EXPERIMENTAL PROCEDURES

Preparation of Recombinant Protein – FUT8 was expressed in soluble form, with a C-terminal polyhistidine tag, and purified by Ni^{2+} chelating affinity chromatography as described previously (Ihara *et al.*, 2006). The protein was expressed using a baculovirus/insect cell expression system and purified by Ni^{2+}-chelating affinity chromatography, as described previously (Ihara *et al.*, 2006). Solutions of the purified proteins, at a concentration of 10mg/ml, in 50 mM Tris-HCl buffer at pH8.3, were used for crystallization. Protein concentrations were determined using a BCA Kit (PIERCE, IL, USA), with bovine serum albumin (BSA) as a standard.

Crystallization -- Crystallization of the recombinant FUT8 was performed at 27°C using the hanging drop vapor diffusion method. The drop contained protein at a concentration of 2.5-6.0 mg/ml in 50 mM HEPES-Na, 0.75 M lithium sulfate monohydrate, pH 7.5, and the reservoir solution contained 100 mM HEPES-Na, 1.5 M lithium sulfate monohydrate, pH 7.5. To prepare heavy atom derivatives of protein crystals, the crystals were soaked for 5 hours in the presence of 10 mM potassium tetrachloroplatinate (II) in the same buffer that was used in the reservoir solution. The crystals were then transferred to a cryoprotectant solution containing 20 % glycerol in the reservoir solution to prevent the formation of ice under the cryogenic environment.

X-ray data collection and structure determination -- Crystals were flash-frozen in cryogenic nitrogen gas at 100K, and data sets were then collected at the same temperature. Diffraction data for Native and Pt derivative were collected using an imaging plate detector at the beamline BL44XU at SPring-8. The obtained data were indexed, integrated and scaled using DENZO/SCALEPACK (Otwinowski *et al.*, 1997). There were two non-isomorphous crystals, native1 and native2. The calculated R_{iso} between them was 38.5% (100-2.8Å). The FUT8 crystal used in this study belongs to the $P6_522$ space group with one molecule per asymmetric unit. The unit cell dimensions for the native1 FUT8 crystal using structure determination are: a = 90.46Å, b = 90.46Å, c = 381.9Å. Data

collection and processing statistics for the data sets are summarized in Table 1. Phases were determined by a single isomorphous replacement with anomalous scattering (SIRAS) using the data sets for native1 and Pt derivative. One clear platinum position was found on Harker sections from both the calculated difference and the anomalous Patterson map. The determined platinum position was refined using the program SHARP (de La Fortelle *et al.*, 1997). Density modification with solvent flattening was performed with the program SOLOMON (Abrahams and Leslie, 1996) implemented in SHARP. The resultant experimental map using native1 data was traced and the model building was performed with the program O (Jones *et al.*, 1991). The final model was built using native2 data and refined with program REFMAC5 (Murshudov *et al.*, 1997) and ARP/wARP (Lamzin *et al.*, 2001) in CCP4 suite (Collaborative Computational Project Number 4, 1994). The Ramachan plot was also defined by PROCHECK (Lakowsky *et al.*, 1993). Figures were produced using the UCSF Chimera program (Pettersen *et al.*, 2004).

Cell culture -- COS-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 5 g/l glucose under a humidified atmosphere of 95 % air and 5 % CO_2.

Construction of expression plasmids -- For transient expression in COS-1 cells, a cDNA encoding human FUT8 (Yanagidani *et al.*, 1997) was subcloned into the EcoRI sites of an expression vector, pCXNII (Niwa *et al.*, 1991). In this vector, FUT8 was expressed under the control of the β-actin promoter and the CMV enhancer.

Site-directed mutagenesis -- Site-directed mutagenesis experiments were carried out using the QuikChange site directed mutagenesis kit (Stratagene, La Jolla, CA), as described previously (Ihara *et al.*, 2004). The primers used in this study were 5'-GTCAGACGCACAGCGAAAGTGGGAACAG-3' and

5'-CTGTTCCCACTTTCGCTGTGCGTCTGAC-3' for replacement of Asp-368 (designated as D368A), 5'- GACGCACAGACGCAGTGGGAACAGAAGC-3' and 5'-GCTTCTGTTCCCACTGCGTCTGTGCGTC -3' for replacement of Lys-369 (K369A), and 5'-AAAGTGGGAACAGCAGCTGCCTTCCAT-3' and 5'-ATGGAAGGCAGCTGCTGTTCCACTTT-3' for replacement of Glu-373 (E373A), 5'-CCCATTGAAGAGGCAATGGTGCATGTT-3' and 5'-AACATGCACCATTGCCTCTTCAATGGG-3' for replacement of Tyr-382 (Y382A), 5'-GTATTTGGCCACAGCGGACCCTTCTTTATTAAAGG-3' and 5'-CCTTTAATAAAGAAGGGTCCGCTGTGGCCAAATAC-3' for replacement of Asp-409 (D409A), 5'-GTATTGGCCACAGATGCGCCTTCTTTATTAAAGG-3' and 5'-CCTTTAATAAAGAAGGCGCATCTGTGGCCAAATAC-3' for replacement of Asp-410 (D410A), 5'-GGAGTGATCCTGGCGATACATTTTCTCTC-3' and 5'-GAGAGAAAATGTATCGCCAGGATCACTCC-3' for replacement of Asp-453 (D453A), and 5'-TGTACTTTTTCAGCACAGGTCTGTCGA-3' and 5'-TCGACAGACCTGTGCTGAAAAAGTACA-3' for replacement of Ser-469 (S469A). The resulting mutations were verified by using BigDye® Terminator v3.1 Cycle Sequencing Kit and a DNA sequencer, ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, CA, USA).

DNA transfection -- Expression plasmids were transfected into cells by electroporation (Chu *et al.*, 1987) using a Gene Pulser (Bio-Rad, CA, USA), as described previously (Ihara *et al.*, 2002). In a typical experiment, the cells were washed with HEPES-buffered saline and resuspended in the same solution. Plasmids (30 μg) were added to the cell suspension, followed by electrification. For the transient expression in Huh-6 cells, transfected cells were harvested after an appropriate growth period. The expression of FUT8 was verified by immunoblot analysis and enzyme activity assay for FUT8.

Electrophoresis and Immunoblot analysis -- Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on 8 % gels, according to Laemmli (1970). Immunoblot analyses were performed as described previously (Ihara *et al.*, 2002). The separated proteins were transferred onto a nitrocellulose membrane (PROTORAN, Schleicher & Schuell Inc., NH, USA), and the resulting blot was blocked with 5 % skim milk and 0.5 % BSA in phosphate-buffered saline (PBS) containing 0.05 % Tween-20. The resulting membrane was incubated with the 15C6 antibody against FUT8. The 15C6 antibody is a monoclonal antibody against human and porcine FUT8, and was obtained from Fujirebio Inc. (Japan). After washing with PBS containing 0.05 % Tween-20, the membrane was reacted with a horseradish peroxidase (HRP)-conjugated rabbit anti-(mouse IgG) Ig from Promega (WI, USA). The immuno-reactive protein bands were visualized by HRP reaction using 4-chloro-1-naphtol and 3,3'-diaminobenzidine tetrahydrochloride (Pirece, IL, USA), as substrates.

FUT8 activity assay -- α1,6-Fucosyltransferase activity was assayed using a fluorescence-labeled sugar chain substrate, as described previously (Uozumi *et al.*, 1996; Ihara *et al.*, 2006). Cell lysates were incubated at 37°C with 10 μM of the acceptor substrate and 0.5 mM GDP-fucose as a donor in 0.1 M MES-NaOH, 1% Triton X-100 (pH 7.0). The reactions were terminated by boiling after an appropriate reaction time, and the reaction mixtures were centrifuged at 15,000 x *g* in a microcentrifuge for 10 min. The resulting supernatants were injected to a reversed phase HPLC equipped with TSKgel, ODS 80TM (4.6 x 150 mm) (Tosoh, Tokyo, Japan). The product and substrate were separated isocratically with 20 mM ammonium acetate buffer (pH 4.0) containing 0.15% n-butanol. The fluorescence of the column elute was detected with fluorescence detector (2475 Multi λ Fluorescence Detector, waters, MA, USA) at excitation and emission wavelengths of 315 nm and 380 nm, respectively.

ACKNOWLEDGMENT

We thank Dr. Pradman K. Qasba for his critical reading of the manuscript. This research was supported by The National Project on Protein Structural and Functional Analyses (Priority Research Program, Protein 3000 Project) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by The 21st Century Center of Excellence Program.

ABBREVIATIONS

FUT8, mammalian α1,6-fucosyltransferase; GDP, guanosine 5'-diphosphate; *N*-glycan, asparagines-linked oligosaccharide.

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LEGENDS TO FIGURES

Fig.1. **Reaction catalyzed by FUT8.** FUT8 transfers a fucose residue from GDP-β-L-fucose to the innermost GlcNAc of an Asn-linked oligosaccharide.

Fig.2. **Overall structure of human FUT8.** Stereo view of the overall structure of the catalytic region of FUT8 (PDB No. 2DE0) is indicated by a ribbon diagram. The secondary structures are highlighted, and helices and β-strands are shown in orange and blue respectively. Thr-367 and Glu-373 colored in red means that the residues, Asp-368 to Thr-372 are disordered. All figures in this study were produced using the program, UCSF Chimera (Pettersen *et al.*, 2004).

Fig.3. **The amino acid sequence and secondary structure of human FUT8.** Amino acid residues 68-575, which were examined by structural analysis are shown. Residues 358-370, 403-416 and 451-477, underlined and in red, indicate the three conserved regions among the α1,2-, α1,6- and protein *O*-fucosyltransferases, as shown in Fig.5A. As illustrated in the diagram of the secondary structures above the sequence, the cylinder and arrow denote the helix and β-strand respectively. Some residues indicated in light blue and italic are additional residues for recombinant expression at the N- and C-terminus. The dashed line denotes regions that are disordered in the structural analysis (residues 68-117, 368-372 and 573-575). Helices, α1–11 are α-helices, and 3H1-4 mean 3_{10}-helices.

Fig. 4. **Overlapping of GT-B glycosyltransferase on FUT8.**

Two GT-B glycosyltransferases, in which the Rossmann fold is similar to that of FUT8, were overlapped on FUT8. The main-chains of the enzymes are indicated. Regions with similarities are shown as a ribbon model. ADP-heptose lps heptosyltransferase II (A, PDB No. 1PSW) and Gtfb,