Purification and Characterization of UDP-N-acetylgalactosamine: Globotriaosylceramide $\beta$-3-$N$-Acetylholactosaminitransferase, a Synthase of Human Blood Group P Antigen, from Canine Spleen*

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A UDP-N-acetylgalactosamine:globotriaosylceramide $\beta$-3-$N$-acetylholactosaminitransferase which catalyzes the conversion of human blood group $P^k$ antigen into P antigen has been purified over 18,000-fold in 4% yield from a Triton X-100 extract of canine spleen microsomes by affinity chromatography on UDP-hexanolamine-Sepharose and globotriaosylceramide acid-Sepharose. The purified enzyme migrates as two major bands with apparent molecular weights of 64,000 and 57,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A single band, with enzyme activity, was observed in non-denaturing acrylamide gels containing Triton X-100. $M^+$ values for UDP-GalNAc and globotriaosylceramide were 14 and 2.5 $\mu$m, respectively. Studies on substrate specificities indicate that the preferred substrates have the general structure $Gal$1-$4Gal$-OR in which the $R$ moiety has relatively little effect on activity. An antibody against the purified enzyme eliminated the activity of the enzyme, but did not neutralize the $\alpha$-3-$N$-acetylgalactosaminitransferase involved in the biosynthesis of Forssman glycolipid.

Glycosyltransferases which transfer sugar residues from specific nucleotides to oligosaccharide chains play an essential role in the biosynthesis of the carbohydrate moiety of glycoproteins and glycolipids. Most of the enzymes are presumed to exist in the Golgi membranes and require a detergent for solubilization.

Glycosyltransferases involved in glycolipid biosynthesis are quite unstable and their activities toward glycolipid substrate are very low. Therefore, until recently most of the kinetic and physicochemical properties have been obtained from data on crude or partially purified enzyme preparations. Recently several specific ligands for affinity chromatography have been successfully employed for the purification of glycosyltransferases involved in glycoprotein synthesis (1). This technique will make it possible to purify the enzymes involved in glycolipid synthesis and characterize further their enzymatic properties.

N-Acetylholactosaminitransferases involved in the biosynthesis of glycolipids such as globoside† (2, 3), Forssman glycolipid (4, 5), $GgOsc$Cer (6) and $G_M^2$ ganglioside (7) have been reported in several mammalian tissues. In previous studies from our laboratory, various mammalian tissues such as guinea pig kidney (5, 4), canine spleen (8), human lymphoblastic cells (6), and human lung (9) have been shown to contain both the $\alpha$-4-galactosidase $\beta$-3-$N$-acetylholactosaminyl transferase ($\beta$-GalNAc transferase) and the $\beta$-3-$N$-acetylholactosaminidase $\alpha$-3-$N$-acetylholactosaminyl transferase ($\alpha$-GalNAc transferase) that synthesize globoside and Forssman glycolipid, respectively. The latter enzyme has been successfully purified from canine spleen particulates by affinity chromatography on globoside acid-Sepharose, and several enzymatic properties have been delineated (10). The former enzyme, UDP-$N$-acetylgalactosamine:globotriaosylceramide $\beta$-3-$N$-acetylholactosaminitransferase (EC 2.4.1.88), catalyzes the reaction UDP-GalNAc + $GgOsc$Cer $\rightarrow$ UDP + globoside. Two glycolipids, globotriaosylceramide and globotriaosylceramide, were identified as $P^k$ and $P$ antigens, respectively, in the human blood group P systems (11). Individuals with blood group $P^k$ phenotype, who do not express the $P$ antigenicity on their erythrocytes or fibroblasts, lack the $\beta$-GalNAc transferase (12). The present study was undertaken in an effort to purify the transferase from canine spleen particulates by affinity chromatography on UDP-hexanolamine-Sepharose and globotriaosylceramide acid-Sepharose. The study is on a series of experiments aimed at elucidating the mechanisms of glycolipid biosynthesis. A highly active and apparently homogeneous enzyme preparation has been obtained.

1 The abbreviations and trivial names used are: globoside (or P antigen), globotetraosylceramide, GalNAc$G_4$-Gal$\beta$1-4Gal$\beta$1-4Glc-Cer; $GbobOsc$Cer, globotriaosylceramide (or $P^k$ antigen), trihexosylceramide, Gal$\beta$1-4Gal$\beta$1-4Glc-Cer; $GgOsc$Cer, gangliotriaosylceramide, GalNAc$G_4$-Gal$\beta$1-4Glc-Cer; $G_M^2$ II, NeuAcGgOscCer, GalNAc$G_4$-4(NeuAc2-3)Gal$\beta$1-4Glc-Cer; GM$\alpha$ IV, NeuAcGgOscCer, GalNAc$G_4$-4(NeuAc2-3)Gal$\beta$1-4Glc-Cer; Gal$\beta$1-4(NeuAc2-3)Gal$\beta$1-4Glc-Cer; GalNAc, N-acetylgalactosaminidase; Forssman glycolipid, GalNAc$G_4$-GalNAc$G_4$-Gal$\beta$1-4Glc-Cer; $G_{MS}$ II, NeuAcLacCer, NeuAc$G_4$-Gal$\beta$1-4Glc-Cer; UDP-GalNAc, uridine 5'-diphospho-$N$-acetylgalactosamine; $G_{MS}$ IV, NeuAcLacCer, NeuAc$G_4$-4(NeuAc2-3)Gal$\beta$1-4Glc-Cer; 2'-fucosyllactose, $FucOsc$ Gal$\beta$1-4Glc-Cer; $\alpha$-GalNAc transferase, UDP-GalNAc:globoside $\alpha$-3-$N$-acetyl-$\beta$-galactosaminyl transferase, UDP-$N$-acetylgalactosaminyltransferase

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UDP-GalNAc:GbOse₃Cer GalNAc Transferase

**EXPERIMENTAL PROCEDURES**

**Materials**

UDP-[1-¹⁴C]GalNAc (46-63 Ci/mmol), UDP-[U-¹⁴C]Gal (250-360 Ci/mmol), and UDP-[1-³²P]GalNAc (30-50 Ci/mmol) were obtained from New England Nuclear. Phosphorylase, bovine serum albumin, ovalbumin, soybean trypsin inhibitor, lysozyme, carboxy anhydrase, and cytochrome c were obtained from Sigma. Aminohexyl Sepharose 4B, DEAE-Sephadex A-25, and PD-10 column (Sephadex G-25) were obtained from Pharmacia. UDP-hexanamine-Sepharose was prepared as described (13). Glycolipids were purified in our laboratory (14). Globoside and GbOse₃Cer were prepared from human erythrocytes. Gb₃, Forsmann glycolipid, and fucosyloceramide were prepared from equine spleen.

Glucosyl- and galactosylceramides were obtained from spleen tissue of a patient with Gaucher disease and from bovine brain, respectively. Gal₃-4Gal-Cer was obtained from kidney tissue of a patient with Tay-Sachs disease, respectively. Gb₃0 was obtained from bovine brain. The oligosaccharide of Gb₀₃ was prepared from globotriaosylceramide by ozonolysis following the method previously described (20). Silver staining of gels was carried out by the method of Morrissey (21). The enzyme sample was treated with BioBeads SM-2 to remove Triton X-100 and then heated with sample buffer in 1% sodium dodecyl sulfate and 2-mercaptoethanol. Silver staining of gels was performed as described previously (22). Gels were scanned by using a Shimadzu CS-910 Chromatoscanner.

**Methods**

**Enzyme Assays**

Method 1 was routinely used to quantitate UDP-GalNAc:GbOse₃Cer β-3-GalNAc transferase activities. Method 2 was used for various glycolipid acceptor substrates. Methods 3 and 4 were used for oligosaccharides and glycoprotein acceptor substrates. One unit of activity is defined as the amount of enzyme that transfers 1 pmol of GalNAc/min under the standard assay conditions.

Method 1—The procedure was the same as that used for determination of UDP-GalNAc globoside α-3-GalNAc transferase activity (9, 10) except that GbOse₃Cer and antibody against globoside were employed instead of globoside and antibody against Forsmann glycolipid, respectively. Reaction mixtures contained, in order of addition, Triton X-100, 500 pg; GbOse₃Cer, 50 μg; sodium cacodylate (pH 6.9), 100 μmol; MnCl₂, 10 μmol; UDP-[1-¹⁴C]GalNAc, 10 nmol; protein, 1-500 ng; in a total volume of 100 μl. For kinetic studies, 50 nmol of UDP-[1-¹⁴C]GalNAc were used per assay. In both cases, control incubation was carried out by the method previously described (17). Assay of α-GalNAc Transferase—This was carried out by the method described previously (9, 10).

**Protein Concentration**

Protein was determined by an Amino-Schwartz dye binding method (18).

**Polyacrylamide Gel Electrophoresis**

Polyacrylamide gel electrophoresis on 7.5% gels at pH 8.5 was conducted by the method previously described (19) in the presence of 1% Triton X-100. Electrophoresis in the presence of sodium dodecyl sulfate and 2-mercaptoethanol was performed by the method previously described (20). Silver staining of gels was carried out by the method of Morrissey (21). The enzyme sample was treated with BioBeads SM-2 to remove Triton X-100 and then heated with sample buffer in 1% sodium dodecyl sulfate and 2% mercaptoethanol (5 min at 100 °C). The standard proteins were (subunit molecular weight) phosphorylase (92,500), bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (29,000), soybean trypsin inhibitor (21,500), and lysozyme (14,500). 5-Carboxymethylated enzyme was prepared as described previously (23). Gels were scanned by using a Shimadzu CS-910 Chromatoscanner.

**Preparation of Glycolipid Acids and Coupling to Aminohexyl-Sepharose**

The GbOse₃Cer acid was prepared as described previously (23). Coupling of GbOse₃Cer acid to aminohexyl-Sepharose 4B was carried out by the method previously described (24), using carbodiimide solution. A GbOse₃Cer acid column was also prepared as described previously (10).

**Preparation of Antiserum and Purification of Antibody**

Antiserum against the purified UDP-GalNAcGbOse₃Cer β-3-GalNAc transferase was raised in a rabbit. A rabbit weighing 3 kg was injected subcutaneously with 50 μg of enzyme emulsified with complete adjuvant. A booster injection of 25 μg of enzyme was given after 1 week. The animal was bled 7 days after the booster injection. Rabbit antisera against globoside and protein purifications were carried out by the method described previously (23) and found to be monospecific by immunoelectrophoretic analysis.

**Ouchterlony’s immunodiffusion** (25) was performed in 1.2% agarose.

**RESULTS**

**Purification of the Enzyme**

**Step 1: Homogenate**—Mongrel dogs were exsanguinated under light isoanbarburate anesthesia. The spleens (794 g) were removed and immediately homogenized in 4 volumes of a solution containing 0.25 M sucrose and 25 mM sodium cacodylate (pH 6.9) with a Polytron homogenizer (Brinkmann Instruments). All fractionation steps were carried out at 0 °C. After centrifugation at 0 °C for 10 min at 5000 X g, the supernatant was filtered through a layer of glass wool.

**Step 2: Triton X-100 Extraction**—The solution was centrifuged at 160,000 X g for 60 min. The pellet was resuspended in one-half volume of the preparation buffer using a tight fitting Teflon-glue homogenizer, and 10% (w/v) Triton X-100 was added to a final concentration of 1% (w/v). After thorough homogenization, the suspension was gently stirred overnight and then centrifuged at 105,000 X g for 60 min. The supernatant was removed, and the pellet was again treated as above. The second supernatant was combined with the first to give the final Triton extract.
Step 3: Chromatography on UDP-hexanamine-Sepharose—The above extract was diluted 5-fold by addition of 4 volumes of 25 mM sodium cacodylate (pH 6.9), and solid NaCl was added to the enzyme solution to give a concentration of 0.1 M. The solution was then applied to a column (2.5 x 15 cm) of UDP-hexanamine-Sepharose which had been equilibrated with 25 mM sodium cacodylate (pH 6.9) containing 1.0% Triton X-100 and 0.1 M NaCl. The column was washed with this buffer (flow rate 40 ml/h) until the eluate was essentially free of protein. Elution was carried out with a linear gradient established between 250 ml of the same buffer and 250 ml of similar buffer containing 0.5 M NaCl. The enzyme was eluted at 0.15 M NaCl (Fig. 1).

Step 4: Rechromatography on UDP-hexanamine-Sepharose II—The enzyme-containing fractions were diluted 2-fold to adjust ionic strength and loaded on the column of UDP-hexanamine-Sepharose, which had been previously equilibrated with 25 mM sodium cacodylate (pH 6.7) containing 1% Triton X-100 and 0.1 M NaCl. The column was washed with the same buffer. The enzyme was eluted with equilibration buffer containing 1 mM UDP. The fractions exhibiting high specific activity were pooled. After addition of glycerol to obtain a 20% solution, the solution was loaded on a small column (1 x 5 cm) of DEAE-Sephadex A-25 which had been equilibrated with the original buffer containing 20% glycerol to remove UDP. The eluted fractions were pooled.

Step 5: Chromatography on Globoside Acid-Sepharose—The enzyme-containing fractions (200 ml) obtained at Step 4 were applied to a column (1 x 8 cm) of globoside acid-Sepharose, which had been equilibrated with 25 mM sodium cacodylate (pH 6.9) containing 1% Triton X-100, 20% glycerol, and 0.1 M NaCl. The flow rate during loading was 2 ml/h. The column was washed with the above buffer. At this step a-GalNAc transferase was adsorbed to the column (10), but β-GalNAc transferase was eluted with the equilibration buffer.

Step 6: Chromatography on GbOse₃Cer Acid-Sepharose—The flow through fraction obtained at Step 5 was directly subjected to GbOse₃Cer acid-Sepharose affinity separation on a column (1 x 6 cm) which had been equilibrated with the equilibration buffer described above. The flow rate during loading was 2 ml/h. After washing the column with the same buffer at a flow rate of 30 ml/h, the enzyme was eluted with a solution consisting of the equilibration buffer containing 1 mM UDP (Fig. 2). At this step two fractions with a high and low specific activity were obtained. The two fractions contain enzyme forms with different affinities for GbOse₃Cer acid-Sepharose or for UDP. The fractions were pooled, concentrated by PM-10 because of the low recovery, and stored at -80 °C. About 80% of the activity applied to the column was recovered at this step and, as indicated in Table I, a purification of more than 18,000-fold was obtained with an overall yield of about 4%.

Purity and Molecular Weight of the Enzyme
The purified enzyme gave a single broad band upon electrophoresis in 7.5% polyacrylamide gels in the presence of 1% Triton X-100. In these studies a duplicate gel was used for the detection of enzyme activity. Detection was made by cutting out 2-mm slices of gel and incubating in an assay mixture. The enzyme activity was localized to the area staining for protein (see Fig. 3A). The preparation showed two components (Fig. 3B) with apparent molecular weights of 64,000 and 57,000 on polyacrylamide gels in the presence of sodium dodecyl sulfate (Fig. 4). When denatured enzyme was analyzed on sodium dodecyl sulfate gels without prior reduction, a major band was discerned corresponding to an apparent molecular weight of about 120,000. This result indicates that the subunits are linked through disulfide bonds. The reduced and alkylated form of the enzyme gave essentially the same pattern as nontreated enzyme in contrast to the case of a-GalNAc transferase in which three bands were observed for the treated enzyme as previously described (10).

Stability and Storage
The purified enzyme was stored at a concentration greater than 0.1 mg/ml in 25 mM sodium cacodylate (pH 6.9) containing 1% Triton X-100 and 20% glycerol. Storage at lower protein concentration and ionic strength resulted in marked loss of activity. The enzyme could be frozen at -80 °C for at least 3 months without detectable loss of activity on repeated freezing and thawing.

Metal Requirement
The purified enzyme showed no activity in the standard assay mixture in the presence of 10 mM EDTA, but EDTA-treated enzyme was fully reactivated by divalent metals. The activity was maximal with Mn²⁺ as shown in Table II, and Fe²⁺ and Co²⁺ showed some activity.

Effect of Sulphydryl Reagents
The effects of several sulhydryl reagents and thiols on the enzyme activity are summarized in Table III. Substantial inhibition was observed with iodoacetic acid, iodoacetamide, and N-ethylmaleimide. 0.5 mM of 4-hydroxymercuribenzoate...
inhibited the enzyme completely. These results indicate that the sulfhydryl group is essential for enzyme activity.

**pH Optimum**

The enzyme showed maximum activity between pH 6.7 and 7.2 with an optimum near 6.9.

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**TABLE I**

Purification of the UDP-GalNAc:GbOse3Cer β-GalNAc transferase from canine spleen microsomes

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Total</th>
<th>Total</th>
<th>Specific</th>
<th>Yield</th>
<th>Total purification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>mg</td>
<td>unit</td>
<td>unit X 10^6</td>
<td>%</td>
<td>-fold</td>
</tr>
<tr>
<td>1.  5,000 X g supernatant</td>
<td>2,000</td>
<td>172,000</td>
<td>4,310</td>
<td>0.03</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>2.  Triton extract</td>
<td>310</td>
<td>24,900</td>
<td>1,642</td>
<td>0.066</td>
<td>38</td>
<td>2.2</td>
</tr>
<tr>
<td>3.  UDP-Sepharose I</td>
<td>150</td>
<td>615</td>
<td>363</td>
<td>0.59</td>
<td>8.4</td>
<td>19.6</td>
</tr>
<tr>
<td>4.  UDP-Sepharose II</td>
<td>200</td>
<td>8</td>
<td>8.4</td>
<td>19.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.  Globoside and GbOse3Cer-Sepharose</td>
<td>15</td>
<td>0.278</td>
<td>157</td>
<td>565</td>
<td>3.6</td>
<td>18,800</td>
</tr>
</tbody>
</table>

**TABLE II**

Cation requirement of UDP-GalNAc:GbOse3Cer β-GalNAc transferase

<table>
<thead>
<tr>
<th>Cation added</th>
<th>Relative activity</th>
</tr>
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<tr>
<td>mM</td>
<td>%</td>
</tr>
<tr>
<td>Mn^2+, 10</td>
<td>100</td>
</tr>
<tr>
<td>Zn^2+, 10</td>
<td>3</td>
</tr>
<tr>
<td>Co^2+, 10</td>
<td>93</td>
</tr>
<tr>
<td>Fe^2+, 10</td>
<td>19</td>
</tr>
<tr>
<td>Cu^2+, 10</td>
<td>0</td>
</tr>
<tr>
<td>Mg^2+, 10</td>
<td>120</td>
</tr>
<tr>
<td>Ca^2+, 10</td>
<td>120</td>
</tr>
<tr>
<td>Ni^2+, 10</td>
<td>6</td>
</tr>
<tr>
<td>Li^+, 10</td>
<td>11</td>
</tr>
<tr>
<td>EDTA, 10</td>
<td>0</td>
</tr>
</tbody>
</table>

*β-GalNAc transferase containing no Mn^2+ was assayed in the standard assay mixture described under "Experimental Procedures" using GbOse3Cer as acceptor substrate with EDTA or metal chlorides listed above substituted for Mn^2+. Activity is expressed as per cent of activity observed in the presence of Mn^2+.

**TABLE III**

Effect of sulfhydryl reagents on UDP-GalNAc:GbOse3Cer β-GalNAc transferase

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>%</td>
</tr>
<tr>
<td>4-Hydroxymercuribenzoate</td>
<td>0.5</td>
</tr>
<tr>
<td>Iodoacetic acid</td>
<td>1</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>1</td>
</tr>
<tr>
<td>N-Ethylmaleimide</td>
<td>1</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>10</td>
</tr>
<tr>
<td>Glutathione</td>
<td>10</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
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</table>
UDP-GalNAc:GbOse₃Cer GalNAc Transferase

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Identification of the Reaction Product

Radiolabeled enzymatic product obtained with the use of the purified transferase had the same Rₚ on thin layer chromatography as authentic globoside (data not shown). The products gave radioactive precipitates with anti-globoside IgG in an immunodiffusion plate experiment (Fig. 5).

Donor

The donor specificity of the enzyme was examined, and UDP-GalNAc was shown to be the only sugar donor used efficiently by the enzyme. UDP-Gal and UDP-GlcNAc had very poor activity (data not shown).

Acceptor

The acceptor substrate specificity was examined with a number of glycolipids and glycoproteins. Comparisons of the relative rates of product formation to that observed using GbOse₃Cer are presented in Table IV. GbOse₃Cer and its oligosaccharide were good acceptors, while 2'-fucosyllactose and blood group H-substance were not active. This indicates that the enzyme is distinct from the α-N-acetylgalactosaminyltransferase involved in the formation of blood group A antigenic determinant (26). The enzyme exhibited no activity toward globoside and deglycosylated bovine submaxillary mucin. This indicates that the enzyme is distinct from the α-N-acetylgalactosaminyltransferases involved in the biosynthesis of Forssman glycolipid (10) and mucin-type glycoprotein (27), respectively. The enzyme also had no activity toward Ga₃ and Galβ1-4Glc-Cer, which indicates that the enzyme is different from the β-N-acetylgalactosaminyltransferases involved in the biosynthesis of GM₂ ganglioside (7) and GgOse₃Cer (6), respectively.

The fact that the oligosaccharide of GbOse₃Cer, globotriaose, was found to be a good acceptor suggests that the size and hydrophobic character of the acceptor have little effect on the activity of the enzyme. Galα₁-4Gal-Cer which has a nonreducing terminal galactose in α₁-4 linkage to galactose was a fairly active substrate. These results strongly suggest that the enzyme forms the β₁-3 linkage with any oligosaccharide or glycolipid containing the Galα₁-4Gal sequence at its nonreducing terminus.

Kinetic Studies

The Kₘ values calculated for UDP-GalNAc and GbOse₃Cer from Lineweaver-Burk plots of initial rate data were 14 and 2.5 μM, respectively. UDP was found to be a competitive inhibitor with respect to UDP-GalNAc and a noncompetitive inhibitor with respect to GbOse₃Cer. Ishibashi et al. (28) reported that UDP did not inhibit the β-GalNAc transferase. The discrepancy between their data and ours can be explained in terms of the degree of enzyme purity.

Immunological Properties

Rabbit antisera directed against the purified enzyme caused inhibition of β-GalNAc transferase activity but not the α-GalNAc transferase activity when preincubated with the enzymes (Fig. 6). This indicates that these two GalNAc transferases are immunologically distinct.

DISCUSSION

The mechanism of metabolic regulation of glycolipid synthesis in mammalian tissues remains unknown. Most of the glycosyltransferases involved in the biosynthesis of glycolipids have not yet been purified, and hence in the earlier studies the data were obtained on the crude or partially purified enzymes or glycolipid containing the Galα₁-4Gal sequence at its nonreducing terminus.

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enzyme preparations (29, 30). In a previous study (10) we were able to purify the α-GalNAc transferase using globoside acid-Sepharose as an immobilized acceptor adsorbent. This was possible because of the remarkably low \( K_m \) of the transferase for the acceptor substrate. In the present study the \( K_m \) value of the β-GalNAc transferase for GbOse\(_3\)Cer was also found to be quite low. Therefore, the same type of affinity chromatography was applicable for the purification of the enzyme. In the present investigation, UDP-GalNAc:GbOse\(_3\)Cer β-3-GalNAc transferase involved in the biosynthesis of globoside has been purified about 18,000-fold from canine spleen microsomes by affinity chromatography on UDP-hexanolamine-Sepharose and GbOse\(_3\)Cer acid-Sepharose, and an apparently homogenous preparation has been obtained with an overall yield of 4%. The success of this type of affinity chromatography, particularly on GbOse\(_3\)Cer acid-Sepharose, has opened the way for the use of new approaches for the isolation of the glycosyltransferases involved in glycolipid metabolism. This is one of the few cases in which a glycosyltransferase has been purified on an immobilized acceptor adsorbent (1).

In this work, we have been able to extend our knowledge of the specificity of the enzyme. The data (Table IV) demonstrate that the enzyme acts effectively on GbOse\(_3\)Cer and its oligosaccharide and that it exhibits substantial activity toward Galα1-4GalCer. Based on the product characterization in Fig. 5, the enzyme is presumably an α-1 galactoside β-3-N-acetylgalactosaminyltransferase. Thus, the preferred substrates seem to have the structure Galα1-4Gal-OR in which the nature of the R moiety and its size and hydrophobic character have relatively little effect on activity. The purified enzyme had a specific activity of approximately 570 pmol/mg of protein/min. This specific activity is about 10\(^7\) lower than that found for the glycosyltransferases that act primarily in glycoprotein biosynthesis. This may reflect a general property of the enzymes involved in glycolipid biosynthesis.

Inhibition patterns by antisera against β-GalNAc transferase and various data on substrate specificity as described above again indicate that the α- and β-GalNAc transferases are immunologically and enzymatically distinct proteins.

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REFERENCES

15. Wiegandt, H., and Boschung, G. (1965) Z. Naturforschung 20b, 164-166
Purification and characterization of UDP-N-acetylglactosamine: globotriaosylceramide beta-3-N-acetylglactosaminyltransferase, a synthase of human blood group P antigen, from canine spleen.

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