A new \(\beta_1,4\)-N-acetylglucosaminyltransferase (GnT) which involves in branch formation of Asn-linked complex-type sugar chains has been purified 224,000-fold from bovine small intestine. This enzyme requires divalent cations, such as \(\text{Mn}^{2+}\), and catalyzes the transfer of GlcNAc from UDP-GlcNAc to biantennary oligosaccharide with the \(\beta_1-4\)-linked mannosyl residue on the Man\(a_1-3\) arm. The purified enzyme shows a single band of \(M_r 58,000\) and behaves as a monomer. The substrate specificity demonstrated that the \(\beta_1-2\)-linked GlcNAc residue on the Man\(a_1-3\) arm (GnT-I product) is essential for the enzyme activity. \(\beta_1-4\)-Galactosylolloan to this essential \(\beta_1-2\)-linked GlcNAc residue or N-acetylglucosaminylation to the \(\beta_1\)-linked Man residue (bisecting GlcNAc, GnT-III product) blocks the enzyme action, while \(\beta_1-6\)-N-acetylglucosaminylation to the Man\(a_1-6\) arm (GnT-V product) increases the transfer. Based on these findings, we conclude that the purified enzyme is UDP-N-acetylglucosamine: \(\alpha_3\)-d-mannoside \(\beta_1,4\)-N-acetylglucosaminyltransferase IV (GnT-IV), that has been a missing link on biosynthesis of complex-type sugar chains.

The complex-type of oligosaccharides are synthesized through elongation by glycosyltransferases after trimming of the precursor oligosaccharides transferred to proteins in the endoplasmic reticulum. N-Acetylgalactosaminyltransferases (GnTs)\(^1\) take part in the formation of branches in the biosynthesis of complex-type sugar chains. In vertebrates, six GnTs, designated as GnT-I to -VI, which catalyze the transfer of GlcNAc to the core mannosyl residues of Asn-linked sugar chains, have been identified (1).

**INTERACTION I**

The complex-type sugar chains play important roles in many biological phenomena, such as control of glycoprotein hormone activity and cell-cell interactions. Some of the functions of sugar chains are closely related to their branching structure. For example, highly branched complex-type sugar chains are essential for the effective expression of in vivo biological activity of human erythropoietin since the activity correlates to the ratio of tetra-antennary to biantennary oligosaccharides (2). It has been reported that the branching of sugar chains increases with the malignancy of tumor cells (3, 4). Recently Yoshimura et al. (5) demonstrated that the sugar chains produced by the action of GnT-V are involved in invasion and cell attachment in the extravasation stage of lung metastasis (5).

To elucidate the regulation and the roles of branching structure of complex-type sugar chains, proteins or genes of GnT-I, -II, -III, and -V, have been isolated from mammalian tissues and cells (6–16). No sequence homology was found among these GnTs (17). Among known mammalian GnTs, only GnT-IV has not been purified, and its gene structure remains unknown.

GnT-IV (UDP-N-acetylglucosamine: \(\alpha_3\)-d-mannoside \(\beta_1,4\)-N-acetylglucosaminyltransferase, EC 2.4.1.145) catalyzes the transfer of GlcNAc from UDP-GlcNAc to the GlcNAc\(\beta_3\)-GlcNAc\(\beta_1\)-Man\(a_1-3\) arm of core oligosaccharide (Gn\(\alpha_3\)(2′,2′)core oligosaccharide) and forms GlcNAc\(\beta_3\)-GlcNAc\(\beta_1\)-2Man\(a_1-3\) structure on the core oligosaccharide (Gn\(\alpha_3\)(2′,2′,2′)core oligosaccharide). The activity of GnT-IV was first identified in the hen ovoviduct by Glesson and Schachter (18). In the rat, most tissues express GnT-IV activity, which is relatively high in the spleen and small intestine (19). The products of GnT-IV (tria- and tetra-antennary sugar chains) are normally found in many glycoproteins, such as fetuin (20), thyroglobulin (21), orosomucoid (22), and erythropoietin (23). Kobata and co-workers (24, 25) reported that human chorionic gonadotropin (hCG) from chorionic carcinoma patients carried GnT-IV products that had GlcNAc\(\beta_3\)-2Man\(a_1-6\)[GlcNAc\(\beta_3\)-4(GlcNAc\(\beta_1\)-2)Man\(a_1-3\)] attached to \(\beta_1\)-mannose of the core oligosaccharide (Gn\(\alpha_3\)(2′,4′)-core structure) and GlcNAc\(\beta_3\)-4(GlcNAc\(\beta_1\)-2) attached to \(\alpha_1-
3-mannose of the core oligosaccharide (Gn\(_2\)(4,2)core structure), which were not found in normal hCG (24, 25). These results suggest an ectopic expression of GnT-IV. Although these phenomena were reported, the mechanism of the regulation of GnT-IV activity is unknown. We report here isolation and characterization of GnT-IV from the bovine small intestine.

**EXPERIMENTAL PROCEDURES**

**Materials**—Uridine, UMP, UDP, UTP, TDP, CDP, UDP-hexoseamine agaroose, UDP-GlcNAc, UDP-glucose, UDP-galactose, UDP-glucuronic acid, 2′-deoxy-UDP, and UDP-N-acetylgalactosamine were obtained from Sigma. Q-Sepharose FF, chelating-Sepharose FF, and Superdex 200 were products of Pharmacia (Uppsala, Sweden). Sialidase from Arthrobacter ureafaciens was purchased from Nacalai Tesque (Kyoto, Japan). β-Galactosidase from Aspergillus sp. was purchased from Toyobo (Tokyo, Japan). β-N-acetylgalactosaminidase from jack bean homogenate was purchased from Boehringer Mannheim (Mannheim, Germany).

**Determination of Activities of GnT-IV**—GnT-IV activity assay was assayed using fluorescence-labeled substrate according to the method of Nishikawa et al. (19) with some modifications (26). Preparation of the substrate Gn\(_3\)(2,core-PA (Fig. 1) was done as reported (26). Enzyme solution (15 μl) was incubated at 37 °C for 4 h with 125 μM MOPS buffer, pH 7.3, containing 0.8 mM substrate, 20 mM UDP-GlcNAc, 7.5 mM MnCl\(_2\), 200 mM GlcNAc, 0.5% (w/v) Triton X-100, 10% glycerol, and 5 mg/ml bovine serum albumin in a total volume of 50 μl. After the incubation, 50 μl of water was added and the enzyme reaction was stopped by boiling for 2 min. After filtration, 5 μl of the reaction mixture was applied to a reverse phase column (Vydac 218TP152010, 10 × 250 mm, Hesperia, CA). Gn\(_3\)(6,2,core-PA was eluted at 50 °C with 50 mM ammonium acetate buffer, pH 4.0, containing 15% 1-butanol at a flow rate of 2.5 ml/min. Gn\(_3\)(2,2,2,core-PA was prepared from the reaction mixture after measurement of GnT-IV activity in the bovine small intestine homogenate.

**Analytical Methods**—500 MHz 1H NMR measurement of the enzymatic product was performed with a JEOL JMG-GSX500 at 30 and 60 °C using acetone as an internal standard at δ 2.225 ppm. Smith’s degradation was carried out according to the method described by Kobata and Takasaki (27). Molecular mass of pyridylaminated sugar chains was determined with MALDI-TOF-MS (LASER MAT 2000, Finnigan MAT, Hemel Hempstead, United Kingdom).

**SDS-Polyacrylamide Gel Electrophoresis**—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) was done by the method of Laemmli (28) using 12% gel. The molecular weight marker kit (Bio-Rad) was used for size standards. Native PAGE was carried out at pH 8.8 on a 7.5% gel containing 0.1% Triton X-100 by the method of Davis (29). Proteins in the gels were stained with silver (Silver Stain Plus, Bio-Rad).

**Purification of GnT-IV**—Buffers used in this purification were as follows: Buffer A (10 mM Tris-HCl, pH 7.4, 0.25 mM sucrose, 1 mM phenylmethylsulfonyl fluoride), Buffer C (20 mM Tris-HCl, pH 7.4, 0.15 mM MnCl\(_2\), 1 mM benzamidine hydrochloride, 0.1% Triton X-100, and 20% glycerol), Buffer D (20 mM Tris-HCl, pH 7.4, 0.15 mM KCl, 10 mM MnCl\(_2\), 1 mM benzamidine hydrochloride, 0.05% Triton X-100, and 20% glycerol), Buffer E (20 mM Tris-HCl, pH 8.0, 10 mM MnCl\(_2\), 0.05% Triton X-100, and 20% glycerol), Buffer F (20 mM Tris-HCl, pH 8.0, 1 mM KCl, 0.05% Triton X-100, and 20% glycerol), Buffer G (20 mM Tris-HCl, pH 7.4, 10 mM MnCl\(_2\), 0.05% Triton X-100, and 20% glycerol), and Buffer H (20 mM Tris-HCl, pH 7.4, 0.05% Triton X-100, and 20% glycerol).

**Preparation of Microsome Fraction**—All procedures were carried out at 4 °C. Frozen bovine small intestine (2 kg) was minced and homogenized in 4 volumes of Buffer A with a Polytron homogenizer (Kinetica AG, Luzern, Switzerland) for 30 s at four times in 1-min intervals. The homogenate was centrifuged at 900 × g for 10 min. The superna-
tand was centrifuged further at 105,000 \( \times g \) for 60 min and the microsomal fraction was obtained as a precipitate.

**Solubilization of GnT-IV**—The microsomal fraction was suspended with 3 volumes of Buffer A containing 1% (w/v) Triton X-100. The suspension was gently stirred for 1 h and then centrifuged at 105,000 \( \times g \) for 1 h. The supemantant fraction was collected and the residual pellet was suspended again in 1.5 volumes of the same buffer above. The first and second Triton extracts were combined (9 liters).

**Q-Sepharose FF Column Chromatography**—The combined Triton X-100 extracts were applied to the Q-Sepharose FF column (5 \( \times \) 30 cm) equilibrated with Buffer B. The column was washed with 3 liters of Buffer B until the protein concentration in the eluate was reduced to a linear gradient between 1 liter of Buffer C and 1 liter of Buffer D. Half of the dialyzed sample (50 ml) was applied to a UDP-hexanolamine-agarose column (1.2 \( \times \) 4.5 cm) equilibrated with Buffer D. The column was washed successively with 30 ml of Buffer D and 30 ml of Buffer E. GnT-IV was eluted with Buffer F. The fractions containing GnT-IV activity (540 ml) were pooled and concentrated to 10 ml on a YM 30 membrane with a Diaflow Ultrafiltrator (Amicon, Beverly, MA).

**UDP-Hexanolamine-Agarose Affinity Column Chromatography I**—*Elution with KCl*—The concentrate was dialyzed extensively against Buffer D. The dialyzed sample (50 ml) was applied to a UDP-hexanolamine-agarose column (1.0 \( \times \) 6.5 cm) equilibrated with Buffer G. After washing the column with 20 ml of the same buffer, GnT-IV was eluted with 20 ml of Buffer H at a flow rate of 0.2 ml/min. The fractions containing GnT-IV activity (6 ml) were pooled.

**Superdex 200 Gel Filtration Column Chromatography**—The pooled fractions were concentrated to 0.5 ml with a small column of Q-Sepharose FF. The concentrate was applied to a column of Superdex 200 HR10/30 (1 \( \times \) 30 cm) equilibrated with Buffer I. GnT-IV was eluted at a flow rate of 0.25 ml/min. After measurement of the protein concentration of the fractions, MnCl\(_2\) was added to give a final concentration of 10 mM. An low molecular weight kit (Pharmacia, Uppsala, Sweden) was used for molecular weight calibration.

**Protein Assay**—The protein in the eluate of Q-Sepharose, Cu\(^{2+}\)-chelating Sepharose, and UDP-hexanolamine-agarose column chromatographies were monitored by a Bio-Rad protein assay kit. The protein assays shown in Table I and the protein of the eluate of Superdex 200 column chromatography were measured by the more sensitive BCA protein assay kit (Pierce), using bovine serum albumin as a standard.

**RESULTS**

**Purification of GnT-IV**—Activity of GnT-IV was assayed using the fluorescence-labeled biantennary sugar chain, Gn\(_2\)(2\(^3\)Man\(_9\)GlcNAc\(_2\)/core-PA, as an acceptor substrate (19). The assay method was originally developed to measure GnT-III, -IV, and -V activity at one time, which enabled the purification of GnT-III and -V (12, 14). However, the sensitivity of this method for GnT-IV activity did not appear sufficient for purification. The substrate concentration used in the previous assay protocol (0.3 mM, Ref. 19) was not high enough to detect GnT-IV activity; moreover, GnT-III and -V which did not exist in the crude enzyme preparation, competed with GnT-IV against the same substrate. To compensate for these problems, the substrate concentration was raised to 0.8 mM. The addition of 10% glycerol and 1% bovine serum albumin in the assay solution were also effective to stabilize GnT-IV activity. As a result of these improvements, the sensitivity for detection of GnT-IV activity in the crude enzyme preparation was eight times higher than that under the previous assay condition.

Using the method to survey the distribution of GnT-IV activity in rat tissues (19), we surveyed GnT-IV activity in commercially available bovine tissues and cultured cells. Since the relative activity of GnT-IV against that of GnT-III and -V in the bovine small intestine was the highest among samples examined, we chose bovine small intestine as an enzyme source.

Like other GnTs, GnT-IV was enriched in microsomes. However, about 20% of the GnT-IV activity in the homogenate was found in the cytosol fraction, suggesting that limited hydrolysis might occur. GnT-IV activity was successfully solubilized from the microsome fraction by Triton X-100 extraction. CHAPS and n-octyl-\(\beta\)-D-thioglucoside were less effective than Triton X-100 (data not shown). The addition of benzamidine hydrochloride, a trypsin inhibitor, was effective for maintenance of GnT-IV activity during the whole procedure. The majority of proteins in the Triton extract were removed from the GnT-IV activity by the Q-Sepharose FF column (Fig. 2A) and Cu\(^{2+}\)-chelating Sepharose FF column chromatography (Fig. 2B) steps. After the two chromatographies, the GnT-IV active fractions still contained GnT-III and -V activities as well (data not shown). Separation of GnT-IV activity from other GnTs was carried out by affinity column chromatography (Fig. 2C), using an analogue of the common donor substrate for GnTs (UDP-hexanolamine) as ligands. Activities of GnT-III and -V passed through the column under the chromatographic conditions as described under “Experimental Procedures.” GnT-IV activity was bound completely to the column and eluted with 1 M KCl at pH 8.0. Since the eluted fractions still contained several proteins of various molecular weights (Fig. 3, lane 1), another chromatography was performed. The GnT-IV activity was retarded on an affinity column (12) to which Gn\(_2\)(2\(^3\)Man\(_9\)GlcNAc\(_2\)/core-Asn was covalently bound, however, the GnT-IV activity could not be isolated from other proteins (data not shown). Neither blue, red, Q-, nor Cu\(^{2+}\)-chelating Sepharose columns were effective for purification in this step. The separation of GnT-IV from other proteins was accomplished by the second UDP-hexanolamine column chromatography with modified elution conditions. The bound GnT-IV was eluted immediately from the column by the buffer lacking MnCl\(_2\) (Fig. 2D). The fraction containing GnT-IV was further purified by gel filtration (Fig. 2E). The purified GnT-IV showed a single band with a molecular weight of 58,000 on SDS-PAGE (Fig. 3, lane 6). It also showed a single band on native PAGE (Fig. 4A), and GnT-IV activity was accompanied by a protein band (Fig. 4B). No activity of GnT-I, -II, -III, or -V was detected in the purified GnT-IV preparation (data not shown). Table I summarizes the purification of GnT-IV. GnT-IV was purified to homogeneity and over 224,000-fold by these procedures. A protein with a molecular weight of 45,000 in lane 4 (Fig. 3) was identified as bovine \(\alpha\)-1,3-galactosyltransferase (30) by sequence analyses of its peptide fragments.

**Protein Chemistry of GnT-IV**—SDS-PAGE analysis of purified GnT-IV before (lane 1) and after (lane 2) digestion with peptide \(\beta\)-N-glycosidase F is shown in Fig. 5. Apparent molecular weight was reduced by 3,000 as a result of glycosidase treatment indicating that GnT-IV is a glycoprotein with Asn-linked sugar chains. As shown Fig. 2E, the apparent molecular weight of GnT-IV was estimated to be 73,000 by gel filtration in the presence of Triton X-100. This estimated molecular weight may be larger than the actual size, since membrane proteins migrate as protein-detergent complexes on gel filtration columns. These observations together suggest that GnT-IV is a monomer with a molecular weight of 58,000.

**Optimum pH**—GnT-IV was the most active between pH 7.0 and 8.0 with an optimum of pH 7.3 (Fig. 6).

**Effect of Divalent Cations and 2-Mercaptoethanol on GnT-IV**
Activity—GnT-IV activity depends on MnCl₂ concentration, and the optimum concentration of MnCl₂ was 10 mM. When MnCl₂ concentration was increased, the activity gradually decreased and was suppressed by 50% at 60 mM (data not shown). The effects of other divalent cations at 10 mM concentration were examined (Table II). Each divalent cation was added to the GnT-IV preparation which had been treated with 1 mM EDTA followed by dialysis against 20 mM Tris-HCl buffer, pH 7.4, containing 20% glycerol and 0.05% Triton X-100. GnT-IV maintained slight activity without the addition of any metal but lost activity completely by the addition of EDTA and CuCl₂. The activity was maximal with MnCl₂, while CoCl₂ and MgCl₂ were less effective. CaCl₂ and FeCl₂ had no effect. The addition of 2-mercaptoethanol (10 mM) to the enzyme had no effect on the enzyme activity (data not shown).

Product Identification—To identify the sugar chain structure produced by the enzyme, the product of GnT-IV using Gnt₂-(2',3')core-PA as a substrate was subjected to Smith's degradation. The molecular weight of the degradation fragments were monitored with a MALDI-TOF mass spectrometer (data not shown). Before Smith's degradation, the molecular weight of the enzymatic products was 1598.2. After the first and second
cycle of Smith’s degradation, the molecular weights of the fragments were 795.3 and 634.7, respectively. This degradation pattern was consistent with that of the GnT-IV product shown in Fig. 7. 1H NMR analysis of the enzymatic product showed peaks of chemical shifts at 4.53, 4.54, and 4.55 ppm, which correspond to those of anomeric protons of GlcNAc 7, GlcNAc 5, and GlcNAc 5’ (31) in Fig. 7, respectively. GlcNAc 7 should link to mannose 4 through β-linkage, because its spin coupling constant ($J_{1,2}$) is 7.9 Hz. These data indicate that the enzymatic product has the structure of Gn$_2$(2',4,2')core-PA (Fig. 1).

**Effect of UDP-GlcNAc Analogues on GnT-IV Activity**—To understand the relative effects of nucleotides and sugar nucleotides against UDP-GlcNAc, the activities of GnT-IV were measured in the presence of 2 mM UDP-GlcNAc analogues (Table III). UDP was the most potent inhibitor. The uracil moiety appeared to be essential for the inhibition of nucleotides, since TDP and CDP did not inhibit enzyme activity. A comparison of uridine, UMP, UDP, and UTP suggested that the number of phosphoesters should be important for inhibition. The sugar nucleotides having the UDP moiety also inhibited enzyme activity, including UDP-hexanolamine which was used as an affinity ligand for enzyme purification.

**Acceptor Substrate Specificity**—Acceptor substrate specificity of GnT-IV was examined with a set of complex-type sugar chains as listed in Fig. 1. Purified GnT-IV appeared to select its substrate clearly (Table IV). It transferred GlcNAc to Gn(2)-core-PA (GnT-I and α-mannosidase II product) and Gn(2',2')-core-PA (GnT-II product) structures. The addition of a GlcNAc-β1–6 at Manα1–6 residue of the core-PA structure (i.e. GnT-V product) increased the transfer rate about 150% (Gn$_2$(2',2')core-PA against Gn$_2$(2')core-PA), while the addition of a GlcNAcβ1–4 at the Manβ1–4 residue of the core-PA structure (Gn$_2$(2',2')bis-core-PA, GnT-III products) prevented the transfer. The addition of fucose at the chitobiose portion through α1–6 linkage reduced the transfer rate about 50% (Gn$_2$(2')core-F-PA against Gn$_2$(2')core-PA). The addition of galactose to GlcNAC(2') of the Gn$_2$(2')core-PA (GGn(2')Gn(2')-core-PA) reduced the transfer rate significantly, and the addition of a galactose to Gn(2)NAC of the Gn$_2$(2')core-PA (Gn(2')GGn(2')NAC-core-PA) prevented the transfer. GnT-IV did not react with either of the sugar chains without GnT-I product, a GlcNAcβ1–2 at Manα1–3 residue of the core structure (Core-PA and Gn(2')core-PA).

**Kinetic Analysis**—Lineweaver-Burk plots of kinetic data obtained with the purified GnT-IV preparation at a 40 mM UDP-GlcNAc concentration were linear, and gave the following kinetic constants: $K_m$ values for Gn$_2$(2',2')core-PA and Gn$_2$(6',2')core-PA were 0.73 and 0.13 mM, respectively, and $V_{max}$ values for the same substrates were 3.23 and 1.75 μmol/min, respectively. The $K_m$ value for UDP-GlcNAc was 0.22 mM in 0.8 mM Gn$_2$(2',2')core-PA as an acceptor.

**DISCUSSION**

A new β1,4-GlcNAc transferase was purified from the membrane fraction of bovine intestine. The enzyme transfers GlcNAc from UDP-GlcNAc to Gn$_2$(2',2')core-PA and produces Gn$_2$(2',4,2')core-PA. This enzyme also acts on Gn$_2$(2')core-oligosaccharides attached to glycoproteins (data not shown), suggesting that the enzyme belongs to the branch-forming GlcNAc transferases for Asn-linked sugar chains. Schachter et al. (18) classified such GlcNAc transferases from GnT-I to GnT-VI (1). Among them, GnT-III, -IV, and -VI are β1,4-GlcNAc trans-

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**TABLE I**

**Purification of GnT-IV from bovine small intestine**

| Step           | Protein | Total activity | Specific activity | Yield | Purification |
|----------------|---------|----------------|-------------------|-------|--------------|
| Homogenate     | 112,900 | 49,500         | 0.44              | 100   | 1            |
| Triton extract | 24,100  | 14,500         | 0.60              | 29    | 1.4          |
| Q-Sepharose    | 4,000   | 7,200          | 1.80              | 14    | 4.1          |
| Cu-chelate Sepharose | 450 | 3,670          | 8.31              | 7.4   | 18.4         |
| First UDP-hexanolamine | 0.59 | 1,950          | 3,310             | 3.9   | 7,510        |
| Second UDP-hexanolamine | 0.035 | 1,420          | 40,600            | 2.9   | 92,200       |
| Superdex 200   | 0.008   | 790            | 98,800            | 1.6   | 224,000      |

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**FIG. 3.** Analysis of aliquots from each step of GnT-IV purification by SDS-PAGE. GnT-IV active fractions of chromatography were analyzed on a 12% SDS-PAGE and stained with silver. Lane 1, Triton extract (4 μg); lane 2, Q-Sepharose FF (4 μg); lane 3, Cu$^{2+}$-chelating Sepharose FF (4 μg); lane 4, UDP-hexanolamine agarose column chromatography I (1 μg); lane 5, UDP-hexanolamine agarose column chromatography II (0.2 μg); lane 6, Superdex-200 column chromatography (0.09 μg). The position of GnT-IV is indicated by an arrowhead. Size standards are shown in lane M. 

**FIG. 4.** Native-PAGE analysis of purified GnT-IV. Purified GnT-IV was separated on a 7.5% native gel electrophoresis containing 0.1% Triton X-100 at 4 °C. After electrophoresis, one lane (0.15 μg of sample) was stained with silver (A) and the other lane (0.06 μg of sample) was sliced in 30 pieces at a width of 2 mm. Each piece was used for enzymatic assays (B).
ferases which produce b1–4GlcNAc branches on the b-linked 
Man residue, the Mana1–3 residue, and the Mana1–6 residue 
of the core oligosaccharides, respectively (1, 32). The b1,4-
GlcNAc transferase newly purified in this paper was identified 
as GnT-IV, because the 1H NMR of the product oligosaccharide 
clearly indicated that the enzyme produced Gn3(29,4,2)core-PA 
exclusively.

According to the Km values, GnT-IV prefers 
Gn3(69,29,29)core-PA (0.13 mM) rather than 
Gn2(29,2)core-PA (0.73 mM). On 
the other hand, GnT-V prefers 
Gn3(29,4,2)core-PA as was re-
ported by Nishikawa 
et al. (12). These substrate specificities of 
GnT-IV and -V suggest that triantennary sugar chains are 
always promoted to tetra-antennary ones. However, glycopro-

FIG. 7. Scheme of degradation patterns of triantennary sugar 
chains by Smith’s degradation. Numbers marked on each monosac-
charide of GnT-IV product are as used in the text. Xn, N-acetylxy-
losamine.

TABLE III
Effects of nucleotides on GnT-IV activity
As described under “Experimental Procedures,” purified enzyme (3 
µg/ml) was incubated in the reaction mixture with 0.5 mM UDP-GlcNAc 
and 2 mM inhibitors. Activity is expressed as percent of b1,4-GlcNAc 
transfer observed in the absence of inhibitor.

| Addition                        | Activity % |
|---------------------------------|------------|
| None                            | 100        |
| Uridine                         | 115        |
| UMP                             | 97.3       |
| UDP                             | 27.3       |
| UTP                             | 88.2       |
| TDP                             | 110        |
| CDP                             | 112        |
| 2′-Deoxy-UDP                    | 67.4       |
| UDP-hexanolamine                 | 73.6       |
| UDP-glucose                     | 56.6       |
| UDP-galactose                    | 87.3       |
| UDP-glucuronic acid             | 92.3       |
| UDP-N-acetylgalactosamine        | 59.7       |

FIG. 6. Effect of pH on GnT-IV activity. GnT-IV activity was 
asayied in the assay solution as described under "Experimental Proce-
dures” except for the pH of the buffer. Assay solution containing 125 mM 
MOPS at pH 6.0–8.0 (●) and 125 mM Tris-HCl at pH 8.5–9.5 (○) were 
used.
Acceptors Relative activity

| Acceptor              | %    |
|-----------------------|------|
| Core-PA               | 90   |
| Gn(2)core-PA          | 100  |
| Gn(2,2)core-PA        | 100  |
| Gn(2,2,2)core-PA      | 0    |
| Gn(2,2,2,2)core-PA    | 0    |
| Gn(6,2,2,2)core-PA    | 0    |

GnT-IV potentially contributes to the production of glycoprotein hormones sharing highly branched sugar chains. The specific activity of GnT-IV in CHO cells used widely for protein production is relatively low (20 pmol/h/mg), which is about 5% of GnT-V specific activity. It is expected that CHO cells with elevated GnT-IV activity by incorporation of the GnT-IV gene will increase the production of tetra-antennae. Cloning of the GnT-IV gene is now in progress in our laboratory.

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Table IV

| TABLE IV                      |
|-------------------------------|
| Purification and Characterization of N-Acetylglucosaminyltransferase IV |

Substrate specificity of GnT-IV

| Table IV | Purification and Characterization of N-Acetylglucosaminyltransferase IV |
|----------|------------------------------------------------------------------------|
| Acceptors Relative activity |
| Core-PA | 90 |
| Gn(2)core-PA | 100 |
| Gn(2,2)core-PA | 0 |
| Gn(2,2,2,2)core-PA | 0 |
| Gn(6,2,2,2)core-PA | 0 |

Teins with tetra-antennary sugar chains are relatively rare. There must be either an imbalance of those enzymes or some factors in glycoproteins which prevent or promote the access of those enzymes (33, 34). It should be quite interesting to investigate the relationship between branch structure of complex-type sugar chains and activity valance among GnT-III, -IV, -V, and β1,4-GalT.

Our result clearly showed that purified GnT-IV could act on the mono-antennary Gn(2)core-PA (product of GnT-I and α-mannosidase II) and produced the Gn(4,2)core sugar chain known as an abnormal biantennary type. Such an abnormal sugar chain appears on hCG from choriocarcinoma (24) and γ-glutamyltranspeptidase from human hepatocellular carcinoma (35). It would be quite interesting to examine if GnT-IV activity correlates with the advances of malignancy on those cancer cells.

GnT-IV potentially contributes to the production of glycoprotein hormones sharing highly branched sugar chains. The specific activity of GnT-IV in CHO cells used widely for protein production is relatively low (20 pmol/h/mg), which is about 5% of GnT-V specific activity. It is expected that CHO cells with elevated GnT-IV activity by incorporation of the GnT-IV gene will increase the production of tetra-antennae. Cloning of the GnT-IV gene is now in progress in our laboratory.

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