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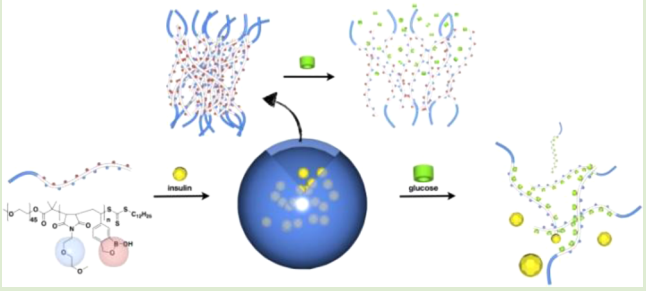
Glucose-Responsive Disassembly of Polymersomes of Sequence-Specific Boroxole-Containing Block Copolymers under Physiologically Relevant Conditions

Hyunkyu Kim, Young Ji Kang, Eun Sun Jeong, Sebyung Kang, and Kyoung Taek Kim*

School of Nano-Bioscience and Chemical Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST Road, Ulsan 698-798, Korea

Supporting Information

ABSTRACT: Polymers containing organoboronic acids have recently gained interest as sugar-responsive materials owing to the reversible binding of saccharides to boronic acids, which triggers a change in the physical and chemical properties of these polymers, such as their water solubility. In particular, the ability of these polymers to bind glucose has attracted considerable attention because of the promise of these materials for the development of sensors and drug delivery systems for glucose-related human diseases, such as diabetes. We report here a new class of sugar-responsive polymers that are based on a sequence-specific copolymer of styreneboroxole and *N*-functionalized maleimide. The reversible addition−fragmentation and chain transfer (RAFT) polymerization of this pair of monomers ensured that a glucose receptor alternates with a nonresponsive solubilizing group throughout the sugar-responsive polymer chain. Due to the presence of hydrophilic solubilizing groups beween the solubility-switching boroxole moieties in the membrane-forming block, the polymersomes of the block copolymers responded to a lower level of glucose in the medium, resulting in diassembly of the bilayer membrane under a physiologically relevant pH and glucose level.



Polymers containing organoboronic acids recognize monosaccharides and saccharides by way of the reversible covalent bond formation between boronic acid and 1,2- and 1,3-diol compounds.[1] In particular, the ability of these polymers to bind glucose has attracted considerable attention because of the promise these materials hold for the development of sensors and drug delivery systems in the treatment of glucose-related human diseases such as diabetes.[2] Controlled radical polymerization of boronic acid-containing vinyl monomers has greatly improved the synthesis of sugar-responsive block copolymers with a well-defined architecture and molecular weight.[3] These block copolymers self-assemble into polymeric nanostructures, such as micelles and vesicles,[4,5] which, along with self-regulating hydrogels containing boronic acids as glucose receptors,[6] exhibit great potential as drug delivery vehicles that can regulate drug release in accordance with the glucose level in the medium.

Of our particular interest is the use of polymer vesicles (polymersomes)[7] formed by self-assembly of sugar-responsive block copolymers as an insulin delivery vehicle. Insulin encapsulated within the water-filled inner compartment of such vesicles can be released via sugar-responsive disassembly of polymersomes only when monosaccharides abound in the surrounding solution. We recently synthesized a new class of monosaccharide-responsive polymers constructed by the reversible addition−fragmentation and chain transfer (RAFT) polymerization of styrene-boroxole.[8] Benzoboroxole (*o*-hydroxymethylphenylboronic acid) is capable of binding with six-membered ring sugars (pyranosides) in aqueous solution at a neutral pH, such as the major isomer of glucose in water.[9] We demonstrated that these well-defined benzoboroxole-containing block copolymers self-assembled into polymersomes, which showed monosaccharide-triggered disassembly of the bilayer membrane, resulting in release of the encapsulated insulin in phosphate buffer at a neutral pH. Despite the enhanced binding of benzoboroxole to glucose at a neutral pH, however, the polymersomes made of boroxole-containing block copolymers only exhibited disassembly in the presence of a high concentration of glucose (>0.3 M).[8] This requirement of a high concentration of glucose, an order of magnitude higher than the concentration of glucose typically found in hyperglycemia (11−20 mM), prevented further investigation of these polymersomes as a potential candidate for smart delivery vehicles under physiological conditions.

In this letter, we report a new class of sugar-responsive block polymers that are based on a sequence-specific copolymer of styreneboroxole and *N*-functionalized maleimide. The RAFT copolymerization of this pair of monomers in the presence of a poly(ethylene glycol)-chain transfer agent ensured that, in the resulting stimuli-responsive block polymer, a glucose receptor

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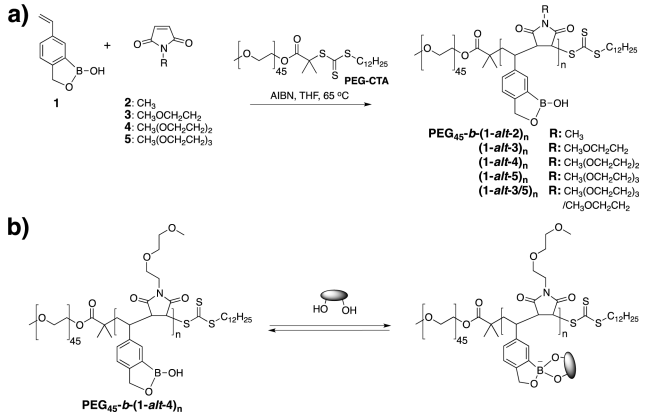
boroxole alternates with a nonresponsive solubilizing group throughout the sugar-responsive polymer block. Due to the presence of hydrophilic solubilizing groups between the solubility-switching boroxole moieties in the membrane-forming block,[13] the polymersomes of the resulting block copolymers exhibited disassembly at a lower glucose level, close to the physiologically relevant concentration under neutral pH conditions. Given their low toxicity to cells and compatibility with serum, the polymersomes of our block copolymers may find application as smart insulin delivery vehicles of which the release behavior is regulated by the glucose level in the surrounding medium.

To implement solubilizing groups in the sugar-responsive backbone in a controllable manner we adopted an alternating copolymerization of **1** and maleimide **2**−**5** with an *N*-functional group of varying degrees of hydrophilicity (Scheme 1). (For full synthetic details and characterization, see Supporting Information.) The RAFT polymerization of these pairs of monomers with PEG-CTA (M_n(PEG) = 2000 g/mol) yielded block copolymers in which the stimuli-responsive block was composed of an alternating sequence of **1** and maleimide **2**−**5**.[10,11] ^{1}H NMR analysis of the resulting block copolymers PEG$_{45}$-*b*-(1-*alt*-4)$_n$ indicated equimolar incorporation of **1** and **4** into the stimuli-responsive block, suggesting a strong tendency for alternating polymerization of these monomers (Figure 1 and Figure S1, Supporting Information). The controlled nature of the RAFT polymerization of **1** and **4** was also observed by quenching the polymerization at different reaction times, resulting in a gradual increase in the DP$_n$ of **1** and **4** (Figure 1) over the reaction time. Gel permeation chromatography (GPC) experiments of the block copolymer samples after purification showed a unimodal peak without any trace of a residual PEG-CTA. The molecular weight determined by GPC was compared to the value calculated from ^{1}H NMR integration, which showed a consistent overestimation, as we previously observed with GPC results of homopolymers of **1** and their block copolymers[8] (Table S1, Supporting Information).

All synthesized block copolymers self-assembled to polymersomes when the chain length of a sugar-responsive block was appropriately controlled by virtue of the controlled nature of polymerization. For example, a series of block copolymers PEG$_{45}$-*b*-(1-*alt*-2)$_n$ exhibited a morphological transition from spherical/cylindrical micelles to polymersomes as the DP$_n$ of **1** and **2** in the sugar-responsive block increases from 30 to 50. The block copolymers prepared by the alternating copolymerization of **1** and maleimide having a hydrophilic oligo(ethylene glycol) unit as a *N*-functional group (methoxyethyl for **3**, methoxyethoxyethyl for **4**, and methoxyethoxyethoxyethyl for **5**) exhibited a similar morphological transition from micelles to polymersomes with increasing DP$_n$ of a sugar-responsive block (Figure 2a and b, Figure S3 and S4, Supporting Information). By observing the morphology of the self-assembled structures on transmission electron microscopy (TEM) and dynamic light scattering (DLS), we identified the optimal DP$_n$ of **1** and maleimide for the formation of polymersomes upon self-assembly for all series of block copolymers (Figure S4 and S5, Supporting Information). The encapsulation of water-soluble molecules within the polymersomes was demonstrated using fluorescein-labeled insulin (F-insulin) as a guest. The polymersomes of PEG$_{45}$-*b*-(1-*alt*-4)$_{45}$ encapsulating F-insulin were visualized using confocal laser fluorescence microscopy (CLFM), which confirmed that the fluorescent guest molecules were well-contained within the polymersomes (Figure 2c and d). The purified polymersomes of PEG$_{45}$-*b*-(1-*alt*-4)$_{45}$ encapsulating F-insulin were stable for a period of 4 weeks at ambient temperature, as judged based on DLS and TEM observations.

The sugar-responsive disassembly of the polymersomes made of block copolymers was studied by measuring the turbidity of the polymersome solution in the presence of glucose in solution by using a UV−vis spectrometer. We examined the effect of the solubilizing group positioned between boroxole

Scheme 1. (a) Synthesis of Sequence-Specific Block Copolymers of Styreneboroxole and *N*-Functionalized Maleimide by the RAFT Polymerization and (b) A Schematic Representation of Sugar-Responsive Behavior of Block Copolymers in Water



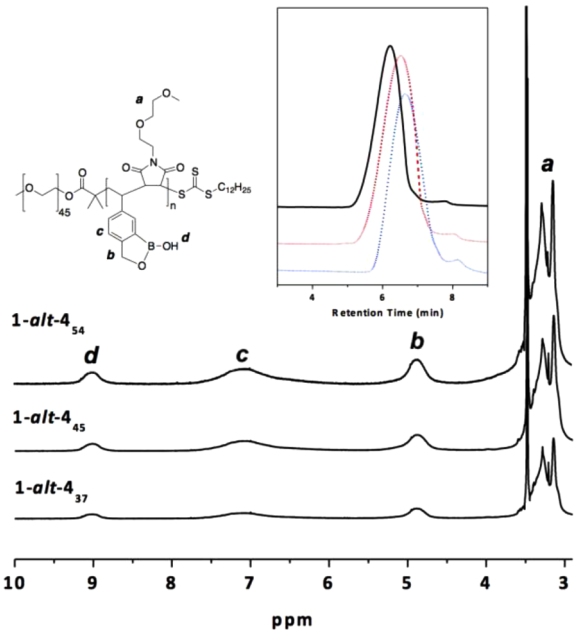


Figure 1. ^{1}H NMR spectra of a series of block copolymers PEG$_{45}$-*b*-(1-*alt*-4)$_n$ where the subscript *n* denotes the calculated degree of polymerization from the comparison of the integration of *a* and *b* by assuming the value of PEG (M_n = 2000 g/mol, DP$_n$ = 45) signal (3.45 ppm). The inset shows GPC traces of purified PEG$_{45}$-*b*-(1-*alt*-4)$_n$ block copolymers (*n* = 37 (dotted line), 45 (dashed line), 54 (solid line)). All block copolymer samples were purified by precipitation before analysis.

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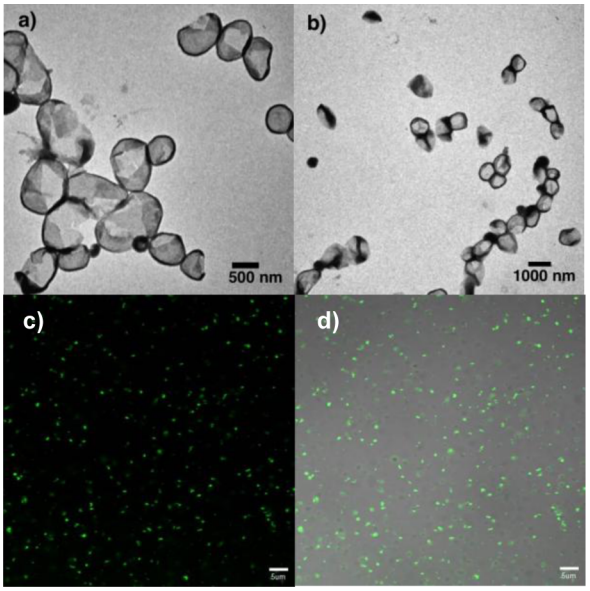

Figure 2. TEM images of polymersomes of (a) PEG_{45}-*b*-(1-*alt*-4)$_{45}$ and (b) PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ (average diameter of polymersomes of PEG_{45}-*b*-(1-*alt*-4)$_{45}$: 484 nm, PDI 0.207; and PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$: 657 nm, PDI 0.153). Confocal laser fluorescence microscopy images of polymersomes of PEG_{45}-*b*-(1-*alt*-4)$_{45}$ encapsulating fluorescein-labeled insulin. (c) A dark-field image and (d) a merged image with a bright-field image. Scale bars represent 5 μm.

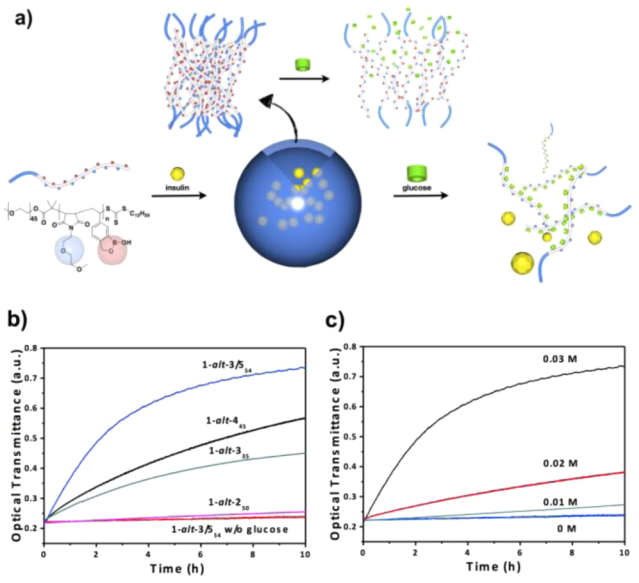

Figure 3. (a) Schematic illustration of glucose-triggered disassembly of polymersomes of sugar-responsive block copolymers. (b) Optical transmittance change of polymersome solutions of block copolymers in the presence of 0.03 M glucose (phosphate buffer, pH 7.6, 36 °C). (c) Optical transmittance change of the polymersome solutions of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ at different concentrations of glucose (PBS, pH 7.6, 36 °C).

units in the sugar-responsive block by changing the solubilizing group from the least hydrophilic (methyl for PEG_{45}-*b*-(1-*alt*-2)$_{40}$) to the most hydrophilic group (methoxytethoxyethoxyethyl for PEG_{45}-*b*-(1-*alt*-5)$_{65}$). In all cases, no change in optical transmittance was observed for more than 48 h at 36 °C in the abscence of glucose in the solution, with the exception of the polymersomes of PEG_{45}-*b*-(1-*alt*-5)$_{65}$, which disassembled completely by 24 h in phosphate buffer lacking glucose. As shown in Figure 3b, at 0.03 M glucose in buffer (pH 7.6), the polymersome solution of PEG_{45}-*b*-(1-*alt*-2)$_{50}$ exhibited virtually no disassembly after 24 h, indicating that the hydrophobic methyl group on maleimide had little effect on increasing the glucose responsiveness and consequential disassembly of the polymersomes. In contrast, when the solubilizing group was switched to more hydrophilic oligo(ethylene glycol) groups, disassembly of the polymersome solutions of the corresponding block copolymers became noticeably faster. For comparison, the response time (t_R) was defined as the time required for reaching 50% transmittance at 580 nm at the given glucose concentration. As the number of ethylene glycol units increased, the t_R decreased from 24.1 h for PEG_{45}-*b*-(1-*alt*-3)$_{45}$ to 6.94 h for PEG_{45}-*b*-(1-*alt*-4)$_{45}$.

The most marked effect was observed with the polymersome solution of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$, the block copolymer that was synthesized using an equimolar mixture of **3** and **5** as a maleimide in pair with **1** to prevent complete dissolution of the block copolymer in buffer without glucose. The polymersome solution of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ showed a rapid increase of transmittance at 580 nm at a concentration of 0.03 M glucose (t_R = 2.15 h at 36 °C), indicating the enhanced binding between boroxole groups in the sugar-responsive block and glucose (Figure 3b). Compared to the glucose-triggered disassembly of polymersomes of amphiphilic block copolymers containing a homopolymer of **1** as a sugar-responsive block (1.84 h at 0.5 M glucose in phosphate buffer at pH 7.4),[8] the polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ exhibited disassembly at a concentration of glucose an order of magnitude lower than that at which the block copolymers with a sugar-responsive block consisting of homopolymer of **1** showed a glucose-responsive behavior. The turbidity test of the polymersome solution of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ revealed that the polymersomes respond to a glucose concentration as low as 0.01 M in phosphate buffer (pH 7.6) (Figure 3c). The polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ also responded to fructose and non-reducing sugars (Figure S6, Supporting Information). As suggested by the large K_a value of the block copolymers to fructose (Table S1, Supporting Information), the polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ disassembled rapidly (t_R = 0.06 h) upon addition of 0.01 M fructose in buffer.

Considering that the measured K_a value of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ to glucose[12] (13.2 M^{-1}; for details, see Table S1 and Supporting Information) was nearly identical to the value reported for benzoboroxole and the homopolymer of **1**,[8] we suspected that this enhanced disassembly of polymersomes arose from the alternating arrangement of a glucose receptor and a hydrophilic solubilizing group in the glucose-responsive block. The binding of glucose to boroxole groups surrounded by hydrophilic solubilizing groups may unwind the polymer chains by imparting additional hydrophilicity to the sugar-responsive block. This, in turn, would promote further diffusion of glucose through the bilayer membrane and essentially induce disassembly of the membrane into individual block polymers (Figure 3a).

To assess the feasibility of using these polymersomes as drug delivery vehicles that can respond to glucose levels under physiologically relevant conditions, we performed the turbidity test using rabbit serum (Sigma). Without glucose in serum, the polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ showed excellent stability for 24 h at 36 °C; no indications of aggregation or disassembly of the polymersomes were found in the turbidity

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test and DLS experiments (Figure 4a). In the presence of 0.03 M glucose, in contrast, disassembly of the polymersome was

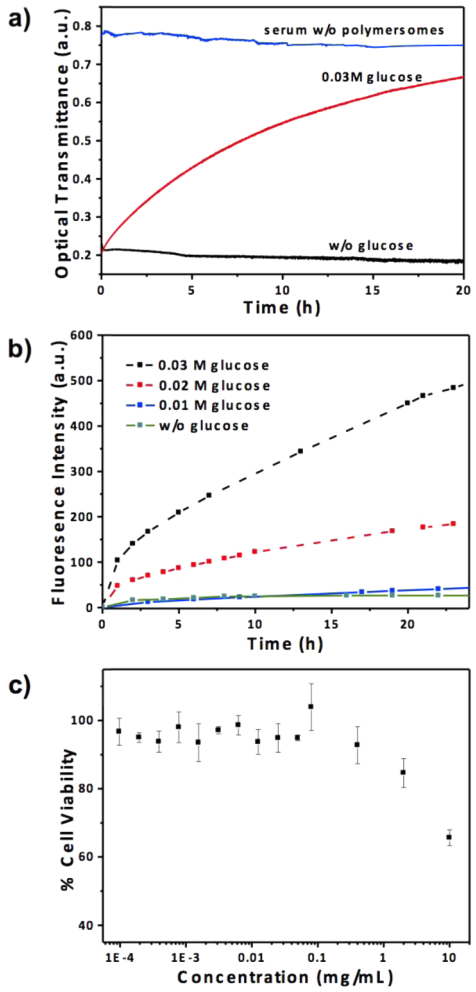

Figure 4. (a) Disassembly of the polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ in serum (rabbit serum, 36 °C) in the presence of 0.03 M glucose. (b) Release profiles of insulin from the polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ at different concentrations of glucose in phosphate buffer (pH 7.4). (c) Results of the cytotoxicity tests of solutions of PEG_{45}-*b*-(1-*alt*-5)$_{65}$ in HeLa cells after 18 h incubation. N = 3, mean ± SD.

observed at a reduced rate (t_R = 7.71 h) compared to the glucose-responsive disassembly in buffer. The release of encapsulated guest F-insulin from the polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ was also investigated at different glucose concentrations in buffer (pH 7.4) (Figure 4b). The insulin-encapsulating polymersomes of PEG_{45}-*b*-(1-*alt*-3/5)$_{54}$ were contained in a semipermeable membrane (MW cutoff 12 kDa), which was dialyzed against buffer containing the desired concentration of glucose for 24 h. As suggested by the results of the turbidity tests, encapsulated insulin was released from the polymersomes via glucose-triggered disassembly. The rate of release was proportional to the concentration of glucose in the medium as shown in Figure 4b. We also assessed the cytotoxicity of the block copolymer by using PEG_{45}-*b*-(1-*alt*-5)$_{65}$ as a model copolymer due to its solubility in the medium lacking monosaccharides. HeLa cells were incubated with an aqueous solution of the block copolymer at varying concentrations for 18 h. Higher than 90% cell viability was observed with up to 0.3 mg/mL of PEG_{45}-*b*-(1-*alt*-5)$_{65}$, suggesting that the block copolymer has low cytotoxicity (Figure 4c).

In summary, we synthesized a new type of glucose-responsive polymers possessing a sequence-specific arrangement of styreneboroxole and *N*-functionalized maleimide by using alternating copolymerization of these monomers under the RAFT condition. Introduction of hydrophilic solubilizing groups, such as oligo(ethylene glycol) at positions adjacent to glucose-binding boroxole groups in the polymer chain, caused the resulting sequence-specific polymer to demonstrate a glucose-responsive solubility change in water at a reduced concentration of glucose (~0.02 M) in aqueous solution at neutral pH. Amphiphilic block copolymers constructed using this sequence-specific sugar-responsive polymer block self-assembled into polymersomes in water, which could encapsulate water-soluble molecules, such as fluorescein-labeled insulin, within their inner compartment. The encapsulated insulin was released only when glucose was present in the medium such as neutral pH buffer and serum, via the glucose-triggered disassembly of the polymersomes. Given their low cytotoxicity, serum compatibility, and sugar-reponsive behavior at a glucose level close to physiologically relevant conditions, our block copolymers and polymersomes may find application, for example, as smart delivery vehicles for glucose-related diseases such as diabetes.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and descriptions of the synthesis and characterizations of block copoymers; additional TEM images; and procedures for Wang's competitive binding assay. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ktkim@unist.ac.kr.

Notes

The authors declare no competing financial interest.

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