Ganglioside biosynthesis. Characterization of uridine diphosphate galactose: GM_2 galactosyltransferase in Golgi apparatus from rat liver

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Abstract An enzyme that transfers galactose from UDP-Gal to ganglioside GM_2 (Tay-Sachs ganglioside) was concentrated 50 times in Golgi apparatus from rat liver relative to total homogenates. This enzyme required detergents or phospholipids as dispersing agents. Of the numerous detergents tested, sodium taurocholate and Triton CF-54 were most effective in stimulating the reaction. Cardiolipin alone was more effective than any of the detergents tested in stimulating enzyme activity. The pH optimum for the reaction varied with the nature of the dispersing agent. With sodium taurocholate, Triton CF-54 and cardiolipin, the pH optima were 6.2, 5.9, and 5.6, respectively. The enzyme had a nearly absolute requirement for Mn^{2+}, with maximum activity being attained at a concentration of 15 mM Mn^{2+}. Other divalent or trivalent cations were either less effective than Mn^{2+} or inhibited the transferase reaction. The K_m values calculated for UDP-Gal and GM_2 were 1.1×10^{-4} M and 9.9×10^{-5} M, respectively. The enzyme could not be dissociated from Golgi apparatus fractions by treatment with ultrasound, indicating that it is tightly associated with the membrane and not part of the luminal contents. The newly synthesized GM_1, the product of the reaction, was incorporated into or became tightly associated with the membranes of the Golgi apparatus.

Supplementary key words detergents • phospholipids • glycolipids • endomembranes • plasma membrane • glycosyltransferases

Gangliosides, a class of glycosphingolipids that contain sialic acids, were first recognized as constituents of bovine brain (1). Basu, Kaufman, and Roseman (2–4) have shown gangliosides of embryonic chicken brain to be synthesized in a stepwise manner by transfer of carbohydrates from sugar nucleotides to growing glycolipid acceptors. In this biosynthetic pathway the product of one glycosyltransferase becomes the substrate for the next enzyme in the pathway, a situation termed "cooperative sequential specificity" by Roseman (3).

More recently gangliosides have been identified as constituents of numerous extraneural tissues (5–12) and it would seem that gangliosides will prove to be ubiquitous, at least among mammalian tissues. While extraneural gangliosides have received lesser study than those of brain, the structures of at least 12 extraneural gangliosides are known (see, for example, ref. 12). For a number of years it was believed that gangliosides were specifically localized in and synthesized by cellular surface membranes (2, 11, 13–16). More recently it was demonstrated that although gangliosides are concentrated in plasma membranes they are also present in intracellular endomembranes, notably in Golgi apparatus (17–19). Following these observations it was found that plasma membranes from rat liver and bovine mammary gland are devoid of significant glycosphingolipid glycosyltransferase activity (20, 21). Instead, these transferase enzymes were found in Golgi apparatus and endoplasmic reticulum fractions which, in toto, accounted for about 85% of the total cellular glycolipid glycosyltransferase activities (20). In Golgi apparatus fractions, these transferases were concentrated nearly 50-fold relative to total homogenates.

This report gives the characteristics of one enzyme involved in ganglioside synthesis in rat liver Golgi apparatus, UDP-Gal:GM_2 galactosyltransferase. This transferase has been characterized previously in embryonic chicken (22), rat (23–25), and frog (26) brains. Yip (27) found this enzyme in several rat tissues under conditions reported to be optimum for chicken brain (22). It will be shown that the optimum conditions for rat liver Golgi apparatus are markedly different than those for crude synaptosomal fractions from chicken brain. These studies appear timely in view of the accumulating literature showing depression of certain glycosphingolipid glycosyltransferase activities, including UDP-Gal:GM_2 galactosyltransferase (9, 28), in malignant tumors (9, 28) and tumorigenically transformed cell lines (28, 30, 31).

Abbreviations: UDP, uridine-5′-diphosphate; Cer, ceramide (*N*-acylsphingosine); Gal, galactose; Glc, glucose; GalNAc, *N*-acetylgalactosamine; AcNeu, *N*-acetylneuraminic acid; Cer-Hex_3, lactosyl ceramide; GM_3, AcNeu-Gal-Glc-Cer; GM_2, GalNAc-(AcNeu)-Gal-Glc-Cer; GM_1, Gal-GalNAc-(AcNeu)-Gal-Glc-Cer.

MATERIALS AND METHODS

New England Nuclear, Boston, Mass. supplied UDP-[^{14}C]-Gal (298 Ci/mole). Unlabeled UDP-Gal was from Sigma, St. Louis, Mo. Reference Cer-Hex$_2$ and GM$_3$ were isolated from bovine milk fat globule membranes and canine erythrocytes (32). Reference GM$_2$ and GM$_1$, obtained from Tay-Sachs or normal human brain were gifts from Dr. Subhash Basu, University of Notre Dame. Authentic phospholipids were from Supelco, Inc., Bellefonte, Pa. Brij 35 was from Pierce Chemical Co., Rockford, Ill. Cholic acid, sodium cholate, sodium taurodeoxycholate, sodium dodecyl sulfate, Triton CF-54, Triton X-100, Triton X-114, Tween 80 and alkaline phosphatase from calf intestine were from Sigma. Cutscum and sodium deoxycholate were from E. H. Sargent Co., Skokie, Ill., and digitonin and Tween 20 were obtained from Nutritional Biochemicals Co., Cleveland, Ohio. The Holtzman Co., Madison, Wis. supplied 150–200 g male rats. Unisil silicic acid was from Clarkson Chemical Co., Williamsport, Pa. Precoated Silica gel G thin-layer plates were purchased from Analtech, Wilmington, Del. Brain tissue, taken at autopsy from an infant with Tay-Sachs disease was a gift from Dr. W. Zeman, Indiana University School of Medicine.

Purification of GM$_2$

Brain tissue was minced and homogenized into 19 volumes of chloroform–methanol 2:1 (all solvent ratios are on a volume basis) and the suspension was stirred overnight. Insoluble material, recovered by filtration, was dispersed into 10 volumes of chloroform–methanol 1:1 and stirred for 10 hr. Insoluble material was removed by filtration and discarded. The two filtrates were combined and gangliosides were recovered by solvent partitioning (33). Combined upper phases were evaporated in vacuo and the resultant solids were saponified to degrade contaminating glycerolipids (34). Ganglioside GM$_2$ was separated on a 2.5 × 20 cm column packed with silicic acid (35). Fractions were examined by thin-layer chromatography and those exhibiting a single constituent with mobility identical to authentic GM$_2$ were combined.

Isolation of Golgi apparatus

Rat liver Golgi apparatus was isolated by the method of Morré (36). Rigorous enzymatic and morphological analysis have shown such Golgi apparatus fractions to be contaminated to an extent of less than 10% with other cellular membranes (20, 37). Golgi apparatus fractions were resuspended in 0.32 M sucrose–14 mM 2-mercaptoethanol and stored at −20°C until assay.

Thin-layer chromatography

Precoated 250 μm thick silica gel G plates were activated at 110°C for at least 30 min before use. Plates were routinely developed in chloroform–methanol–20% ammonia 12:7:2. As an aid in identification of the product of the enzymatic reaction, the *n*-propanol–1 N ammonium hydroxide 7.3 solvent system of Svennerholm (38) was also used. Gangliosides were detected by spraying plates with resorcinol reagent (39), covering the layer with a clean glass plate and heating in an oven at 110°C until the characteristic blue color of the gangliosides appeared. To detect non-sialic acid containing compounds, plates were sprayed with 50% sulfuric acid and heated at 150°C.

Chemical determinations

Sialic acids were measured spectrophotometrically by the periodate resorcinol method (40) using AcNeu as the standard. Protein was determined according to Lowry et al. (41) with crystalline bovine serum albumin as the standard.

Enzyme assay

Exact compositions of reaction mixtures are given in the figures and tables. Glycolipid acceptor, detergent and phospholipid were added as chloroform–methanol solutions to 6 × 50 mm glass tubes and the solvents were removed under a stream of nitrogen. Buffer, cations and UDP-[^{14}C]-Gal were added in a volume of 50 μl and the tubes were mixed thoroughly with a Vortex mixer. Reactions were initiated by addition of 50 μl of Golgi apparatus suspension and incubation was at 37°C in a shaking water bath. Reactions were terminated by addition of 10 μmoles of EDTA, after which reaction mixtures were quantitatively streaked onto 4 × 30 cm strips of Whatman 3MM chromatography paper 10 cm from the origin. Papers were developed (descending) for 4 or more hr with 1% sodium tetraborate. This effectively removes sugar nucleotides and their degradation products from the glycolipid, which remains at the origin (42). Papers were dried and the regions to which samples were applied were cut out and transferred to scintillation vials containing 15 ml of chloroform–methanol–water 2:1:0.2. After agitation for 1 hr at 37°C, the papers were removed, the solvents were evaporated and the residue was taken up in hyamine hydroxide (1.0 ml). Scintillation fluid (a solution of PPO, 5.0 g, and POPOP, 200 mg, in toluene, 1 liter) was added and the radioactivity was determined in a Packard 574 Tricarb scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Specific activity values were calculated from the amount of radioactive sugar transferred to lipids. In all cases blanks without added acceptor were assayed in parallel to determine the amount of galactose incorporated into endogenous acceptors; this value was subtracted to determine incorporation into exogenous acceptor. Unless otherwise stated, all values reported are representative of several trials and were obtained under conditions of linear kinetics with respect to incubation time and enzyme concentration.

For assay of UDP-Gal pyrophosphatase activity, reaction mixtures contained 10 μmoles cacodylate-HCl, pH 6.0, 2.5 moles $MnCl_2$, 50 nmoles UDP-[^{14}C]-Gal, 100 μg of cardiolipin or detergent (sodium taurocholate or Triton CF-54) and 100 μg of Golgi apparatus protein in a final volume of 100 μl. After incubation at 37°C for periods up to three hours, reactions were stopped by addition of 100 μl of ethanol and precipitated protein was removed by centrifugation. Ethanol–water was evaporated under nitrogen at room temperature

TABLE 1. Effect of detergents on UDP-Gal:GM_2 galactosyltransferase activity of Golgi apparatus from rat liver

Detergent	Concentration	[^{14}C]Galactose Incorporated
	μg/100 μl	*pmoles*
None		753
Triton CF-54	100	1,200
Triton X-100	100	945
Triton X-114	100	1,270
Sodium taurocholate	100	1,500
Sodium deoxycholate	100	658
Sodium deoxytaurocholate	25	297
Sodium deoxytaurocholate	100	662
Sodium cholate	100	827
Sodium dodecyl sulfate	25	1,920
Sodium dodecyl sulfate	100	0
Cholic acid	100	724
Tween 20	100	659
Tween 80	100	524
Brij 35	100	279
Cutscum	100	972
Digitonin	100	524

Reaction mixtures contained in final volumes of 100 μl: GM_2, 0.05 μmoles; UDP-Gal (8 × 10^5 cpm/μmole), 0.05 μmoles; $MnCl_2$, 2.5 μmoles; cacodylate-HCl, pH 6.2, 10 μmoles; and 100 μg of Golgi apparatus protein. Incubation was at 37°C for 3 hr.

and 200 μl of 50 mM glycine buffer, pH 10.0, containing 1 IU of alkaline phosphatase was added to the residue. After incubation at 37°C for 30 min to convert galactose-1-phosphate to galactose (43), reaction mixtures were transferred to small columns of Bio-Rad AG2-1B; free galactose was eluted and measured by liquid scintillation counting (36). Nonenzymatic hydrolysis of UDP-Gal was measured in reaction mixtures run in parallel with boiled enzyme.

RESULTS

Effect of dispersing agents on reaction velocity

Several common detergents were evaluated for their ability to stimulate enzymatic synthesis of GM_1 (**Table 1**). At concentrations of 1 mg/ml, Cutscum, Tritons CF-54, X-100 and X-114, sodium taurocholate and sodium cholate stimulated enzyme activity. Other detergents, such as Brij 35 and Tween 80, depressed reaction velocities below the level obtained in the absence of exogenous detergent. At 1 mg/ml, sodium dodecyl sulfate completely abolished activity, but at 250 μg/ml this detergent was the most effective of the agents tested in stimulating enzyme activity. This effect of sodium dodecyl sulfate was confirmed in further experiments (**Fig. 1**). The optimum concentrations of Tritons X-114 and CF-54 were about 1 mg/ml with Triton X-114 being slightly more effective than Triton CF-54 (Fig. 1). No additional enhancement of enzyme activity was obtained on addition of mixtures of Tritons X-114 and CF-54 (not shown).

Phospholipids, in particular cardiolipin and phosphatidylglycerol, have been reported to stimulate activity of CMP-AcNeu:glycolipid sialyltransferases (20, 44) and phosphatidylglycerol reportedly stimulates UDP-Gal:Cer-Hex galactosyltransferase (21). Accordingly, various phospholipids were tested for their ability to stimulate the UDP-Gal:GM_2 galactosyltransferase (**Table 2**). Cardiolipin was markedly stimulatory to the activity, increasing the incorporation of Gal to 2.4 times the level obtained with no dispersing agent. Addition of Triton CF-54 along with cardiolipin had little additional effect on reaction velocity. Cardiolipin was effective over a wide concentration range, with the optimum activity being attained at concentrations ranging from 1 to 2 mg/ml (**Fig. 2**). Phosphatidylethanolamine alone also had a stimulatory effect on reaction velocity, although it was less effective than was cardiolipin (Table 2). When Triton CF-54 and phosphatidylethanolamine were added together, the stimulatory effect was nearly as great as that achieved with cardiolipin. Phosphatidylglycerol was only slightly stimulatory to enzyme activity; combining Triton CF-54 with phosphatidylglycerol further stimulated the reaction, but not to as great an extent as did cardiolipin (Table 2).

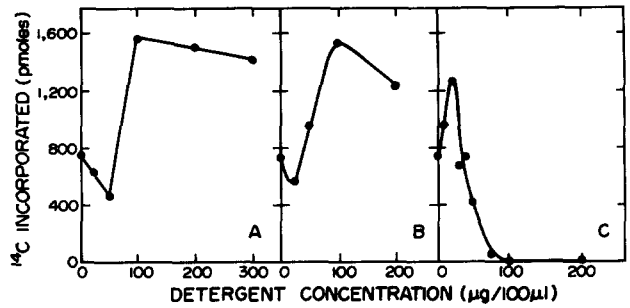

Fig. 1. Effect of detergents on UDP-Gal:GM_2 galactosyltransferase activity of Golgi apparatus from rat liver. Reaction mixtures contained in final volumes of 100 μl: GM_2, 0.05 μmoles; UDP-Gal (8 × 10^5 gpm/μmole), 0.05 μmoles; cacodylate-HCl, pH 6.2, 10 μmoles; $MnCl_2$, 2.5 μmoles; detergent as indicated (A, Triton X-114; B, Triton CF-54; C, sodium dodecyl sulfate); and 100 μg of Golgi apparatus protein. Incubation was for 3 hr at 37°C.

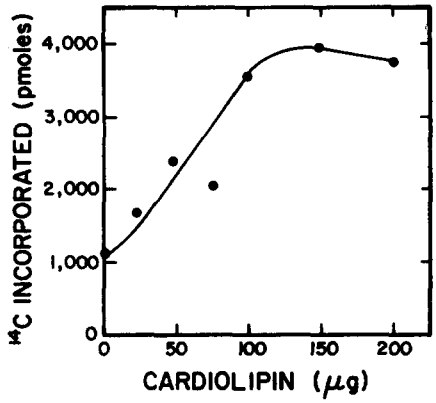

Fig. 2. Effect of cardiolipin on UDP-Gal:GM_2 galactosyltransferase activity of Golgi apparatus from rat liver. Reaction mixtures contained in final volumes of 100 μl: GM_2, 0.05 μmoles; UDP-Gal (8 × 10^5 cpm/μmole), 0.05 μmoles; cacodylate-HCl, pH 5.9, 10 μmoles; $MnCl_2$, 1.5 μmoles; cardiolipin as indicated (μmoles); and 100 μg of Golgi apparatus protein. Incubation was at 37°C for 3 hr.

TABLE 2. Effect of phospholipids on UDP-Gal:GM_2 galactosyltransferase activity of Golgi apparatus from rat liver

Phospholipid	Concentration	[^{14}C]Galactose Incorporated
	μg/100 μl	*pmoles*
None		1,150
Cardiolipin	100	2,750
Phosphatidylglycerol	100	1,310
Phosphatidylethanolamine	100	1,680
Cardiolipin *plus*	100	2,790
Triton CF-54	100	
Phosphatidylglycerol *plus*	100	2,220
Triton CF-54	100	
Phosphatidylethanolamine	100	2,690
plus Triton CF-54	100	
Triton CF-54	100	1,840

Incubation conditions and reaction mixtures are identical to those given in Table 1, except that the mixture contained 1.5 μmoles of $MnCl_2$ (instead of 2.5 μmoles).

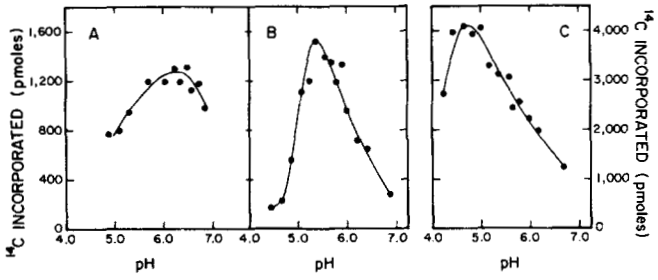

Fig. 3. Effect of pH on UDP-Gal:GM_2 galactosyltransferase activity of Golgi apparatus from rat liver. Reaction mixtures were identical to those described in Fig. 2 except that the pH of the buffer was varied as indicated and different dispersing agents were used. A, 25 μg of sodium taurocholate; B, 100 μg of Triton CF-54; C, 150 μg of cardiolipin. In C, $MnCl_2$ was present at a level of 1.5 μmoles. Incubation was at 37°C for 3 hr.

pH Optima and linearity of product formation

With sodium taurocholate as the dispersing agent, maximum activity was attained at a pH of 6.2 with cacodylate buffer (**Fig. 3**). A more acidic pH optimum, 5.9, was observed when Triton CF-54 was used as the dispersing agent (Fig. 3). An even lower pH optimum, 5.6, was observed when cardiolipin was used to stimulate the reaction. With both cardiolipin and Triton CF-54, a sharp maximum in activity was attained as the pH was varied, whereas with sodium taurocholate the enzyme was more nearly maximally active over a broader pH range. Thus, the pH optimum varied, albeit over a narrow range, when different dispersing agents were used.

Product formation was linear with Golgi apparatus protein levels of up to at least 100 μg and with incubation times up to at least 3 hr.

Ion requirements

Multivalent cations were essential for enzyme activity. Of the several ions tested, Mn^{2+} was by far the most effective. Only Co^{2+} and Fe^{2+} could replace Mn^{2+} to any significant extent. Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Ni^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Sn^{2+}, Fe^{3+}, and Sb^{3+} had no effect or were inhibitory to the reaction (not shown). Product information was maximum at Mn^{2+} concentrations of 15 mM (**Fig. 4**); higher levels of this ion depressed activity to below the maximum. In contrast to the

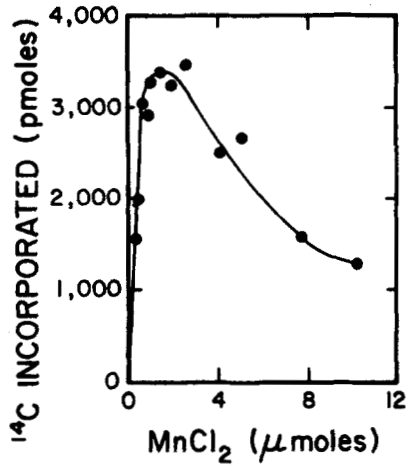

Fig. 4. Effect of $MnCl_2$ concentration on UDP-Gal:GM_2 galactosyltransferase activity of Golgi apparatus from rat liver. Incubation mixtures contained in final volumes of 100 μl; GM_2, 0.05 μmoles; UDP-Gal (8 × 10^5 cpm/μmole), 0.05 μmoles; cacodylate-HCl, pH 5.6, 10 μmoles; $MnCl_2$ (μmoles) as indicated; cardiolipin, 150 μg; and 100 μg of Golgi apparatus protein. Incubation was for 3 hr at 37°C.

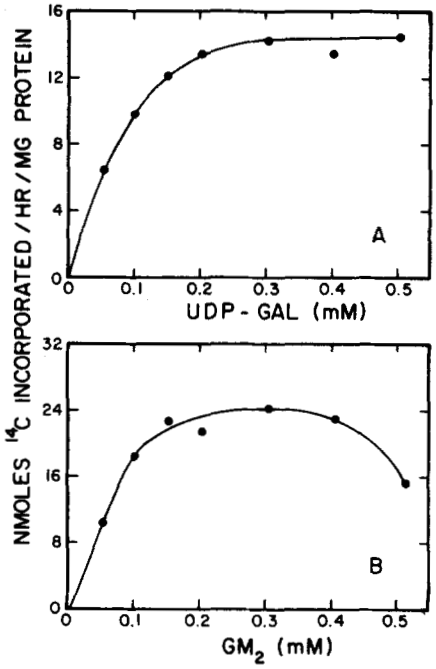

Fig. 5. Effects of substrate concentration on initial velocities of UDP-Gal:GM_2 galactosyltransferase of Golgi apparatus from rat liver. Reaction mixtures are identical to those described in Fig. 3 C except that the concentrations of UDP-Gal (A) or GM_2 (B) were varied as indicated. Incubation was at 37°C for 1 hr. Initial velocities were calculated by regression analysis.

TABLE 3. Association of UDP-Gal:GM_2 galactosyltransferase activity with Golgi apparatus membranes

Fraction	Protein	[^{14}C]Galactose Incorporated
	μg	*pmoles/mg protein*
Untreated Control	168	5,750
Treated Supernatant	57	230
Treated Pellet	200	5,900

Golgi apparatus was isolated from rat liver and divided into two portions, one of which was untreated and served as a control. The other portion was treated with ultrasound as indicated in the text and then centrifuged for 30 min at 19,000 g. Supernatant and pellet fractions were collected and assayed for transferase activity. Reaction mixtures contained in final volumes of 100 μl: GM_2, 0.05 μmoles; UDP-Gal (8×10^5 cpm/μmole), 0.05 μmoles; sodium taurocholate, 25 μg; $MnCl_2$, 2.5 μmoles; cacodylate-HCl, pH 5.9, 10 μmoles; and the indicated amount of protein. Incubation was at 37°C for 2 hr.

TABLE 4. Association of newly formed GM_1 with Golgi apparatus membranes

Fraction	Detergent	[^{14}C]Galactose Incorporated
		pmoles
Pellet	None	4,950
Supernatant	None	59
Pellet	Triton CF-54	13,500
Supernatant	Triton CF-54	453

Golgi apparatus fractions were incubated in duplicate reaction mixtures containing 30 μmoles cacodylate-HCl, pH 6.2; 0.15 μmole of GM_2, 0.15 μmole of UDP-Gal (8×10^5 cpm/μmole); 2.5 μmoles of $MnCl_2$; and 300 μg of Golgi apparatus protein in final volumes of 300 μl. In addition, one of the reaction mixtures contained 100 μg of Triton CF-54. After incubation at 37°C for 380 min, reactions were terminated by addition of 30 μmoles of EDTA. Reaction mixtures were then centrifuged at 19,000 g for 30 min and supernatants and pellets were collected separately. Pellets were suspended in 0.5 ml water and treated with ultrasound for 1 min (Branson Model W 140, amplitude 4). After centrifugation as above, pellets and the combined supernatants were assayed for radioactive lipid product as described in the text.

pH optima, there was no measurable change in optimum Mn^{2+} levels when sodium taurocholate, Triton CF-54, or cardiolipin was used as the dispersing agent.

Effect of substrate concentration on reaction velocity

At a concentration of 0.5 mM GM_2, the enzyme became saturated at UDP-Gal concentrations of 0.3 mM (**Fig. 5**). There was no effect on velocity when UDP-Gal concentration was raised to 0.5 mM. The K_m for UDP-Gal, calculated from reciprocal plots of substrate versus velocity, was 1.1×10^{-4} M. At a concentration of 0.5 mM UDP-Gal, the enzyme became saturated at a GM_2 concentration of 0.15 mM (Fig. 5). Concentrations of GM_2 higher than 0.4 mM were inhibitory to the reaction. The K_m for GM_2, calculated from initial velocities, was 9.9×10^{-5} M.

Coleman et al. (43) and Mookerjea and Yung (45) have observed appreciable hydrolysis of nucleotide sugars in glycosyltransferase assay systems. When assayed in reaction mixtures similar to those used for transferase assays but without acceptor GM_2 (see Methods), UDP-Gal was partially degraded by our Golgi apparatus fractions. The extent of degradation was influenced by the detergent used, but was never greater than 26% of the total UDP-Gal. With cardiolipin the total enzymatic plus nonenzymatic degradation of UDP-Gal in 3 hr averaged about 13%; with sodium taurocholate and Triton CF-54 the average degradation was 16% and 24%, respectively. Thus, at the uniform level of 50 nmoles of UDP-Gal used through these studies, saturating quantities of the sugar nucleotide (30 nmoles; Fig. 5) would still be present after 3-hr incubations.

Identification of product

In the absence of added GM_2, incorporation of galactose into lipids was less than 3% of the incorporation levels achieved with exogenous acceptor. On thin-layer chromatography in a propanol–ammonia solvent system, about 85% of the chloroform–methanol soluble radioactivity was recovered in a band that migrated with authentic GM_1. When the lipid products of the reaction were chromatographed on thin-layer plates developed with chloroform–methanol–ammonia, 88% of the radioactivity applied to plates was recovered in a band that migrated with GM_1. Thus, the major product of the reaction contains galactose, is formed to a significant extent only in the presence of exogenously supplied GM_2, and migrates in two thin-layer solvent systems with GM_1 mobility.

Association of enzyme and product with membranes

Golgi apparatus fractions were treated with ultrasound (Branson Model W 140, amplitude 4, Branson Instrument Co., Stamford, Conn.) at intervals for a total time of one minute with cooling. The suspension was then separated into supernatant and particulate fractions by centrifugation. This treatment released 22% of the total protein from the fraction. In contrast, 99% of the total GM_2 galactosyltransferase activity recovered remained associated with the particulate fraction (**Table 3**). Under these conditions the enzyme was stable and more than 90% of the original activity was accounted for in the resultant fractions. It thus appears that this enzyme is a firmly associated constituent of the membrane and is not simply loosely associated with the membrane or a constituent of the cisternal lumina or forming secretory vesicle contents.

To establish the degree of product association with membranes, Golgi apparatus fractions were incubated in the standard reaction mixture either with or without detergent. After termination of the reaction, soluble and membrane fractions were separated by centrifugation and the particulate fraction was resuspended in water and treated with ultrasound as above. Following centrifugation, product was recovered from the particulate and combined supernatant fractions and assayed for radioactivity. In the incubation mix-

ture without detergent, nearly 99% of the incorporated radioactivity was recovered in the particulate fraction (**Table 4**). In the reaction mixture containing detergent, about 97% of the incorporated radioactivity was recovered in the particulate fraction. Thus, the product of the reaction, GM_1, became tightly associated with Golgi apparatus membranes. Since GM_1 is freely water soluble, these treatments would be expected to cause its release if it were loosely associated with the membrane or part of the cisternal or vesicle contents.

DISCUSSION

Results of this study confirm the presence of UDP-Gal:GM_2 galactosyltransferase in Golgi apparatus fractions from rat liver (20). Optimum conditions for enzyme activity are a pH of 5.6, 1.5 mg/ml of cardiolipin, 0.3 mM UDP-Gal, 0.15 mM GM_2, and 15 mM Mn^{2+}. These conditions differ markedly from those used in the original study of Basu, Kaufman, and Roseman (22), where activity of this enzyme was measured in crude synaptosomal fractions from embryonic chicken brain. In these synaptosomal fractions this activity is enriched about 4-fold relative to total homogenates whereas with Golgi apparatus fractions from liver this enrichment factor is at least 50-fold (20). A similarity of the two systems is that both require about 15–20 mM Mn^{2+} for optimum activity; this ion can be partially replaced by Co^{2+} but not by Ca^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+} or Zn^{2+}. The specific activity of UDP-Gal:GM_2 galactosyltransferase under optimum conditions is 22.4 nmoles of galactose incorporated/hr/mg of protein. This is 5.5 times higher than the specific activity previously obtained with rat liver Golgi apparatus using conditions reported to be optimum for the brain enzyme (20).

An unexpected finding was that the pH optimum varied depending on the dispersing agent used. Stimulation with sodium taurocholate was optimal at pH 6.2, whereas Triton CF-54 was optimal at pH 5.9. Cardiolipin required a more acidic pH, 5.6, than did the detergents. The mode of action of these agents in stimulating the reaction is unknown. One plausible explanation is that solubilizing agents in some manner facilitate interaction of substrate with the active site of the enzyme. For the galactosyltransferase of Golgi apparatus functioning in lactose synthesis, there is evidence to indicate that the active site is oriented along the internal face of cisternal membranes (46, 47). If such localization of glycolipid glycosyltransferases proves to be true, the necessity for dispersing agents could be explained as aiding in penetration of the substrate to the active site of the enzyme.

Studies of glycolipid synthesis in bacteria suggest a mode of interaction of phospholipids with acceptors and enzymes. Rothfield and Pearlman (48) have found that two purified microbial glycosyltransferases have an absolute requirement for phospholipids. Further studies have revealed that a glycolipid–phospholipid complex is formed which then interacts with the enzyme (49), and it has been suggested that this complex provides a hydrophobic environment which favors the transfer of sugars to glycolipids (50). While cardiolipin was the most effective of the agents tested in promoting GM_1 formation, it should be noted that this is an artificial situation. There is no significant amount of cardiolipin in Golgi apparatus fractions from rat liver (51).

Gammach (52) found that gangliosides form micelles of 2.5–4 $\times$ 10^5 daltons in aqueous solutions, with the critical micellar concentration being 0.02% by weight. With GM_2, this would correspond to about 0.15 mM, a concentration which is strikingly close to the saturation concentration of GM_2 in the galactosyltransferase reaction. It is possible that detergents or phospholipids change micellar size or alter the orientation of GM_2 in the micelle in such a manner as to facilitate interaction between enzyme and substrate.

The fact that enzyme activity remained associated with membranes after treatment with ultrasound shows that the enzyme is firmly associated with the membrane and not simply loosely bound or a constituent of the cisternal lumina or forming secretory vesicle contents. More surprising was the fact that the product also became firmly associated with the membrane. This corresponds to the in vivo situation, where it appears that sugars are added to glycolipids of membranes at the level of Golgi apparatus and endoplasmic reticulum (20, 21). Presumably, newly formed ceramides are incorporated into endoplasmic reticulum membrane and these are progressively glycosylated as the membrane flows through the Golgi apparatus. This and previous studies clearly suggest that glycolipids (20, 21) and both membrane and secretory glycoproteins (53–56) are glycosylated before they reach the surface membrane of the cell. The avidity of Golgi apparatus membranes for the exogenously supplied GM_2 or newly synthesized GM_1 correlates with recent demonstrations that exogenously supplied neutral glycosphingolipids (57, 59) and gangliosides (58, 59) are rapidly accumulated by cellular membranes.

This work was supported by grants from the National Science Foundation (GB 25110) and the National Cancer Institute, NIH (1 R01 CA 13145). T. W. K. is supported by Public Health Service Research Career Development Award 1 K04 GM-70596 from the National Institute of General Medical Science. Purdue University AES Journal Paper No. 5857.

Manuscript received 14 April 1975 and in revised form 16 September 1975; accepted 17 December 1975.

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