Biochimica et Biophysica Acta, 643 (1981) 292—305
Elsevier/North-Holland Biomedical Press

BBA 79208

TRANSLOCATION OF CYTIDINE 5'-MONOPHOSPHOSIALIC ACID ACROSS GOLGI APPARATUS MEMBRANES

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(Received Ocotber 7th, 1980)

Key words: Cytidine 5'-monophosphosialic acid, Golgi apparatus, Glycolipid, Glycoprotein; (Rat liver)

Summary

Golgi apparatus, isolated from rat liver, incorporate [^{14}C]sialic acid from CMP[^{14}C]sialic acid into endogenous glycolipid and glycoprotein acceptors. Incorporation of [^{14}C]sialic acid into endogenous glycoprotein acceptors was stimulated an average of 3-fold by Triton X-100 at an optimal concentration of 0.05% and was inhibited at higher concentrations. Incorporation of [^{14}C]sialic acid into endogenous glycolipid acceptors was not stimulated by detergent. The major glycolipid product was identified by thin-layer chromatography as the ganglioside G_{D3}. SDS-polyacrylamide gel electrophoresis of the glycoprotein products demonstrated incorporation of [^{14}C]sialic acid into 6—7 major bands. Neuraminidase studies determined that approximately 60% of the [^{14}C]sialic acid incorporated into endogenous acceptors in the absence of detergent had a luminal orientation. Furthermore, electron microscopy studies showed that the isolated Golgi apparatus fraction consisted of intact membrane cisternae. Our results demonstrate that sialylation of cisternal acceptors located on the inside of the membrane occurs in the absence of detergent. They are consistent with carrier-mediated transport as a mechanism to allow CMPsialic acid to traverse the Golgi apparatus membrane and to be used to glycosylate endogenous glycoprotein and glycolipid acceptors.

Abbreviations: G_{M1}, galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosyl-glucosyl-ceramide; G_{M2}, *N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosyl-glucosyl-ceramide; G_{M3}, *N*-acetylneuraminyl-galactosyl-glucosyl-ceramide; G_{D3}, (*N*-acetylneuraminyl)$_2$-galactosyl-glucosyl-ceramide; G_{D1a}, *N*-acetylneuraminyl-galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosyl-glucosyl-ceramide, G_T, trisialoganglioside; PPO, 2,5-diphenyloxazole; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography; GlcNAc, *N*-acetylglucosamine; POPOP, 1,4-bis(5-phenyloxazolyl-2)benzene.

0005-2736/81/0000—0000/$02.50

Introduction

The subcellular site of synthesis of the sugar nucleotide CMPsialic acid in rat liver is the nucleus [1]. However, it is clear that the addition of sialic acid to lipids and proteins is catalyzed by sialyltransferase found predominantly in the lumens of the Golgi apparatus cisternae [2—9]. Therefore, the CMPsialic acid must cross endomembranes in order to react with the sialyltransferases.

Diffusion or facilitated transport are mechanisms that would allow the passage of sugar nucleotides through membranes. However, it is generally assumed that microsomal membranes are not penetrated by negatively charged molecules such as sugar nucleotides and, therefore, simple diffusion would seem unlikely [10,11]. Some studies support carrier-mediated or -facilitated transport of certain molecules across microsomal membranes. For example, Arion and co-workers [12,13] have shown that glucose 6-phosphate but not mannose 6-phosphate is transported across endoplasmic reticulum isolated from rat liver by a carrier-mediated system. Also, Kuhn and White [14,15] have provided evidence for the specific, probably facilitated, transport of UDPgalactose across Golgi apparatus membranes isolated from mammary gland of the rat. These studies were conducted to determine whether a similar mechanism is involved in the transport of the sugar nucleotide CMPsialic acid across Golgi apparatus membranes isolated from rat liver. During the course of these studies a report appeared by Carey et al. [16] that provided evidence of a specific transport system for CMPsialic acid in microsomes isolated from mouse liver.

Materials and Methods

Materials. UDP[^{14}C]galactose (347 mCi/mmol), CMP[^{14}C]sialic acid (304 mCi/mmol) and NCS tissue solubilizer were from Amersham-Searle (Arlington Heights, IL). Neuraminidase Type V, purified from *Clostridium perfringens*, and Triton X-100 were from Sigma Chemical Co. (St. Louis, MO). Precoated plates of silica gel G-60 (F-254) were from E. Merck, Darmstadt, F.R.G. (Scientific Products, McGaw Park, IL). Kodak RP Royal X-Omat medical X-ray film was from General Electric Co. (Indianapolis, IN). PPO was from Research Products International Corp. (Elk Grove Village, IL) and dimethyl POPOP from Packard Instrument Co., Inc. (Downers Grove, IL). Dimethylsulfoxide was from Fischer Scientific Co. (Itasca, IL). Ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine were from Bio-Rad Laboratories (Richmond, CA). Acrylamide, *N,N'*-methylenebisacrylamide and SDS were from Eastman Kodak (Rochester, NY). Ganglioside standards were gifts of Dr. T.W. Keenan, Department of Animal Sciences, Purdue University.

Isolation of Golgi apparatus. Golgi apparatus were isolated from the livers of male Holtzman rats (Holtzman Co., Madison, WI), given food and water ad libitum, by the method of Morré et al. [17]. The Golgi apparatus pellet was carefully resuspended in a minimal volume of TKM buffer [18] (50 mM Tris-HCl, pH 7.5/50 mM KCl/5 mM $MgCl_2$) and protein determined by the method of Lowry et al. [19].

Electron microscopy. Fractions were fixed overnight in 2% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, at 4°C. Fractions were then post-fixed in 1%

osmium tetroxide in 0.05 M phosphate buffer, pH 7.2, for 2 h. Samples were rinsed and dehydrated through an acetone series and embedded in Epon [20]. Thin sections were examined and photographed with a Philips EM/200 electron microscope.

CMP[^{14}C]sialic acid to endogenous acceptor sialyltransferase assay. Isolated Golgi apparatus membranes (0.2—0.4 mg protein) were incubated in reactions containing 50 mM Tris-HCl/10 mM $MnCl_2$/1 mM AMP, pH 7.5, and 100 000 cpm of CMP[^{14}C]sialic acid (0.33 μM) of UDP[^{14}C]galactose (0.29 μM), in a final volume of 0.5 ml at 25°C. Reactions contained Triton X-100 where indicated. Incubations were for the times indicated and initiated with sugar nucleotide. Reactions were stopped with 0.5 ml of ice-cold 12% trichloroacetic acid/1% phosphotungstic acid followed by 1 ml of ice-cold trichloroacetic acid/0.5% phosphotungstic acid. Incorporation of ^{14}C-labeled sugar into endogenous glycoprotein and glycolipid acceptors was as described by Tkacz et al. [21]. Briefly, reactions were allowed to precipitate for at least 30 min at 4°C, centrifuged, and the supernatants discarded. Each precipitate was washed three times with 2 ml of ice-cold 6% trichloroacetic acid/0.5% phosphotungstic acid, extracted twice with 2 ml of chloroform/methanol, 1 : 1 (v/v), and twice with 2 ml of chloroform/methanol, 2 : 1 (v/v). The chloroform/methanol extracts from each sample were combined, dried under a stream of nitrogen, and radioactivity determined in 15 ml of a toluene-based scintillation cocktail (0.5% PPO/0.01% dimethyl POPOP). The chloroform/methanol extracts measure incorporation of ^{14}C-labeled sugar into glycolipid. The residual precipitate, which measures incorporation into glycoprotein, was dissolved in 0.5 ml of NCS tissue solubilizer and added to 15 ml of toluene-based scintillation cocktail. Glacial acetic acid (0.035 ml) was added to neutralize the digest and eliminate chemiluminescence prior to determination of radioactivity. Zero time controls were prepared by the simultaneous addition of sugar nucleotide and 0.5 ml of 12% trichloroacetic acid/1% phosphotungstic acid.

The results are the averages of duplicate determinations after subtraction of zero time controls.

Neuraminidase assay. Reactions containing Golgi apparatus membranes (0.3—0.4 mg protein) and CMP[^{14}C]sialic acid were incubated for 30 min at 25°C, in the absence of Triton X-100, in the standard incubation mixture. Reactions were stopped by dilution with 3 ml of TKM buffer [18] and the [^{14}C]sialic acid-labeled Golgi apparatus membranes were reisolated by centrifugation in a clinical centrifuge. A neuraminidase preparation (0.2 ml) was added to each tube containing the [^{14}C]sialic acid-labeled membranes, the membranes were resuspended by mixing with a Vortex mixer, and subsequently incubated at 25°C for the times indicated. The neuraminidase solution was prepared by mixing 3.5 mg of neuraminidase, 8.2 mg of sodium acetate and 1.7 g of sucrose in a total volume of 10 ml of water. The final pH was 5.5. In neuraminidase preparations containing Triton X-100 the sucrose was omitted. Neuraminidase reactions were stopped by the addition of 0.2 ml of ice-cold 12% trichloroacetic acid/1% phosphotungstic acid, followed by 1 ml of ice-cold 6% trichloroacetic acid/0.5% phosphotungstic acid. Reactions were allowed to precipitate for 30 min and the pellets collected by centrifugation. The pellets were subsequently washed 3 times with 2 ml of ice-cold 6% trichloroacetic acid/0.5%

phosphotungstic acid. The washed pellets were solubilized in 0.5 ml of NCS tissue solubilizer and radioactivity determined in 15 ml of toluene-based scintillation fluid containing 0.035 ml of glacial acetic acid.

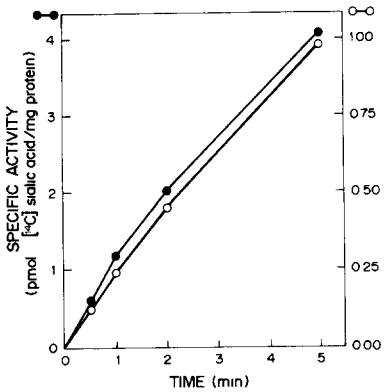
SDS-polyacrylamide gel electrophoresis. Endogenous protein acceptors of [^{14}C]sialic acid from CMP[^{14}C]sialic acid were labeled by incubating Golgi apparatus membranes (1.6 mg protein) for 5 min in the presence or absence of 0.05% Triton X-100, in a 2-fold scale-up of the standard endogenous acceptor sialyltransferase assay mixture described. Reactions were terminated by the addition of 5 ml of distilled water and the [^{14}C]sialic acid-labeled Golgi apparatus membranes were reisolated by centrifugation at 100 000 × *g* for 30 min (SW 50.1 rotor). The protein pellet was solubilized in 2% SDS/1% 2-mercaptoethanol/10% glycerol by heating to 100°C for 3 min prior to application to the gel. Samples and standards were applied to a 10% polyacrylamide slab gel and electrophoresed overnight by the method of Studier [22] using a model 220 slab gel electrophoresis apparatus (Bio-Rad Laboratories, Richmond, CA). Molecular weight standards used to calibrate the gels were albumin, heavy chain immunoglobulin, ovalbumin, light chain immunoglobulin and cytochrome *c*. These proteins have monomeric molecular weights of 68 000, 50 000, 43 000, 23 500 and 12 000, respectively. The gels were stained overnight in an aqueous solution of 0.05% Coomassie blue/25% isopropanol/10% acetic acid. The gels were destained for 5—7 h in an aqueous solution of 10% isopropanol/10% acetic acid and then photographed. [^{14}C]Sialic acid-labeled proteins were visualized by fluorography. Gels were fixed, impregnated with PPO, and dried according to the procedure of Bonner and Laskey [23]. Dried gels were exposed to Kodak RP Royal X-Omat medical X-ray film at —70°C for approximately 30 days.

Results

Characterization of CMPsialic acid sialyltransferase for endogenous acceptor

Isolated Golgi apparatus membranes incubated with CMP[^{14}C]sialic acid in the absence of detergent incorporated [^{14}C]sialic acid into endogenous glycolipid and glycoprotein acceptors. Incorporation was first-order with respect to time during incubations of 5 min or less (Fig. 1). For endogenous protein acceptors, incorporation in the absence or the presence of 0.05% Triton X-100 was first-order with respect to protein concentration between 0.2—0.6 mg protein per assay (Fig. 2).

The concentration of the detergent Triton X-100 in incubations affected the incorporation of [^{14}C]sialic acid from CMP[^{14}C]sialic acid into endogenous glycolipid and glycoprotein acceptors (Fig. 3). Concentrations of Triton X-100 below the critical micelle concentration inhibited incorporation of [^{14}C]sialic acid into lipid products, as did higher concentrations of the detergent. Triton X-100 concentrations of 0.2% or greater completely inhibited incorporation of [^{14}C]sialic acid into endogenous glycolipid. However, incorporation of [^{14}C]-sialic acid into glycoprotein was stimulated an average of 2.75-fold at a Triton X-100 concentration of 0.05% (Fig. 3). The stimulation by Triton X-100 occurred over a very narrow range of detergent concentrations. Concentrations above 0.1% significantly inhibited incorporation of [^{14}C]sialic acid into endogenous



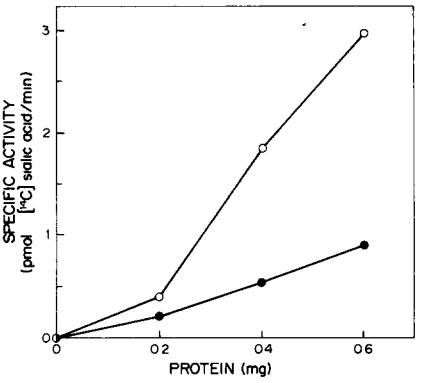
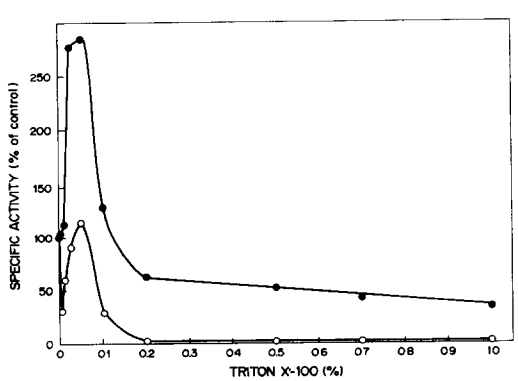


Fig. 1. Incorporation of [^{14}C]sialic acid from CMP[^{14}C]sialic acid into endogenous glycolipid (○) and glycoprotein (●) acceptors was first-order with time. Isolated Golgi apparatus (0.41 mg protein) were incubated for the times indicated in the absence of Triton X-100.

Fig. 2. Incorporation of [^{14}C]sialic acid from CMP[^{14}C]sialic acid into endogenous glycoprotein acceptors was first-order with protein. Isolated Golgi apparatus were incubated for 3 min, at the protein concentrations indicated, in the absence (●) or the presence (○) of 0.05% Triton X-100.

protein acceptors. The stimulation by Triton X-100 of the incorporation of [^{14}C]sialic acid into endogenous glycoprotein acceptors occurred whether incorporation of [^{14}C]sialic acid was measured over short (Fig. 4) or long (Fig. 5) periods of incubation. Even after 90 min of incubation, [^{14}C]sialic acid was still being incorporated into endogenous protein acceptors, independent of the presence or absence of Triton X-100 (Fig. 5). During long periods of incubation, the amount of [^{14}C]sialic acid incorporated into endogenous protein acceptors in the absence of detergent never reached the amount incorporated in



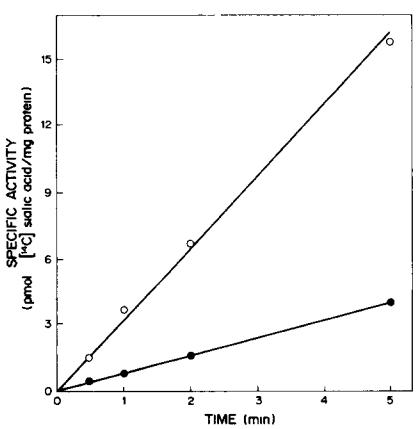


Fig. 3. Triton X-100 concentration affects incorporation of [^{14}C]sialic acid from CMP[^{14}C]sialic acid into endogenous glycolipid (○) and glycoprotein (●) acceptors. Isolated Golgi apparatus (0.30—0.40 mg protein) were incubated for 5 min in assays containing varying concentrations of Triton X-100.

Fig. 4. Triton X-100 stimulates incorporation of [^{14}C]sialic acid from CMP[^{14}C]sialic acid into endogenous glycoprotein acceptors during short times of incubation. Isolated Golgi apparatus (0.35 mg protein) were incubated for the times indicated in the absence (●) or the presence (○) of 0.05% Triton X-100.

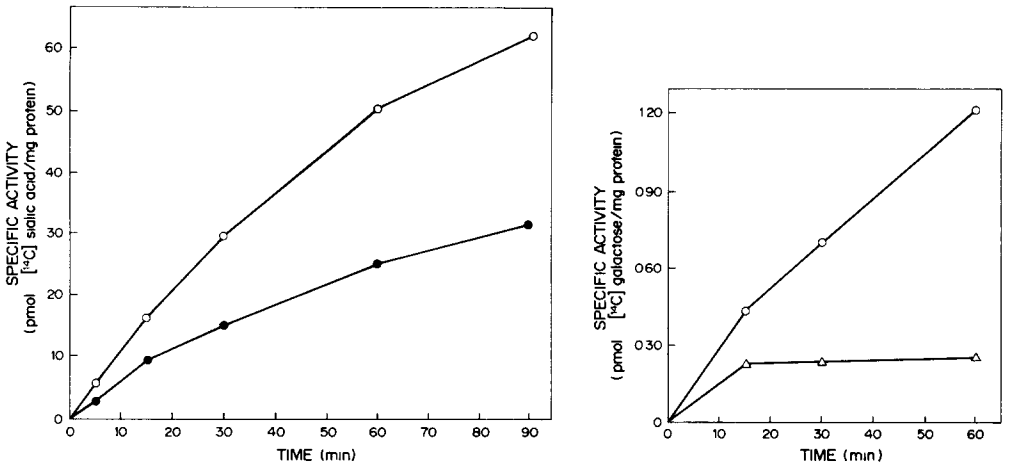


Fig. 5. Triton X-100 stimulates incorporation of [^{14}C]sialic acid from CMP[^{14}C]sialic acid into endogenous glycoprotein acceptors during long times of incubation. Isolated Golgi apparatus (0.32 mg protein) were incubated for the times indicated in the absence (●) or the presence (○) of 0.05% Triton X-100.

Fig. 6. Triton X-100 inhibits incorporation of [^{14}C]galactose from UDP[^{14}C]galactose into endogenous glycolipid acceptors. Isolated Golgi apparatus (0.45 mg protein) were incubated for the times indicated in the absence (○) or the presence (△) of 0.05% Triton X-100.

the presence of detergent. Therefore, under either of these assay conditions all of the endogenous acceptor sites were never saturated with [^{14}C]sialic acid.

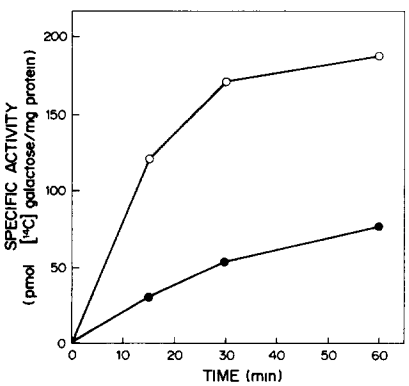
Incorporation of [^{14}C]galactose from UDP[^{14}C]galactose into endogenous acceptors of isolated Golgi apparatus

Isolated Golgi apparatus membranes incorporated [^{14}C]galactose from UDP-[^{14}C]galactose into endogenous glycolipid and glycoprotein acceptors in the absence of detergent (Figs. 6 and 7). Similar to the incorporation of [^{14}C]sialic acid, 0.05% Triton X-100 stimulated 2- to 3-fold the incorporation of [^{14}C]-galactose into endogenous glycoprotein acceptors (Fig. 7). Triton X-100 at 0.05% inhibited incorporation of galactose into endogenous glycolipid acceptors (Fig. 6).

Isolation and characterization of the glycolipid and glycoprotein products of the CMPsialic acid to endogenous acceptor sialyltransferase reaction

Isolated Golgi apparatus membranes were incubated with CMP[^{14}C]sialic acid for 1 h in the absence or presence of 0.05% Triton X-100. The ^{14}C-labeled glycolipids were extracted with chloroform/methanol and purified by TLC. The major glycolipid product formed in the absence of 0.05% Triton X-100 comigrated with ganglioside standard G_{D3} (Fig. 8). Small amounts of glycolipid product that comigrated with ganglioside standards G_{M3} and G_T were formed. The addition of 0.05% Triton X-100 to the incubation medium reduced incorporation of [^{14}C]sialic acid into glycolipids (Fig. 8).

In another experiment, isolated Golgi apparatus membranes were incubated for 5 min with CMP[^{14}C]sialic acid in the presence or absence of 0.05% Triton X-100. The ^{14}C-labeled Golgi apparatus membranes were reisolated by centrifugation, solubilized in SDS, and individual polypeptides separated by SDS-poly-



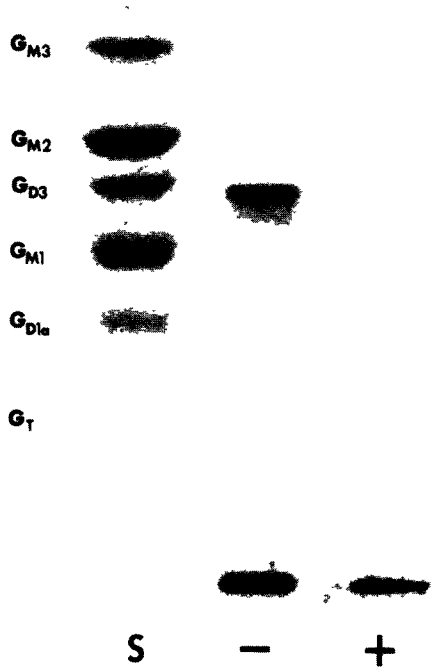


Fig. 7. Triton X-100 stimulates incorporation of [^{14}C]galactose from UDP[^{14}C]galactose into endogenous glycoprotein acceptors. Isolated Golgi apparatus (0.45 mg protein) were incubated for the times indicated in the absence (●) or the presence (○) of 0.05% Triton X-100.

Fig. 8. Identification of [^{14}C]sialic acid-labeled endogenous glycolipid products formed during incubations with CMP[^{14}C]sialic acid and Golgi apparatus. Isolated Golgi apparatus (0.35 mg protein) were incubated for 60 min with CMP[^{14}C]sialic acid in the absence (—) or the presence (+) of 0.05% Triton X-100. Reactions were stopped by dilution with 10 ml water and the membranes pelleted. The membranes were washed a second time with 2 ml water and pelleted. Glycolipids were extracted from the pellets, first with 2 ml of chloroform/methanol, 2 · 1, (v/v) and then 2 ml of chloroform/methanol, 1 : 1, (v/v). The chloroform/methanol washes were combined and dried under a stream of nitrogen. The dried glycolipid pellets were resuspended in a small volume of chloroform/methanol, 1 : 1, (v/v) and spotted on a TLC plate along with authentic ganglioside standards (S). The plate was developed in chloroform/methanol/ammonium hydroxide/water, 60 : 35 : 7 : 3. Ganglioside standards were visualized by spraying with resorcinol reagent [24] and ^{14}C-labeled glycolipids by exposure to Kodak RP Royal X-Omat medical X-ray film for 30 days.

acrylamide gel electrophoresis. A Coomassie blue stain of the gel showed that there was virtually no change in the total protein banding pattern whether or not the Golgi apparatus membranes were incubated in the presence or absence of 0.05% Triton X-100 (Fig. 9). A fluorogram of the gel showed that [^{14}C]-sialic acid was incorporated into proteins that ran as 6—7 distinct bands (Fig. 9). Interestingly, the stimulation by Triton X-100 of the incorporation of [^{14}C]-sialic acid into glycoproteins was not the same for individual bands. Triton X-100 stimulated incorporation of [^{14}C]sialic acid into glycoproteins of M_r

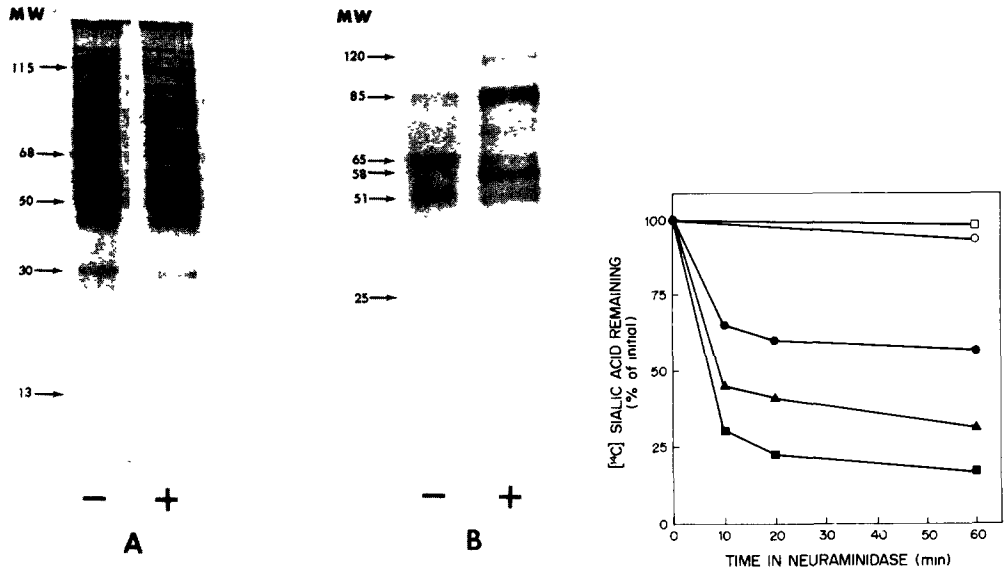


Fig. 9. Separation of [^{14}C]sialic acid-labeled endogenous glycoprotein products formed during incubations with CMP[^{14}C]sialic acid and Golgi apparatus. Isolated Golgi apparatus (1.6 mg protein) were incubated for 5 min with CMP[^{14}C]sialic acid in the absence (—) or the presence (+) of 0.05% Triton X-100. Golgi apparatus membranes were reisolated by centrifugation and proteins separated by SDS-polyacrylamide gel electrophoresis. Total proteins (0.15 mg protein per lane) were visualized by staining with Coomassie blue (A) and [^{14}C]sialic acid-containing glycoproteins (0.65 mg protein per lane) by fluorography (B). The numerical designations refer to apparent molecular weights ($\times 10^{-3}$) as determined from molecular weight standards.

Fig. 10. Effect of neuraminidase and Triton X-100 on the release of [^{14}C]sialic acid from [^{14}C]sialic acid-labeled Golgi apparatus membranes. Isolated Golgi apparatus membranes (0.3—0.4 mg protein) were incubated for 30 min in the absence of Triton X-100, with CMP[^{14}C]sialic acid to label endogenous glycolipid and glycoprotein acceptors with [^{14}C]sialic acid. The membranes were reisolated by centrifugation and resuspended in buffer containing no neuraminidase and no Triton X-100 (○), no neuraminidase and 0.5% Triton X-100 (□), neuraminidase and no Triton X-100 (●), neuraminidase and 0.05% Triton X-100 (▲) or neuraminidase and 0.5% Triton X-100 (■) and incubated for the times indicated. The amount of [^{14}C]sialic acid remaining incorporated into endogenous acceptors was measured as described. The results are expressed as the percent of [^{14}C]sialic acid remaining following the neuraminidase treatment as compared to the [^{14}C]sialic acid present following the initial 30 min incubation with CMP[^{14}C]sialic acid.

120 000, 85 000 and 58 000. On the other hand, incorporation of [^{14}C]sialic acid into glycoproteins of M_r 65 000, 51 000 and 25 000 was not stimulated by incubating the Golgi apparatus membranes in the presence of 0.05% Triton X-100 (Fig. 9). This observation may be the result of differences in the topology of the endogenous protein acceptors being labeled with the [^{14}C]sialic acid.

Topology of the endogenous acceptors of [^{14}C]sialic acid in Golgi apparatus membranes

Experiments were then conducted to determine the topology of the endogenous acceptors in Golgi apparatus that were labeled with [^{14}C]sialic acid in incubations without detergent. Isolated Golgi apparatus membranes were incu-

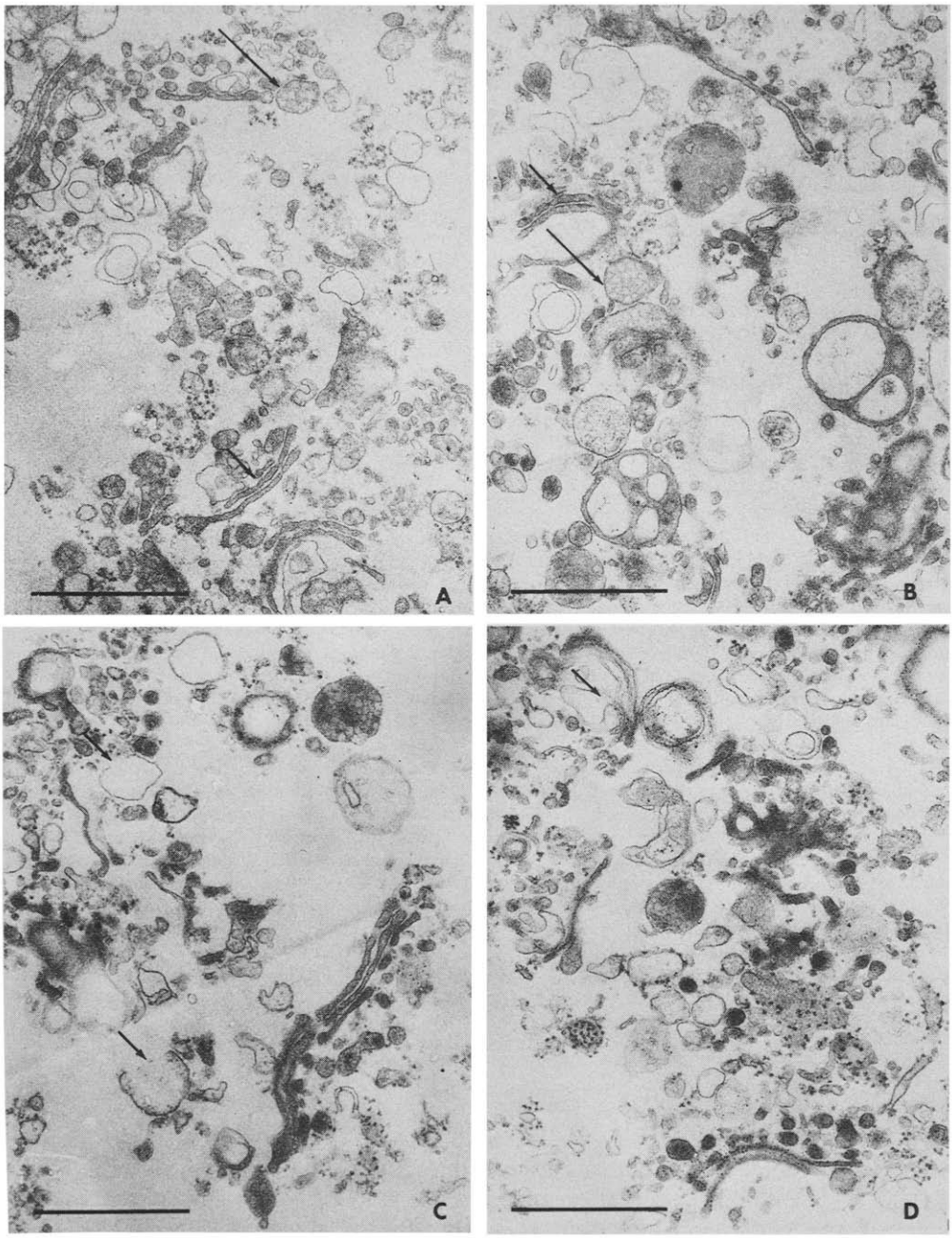


Fig. 11.

bated for 30 min with CMP[^{14}C]sialic acid in the absence of detergent to label endogenous acceptors with [^{14}C]sialic acid. The [^{14}C]sialic acid-labeled membranes were reisolated by centrifugation and resuspended in the presence or absence of detergent and the sialic acid-cleaving enzyme, neuraminidase. When [^{14}C]sialic acid-labeled Golgi apparatus membranes were resuspended in neura-

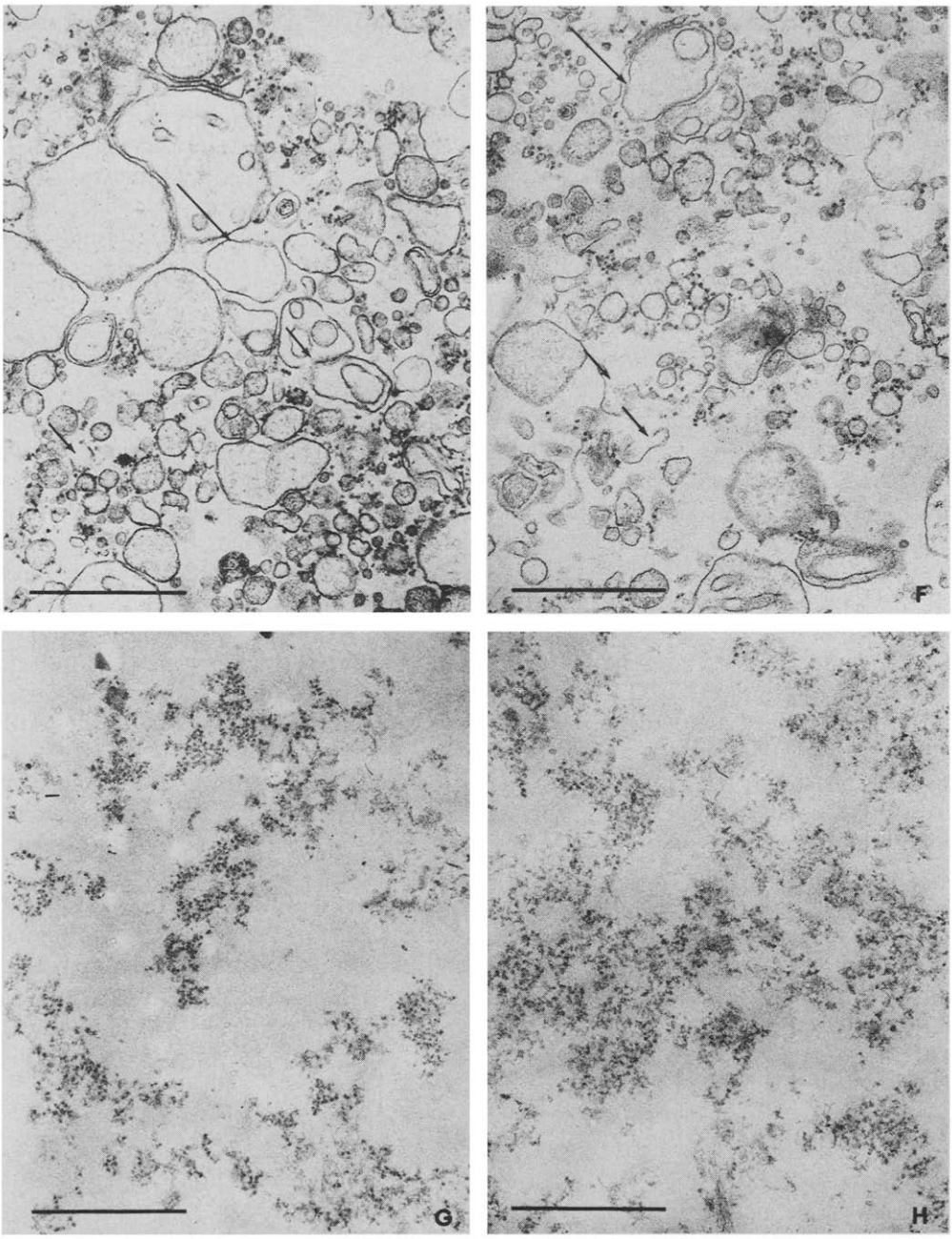


Fig. 11. Electron micrographs of isolated Golgi apparatus membranes treated with Triton X-100 and neuraminidase. Initial Golgi apparatus fraction (A) consisted of stacks of cisternae (short arrow) and associated secretory vesicles (long arrow). Golgi apparatus membranes isolated following a 30 min incubation with CMP[^{14}C]sialic acid (B) still showed stacks of cisternae (short arrow) and associated secretory vesicles (long arrow). The [^{14}C]sialic acid-labeled Golgi apparatus membranes incubated in the presence (C) or absence (D) of neuraminidase had some breaks in the membranes (arrows). The [^{14}C]sialic acid-labeled membranes incubated with 0.05% Triton X-100 in the presence (E) or the absence (F) of neuraminidase had swollen cisternae (long arrows), some with breaks in the membranes (short arrows). The [^{14}C]sialic acid-labeled membranes incubated with 0.5% Triton X-100 in the presence (G) or absence (H) of neuraminidase were solubilized and only membrane fragments and ribosomes were pelleted. Scale marker, 1.0 μm.

minidase containing no Triton X-100, about 35—40% of the [^{14}C]sialic acid incorporated into glycolipid and glycoprotein was removed (Fig. 10). As the membranes were solubilized in increasing concentrations of Triton X-100, to expose the cisternal surface of the vesicles to neuraminidase, 80% of the [^{14}C]-sialic acid incorporated into endogenous acceptors was removed (Fig. 10). These results demonstrated that up to 60% of the [^{14}C]sialic acid incorporated into endogenous acceptors of Golgi apparatus membranes in the absence of detergent was accessible to neuraminidase only in the presence of detergent and suggested a luminal orientation of at least some of the [^{14}C]sialic acid incorporated into endogenous acceptors.

Electron microscopy studies of neuraminidase- and Triton X-100-treated Golgi apparatus membranes

Electron micrographs of the initial Golgi apparatus pellet showed that the fraction consisted of intact Golgi apparatus stacks and associated vesicles (Fig. 11A). The Golgi apparatus membranes were intact, with no apparent breaks in the membranes. The Golgi apparatus fraction showed about 5% contamination with endoplasmic reticulum. Micrographs of Golgi apparatus membranes that were pelleted following a 30 min incubation period with CMP[^{14}C]sialic acid showed that the membranes remained intact and that some existed as stacks of Golgi apparatus cisternae (Fig. 11B). Compared to the initial Golgi apparatus pellet some of the cisternae appeared to be slightly swollen but still intact. Electron micrographs of these [^{14}C]sialic acid-labeled Golgi apparatus membranes, following a 10 min incubation with neuraminidase in the absence of any detergent, showed that most of the vesicles were still intact but that a few had breaks (Fig. 11C). Controls incubated without neuraminidase appeared similar (Fig. 11D). [^{14}C]Sialic acid-labeled Golgi apparatus membranes incubated in 0.05% Triton X-100 in the presence or absence of neuraminidase showed complete unstacking of Golgi apparatus cisternae. Swollen cisternae, some with broken membranes, were present (Fig. 11E and F). Incubation of Golgi apparatus membranes with 0.5% Triton X-100 in the presence or absence of neuraminidase resulted in complete solubilization of the membranes which allowed pelleting of only ribosomes and a few membrane fragments (Fig. 11G and H).

Discussion

The transfer of sialic acid from CMPsialic acid to glycoprotein and glycolipid acceptors is catalyzed by sialyltransferases predominantly in the Golgi apparatus [2,5,8]. Since sialyltransferase activity is enhanced by detergents, it is assumed that the active site of the transferases is on the luminal side of the membranes [2,5,6]. The enhancement of glycosyltransferase activity by detergents is believed to result from a removal of the permeability barrier imposed by microsomal membranes toward sugar nucleotides. However, in this report we showed that sialylation of endogenous glycolipid and glycoprotein acceptors in Golgi apparatus occurred to a significant extent even in the absence of detergent. These results suggest that CMPsialic acid is traversing the Golgi apparatus membranes in the absence of detergent disruption.

Initially we showed that the CMPsialic acid to endogenous acceptor sialyltransferase assay was valid since the formation of product was linear with time and protein concentration. To see what effect disruption of the membranes had on the sialyltransferase activity we determined the enzymatic activity over a wide range of Triton X-100 concentrations. A complex interaction between the detergent Triton X-100 and the incorporation of [^{14}C]sialic acid into endogenous glycolipid acceptors was found. Similar to results by McEvoy et al. [25] on a glycolipid galactosyltransferase, we found that concentrations of detergent near or below the critical micelle concentration for Triton X-100 [26] inhibited the transfer of sialic acid to endogenous glycolipid acceptors. Concentrations above 0.2% were completely inhibitory. Therefore, the detergent was inhibitory in both its monomer and its micellar forms. In contrast, Triton X-100 at 0.05% stimulated by 3-fold the incorporation of [^{14}C]sialic acid into endogenous glycoprotein acceptors. This stimulation occurred over a very narrow range of detergent concentrations and higher concentrations were inhibitory. It was difficult to precisely determine whether this stimulation was due to a breakdown in the permeability barrier of the membrane to the sugar nucleotide CMPsialic acid or whether the detergent activates the enzyme by modifying its interaction with other components of the membranes.

In previous studies, Triton X-100 was reported to inhibit the incorporation of GlcNAc [27] and galactose [28] into endogenous protein acceptors of isolated Golgi apparatus. However, we found that incorporation of galactose into endogenous glycoprotein acceptors was stimulated 2- to 3-fold at a Triton X-100 concentration of 0.05%. Our data were consistent with results of Kuhn et al. [29] where suitable concentrations of Triton elevated the rate of lactose synthesis by Golgi apparatus membranes isolated from rat mammary gland. Triton X-100 concentrations above 0.4% stimulated incorporation of mannose into endogenous glycoprotein acceptors in an isolated Golgi apparatus fraction [30]. In a recent study Andersson and Eriksson [31] reported that Triton X-100 did not stimulate the transfer of galactose from UDPgalactose to the exogenous acceptor asialomucin. This is not consistent with previous reports that found large stimulations, by Triton X-100, of galactosyl transfer to ovalbumin [32] and ovomucoid [33,34].

To demonstrate conclusively that the CMPsialic acid did cross the membranes of the Golgi apparatus it was necessary to determine the topology of the endogenous acceptor. This was accomplished by treating Golgi apparatus membranes, the endogenous acceptors of which have been pre-labeled with [^{14}C]-sialic acid in the absence of detergent, with neuraminidase. These studies showed that approximately 60% of the incorporated sialic acid had a luminal orientation. Furthermore, electron microscopy studies showed that the isolated Golgi apparatus fractions consisted of intact membrane cisternae. Therefore, it was unlikely that the incorporation of sialic acid in the absence of detergent was due entirely to 'leaky' or broken cisternae.

To demonstrate that we were measuring incorporated sialic acid and not residual or trapped sugar nucleotide, we isolated and characterized the [^{14}C]sialic acid containing glycolipid and glycoprotein products. The major endogenous glycolipid product formed was the ganglioside G_{D3}, while some [^{14}C]sialic acid was incorporated into the gangliosides G_{M3} and G_T. SDS-polyacrylamide gel

electrophoretic analysis of the [^{14}C]sialic acid-containing glycoprotein products demonstrated incorporation of [^{14}C]sialic acid into some of the glycoproteins was stimulated in incubations containing 0.05% Triton X-100 while incorporation of [^{14}C]sialic acid into other proteins appeared unaffected by detergent disruption. These results suggested that some of the sialylation may take place on the cytoplasmic surface of the cisternae and that the Triton X-100 relaxes permeability restraints of the CMPsialic acid so that glycosylation was enhanced on acceptors with a luminal orientation. These results agreed with the neuraminidase data which suggested that some of the sialic acid incorporated into endogenous acceptors had a cytoplasmic orientation. But, important to the aims of this investigation, glycosylation of cisternal acceptors did occur in the absence of detergent.

While this study demonstrated that sialic acid was incorporated into endogenous acceptors that have a luminal orientation in incubations with intact Golgi apparatus cisternae, it left some questions unanswered. While it was assumed that CMPsialic acid was translocated across the membranes intact this was not demonstrated. If CMPsialic acid was broken down by pyrophosphatases and entered the membranes as sialic acid and CMP, or other metabolites, it would have to be resynthesized inside the cisternae. Since CMPsialic acid synthetase was absent from the lumens of the Golgi apparatus cisternae [35], this mechanism of transport is unlikely. Further studies are necessary to demonstrate conclusively the mechanisms to allow CMPsialic acid to cross the membrane and be used to glycosylate endogenous acceptors. Recent results of Carey and Hirschberg [8] would favor a carrier-mediated process.

Acknowledgements

We thank Keri Safranski and Dorothy Werderitsh for technical assistance and Ed Croze for carrying out the gel separations. This work was supported in part by a grant from the National Institute of Health, CA 18801.

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