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Large-scale production of GDP-fucose and Lewis X by bacterial coupling

S Koizumi, T Endo, K Tabata[1], H Nagano[1], J Ohnishi and A Ozaki[1]

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3-6-6, Asahi-machi, Machida, Tokyo 194-8533, Japan

A large-scale production system of GDP-fucose (GDP-Fuc) and fucosylated oligosaccharides was established by the combination of recombinant *Escherichia coli* cells overexpressing GDP-Fuc biosynthetic genes and *Corynebacterium ammoniagenes* cells. *E. coli* cells overexpressed the genes for glucokinase, phosphomannomutase, mannose-1-phosphate guanylyltransferase, GDP-mannose (GDP-Man) dehydratase, and GDP-4-keto-6-deoxy-mannose (GKDM) epimerase/reductase as well as phosphoglucomutase and phosphofructokinase. *C. ammoniagenes* contributed to the formation of GTP from GMP. GDP-Fuc accumulated to 29 mM (18.4 g l^{-1}) after a 22-h reaction starting with GMP and mannose through introducing the two-step reaction to overcome the inhibition of GDP-Fuc on GDP-Man dehydratase activity. When *E. coli* cells overexpressing the α1,3-fucosyltransferase gene of *Helicobacter pylori* were put into the GDP-Fuc production system, Lewis X [Galβ1–4(Fucα1–3)GlcNAc] was produced at an amount of 40 mM (21 g l^{-1}) for 30 h from GMP, mannose, and *N*-acetyl lactosamine. The production system through bacterial coupling can be applied to the industrial manufacture of fucosylated oligosaccharides.
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Keywords: enzymatic synthesis; oligosaccharide; metabolic engineering; sugar nucleotide; GDP-fucose; Lewis X

Introduction

The Lewis blood group antigens, Lewis X (Le^x), Lewis Y (Le^y), Lewis a, Lewis b, and their sialylated derivatives such as sialyl Lewis X (sLe^x) that contain fucose moieties play important roles in various types of biochemical recognition processes [10,21]. In mammals, Le^x is a stage-specific embryonic antigen, and Le^x, sLe^x, and Le^y are all regarded as tumor-associated markers [4,7]. sLe^x mediates cell to cell adhesion through interaction with selectins [17]. It has been proposed that Le^x plays a similar function during physiological and pathological processes [8].

Even now, large-scale syntheses of oligosaccharides containing fucose remain extraordinarily difficult. Chemical synthesis of Le^x was carried out at a gram scale; however, it required multiple protection and deprotection steps [12]. Enzymatic synthesis using fucosyltransferases circumvents the drawbacks; nevertheless, it requires GDP-fucose (GDP-Fuc) as a substrate [9,13]. GDP-Fuc is one of the most expensive and unavailable sugar nucleotides, although chemical, enzymatic, and microbiological methods have been reported [1,9,22]. Although an efficient chemoenzymatic synthesis of Le^x and sLe^x with *in situ* regeneration of GDP-Fuc was reported, it required expensive starting materials, such as phosphoenolpyruvate, and several enzyme preparations [9].

We described a system for large-scale production of sugar nucleotides such as UDP-galactose [11], UDP-*N*-acetylglucosamine [20], and CMP-*N*-acetylneuraminic acid [3] using recombinant *Escherichia coli* cells that overexpressed the sugar nucleotide biosynthetic genes and *Corynebacterium ammoniagenes* cells. High level accumulations of oligosaccharides were also confirmed when *E. coli* cells expressing bacterial glycosyltransferase genes were put into the sugar nucleotides production system [2,3,11].

In this paper, we describe the strategy for GDP-Fuc production from inexpensive and readily available starting materials through the combination of metabolically engineered *E. coli* and *C. ammoniagenes*. *E. coli* cells were engineered to overexpress GDP-Fuc biosynthetic genes, whereas *C. ammoniagenes* contributed to the formation of GTP from GMP. Moreover, Le^x [Galβ1–4(Fucα1–3)GlcNAc] was produced in a large quantity from GMP, mannose, and *N*-acetyl lactosamine by coupling *E. coli* cells that expressed the α1,3-fucosyltransferase gene of *Helicobacter pylori* with the GDP-Fuc production system.

Materials and methods

Bacterial strains and culture conditions

E. coli NM522 (Stratagene, La Jolla, CA) was used for DNA manipulation in *E. coli*. The cultivations of *E. coli* and *C. ammoniagenes* DN510 in a jar fermenter were carried out as described before [5,11]. *E. coli* cells harboring a plasmid containing P_L promoter from phage lambda were grown at 30°C for 5 h and followed at 40°C for 3 h, and cells harboring a plasmid containing the tryptophan promoter were grown at 37°C. Cells were collected by centrifugation (12,000×*g* for 15 min) and stored at −20°C.

Plasmids and DNA manipulation

The plasmid pPA31 and its derivative, pPAC31 [11], which contains the replication origin and ampicillin resistance gene from pBR322 and P_L promoter, and pTrS31 that is a derivative of pKYP200 [16] containing the tryptophan promoter were used for the construction of the expression plasmids. The *Eco*RI–*Xho*I

Correspondence: S Koizumi, Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3-6-6, Asahi-machi, Machida, Tokyo 194-8533, Japan

[1]Present address: Technical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1-1 Kyowa-cho, Hofu, Yamaguchi 747-8522, Japan
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fragment of pPAC31 containing the temperature-sensitive cI857 repressor from phage lambda was inserted between *Eco*RI and *Sal*I sites of plasmid pSTV28 that contains the replication origin of p15A and chloramphenicol resistance gene (Takara Shuzo, Japan) to form pNT40. The phosphomannomutase gene (*manB*) and mannose-1-phosphate guanylyltransferase gene (*manC*) were amplified by polymerase chain reaction (PCR) using *E. coli* W3110 chromosomal DNA as a template, and 5′-CGTCAATCGATAAGCTTAAATGATATTCGGGGATAAT-3′ (*Cla*I cleavage site underlined) and 5′-AGGGAGGATCCGACATTACTCGTTC-3′ (*Bam*HI cleavage site underlined) as primers [18]. The 3.0-kb PCR product was digested with *Cla*I and *Bam*HI and inserted between the *Cla*I and *Bam*HI sites of pPAC31 to give pNK7. The glucokinase gene (*glk*) was amplified by PCR from the plasmid pNT46 using 5′-CCGCAAGATCTCGTAAAAAGGGTATCGATAAGC-3′ (*Bgl*II cleavage site underlined) and 5′-GAGCTGACTGGGTTGAAGGC-3′ as primers [20]. The PCR product was digested with *Bgl*II and *Sal*I and inserted between the *Bam*HI and *Sal*I sites of pNK7 to give pNK11 that overexpressed *manB*, *manC*, and *glk*. The GDP-mannose (GDP-Man) dehydratase gene (*gmd*) and GKDM epimerase/reductase gene (*wcaG*) were amplified by PCR from *E. coli* W3110 using 5′-TTGGGAAGCTTCCGGCAAATGTGGTTT-3′ (*Hin*dIII cleavage site underlined) and 5′-ATAAACTCGAGAGAGACAAGCGGAG-3′ (*Xho*I cleavage site underlined) as primers [18]. The 2.6-kb PCR product was digested with *Hin*dIII and *Xho*I and inserted between the *Hin*dIII and *Sal*I sites of pPA31 to give pNK8. The GDP-Man dehydratase gene (*gmd*) was amplified by PCR from *E. coli* W3110 using 5′-GAATCTAGAATGTCAAAAGTCGCTCTC-3′ (*Xba*I cleavage site underlined) and 5′-CTCAAGCTTATGACTCCAGCGCGAT-3′ (*Hin*dIII cleavage site underlined) as primers [18]. The 1.1-kb PCR product was digested with *Xba*I and *Hin*dIII. The tryptophan promoter was amplified by PCR from plasmid pTrS31 using 5′-CAAGAATTCTCATGTTTGACAGCT-3′ (*Eco*RI cleavage site underlined) and 5′-CATTCTAGACCTCCTTAATTCGCGAAAATGGATCGATACCCTTTTTAC-3′ (*Xba*I cleavage site underlined) as primers. The 0.4-kb PCR product was digested with *Eco*RI and *Xba*I. The above two DNA fragments were inserted between the *Eco*RI and *Hin*dIII sites of pBluescriptII SK+ to give pGE19. The GKDM epimerase/reductase gene (*wcaG*) was amplified by PCR from *E. coli* W3110 using 5′-GTCATCGATATGAGTAAACAACGAGTT-3′ (*Cla*I cleavage site underlined) and 5′-ATAAACTCGAGAGAGACAAGCGGAG-3′ (*Xho*I cleavage site underlined) as primers [18]. The 1.0-kb PCR product was digested with *Cla*I and *Xho*I and inserted between the *Cla*I and *Sal*I sites of pPAC31 to give pGE8. The plasmid pNT24 [20] containing the phosphoglucomutase gene (*pgm*) was digested with *Xho*I and *Bam*HI and inserted between the *Sal*I and *Bam*HI sites of pSTV28 to give pNT53. The phosphofructokinase gene (*pfkB*) was amplified by PCR from the plasmid pNT47 [7] using 5′-CCGCAAGATCTCGTAAAAAGGGTATCGATAAGC-3′ (*Bgl*II cleavage site underlined) and 5′-TTTTTGATATCCCCAATGCTGGGGGTTTTTG-3′ (*Eco*RV cleavage site underlined) as primers. The 1.3-kb PCR product was digested with *Bgl*II and *Eco*RV and inserted between the *Bam*HI and *Eco*RV sites of pNT53 to form pNT55. The α1,3-fucosyltransferase gene (*fucT*) was amplified by PCR from *H. pylori* (NCTC11637, National Collection of Type Cultures, UK) using 5′-AGGAAGCTTATGTTCCAACCCCTATTAGAC-3′ (*Hin*dIII cleavage site underlined) and 5′-TAGGGATCCGGGTTTGATGGGTTTGTT-3′ (*Bam*HI cleavage site underlined) as primers [6,14]. The 1.4-kb PCR product was digested with *Hin*dIII and *Bam*HI and inserted between the *Hin*dIII and *Bam*HI sites of pPA31 to give pPFT7.

GDP-Fuc production

The production of GDP-Fuc was carried out on a 30-ml scale in a 200-ml vessel containing the ingredients (g l^{-1}): *C. ammoniagenes* cells, 150 (cell concentrations were calculated by wet weight); NM522/pNK11/pNT55, 25; NM522/pGE19, 15; NM522/pGE8, 25; fructose, 60; mannose, 30; KH_2PO_4, 25; $MgSO_4 \cdot 7H_2O$, 5; GMP (2Na, $7H_2O$), 30; phytic acid, 5; Nymeen S-215 (polyoxyethylene octadecylamine; Nippon Oil and Fats, Tokyo, Japan), 4; and xylene, 10. The reaction was carried out at 32°C with agitation (900 rpm) using a magnetic stirrer, and the pH was kept at 7.2 with 4 N NaOH.

Isolation of GDP-Fuc

The reaction mixture was centrifuged at 12,000×*g* for 20 min at 4°C to remove the cells. The supernatant containing 100 mg of GDP-Fuc was applied to an activated charcoal column (14×70 mm). The column was washed with 15 bed volumes of H_2O and then washed again with 18 bed volumes of 5% ethanol to wash out inorganic salts, GMP, and monosaccharides. GDP-Fuc was eluted with five bed volumes of 40% ethanol. Nucleotides other than GDP-Fuc were further removed by means of gel filtration with Bio-Gel P-2 (25×900 mm, BioRad, Hercules, CA). Fractions containing GDP-Fuc (17 mg) were collected and freeze-dried.

Le^x production

The production of Le^x was carried out on a 30-ml scale in a 200-ml vessel containing the ingredients (g l^{-1}): *C. ammoniagenes* cells, 150 (cell concentrations were calculated by wet weight); NM522/pNK11/pNT55, 25; NM522/pGE19, 15; NM522/pGE8, 15; NM522/pPFT7, 25; fructose, 60; mannose, 30; *N*-acetyl lactosamine (LacNAc), 40; KH_2PO_4, 25; $MgSO_4 \cdot 7H_2O$, 5; GMP (2Na, $7H_2O$), 30; phytic acid, 5; Nymeen S-215, 4; and xylene, 10. NM522/pGE8 and NM522/pPFT7 were added to the reaction mixture after 12 h. The reaction was carried out at 32°C with agitation (900 rpm) by the magnetic stirrer, and the pH was kept at 7.2 with 4 N NaOH.

Isolation of Le^x

The reaction mixture was centrifuged at 12,000×*g* for 20 min at 4°C to remove the cells. The supernatant containing 237 mg of Le^x was applied to an activated charcoal column (14×70 mm). The column was washed with 10 bed volumes of H_2O and then washed again with 10 bed volumes of 5% ethanol to wash out inorganic salts, GMP, monosaccharides, and LacNAc. Le^x was eluted with five bed volumes of 25% ethanol. Carbohydrates other than Le^x were further removed by means of gel filtration with Bio-Gel P-2 (25×900 mm). Fractions containing Le^x (75 mg) were collected and freeze-dried.

Analytical procedures

The concentrations of GDP-Fuc, GDP-Man, GKDM, and GMP in the reaction mixture were determined by the method described before [20]. Le^x and other carbohydrates were measured by means of high-performance anion exchange chromatography with pulsed

amperometric detection (HPAEC/PAD) using a Dionex DX-500 system on a carbopac PA10 column (Dionex, Sunnyvale, CA). A sample (20 μl) was injected and eluted with a gradient of sodium hydroxide from 40 to 200 mM in 21 min with a flow rate of \$ min^{-1}. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in D_2O with a JEOL JNM-A400, operated at a frequency of 400 and 100 MHz, respectively, and fast atom bombardment (FAB) mass spectroscopy was performed with a JEOL JMS-HX/HX110A.

Results and discussion

Production of GDP-Fuc

The production of GDP-Fuc was carried out by the combination of *C. ammoniagenes* cells and *E. coli* cells expressing *glk*, *manB*, *manC*, *gmd*, *wcaG*, *pgm*, and *pfkB* as shown in Figure 1. The genes of *manB*, *manC*, *gmd*, and *wcaG*, which are involved in the biosynthesis of GDP-Fuc, and part of the biosynthetic genes of extrapolysaccharides colanic acid of *E. coli* [18] were isolated from chromosomal DNA of *E. coli*. Phosphomannomutase requires glucose-1,6-diphosphate (Glc-1,6-P_2) as a cofactor to express its activity, as is generally observed in hexose phosphate mutases [15]. Glc-1,6-P_2 was supplied by the activities of phosphoglucomutase and phosphofructokinase [20]. *C. ammoniagenes* has a strong activity for conversion of GMP to GTP. The conversion of GMP to GTP is an energy-dependent reaction; therefore, fructose was added to the reaction mixture as an energy source, as was potassium dihydrogen phosphate and magnesium sulfate. Phytic acid was added as a chelator to avoid precipitation from magnesium and phosphate. Both *C. ammoniagenes* cells and *E. coli* cells were permeabilized with a surfactant (polyoxyethylene octadecylamine, Nymeen S-215, 4 g l^{-1}) and xylene added to the reaction mixture. Starting with 56 mM GMP and 167 mM mannose, 5.9 mM (3.7 g l^{-1}) of GDP-Fuc was accumulated in the reaction mixture after 22 h, whereas the accumulation of GDP-Man reached 15.6 mM (Figure 2a). The high accumulation of GDP-Man was thought to be due to inhibition of GDP-Man dehydratase by GDP-Fuc [19]. Therefore, in order to overcome inhibition by GDP-Fuc on GDP-Man dehydratase, a two-step reaction that consisted of the formation of GKDM, a precursor of GDP-Fuc, and the conversion to GDP-Fuc from GKDM was proposed. Along with the proposed scheme, firstly, GKDM accumulated to 29 mM for 12 h by the activities of *C. ammoniagenes* cells and *E. coli* cells expressing *glk*, *manB*, *manC*, *gmd*, *pgm*, and *pfkB*. After *E. coli* cells that overexpressed the *wcaG* gene were put into the reaction mixture, the accumulated GKDM was converted to GDP-Fuc. In the two-step reaction, GDP-Fuc accumulated at a level of 29 mM (18.4 g l^{-1}) after 22 h (Figure 2b). The yield of GDP-Fuc was 52% from GMP and 17% from mannose. When the reaction was carried out

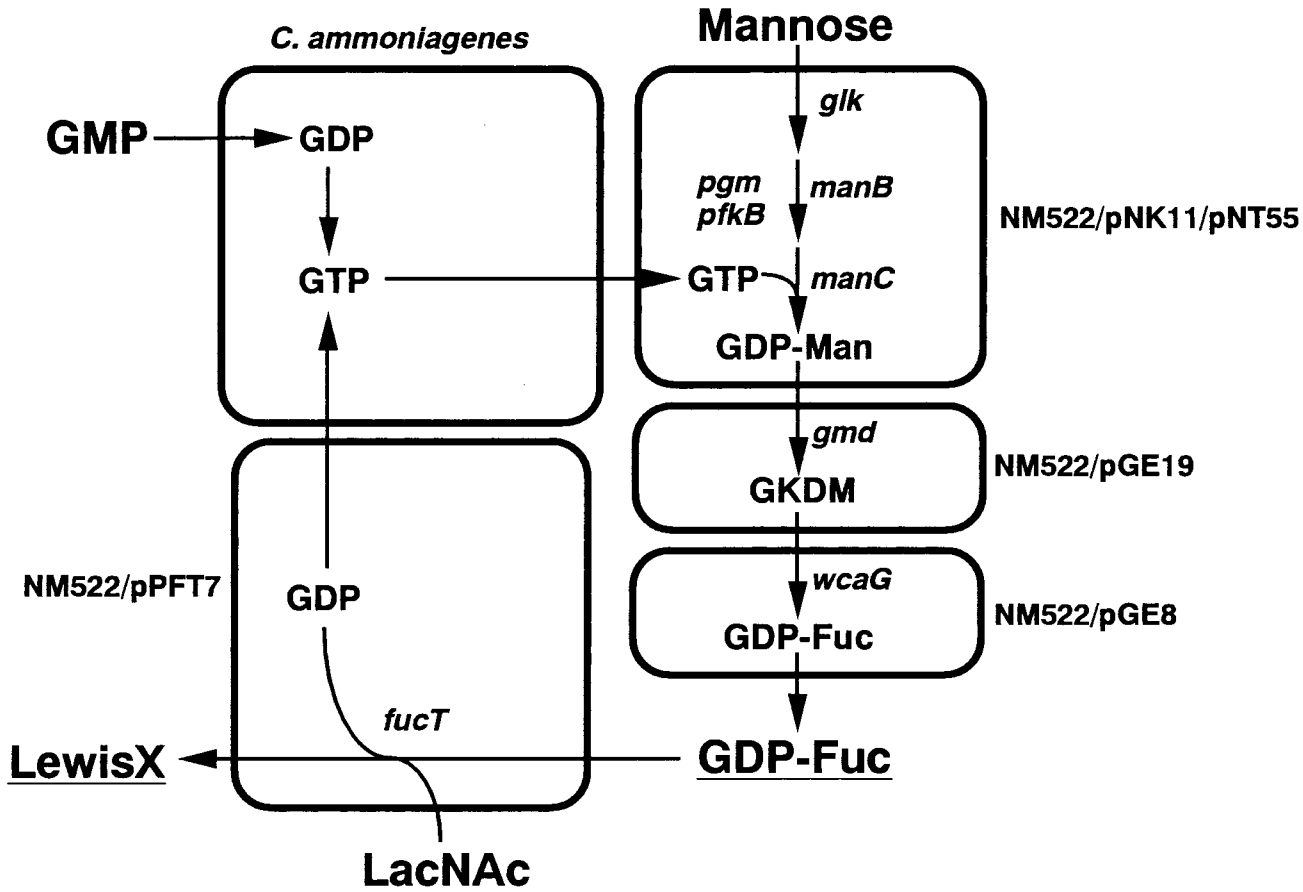

Figure 1 The production system of GDP-Fuc and Le^x. *E. coli* cells expressed glucokinase (*glk*), phosphomannnomutase (*manB*), mannose-1-phosphate guanylyltransferase (*manC*), phosphoglucomutase (*pgm*), phosphofructokinase (*pfkB*), GDP-Man dehydratase (*gmd*), GKDM epimerase/reductase (*wcaG*), and the α1,3-fucosyltransferase gene (*fucT*). *C. ammoniagenes* cells produce GTP from GMP.

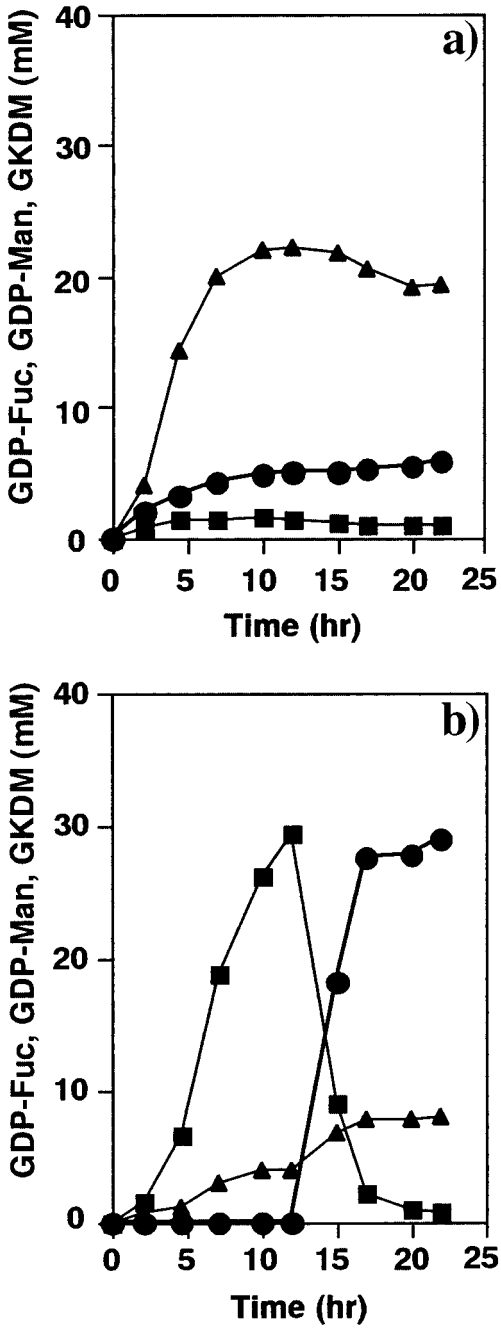

Figure 2 Time course for GDP-Fuc production using *C. ammoniagenes* cells and *E. coli* cells that expressed the genes involved in the biosynthesis of GDP-Fuc from GMP, mannose, and fructose. (a) One-step reaction. (b) Two-step reaction. The values represent the accumulation of GDP-Fuc (●), GDP-Man (▲), and GKDM (■).

on a 15-l scale in a 30-l fermenter, 12.5 g l^{-1} of GDP-Fuc was produced after 25 h.

Structural analysis of GDP-Fuc

The negative ion mode FAB mass spectrum of GDP-Fuc isolated from the reaction mixture showed an intense signal at m/z 588.0, corresponding to GDP-Fuc (calculated mass 589.4). The structure of GDP-Fuc was identified by comparison to previously described ^{1}H NMR and ^{13}C NMR data [12]: ^{1}H NMR: 8.15 (s, 1H, H-8), 5.96 (d, 1H, $J_{1',2'}$ 6.1 Hz, H-1′), 4.83 (br. dd, 1H, $J_{1',2'}$ 6.1 Hz, $J_{2',3'}$ 5.3 Hz, H-2′), 4.56 (dd, 1H, $J_{2',3'}$ 5.3 Hz, $J_{3',4'}$ 3.3 Hz, H-3′), 4.38 (m, 1H, H-4′), 4.24 (dd, 2H, $J_{4',5'}$ 3.4 Hz, $J_{5',P}$ 5.4 Hz, H-5′), 4.95 (dd, 1H, $J_{1'',2''}$ 7.9 Hz, $J_{1'',P}$ 8.1 Hz, H-1″), 3.59 (dd, 1H, $J_{1'',2''}$ 7.9 Hz, $J_{2'',3''}$ 10.0 Hz, H-2″), 3.69 (dd, 1H, $J_{2'',3''}$ 10.0 Hz, $J_{3'',4''}$ 3.4 Hz, H-3″), 3.74 (br. d, 1H, $J_{3'',4''}$ 3.4 Hz, H-4″), 3.80 (dq, 1H, $J_{4'',5''}$ 0.9 Hz, $J_{5'',6''}$ 6.5 Hz, H-5″), 1.26 (d, 3H, $J_{5'',6''}$ 6.5 Hz, H-6″); ^{13}C NMR: 154.8 (s, C-2), 152.7 (s, C-4), 117.2 (s, C-5), 159.8 (s, C-6), 138.6 (d, C-8), 87.6 (d, C-1′), 74.4 (d, C-2′), 71.3 (d, C-3′), 84.7 (d, J_{CP} 9.1 Hz, C-4′), 66.2 (t, J_{CP} 5.8 Hz, C-5′), 99.3 (d, J_{CP} 5.8 Hz, C-1″), 71.9 (d, J_{CP} 7.4 Hz, C-2″), 73.4 (d, C-3″), 72.3 (d, C-4″), 72.0 (d, C-5″), 16.3 (q, C-6″).

Production of Le^{x}

The production of Le^{x} was examined by adding *E. coli* cells harboring pPFT7 that expressed the α1,3-fucosyltransferase gene of *H. pylori* to the GDP-Fuc production system (Figure 1). LacNAc was added to the reaction mixture as a substrate. As the result of the cellular reaction for 30 h, 40 mM (21 g l^{-1}) of Le^{x} was produced from 167 mM mannose and 100 mM LacNAc (Figure 3). The yield of Le^{x} was 24% from mannose and 40% from LacNAc. Considering the remaining sugars in the reaction mixture, the actual yield of Le^{x} was 75% from LacNAc, which was the most expensive starting material. In the case of Le^{x} production, the accumulation of Le^{x} continued until 30 h unlike the GDP-Fuc production because the biosynthesis of GDP-Fuc was not inhibited due to the relatively low level of GDP-Fuc. Almost no peaks other than mannose, LacNAc, fructose, and Le^{x} were observed after 30 h when analyzed by HPAEC/PAD.

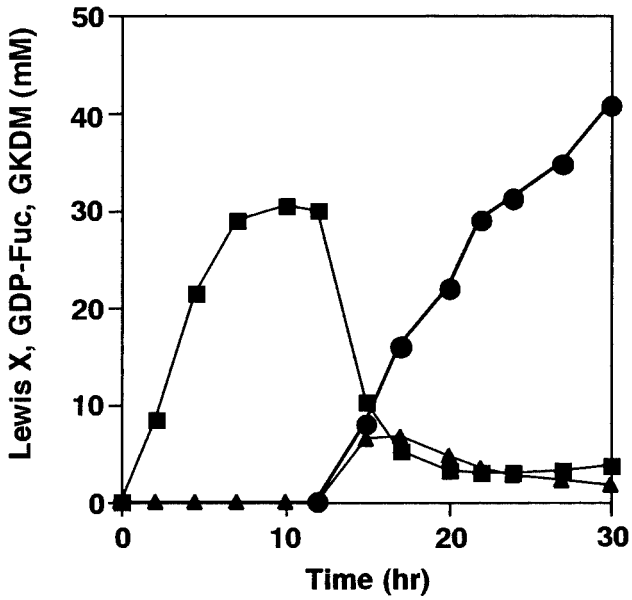

Figure 3 Time course for Le^{x} production using *C. ammoniagenes* cells and *E. coli* cells that expressed the genes involved in the biosynthesis of GDP-Fuc and the α1,3-fucosyltransferase from GMP, mannose, fructose, and *N*-acetyl lactosamine. The values represent the accumulation of Le^{x} (●), GDP-Fuc (▲), and GKDM (■).

Structural analysis of Le^x

The negative ion and positive ion mode FAB mass spectra of Le^x isolated from the reaction mixture showed an intense signal at m/z 528 and 530, respectively, corresponding to Le^x (calculated mass 529). The NMR data of Le^x were in agreement with literature values [1]: ^{1}H NMR: α-anomer, 5.12 (d, 1H, $J_{1,2}$ 3,4 Hz, H-1), 4.17 (dd, 1H, $J_{1,2}$ 3.4 Hz, $J_{2,3}$ 10.4 Hz, H-2), 4.03 (m, 1H, H-3), 3.99 (m, 1H, H-4), 4.03 (m, 1H, H-5), 3.95 (m, 2H, H-6a, H-6b), 2.05 (s, 3H, CH_3CO-), 4.48 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1′), 3.53 (dd, 1H, $J_{1',2'}$ 7.8 Hz, $J_{2',3'}$ 9.9 Hz, H-2′), 3.67 (m, 1H, H-3′), 3.92 (d, 1H, $J_{3',4'}$ 3.0 Hz, H-4′), 3.62 (m, 1H, H-5′), 3.76 (m, 2H, H-6a′, H-6b′), 5.13 (d, 1H, $J_{1'',2''}$ 3.7 Hz, H-1″), 3.71 (m, 1H, H-2″), 3.92 (m, 1H, H-3″), 3.81 (d, 1H, $J_{3'',4''}$ 2.9 Hz, H-4″), 4.85 (q, 1H, $J_{5'',6''}$ 6.6 Hz, H-5″), 1.20 (d, 3H, $J_{5'',6''}$ 6.6 Hz, H-6″); β-anomer, 4.75 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 3.89 (m, 1H, H-2), 3.89 (m, 1H, H-3), 3.99 (m, 1H, H-4), 3.61 (m, 1H, H-5), 3.89 (m, 1H, H-6a), 4.01 (m, 1H, H-6b), 2.05 (s, 3H, CH_3CO–), 4.47 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1′), 3.51 (dd, 1H, $J_{1',2'}$ 7.8 Hz, $J_{2',3'}$ 9.9 Hz, H-2′), 3.67 (m, 1H, H-3′), 3.92 (d, 1H, $J_{3',4'}$ 3.0 Hz, H-4′), 3.62 (m, 1H, H-5′), 3.76 (m, 2H, H-6a′, H-6b′), 5.13 (d, 1H, $J_{1'',2''}$ 3.7 Hz, H-1″), 3.71 (m, 1H, H-2″), 3.92 (m, 1H, H-3″), 3.81 (d, 1H, $J_{3'',4''}$ 2.9 Hz, H-4″), 4.85 (q, 1H, $J_{5'',6''}$ 6.6 Hz, H-5″), 1.19 (d, 3H, $J_{5'',6''}$ 6.6 Hz, H-6″). ^{13}C NMR: α-anomer, 91.88 (d, C-1), 54.89 (d, C-2), 73.63 (d, C-3), 74.13 (d, C-4), 72.14 (d, C-5), 60.52 (t, C-6), 175.02 (s, CH_3CO–), 22.81 (q, *C*H_3CO–), 102.61 (d, C-1′), 71.87 (d, C-2′), 73.28 (d, C-3′), 69.15 (d, C-4′), 75.71 (d, C-5′), 62.30 (t, C-6′), 99.38 (d, C-1″), 68.53 (d, C-2″), 70.09 (d, C-3″), 72.73 (d, C-4″), 67.47 (d, C-5″), 16.09 (q, C-6″); β-anomer, 95.52 (d, C-1), 57.75 (d, C-2), 75.78 (d, C-3), 74.16 (d, C-4), 76.26 (d, C-5), 60.61 (t, C-6), 175.24 (s, CH_3CO–), 23.06 (q, *C*H_3CO–), 102.64 (d, C-1′), 71.84 (d, C-2′), 73.28 (d, C-3′), 69.15 (d, C-4′), 75.71 (d, C-5′), 62.30 (t, C-6′), 99.43 (d, C-1″), 68.50 (d, C-2″), 70.04 (d, C-3″), 72.73 (d, C-4″), 67.47 (d, C-5″), 16.09 (q, C-6″).

Conclusions

The coupling of *C. ammoniagenes* cells and recombinant *E. coli* cells that overexpressed the genes involved in GDP-Fuc biosynthesis as well as Glc-1,6-P_2 formation resulted in efficient production of GDP-Fuc, especially in the case of the two-step reaction to overcome the inhibition of GDP-Fuc. When adding *E. coli* cells that expressed the α1,3-fucosyltransferase gene of *H. pylori*, Le^x was produced in a large quantity from cheap starting materials. The strategy of producing GDP-Fuc and Le^x by combining metabolically engineered recombinant *E. coli* with a GTP-producing microorganism should be applied to the manufacture of other fucosylated oligosaccharides and facilitate the research in the field of glycobiology.

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