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RESEARCH ARTICLE

Fluorometric Detection of Lectin with Water-Soluble Hyperbranched Conjugated Polymer Using Mannose Mediation

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A water-soluble hyperbranched polymer containing boronic acid groups at the ends of the polymer, which are capable of binding to diol-containing mannose, was syntheized. The hyperbranched polymer was prepared by a palladium-catalyzed Suzuki cross-coupling reaction using the tribromo monomer for the hyperbranched type structure. The water-soluble hyperbranched polymer (**HP**) exhibited enhanced fluorescence intensity upon exposure to lectin in the presence of mannose compared to other proteins, such as lysozyme and cytochrome *c*, because mannose plays a key role in binding both lectin and **HP** resulting in selective sensing toward lectin.

Keywords: Fluorescence, Lectin Sensor, Water-Soluble Hyperbranched Polymer.

1. INTRODUCTION

Recently, conjugated polymers have attracted considerable interest because of their interesting properties derived from a π-conjugated system.[1–4] Linear conjugated ones, such as poly(*p*-phenylenevinylene), polyfluorene, and their derivatives, are the most extensively studied conjugated polymers. However, there are some drawbacks that limit their potential applicability, such as low solubility and high viscosity.[5,6]

To solve these problems, several attempts have been made to utilize longer and branched side chains or bulky substituents, copolymerization techniques, and dendrimer attachments.[7,8] Among them, the hyperbranched system has advantage over its linear counterpart in terms of high solubility, good processability, and fewer unfavorable intermolecular interactions and crystallization.[9] In addition, it is possible to endow these hyperbranched architectures with functions such as enhanced adhesion, energy harvesting and optoelectronic characteristics by controlling the nature of the end groups.[10]

Boronic acids have been known for a few decades for their ability to interact with diol-containing compounds such as carbohydrates.[11,12] They have been used for the development of receptor and fluorescent probes for sugars.[13,14] The main advantage of using boronic acid as a ligand group for sugars are the fast and reversible interaction with sugars.[15,16]

Carbohydrate-protein interactions, which play a role in the interactions of proteins, viruses, and bacteria, are among the most important events or mechanisms in biological systems.[17,18] Lectins are carbohydrate-binding proteins that mediate important biological processes such as cell growth, the inflammatory response and viral infections.[19–21] A range of sensors for lectins have been developed using different principles in the sensor design. Among them, fluorescence-based lectin sensors employ a sugar-containing probe molecule. In the present study, water-soluble conjugated polymer containing mannose group to use as a lectin sensor was designed and synthesized.[22]

This paper reports the first water-soluble hyperbranched polymer containing boronic acid, which shows high binding properties with mannose. The number of mannose-binding ligands, boronic acid, was maximized through the synthesis of a hyperbranched polymer structure, which has

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comparable number of polymer end groups. The mannose-mediated hyperbranched polymer synthesized has high selectivity to lectin.

2. EXPERIMENTAL DETAILS

2.1. Instrumentation

The ^{1}H NMR spectra were obtained on a Bruker DRX-300 spectrometer (Korea Basic Science Institute). Elemental analyses were performed using a CE Instruments EA-1110 elemental analyzer. The FT-IR spectra were obtained using a Mattson Genesis II spectrometer. UV-vis absorption spectroscopy was carried out using a PerkinElmer Lambda 35 spectrometer. The photoluminescence spectra were obtained using a Varian Cary Eclipse equipped with a xenon lamp excitation source.

2.2. Synthesis of HP

2 (0.5 g, 0.925 mmol), benzene-1,4-diboronic acid (0.233 g, 1.406 mmol), **3** (0.027 g, 0.0925 mmol), tris(4-bromophenyl)amine (0.037 g, 0.154 mmol), an aqueous 2 M Na_2CO_3 solution (8 ml), and dry DMF (18 ml) under nitrogen was added to a 100 ml round-bottomed flask. After adding Pd(0) (5 mol%) as a catalyst the mixture was heated to 90 °C. The mixture was stirred for 40 h. The reaction mixture was poured into acetone. The precipitate was re-dissolved in deionized water and the solution was dialyzed using a membrane (3500 cutoff) for 3 days. The polymer was obtained after drying in freeze drier. Yield 0.21 g (40.2%). ^{1}H NMR (300 MHz, D_2O): δ 8.1–6.8 (*m*, 8 H), 4.1–3.8 (*m*, 6 H), 3.3–2.8 (*t*, 6 H), 2.3–1.8 (*d*, 12 H) ppm. FT-IR (cm^{-1}): 724 (S–O), 1151 (aryl C–N), 1372 (S═O), 1618 (C═C), 2939 (C–H), 3418 (OH).

3. RESULTS AND DISCUSSION

Scheme 1 illustrates the synthetic procedures for the preparation of the monomers and polymer. Monomers **1** and **2** for the preparation of the polymer were synthesized using the literature procedures.[23] Monomer **3** was obtained easily in good yield by the bromination of commercially available 2,1,3-benzothiadiazole according to the published methods.[24] The hyperbranched polymer was typically prepared by the palladium-catalyzed Suzuki cross-coupling of diboronic acid, dibromo compound and tribromo compound. For the preparation of **HP**, a slight excess of 1,4-phenylenediboronic acid was used in the presence of an additional catalytic amount of Pd(0) to produce a high abundance of boronic acid end groups at the polymer branches.

The reaction time for polymerization was determined experimentally before the appearance of the precipitates. **HP** obtained before precipitation had good solubility in water. However, the product precipitated was insoluble in water due mainly to its crosslinked structure resulting from trifunctional monomer. Therefore, precise control of the reaction time and tribromo monomer, tris(4-bromophenyl)amine content is essential for obtaining a water-soluble hyperbranched polymer. **HP** was characterized by ^{1}H NMR, IR, and elemental analysis. The ^{1}H NMR spectrum of **HP** showed chemical shifts of the phenylene group, benzothiadiazole group and alkylene group at 8.1–7.7, 7.5–6.8, and 4.1–1.8 ppm, respectively.

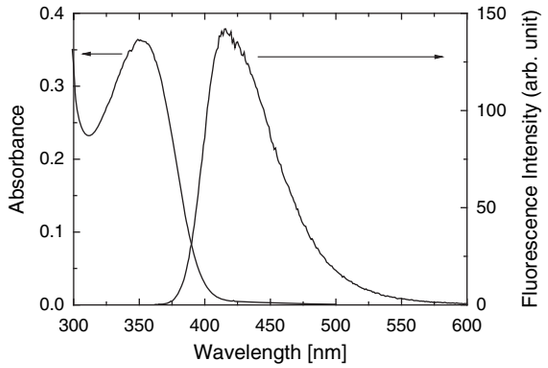


Fig. 1. Absorption and emission spectra of **HP** (excited at 352 nm) in aqueous solution; $[\mathbf{HP}] = 1.0 \times 10^{-5}$ M for absorption spectra.

The optical properties of the polymer were examined by a UV-vis and fluorescence spectroscopy in 6 mM sodium phosphate buffer solution at a physiological pH of 7.4. The polymer showed good solubility in the buffer solution due to the presence of water-soluble sulfonic acid group at the side chain of **HP**. Figure 1 shows the absorption and photolumine-scence (PL) spectra of the hyperbranched polymer in the solution state.

The absorption maximum of **HP** in a buffer solution was observed at 352 nm. This absorption corresponds to the push-pull structure of **HP**, which has an electron-donating triphenylamine group and an electron-accepting benzothidaizole group. On the other hand, the maximum emission of **HP** was observed at 418 nm. This was

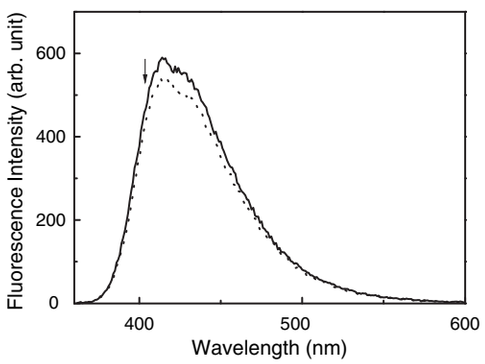
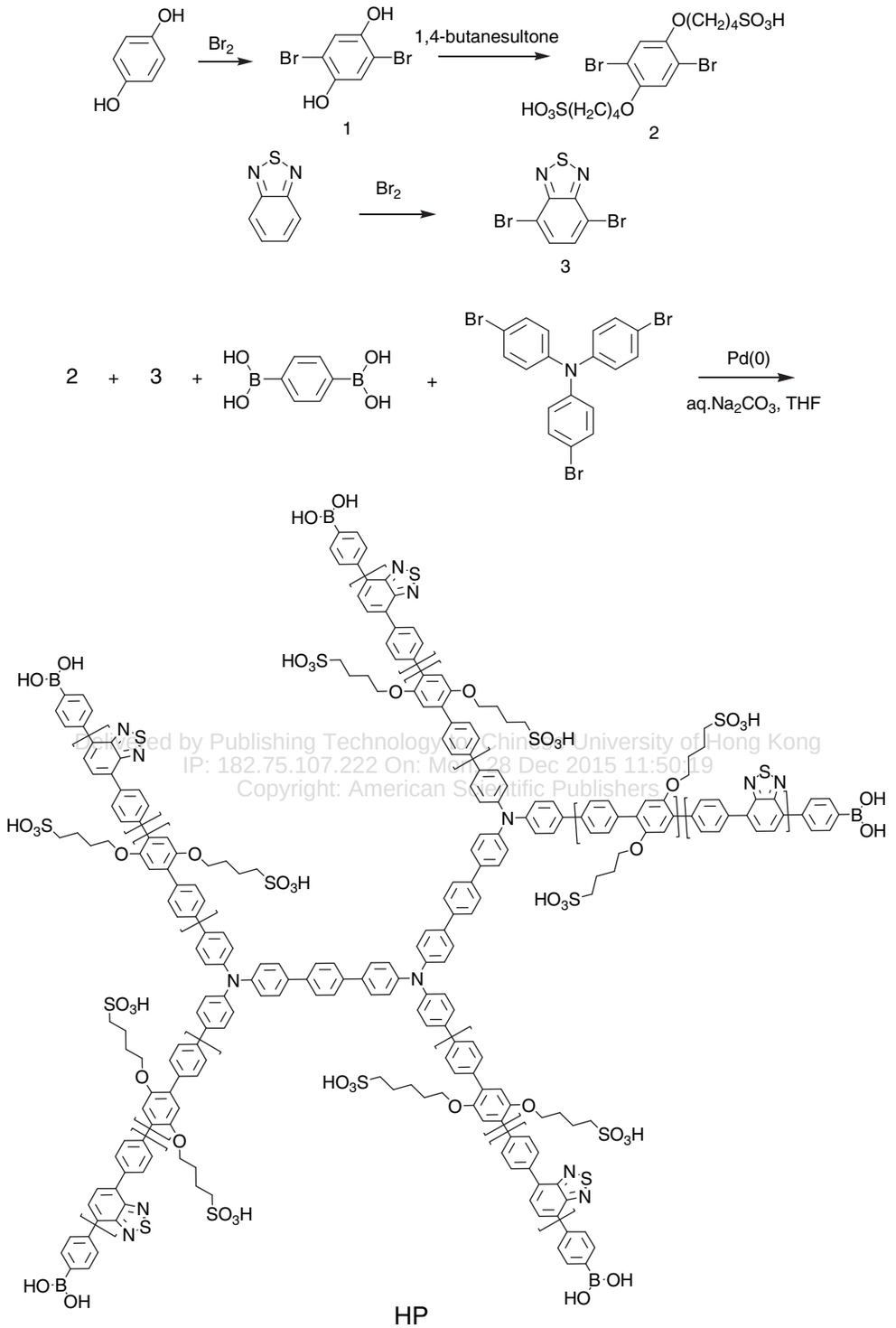


Fig. 2. Fluorescence spectra of **HP** (1.0×10^{-5} M) upon the addition of mannose (2.0×10^{-4} M) in 6 mM aqueous sodium phosphate buffer at pH 7.4; $\lambda_{ex} = 352$ nm.



Scheme 1. Synthetic routes for the monomers and **HP**.

attributed to effective excitation energy transfer from the short conjugated species to the larger ones, which are the main emitting species.

Based on the well-known mannose-boronic acid interaction, mannose-modified **HP** was prepared from a reaction with boronic acid functionalized **HP** and mannose at room temperature. The fluorescence intensity of **HP** (1.0×10^{-5} M) decreased slightly upon the addition of mannose, as shown in Figure 2. This suggests that the introduction of mannose at the ends of **HP** did not affect

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the π-conjugated structure because the fluorescence property of the polymer had not been altered considerably. Based on the complex formation between boronic acid and diol groups in mannose, **HP** has a great advantage over its linear counterpart, because a large number of end groups (boronic acid) can be incorporated in the backbone.

Lectins have specific binding affinity to glucose and mannose. Based on this binding property, an attempt was made to add some proteins (5.0×10^{-7} M) in the mannose-mediated **HP** buffer solution. Mannose-mediated **HP** showed the most sensitive fluorescence intensity change to lectin even in the μM range (Fig. 3(a)).

Compared to lysozyme and cyt *c* (Figs. 3(b) and (c)), a consecutive increase in fluorescence intensity was observed in **HP** with increasing lectin concentration. On the other hand, the change in fluorescence intensity was negligible in the case of lysozyme due mainly to the low binding affinity between lysozyme and mannose-mediated **HP**. In the case of cyt *c*, the fluorescence was quenched completely in the buffer solution as shown in Figure 3(c). It was reported that the heme group in cyt *c*, which is a complex compound with Fe^{2+} ions, can result in fluorescence quenching.[25, 26] This suggests that a number of boronic acid functional group on **HP** provide a potential binding with mannose, which can have a high selectivity with lectin over other proteins as shown in Figure 4.

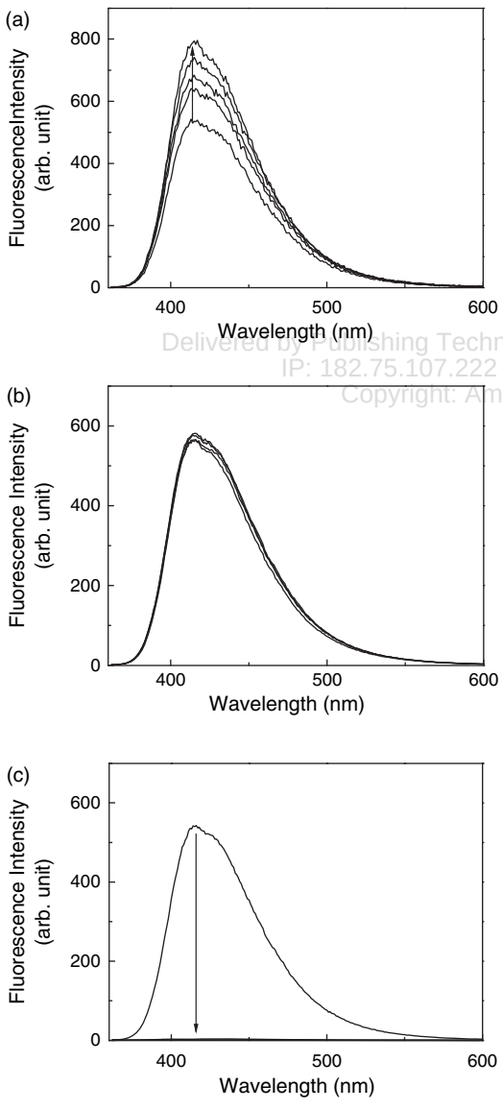

Fig. 3. Fluorescence spectra of mannose-mediated **HP** (1.0×10^{-5} M) upon the addition of (a) lectin; (b) lysozyme; (c) cytochrome *c* (0, 10, 50, 100, 500 nM) in 6 mM aqueous sodium phosphate buffer at pH 7.4; $\lambda_{ex} = 333$ nm.

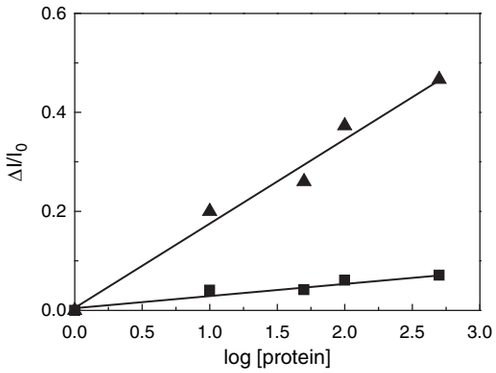

Fig. 4. Emission intensity changes (I/I_0) of mannose-mediated **HP** as a function of the proteins concentration; 1.0×10^{-5} M in 6 mM aqueous sodium phosphate buffer at pH 7.4; $\lambda_{ex} = 352$ nm and $\lambda_{em} = 418$ nm for **HP**; ▲ lectin; ■ lysozyme; I_0: fluorescence intensity before the addition of the protein; ΔI: fluorescence intensity change before and after the addition of the protein.

4. CONCLUSION

A blue-emitting hyperbranched water-soluble conjugated poly(*p*-phenylene-benzothiadiazole) with a number of boronic acid functional groups in its ends was synthesized via Suzuki coupling polymerization. After binding to boronic acid end-groups in **HP** and mannose, the polymer played a role in the selective sensing of the mannose-binding glycoprotein, lectin. According to the investigations on its sensing properties toward lectin, the mannose-modified hyperbranched polymer can be used as a selective and sensitive sensor for lectin.

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