructose were measured.[30] As a result, the thermal transition behaviour of **Prop-P*i*PrOx-Prop** was not changed after the addition of fructose. The T_{CP} of **B-P*i*PrOx-B** was not changed upon incubation for 24 h in 10 mM PBS (150 mM NaCl, pH 7.4) at 25 °C, indicating the sufficient stability of pinacol boronic ester (Fig. S8).

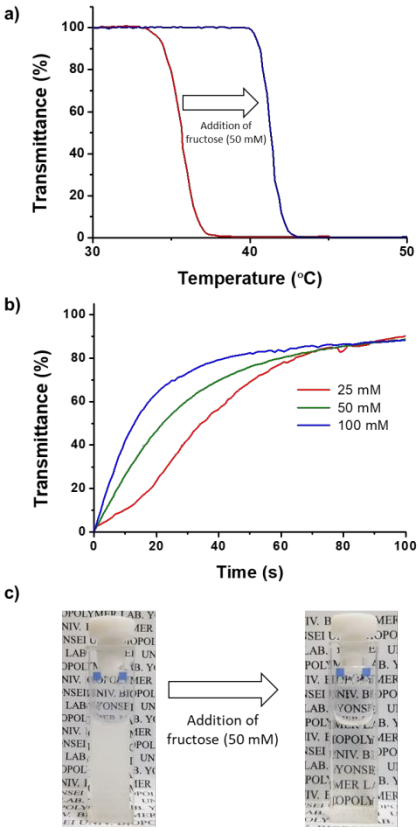

Figure 2. Thermoresponsiveness of **B-PiPrOx-B** in pH 7.4 PBS solutions containing 50 mM glucose: a) Temperature-dependent transmittance changes of **B-PiPrOx-B** solutions before and after fructose addition (50 mM), b) time- course transmittance measurement at 38 °C of **B-P*i*PrOx-B** solutions after addition of various concentrations of fructose, c) photographs of **B-P*i*PrOx-B** solutions before and after fructose addition (50 mM).

Because fructose is the most reactive substrate for **B-P*i*PrOx-B**, T_{CP} of **B-P*i*PrOx-B** (4.0 g/L) in 10 mM PBS (150 mM NaCl, pH 7.4) solution containing various concentrations of fructose (1–200 mM) was again measured (Fig. 1e,f). As the concentration of fructose was increased, T_{CP} gradually increased, with saturation behaviour. At a fructose concentration of 200 mM, T_{CP} of **B-P*i*PrOx-B** became 42.0 °C, which is 6.3 °C higher than the original T_{CP} of **B-P*i*PrOx-B** without fructose. At very low concentrations of fructose (1 and 2 mM), T_{CP} continuously varied for 19 h, indicating that a long reaction time is needed to reach equilibrium state. In contrast, T_{CP} immediately reached to equilibrium state when the concentration of fructose was higher than 5 mM. Notably, the **B-P*i*PrOx-B** solution containing only 5 mM fructose showed a T_{CP} increment of 2.1 °C. Therefore, we can conclude that **B-P*i*PrOx-B** has significantly higher sensitivity to fructose than to other saccharide molecules.

Because **B-P*i*PrOx-B** showed remarkably higher sensitivity to fructose than to other saccharide molecules, we examined fructose-induced T_{CP} changes in the presence of glucose. For this experiment, a solution containing **B-P*i*PrOx-B** (4.0 g/L, pH 7.4 PBS buffer) with 50 mM of glucose was prepared and stirred for 24 h to allow the system to reach equilibrium. Then, we confirmed the T_{CP} value of the initial glucose-containing **B-P*i*PrOx-B** at 36.5 °C. To the glucose-containing **B-P*i*PrOx-B** solution, fructose was added to a final concentration 50 mM. As a result, T_{CP} of **B-P*i*PrOx-B** gradually increased and finally reached 41.4 °C, which is the same that of **B-P*i*PrOx-B** with 50 mM fructose but without glucose (Fig. 2a). Because the T_{CP} of **B-P*i*PrOx-B** with 50 mM fructose has the same value, regardless of the presence or absence of glucose, we can conclude that the interaction between boronic acid and fructose is quite strong, and that all of the glucose molecules conjugated to **B-PiPrOx-B** are replaced with fructose under these conditions. Because the normal blood sugar level of human is about 5mM, the competition experiment between fructose and glucose was performed under that condition (Fig. S9).[44] When 5 mM of fructose was added to **B-P*i*PrOx-B** solution having T_{CP} at 36.1 °C with 5 mM of glucose, the T_{CP} was changed to 37.9 °C, implying that the sensitivity of **B-P*i*PrOx-B** to the fructose is sufficient in the range of the normal blood sugar level, which is comparatively low sugar concentration.

As mentioned previously, the boronic ester exchange from pinacol to fructose was quite fast under the 50 mM fructose conditions. In contrast, the exchange from glucose to fructose is slower than that from pinacol to fructose. To confirm the rate of boronic ester exchange from glucose to fructose, we monitored the hydrophilic-hydrophobic transition of **B-P*i*PrOx-B** at a specific temperature (38.0 °C). The solution containing **B-P*i*PrOx-B** with 50 mM glucose is opaque at 38.0 °C because T_{CP} corresponds to 36.5 °C. After the addition of fructose, the solution gradually becomes transparent because T_{CP} of **B-P*i*PrOx-B** with 50 mM fructose is 41.4 °C (Fig. 2b). In the time-course measurements, the transmittance of the **B-P*i*PrOx-B** solution at 38.0 °C reaches approximately 90% within 100 s, where the rate of the exchange reaction depends on the fructose concentration (Fig. 2c). Throughout the competition experiment, we again confirm the high binding affinity of fructose to **B-P*i*PrOx-B**. Moreover, we can control the solubility of **B-P*i*PrOx-B** by the addition of saccharides near human-body temperature.

In summary, boronic ester-bearing telechelic poly(2-isopropyl-2-oxazoline) was prepared as a dual stimuli-responsive functional polymer, which showed high sensitivity and selectivity to fructose. At a certain temperature, **B-P*i*PrOx-B**

exhibited a hydrophobic-to-hydrophilic phase transition through boronic ester exchange from glucose to fructose. Using the transmittance change of the **B-P*i*PrOx-B** solution, the binding affinities of two different saccharides to boronic acid can be directly compared. In addition, the rate of the exchange reaction can be evaluated on the basis of time-course transmittance changes. Because the solubility of **B-P*i*PrOx-B** can be controlled by adjusting the fructose concentration, the design of a fructose-mediated delivery system is feasible.

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Conflicts of interest

There are no conflicts to declare.

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