Communications to the Editor

Sensitive Glucose-Induced Change of the Lower Critical Solution Temperature of Poly[*N,N*-dimethylacrylamide-*co*-3-(acrylamido)phenylboronic acid] in Physiological Saline

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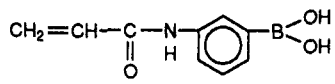
Introduction. Recently, considerable impetus has been given to the research on intelligent polymers which change their properties responding to external stimuli such as heat,[1] light,[2] electric field,[3] and pH.[4] However, few examples are known of synthetic polymers, in both solution and gel form, specifically responding to a small concentration change of a chemical species in the milieu. In this paper, we report the first preparation of a polymer which changes the lower critical solution temperature (LCST) in aqueous solution responding to glucose concentration. Our system is based on the shift in the equilibrium between the uncharged and anionic form of phenylboronic acid moieties in the polymer chain through complex formation with glucose (Figure 1). This concept originated from our studies of a glucose-responsive insulin delivery system[5] and a synthetic mitogen for lymphocytes.[6]

Experimental Part. A copolymer (DB-15) of *N,N*-dimethylacrylamide containing 15 mol % of 3-(acrylamido)phenylboronic acid (APBA) of the chemical formula



was prepared by the radical copolymerization of the corresponding monomers in ethanol at 45 °C using 2,2′-azobis(2,4-dimethylvaleronitrile) as initiator.[7] The APBA content in DB-15 was determined by atomic absorption spectrometry (AA-670; Shimadzu Co.). The molecular weight of DB-15 was determined to be 9×10^4 by static light scattering (DLS-700; Otuka Electronics). LCST determination was carried out spectrophotometrically (500 nm) by dissolving DB-15 in HEPES[8]-buffered physiological saline (pH 7.4, NaCl; 8.0 g/L, KCl; 0.2 g/L, HEPES; 25 mM) at 0.1 w/v %. The pH change with temperature during LCST measurement was compensated by adding a small amount of NaOH aqueous solution.

3-(Propionamido)phenylboronic acid, a model compound of APBA units in DB-15, was prepared by the condensation reaction of propionic acid with 3-aminophenylboronic acid hemisulfate using [1-ethyl-3-(dimethylamino)propyl]carbodiimide hydrochloride as a condensation reagent through a similar to procedure APBA synthesis.[5] Acid–base titrations of 3-(propionamido)-phenylboronic acid were performed at varying concentrations of glucose to determine apparent pK_a values. A total of 4 mg of 3-(propionamido)phenylboronic acid was dissolved in 30 mL of water with varying concentrations of glucose and titrated with 0.05 N NaOH at 37 °C after all boronic acid groups were nonionized by adding small amounts of 0.1 N HCl.

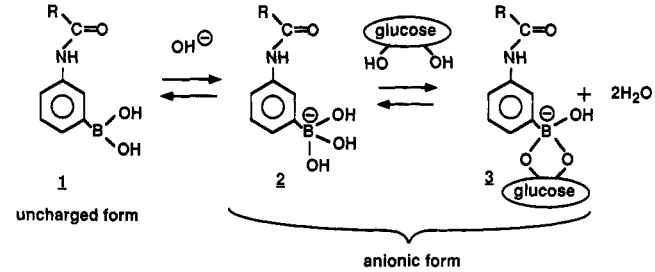


Figure 1. Equilibria of (alkylamido)phenylboronic acid (**1**) in an aqueous solution in the presence of glucose. R: CH_3CH_2– or $-(CH_2\underset{|}{C}H)-_n$.

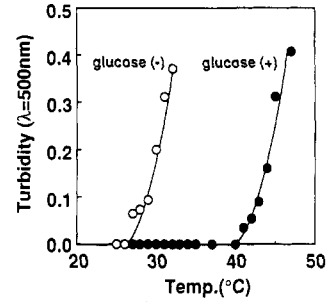


Figure 2. Change in LCST of DB-15 with glucose concentration in HEPES-buffered physiological saline (pH 7.4): (○) without glucose; (●) with glucose (16.7 g/L). Polymer concentration: 0.1 wt %.

Results and Discussion. As shown in Figure 2 (open circles), DB-15 has a LCST of around 27 °C in HEPES-buffered saline without glucose. Since no LCST was observed for the homopolymer of *N,N*-dimethylacrylamide in this temperature region, the phenylboronic acid moieties in DB-15 must play a crucial role in the appearance of LCST. On the basis of the pK_a value of a model compound of the APBA unit in DB-15, 3-(propionamido)phenylboronic acid (pK_a = 8.6), only 6% of the APBA units in DB-15 exist as the borate anion (**2** in Figure 1) at pH 7.4, with the rest of the APBA units present in the uncharged trigonal form (**1** in Figure 1). Uncharged APBA units are hydrophobic and induce phase separation from aqueous solution close to ambient temperature. Indeed, an increase in LCST is observed by raising the pH to increase the fraction of borate anion **2** in the polymer chain (data not shown).

There is a significant increase in LCST on the addition of small amounts of glucose to the solution, i.e., an approximately 15 °C increase in the presence of 16.7 g/L of glucose (closed circles in Figure 2). This change in turbidity was reversible as shown in Figure 3, where the turbid solution of DB-15 at pH 7.4 and 27 °C became clear on the addition of glucose (16.7 g/L).

This phenomenon of a glucose-induced change in the solubility can be explained as follows. As shown in Figure

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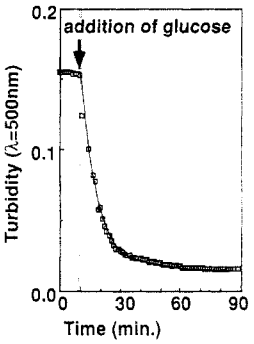

Figure 3. Recovery of transmittance of DB-15 solution by the addition of glucose (16.7 g/L) at 27 °C, LCST of DB-15 in HEPES-buffered saline at pH 7.4.

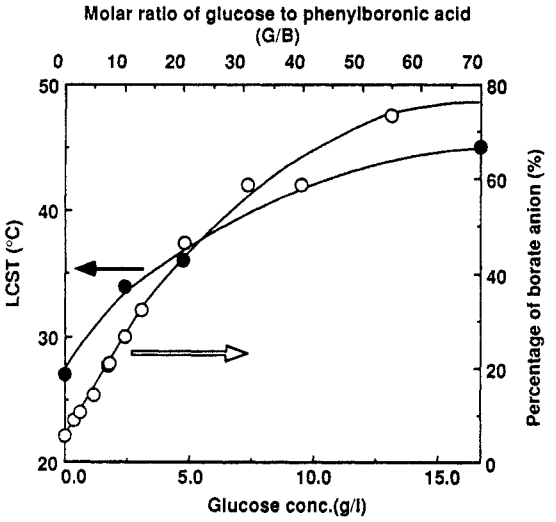

Figure 4. Glucose-dependent change in LCST of DB-15 (●) and percentage of borate form (2 + 3) in phenylboronic acid fractions (1 + 2 + 3) (○) at pH 7.4 in HEPES-buffered saline.

1, phenylborate (2) forms a considerably stable complex (3) with polyol compounds including glucose in aqueous solution.[9] Direct complexation of boronic acid in trigonal form (1) with a polyol compound is known to be unstable in water, and thus its contribution to the equilibrium can be neglected under physiological conditions.[10] Figure 1 shows that an increase in the concentration of glucose increases the concentration of the borate anions (2 + 3), decreasing the apparent pK_a. Consequently, the fraction of borate anions in DB-15 increases with increasing glucose concentration, increasing the hydrophilicity of the polymer, and raising the LCST. Indeed, the apparent pK_a of 3-(propionamido)phenylboronic acid decreases from 8.63 to 6.96 on addition of a 55-fold molar excess of glucose. An increased hydrophilicity of polymer chain due to the glucose complexation may also contribute to the increase of the LCST.

The change in LCST of DB-15 with glucose concentration is summarized in Figure 4 together with the percentage of borate anions (2 + 3) calculated from the apparent pK_a of 3-(propionamido)phenylboronic acid determined from acid–base titration in the presence of varying concentrations of glucose. A good correlation between LCST of DB-15 and the borate percentage is observed. The concept of utilizing phenylboronic acid groups as glucose-sensitive command moieties should be applicable to other LCST polymers and may find applications in various fields including drug delivery systems, sensor and actuator systems, and bioreactors. Introducing boronic acid groups into LCST gels may open a way to prepare totally synthetic gels showing volume phase transitions responding to glucose, which could be used for self-regulating insulin delivery systems.[5,11]

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References and Notes

(1) (a) Okano, T.; Bae, Y. H.; Kim, S. W. In *Pulsed and Self Regulated Drug Delivery*; Kost, J., Ed.; CRC Press: Boca Raton, FL, 1990; Chapter 2, p 17 and references therein. (b) Shild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163 and references therein. (c) Urry, D. W. *Prog. Biophys. Mol. Biol.* **1992**, *57*, 23 and references therein. (d) Okano, T.; Yoshida, R. In *Biomedical Applications of Polymeric Materials*; Tsuruta, T., Hayashi, T., Ishihara, K., Kataoka, K., Kimura, Y., Eds.; CRC Press; Boca Raton, FL, 1993; p 407 and references therein.
(2) Irie, M. *Adv. Polym. Sci.* **1990**, *62*, 1495 and references therein.
(3) (a) Kwon, I. C.; Bae, Y. H.; Kim, S. W. *Nature* **1991**, *354*, 291. (b) Osada, Y.; Okazaki, H.; Hon, H. *Nature* **1992**, *355*, 242.
(4) (a) Tanaka, T. In *Encyclopedia of Polymer Science and Engineering*; Mark, H., Bikales, N. M., Overberger, C. G., Menges, G., Kroschwitz, J. I., Eds.: Wiley-Interscience: New York, 1987; Vol. 7, p 514 and references therein. (b) Dong, L.-C.; Hoffman, A. S. *J. Controlled Release* **1991**, *15*, 141.
(5) (a) Kitano, S.; Kataoka, K.; Koyama, Y.; Okano, T.; Sakurai, Y. *Makromol. Chem., Rapid Commun.* **1991**, *12*, 227. (b) Kitano, S.; Koyama, Y.; Kataoka, K.; Okano, T.; Sakurai, Y. *J. Controlled Release* **1992**, *19*, 162.
(6) Miyazaki, H.; Kikuchi, A.; Koyama, Y.; Okano, T.; Sakurai, Y.; Kataoka, K. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 829.
(7) Miyazaki, H.; Kikuchi, A.; Kitano, S.; Kataoka, K.; Koyama, Y.; Okano, T.; Sakurai, Y. *Proceedings of the First International Conference on Intelligent Materials*; Takagi, T., Takahashi, K., Aizawa, M., Miyata, S., Eds.; Technomic: Lancaster, PA, 1993; p 481.
(8) HEPES: *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid.
(9) (a) Böeseken, J. *Adv. Carbohydr. Chem.* **1949**, *47*, 204. (b) Aronoff, S.; Chen, T.; Cheveldayoff, M. *Carbohydr. Res.* **1975**, *40*, 299.
(10) (a) Lorand, J. P.; Edwards, J. D. *J. Org. Chem.* **1959**, *24*, 769. (b) Yoon, J. D.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.
(11) (a) Kim, S. W.; Pai, C. M.; Makino, K.; Seminoff, L. A.; Holmberg, D. L.; Gleeson, J. M.; Wilson, D. E.; Mack, E. J. *J. Controlled Release* **1990**, *11*, 193. (b) Kokufuta, E.; Tanaka, T. *Macromolecules* **1991**, *24*, 1605. (c) Kokufuta, E.; Zhang, Y.-Q.; Tanaka, T. *Nature* **1991**, *351*, 302.