Pork Liver Guanosine Diphosphate-L-Fucose Glycoprotein Fucosyltransferases*

INDIRJIT JABBAL AND HARRY SCHACHTER
From the Department of Biochemistry, University of Toronto, Toronto 8, Canada

SUMMARY

Two fucosyltransferases have been found in pork liver which transfer L-fucose from GDP-L-fucose into various derivatives of human plasma α1-acid glycoprotein. One of these enzymes incorporates L-fucose into the terminal positions of α1-acid glycoprotein prosthetic groups and into the disaccharide, galactosyl (β,1 → 4)-N-acetylglucosamine. This enzyme does not transfer fucose to lactose, galactosyl(β,1 → 3)-N-acetylglucosamine, galactosyl(β,1 → 6)-N-acetylglucosamine, and the α- and β-methyl-D-galactopyranosides. The substrate specificity indicates that the fucosyltransferase is responsible for the incorporation of L-fucose into plasma glycoproteins in vivo. The enzyme is membrane-bound and requires Triton X-100 and divalent cation for optimum activity. The other pork liver fucosyltransferase incorporates L-fucose into a position on the oligosaccharide prosthetic group of α1-acid glycoprotein at which L-fucose does not occur in the native glycoprotein; the function of this enzyme in pork liver is not known.

L-Fucose (6-deoxy-L-galactose) is widely distributed in bacteria, plants, and animals as a component of various macromolecules such as milk oligosaccharides (1), human and porcine blood group substances (2-5), plasma glycoproteins (6, 7), and glycolipids (8, 9). The L-fucose is incorporated into macromolecules by a family of enzymes called the fucosyltransferases which transfer L-fucose from GDP-L-fucose into specific acceptor molecules. Fucosyltransferases have been described which transfer L-fucose from GDP-L-fucose into galactosyl residues (blood group H antigen), into (α,1 → 4) linkage to N-acetylgalactosaminyl residues (blood group Lea antigen) and into (α,1 → 3) linkage to β-glucosyl or N-acetylgalactosaminyl residues (10-16). Plasma glycoproteins such as α1-acid glycoprotein contain variable amounts of L-fucose at terminal positions on the oligosaccharide chains; the fucose residues of native α1-acid glycoprotein appear to be linked (α,1 → 3) to galactosyl residue (17, 18). The fucosyltransferase responsible for the incorporation of these fucose residues has not yet been described. This report shows the presence of at least two fucosyltransferases in pork liver, one of which is probably the enzyme responsible for incorporation of fucose into plasma glycoproteins in vivo; the other pork liver fucosyltransferase is similar to an enzyme of unknown function previously reported in HeLa cells (19, 20) and may be related to one of the fucosyltransferases which transfer fucose to N-acetyl glucosaminyl residues in the process of blood group glycoprotein synthesis.

EXPERIMENTAL PROCEDURE

Materials—Porcine liver was obtained fresh from Canada Packers, Toronto, and kept on ice until used. L-Fucose-1-14C, 48.6 mCi per mmole, was purchased from Calbiochem and was diluted with nonradioactive L-fucose (Sigma) to give a specific activity of 4.35 mCi per mmole.

Dr. Y. C. Lee, Johns Hopkins University, provided samples of α-methyl-D-galactoside and a mixture of α- and β-methyl-D-galactoside. Dr. E. J. McGuire, Johns Hopkins University, donated samples of galactosyl(β,1 → 3)-N-acetylglucosamine, galactosyl(β,1 → 6)-N-acetylglucosamine, and fetuin. Dr. G. W. Jourdain, University of Michigan, provided generous amounts of galactosyl(β,1 → 4)-N-acetylglucosamine. Supernatant remaining after the preparation of Cohn Fraction V from pooled human plasma was supplied by Dr. G. A. McVicar, Connaught Laboratories, University of Toronto, and was used to prepare α1-acid glycoprotein by the method of Schmid (21). Fetuin and α1-acid glycoprotein were treated with a highly purified preparation of sialidase from Clostridium perfringens (22) and the sialidase was removed by column chromatography as previously described (23). After sialidase treatment, α1-acid glycoprotein had 0.42 μmole of potential acceptor sites for sialic acid per mg of glycoprotein. The protein was further treated with a partially purified β-galactosidase from C. perfringens (24) to expose 0.44 μmole of potential acceptor sites for N-acetylglucosamine per mg of glycoprotein and with a mixture of β-galactosidase and β-N-acetylgalactosaminidase from C. perfringens (24) to expose 0.45 μmole of potential acceptor sites for N-acetylglucosaminyl per mg of glycoprotein. The glycosidases were removed by passage through columns of Sephadex G-100. α1-acid glycoprotein was hydrolyzed with 0.4 N H2SO4 at 70° for 1 hour; this treatment removed all of the sialic acid and most of the fucose, exposing 0.48 μmole of terminal galactosyl residues per mg of glycoprotein. Acid was removed by neutralization with sodium hydroxide followed by dialysis against water. Submaxillary mucins were prepared from ovine, bovine, and porcine submaxillary glands with the detergent hexadecyltrimethylammonium bromide (25).
The mucins were treated with sialidase from *C. perfringens* (22) to remove over 95% of the bound sialic acid and sialidase was inactivated by heating at 60° for 30 min (28). Fetuin and porcine submaxillary mucin were subjected to mild acid hydrolysis (1 N HClO4 at 70° for 3 hours) to remove protein-bound sialic acid and, in the case of the mucin, protein-bound fucose; fetuin does not contain fucose. The release of sugars during the preparation of the above compounds was monitored by assaying for free sialic acid (27), *N*-acetylgalactosamine (28), fucose (29), and galactose (29). All other chemicals were of commercial origin.

Preparation of GDP-β-[1-14C]-fucose-The method of Ishihara et al. (30, 31) was modified as follows. All procedures were carried out at 4°. Pork liver L-fucose kinase was purified up to the protamine sulfate step as previously described (30). Solid ammonium sulfate was added to the protamine sulfate supernatant to bring the saturation to 30% and the solution was centrifuged at 20,000 g for 20 min. The ammonium sulfate pellet obtained from 250 g of pork liver was dissolved in about 15 ml of 0.13 M sodium phosphate buffer at pH 7.4. An incubation mixture was prepared which contained the following (in micromoles): GDP-β-[1-14C]-fucose, 67 (8.7 × 10^6 cpm per μmole); ATP, 2,000; MgCl2, 1,000; KF, 1,000; Tris, pH 8.0, 6,700; and 10 μl of the crude L-fucose kinase preparation described above. After incubation at 37° for 2 hours, the reaction was stopped by the addition of 2 volumes of ethanol and β-L-fucose-1-[14C]-1-phosphate was isolated as previously described (30). The pork liver supernatant remaining after precipitation of L-fucose kinase activity at 30% ammonium sulfate was adjusted to an ammonium sulfate concentration of 50%, centrifuged at 20,000 g for 20 min. The resulting pellet was dissolved in about 10 ml of 0.03 M sodium phosphate buffer at pH 7.4 containing 0.1 mM dithiothreitol. This crude preparation of GDP-β-L-fucose-[14C]- was further purified by gel filtration on a Sephadex G-100 column (5 × 85 cm) equilibrated with 0.03 M sodium phosphate buffer at pH 7.4 containing 0.1 mM dithiothreitol. An incubation was prepared which contained the following (in micromoles) in a final volume of 100 ml: L-fucose-1-[14C], 67 (8.7 × 10^6 cpm per μmole); ATP, 2,000; MgCl2, 1,000; KF, 1,000; Tris, pH 8.0, 6,700; and 10 μl of the crude L-fucose kinase preparation described above. After incubation at 37° for 2 hours, the reaction was stopped by the addition of 2 volumes of ethanol and β-L-fucose-1-[14C]-1-phosphate was isolated as previously described (30). The pork liver supernatant remaining after precipitation of L-fucose kinase activity at 30% ammonium sulfate was adjusted to an ammonium sulfate concentration of 50% and was centrifuged at 20,000 g for 20 min. The resulting pellet was dissolved in about 10 ml of 0.03 M sodium phosphate buffer at pH 7.4 containing 0.1 mM dithiothreitol. This crude preparation of GDP-β-L-fucose-1-[14C]- was further purified by gel filtration on a Sephadex G-100 column (5 × 85 cm) equilibrated with 0.03 M sodium phosphate buffer at pH 7.4 containing 0.1 mM dithiothreitol. An incubation was prepared which contained the following (in micromoles) in a final volume of 300 ml: β-L-fucose-1-[14C]-1-phosphate, 97; GTP, 210; MgCl2, 1,900; KF, 2,400; Tris, pH 8.0, 12,000; and 150 ml of GDP-β-L-fucose-1-phosphorylase from the Sephadex G-100 column. After incubation at 37° for 2 hours, the reaction was stopped by addition of 2 volumes of ethanol. The solution was centrifuged at 4,000 × g for 15 min and the pellet was re-extracted with ethanol. The combined ethanol extracts were flash evaporated at 37° and 300 ml of water were added. The solution was loaded on a column (5 × 2 cm) of Dowex 1-X2, chloride form, 50 to 100 mesh, and the column was washed with 400 ml of 0.005 M Tris-HCl buffer, pH 7.5. The adsorbed materials were fractionated by elution with a linear gradient (3000 ml) of 0 to 1.0 M KC1 in 0.005 M Tris-HCl, pH 7.5. Fractions were assayed for radioactivity and for ultraviolet light-absorbing materials; GDP-β-L-fucose-[14C] was eluted after GDP-β-fucose-1-phosphate and the main ultraviolet light-absorbing peak. The GDP-fucose peak was pooled, flash evaporated at 37°, and desalted by passage through a column (5 × 80 cm) of Sephadex G-100 equilibrated with 0.05 M triethylamine-bicarbonate, pH 7.5. Yields of GDP-β-fucose-[14C] were usually better than 60%. The final GDP-β-fucose-[14C] preparation had a specific activity of 8.3 × 10^6 cpm per μmole, gave an ultraviolet spectrum characteristic of a guanosine nucleotide, and ran as a single radioactive peak on high voltage electrophoresis at pH 6.5 in pyridine acetate (32) and on paper chromatography in Solvent Systems I and II (see below). Hydrolysis of the nucleotide sugar with 0.01 M HCl for 10 min at 100° released a radioactive compound which migrated with standard L-fucose on high voltage electrophoresis in 1% sodium tetaborate and on paper chromatography in Solvent Systems I and III (see below).

Preparation of Pork Liver Fucosyltransferase—All procedures were conducted at 4°. Three pieces of fresh pork liver, about 200 g each, were obtained from three different animals and were ground in a meat grinder. The mince was stirred thoroughly with a glass rod and a 7-g portion was homogenized in 28 ml of 0.25 M sucrose in 0.05 M Tris-HCl buffer at pH 8.0, containing 0.001 M dithiothreitol, with three strokes of a motor-driven glass-Teflon Potter-Elvejem homogenizer (A. H. Thomas, Size C). The homogenate was filtered through four layers of gauze and centrifuged at 14,000 × g for 20 min. The pellet was discarded and the supernatant was centrifuged at 48,000 × g for 30 min. The supernatant was discarded and the pellet was suspended in 7 ml of 0.95 M sucrose containing 0.001 M dithiothreitol; the suspension was homogenized with two or three strokes of a hand-driven Duall type ground glass homogenizer. The resulting preparation had about 50% of the fucosyltransferase activity present in the crude homogenate and was used in all experiments reported below. The protein concentrations of these preparations varied between 7 and 17 mg of protein per ml. The high speed supernatant derived from the above pork liver homogenate had negligible amounts of enzyme activity, indicating the fucosyltransferase to be a membrane-bound enzyme as are the other known mammalian liver glycosyltransferases (23).

Fucosyltransferase Assays—Enzyme activities were determined by measuring incorporation of 3H-L-fucose from GDP-3H-L-fucose into various acceptors. Concentrations of acceptors are expressed in terms of available sites for fucose incorporation. The incubation mixture for the pork liver enzyme contained (in micromoles): α-acid glycoprotein treated with sialidase, 0.29; GDP-3H-L-fucose, 0.012; Tris, pH 8.0, 2.5; MgCl2, 2.0; GTP, 0.125; and 0.5 μl of Triton X-100 and from 0.2 to 0.3 mg of enzyme protein in a final volume of 0.05 ml. The above mixture was incubated at 37° for 1 hour and the reaction was stopped by addition of 0.020 ml of 2% sodium tetraborate containing 0.50 M EDTA. Aliquots (0.100-ml) were then subjected to high voltage paper electrophoresis in 1% sodium tetaborate at 2.5 kv for 45 min. Acceptor-bound radioactivity remained near the origin and was counted by liquid scintillation techniques as previously described (32). The product was well separated from 3H-L-fucose and GDP-3H-fucose which migrated away from the origin on high voltage electrophoresis.

Control incubations were conducted on all samples in the absence of acceptor and the endogenous incorporation so obtained was subtracted from the incorporation in the presence of exogenous acceptor in the calculation of enzyme activities. The endogenous values were always less than 10% of the values obtained with exogenous acceptor.

Paper Chromatography—Paper chromatography by the descending technique was performed with Whatman No. I paper with the following solvent systems: I, ethanol-1 M ammonium acetate at pH 7.3; II, ammonium hydroxide-water-ethanol (1:19:80); III, isobutyric acid-ammonium...
by addition of 3 g of Dowex 50W-X8, in the hydrogen ion form, Dowex 1-X2 in the bicarbonate form, 50 to 100 mesh, followed and were desalted by passage through a column (1 x 5 cm) of 14C-fucose. The hydrolysates were neutralized with NaOH gels was 37%. Aliquots (0.4-ml) of the radioactive products 2,5-diphenyloxazole and 100 mg of p-bis[2-(5-phenyloxazolyl)]-scintillation counter. The recovery of radioactivity from the benzene per liter and the solution was counted with a liquid this suspension were added 15 ml of toluene containing 4 g of this table have not been corrected for activity in the absence of added glycoprotein acceptor.

| Omission from complete incubation mixture | Fucosyltransferase activity % |
|------------------------------------------|-------------------------------|
| None                                     | 100                           |
| Glycoprotein acceptor                    | 11                            |
| Active enzyme replaced by heat-inactivated enzyme (80° for 3 min) | 12                            |
| MgCl₂                                    | 36                            |
| MgCl₂ replaced by 2.5 μmoles of EDTA     | 25                            |
| None, but 2.5 μmoles of EDTA added       | 35                            |
| GTP                                      | 48                            |
| Triton X-100                             | 29                            |

hydroxide-water (57:4:39). Radioactivity was detected on chromatograms by use of a Packard strip scanner. Sugars were detected with a periodate-benzidine stain (33).

Protein Assay—Protein was determined by the method of Lowry et al. (34) with bovine serum albumin as standard.

Preparation of Fucosyltransferase Reaction Products—The following preparative scale incubations were set up to obtain sufficient reaction products for characterization studies. (a)

In a total volume of 0.25 ml, the incubation contained (in micromoles) α1-acid glycoprotein treated with sialidase, 1.10; GDP-14C-fucose, 0.060; Tris, pH 8.0, 12.5; MgCl₂, 10.0; GTP, 0.625; and 2.5 μl of Triton X-100 and 1.20 mg of pork liver fucosyltransferase. (b) As in except that the acceptor was 1.15 μmoles of α1-acid glycoprotein which had been previously treated with sialidase and β-galactosidase. The mixtures were incubated at 37° for 6 hours and were then diluted 10-fold with distilled water and dialyzed at 4° against 0.1 M KCl followed by frequent changes of distilled water. The dialyzed solutions were lyophilized and each of the residues was taken up in 2.0 ml of water. The radioactive products were subjected to polyacrylamide disc gel electrophoresis at pH 9.3 with the apparatus and buffer system originally described by Davis and Ornstein (35, 36). After electrophoresis, the gels were sliced longitudinally and one-half was stained with Amido black whereas the other half was cut into small segments (about 2 mm long). The unstained segments were finely minced with a sharp knife and suspended in

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Requirements for GDP-fucose:glycoprotein fucosyltransferase

The complete incubation mixture contained (in micromoles): α1-acid glycoprotein treated with sialidase, 0.22; GDP-14C-fucose, 0.012; Tris, pH 8.0, 2.5; MgCl₂, 2.0; GTP, 0.128; and 0.5 μl of Triton X-100 and 0.24 mg of enzyme protein in a total volume of 0.050 ml. After 60 min at 37°, the reaction was stopped and assayed as described in the text. The enzyme activities listed in this table have not been corrected for activity in the absence of added glycoprotein acceptor.

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Table II

Acceptor specificity studies

Pork liver fucosyltransferase was assayed as described in the text except that α1-acid glycoprotein treated with sialidase was replaced by various other acceptors.

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FIG. 2 (left). Effect of incubation time on the incorporation of $^{14}$C-fucose into various acceptors by pork liver and submaxillary gland fucosyltransferases. (See the text, footnote 1.) The pork liver enzyme incubations contained the following acceptors (in milligrams of acceptor per 0.010 ml of incubation mixture): α1-acid glycoprotein, treated with sialidase, 0.12, □; α1-acid glycoprotein, treated with sialidase and β-galactosidase, 0.10, ●; N-acetyllactosamine, 0.05, Δ, and porcine submaxillary mucin hydrolyzed with mild acid, 0.10, △. The pork submaxillary gland enzyme incubation contained 0.10 mg of porcine submaxillary mucin hydrolyzed with mild acid per 0.010 ml of incubation mixture, ○. Incubations were carried out as described in the text.

the various papers corresponding to free fucose were cut out and counted in a liquid scintillation counter.

RESULTS

Requirements for Assaying Pork Liver Fucosyltransferase—Table I lists the requirements for assaying the pork liver fucosyltransferase. It is evident that omission of any of the components of the incubation mixture resulted in a significant loss of enzyme activity. The value obtained in the absence of added acceptor was identical with the value obtained with heat-inactivated enzyme. The transferase showed a partial requirement for Mg$^{2+}$ and for Triton X-100. The stimulation of enzyme activity by GTP was probably due to a protective effect on GDP-fucose (10).

Stability—The pork liver fucosyltransferase lost 18% of its activity on storage at -20° for 4 days. Most of the experiments reported below were therefore carried out with enzyme preparations which were no older than 2 days and which had been stored at 4°.

Effect of Time, Protein Concentration, pH, and Temperature—Under the standard assay conditions, product formation by the pork liver fucosyltransferase was linear with time for at least 2 hours, and with enzyme concentration to at least 0.5 mg of enzyme protein in 0.050 ml of incubation. The pork liver fucosyltransferase had a fairly broad pH optimum between 6.5 and 8.5. The temperature optimum for the standard assay was 37°.

Effect of Cation, GTP, and Detergent Concentrations—The enzyme showed a partial requirement for cation (Table I). Both Mg$^{2+}$ and Mn$^{2+}$ were equally effective in stimulating enzyme activity. Both cations achieved optimum stimulation at about 0.020 to 0.040 M and further increases of cation concentration to values as high as 0.12 M had relatively little added effect on enzyme activity.

The stimulatory effect of GTP showed an optimum between 0.0005 M and 0.0025 M GTP. Triton X-100 showed its maximum stimulatory effect at a concentration of 0.2% (v/v) and increases of concentration to values as high as 2% had no added effect on enzyme activity.

Effect of GDP-fucose Concentration—Fig. 1 shows the effect of varying the concentration of GDP-fucose$^{14}$C on pork liver fucosyltransferase activity using as acceptor α1-acid glycoprotein treated with sialidase. It is apparent that the enzyme obeyed Michaelis-Menten kinetics for this substrate. The $K_m$ was calculated to be $7.8 \times 10^{-5}$ M for the nucleotide sugar.

Acceptor Specificity Studies—Table II shows the abilities of acceptors other than α1-acid glycoprotein treated with sialidase to accept fucose from GDP-fucose under the influence of pork liver fucosyltransferase. α1-acid glycoprotein, which has lost both fucose and sialic acid by hydrolysis in mild acid, was as effective an acceptor as α1-acid glycoprotein treated with sialidase. It can therefore be concluded that the galactosyl residues which have fucose attached in the native glycoprotein do not have enhanced affinity for fucose but rather that the sialyl- and
Pork liver fucosyltransferase was assayed as described in the text, with 0.26 mg of enzyme protein in the standard assay, in the presence of either one or two acceptors, every acceptor was tested at three different concentrations. The calculated velocity for an incubation in the presence of two acceptors, each of which is being acted upon by a different fucosyltransferase, is obtained by summing the activities obtained in the presence of each acceptor alone. The calculated velocity for an incubation in the presence of two acceptors, each of which is competing for the same enzyme, is obtained from the formula below (39); where \( v \) is the velocity in the presence of both acceptors, \( A_1 \) and \( A_2 \), are the concentrations of the two acceptors, \( V_1 \) and \( V_2 \) are the respective maximum velocities, and \( K_1 \) and \( K_2 \) are the Michaelis constants. The values for \( V_{\max} \) and \( K_m \) are, respectively: \( \alpha_1 \)-acid glycoprotein, hydrolyzed with mild acid, 1,970 cpm, 1.97 mM; \( \alpha_1 \)-acid glycoprotein, treated with sialidase and \( \beta \)-galactosidase, 21,000 cpm, 17.0 mM; and N-acetyllactosamine, 3,890 cpm, 3.1 mM.

\[
v = \frac{V_1 \left( \frac{A_1}{K_1} \right) + V_2 \left( \frac{A_2}{K_2} \right)}{1 + \frac{A_1}{K_1} + \frac{A_2}{K_2}}
\]

### Table III

**Competition studies**

| Acceptor | \( \alpha_1 \)-Acid glycoprotein treated with sialidase | Calculated velocity for One enzyme | Calculated velocity for Two enzymes | \( \alpha_1 \)-Acid glycoprotein treated with sialidase and \( \beta \)-galactosidase | Conclusion: no. of enzymes |
|----------|-----------------------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------------------------|-----------------------------|
|          | cpm/hr                                              |                                   |                                   | cpm/hr                                              |                             |
| m.w      | m.w                                                 |                                   |                                   | m.w                                                 |                             |
| 2.2      | 1006                                                | 2190                              | 2976                              | 2                                                   |                             |
| 2.2      | 1006                                                | 2190                              | 2976                              | 2                                                   |                             |
| 4.4      | 1226                                                | 4340                              | 6.5                               | 6                                                   |                             |
| 4.4      | 1226                                                | 4340                              | 6.5                               | 6                                                   |                             |
| 8.8      | 7420                                                | 12.0                              | 1512                              | 12.9                                                |                             |
| 8.8      | 7420                                                | 12.0                              | 1512                              | 12.9                                                |                             |

| Acceptor | \( \alpha_1 \)-Acid glycoprotein hydrolyzed with acid | Calculated velocity for One enzyme | Calculated velocity for Two enzymes | \( \alpha_1 \)-Acid glycoprotein treated with sialidase and \( \beta \)-galactosidase | Conclusion: no. of enzymes |
|----------|-----------------------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------------------------|-----------------------------|
|          | cpm/hr                                              |                                   |                                   | cpm/hr                                              |                             |
| m.w      | m.w                                                 |                                   |                                   | m.w                                                 |                             |
| 2.2      | 1006                                                | 1352                              | 2008                              | 1                                                   |                             |
| 4.4      | 1226                                                | 1530                              | 2556                              | 1                                                   |                             |
| 8.8      | 1512                                                | 1718                              | 3262                              | 1                                                   |                             |

| Acceptor | \( \alpha_1 \)-Acid glycoprotein treated with sialidase | Calculated velocity for One enzyme | Calculated velocity for Two enzymes | \( \alpha_1 \)-Acid glycoprotein treated with sialidase and \( \beta \)-galactosidase | Conclusion: no. of enzymes |
|----------|-----------------------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------------------------|-----------------------------|
|          | cpm/hr                                              |                                   |                                   | cpm/hr                                              |                             |
| m.w      | m.w                                                 |                                   |                                   | m.w                                                 |                             |
| 2.2      | 1006                                                | 2295                              | 3421                              | 1                                                   |                             |
| 4.4      | 1226                                                | 2358                              | 4206                              | 1                                                   |                             |
| 8.8      | 3360                                                | 2845                              | 5110                              | 1                                                   |                             |

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|          | cpm/hr                                              |                                   |                                   | cpm/hr                                              |                             |
| m.w      | m.w                                                 |                                   |                                   | m.w                                                 |                             |
| 2.2      | 1006                                                | 2295                              | 3421                              | 1                                                   |                             |
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| 8.8      | 3360                                                | 2845                              | 5110                              | 1                                                   |                             |
fucoysltransferases appear to compete for available galactosyl residues in a random manner during the biosynthesis of \( \alpha \)-acid glycoprotein in pork liver. \( \alpha \)-acid glycoprotein, which has terminal N-acetyllactosaminyl residues exposed by treatment with sialidase and \( \beta \)-galactosidase, was also an excellent fucose acceptor; however, both the \( V_{\text{max}} \) and \( K_m \) for this acceptor were appreciably higher than the values for \( \alpha \)-acid glycoprotein treated with either mild acid or sialidase, indicating that a different fucoysltransferase might be involved. The existence of the two fucoysltransferases was in fact proved by competition studies below. \( \alpha \)-acid glycoprotein treated with sialidase, \( \beta \)-galactosidase, and \( \beta \)-N-acetyllactosaminidase accepted fucose at about one-tenth of the rate of the protein treated with sialidase and \( \beta \)-galactosidase; this finding is compatible with the fact that \( \beta \)-N-acetylgalactosaminidase did not remove all the terminal N-acetyllactosamine residues (23) and those residues remaining were therefore available for fucose incorporation. It cannot, however, be ruled out that mannose residues exposed by \( \beta \)-N-acetylgalactosaminidase did not also accept fucose. Fetuin treated with either sialidase or mild acid has terminal galactosyl residues exposed as a result of the removal of sialic acid and both proteins were excellent fucose acceptors. Porcine submaxillary mucin, hydrolyzed with mild acid to remove both fucose and sialic acid, was a relatively poor fucose acceptor with the pork liver fucoysltransferase but a highly active acceptor with pork submaxillary gland enzyme; porcine submaxillary mucin treated with sialidase was totally ineffective with either enzyme and it can be therefore concluded that fucose becomes attached to the galactosyl residue exposed by mild acid hydrolysis rather than to the N-acetyllactosaminyl residue exposed by both sialidase and mild acid treatment (5).

Table II also shows the results of acceptor studies with low molecular weight compounds. The only effective acceptor was the disaccharide galactosyl-(\( \beta \), 1 \( \rightarrow \) 4)-N-acetyllactosamine (N-acetyllactosamine). Ineffective were lactose, the (\( \beta \), 1 \( \rightarrow \) 3) and (\( \beta \), 1 \( \rightarrow \) 6) isomers of N-acetyllactosamine and various other sugars and galactosides. This substrate specificity resembles that for goat colostrum sialyltransferase (27) and porcicoid liver and serum sialyltransferases (28) and indicates that a terminal galactosyl residue, a penultimate N-acetyllactosaminyl residue, and a (\( \beta \), 1 \( \rightarrow \) 4) linkage between the two are all essential components of the substrate requirements for both the sialyl- and fucoysltransferases in pork liver.

Fig. 2 shows the time course of \( ^{14} \)C-fucose incorporation into four different acceptors, catalyzed by pork liver fucoysltransferase. Fig. 2 also shows the time course for pork submaxillary gland enzyme using as acceptor porcine submaxillary mucin hydrolyzed with mild acid. It is evident that the incorporation catalyzed by the pork liver enzyme achieved a maximum at about 6 hours and then began to decrease whereas the submaxillary gland enzyme catalyzed incorporation continuously for at least 24 hours. This finding indicates the presence in pork liver of a fucoyslase capable of removing \( ^{14} \)C-fucose from the various products of the fucoysltransferase reaction.

Effect of Acceptor Concentration—Figs. 3 and 4 show the effects on fucoysltransferase activity of varying the concentrations of some of the acceptors. The only acceptors used for these studies were those containing galactosyl residues exposed by mild acid hydrolysis.

\( \alpha \)-acid glycoprotein treated with sialidase alone and with both sialidase and \( \beta \)-galactosidase. Similar data have been obtained for other acceptors. There was reasonable adherence to Michaelis-Menten kinetics at low acceptor concentrations but high concentrations of some of the acceptors resulted in inhibition of enzyme activity.

Competition Studies—Since the pork liver fucoysltransferase preparation used in this study was a crude membrane-bound preparation, the only techniques available to determine the number of enzymes in the preparation were indirect ones such as competition studies. Table III shows the results of acceptor competition studies; every acceptor was tested at three concentrations against every other acceptor. Comparison of the experimental values for \( ^{14} \)C-fucose incorporation with calculated values (Table III) indicates that \( \alpha \)-acid glycoprotein treated with sialidase, \( \alpha \)-acid glycoprotein hydrolyzed with mild acid, and N-acetyllactosamine competed with one another for a common active site; \( \alpha \)-acid glycoprotein treated with sialidase and \( \beta \)-galactosidase, however, did not compete with any of the above acceptors, indicating the presence of a second fucoysltransferase. Thus, pork liver has at least two fucoysltransferases, one acting on acceptors with terminal galactosyl residues and the other acting on acceptors with terminal N-acetyllactosaminyl residues.

Characterization of Reaction Products—The products obtained by the action of pork liver fucoysltransferase on \( \alpha \)-acid glycoprotein derivatives showed one major and two minor bands on polyaerylamide disc gel electrophoresis. Radioactivity corresponded to the major protein band in all cases indicating incorporation of \( ^{14} \)C-fucose into glycoprotein. Also, acid hydrolysates of the reaction products all showed release of at least 90% of radioactivity as free \( ^{14} \)C-fucose.

**Discussion**

\( \alpha \)-Fucose is known to occur in human blood group substances and human milk oligosaccharides (1-4) in the following linkages: (1) (\( \alpha \), 1 \( \rightarrow \) 2) to galactose, the blood group H antigenic determinant, (b) (\( \alpha \), 1 \( \rightarrow \) 4) to N-acetyllactosamine, the Lewis blood group antigenic determinant, and (c) (\( \alpha \), 1 \( \rightarrow \) 3) to either glucose or N-acetyllactosamine. Fucosyl-(\( \alpha \), 1 \( \rightarrow \) 2)-galactosyl also occurs in porcine submaxillary mucin (5). Fucoysltransferases have been described which are involved in the biosynthesis of all the above human oligosaccharides (10-16) and of porcine submaxillary mucin. The structure of a glycopeptide isolated from human \( \alpha \)-acid glycoprotein has been shown to contain fucose linked (\( \alpha \), 1 \( \rightarrow \) 3) to galactose (18). The present study was undertaken to demonstrate the fucoysltransferase in mammalian liver responsible for incorporating this latter fucose into \( \alpha \)-acid glycoprotein.

The experiments reported above indicate that pig liver contains at least two fucoysltransferases, a GDP-fucose:galactoside fucoysltransferase and a GDP-fucose:N-acetyllactosaminidase fucoysltransferase. The latter enzyme is similar to a fucoysltransferase previously described in HeLa cells by Bosmann et al. (19, 20) which incorporates fucose into fetuin treated with sialidase and \( \beta \)-galactosidase, presumably into linkage with a terminal N-acetyllactosaminyl residue. The linkage between fucose and N-acetyllactosamine in the products from either of these enzymes is not known nor is it known whether these enzymes are the same as one of the human blood group fucoysltransferases involved in incorporating fucose into either (\( \alpha \), 1 \( \rightarrow \) 4) or (\( \alpha \), 1 \( \rightarrow \) 3) linkages.
3) or (α, 1 → 4) linkage to N-acetylglucosamine (12-16). These questions require more detailed study. The main emphasis of the present report has been to demonstrate that the pork liver GDP-fucose: galactoside fucosyltransferase is a previously unreported enzyme which is probably responsible for incorporating fucose into α1-acid glycoprotein and other plasma glycoproteins in vivo.

Several GDP-fucose: galactoside fucosyltransferases have been described. Bosmann et al. (19, 20) have described such an enzyme in HeLa cells using as acceptor porcine submaxillary mucin hydrolyzed with mild acid. The HeLa cell extract is incapable of transferring fucose to either sialic acid-free α1-acid glycoprotein or fetuin and is therefore different from the pork liver enzyme which can utilize both these proteins as acceptors. Porcine submaxillary gland also contains a fucosyltransferase capable of transferring fucose to the terminal galactosyl residues of porcine submaxillary mucin hydrolyzed with mild acid. The following evidence indicates that this enzyme is different from the pork liver enzyme. For the pork liver enzyme, α1-acid glycoprotein treated with sialidase is 3 times more effective as a fucose acceptor than porcine submaxillary mucin hydrolyzed with mild acid; the mucin is 4 times more effective than the glycoprotein acceptor for the pork submaxillary gland enzyme. Table II shows that N-acetyllactosamine is an excellent acceptor for the pork liver enzyme while lactose and the (β, 1 → 3) and (β, 1 → 6) isomers of N-acetyllactosamine are ineffective; the porcine submaxillary gland fucosyltransferase, however, is most effective with galactosyl-(β, 1 → 3)-N-acetylglucosamine and is relatively inactive toward lactose, N-acetyllactosamine and galactosyl-(β, 1 → 6)-N-acetylglucosaminyl. It is interesting that the galactosyl-(β, 1 → 4)-N-acetyllactosaminyl structure is present at the termini of plasma glycoproteins whereas the galactosyl-(β, 1 → 3)-N-acetylgalactosaminyl structure is present in porcine submaxillary mucin (5); it appears, therefore, that the porc liver and submaxillary gland fucosyltransferases are different enzymes and that the former is probably involved in plasma glycoprotein synthesis whereas the latter is involved in mucin synthesis. Finally, human milk and other tissues (13, 15) contain a GDP-fucose: galactoside fucosyltransferase involved in synthesis of the blood group H antigenic determinant. The human H-specific transferase can be distinguished from the pork liver enzyme by the ability of the former to transfer fucose not only to N-acetyllactosamine but also to lactosamine and galactosyl-(β, 1 → 3)-N-acetylgalactosaminyl (15). Thus on the basis of substrate specificity, it is evident that the pork liver fucosyltransferase is different from all previously described fucosyltransferases. The actual linkage catalyzed by the porc liver enzyme is not yet known. If the porc liver enzyme is in fact concerned with the incorporation of fucose into plasma glycoprotein in vivo, as the substrate specificity studies suggest, then the expected linkage would be (α, 1 → 3).

It is of interest to note that both pork liver fucosyltransferase (Table II) and pork liver sialyltransferase (38) have identical substrate specificities in that they require a galactosyl-(β, 1 → 4)-N-acetylgalactosaminyl terminus to function. Further, since α1-acid glycoprotein treated with sialidase and α1-acid glycoprotein hydrolyzed with mild acid show identical kinetic parameters with the fucosyltransferase (Table II), it appears that the galactosyl residues which carry fucose in the native glycoprotein have no enhanced affinity for fucose. Thus, whenever a galactosyl-(β, 1 → 4)-N-acetylgalactosaminyl terminus presents itself during the process of plasma glycoprotein synthesis in liver (23) both the sialyl- and fucosyltransferases have the ability to incorporate their respective glycosic residues. What, then, determines the fucose to sialic acid ratios characteristic of individual glycoproteins? The answer to this question is not known but a number of different factors can presumably be involved. For example, the relative activities of the two transferases must be important; using modified α1-acid glycoprotein as acceptor, the activity of pork liver fucosyltransferase is about 1 n mole per mg of protein per hour and the activity of pork liver sialyltransferase is at least 10 times higher (38). Other factors are the relative affinities for nucleotide-sugar, the concentrations of nucleotide-sugar available to the transferases, and the sequence in which the transferases are arranged along the membranes that constitute the assembly line of glycoprotein biosynthesis (20). If all the plasma glycoproteins of a particular species pass through the same assembly line in the liver, then the above factors cannot explain differences in the fucose to sialic acid ratios between these glycoproteins. Such differences may be due to differences in the rates of migration of different glycoproteins through the assembly line or to the existence of separate glycosyltransferases for every glycoprotein. The latter hypothesis would require the existence of many fucosyltransferases in liver, each one specific for a particular glycoprotein. Preliminary competition studies with pork liver sialyltransferases (38) have shown that the same transferase acts on sialidase-treated human α1-acid glycoprotein, sialidase-treated calf fetuin, and N-acetyllactosamine. Much further work is required before this observation can be extended to other glycoproteins, other glycosyltransferases, and other species.

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REFERENCES

1. Stacey, M., and Barker, S. A., Carbohydrates of living tissues, Van Nostrand, London, 1962, p. 122.
2. Watkins, W. M., Science, 152, 172 (1965).
3. Marcus, D. M., N. Engl. J. Med., 250, 994 (1959).
4. Kabat, E. A., in D. Aminoff (Editor), Blood and tissue antigens, Academic Press, New York, 1970, p. 187.
5. Carlsson, D. M., J. Biol. Chem., 253, 5160 (1980).
6. Witzke, R. J., in E. A. Balazs and R. W. Jeanloz (Editors), The amino sugars, Vol. IIA, Academic Press, New York, 1968, p. 337.
7. Spilo, R. G., N. Engl. J. Med., 281, 991, 1043 (1969).
8. Hakomori, S., and Jeanloz, R. W., J. Biol. Chem., 239, PC 6005 (1964).
9. Hakomori, S., and Jeanloz, R. W., in D. Aminoff (Editor), Blood and tissue antigens, Academic Press, New York, 1970, p. 149.
10. Grollman, A. P., Hall, C. W., and Ginsburg, V., J. Biol. Chem., 240, 975 (1965).
11. Grollman, A. P., and Marcus, D. M., Biochem. Biophys. Res. Commun., 25, 542 (1966).
12. Jankovsky, Z., Marcus, D. M., and Grollman, A. P., Biochemistry, 5, 1123 (1970).
13. Shen, L., Grollman, E. F., and Ginsburg, V., Proc. Nat. Acad. Sci. U. S. A., 59, 224 (1968).
14. Geollman, E. F., Kobata, A., and Ginsburg, V., J. Clin. Invest., 48, 1499 (1969).
15. Chester, M. A., and Watkins, W. M., Biochem. Biophys. Res. Commun., 34, 835 (1969).
16. Watkins, W. M., in D. Aminoff (Editor), Blood and tissue antigens, Academic Press, New York, 1970, p. 441.
17. Hughes, R. C., and Jeanloz, R. W., Biochemistry, 5, 253 (1966).
18. Sato, T., Yoshizawa, Z., Masubuchi, M., and Yamanchi, F., Carbohydr. Res., 5, 387 (1967).
19. Roosmann, H. R., Haidopian, A., and Eylar, E. H., J. Cell. Physiol., 72, 81 (1969).
20. Roosmann, H. B., Haidopian, A., and Eylar, E. H., Arch. Biochem. Biophys., 128, 470 (1968).
21. Schmid, K., J. Amer. Chem. Soc., 75, 60 (1953).
22. Cassidy, J. T., Jourdain, G. W., and Roseman, S., J. Biol. Chem., 240, 3501 (1965).
23. Schachter, H., Jabbar, I., Hudgin, R. L., Pinteric, L., McGuire, E. J., and Roseman, S., J. Biol. Chem., 245, 1090 (1970).
24. Chipowsky, S., and McGuire, E. J., Fed. Proc., 28, 606 (1969).
25. Hashimoto, Y., Hashimoto, S., and Pigman, W., Arch. Biochem. Biophys., 104, 282 (1964).
26. Carlson, D. M., McGuire, E. J., Jourdain, G. W., and Roseman, S., in E. F. Neufeld and V. Ginsburg (Editors), Methods in enzymology, Vol. VIII, Academic Press, New York, 1966, p. 361.
27. Warren, L., J. Biol. Chem., 234, 1971 (1959).
28. Reissig, J. L., Strominger, J. L., and Leib, L. F., J. Biol. Chem., 217, 905 (1955).
29. Finch, P. R., Yuen, R., Schachter, H., and Moscarello, M. A., Anal. Biochem., 31, 256 (1969).
30. Ishihara, H., Massaro, D. J., and Heath, E. C., J. Biol. Chem., 243, 1103 (1968).
31. Ishihara, H., and Heath, E. C., J. Biol. Chem., 243, 1110 (1968).
32. Efron, M., in I. Smith (Editor), Chromatographic and electrophoretic techniques, Vol. II, Interscience Publishers, New York, 1960, p. 158.
33. Smith, I., in I. Smith (Editor), Chromatographic and electrophoretic techniques, Vol. I, Ed. 2, Interscience Publishers, New York, 1969, p. 252.
34. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
35. Davis, B. J., Ann. N. Y. Acad. Sci., 121, 404 (1964).
36. Orenstein, L., Ann. N. Y. Acad. Sci., 121, 321 (1964).
37. Bartholomew, B. A., Jourdain, G. W., and Roseman, S., in E. F. Neufeld and V. Ginsburg (Editors), Methods in enzymology, Vol. VIII, Academic Press, New York, 1966, p. 698.
38. Hudgin, R. L., and Schachter, H., Can. J. Biochem., 49, 829 (1971).
39. Dixon, M., and Webb, E. C., Enzymes, Ed. 2., Academic Press, New York, 1964, p. 84.
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