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Glucose-Sensitive Lower Critical Solution Temperature Changes of Copolymers Composed of *N*-Isopropylacrylamide and Phenylboronic Acid Moieties

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Glucose concentration in blood is well maintained at approximately 5 mM *via* secretion of insulin from Langerhans islets of pancreas. Deterioration of islet function results in diabetes. For treatment of diabetes mellitus, monitoring and accurate control of blood glucose level is required. To date, various systems for monitoring glucose concentrations have been proposed based on electrochemical[1-3] or spectroscopic[4-7] methods. The sensors should transduce the amount of glucose into different signals such as electric current, optical absorbance and fluorescence intensity. These conversions should be large with high sensitivity and fast response.

Poly(*N*-isopropylacrylamide) (PIPAAm) is a water-soluble polymer having a lower critical solution temperature (LCST) at 32°C due to the phase transition behavior between dehydration and hydration states of the polymer side chains.[8] As the dehydration/hydration changes trigger drastic and rapid solubility change of PIPAAm in response to temperature changes, PIPAAm molecule can be considered as a "molecular switch" operated rapidly by the temperature changes. Thus, the structural changes of PIPAAm chains induce functional changes which open new modulation systems for biomedical fields.[9-14]

It is known that phenylboronic acid groups in tetrahedral form bind to diol compounds such as glucose in aqueous media.[15] In addition, the incorporation of amino groups in the copolymer having phenylboronic acid groups promotes complexation between phenylboronic acid groups and glucose under physiological pH conditions.[16] Hence, it is anticipated that a ternary copolymer composed of 3-acrylamidophenylboronic acid (PBA), *N*-(3-dimethylaminopropyl)acrylamide (DMAPAA), and *N*-isopropylacrylamide (IPAAm) forms a stable complex with glucose at pH 7.4, converting the changes of glucose concentrations into hydrophilic/hydrophobic property changes of macromolecular chains. As a consequence, the ternary copolymer is regarded as a "macromolecular glucose sensor". In fact, a binary copolymer composed of *N*,*N*-dimethylacrylamide and PBA exhibiting LCST near 30°C showed LCST shifts to higher temperature with increasing glucose concentration in aqueous solution.[17] This LCST change is explained by increasing hydrophilicity of polymer side chains due to complexation of phenylboronic acid with glucose, stabilizing boronate anions.

This paper describes glucose-induced LCST changes of the IPAAm copolymer having glucose-binding phenylboronic acid moieties. Opposite and larger LCST shifts of the ternary copolymer responding to glucose concentrations in aqueous solution were observed at pH 7.4 when compared with LCST changes of the binary copolymer comprising IPAAm and PBA.

EXPERIMENTAL

Materials

N-Isopropylacrylamide (IPAAm) and 3-acrylamidophenylboronic acid (PBA) were supplied from Kohjin Co., Ltd. and NOF Corp., respectively. *N*-(3-dimethylaminopropyl)acrylamide (DMAPAA) was purchased from Tokyo Chemical Industry Co., Ltd. Other reagents were purchased from Wako Pure Chemical Industries, Co., Ltd. PBA and 2,2′-azobis(isobutyronitrile) (AIBN) were purified by recrystallization from hot water and methanol, respectively. IPAAm was purified by precipitation from toluene to petroleum ether. DMAPAA (bp 135°C at 7 mmHg) and *N*,*N*-dimethylformamide (DMF) (bp 80°C at 39 mmHg) were distilled before use. Other reagents were used as received.

Preparation of Polymers

Abbreviations for polymers used in this work are as follows: poly(IPAAm), PIPAAm; poly(IPAAm-*co*-PBA) binary copolymer, IB; poly(IPAAm-*co*-PBA-*co*-DMAPAA) ternary copolymer, IAB. The structural formulae of the copolymers are shown in Figure 1. These polymers

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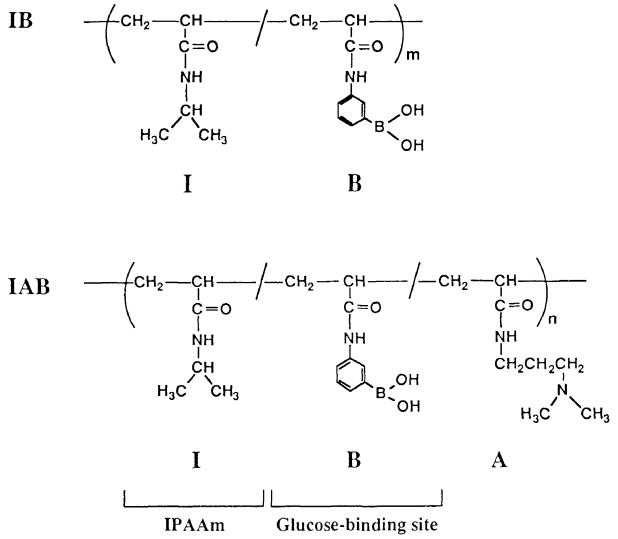


Figure 1. Structural formulae of copolymers.

were each synthesized in 100 cm^3 DMF at 70°C for 2 h using 0.13 mM of AIBN as a free radical initiator. IB was synthesized using 5 wt% PBA in the feed, and IAB with 5 wt% DMAPAA, and 5 wt% PBA in the feed, respectively. The polymers were recovered as precipitates from diethyl ether. Copolymer composition was calculated from amine and boron contents determined by non-aqueous acid-base titration using 0.1 M $HClO_4$/CH_3COOH solution and atomic absorption spectroscopy, respectively. Boron content in IB copolymer is 2.42 mol% and boron and amine contents in IAB copolymer were 2.20 mol% and 4.41 mol%, respectively.

Transmittance Measurements

Polymers were dissolved in 0.1 M phosphate buffered (PB) solutions (pH 7.4; Na_2HPO_4; 0.081 mol l^{-1}, NaH_2PO_4; 0.019 mol l^{-1}) at 1 w/v%. Optical transmittance of the polymer aqueous solutions at various temperatures was monitored at 500 nm by means of a spectrophotometer (JASCO, Ubest 30 LCD). The quartz cell was thermostated with a circular water jacket equipped with a temperature controller (JASCO, EHC-441). LCST was defined as the temperature at 50% optical transmittance.

RESULTS AND DISCUSSION

Transmittance changes for IB binary and IAB ternary copolymers in 0.1 M PB aqueous solutions (pH 7.4) are shown in Figure 2. The copolymers were water-soluble and showed characteristic LCSTs. The IAB ternary copolymer exhibited LCST near 32°C and IB binary copolymer demonstrated lower LCST of approximately 18°C. As described previously,[12,18] LCSTs of PIPAAm chains are affected by hydrophobic or hydrophilic nature of comonomers. LCSTs for IPAAm oligomers can be controlled to decrease below 32°C by incorporating *n*-butyl methacrylate as a hydrophobic comonomer and increase above 32°C with increasing composition of *N*,*N*-dimethylacrylamide in the copolymer as a hydrophilic segment.[12] Since a phenylboronic acid has pK_a of 8.6,[17] only 6% of phenylboronic acid moieties exists in tetrahedral anionic form at pH 7.4 and most of these

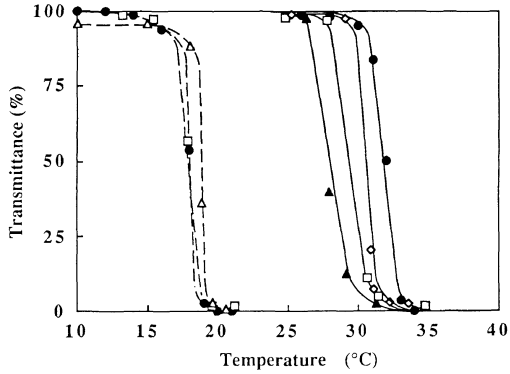
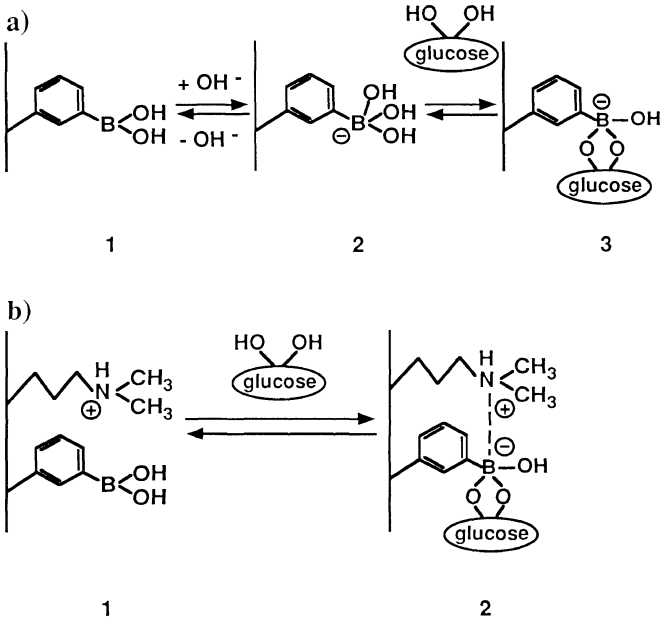


Figure 2. Temperature dependence of optical transmittance for IB (dotted line) and IAB (solid line) copolymers in 100 mmol l^{-1} phosphate buffered solutions (pH 7.4) in the absence and presence of glucose. Glucose concentration: 0 mg ml^{-1}; (●); 1 mg ml^{-1}; (◇); 2 mg ml^{-1}; (□); 4 mg ml^{-1}; (▲); 10 mg ml^{-1}; (△).



Scheme 1. Schematic views of the equilibrium between phenylboronic acid moiety and glucose in aqueous media. (a) without amino groups; (b) with amino groups.

groups exhibited hydrophobicity. Therefore, the IB copolymer showed lower LCST near 18°C. As amino groups in IAB copolymer are protonated under physiological pH conditions (pH 7.4), the protonated amino groups serve as hydrophilic components. A binary copolymer composed of IPAAm and DMAPAA showed higher LCST of around 35°C in water (data not shown). The hydrophobic effects of phenylboronic acid moieties were compensated for by incorporating the hydrophilic comonomer, DMAPAA, in the copolymer and as a consequent, IAB copolymer showed higher LCST than IB copolymer.

The phenylboronic acid in tetrahedral anionic form binds to glucose, shifting equilibrium to right (Scheme 1a-3). Glucose addition may have influence on LCSTs for IB and IAB copolymer aqueous solutions, since these copolymers contain both thermo-responsive IPAAm units and glucose-binding phenylboronic acid moieties. The influence of glucose concentrations in PB solutions (pH 7.4) on the phase transition behavior of IB and IAB

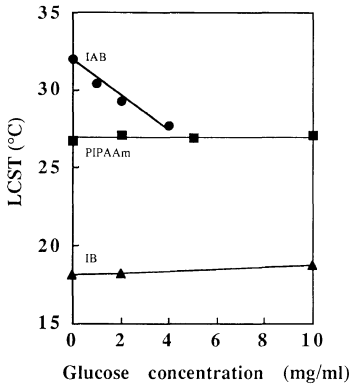


Figure 3. Relationship between glucose concentrations in aqueous media and LCST of PIPAAm (■), IAB (●), and IB (▲) copolymers in phosphate buffered solution at pH 7.4. LCST is defined as the temperature at 50 % optical transmittance.

copolymers was investigated. Figure 2 also shows temperature-dependent optical transmittance changes for both copolymers in PB solutions. Transmittance of the copolymer solutions was almost 100% at lower temperatures and 0% at higher temperatures regardless of glucose addition. The transition of IAB copolymer responding to glucose concentrations was quite different from that of IB copolymer. While the transition temperature from hydration to dehydration states of IB copolymer slightly increased with increasing glucose concentrations, that for IAB copolymer apparently decreased.

Figure 3 summarizes LCST changes for PIPAAm, IB, and IAB copolymers in the buffered solutions as a function of glucose concentration. PIPAAm exhibited LCST of approximately 27°C in PB solution and no influence of glucose concentration on LCST for PIPAAm aqueous solutions was observed. For the IB copolymer, LCST slightly increased from 18.1°C to 18.7°C at glucose concentration of 0 mg ml^{-1} and 10 mg ml^{-1}, respectively. In contrast, IAB copolymer exhibited relatively large decrease in LCST by the addition of a small amount of glucose in the medium. An approximately 4°C decrement was seen in the presence of 4 mg ml^{-1} glucose. The boron content in IB and IAB copolymers was 2.4 mol% and 2.2 mol%, respectively, indicating that both copolymers contain similar amounts of boron. Nonetheless, IAB showed opposite and large LCST changes compared to LCST changes of IB copolymer. These glucose-induced LCST changes of the polymers can be explained as follows: a phenylboronic acid group exists in equilibrium between the uncharged trigonal form (Scheme 1a-1) and tetrahedral anionic form (Scheme 1a-2) with pK_a of 8.6.[17] The phenylboronic acid group in the tetrahedral anionic form can reversibly interact with diol compounds to form stable complexes in aqueous solution (Scheme 1a-3).[15] As previously reported,[17] glucose addition decreases apparent pK_a of phenylboronic acid to increase LCST of the binary copolymer, poly(*N*,*N*-dimethylacrylamide-*co*-PBA), due to stabilization of phenylboronate anion (Scheme 1a-2,3). This means that glucose addition results in increased anionic phenylboronate content, and thus, increased hydrophilicity of the binary copolymer. Although poly(*N*,*N*-dimethylacrylamide) also shows a negative heat of dilution,[19] the polymer chain is in sufficiently hydrated state below the water boiling point, judging from the equilibrium swelling of poly(*N*,*N*-dimethylacrylamide) hydrogel in water as a function of temperature.[20] Phenylboronic acid groups in hydrated polymeric chains can interact easily with glucose in the medium, which results in decreased apparent pK_a with increasing the glucose concentration. PIPAAm in distilled water demonstrates the drastic phase transition behavior at 32°C and thus isopropyl groups of the polymer side chain work as hydrophobic groups. Therefore, it is considered that cooperative hydrophobic effects of isopropyl and phenylboronic acid groups in the IB copolymer enhance hydrophobic environment around phenylboronic acid groups in aqueous milieu, suppressing decrease in apparent pK_a value of phenylbornonic acid groups in the copolymer. Urry reports that increasing hydrophobicity of a polymer chain results in increase in pK_a of dissociating groups.[21] Therefore, it is plausible that less phenylboronic acid content in the IB copolymer transformed into phenylboronate anions by the addition of glucose. This may result in a small increase in LCST of the IB copolymer with increasing glucose concentration.

Despite the low content of boron unit in IAB copolymer, LCST of IAB aqueous solution significantly shifted to lower temperature with increasing glucose concentration. The incorporation of amino groups in the vicinity of phenylboronic acid moieties increases microenvironmental pH, stabilizing phenylboronate in tetrahedral anionic form, which should enhance the hydrophilicity of polymer chains. Therefore, complexation of phenylboronate with glucose results in a shift to right in the equilibrium in Scheme 1b-2. Moreover, the amounts of amino groups in the ternary copolymer was twice boron content and some amino groups exist in protonated form at physiological pH. These protonated amino groups counteract with complex-formed phenylboronate anions in the copolymer. The neutralization of charge might reduce net hydrophilicity of copolymer, demonstrating the lower LCST of terpolymer by glucose addition. It is considered that amino groups not only promote complexation between boronic acid groups and glucose at pH 7.4 but also neutralize boronate anions formed by complexation.

In conclusion, a glucose-sensitive ternary polymer was obtained by copolymerizing IPAAm, PBA, and DMAPAA. This copolymer showed the lowering LCST in response to increasing glucose concentration. LCSTs of the copolymer can be controlled easily by change of external glucose concentration and thus IAB copolymer is regarded as a "molecular switch" which equips both sensor function to glucose and actuator function in aqueous milieu. Hydrogels consisting of these comonomers might exhibit reversible swelling/deswelling behavior at physiological pH in response to glucose, which will be applied to glucose-responsive insulin release as well as chemo-mechanical actuation.

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