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pH and Sugar Responsive Host Polymer Hydrogels Designed Based on Sugar and Boronic Acid Interaction

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By mixing boronic acid-modified poly(acrylic acid) (pAA-BA) and β-1,3-glucan Schizophyllan (SPG) in water at pH 9.9, hydrogel was readily formed with the aid of the sugar and boronic acid interaction. This hydrogel showed the reversible transformation between sol and gel depending on the medium pH. Moreover, this hydrogel stability was sensitive to fructose concentration. As one potential output, we prepared the hydrogel including single-walled carbon nanotubes, utilizing the nanotube-wrapping property inherent to SPG.

So far, much attention has been paid to various functions created by high-water-content materials, especially hydrogels because of their low environmental burden and high biocompatibility.[1] Today, hydrogels are expected to be applicable even to the medical field, such as drug delivery, by encapsulating the drugs into their network structures.

To use hydrogel as a practical drug carrier, some contrivance for stimuli responsiveness is indispensable because the drug should be released after reaching the objective place in a controlled manner. Nevertheless, reports on such stimuli-responsive hydrogels[2] have been limited in the bioinspired fields such as sensor systems, artificial muscles, and self-healing materials.

Now, carbon nanotubes have emerged as new materials useful to design such functions.[3] However, their strong aggregation property and poor solvent solubility cause many troubles, which have made their manipulation very difficult. Therefore, many researchers have devoted their efforts to carbon nanotubes soluble discretely into solvents.[4] For example, some helical polymers such as DNA and amylose are able to solubilize single-walled carbon nanotube (SWCNT) into water by wrapping SWCNT into their helical frameworks.[5,6] However, it is difficult to modify the outside surface of SWCNT/DNA or SWCNT/amylose composites to acquire some desired functions. Schizophyllan (SPG) is one kind of β-1,3-glucan polysaccharide produced by the fungus *Shizophyllum commune*. The repeating unit of SPG consists of three β-1,3-glucose units and one β-1,6-glucose side chain every three main chain glucose units as shown in Figure 1. SPG forms a triple-stranded helical assembly in nature, which can be dissociated into a single chain in dimethyl sulfoxide (DMSO). It is possible to regenerate the SPG triple-stranded helix by exchanging the solvent from DMSO to water. Previously, we demonstrated that SPG entraps various kinds of hydrophobic monomers and polymers,[7] including SWCNT,[8] into the one-dimensional cavity constructed in its helical structure and discretely solubilizes them into water.

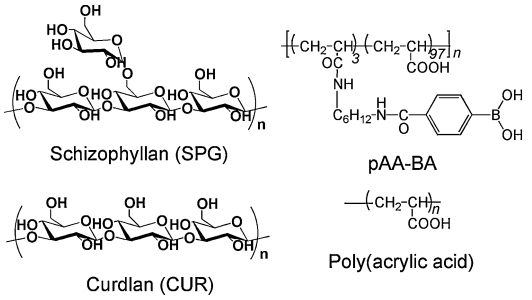

Figure 1. Structures of the chemicals used in this research.

Moreover, it is possible to modify the outside surface of those composites through periodate oxidation of the glucose side chain. For instance, β-lactoside-modified SPG thus synthesized can be used as a DNA carrier which conveys the DNA selectively to liver.[9] Herein, we report molecular design of a new pH-responsive hydrogel based on the interaction between the glucose side chain and the polymer-appended boronic acid, which is expected to undergo the sol–gel phase transition depending on the physiological pH change and to be useful to prepare a new SWCNT hydrogel.

It is well-known that 4- and 6-hydroxy groups in glucose reversibly form covalent complexes with boronic acids at from neutral to basic pH conditions.[10] Taking advantage of this, we recently demonstrated that SWCNT wrapped in the helix of SPG can be aligned into one direction with the aid of the side chain glucose/boronic acid interaction.[11] To further extend this toward the formation of hydrogel materials, we employed boronic acid-modified poly(acrylic acid) (pAA-BA, the number average molecular weight of the main chain was 250000) instead of monomeric boronic acid, which is expected to link not only SPG but also SPG/SWCNT composite through the side chain glucose/pendent boronic acid interaction, as shown in Figure 2.

At first, the carboxylic acid groups of poly(acrylic acid) were partially modified with boronic acid utilizing the condensation reaction as shown in Figure 1. The introduced percentage of boronic acid was estimated to be 3% from the peak intensity of ^{1}H NMR spectrum (see Supporting Information[15]).

The hydrogel was prepared according to the following procedure. SPG and pAA-BA were mixed in 0.40 mL of aqueous $Na_2CO_3/NaHCO_3$ buffer solution ([SPG] = 5 g L^{-1}, [pAA-BA] = 3.5 g L^{-1}, pH 9.9). The obtained aqueous solution was further sonicated for 5 min and left for 15 min at room temperature. These treatments gave the hydrogel as shown in

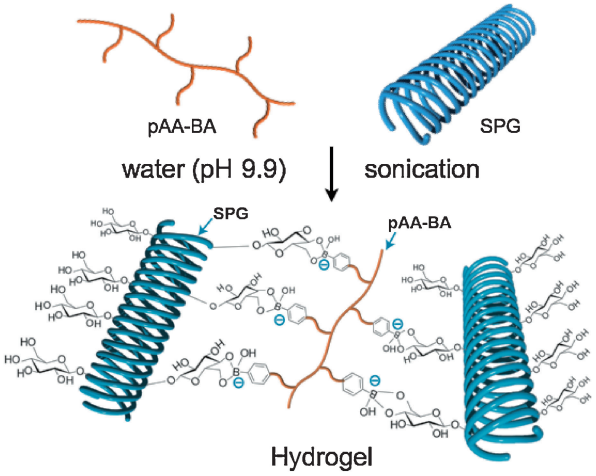

Figure 2. Schematic illustration of hydrogel formation.

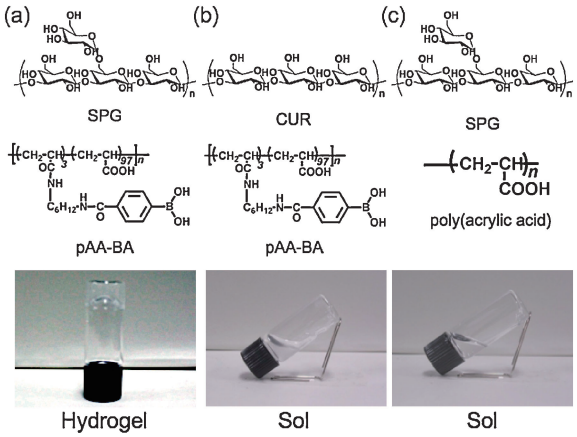

Figure 3. Photographs of the aqueous mixtures of SPG and pAA-BA (a), CUR and pAA-BA (b), and SPG and poly(acrylic acid) (c). The concentration of each component was [SPG] = 5 g L^{-1}, [CUR] = 3.8 g L^{-1}, [pAA-BA] = 3.5 g L^{-1}, and [poly(acrylic acid)] = 3.3 g L^{-1}. The monomer unit ratios are same between SPG and CUR, and between pAA-BA and poly(acrylic acid).

Figure 3a. As a reference experiment, we used curdlan (CUR) bearing the same β-1,3-glucan main chain but without side chain glucose unit, instead of SPG. Then, we could not obtain any hydrogel through the same experimental procedure (Figure 3b). Along the same line, when poly(acrylic acid) was used instead of pAA-BA, the hydrogel was not obtained either, as shown in Figure 3c. These results clearly indicate that the glucose/boronic acid interaction is indispensable for formation of the stable hydrogel.

To confirm the influence of SPG on the stability of the obtained hydrogel, an aqueous solution of SPG (200 µL) was mixed with a buffer solution of pAA-BA (7 g L^{-1}, 200 µL, pH 9.9, Na_2CO_3/$NaHCO_3$) with changing the SPG concentration in a range from 0 to 7.5 g L^{-1}. Consequently, the viscoelasticity of the aqueous mixture was increased with the increase in the SPG concentration as shown in Figure 4. On the contrary, the aqueous buffer solution containing pAA-BA (200 µL, pH 9.9, Na_2CO_3/$NaHCO_3$) was added to the 10 g L^{-1} of SPG aqueous solution (200 µL) with changing the concentration from 0 to 2.65 g L^{-1}. With the increase in the concentration of pAA-BA, formation of the hydrogel was also observed as shown in Figure S2.[15] These results imply that the hydrogelation was affected by the concentration of both SPG and pAA-BA, supporting the view that the glucose/boronic acid interaction plays a crucial role for the gel formation.

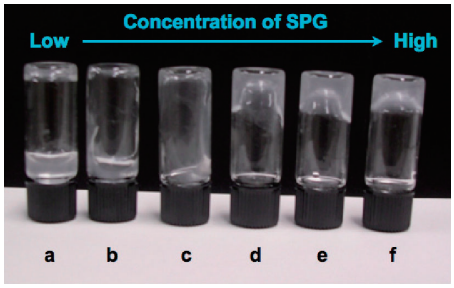

Figure 4. Hydrogelation depending on the concentration of SPG. In this aqueous mixture, the concentration of pAA-BA is 3.5 g L^{-1} and that of SPG is (a) 0, (b) 1.25, (c) 2.5, (d) 3.75, (e) 5, and (f) 7.5 g L^{-1}.

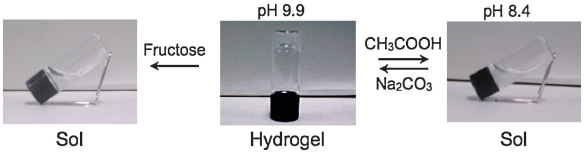

Figure 5. pH and sugar responsiveness of the hydrogel prepared from pAA-BA and SPG. The hydrogel was transformed to sol after the addition of fructose. Also, the hydrogel was transformed to sol depending on pH. The mixing ratio is SPG/pAA-BA = 5 g L^{-1}/3.5 g L^{-1}.

It was already reported that fructose can interact with boronic acid more strongly than glucose.[12] Utilizing this affinity trend, an aqueous solution of fructose (0.2 mol L^{-1}, 10 µL) was added to the hydrogel prepared by the procedure described above. As a result, immediately after the addition of fructose, the hydrogel was transformed to the sol state as shown in Figure 5. This interconversion induced by added fructose indicates that this hydrogel formation can be controlled by the sugar concentration.

Furthermore, it is also known that the glucose/boronic acid interaction effectively works at pH >9, because the stable covalently bonded complex can exist above the pK_a value of the phenylboronic acid (ca. 9).[13] In the present hydrogel system, the pH decrease to 8.4 by addition of 1 mol L^{-1} acetic acid solution transformed the hydrogel to the sol state. After that, when the pH value was increased to 9.8 using 2 mol L^{-1} aqueous solution of sodium carbonate, the hydrogel was reformed again. From these results, one can confirm that the present hydrogel prepared from pAA-BA and SPG has not only the sugar responsiveness but also the pH responsiveness, as shown in Figure 5.

As described before, we have already reported that various hydrophobic guest polymers, such as SWCNT, can be entrapped into the one-dimensional cavity constructed by SPG during its renature/denature processes, leading to the creation of sugar-coated water-soluble composites.[7,8] In order to prepare the hydrogel material containing SWCNT, we used this SWCNT/

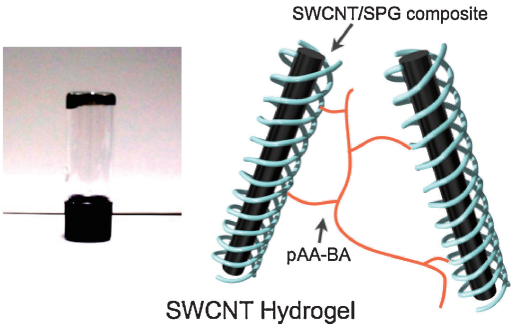

Figure 6. Photograph and schematic illustration of hydrogel including SWCNT entrapped into the helical structure of SPG.

SPG composite instead of SPG. The composite was basically prepared according to the literature previously reported by us.[8,11] Thus, the SWCNT/SPG composites were prepared according to the following procedure. To a DMSO solution of SPG (15 g L^{-1}, 400 µL), an aqueous solution of cut single-walled carbon nanotubes (2000 µL) was added at once with sonication. This clear black aqueous mixture was left for 3 days to let SPG form the stranded helix. The SWCNT/SPG composites were collected as black paste by ultrafiltration (3000 rpm, 60 min), which was dissolved into 100 µL of water again. To this aqueous solution of SWCNT/SPG composite, an aqueous solution of pAA-BA (7 g L^{-1}, 50 µL, pH 9.9, Na_2CO_3/$NaHCO_3$) was added and sonicated for 5 min. After leaving for 15 min, the hydrogel including SWCNT was formed as shown in Figure 6.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images of SWCNT/SPG composites before and after the addition of pAA-BA were quite different as shown in Figures S3 and S4.[15] These morphological changes clearly indicate that the SWCNT/SPG composites construct a polymer-network crosslinked by pAA-BA in water.

In summary, we have demonstrated that the novel hydrogel materials can be prepared from the boronic acid-modified poly(acrylic acid) and SPG by using the glucose/boronic acid interaction. Furthermore, the inherent SWCNT-wrapping property of SPG is applicable to formation of the hydrogel including carbon nanotubes. The SWCNT-containing hydrogel is so stable that it has the high potential to apply it to biosensors, biomineralization, artificial muscles, etc. Since SPG can entrap various other guest molecules,[7,14] one can combine this stimuli-responsive hydrogel with other functions inherent to the wrapped guest molecules, which would lead, for example, to drug delivery, chemical sensors, self-healing materials, and so on.

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References and Notes

1 a) L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201. b) Q. Wang, J. L. Mynar, M. Yoshida, E. Lee, M. Lee, K. Okuro, K. Kinbara, T. Aida, *Nature* **2010**, *463*, 339.
2 a) Y.-J. Lee, P. V. Braun, *Adv. Mater.* **2003**, *15*, 563. b) N. M. Sangeetha, U. Maitra, *Chem. Soc. Rev.* **2005**, *34*, 821. c) A. Sidorenko, T. Krupenkin, A. Taylor, P. Fratzl, J. Aizenberg, *Science* **2007**, *315*, 487. d) S. Tamesue, Y. Takashima, H. Yamaguchi, S. Shinkai, A. Harada, *Angew. Chem., Int. Ed.* **2010**, *49*, 7461. e) M. Ikeda, T. Tanida, T. Yoshii, I. Hamachi, *Adv. Mater.* **2011**, *23*, 2819. f) P. Froimowicz, H. Frey, K. Landfester, *Macromol. Rapid Commun.* **2011**, *32*, 468.
3 S. Iijima, T. Ichihashi, *Nature* **1993**, *363*, 603.
4 a) W. Zhou, Y. H. Ooi, R. Russo, P. Papanek, D. E. Luzzi, J. E. Fischer, M. J. Bronikowski, P. A. Willis, R. E. Smalley, *Chem. Phys. Lett.* **2001**, *350*, 6. b) H. Murakami, T. Nomura, N. Nakashima, *Chem. Phys. Lett.* **2003**, *378*, 481. c) T. Ogoshi, Y. Takashima, H. Yamaguchi, A. Harada, *J. Am. Chem. Soc.* **2007**, *129*, 4878.
5 a) N. Nakashima, S. Okuzono, H. Murakami, T. Nakai, K. Yoshikawa, *Chem. Lett.* **2003**, *32*, 456. b) M. Zheng, A. Jagota, E. D. Semke, B. A. Diner, R. S. Mclean, S. R. Lustig, R. E. Richardson, N. G. Tassi, *Nat. Mater.* **2003**, *2*, 338.
6 A. Star, D. W. Steuerman, J. R. Heath, J. F. Stoddart, *Angew. Chem., Int. Ed.* **2002**, *41*, 2508.
7 a) C. Li, M. Numata, A.-H. Bae, K. Sakurai, S. Shinkai, *J. Am. Chem. Soc.* **2005**, *127*, 4548. b) M. Numata, S. Tamesue, T. Fujisawa, S. Haraguchi, T. Hasegawa, A.-H. Bae, C. Li, K. Sakurai, S. Shinkai, *Org. Lett.* **2006**, *8*, 5533.
8 M. Numata, M. Asai, K. Kaneko, A.-H. Bae, T. Hasegawa, K. Sakurai, S. Shinkai, *J. Am. Chem. Soc.* **2005**, *127*, 5875.
9 T. Hasegawa, T. Fujisawa, M. Numata, T. Matsumoto, M. Umeda, R. Karinaga, M. Mizu, K. Koumoto, T. Kimura, S. Okumura, K. Sakurai, S. Shinkai, *Org. Biomol. Chem.* **2004**, *2*, 3091.
10 a) S. Shinkai, M. Takeuchi, *Bull. Chem. Soc. Jpn.* **2005**, *78*, 40. b) Y. Kanekiyo, Y. Ono, K. Inoue, M. Sano, S. Shinkai, *J. Chem. Soc., Perkin Trans. 2* **1999**, 557.
11 S. Tamesue, M. Numata, K. Kaneko, T. D. James, S. Shinkai, *Chem. Commun.* **2008**, 4478.
12 T. D. James, K. R. A. S. Sandanayake, R. Iguchi, S. Shinkai, *J. Am. Chem. Soc.* **1995**, *117*, 8982.
13 N. DiCesare, M. R. Pinto, K. S. Schanze, J. R. Lakowicz, *Langmuir* **2002**, *18*, 7785.
14 K. Sakurai, M. Mizu, S. Shinkai, *Biomacromolecules* **2001**, *2*, 641.
15 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

