A solubilized α-fucosyltransferase activity has been isolated from a bovine spleen Golgi-rich membrane fraction. The enzyme transfers fucose from GDP-β-L-fucose to a blood group B-active pentaglycosylceramide acceptor (Galα(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc-ceramide) isolated from rabbit erythrocytes. Treatment of the membranes with 0.2% (final concentration) sodium taurodeoxycholate detergent produced maximal recovery (90%) of activity. A cationic detergent, G-3634-A, is required for optimal activity and the enzyme does not require addition of exogenous metal ion for activation. The purified 14C-labeled product of the reaction migrated with human blood group B-active hexaglycosylceramide on Silica Gel G thin layer plates. After treatment with α-galactosidase, the radioactive pentaglycosylceramide migrated with human H-active glycosphingolipid. The 14C-labeled product inhibited the hemagglutination reaction of B-type erythrocytes with Bandeiraea simplicifolia lectin and anti-B serum and formed a precipitin line with Euonymus europaeus lectin. Treatment of the 14C-labeled product with α-fucosidase (Venus mercenaria) or weak acid at 100 °C for 2 h released 80-90% of the bound radioactive fucose.

The glycoconjugate constituents of animal cell membranes with ABO(H) and Lewis blood group specificities are characterized by the presence of fucose residues as nonreducing terminals. A family of enzymes called fucosyltransferases transfer L-fucose residues from GDP-L-fucose into specific glycoconjugate acceptor molecules. Blood group glycoprotein: fucosyltransferases have been detected in milk (1), gastrointestinal mucosa (2-4), submaxillary glands (3), human serum (5, 6), and bone marrow (7). However, very little work has been published on the biosynthesis in vitro of blood group-active glycosphingolipids.

Lactotriaosylceramide (GlcNAcβ(1-3)Galβ(1-4)Glc-cer) serves as substrate for the synthesis of the core structures of the blood group-related glycosphingolipids such as nLcOse4Cer (Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc-cer) (8-11) and nLcOse4Cer (Galα(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc-cer) (8, 10-13). Biosynthesis in vitro of H blood group-active glycosphingolipid (FucαGalβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc-cer) using Golgi-rich membrane fraction has been achieved previously (14). Employing human serum as the source of fucosyltransferase, the biosynthesis of an H-active glycolipid has also been achieved (15). Fucosyltransferases involved in the biosynthesis in vitro of types H and B glycolipids have been detected in a membrane preparation isolated from a human neuroblastoma-derived clonal cell line, IMR-32 (16). However, structures of these biosynthesized products have yet to be determined.

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

In the present report we have shown that an α-L-fucosyltransferase activity solubilized from a bovine spleen Golgi-rich membrane fraction catalyzes the transfer of α-L-[14C]fucose from GDP-β-L-[14C]fucose to pentaglycosylceramide (nLcOse4Cer) acceptor. The product of the reaction is a human B-active fucose-containing hexaglycosylceramide (Galα(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc-cer) + GDP-β-L-[14C]fucose → Galα(1-3)[Fucα]Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glc-cer + GDP.

Treatment of a bovine spleen Golgi-rich membrane fraction (14) with 0.2% sodium taurodeoxycholate produced maximum recovery of α-L-fucosyltransferase activity in the 100,000 × g supernatant fraction. A 10-fold increase in the specific activity of the enzyme was obtained relative to the total homogenate. Endogenous acceptor activity of the system was almost completely eliminated (1-2 nmol/mg of protein in...
biosynthesis in vitro of blood group B-active glycolipid

49) to the accepted biosynthetic schemes for blood group agarose likewise do not have metal ion requirement. The residues attached to penultimate N-acetylglucosamine, far, from mouse brain (28) and HeLa cells (52), in that addition identical. These results suggest an alternative pathway (14, bovine spleen enzyme transfers fucose to terminal galactose purified by affinity chromatography on GDP-hexanolamine-samine moiety. It is therefore not surprising that the activities charides, led to the conclusion that the structural determinants of exogenous metal ion.

The radioactive hexa- and pentaglycosylceramides, thus, not polypeptide Glyco-15,51) cannot be ruled out at the present time. The solubilized fucosyltransferase is similar to the only other solubilized mammalian fucosyltransferases studied thus far, from mouse brain (28) and HeLa cells (52), in that addition of exogenous metal ion is not required for enzyme activation (Table 1). The human milk a-fucosyltransferases (27, 53) purified by affinity chromatography on GDP-hexanolamine-agarose likewise do not have metal ion requirement. The bovine spleen enzyme transfers fucose to terminal galactose residues attached to penultimate N-acetylglucosamine, whereas the brain and HeLa cell enzymes require penultimate N-acetylgalactosamine residues. However, the a-fucosyltransferase activities isolated from bovine spleen and rat small intestinal mucosa (29) both are inhibited competitively by GDP, the product of the reaction. In addition to GDP, GMP and GTP also inhibit these enzyme activities. Whether this inhibition is due to the attachment of any specific groups of the guanosine moiety at the GDP-fucose binding site has not been established unambiguously.

The radioactive hexa- and pentaglycosylceramides of the reaction is most probably a mixture of Gal-[\(^{14}C\)Fuc]Gal-GlcNAc-Gal-Glc-Cer and Gal-[\(^{14}C\)Fuc]Gal-GlcNAc-Gal-Glc-Cer, since two bands of similar R\(_f\) values (Fig. 4) have been observed on a radioautogram. The first reaction product co-migrated with human B-type glycosphingolipid, and the second product migrated in the region of fucose-containing hexa- and pentaglycosylceramides, but slightly below the B-active glycolipid, resembling the chromatographic mobility of glycolipids in which fucosyl residues are attached to N-acetylgalactosamine (45). Treatment of the \(\alpha\)-fucosyltransferase-catalyzed glycosphingolipids with endoglycosidases such as Charonia lampas and Venus mercenaria cleaved 40% and 80%, respectively. However, reaction conditions might have prevented complete hydrolysis.

Lactosylceramide is a poor acceptor with this enzyme preparation. Perhaps the fucose derivative extract contains another specific fucosyltransferase that catalyzes the transfer of L-fucose to either position C-3, C-4, or C-6 of the N-acetylgalactosamine residue. The presence of glycolipid (\(\alpha\)-1,3)fucosyltransferase activity in human serum has been reported by Pauszuska and Koscielek (15). The occurrence of two specific glycoproteins:fucosyltransferases in human serum and in rat liver microsomal membranes has been investigated by Schachtler and his co-workers (6, 54, 55). Since assay conditions can be better controlled with a solubilized enzyme system, our present report should be useful for further purification of specific fucosyltransferases and studies of the biosynthetic mechanisms of various blood group-active glycosphingolipids. Recently, Hill and co-workers (56, 57) reported the purification and substrate specificity of an H blood group \(\beta\)-galactoside \(\alpha\)-fucosyltransferase from porcine submaxillary glands. Unlike bovine spleen (\(\alpha\)-1,2)fucosyltransferase, this porcine \(\alpha\)-fucosyltransferase shows a preference for acceptors with nonreducing terminal galactose in a \(\beta\)-1 \(\rightarrow\) 3 linkage to an N-acetylgalactosamine (GlcNAc) or GalNAc). However, these differences in the substrate specificity between the two (\(\alpha\)-1,2)fucosyltransferases, isolated from two different species, might be due to the presence of two different proteins. Acknowledgment—We wish to thank Dr. Donald M. Huse for the generous gift sample of H-cyst glycoprotein.

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Supplementary Material to

Biosynthesis in Vitro of Blood Group B-active Glycolipid

B. E. Creese, R. M. Greaves, and M. H. Close

EXPERIMENTAL PROCEDURES

The following materials were purchased from commercial sources: bovine spleen (Centaur A) and porcine spleen (Gentner-Packling Co., St. Louis, Mo.), bovine erythrocyte (Venus Blood Products, Ltd., London, England), glycosphingolipid acceptors (142), and lacto-N-fucopentaose from the Gentner-Packling Co. (St. Louis, Mo.). Sodium tauro-deoxycholate (Sigma Chemical Co.) and sodium taurodeoxycholate (Sigma Chemical Co.); lacto-N-biose 15-w. Schumacher Co.); G-3634-A (Atlas Chemical Industries); and sodium tauro-deoxycholate (Sigma Chemical Co.).

The following materials were obtained as gift samples: B-2 glycosphingolipid (Dr. C. E. Thomas, The Upjohn Co.), B-3 glycosphingolipid (Dr. G. W. Schachter, Columbia University), and B-4 glycosphingolipid (Dr. W. O. Clark, Harvard University). The following materials were obtained as gift samples: B-2 glycosphingolipid (Dr. C. E. Thomas, The Upjohn Co.), B-3 glycosphingolipid (Dr. G. W. Schachter, Columbia University), and B-4 glycosphingolipid (Dr. W. O. Clark, Harvard University).

Sodium tauro-deoxycholate was isolated from bovine serum. The B-2 and B-3 glycosphingolipids were isolated from small-intestinal and horse erythrocyte (Venus Blood Products, Ltd., London, England), glycosphingolipid acceptors (142), and lacto-N-fucopentaose from the Gentner-Packling Co. (St. Louis, Mo.). Lacto-N-biose was obtained from the G. W. Schachter Co. The following materials were obtained as gift samples: B-2 glycosphingolipid (Dr. C. E. Thomas, The Upjohn Co.), B-3 glycosphingolipid (Dr. G. W. Schachter, Columbia University), and B-4 glycosphingolipid (Dr. W. O. Clark, Harvard University).

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Table I

| Requirement for α-1,2-fucotransferase activity |
|-----------------------------------------------|
| The complete incubation mixture contained the following components (in micromoles unless otherwise stated) in final volumes of 0.04 ml: nLcOse5Cer, 0.05; detergent G-3634-A, 0.1 mg; cacodylate-HCl buffer (pH 6.35), 10; GDP-B-L-14Cfucose, 0.01 (2.3 x 10^6 cpm/μmol); and enzyme fraction TDCS, 0.05 mg of protein. After incubation at 37°C for 45 min, the mixtures were assayed by the double chromatographic method described under "Enzyme Assay". |

Table II

| Glycosphingolipid acceptor specificity with the bovine spleen Galβ1-3Galβ1-4GlcNAcR acceptor fraction |
|---------------------------------------------------------------------------------------------------|
| Conditions were the same as described in Table I, except that the different potential acceptor glycolipids were used. Samples were incubated at 37°C for 45 min and assayed by the double chromatographic method described in the text. |

Table III

| Oligosaccharide acceptor specificity studies with the bovine spleen Galβ1-3Galβ1-4GlcNAcR acceptor fraction |
|----------------------------------------------------------------------------------------------------------|
| Conditions were the same as those described in Table II, except that the different potential oligosaccharide acceptors were used. After incubation at 37°C for 45 min, the mixtures were assayed by high voltage paper electrophoresis (18 volts, 45 min) followed by chromatography in 80% ethanol. |

Table IV

| Microagglutination inhibition reaction |
|----------------------------------------|
| The hemagglutination inhibition technique is described in detail in the text. Reaction mixtures contained a standard concentration of 1.29 μg of protein, human B-type red blood cells (100,000), and the indicated amounts of oligosaccharide (10 final volumes of 0.04 ml). +++ indicates 100% inhibition (10 to 20 cells/clone); ++ indicates 5 to 10 cells/clone; and -- indicates no agglutination. |

Fig. 3. Effect of glycolipid acceptor concentration on the rate of reaction. Incubation mixtures were the same as in Table II, except that varied concentrations of glycolipid acceptor, nLcOse5Cer, McI2, 0.12% saline; and GDP-BL-14Cfucose, 0.02 μmol (2.3 x 10^6 cpm/μmol), were used. Incubations were conducted at 37°C for 45 min, and the double chromatographic method described under "Enzyme Assay" was used. The inset shows a Hanes-Woolf plot of the same data.

Fig. 4. Radioautogram of 14C-labeled product. 1, 14C-labeled product (10,000 cpm, exposed to X-ray film for 4 months); 2, human B-type red blood cells (100,000); 3, and 4, human B-type red blood cells (100,000) exposed to X-ray film for 4 months. Spots in lanes 1 and 2 were developed by spraying with diphenylamine reagent.
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