Chemical Communications

Supporting Information

Anomalous glucose-responsive rheological changes in a boronic acid-modified hyaluronan

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Materials

Sodium hyaluronate (HA, 50-110 kDa) was purchased from Kikkoman Biochemifa (Tokyo, Japan). Glucose (Glc), fructose (Fru), sorbitol (Sor), sodium hydroxide (NaOH; 8 mol/L), and sodium hydrogen carbonate were obtained from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Anhydrous galactose (Gal), pentaerythritol (PE), and 3-aminophenylboronic acid monohydrate (BA) were obtained from Tokyo Chemical Industry (Tokyo, Japan). Deuterium oxide (D_2O) was acquired from Kanto Chemical Co., Inc. (Tokyo, Japan). Sodium deuteroxide (NaOD) solution (40% (w/w)) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) were purchased from Sigma-Aldrich (Tokyo, Japan). Cellulose tubes (36/32, molecular weight cutoff: 14 kDa) were obtained from Viskase Companies, Inc. (IL, USA).

Apparatus

NMR spectroscopy

^{1}H NMR spectroscopy was conducted using a 600 MHz Bruker AVANCE NEO 600 spectrometer (Bruker Japan KK, Kanagawa, Japan).

Modification of HA using BA

BA (234.8 mg, 1.515 mmol) was added to 100 mL of distilled water and dissolved using ultrasound. Subsequently, HA (1.0 g, 5.05 mmol as repeating units) was dissolved. DMT-TT (628.9 mg, 2.273 mmol) was added, and the pH of the solution was adjusted to 6.5 using NaOH solution. The reaction mixture became cloudy after stirring at room temperature for 24 h. To completely dissolve the reaction mixture, an adequate amount of 8 M NaOH solution was added. Subsequently, the reaction solution was dialysed against water at room temperature with a cellulose tube, followed by freeze-drying.

^{1}H NMR analysis of BA-modified HA (BA-HA)

NaOD deuterium oxide solution (0.1 M) was used as solvent. BA-HA was used at a concentration of 8 mg/mL. The chemical shifts were relative to the solvent peaks of HDO (4.63 ppm). The ^{1}H NMR spectra are shown in Fig. S1.

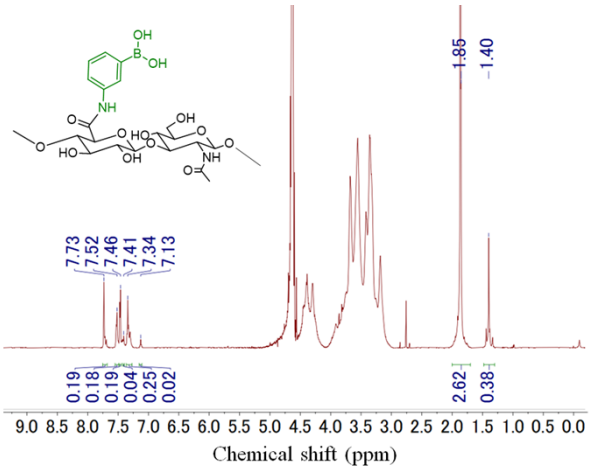

Fig. S1. ^{1}H NMR spectra of 8 mg/mL BA-HA.

Preparation of native HA or BA-HA samples

Preparation of native HA or BA-HA samples were prepared to a concentration of 12 mg/mL using 0.2 M sodium carbonate buffer (pH 10.5) as a solvent.

Visual observation of native HA or BA-HA samples

Samples were stored at 25 °C above 12 h before use. Native HA or BA-HA (1.02 mL of 12 mg/mL) with or without polyols was placed in 3.5–mL glass vials. The images were captured 30 s after the vials were inverted.

For the injection test, BA-HA without polyols (12 mg/mL) was injected through a 26G injection needle (outer diameter: 0.45 mm, inner diameter: 0.23 mm).

For the movies, 20 μL sodium carbonate buffer (0.2 M, pH 10.5) or 42.5 mM Glc in 0.2 M sodium carbonate buffer were put into 150 μL of 13.6 mg/mL BA-HA in a 1-mL microtube. Immediately after placement, the samples were mixed for 30 s with a pipette tip, followed by handling with a pipette tip and tweezers.

Rheological measurements

A stress-controlled rotational rheometer (MCR-102, Anton Paar, Ostfildern, Germany) with solvent trap was used for dynamic rheological measurements at 25 °C. Strains (γ) were fixed at 10%. A conical plate of 25 mm diameter was used. Samples were stored at 25 °C above 12 h before use. Aliquot of 170 µL samples were loaded onto the stage of the rheometer, and measurements were conducted after 5 min.

Chemical structures of Gal, PE, Fru, and Sor

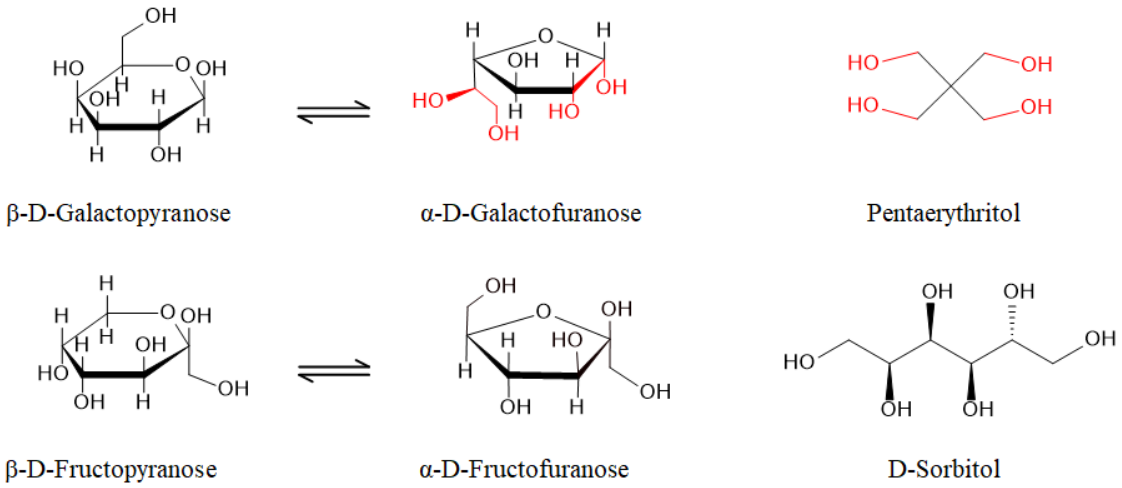

Fig. S2. Chemical structures of Gal, PE, Fru, and Sor. For α-D-galactofuranose and PE, the potential boronic acid binding OH-groups are shown in red.