Electronic Supplementary Material (ESI) for Analyst.

Supplementary material

Paper-based 1,5-anhydroglucitol quantification using enzyme-based glucose elimination

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Experimental section

Materials

D-glucose, 1,5-anhydroglucitol (1,5-AG), chitosan oligosaccharide lactate, catalase, and 4-aminoantipyrine (4-AAP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). GOx and HRP were purchased from Toyobo Co., Ltd. (Osaka, Japan). Pyranose oxidase was purchased from Creative Enzymes (Shirley, NY, USA). Mutarotase was obtained from Fitzgerald (Acton, MA, USA). N,N-Bis(4-sulfobutyl)-3,5-dimethylaniline disodium salt (MADB) was purchased from Dojindo Molecular Technologies, Inc. (Tokyo, Japan). Pooled normal human urine was purchased from Innovative Research (Novi, MI, USA). 1× phosphate buffer saline was obtained from LPS Solution (Daejeon, South Korea). Immuno RP membrane (nitrocellulose) was purchased from Whatman Inc. (Seoul, South Korea). Absorbent pads (Grade 222) were purchased from Boreda Biotech (Gyeonggi-do, South Korea). Deionized water (DW), with resistivity of 18.2 MΩ cm, was obtained using a PURELAB Option-Q water purification system (Atlanta, UK).

Preparation of enzyme mixtures

The enzymatic colorimetric assay, used to detect glucose and 1,5-AG, was performed as follows. GOx and HRP were adjusted to 10 and 1 kU/mL in PBS (pH 7.0). MADB and 4-AAP were dissolved in DW to obtain 200 mM concentration. Catalase was adjusted to 50 kU/mL in PBS (pH 7.0), and mutarotase was prepared to 2.8 kU/mL in PBS (pH 7.0). To prepare the color focusing reagent, chitosan oligosaccharide lactate (COL) was adjusted to 5 wt% in DW. For sensing glucose, 1 kU/mL of GOx–HRP (2 μL) and 200 mM 4-AAP–MADB (2 μL) solutions were mixed with 5 wt% (6 μL) solution of COL. For sensing 1,5-AG, 100 U/mL of PROD (2 μL), 1 kU/mL of HRP (2 μL), and 200 mM 4-AAP–MADB (2 μL) solutions were mixed with 5 wt% (6 μL) solution of COL. To fabricate the elimination pad, 10 kU/mL of GOx (100 μL), 50 kU/mL of catalase (100 μL), and 2.8 kU/mL of mutarotase (100 μL) were mixed with PBS (80 μL) and 5% COL (20 μL).

Fabrication of sensor

The immunopore RP membrane and absorbent pad was sized to 2 × 100 mm. The 40-μL elimination solution was loaded on the cut absorbent pad, and dried in a desiccator for 25 min. The detection solution for glucose and 1,5-AG was spotted on to immunopore RP membrane, and dried in a desiccator for 2 min. As the sample-loading zone, the absorbent pad firstly located to backing card. The immuno RP membrane, treated with the detecting solution, was located at 1.2 mm from the sample pad. Finally, the elimination pad was overlapped on the sample pad.

Optimization of enzyme concentration and passing time

The elimination pad was treated with various conditions of enzyme mixture to optimize the elimination efficiency. The concentration of GOx and catalase was adjusted to 10, 25, and 50 kU/mL and 10, 50, 100, and 200 kU/mL, respectively. Additionally, 80 μL of 50 mg/dL glucose was injected to the fabricated sensors with the various conditions of enzyme mixtures, and the elimination efficiency was measured using Bio-Rad Universal Hood III instrument (Bio Rad Laboratories, Inc., Hercules, CA).

Efficiency measurement of glucose elimination

The glucose solution was adjusted to 0, 5, 10, 15, 30, and 50 mg/dL in DW, and 80 μL of sample was loaded onto a 96-well plate. The fabricated paper sensors, prepared with elimination pad or without, were dipped into the plate wells. To reduce the fluctuation of signal intensity in this sensor, the temperature of the Bio-Rad Universal Hood III instrument (Bio Rad Laboratories, Inc., Hercules, CA) was adjusted to 24℃, and the sample was located center of tray to prevent the fluctuation of the light source. After adjusting, the exposing time of light was fixed to 0.12 s and the colorimetric signal was measured at 10 min.

Calibration of glucose, 1,5-AG, and mixture

Solutions of 1,5-AG were prepared at 0, 5, 10, 15, 30, and 50 μg/mL in DW. To detect the targets in mixture, glucose and 1,5-AG were mixed and adjusted 0–50 mg/dL and 0–50 μg/mL, respectively. In the sample hole of the 3D case, 80 μL each of glucose, 1,5-AG, and mixture were loaded. After 2 and 10 min, the color signal was measured for glucose and 1,5-AG quantification.

Test in spiked human urine

The base concentration of glucose and 1,5-AG in human urine was determined to be 0 mg/dL and 2.2 μg/mL, using Hitachi 7020 automatic analyzer (Hitachi, Tokyo, Japan) and 1,5-AG competitive ELISA kit (Cloud-Clone Corp. TX, USA). Both glucose and 1,5-AG were adjusted to 15 mg/dL and 6.5, 11.5, 16.5, 31.5, and 51.5 μg/mL in human urine. Finally, the 80 μL spiked human urine was loaded on to the sample hole of the fabricated sensor.

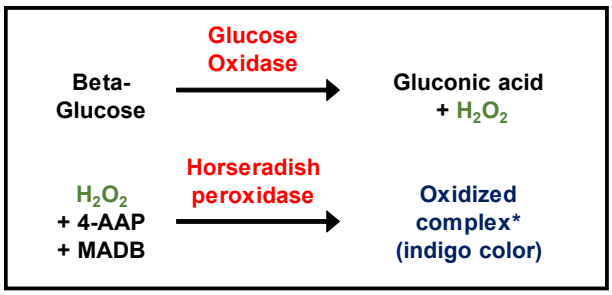

Figure S1. Mechanism of glucose detection

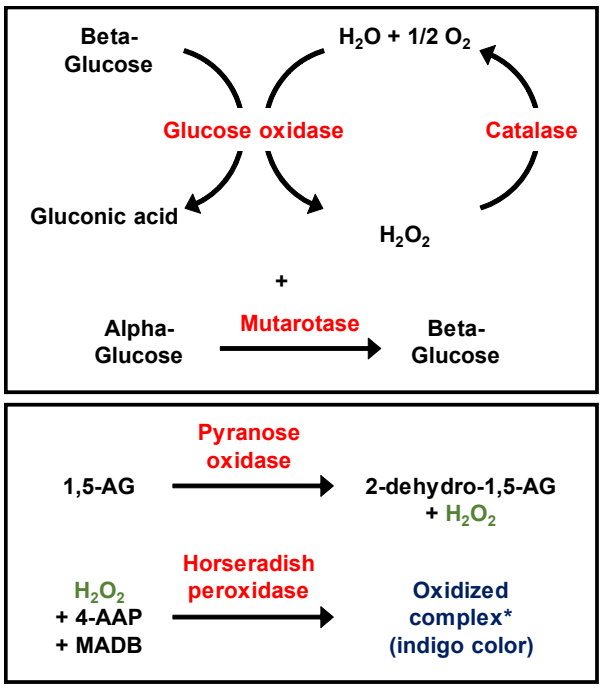

Figure S2. Mechanism of 1,5-AG detection

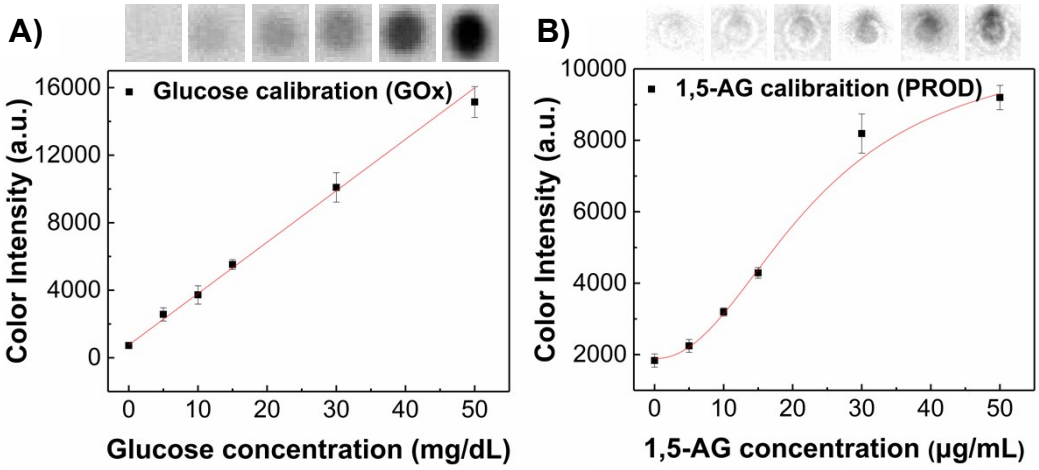

Figure S3. The plots indicate the measured intensity of color signal on this sensor. They were fitted to linear and Hill 1 equations for glucose (A) and 1,5-AG (B) (n = 5), respectively. The images show the color intensity following the concentration of targets.

Catalase (kU/mL) \ GOx (kU/mL)	10	25	50
10	15,526.92/(30 s)	12,895.25/(1 min)	9,005.52/(3 min 30 s)
50	7,609.45/(8 min)	7,019.26/(9 min 30 s)	6,927.12/(10 min)
100	6,753.58/(11 min 30 s)	5,890.86/(12 min)	5,588.68/(15 min 30 s)
200	5,661.65/(15 min)	5,230.83/(15 min)	5,579.26/(17 min)

Table S1. Optimization of enzyme concentration and passing time (signal intensity/passing time).

1,5-AG conc. (spiked, µg/mL)	6.5	11.5	16.5	31.5	51.5
1,5-AG conc. (measured, µg/mL)	5.7	12.5	18.1	31.6	47.6
Recovery (%)	82	109	110	100	92

Table S2. Recovery rate of spiked and measured concentrations of 1,5-AG.