Wood, H. G., Ragsdale, S. W., & Pezacka, E. (1986a) *FEMS Microbiol. Rev. 39*, 345–362.
Wood, H. G., Ragsdale, S. W., & Pezacka, E. (1986b) *Trends Biochem. Sci. 11*, 14–18.
Wood, J. M., Kennedy, F. S., & Wolfe, R. S. (1968) *Biochemistry 7*, 1707–1713.
Zehnder, A. J. B., & Wuhrmann, K. (1976) *Science 194*, 1165–1166.

Localization in the Golgi Apparatus of Rat Liver UDP-Gal:Glucosylceramide β1→4Galactosyltransferase†

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ABSTRACT: The presence and subcellular localization of UDP-Gal:glucosylceramide β1→4galactosyltransferase (GalT-2) was investigated in rat liver. For this purpose, purified Golgi apparatus, endoplasmic reticulum, and plasma membrane fractions were prepared from the liver and used as the enzyme source for detecting GalT-2. A pure Golgi apparatus, highly enriched in many glycosyltransferases, was the only fraction where GalT-2 was measurable. The reaction product formation rate under appropriate assay conditions, which requires high detergent concentration and Mn^{2+}, was low but comparable with that of other glycosyltransferases. The product formation was stimulated by exogenously added acceptor GlcCer, donor UDP-Gal, and Golgi protein. The reaction product was a single spot that was identified by chromatographic behavior, sensitivity to β-galactosidase, and permethylation studies as Galβ1→4Glcβ1→1′Cer (lactosylceramide). A metabolic experiment, performed by determining the glycosphingolipids which became radioactive in the above subcellular fractions prepared from the liver of animals treated with glucose-labeled glucosylceramide, further indicated that the in vivo glycosylation of glucosylceramide takes place in the Golgi apparatus.

Glycosyltransferases involved in the elongation and termination of the oligosaccharide chain of glycosphingolipids and glycoproteins have been extensively studied, and much information is available now on their catalytic properties, specificity, and subcellular localization. For some of them, details are also known at the protein and gene level (Paulson & Colley, 1989).

Many results were also generated on the initial assembly of the oligosaccharide chain of Asn-linked glycoproteins (Hirschberg & Snider, 1987), and some reports also recently investigated the first biosynthetic steps occurring in the biosynthesis of the oligosaccharide chain of O-linked glycoproteins (Abeijon & Hirschberg, 1987; Piller et al., 1990). However, the activities involved in the initial glycosylation of glycosphingolipids were poorly studied. UDP-Gal:glucosylceramide (GlcCer)[1] β1→4galactosyltransferase, or GalT-2, is the enzyme activity responsible for the biosynthesis of LacCer, a common precursor of many acid and neutral glycosphingolipids. After its first discovery in the nervous system (Basu et al., 1968) and in the rat spleen (Hildebrand & Hauser, 1969), it was reported in other tissues such as bone marrow (Taki et al., 1982) and renal cells (Chatterjee & Castiglione, 1987). In rat liver, the presence of GalT-2 was suggested (Senn et al., 1983), but exogenous GlcCer-dependent formation of true LacCer could not be demonstrated in vitro (Walter et al., 1983). In vivo, the occurrence of glycosylation of exogenous GlcCer (Trinchera et al., 1990b) suggests that GalT-2 may be present in this tissue. Moreover, the subcellular localization of GalT-2 has not yet been investigated in any tissue or cultured cell, even though it is a central point, especially in light of the possible regulatory effect exerted by exogenous substances on its activity (Chatterjee et al., 1988).

In this paper, we attempted to identify and characterize GalT-2 in rat liver, with the aim of establishing its subcellular localization with respect to the other glycosyltransferase activities involved in glycosphingolipid biosynthesis. In addition, we wanted to determine within which subcellular fraction exogenous GlcCer undergoes glycosylation in vivo. For these purposes we tested galactosyltransferase activity toward exogenous GlcCer using different cellular subfractions as the enzyme source. We prepared enough reaction product, radiolabeled in two different positions of the molecule, in order to unambiguously establish its structure. Further, we determined the amount of glycosphingolipids which became radioactive in the above subcellular fractions prepared from the liver of animals treated with glucose-labeled GlcCer ([*Glc*-^{3}H]GlcCer) and sacrificed at two different times after the injection.

Experimental Procedures

Materials. GlcCer and LacCer were prepared by controlled acid hydrolysis (0.5 M HCl, 4 h, 80 °C) of a bovine brain ganglioside mixture (Ghidoni et al., 1980). They were sepa-

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[1] Abbreviations: HPTLC, high-performance thin-layer chromatography; GLC, gas–liquid chromatography. Gangliosides and glycosphingolipids are coded according to the nomenclature of Svennerholm (1964) and the *IUPAC–IUB Recommendations* (1977). Designation of glycosphingolipid glycosyltransferases is according to Basu et al. (1987) with the reported extension (Trinchera et al., 1990a).

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rated by silica gel column chromatography with chloroform (3 volumes) and chloroform/methanol in different ratios (v/v) (9:1, 3 volumes; 6:1, 2 volumes; 4:1, 2 volumes; 3:2, 3.5 volumes; 1:2, 2 volumes; 1:4, 2 volumes) and successively purified in a second chromatographic step with chloroform/methanol (18:1 v/v) (GlcCer) and chloroform/methanol/water (125:30:2 v/v/v) (LacCer) as the eluting solvent systems. Materials were quantitated by the fluorescamine method (Higgins, 1984). The ratio of fluorometrically and gravimetrically determined compounds was 98.7/100 and 98.3/100 for GlcCer and LacCer, respectively, and their purity, assessed by HPTLC (Ghidoni et al., 1986) and GLC (Ghidoni et al., 1980), was better than 99%.

Detergents, ovalbumin (chicken egg albumin grade V), asialofetuin, CDP-choline, nucleotide sugars, bovine testis and Jack bean β-galactosidases, maltose, and isomaltose were obtained from Sigma, and *Vibrio cholerae* sialidase was from Behringwerke (Marburg, West Germany). HPTLC plates, silica gel 100, sucrose for density gradient ultracentrifugation, and common chemicals were obtained from Merck (Darmstadt, West Germany). NaB^3H_4 (7.1 Ci/mmol), UDP-[U-^{14}C]galactose, CMP-*N*-acetyl[4,5,6,7,8,9-^{14}C]neuraminic acid, and [U-^{14}C]galactose were obtained from Amersham International (Amersham, Bucks, United Kingdom).

[*Glc*-^{3}H]GlcCer was prepared by the procedure of McMaster and Radin (1976), which involves oxidation of the C-6 hydroxyl group by the Pfitzner–Moffat reaction followed by NaB^3H_4 reduction. Its radiochemical purity and specific radioactivity were assessed as reported previously (Trinchera et al., 1990a). Standard radiolabeled LacCer (Leskawa et al., 1984) and gangliosides (Gazzotti et al., 1984) were prepared according to the given references.

Animals and Animal Treatment. Male Wistar rats (average body weight 150 g) were purchased from Charles River (Milan). For metabolic experiments, animals were intravenously injected in the tail, without anaesthesia, with 50 μCi of [*Glc*-^{3}H]GlcCer prepared according to Tokoro et al. (1987). At different times from the injection, animals were killed and their livers removed and immediately processed (Ghidoni et al., 1986).

Subcellular Fractionation. The procedure of Morré et al. (1988) was followed, with some modifications, for subcellular fractionation. A single homogenate was obtained from the liver of each treated animal by dispersing it at 6000 rpm for 40 s, with a Polytron homogenizer (20 ST, Kinematica, Lucerne, Switzerland): 5–30 g of minced fresh liver in 2 volumes of 37.5 mM Tris/maleate buffer, pH 7.0, containing 1% dextran, 0.5 M sucrose, and 5 mM $MgCl_2$. The homogenate was then spun at 6000*g* for 15 min, and the upper two-thirds of the obtained pellet was resuspended and layered on 25 mL of 1.2 M sucrose and spun at 100000*g* in a Beckman SW-28 rotor. Material from the homogenate/sucrose interface was collected, made 40% (w/v) in sucrose, placed on the bottom of a nitrocellulose tube, overlayered with 10 mL of both 35% and 20% (w/v) sucrose, and spun at 90000*g* for 1 h. Material at the 35/20% sucrose interface was collected, diluted, and pelleted to obtain the membranes of the Golgi apparatus fraction.

The plasma membrane and endoplasmic reticulum fractions, from the lower one-third of the first pellet and from the supernatant of the first centrifugation, respectively, were prepared without modification of the cited method (Morré et al., 1988).

Enzyme Assays. Glc-6-phosphatase (Nordlie & Aron, 1966), 5′-nucleotidase (Emmelot et al., 1964), and acid phosphatase (Morré et al., 1983) were assayed according to the given references. Glycosphingolipid glycosyltransferases were assayed according to the general procedure of Basu et al. (1987). Adaptations to the rat liver and to the different substrates employed were done in order to obtain optimum detection of enzymatic activities (Trinchera et al., 1990a). GalT-2 was assayed in a reaction mixture containing, in a final volume of 0.04 mL, 0.25 mM acceptor GlcCer, 3.5 mg/mL Triton X-100, 0.2 M cacodylate/HCl buffer, pH 7.0, 15 mM $MnCl_2$, 7.5 mM CDP-choline, 0.8 mM donor UDP-[^{14}C]Gal (specific radioactivity 1.5 mCi/mmol), and the enzyme source, about 1.0 mg/mL in the case of the Golgi apparatus and 1–3 mg/mL in the case of the whole homogenate and other subfractions. GalT-6 was assayed in the same reaction mixture as GalT-2 but containing 10 mM $MnCl_2$, 5.0 mM CDP-choline, 1–3 mg/mL enzyme protein, and 0.4 mM acceptor LacCer. Both incubations were for 2 h at 37 °C.

GalT-3 and GD2-GalT (Trinchera et al., 1990a) and SAT-4 (Trinchera & Ghidoni, 1989) were assayed as reported. Blanks were regularly prepared by omitting the acceptors in the reaction mixture. The whole mixtures were then spotted on Whatman 3MM paper and assayed by descending chromatography in 1% tetraborate (Basu et al., 1987). The radioactivity of the appropriate areas was quantitatively determined by liquid scintillation counting, and blank values were subtracted. UDP-Gal:ovalbumin galactosyltransferase (Brew et al., 1975), or ovalbumin-GalT, and CMP-NeuAc:asialofetuin sialyltransferase (Briles et al., 1977), or asialofetuin-SAT, were assayed according to the given references.

Protein content was determined (Lowry et al., 1951) with bovine serum albumin as standard.

Reaction Product Characterization. In the case of GalT-2, ^{14}C-labeled LacCer was eluted from the paper with chloroform/methanol (2:1 v/v) and washed with 1 volume of water, and the chloroform phase was dried. The final product before and after Jack bean β-galactosidase treatment (Li et al., 1975) was analyzed by HPTLC and visualized by fluorography (Ghidoni et al., 1986). [*Glc*-^{3}H]LacCer was obtained with cold UDP-Gal as the donor and [*Glc*-^{3}H]GlcCer as the acceptor (specific radioactivity 1.2 Ci/mmol), under the same conditions described in the regular assay but in a final volume of 1.0 mL. It was extracted from the whole reaction mixture and partitioned (Tettamanti et al., 1973). It was then purified from the radioactive unreacted acceptor by two-step column chromatography on silica gel 100, as described above for cold LacCer purification. The purified product was submitted to permethylation (Stoffyn et al., 1973), and the obtained radioactive hexoses were analyzed by HPTLC and fluorography (Stoffyn et al., 1973). Reference methylated glucoses were prepared, with the same procedure, as follows: 2,3,6-tri-*O*-methylglucose and 2,3,4,6-tetra-*O*-methylglucose from maltose; 2,3,4-tri-*O*-methylglucose and 2,3,4,6-tetra-*O*-methylglucose from isomaltose.

In the case of GalT-3 and GD2-GalT (Trinchera et al., 1990a), of GalT-6 (Taniguchi et al., 1985), and of SAT-4 (Trinchera & Ghidoni, 1989), reaction product characterization was done as reported.

Extraction and Fractionation of Glycosphingolipids. Total lipids were extracted by the phosphate buffer/tetrahydrofuran method and then partitioned by diethyl ether as reported (Tettamanti et al., 1973). After partitioning, the organic phase, containing mostly neutral glycosphingolipids, was evaporated to dryness. Total gangliosides were purified from the evaporated aqueous phase by a Sepharose CL-6B column (Trinchera & Ghidoni, 1990), dialyzed, and lyophilized.

Table I: Requirements of Rat Liver Golgi GalT-2[a]

assay condition	GalT-2 activity
complete	100.0
minus acceptor	27.4
minus detergent	26.5
minus Mn^{2+}	21.6
minus CDP-choline	81.3
plus Mg^{2+}	84.7

[a] The complete reaction mixture contained acceptor GlcCer, detergent, donor UDP-[^{14}C]Gal, $MnCl_2$, CDP-choline, Golgi protein, and buffer as described under Experimental Procedures. Results are the means for four experiments. Values are expressed as a percent of the "complete" assay condition. One hundred percent activity corresponds to 8.6 nmol of transferred Gal (mg of protein)$^{-1}$ h^{-1}.

During all the procedures, radioactivity was monitored and determined by liquid scintillation counting in solution with Ultima Gold (Packard) as scintillation cocktail and a Packard Tricarb 01900 analyzer. Individual compounds were separated by HPTLC with chloroform/methanol/water (55:20:3 v/v/v) (neutral glycosphingolipids) and chloroform/methanol/0.2% aqueous $CaCl_2$ (50:42:11 v/v/v) (gangliosides) as the eluting solvent systems. Radioactive spots were revealed by fluorography (Ghidoni et al., 1986) and quantitated by radiodensitometry, on an RITA analyzer (Raytest, Essen, West Germany).

Radiochemical Characterization of Glycosphingolipids. Radioactive compounds from the whole homogenate of treated animals were obtained as above, but they were preparatively separated by silica gel column chromatography. Elution was performed, in the case of neutral glycosphingolipids, as described above for GlcCer and LacCer purification and in the case of gangliosides with chloroform/methanol/water (12:7:1 v/v/v) (Ghidoni et al., 1987). Identification of individual radiolabeled glycosphingolipids was accomplished by submitting the isolated compounds to enzyme action, as previously reported. In detail, the radioactive spot found in the organic phase in addition to the injected [*Glc*-^{3}H]GlcCer comigrated, by HPTLC, with reference LacCer and originated radioactive GlcCer but not radioactive galactose upon Jack bean β-galactosidase treatment (Li et al., 1975). In the ganglioside fraction, the spots which corresponded, by HPTLC, to GM3, GD1a, and GD1b were affected by *V. cholerae* sialidase action (Ghidoni et al., 1987), originating radioactive LacCer (GM3) and GM1 (GD1a and GD1b), respectively, and that which corresponded to GM1 was affected by bovine testis β-galactosidase treatment (Cahan et al., 1982), originating radioactive GM2. The intramolecular distribution of radioactivity was determined by radio-GLC analysis, as reported (Trinchera et al., 1990b), and indicated that the radioactivity was located only on the glucose residue.

Results

Detection of GalT-2. On the basis of the obtained metabolic findings (see last paragraph of Results), we chose the Golgi apparatus fraction as the enzyme source for detecting GalT-2. The requirements of the activity toward exogenous GlcCer include detergent and Mn^{2+} (Table I). Triton X-100 was the most effective among the common commercial detergents tested; its concentration is critical since activity was almost undetectable when it was either 25% less than or 20% over the optimal concentration. Maximum activity was found at pH 7.0, and pH values over 7.5 or under 6.5 strongly inhibited GalT-2.

Kinetic Parameters of GalT-2. Activity dependence on the acceptor substrate was clearly demonstrated with GlcCer (Figure 1A). Saturation occurred at values over 0.20 mM,

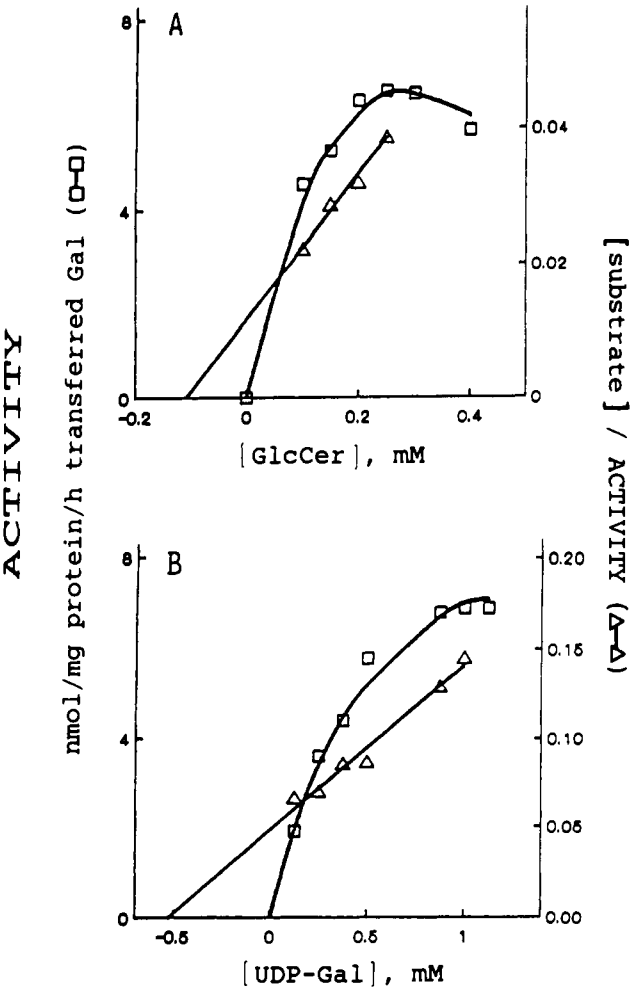

FIGURE 1: Characterization of rat liver Golgi GalT-2. Enzyme activity measurement and Golgi apparatus preparation were carried out as described under Experimental Procedures. The effect of acceptor GlcCer (panel A) and donor UDP-Gal (panel B). Values are the mean for three experiments. Standard deviations were within the size of the symbols used. The right scale shows the transposition in a Hanes–Woolf plot of the activity values.

and concentrations over 0.30 mM progressively inhibited the enzyme activity. Apparent calculated K_m and V_{max} are 0.11 mM acceptor GlcCer and 9.2 nmol of transferred Gal h^{-1} (mg of protein)$^{-1}$, respectively. The effect of donor UDP-Gal on GalT-2 showed that it is saturated only at relatively high concentrations of donor (over 0.8 mM) (Figure 1B). Apparent calculated K_m for UDP-Gal is 0.52 mM. Activity was linear in a protein concentration range from 0.5 to 1.5 mg/mL and increased linearly with time from 1.5 and 4 h. Due to the low specific activity, it was difficult to obtain reliable values over the blank at shorter times.

Reaction Product Characterization. When cold GlcCer was used as the acceptor and UDP-[^{14}C]Gal as the sugar donor, one radioactive spot was obtained after partitioning in the organic phase, which comigrated, by HPTLC, with standard LacCer. No additional spots were detectable with both fluorography and radiodensitometry. This compound disappeared upon Jack bean β-galactosidase treatment, originating a spot comigrating with reference galactose (Figure 2A). When [*Glc*-^{3}H]GlcCer was used as the acceptor and cold UDP-Gal as the donor in the same reaction mixture, two spots were obtained; the first one (about 91%) comigrated with standard GlcCer and the second one (about 7%) with standard LacCer. After purification and permethylation of this second spot, HPTLC analysis of the obtained radioactive hexoses

Table II: Activity of GalT-2 and Other Glycosyltransferases in Rat Liver Subcellular Fractions[a]

enzymatic act.	whole homogenate sp act.	golgi apparatus			endoplasmic reticulum			plasma membrane		
		sp act.	RSA	% recovery	sp act.	RSA	% recovery	sp act.	RSA	% recovery
GalT-2	not detectable	6.2 ± 0.8			not detectable			not detectable		
GalT-3	0.50 ± 0.08	68.1 ± 8.1	136	16.3	0.59 ± 0.09	1.2	0.8	0.51 ± 0.09	1.0	0.9
GD2-GalT	0.32 ± 0.06	45.3 ± 6.4	141	16.9	0.38 ± 0.05	1.2	0.8	0.31 ± 0.05	1.0	0.9
GalT-6	not detectable	2.1 ± 0.3			not detectable			not detectable		
ovalbumin-GalT	1.11 ± 0.18	181.2 ± 24.1	163	19.6	1.82 ± 0.29	1.6	1.1	0.71 ± 0.11	0.6	0.5
SAT-4	0.61 ± 0.10	97.8 ± 10.9	160	19.2	0.8 ± 0.12	1.3	0.9	0.39 ± 0.06	0.6	0.5
asialofetuin-SAT	15.5 ± 2.6	1946 ± 312	126	15.1	16.8 ± 2.42	1.1	0.8	4.3 ± 0.62	0.3	0.3
Glc-6-phosphatase	6.1 ± 1.2	6.4 ± 1.1	1.0	0.1	25.1 ± 3.7	4.1	3.1	4.8 ± 0.7	0.8	0.6
5′-nucleotidase	2.5 ± 0.2	7.1 ± 1.3	2.8	0.3	2.2 ± 0.2	0.9	0.7	37.5 ± 5.2	15.0	13.6
acid phosphatase	2.3 ± 0.2	2.3 ± 0.3	1.0	0.1	3.5 ± 0.4	1.5	1.1	3.0 ± 0.4	1.3	1.2
protein	175.5 ± 18.3[b]	0.21 ± 0.04[b]		0.12	1.3 ± 0.18[b]		0.74	1.5 ± 0.22[b]		0.91

[a] GalT-2 has been monitored by measuring the specific activity in the whole homogenate and subcellular fractions (prepared as described under Experimental Procedures) with respect to other glycosphingolipid glycosyltransferases and to the following marker enzymes: ovalbumin-GalT and asialofetuin-SAT (Golgi apparatus), Glc-6-phosphatase (endoplasmic reticulum), 5′-nucleotidase (plasma membrane), and acid phosphatase (lysosomes). Values are expressed as nmol of transferred sugar (mg of protein)$^{-1}$ h^{-1} for all glycosyltransferases and μmol of released phosphorus (mg of protein)$^{-1}$ h^{-1} for the others. Results are means ± standard deviations for four experiments. RSA (relative specific activity) and recovery were calculated with respect to the whole homogenate. [b] mg/g of fresh tissue.

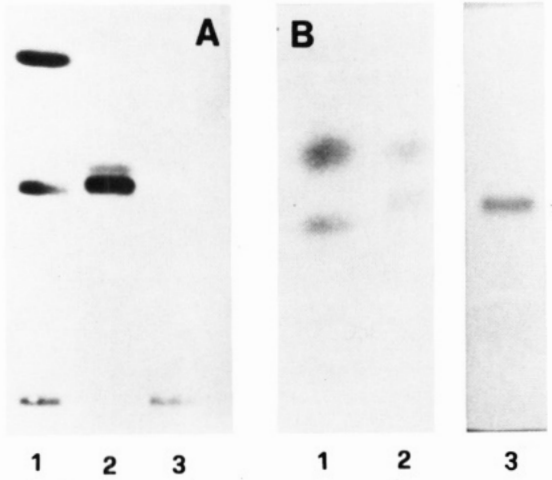

FIGURE 2: Reaction product characterization of GalT-2. (Panel A) The product was obtained with cold acceptor and UDP[^{14}C]Gal as the sugar donor as described under Experimental Procedures. Lane 1, standard radiolabeled GlcCer (upper spot), LacCer (middle spot), and Gal (lower spot); lane 2, reaction product of GalT-2; lane 3, Jack bean β-galactosidase treatment of lane 2. (Panel B) The product was obtained with [*Glc*-^{3}H]GlcCer as the acceptor and cold UDP-Gal as the donor and was purified and permethylated as described under Experimental Procedures. Lane 1, permethylated hexoses from isomaltose; lane 2, permethylated hexoses from maltose; lane 3, permethylated hexoses from the reaction product of GalT-2. HPTLC plates were developed with chloroform/methanol/water (55:20:3 by volume) (panel A) and acetone/5 M ammonia (50:0.9 by volume) (panel B) as the eluting solvent systems and visualized by fluorography (panel A and lane 3 of panel B) or by anisaldehyde spray reagent (lanes 1 and 2 of panel B).

revealed one spot, which was identified as 2,3,6-tri-*O*-methylglucose, by comparison with reference standards (Figure 2B). All these results indicate that GalT-2 elaborated the Galβ1→4Glc sequence of LacCer.

Subcellular Distribution of GalT-2 and Other Galactosyltransferase Activities. Subcellular fractions obtained from a single rat liver homogenate were characterized on the basis of the activity of specific marker enzymes. Table II shows that both endoplasmic reticulum and plasma membrane fractions were of a good degree of purity. In addition, the Golgi apparatus fraction was very enriched in specific markers such as ovalbumin-GalT and asialofetuin-SAT, as well as in the activity of other glycosyltransferases. This Golgi fraction was constituted by intact cisternal stacks and was free from other membranes, as assessed by electron microscopy (M. Trinchera, D. Carrettoni, and R. Ghidoni, unpublished results). With the assay conditions as above, GalT-2 was assayed on the whole liver homogenate and moreover on the above subcellular fractions. The Golgi apparatus was the only fraction where GalT-2 was well detectable; in fact, no reproducible values over the blank were obtained in the whole homogenate or in the other subfractions; analogous results were found for GalT-6. Ovalbumin-GalT, GalT-3, and GD2-GalT, as well as other glycosyltransferases, were detectable in all the fractions: they were about 140-fold enriched in the Golgi apparatus, whereas they were diminished, with respect to the whole homogenate, in the other subfractions.

Glycosylation of Exogenous [Glc-^{3}H]GlcCer in Different Rat Liver Membranes. The same subcellular fractions as above were prepared from the liver of animals treated with [*Glc*-^{3}H]GlcCer and sacrificed at two different times from the injection. In addition to administered GlcCer, the identified radioactive glycosphingolipids, metabolically obtained, were LacCer and gangliosides (see Experimental Procedures). All carried the radioactivity only on the glucose residue. The radioactivity values expressed per milligram of protein (Figure 3, upper panel) indicate the specific radioactivity of the glycosphingolipids in each subfraction, whereas those expressed per gram of fresh tissue (Figure 3, lower panel) represent the radioactive glycosphingolipids per total organelle, since they were calculated with respect to the recovery of positive markers in the respective subfractions (Trinchera & Ghidoni, 1990). In both cases, while radioactive LacCer and gangliosides decreased with time in the Golgi apparatus fraction, they increased in the plasma membrane and endoplasmic reticulum fractions. At 4 h, LacCer assumed high radioactivity values only in the Golgi apparatus, whereas radioactive gangliosides were present in both the Golgi apparatus and plasma membrane fractions in similar amounts. At 20 h, low amounts of radioactive LacCer were found in both the Golgi apparatus and plasma membrane fractions, whereas ganglioside-associated radioactivity was very high on the plasma membrane. Only minor amounts of radioactive LacCer and gangliosides were found in the endoplasmic reticulum fraction, at both the investigated times.

Discussion

In this paper we detected an activity able to elaborate the Galβ1→4Glc sequence of LacCer in rat liver and determined its localization in the Golgi apparatus; moreover, we provided metabolic evidence that such a glycosylation occurs in the Golgi apparatus in vivo.

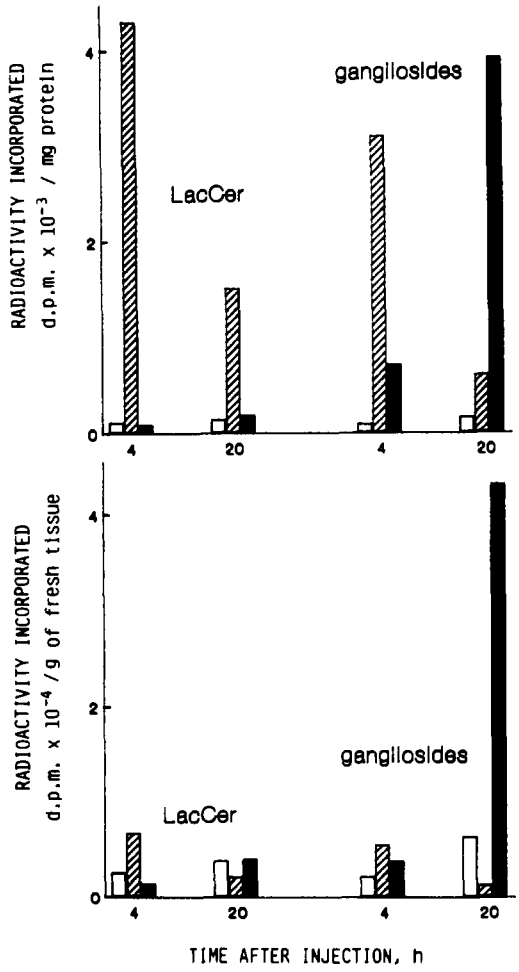

FIGURE 3: Distribution of radioactive glycosphingolipids in the different liver subfractions after administration of 50 μCi of [*Glc*-^{3}H]GlcCer. The endoplasmic reticulum (empty bars), Golgi apparatus (dotted bars), and plasma membrane (full bars) fractions were prepared and analyzed for the distribution of radiolabeled glycosphingolipids as described under Experimental Procedures. Results are the mean for two different experiments. (Upper panel) Specific radioactivity values; (lower panel) radioactivity per total organelle. For details on calculation see text.

The specific activity value determined in vitro for GalT-2 is low and does not permit the clear detection of the activity in the whole homogenate but only in a highly purified Golgi apparatus. The preparation of this subfraction, in good yield, and the availability of [*Glc*-^{3}H]GlcCer allowed us to unambiguously identify its reaction product, LacCer. In fact, sensitivity to Jack bean β-galactosidase demonstrated that a β-galactose is transferred to GlcCer, and the results of the permethylation study confirmed that only the glucose C-4 position of the acceptor is substituted.

Conditions for optimum detection are critical, especially with respect to the concentration of reagents (mainly the detergent), to the purity of the acceptor GlcCer, and to the purity of the enzyme source. Requirements and kinetic parameters are similar to those of other galactosyltransferase activities still present in the same enzyme preparation. Two of these activities elaborate the Galβ1→3GalNAc sequence of gangliosides, one elaborates the Galα1→4Gal sequence of globosides, and another one elaborates the Galβ1→4GlcNAc sequence of glycoproteins, all different from the Galβ1→4Glc sequence of LacCer elaborated by GalT-2. On this basis, it appears reasonable that GalT-2 constitutes a distinct catalytic activity in rat liver, as already suggested for other tissues (Basu et al., 1987). More studies are needed to better elucidate this point. In fact, it has been reported that a soluble galactosyltransferase from bovine milk, which partially copurified with lactose synthase, is able to synthesize LacCer (Bushway & Keenan, 1979). Further, lactose synthase A from human plasma was reported to biosynthesize both LacCer and paragloboside (Yamato & Yoshida, 1982).

GalT-2 distribution in the different subcellular fractions prepared from the liver indicated that the bulk of the activity is localized in the Golgi apparatus. In the other subfractions and also in the whole homogenate, it is under the detection limit of our in vitro assay. GalT-2 specific activity appeared in the range of the other glycosphingolipid glycosyltransferases, suggesting that GalT-2 enrichment in the Golgi apparatus may be similar to that of other such activities. Metabolic data obtained by the in vivo administration of [*Glc*-^{3}H]GlcCer corroborated the hypothesis that GalT-2 is mainly localized in the Golgi apparatus, as is the case for many other rat liver glycosphingolipid glycosyltransferases (Keenan et al., 1974; Trinchera et al., 1990a). In fact, we found both radioactive LacCer and ganglioside in the Golgi apparatus a short time after the injection of labeled GlcCer but not in the endoplasmic reticulum. Their occurrence in the other subfractions at longer time periods indicated that LacCer and gangliosides, once biosynthesized, are not accumulated but quickly processed in the Golgi apparatus. In fact, LacCer was mainly utilized as a substrate for ganglioside biosynthesis and poorly translocated to other membranes, whereas gangliosides were accumulated on the plasma membrane, as expected (Miller-Podraza & Fishman, 1982; Trinchera & Ghidoni, 1990).

In conclusion, our data demonstrated that the bulk of the galactosylation of GlcCer to LacCer occurs in the Golgi apparatus. Since the biosynthesis of the ceramide moiety of glycosphingolipids is considered to occur in the endoplasmic reticulum (Walter et al., 1983), a compound leaves this organelle and reaches the Golgi apparatus for running the glycosylation pathway. By now, it is not possible to establish whether ceramide or glucosylceramide is involved in this process. In fact, one report (Coste et al., 1986) has suggested that the glucosyltransferase acting on the ceramide moiety is localized in the Golgi apparatus (at the cytosolic side), in porcine submaxillary gland. Other studies proposed that GlcCer biosynthesis may not occur in the membranes where further steps of glycosphingolipid biosynthesis occur (Saito et al., 1984; Miller-Podraza & Fishman, 1984). Preliminary results indicate that the same Golgi apparatus fraction utilized in the present study also catalyzes the transfer of glucose from UPD-glucose to ceramide in a ceramide-dependent manner.

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REFERENCES

Abeijon, C., & Hirschberg, C. B. (1987) *J. Biol. Chem. 262*, 4153–4159.

Basu, S., Kaufman, B., & Roseman, S. (1968) *J. Biol. Chem. 243*, 5802–5804.

Basu, M., De, T., Das, K., Kyle, J. W., Chon, H., Schaper, R. J., & Basu, S. (1987) *Methods Enzymol. 138*, 575–607.

Brew, K., Shaper, J. H., Olsen, K. W., Trayer, I. P., & Hill, R. L. (1975) *J. Biol. Chem. 250*, 1434–1446.

Briles, E. B., Li, E., & Kornfeld, S. (1977) *J. Biol. Chem. 252*, 1107–1116.

Bushway, A. A., & Keenan, T. W. (1979) *Biochim. Biophys. Acta 572*, 146–152.
Cahan, L. D., Irie, R. F., Singh, R., Cassidenti, A., & Paulson, S. C. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7629–7633.
Chatterjee, S., & Castiglione, E. (1987) *Biochim. Biophys. Acta 923*, 136–142.
Chatterjee, S., Ghosh, N., Castiglione, E., & Kviterovich, P. O., Jr. (1988) *J. Biol. Chem. 263*, 13017–13022.
Coste, H., Martel, M. B., & Got, R. (1986) *Biochim. Biophys. Acta 858*, 6–12.
Emmelot, P., Bos, C. J., Benedetti, E. L., & Rumke, P. (1964) *Biochim. Biophys. Acta 90*, 126–145.
Gazzotti, G., Sonnino, S., Ghidoni, R., & Tettamanti, G. (1984) *Glycoconjugate J. 1*, 111–121.
Ghidoni, R., Sonnino, S., Tettamanti, G., Baumann, N., Reuter, J., & Schauer, R. (1980) *J. Biol. Chem. 255*, 6990–6995.
Ghidoni, R., Trinchera, M., Venerando, B., Fiorilli, A., Sonnino, S., & Tettamanti, G. (1986) *Biochem. J. 237*, 147–155.
Ghidoni, R., Trinchera, M., Sonnino, S., Chigorno, V., & Tettamanti, G. (1987) *Biochem. J. 247*, 157–164.
Higgins, T. J. (1984) *J. Lipid Res. 25*, 1007–1009.
Hildebrand, J., & Hauser, G. (1969) *J. Biol. Chem. 244*, 5170–5180.
Hirschberg, C. B., & Snider, M. D. (1987) *Annu. Rev. Biochem. 56*, 63–87.
IUPAC–IUB Recommendations (1977) *Lipids 12*, 455–468.
Keenan, T. W., Morrê, D. J., & Basu, S. (1974) *J. Biol. Chem. 249*, 310–315.
Leskawa, K. C., Dasgupta, S., Chien, J.-L., & Hogan, E. L. (1984) *Anal. Biochem. 140*, 172–177.
Li, S.-C., Mazzotta, M. Y., Chien, S.-F., & Li, Y.-T. (1975) *J. Biol. Chem. 250*, 6786–6791.
Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
McMaster, M. C., & Radin, N. S. (1976) *J. Labelled Compd. Radiopharm. 23*, 353–357.
Miller-Podraza, H., & Fishman, P. H. (1982) *Biochemistry 21*, 3265–3270.
Miller-Podraza, H., & Fishman, P. H. (1984) *Biochim. Biophys. Acta 804*, 44–51.
Morrê, D. J., Morrê, D. M., & Heidrich, H.-G. (1983) *Eur. J. Cell Biol. 31*, 263–274.
Morrê, D. M., Morrê, D. J., Bowen, S., Reutter, W., & Windel, K. (1988) *Eur. J. Cell Biol. 46*, 307–315.
Nordlie, R. C., & Aron, W. J. (1966) *Methods Enzymol. 9*, 619–625.
Paulson, J. C., & Colley, K. J. (1989) *J. Biol. Chem. 264*, 17615–17618.
Piller, V., Piller, F., & Fukuda, M. (1990) *J. Biol. Chem. 265*, 9264–9271.
Saito, M., Saito, M., & Rosenberg, A. (1984) *Biochemistry 23*, 1043–1046.
Senn, H.-J., Wagner, M., & Decker, K. (1983) *Eur. J. Biochem. 135*, 231–236.
Stoffyn, P., Stoffyn, A., & Hauser, G. (1973) *J. Biol. Chem. 248*, 1920–1923.
Svennerholm, L. (1964) *J. Lipid Res. 5*, 145–155.
Taki, T., Kamada, R., & Matsumoto, M. (1982) *J. Biochem. 91*, 665–674.
Taniguchi, N., Yanagisawa, K., Makita, A., & Naiki, M. (1985) *J. Biol. Chem. 260*, 4908–4913.
Tettamanti, G., Bonali, F., Marchesini, S., & Zambotti, V. (1973) *Biochim. Biophys. Acta 269*, 160–170.
Tokoro, T., Gal, A. E., Gallo, L. L., & Brady, R. O. (1987) *J. Lipid Res. 28*, 968–972.
Trinchera, M., & Ghidoni, R. (1989) *J. Biol. Chem. 264*, 15766–15769.
Trinchera, M., & Ghidoni, R. (1990) *Biochem. J. 266*, 363–369.
Trinchera, M., Pirovano, B., & Ghidoni, R. (1990a) *J. Biol. Chem. 265*, 18242–18247.
Trinchera, M., Vallenzasca, C., Ghidoni, R., & Tettamanti, G. (1990b) *FEBS Lett. 260*, 23–26.
Walter, V. P., Sweeney, K., & Morrê, D. J. (1983) *Biochim. Biophys. Acta 750*, 346–352.
Yamato, K., & Yoshida, A. (1982) *J. Biochem. 92*, 1123–1127.