Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: http://www.tandfonline.com/loi/tbbb20

Preferential binding of E. coli with type 1 fimbria to d-mannobiose with the Manα1→2Man structure

Katsumi Ajisaka, Kaoru Yuki, Kaori Sato, Nozomi Ishii, Ichiro Matsuo, Ryo Kuji, Tatsuo Miyazaki & Kiyoshi Furukawa

To cite this article: Katsumi Ajisaka, Kaoru Yuki, Kaori Sato, Nozomi Ishii, Ichiro Matsuo, Ryo Kuji, Tatsuo Miyazaki & Kiyoshi Furukawa (2016) Preferential binding of E. coli with type 1 fimbria to d-mannobiose with the Manα1#2Man structure, Bioscience, Biotechnology, and Biochemistry, 80:1, 128-134, DOI: 10.1080/09168451.2015.1075863

To link to this article: http://dx.doi.org/10.1080/09168451.2015.1075863

Published online: 19 Aug 2015.

Bioscience, Biotechnology, and Biochemistry, 2016
Vol. 80, No. 1, 128–134

Preferential binding of *E. coli* with type 1 fimbria to D-mannobiose with the Manα1→2Man structure

Katsumi Ajisaka[1], Kaoru Yuki[2], Kaori Sato[2], Nozomi Ishii[3], Ichiro Matsuo[3], Ryo Kuji[2], Tatsuo Miyazaki[1] and Kiyoshi Furukawa[2,*]

[1]*Department of Food Science, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan;* [2]*Department of Bioengineering, Nagaoka University of Technology, Nagaoka, Japan;* [3]*Division of Molecular Sciences, Faculty of Science and Technology, Gunma University, Kiryuu, Japan*

Received May 14, 2015; accepted July 15, 2015
http://dx.doi.org/10.1080/09168451.2015.1075863

Manα1→2Man, Manα1→3Man, Manα1→4Man, and Manα1→6Man were converted to the glycosylamine derivatives. Then, they were mixed with monobenzyl succinic acid to obtain their amide derivatives. After removing the benzyl group by hydrogenation, the succinylamide derivatives were coupled with the hydrazino groups on BlotGlyco™ beads in the presence of water-soluble carbodiimide. D-Mannobiose-linked beads were incubated with fluorescence-labeled *Escherichia coli* with type 1 fimbria, and the number of the fluorescent dots associated with the beads was counted in order to determine the binding preference among D-mannobiose isomers. The results showed that the bacteria bind strongly to Manα1→2Man1→beads, Manα1→3Man1→beads, Manα1→4Man1→beads, and Manα1→6Man1→beads, in order. In the presence of 0.1 M methyl α-D-mannopyranoside, most of the bacteria failed to bind to these beads. These results indicate that *E. coli* with type 1 fimbria binds to all types of D-mannobiose isomers but preferentially to Manα1→2Man disaccharide.

Key words: *E. coli*; carbohydrates; D-mannobiose; binding preference

Many microorganisms recognize cell surface glycans as receptors for cell attachment and tissue colonization. This is because viruses, bacteria, and protozoa express a large number of glycan-binding proteins so-called lectins (reviewed in Ref. [1]). For example, human influenza viruses recognize the Siaα2→6Gal structure by their hemagglutinins expressed on virus surface, and *Helicobacter pylori* binds Fucα1→2Galβ1→3 (Fucα1→4) GlcNAc1β→R structure (Le^b antigen) by its adhesin BabA expressed on flagella.[2,3] In fact, such glycans are expressed on cell surfaces of human respiratory tract and stomach tissues, respectively. Furthermore, *Escherichia. coli* binds high mannose-type glycans rich in intestinal mucosa and glycans with the Galα1→4Gal group in urinary tract depending on the strains, and the pathogenic strains often cause enterohemorrhagy with verotoxin produced, hemolytic uremic syndrome, acute encephalopathy, pyelonephritis, cystitis, and urethritis for humans.[4–8] *E. coli* is a gram-negative bacterium and covered with fimbria. At the top of the fimbria, there are glycan-binding proteins called adhesins. The most well characterized bacterial adhesin is the type 1 fimbrial FimH adhesin which recognizes α-D-mannose.[9] FimH is displayed on the bacterial surface as a component of the type 1 fimbrial organelle.

Using beads immobilized with α-D-mannose-containing glycans, we found *E. coli* binds strongly to high mannose-type glycans and wondered how many α-D-mannose residues are required for the recognition by the FimH adhesin. Then, we prepared beads immobilized with D-mannose or D-mannobiose with Manα1→2-Man structure, examined the binding activity of *E. coli* (strain W3110) to these beads, and found that the bacteria bind stronger to D-mannobiose-linked beads than α-D-mannose-linked beads. However, in the case of D-mannobiose, there are four isomers with different glycosidic linkages. In this study, we examined the binding preference of *E. coli* among D-mannobiose isomers with Manα1→2Man, Manα1→3Man, Manα1→4Man, and Manα1→6Man structures.

Materials and methods

Reagents. All the chemical reagents used for the preparation of sugar-linked beads were from Wako Pure Chemicals Co. (Osaka). D-Mannobiose isomers, Manα1→2Man, Manα1→3Man, and Manα1→6Man were synthesized by HCl-catalyzed condensation reaction according to the method described previously.[10] Manα1→4Man was obtained from Carbosynth Ltd.

*Corresponding author. Email: furukawa@vos.nagaokaut.ac.jp
Abbreviations: E. coli, *Escherichia coli*; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; PBS, 10 mM phosphate-buffered saline (pH 7.4); WSC, water-soluble carbodiimide.

(Berkshire, UK). A PKH67 green fluorescent cell linker kit for general cell membrane labeling was purchased from Sigma-Aldrich Corp. (St. Louis, MO).

Synthesis of D-mannobiosyl succinylamide derivatives. Since the method for preparing D-mannobiose-linked beads is the same among the isomers, the preparation of Manα1→2-Man1→beads was described here as a representative. The amination of the D-mannobiose was performed in a saturated $(NH_4)_2CO_3$ solution by the method with a slight modification[11] as described in Fig. 1. A disaccharide, Manα1→2Man, (**1**, 51.5 mg, 0.15 mmol) was dissolved in a saturated $(NH_4)_2CO_3$ solution (5 mL), and the mixture was stirred at 40 °C for 4 h. After confirming the disappearance of a starting material spot (Rf: 0.43) by thin layer chromatography ($CH_3CN/H_2O = 3:1$ (v/v) containing 0.1% triethylamine), the solution was subjected to evaporation initially at 45 mm Hg and then at 12 mm Hg.

Monobenzyl succinic acid was synthesized by mixing benzyl alcohol (1.0 mL, 9.6 mmol) and succinic anhydride (1.2 g, 1.2 mmol) in pyridine (2.0 mL) at room temperature for 1 h. The concentrated reaction mixture was dissolved in ethyl acetate, washed with a 1 M HCl solution, brine (a saturated aqueous NaCl solution), a saturated aqueous $NaHCO_3$ solution and brine, dried in the presence of Na_2SO_4, and then concentrated *in vacuo* to obtain monobenzyl succinic acid as a pure compound. All the concentrated D-mannobiosyl amine syrup (**1**) was dissolved in dimethyl formamide (5 mL), to which monobenzyl succinic acid (46.8 mg, 0.225 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride n-hydrate[12] (61.2 mg, 0.225 mmol) were added. The mixture was stirred at 40 °C for 22 h and then concentrated *in vacuo* to obtain syrup (34.5 mg). The syrup was purified by chromatography using a C-18 Sep-Pak™ column (Nihon Waters KK, Japan) and a Mightysil RP-18 GP™ column (1 × 25 cm) (Kanto Chemical Co., Inc., Tokyo). Fractions eluted at the retention time from 12 to 14 min were collected (the retention times were different among D-mannobiose isomers), and the collected fractions were concentrated to obtain a pure D-mannobiosyl benzylsuccinylamide derivative (**3**, 9.8 mg, 18 μmol).

The compound **3** (7.1 mg, 13 μmol) was dissolved in distilled water (2 mL), and PD-C (10 mg) was added to the solution. It was then placed into a flask attached with a H_2 balloon and stirred at room temperature for 3 h. After completing the reaction, the catalyst was separated by centrifugation at 2500 × *g* for 3 min. By concentrating the supernatant, D-mannobiosyl succinylamide derivative (**4**) was obtained quantitatively. The ^{1}H-NHR and ^{13}C-NMR spectra and high-resolution mass spectrometry (HRMS) spectra of D-mannobiosyl succinylamide derivatives were obtained with a Jeol JNM-EC600 spectrometer (JEOL Ltd., Tokyo) and a micrOTOF II spectrometer (Bruker Daltonics Inc., Billerica, MA), respectively.

Analytical data of Manα1→2Man succinylamide derivative. Rf: 0.28 ($CH_3CN/H_2O = 3:1$). ^{1}H-NMR (600 MHz, D_2O): δ_H 5.57 (0.18H, d, $J = 1.91$ Hz, H-1α), 5.15 (0.82H, bs, H-1β), 5.13 (0.16H, s, H-1α′), 4.97 (0.84H, d, $J = 1.81$ Hz, H-1β′), 4.00 (1H, dd, $J = 1.92$, 3.05 Hz, H-2), 3.93 (1H, bd, $J = 3.04$ Hz, H-2′), 3.81 (1H, dd, $J = 1.21$, 11.98 Hz, H-6), 3.78 (1H, dd, $J = 3.08$, 9.51 Hz, H-3′), 3.89 (1H, bd, $J = 2.81$, 11.83 Hz, H-6), 3.70 (1H, dd, $J = 3.08$, 9.66 Hz, H-3), 3.62 (1H, dd, $J = 6.81$, 12.21, H-6′), 3.59 (1H, dd, $J = 5.85$, 12.01 Hz, H-6′) 3.53 (1H, t, $J = 9.461$ Hz, H-4), 3.51 (1H, t, $J = 9.59$ Hz, H-4), 3.34 (1H, ddd, $J = 2.02$, 5.97, 9.67 Hz, H-5), 2.57–2.49 (4H, m, –CH_2–CH_2–), ^{13}C-NMR (150 MHz, D_2O): δ_C 177.53, 175.49 (C=O), 103.10 (C-1′), 81.25, 78.91 (C-1α), 78.58 (C-1β), 78.33 (C-3), 77.75, 74.44, 73.89 (C-5), 70.62 (C-2), 67,39, 67.02, 61.45 (C-6), 61.40, 30.43, 29.51 (–CH_2–CH_2–).

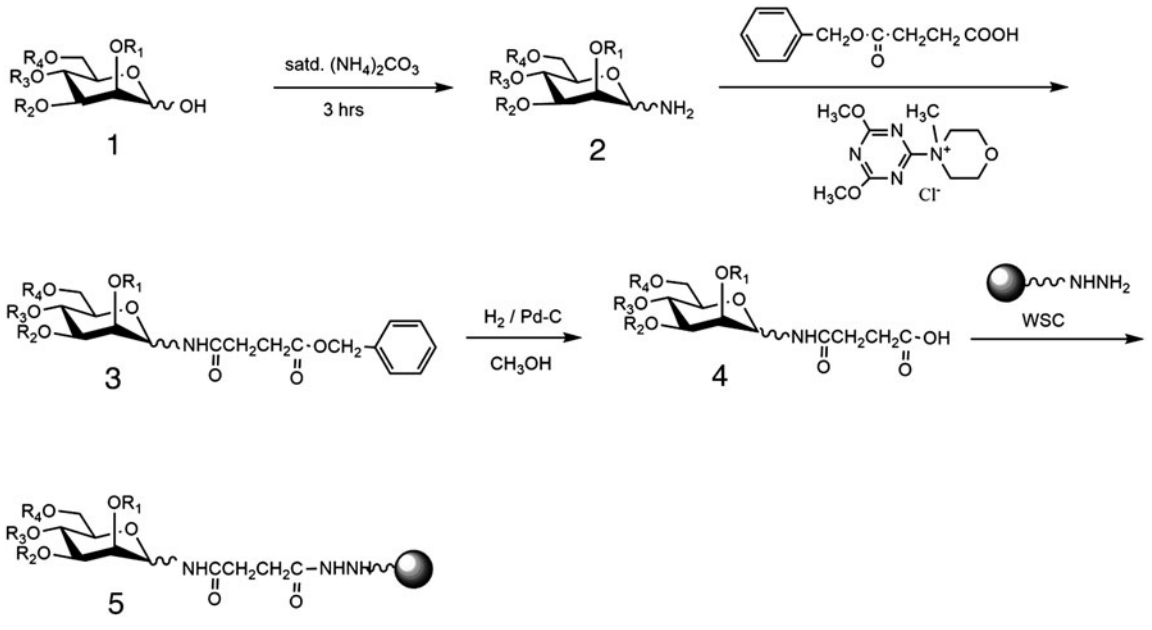

Fig. 1. Synthetic pathways of BlotGlyco™ beads linked with D-mannobiose derivative (one of R_1–R_4 is D-mannose, and the others are H's).

HRMS m/z calcd. for $C_{16}H_{26}NO_{13}$ [M-H$^+$] 440.1399, found 440.1390.

Analytical data of Manα1→3Man succinylamide derivative. Rf: 0.27 ($CH_3CN/H_2O = 3:1$). ^{1}H-NMR (600 MHz, D_2O): δ_H 5.28 (0.09H, d, $J = 1.95$ Hz, H-1α), 5.13 (0.91H, s, H-1β), 5.10 (0.09H, s, H-1α′), 4.97 (0.91H, d, $J = 1.38$ Hz, H-1β′), 3.95 (1H bd, $J = 3.15$ Hz, H-2′), 3.93 (1H, dd, $J = 1.48$, 3.06 Hz, H-2), 3.77–3.72 (3H, m, H-6, H-6, H-3), 3.70 (1H, dd, $J = 3.32$, 9.47 Hz, H-3), 3.67–3.56 (4H, m, H-6′, H-6′, H-5′, H-4), 3.51 (1H, t, $J = 9.66$ Hz, H4), 3.36 (1H, ddd, $J = 2.00$, 5.53, 9.56 Hz, H-5), 2.48 (4H, bs, –CH2–CH2–), ^{13}C-NMR (150 MHz, D_2O): δ_C 178.78, 176.14 (C=O), 102.56 (C-1′), 80.86, 78.19, 78.23, 78.23, 74.44, 73.92, 70.91, 70.57, 70.36, 67,36, 66.52, 61.55 (C-6), 61.30 (C-6′), 31.12, 30.43 (–CH_2–CH_2–). HRMS *m/z* calcd. for $C_{16}H_{26}NO_{13}$ [M-H$^+$] 440.1399, found 440.1393.

Analytical data of Manα1→4Man succinylamide derivative. Rf: 0.25 ($CH_3CN/H_2O = 3:1$). ^{1}H-NMR (600 MHz, D_2O): δ_H 5.31 (0.12H, d, $J = 1.70$ Hz, H-1α), 5.14 (0.88H, d, $J = 1.46$ Hz, H-1β), 5.10 (1H, d, $J = 0.85$ Hz, H-1β′), 3.94 (1H, dd, $J = 1.70$, 2.97 Hz, H-2), 3.79 (1H, dd, $J = 0.81$, 3.09 Hz, H-2′), 3.78–3.74 (2H, m, H-6, H-6), 3.71–3.60 (4H, m), 3.59–3.52 (3H, m), 3.43 (1H, ddd, $J = 1.95$, 5.10, 9.40 Hz, H-5), 2.59–2.50 (4H, m, CH_2–CH_2–), ^{13}C-NMR (150 MHz, D_2O) δ_C 177.17, 175.42 (C=O), 101.57 (C-1′), 80.86, 78.37, 76.83, 74.41, 74.27, 71.23, 70.87, 67,12, 61.54 (C-6), 61.48 (C-6′), 30.04, 28.91 (–CH_2–CH_2–). HRMS *m/z* calcd. for $C_{16}H_{26}NO_{13}$ [M-H$^+$] 440.1399, found 440.1400.

Analytical data of Manα1→6Man succinylamide derivative. Rf: 0.25 ($CH_3CN/H_2O = 3:1$). ^{1}H-NMR (600 MHz, D_2O): δ_H 5.31 (0.11H, d, $J = 1.70$ Hz, H-1α), 5.11 (0.89H, d, $J = 0.97$ Hz, H-1β), 4.77 (0.88H, d, $J = 1.59$ Hz, H-1β′), 4.75 (012H, d, $J = 1.64$ Hz, H-1α′), 3.86 (1H, dd, $J = 1.62$, 3.98 Hz, H-2), 3.83 (1H, dd, $J = 1.02$, 2.98 Hz, H-2′), 3.81 (1H, dd, J = 5.13, 11.46 Hz, H-6), 3.75 (1H, dd, $J = 2.04$, $J = 12.17$ Hz, H-6), 3.71 (1H, dd, $J = 3.18$, 8.93 Hz, H-3′), 3.63 (1H, dd, $J = 5.56$, 12.04, H-6′), 3.59 (1H, dd, $J = 5.47$, 11.64, H-6′), 3.58 (1H, dd, $J = 3.92$, 9.58, H-3), 3.58–3.51 (3H, m, H-4, H-4′, H-5′), 3.47 (1H, ddd, $J = 1.95$, 5.10, 9.40 Hz, H-5), 2.59–2.52 (4H, m, –CH_2–CH_2–),^{13}C-NMR (150 MHz, D_2O) δ_C 177.66, 175.79 (C=O), 100.10 (C-1′), 78.27, 76.34, 74.01, 73.27, 71.04, 74.01, 70.04, 67,23, 66.89, 66.28 (C-6), 61.44 (C-6′), 30.55, 29.40 (–CH_2–CH_2–). HRMS m/z calcd. for $C_{16}H_{26}NO_{13}$ [M-H$^+$] 440.1399, found 440.1394.

Immobilization of D-mannobiose isomers on beads. To D-mannobiosyl succinylamide derivative (**4**, 2 mg, 4.5 μmol) dissolved in distilled water (300 μL), 8 mg of BlotGlyco™ beads (Sumitomo Bakelite Co. Ltd., Tokyo)[13] and water-soluble carbodiimide (WSC) (86.3 mg, 450 μmol) was added (Fig. 1). The immobilization was conducted by rotation of the mixture at 40 °C for 48 h, and then, the suspension was centrifuged at 2500 × *g* for 30 s. The beads were washed with water (300 μL × 3 times) and methanol (500 μL × 3 times) and finally dried *in vacuo* to obtain D-mannobiose-linked BlotGlyco™ beads (**5**) with keeping its cyclic conformation of D-mannose residue at the reducing end (Fig. 1).

Determination of amounts of D-mannose linked to beads. The amount of D-mannobiose linked to BlotGlyco™ beads was determined as follows. The disaccharide-linked beads (**5**, 0.9–2.3 mg) were placed into 4 M CF_3COOH (300 μL) in an Eppendorf tube. After incubating the tube at 100 °C for 3 h, N_2 gas was flashed into the tube for evaporation of CF_3COOH and water. D-Mannose residues released were dissolved in distilled water (200 μL), and the sample was filtered through a Millex filter (0.45 μm) (Merk Millipore Corp., Billerica, MA) at 700 × g for 30 s. The amount of D-mannose was determined by high-performance liquid chromatography (HPLC) equipped with an evaporative light scattering detector using an Asahi-Pak NH_2-P50^{TM} column (Showa Denko K.K., Tokyo).

Culture and labeling with a fluorescent dye of bacteria. *E. coli* (strain W3110)[14] donated by Dr. M. Fukuda of Nagaoka University of Technology was grown in a Luria–Bertani medium at 37 °C for 14–20 h by shaking. After centrifugation, the bacteria were suspended in Diluent C (1 mL) from the cell membrane labeling kit, mixed with 10 μL of PKH67 solution (4 μL of PKH67 mixed with 996 μL of Diluent C), and incubated at 37 °C for 10 min to label the bacteria with the fluorescent dye according to the manufacturer's protocol. In this study, more than 90% of *E. coli* was labeled with the dye and alive during the period of experiments, which was confirmed by determining the growth rate of the fluorescence-labeled *E coli* in culture. Higher percentages of cells labeled with this dye together with a longer survival time of the dye-labeled cells were reported.[15]

Assay for binding of bacteria to D-mannobiose-linked beads. Fluorescence-labeled *E. coli* suspended in a Luria-Bertani medium (OD600 = 0.1, 200 μL) was mixed with 1 mg of D-mannobiose-linked beads suspended in 40 μL of 10 mM phosphate-buffered saline (pH 7.4) (PBS) in a glass-bottom dish and incubated at 37 °C for 1–2 h. Then, beads were washed with PBS (200 μL) three times and suspended in PBS (200 μL) for observation under a confocal laser microscope. In some experiments, the bacteria and the beads were incubated in the presence of 0.1 M methyl α-D-mannopyranoside.

Results and discussion

Preparation of D-mannobiose isomer-linked beads

In order to determine the binding preference of *E. coli* with type 1 fimbria among D-mannobiose

isomers, Manα1→2Man, Manα1→3Man, and Manα1→6-Man were synthesized by HCl-catalyzed condensation reaction,[10] and Manα1→4-Man were commercially obtained. Individual D-mannobiose isomers were mixed with monobenzyl succinic acid in the presence of 4-(4,6-dimethoxy-1,3,5-triazin–2-yl)–4-methylmorpholinium chloride n-hydrate, and then, the benzyl group was removed by hydrogenolysis to obtain D-mannobiosyl succinylamide derivatives. The ^{1}H-NMR spectra of D-mannobiosyl succinylamide derivatives (Fig. 2(a–d)) showed two sets of signals derived from α-anomer (1α) and β-anomer (1β) at the reducing end and at the non-reducing end, the latter of which is indicated with an asterisk shown in Fig. 2. The assignment of H-1 signals was performed by nuclear Overhauser effect techniques in NMR spectroscopy. The H-1 signal giving a cross-peak with H-5 proton signal was assigned to be axial, which indicates that the succinylamide group was conjugated in a β-linkage to the D-mannose residue. Contrary to the empirical rule in the glycosylation of D-mannose, the amount of the β-linked isomer was more than that of the α-linked isomer, and furthermore, the coupling constant between H-1α and H-2 was larger than that between H-1β and H-2 in all the derivatives. For all the succinylamide derivatives of D-mannobiose isomers, the percentages of the β-isomers were 82–93% by calculating the peak areas of the proton signals at individual reducing ends. The α-/β-isomer ratios obtained by ^{1}H-NMR and HPLC analyses are shown in Table 1.

D-Mannobiosyl succinylamide derivatives were mixed with BlotGlyco™ beads. The immobilization of them on the beads was conducted according to the reaction described in Fig. 1. Although the protocol of

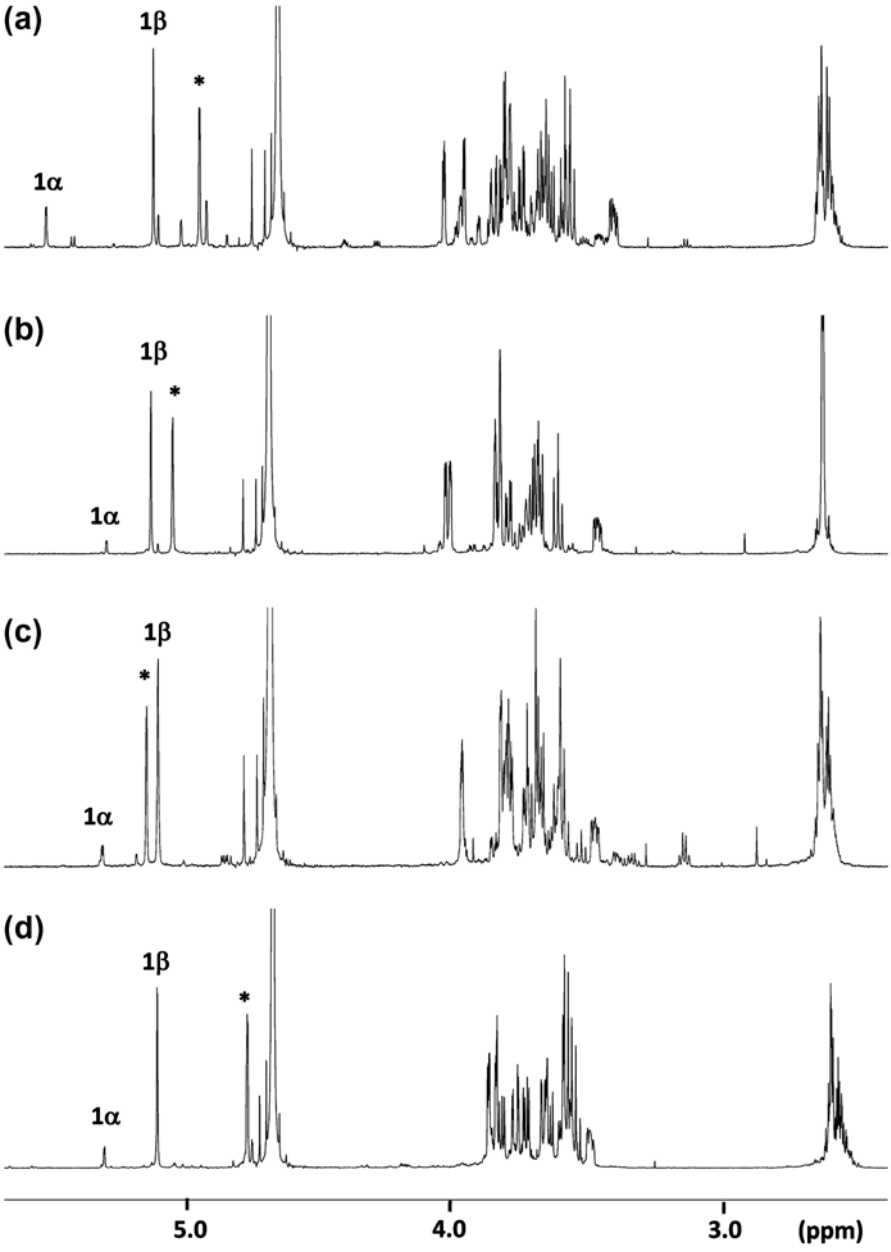

Fig. 2. 600 MHz ^{1}H-NMR spectra of D-mannobiosyl succinyl-amide derivatives. Panels (a), (b), (c), and (d) indicate Manα1→2Man1-, Man-α1→3Man1-, Manα1→4-Man-1-, and Manα1→6Man1-succinyl-amide derivatives, respectively. The 1α and 1β indicate H-1α and H-1β proton signals at the reducing end, respectively. *, a signal from the H-1 proton at the non-reducing end.

Table 1. The α/β percentages of D-mannobiosyl succinylamide derivative **4**'s.

Sugar part of compound **4**	NMR[a]		HPLC[b]	
	α	β	α	β
Manα1→2Man	18	82	11	89
Manα1→3Man	9	91	10	90
Manα1→4Man	12	88	13	87
Manα1→6Man	11	89	10	90

[a]Determined from integral intensity of each H-1 signal in NMR spectra.
[b]Determined from height of each peak in HPLC chart.

BlotGlyco™ beads shows the coupling between a hydrazino group on the bead and an aldehyde group of the reducing end of glycan,[13] the present method was to couple the carboxyl group of D-mannobiosyl succinylamide derivatives to a hydrazino group on the bead with an aid of WSC to establish a hydrazide linkage. As the reaction was to hydrazino groups on the bead, a 100-fold higher amount of WSC than that of bead was used. The amount of D-mannobiose isomers linked to BlotGlyco™ beads was determined, which is described in detail in the *materials and methods* section, and 0.11–0.16 µmol of D-mannose was linked to 1 mg of the beads (Table 2). As the amount of hydrazino group expressed on the bead is shown to be 1–2 µmol/mg bead according to the manufacturer's protocol,[13] the obtained values in Table 2 appeared reasonable.

Binding of E. coli *to D-mannobiose isomer-linked beads*

E. coli with type 1 fimbria is shown to bind to α-D-mannose-containing glycans.[4,5,9] Our preliminary study showed that the bacteria bind to D-mannobiose-linked beads stronger than D-mannose-linked beads. In order to examine whether *E. coli* shows binding preference to a D-mannobiose isomer with a certain structure, the fluorescence-labeled bacteria were incubated with Manα1→2Man-1→beads, Manα1→3-Man1→beads, Manα1→4Man1→beads, or Manα1→6Man-1→beads, and the reaction mixtures were observed under a confocal laser microscope for counting the numbers of the dots associated with the circumferences of the beads. The bacteria bound to all types of D-mannobiose isomer-linked beads (Fig. 3). In the case of Manα-1→2-Man1→beads, the average number of the dots associated with the beads was 52 dots/bead, and more dots whose numbers were unable to be counted were associated with the two beads shown in Fig. 3(a). When the numbers of the fluorescent dots associated with other types of the beads were counted, the average numbers of them were 26 dots/bead in the case of Manα1→3-Man-1→bead (Fig. 3(b)), 16 dots/bead in the case of Manα1→4-Man1→bead (Fig. 3(c)), and 10 dots/bead in the case of Manα1→6Man1→bead (Fig. 3(d)), respectively. The binding assay was conducted three times, and similar binding preference to those shown in Fig. 3 was obtained. When the bacteria were incubated with Manα1→2Man-1→beads in the presence of 0.1 M methyl α-D-mannopyranoside, most of them failed to bind to the beads (Fig. 3(e)). The same results as that shown in Fig. 3(e) were obtained for *E. coli* using Manα1→3Man1→beads, Manα1→4-Man-1→beads, and Manα1→6Man1→beads, respectively, in the presence of the haptenic sugar (data not shown). These results indicate that *E. coli* with type 1 fimbria binds to D-mannobiose-linked beads through its type 1 adhesion and that the bacteria have a preference to bind to Manα1→2Man disaccharide among the isomers. There are many fluorescent dots not bound to the beads in Fig. 3. They can be removed from the reaction mixtures by harsh washings with PBS. However, such washings also take the beads out and reduce the number of the beads to be observed. Another approach to reduce the background fluorescent dots is to decrease the number of *E. coli* mixed with the beads. However, in this case, it also decreases the number of the fluorescent dots associated with the beads. Therefore, the present assay condition is suitable for determining the binding preference of *E. coli* among D-mannobiose isomers. Furthermore, once the bacteria bind to these beads, they are not released from the beads under the present condition unless the haptenic sugar is included in the reaction mixture. Therefore, the fluorescent dots in the background are not from those released from the beads.

High mannose-type glycans attached to proteins contain Manα1→2Man disaccharides at the non-reducing termini,[16] and *E. coli* may bind to these high mannose-type glycans expressed in the large intestine where they reside. *E. coli* is quite often contaminated in water for domestic use to cause us serious enterohemorrhagic diseases.[8] If some filtration systems immobilized with the D-mannobiose or high mannose-type glycans are developed and applied to a water supply system to clean, they can trap specifically *E. coli* with type 1 fimbria in water and provide us with safe water. In some underdeveloped countries, local residents are still facing to serious infectious diseases with *E. coli* contaminated in water for domestic use. It has been reported that around 100 bacteria are contaminated in 1 mL of water.[17] Our preliminary study

Table 2. Amounts of D-mannose liberated from sugar-linked BlotGlyco™ beads.

	Beads[a] (mg)	D-mannose[b] (µg/200 µL)	D-mannose on the beads	
			(µg/mg beads)	(µmol/mg beads)
Manα1→2Man	0.577	32.2	56	0.16
Manα1→3Man	0.576	20.2	37	0.11
Manα1→4Man	0.569	44.0	42	0.12
Manα1→6Man	0.570	28.6	50	0.15

[a]Weights of sugar-linked BlotGlyco™ beads used for the analysis.
[b]Amounts of D-mannose from BlotGlyco™ beads determined by HPLC analysis.

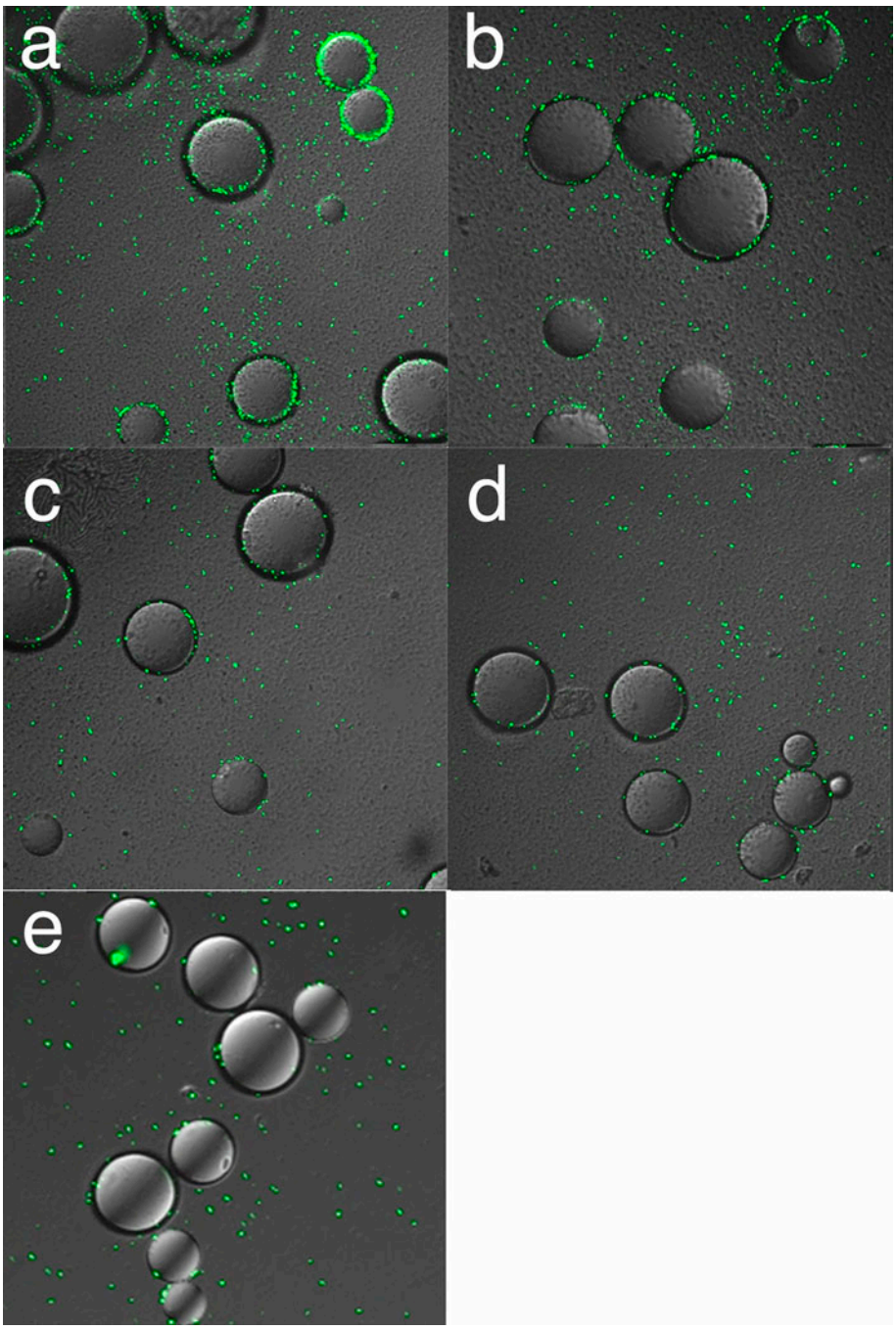

Fig. 3. Incubation of fluorescence-labeled *E. coli* with sugar-linked beads. Panels (a), (b), (c), and (d) indicate the bacteria with Manα1→2-Man1→beads, Manα1→3Man-1→beads, Man-α1→4Man-1→beads, and Manα1→6Man1→beads, respectively, and panel (e) indicates the bacteria with Manα1→2Man1→beads in the presence of 0.1 M methyl α-D-mannopyranoside.

showed that 1 mg of beads immobilized with high mannose-type glycans can trap at least a million of *E. coli*. If such a tool immobilized with Manα1→2Man disaccharide or high mannose-type glycans is used in these countries, it can trap the bacteria in water for domestic use and provide the local residents with safe water.

Author contributions

KA and KF designed the research and wrote the paper. KY, KS, NI, IM, RK, and TM performed the experiments.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the MEXT of Japan under a Grant-in-Aid for Scientific Research [grant number 15K12314] to KF; Grant of Strategic Research Foundation Grant-Aided Project for Private Universities [grant number S1001030] to KA and TM, and by the Japan Science and Technology Agency under a Research Promotion Fund to KF.

References

[1] Varki A, Cummings RD, Esko JD, et al. Essentials of glycobiology. 2nd ed. New York (NY): Cold Spring Harbor Lab Press; 2008.

[2] Couceiro JN, Paulson JC, Baum LG. Influenza virus strains selectively recognize sialyloligosaccharides on human respiratory epithelium; the role of the host cell in selection of hemagglutinin receptor specificity. Virus Res. 1993;29:155–165.

[3] Ilver D, Amrnqvist A, Ogren J, et al. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. 1998;279:373–377.

[4] Ofek I, Beachey EH. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. 1978;22:247–254.

[5] Firon N, Ashkenazi S, Mirelman D, Ofek I, Sharon N. Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated *Escherichia coli* to yeast and intestinal epithelial cells. Infect. Immun. 1987;55:472–476.

[6] Gouin SG, Wellens A, Bouckaert J, Kovensky J. Synthetic multimeric heptyl mannosides as potent antiadhesives of uropathogenic *Escherichia coli*. ChemMedChem. 2009;4:749–755.

[7] Bock K, Breimer ME, Bringnole A, et al. Specificity of binding of a strain of uropathogenic *Escherichia coli* to Galα1-4Gal-containing glycosphingo-lipids. J. Biol. Chem. 1985;260:8545–8551.

[8] Konowalchuk J, Speirs J, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun. 1977;18:775–779.

[9] Klemm P, Schembri MA. Bacterial adhesins: function and structure. Int. J. Med. Microbiol. 2000;290:27–35.

[10] Ajisaka K, Yagura M, Miyazaki T. A novel two-step synthesis of α-linked mannobioses based on an acid-assisted reverse hydrolysis reaction. Carbohydr. Res. 2012;347:147–150.

[11] Likhosherstov LM, Novikova O, Derevitskaja VA, Kochetkov NK. A new simple synthesis of amino sugar β-D-glycosylamines. Carbohydr. Res. 1986;146:C1–C5.

[12] Kunishima M, Kawachi C, Hioki K, Terao K, Tani S. Formation of carboxamides by direct condensation of carboxylic acids and amines in alcohols using a new alcohol- and water-soluble condensing agent: DMT-MM. Tetrahedron. 2001;57:1551–1558.

[13] Protocol for on "BlotGlycoTM Glycan purification and labeling kit". Sumitomo Bakelite Co., Ltd. (2012).

[14] Bachmann BJ. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. 1972;36:525–557.

[15] Poon RYM, Ohlsson-Wilhelm BM, Bagwell CB, Muirhead KA. Use of PKH membrane intercalating dyes to monitor cell trafficking and function. In: Diamond RA, DeMaggio S, editors. In living color. New York (NY): Springer-Verlag; 2000. p. 302–352.

[16] Liang C-J, Yamashita K, Kobata K. Structural study of the carbohydrate moiety of bovine pancreatic ribonuclease B. J. Biochem. 1980;88:51–58.

[17] Lee J-w. World Health Organization. Water, Sanitation, and Hygiene Links to Health, Facts and Figures-updated. November 2004.