Porcine Submaxillary Gland GDP-\(\text{L}-\text{fucose;\(\beta\)-D-Galactoside} \\alpha\text{-2-L-Fucosyltransferase Is Likely a Counterpart of the Human Secretor Gene-encoded Blood Group Transferase*}

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Partial amino acid sequence of GDP-\(\text{L}-\text{fucose;\(\beta\)-D-galactoside} \\alpha\text{-2-L-fucosyltransferase purified from porcine submaxillary glands was determined. Amino acid sequence analysis yielded 100, 93.3, and 84.2\% and 75, 46.6, and 84.2\% sequence identity between 12-, 15-, and 19-amino acid tryptic peptides generated from porcine enzyme and amino acid residues 61-72, 111-125, and 308-326 and 89-100, 139-153, and 338-356 of the human Secretor and H type \(\alpha\text{-2-fucosyltransferases}, respectively. Higher amino acid sequence homology of the porcine enzyme with the predicted sequence for the human Secretor locus as compared with H gene-encoded blood group \(\beta\)-galactoside \(\alpha\text{-2-L-fucosyltransferase suggests that porcine } \alpha\text{-2-fucosyltransferase highly corresponds to the human Secretor gene-encoded enzyme.}

The minimum structure of the H antigenic determinant is a terminal Fuc(1–2)Galβ1. Two distinct GDP-\(\text{L}-\text{fucose;\(\beta\)-D-galactoside} \\alpha\text{-2-fucosyltransferases, encoded by the H and Secretor (Se)}^2\text{ genes, are known to control the biosynthesis of the mono- and difucosylated lactoseries structures in normal tissues and in epithelial carcinomas in humans (1), although evidence from normal and tumor tissues suggests that the human genome contains additional structural genes encoding \(\alpha\text{-2-fucosyltransferases (2–5). A two-loci model for expression of } \alpha\text{-2-fucosyltransferase was proposed based on the presence of } \alpha\text{-2-fucosyltransferase enzymatic activity in the serum of Bombay, Se-positive individuals, who lack H gene expression (6). In this model, both Se and H represent structural genes encoding } \alpha\text{-2-fucosyltransferases, and the enzymes under the control of the Se and H genes could preferentially use type 1, 2, 3, and 4 precursors or type 2 structures and are expressed in cells of endodermal and mesodermal origin, respectively (7). The two-loci model for expression of } \alpha\text{-2-fucosyltransferases was firmly established by cloning H (8) and a candidate for human Secretor locus-encoded } \alpha\text{-2-fucosyltransferase (9). The Se gene-encoded enzyme shares 68\% identity with the human H } \alpha\text{-2-fucosyltransferase protein sequence, which confirms the hypothesis that H and Secretor loci represent two distinct but closely linked } \alpha\text{-2-fucosyltransferase genes.}

Evolutionary evidence has suggested that the Se gene is ancestral in mammals, with the evolutionarily newer H gene present only in humans and anthropoid apes. Indeed, ABO blood group antigens are present in red blood cells, vascular endothelium, and secretions in man and anthropoid apes but only in secretions in lower mammals (10).

The evidence that the \(\beta\)-galactoside \(\alpha\text{-2-fucosyltransferase from porcine submaxillary glands is equivalent to the human serum Se-type and not H-type enzyme is based on striking similarities with the Se enzyme with respect to the kinetic pattern, specificity toward various oligosaccharide acceptors and physicochemical properties. Both, the porcine and human Se } \alpha\text{-2-fucosyltransferase display the preference for type 1 and 3 lactoseries oligosaccharide acceptors, are represented by 55-kDa glycoproteins, and demonstrate binding properties to S-Sepharose (11–16). We postulated that porcine } \alpha\text{-2-fucosyltransferase is equivalent to the human Se gene-encoded enzyme and different from the H blood group } \alpha\text{-2-fucosyltransferase.}

Tryptic peptides generated from the porcine submaxillary gland \(\beta\)-galactoside \(\alpha\text{-2-fucosyltransferase, share higher amino acid sequence homology with human Se blood group enzyme as compared with human H gene-encoded enzyme and results confirm the hypothesis that porcine submaxillary gland enzyme is equivalent to the human Se } \alpha\text{-2-fucosyltransferase.}

EXPERIMENTAL PROCEDURES

Materials

Porcine submaxillary glands were from Pel-Freeze Biologicals, Inc. (Milwaukee, WI); GDP-\(\text{L}-\text{fucose (268 Ci/mol) was from Amersham Corp., Phenyl-\(\beta\)-O-galactoside, GDP, and ATP were from Sigma, and Sep-Pak C18 cartridges were from Waters Associates (Milford, MA). ACS liquid scintillation mixture was from DuPont NEN; S-Sepharose, Sepharose-4B, and Sephadex G-50 (fine) were from Pharmacia Biotech Inc., and protein molecular weight markers for PAGE were from Bio-Rad.

Enzyme Assays

Activity of the \(\beta\)-galactoside \(\alpha\text{-2-L-fucosyltransferase was determined using phenyl-\(\beta\)-galactoside as an acceptor as described previously (13, 14).}

Protein Determination and SDS-PAGE

Protein was determined, and electrophoresis was performed as described previously (13, 14).

Synthesis of GDP-Hexanolamine-Sepharose

GDP-hexanolamine and GDP-fucose were synthesized as described previously (17). GDP-hexanolamine-Sepharose (250 ml) was synthesized as described previously (18) with the following modifications. CNBr-activated Sepharose 4B was freshly prepared using a solution of CNBr in dioxane (1 g/ml) instead of powdered CNBr, and the pH of the reaction was brought to 11.0 instead of 11.5–12.0 with 1.5 M LiOH. The temperature of the reaction was kept at 2–4 °C. The ligand concentra-
The partial amino acid sequence of the β-D-galactoside α-2-fucosyltransferase from porcine submaxillary glands, which was purified to homogeneity, was determined.

**RESULTS**

The 55- and 60-kDa individual proteins were excised from the membrane following SDS-PAGE of fractions obtained from HPLC and GDP-hexanolamine-Sepharose III columns, respectively. Tryptic peptides generated from the both isolated proteins were separated by reverse-phase chromatography. The molecular mass of the selected fractions was established using mass spectrometry, and peptides were subjected to automated amino acid sequence analysis using an Applied Biosystems gas/liquid phase sequencer equipped with a Nelson analytical data analysis system.

### Table I

| Step | Purification | Volume | Total protein | Specific activity | Yield | Yield |
|------|--------------|--------|---------------|------------------|-------|-------|
| 1.   | Triton extract | 61,150 | 1,335,125 | 12.3 | 0.00009212 | 100 |
| 2.   | S-Sepharoseb | 9,850 | 30,241 | 6,817 | 0.0002254 | 55.4 | 24.46 |
| 3.   | GDP-hexanolamine-Sepharose-Ib | 1,730 | 1,255 | 5,181 | 0.0041228 | 42.12 | 447.5 |
| 4.   | Sephadex G-50 (fine) | 2,790 | 878 | 5,061 | 0.00576 | 41.14 | 625.27 |
| 5.   | GDP-hexanolamine-Sepharose-IIc | 490 | 80 | 3,037 | 0.03796 | 24.69 | 412.07 |
| 6.   | Sephadex-G-50 (fine) | 600 | 47 | 3.13 | 0.06659 | 25.44 | 722.86 |
| 7.   | GDP-hexanolamine-Sepharose-IIIc | 25 | 2.5 | 1.312 | 0.524 | 10.66 | 56,882 |
| 8.   | HPLC size-exclusiond | 37.5 | 0.27 | 0.308 | 1.1407 | 2.5 | 123,827 |

a Unit is defined as 1 μmol of product formed/min at saturating concentrations of GDP-fucose and 100 μg of galactose-β-ol-phenyl as acceptor substrate.
b Steps 2–4 were performed with 5-kg batches of porcine submaxillary glands, and values are cumulative for four preparations.
c Values for steps 5–7 were obtained for pooled solutions of fucosyltransferase from 21.5 kg of submaxillary glands.
d The values in this step were extrapolated from the purification of α-2-fucosyltransferase from 100 μl of the fraction obtained from step 7.

**Purification of Porcine Submaxillary Gland β-D-Galactoside α-2-L-Fucosyltransferase**

Purification of porcine submaxillary gland α-2-fucosyltransferase was based on a previously published purification scheme (15). Therefore, only steps representing alterations to the original method are described in detail. Full details of the purification procedure could be obtained directly from the authors.

**Steps 1-4: Triton X-100 Extraction and S-Sepharose Ion-exchange Chromatography**—Triton extraction was performed on 5-kg batches of submaxillary glands and adsorbed with S-Sepharose. Fractions containing α-2-fucosyltransferase eluted from S-Sepharose were applied to a GDP-hexanolamine-Sepharose column I (4 × 15 cm and 5.4–6.6 μmol of ligand/ml of settled gel). α-2-Fucosyltransferase was eluted with 400 ml of a linear NaCl gradient (0.4–2.0 mM NaCl) in 25 mM sodium cacodylate buffer, pH 6.0, and active pooled fractions were desalted on a Sephadex G-50 (fine) column (5 × 50 cm) using 25 mM sodium cacodylate buffer, pH 7.0.

**Steps 5 and 6: GDP-Hexanolamine-Sepharose—**Desalted active fractions were applied at 100 ml/hto a GDP-hexanolamine-Sepharose-II affinity column (4 × 15 cm and 5.4–6.6 μmol of ligand/ml of settled gel) equilibrated with 25 mM sodium cacodylate buffer, pH 7.0, containing 0.15 mM NaCl. α-2-Fucosyltransferase was eluted with 2 mM NaCl, and the active fractions were pooled and desalted exactly as in Step 4.

**Step 7: GDP-Hexanolamine-Sepharose—**Desalted active fractions from GDP-hexanolamine-Sepharose column II were directly applied to a fresh GDP-hexanolamine-Sepharose column III (1.5 × 7 cm and 5.4–6.6 μmol of ligand/ml of settled gel). The column was eluted with 0.5 mM GDP as described previously (15).

**Step 8: High Pressure Size-Exclusion Liquid Chromatography**—HPLC separation was performed on a HPLC LKB 2150 apparatus with a TSKG3000 SW size-exclusion column (Pharmacia) and with an injection loop (100 μl) (13, 14). The HPLC column was pre-equilibrated with 25 mM sodium cacodylate buffer, pH 7.0, containing 0.035 mM NaCl. Pooled fractions eluted from the GDP-hexanolamine-Sepharose column III were concentrated (10-fold) using Speed-Vac, and a sample (100 μl) was subjected to HPLC size-exclusion chromatography at a flow rate 0.5 ml/min. Fractions (0.3 ml) were collected every 36 s. Enzymatic activity was monitored by standard assay using phenyl-β-D-galactoside as an acceptor; protein and GDP were monitored with a wavelength monitor LKB 2141 at 223 nm.

**Amino Acid Sequence Determination**

The 55-kDa enzymatic protein (180 pmol), purified by HPLC size-exclusion chromatography, was submitted to the Wistar Institute Mass Spectrometry/Protein Microchemistry Laboratory for amino acid sequence analysis. Protein sample (50 μg) eluted from the GDP-hexanolamine-Sepharose III affinity chromatography column containing 60–55% and 18-kDa proteins was submitted to determine the amino acid sequence of the 60-kDa protein. Proteins were separated by SDS-PAGE on 5–15% gradient gel and electrotransferred to a polyvinylidene difluoride membrane. The 55- and 60-kDa individual proteins were excised from the membrane following SDS-PAGE of fractions obtained from HPLC and GDP-hexanolamine-Sepharose III columns, respectively. Tryptic peptides generated from the both isolated proteins were separated by reverse-phase chromatography. The molecular mass of the selected fractions was established using mass spectrometry, and peptides were subjected to automated amino acid sequence analysis using an Applied Biosystems gas/liquid phase sequencer equipped with a Nelson analytical data analysis system.
Sepharose II as compared with column I. Again, the active fractions eluted with 2 M NaCl were desalted using a Sephadex G-50 (fine) column, which removes approximately 50% of the total protein at this point without loss of enzymatic activity. Desalted active fractions were directly applied to a third affinity chromatography step, which consisted of small GDP-hexanolamine-Sepharose II column. The enzyme was specifically eluted using 0.5 mM GDP in 25 mM sodium cacodylate buffer (pH 7.0) containing 0.035 mM NaCl. A 14-fold purification and considerable loss of enzymatic activity (60%) due to a low levels of protein occurred at this step (Table I). SDS-PAGE analysis of the pooled fractions eluted from the third affinity column upon reduction with β-mercaptoethanol showed major protein bands of 60 and 55 kDa and a band at 18 kDa (not shown).

This fraction was submitted for tryptic digestion and amino acid sequence analysis of the major 60-kDa protein at the Wistar Institute Mass Spectrometry/Protein Microchemistry Laboratory. Amino acid sequencing of two tryptic peptides generated from this protein band demonstrated 98 and 94% homology with bovine catalase, suggesting that the 60-kDa protein purified by affinity chromatography on GDP-hexanolamine-Sepharose III represents catalase (Table II).

Final purification of the porcine α-2-fucosyltransferase was achieved by HPLC size-exclusion chromatography. The pooled enzymatic fractions eluted from GDP-hexanolamine-Sepharose III were subjected to HPLC size-exclusion chromatography in 25 mM sodium cacodylate buffer (pH 7.0) containing 0.035 mM NaCl. Enzymatic activity was monitored by standard assay using phenyl-β-D-galactoside as an acceptor and absorption at 223 nm (Fig. 1). The HPLC size-exclusion enzymatic activity profile showed a broad peak corresponding to molecular size of 55 kDa. SDS-PAGE analysis of the active fractions in the presence of β-mercaptoethanol revealed the 55-kDa protein representing α-2-fucosyltransferase (Fig. 1, inset).

Amino Acid Sequence Determination of β-D-Galactoside α-2-L-Fucosyltransferase—The HPLC-purified protein of 55 kDa representing porcine β-D-galactoside α-2-fucosyltransferase was subjected to HPLC size-exclusion chromatography and a 2 M NaCl pulse elution, and HPLC size-exclusion steps instead of Sephadex G-50 gel filtration and chromatography on SP-Sephadex steps of the original procedure.

Three proteins, 60, 55, and 18 kDa, were eluted from the third GDP-hexanolamine-Sepharose affinity column by specific elution using 5 mM GDP. Based on a previous analysis suggesting that a 60- and 55-kDa doublet represented β-D-galactoside α-2-L-fucosyltransferase purified from porcine submaxillary glands (15), we analyzed the amino acid sequence of the 60-kDa protein isolated after the last affinity chromatography step. Amino acid sequence analysis of two tryptic peptides of this protein revealed high sequence homology with bovine catalase, consistent with the previous observation that catalase for undetermined reasons shows high binding capacity to GDP-hexanolamine-Sepharose and copurifies with α-2-fucosyltransferase from the affinity column (15). Therefore, to further purify the α-2-fucosyltransferase protein, HPLC size-exclusion chromatography was used as a purification step following the affinity chromatography steps. The HPLC step resulted in the isolation of a single major protein of 55 kDa, which coeluted with enzymatic activity, strongly suggesting that the 55-kDa

**TABLE II**

| Peptide (Protein 55 kDa) | Sequence | Mr | Identity with human enzymes |
|--------------------------|----------|----|-----------------------------|
| HP 94266                 | LGNQMGEMYATLY | 1359.4 | 100% 75.0 |
| HP 94244                 | IPWONYHLDWMEER | 2038.4 | 93.3% 46.6 |
| HP 94278                 | LFKPEAAFLPEWIGEADL | 2699.7 | 84.2% 84.2 |
| HP 94209                 | NPVNYFAEVEQMAFDPSNMPPGIEPSDK | 3218.5 | 98.0% |
| S94075                  | AAQKDILTTGSGNPGIDK | 1876 | 94.0% |

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**Fig. 1. HPLC size-exclusion chromatography of porcine β-D-galactoside α-2-L-fucosyltransferase.** One hundred μl of concentrated fractions eluted from GDP-hexanolamine-Sepharose column III was injected into an HPLC column equilibrated with 25 mM sodium cacodylate buffer, pH 7.0, containing 0.035 mM NaCl. Eluted fractions were monitored by standard enzymatic assay using phenyl-β-D-galactoside as an acceptor (open boxes) and absorption at 223 nm (closed circles). Inset represents α-2-fucosyltransferase protein eluted from HPLC column as determined by SDS-PAGE in the presence of β-mercaptoethanol after silver staining. The fractions correspond to those in the graph.
protein represents porcine submaxillary gland β-α-galactoside α-2-fucosyltransferase. In this purification scheme, catalase was separated from fucosyltransferase as a tetrameric protein in native conditions by the HPLC size-exclusion step.

The isolated porcine enzyme as determined by amino acid sequence analysis is highly homologous to human Se and H type blood group β-α-galactoside α-2-fucosyltransferases (8, 9). As expected, a higher level of homology was observed between the three tryptic peptides generated from the porcine enzyme and a recently cloned human candidate for Se type α-2-fucosyltransferase (100, 93.3, and 84.2%) as compared with the human H gene-encoded enzyme (75, 46.6, and 84.2%). The amino acid sequence of the HP 94266 peptide is identical with the Se type α-2-fucosyltransferase, whereas 75% of homology was observed with respective sequences of H enzyme. Thus, the 75% homology found between the porcine and H type α-2-fucosyltransferases also reflects the level of homology between both human Se and H enzymes. Porcine peptide HP 94278 shares the same degree of homology (84.2%) with both the Se and H type α-2-fucosyltransferases. The amino acid substitutions are in exactly the same positions in both human and porcine α-2-fucosyltransferases, although they are represented by different amino acids in all three enzymes (9). The most striking differences between the protein sequence of porcine and human H enzyme were observed in the amino acid sequence corresponding to HP 94244 peptide, where only 46.6% identity was observed. On the other hand, this peptide shares 93.3% identity with the corresponding amino acid sequence of Se type α-2-fucosyltransferase. These results support the hypothesis that porcine β-α-galactoside α-2-fucosyltransferase is equivalent to the human Se type enzyme and different from the human H blood group fucosyltransferase. Very high sequence homology between the peptides from the porcine enzyme and recently cloned rabbit α-2-fucosyltransferase, RFT-II (19), which likely corresponds to rat FTB enzyme (20), also suggests that they represent equivalent enzymes.

Porcine peptide HP 94244, which shares the lowest degree of sequence homology with the human H enzyme, is derived from the catalytic domain region where the least sequence identity between corresponding amino acid sequences of Se and H type enzymes was determined (9). These results suggest that this region may determine the oligosaccharide substrate specificity, in particular this region may be involved in β-α-galactoside binding since H and Se enzymes greatly differ in affinity to β-α-galactose and its derivatives, as determined by K_i values (11–14). Nine amino acid sequences similar to those of HP 94244 were also found in bacterial phospho-β- and β-galactosidases, sharing 55.6 and 62.5% homology with the peptide (21–23).

The availability of the DNA primary structure of the human H and Se gene-encoded β-α-galactoside α-2-fucosyltransferases (9, 10) and their animal counterparts (19, 20) will enable study of the molecular basis of α-2-fucosylated glycoconjugates expression in different species.

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REFERENCES
1. Watkins, W. M. (1978) Proc. R. Soc. Lond. B. 202, 31–53
2. Langkilde, N. C., Wolf, H., and Ørntoft, T. F. (1991) Br. J. Haematol. 79, 493–499
3. Ørntoft, T. F., Holmes, E. H., Johnson, P., Hakomori, S., and Clausen, H. (1991) Blood 77, 1389–1396
4. Blaszczyk-Thurin, M., Sarnesto, A., Thurin, J., Hindsgaul, O., and Koprowski, H. (1988) Biochem. Biophys. Res. Commun. 151, 100–108
5. Yazawa, S., Nakamura, J., Asao, T., Nagamachi, Y., Sagi, M., Matta, K. L., Tachikawa, T., and Akamatsu, M. (1993) J. Cancer Res. 84, 989–995
6. Le Pendu, J., Cartron, J. P., Lemoine, R. U., and Oriol, R. (1985) Am. J. Hum. Genet. 37, 749–760
7. Ørild, R., Danilovs, J., and Hawkins, B. R. (1981) Am. J. Hum. Genet. 33, 421–431
8. Larsen, R. D., Ernert, L. K., Nair, R. P., and Lowe, J. B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6674–6678
9. Kelly, R. J., Rouquier, S., Giorgi, D., Lennon, G. G., and Lowe, J. B. (1995) J. Biol. Chem. 270, 4640–4649
10. Oriol, R., Cooper, J. E., Davies, D. R., and Keeling, W. N. (1984) Lab. Invest. 50, 514–518
11. Betteridge, A., and Watkins, W. M. (1985) Glycoconjugate J. 2, 61–78
12. Kumazaki, T., and Yoshida, A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4193–4197
13. Sarnesto, A., Köhlin, T., Thurin, J., and Blaszczyk-Thurin, M. (1990) J. Biol. Chem. 265, 15067–15075
14. Sarnesto, A., Köhlin, T., Hindsgaul, O., Thurin, J., and Blaszczyk-Thurin, M. (1992) J. Biol. Chem. 267, 2737–2744
15. Beyer, T. A., Sadler, J. E., and Hill, R. L. (1980) J. Biol. Chem. 255, 5364–5372
16. Beyer, T. A., and Hill, R. L. (1980) J. Biol. Chem. 255, 5373–5379
17. Gokhale, U. B., Hindsgaul, O., and Palic, M. M. (1990) Can. J. Chem. 68, 1063–1071
18. Sadler, J. E., Rearick, J. I., Paulson, J. C., and Hill, R. L. (1979) J. Biol. Chem. 254, 4434–4443
19. Hitoshi, S., Kusunoki, S., Kanazawa, I., and Tsuji, S. (1995) J. Biol. Chem. 270, 8844–8850
20. Plau, J. P., Labarrière, N., Dabouis, G., and Denis, M. G. (1994) Biochem. J. 300, 623–626
21. Breidt, F. J., and Stewart, G. C. (1987) Appl. Environ. Microbiol. 53, 969–973
22. de Vos, W. M., and Gaison, M. J. (1989) J. Gen. Microbiol. 135, 1833–1846
23. Huang, D. C., Novel, M., Huang, X. F., and Novel, G. (1992) Gene (Amst.) 118, 39–46
24. IUB-IUPAC Joint Commission on Biochemical Nomenclature (JCBN) (1982) J. Biol. Chem. 257, 3347–3351