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Mechanisms of glycosylation and sulfation in the Golgi apparatus: Evidence for nucleotide sugar/nucleoside monophosphate and nucleotide sulfate/nucleoside monophosphate antiports in the Golgi apparatus membrane

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ABSTRACT **The mechanism of translocation *in vitro* of sugar nucleotides and adenosine 3′-phosphate 5′-phosphosulfate (PAPS) into the lumen of rat liver Golgi apparatus vesicles has been studied. It has been previously shown that the Golgi apparatus membrane has specific carrier proteins for PAPS and sugar nucleotides. We now report that translocation of the above nucleotide derivatives across Golgi membranes occurs via a coupled equimolar exchange with the corresponding nucleoside monophosphates. An initial incubation of Golgi vesicles with GDP-fucose radiolabeled in the guanidine ring resulted in accumulation within the lumen of radiolabeled GMP. Exit of GMP from these vesicles was specifically dependent on the entry of (additional) GDP-fucose into the vesicles (GDP-mannose and other sugar nucleotides had no effect). GDP-fucose-stimulated exit of GMP was temperature dependent, was blocked by inhibitors of GDP-fucose transport, such as 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid, and appeared to be equimolar with GDP-fucose entry. Preliminary evidence for specific, equimolar exchange of CMP-*N*-acetylneuraminic acid with CMP, PAPS with 3′-AMP, and UDP-galactose and UDP-*N*-acetylglucosamine with UMP was also obtained. These results strongly suggest the existence of different antiport proteins within the Golgi membrane that mediate the 1:1 exchange of sugar nucleotides or PAPS with the corresponding nucleoside monophosphate. Such proteins may have a regulatory role in glycosylation and sulfation reactions within the Golgi apparatus.**

Recent studies from this laboratory have shown that rat liver Golgi-derived vesicles can translocate *in vitro* CMP-*N*-acetylneuraminic acid (AcNeu), GDP-fucose, UDP-*N*-acetylglucosamine (GlcNAc), and adenosine 3′-phosphate 5′-phosphosulfate (PAPS) from an external compartment into a lumenal one (1–5). These reactions were found to be (*i*) saturable at high concentrations of sugar nucleotides and PAPS, (*ii*) temperature dependent, (*iii*) inhibited by treatment of the Golgi vesicles with proteases under conditions where lumenal marker enzymes were not inhibited, and (*iv*) inhibited competitively by the corresponding nucleoside mono-, di-, and triphosphate (6). Since the above sugar nucleotides and PAPS did not inhibit translocation of each other, it was hypothesized that there were different translocator proteins in the membrane of the Golgi apparatus and that portions of these proteins face the cytoplasmic side of the Golgi apparatus membranes. Evidence for translocation of UDP-galactose (Gal) into Golgi vesicles from mammary gland and rat liver (7, 8), CMP-AcNeu into rat liver Golgi (9) and hen oviduct microsomes (10) has also been obtained in other laboratories.

The aim of the present study was to understand the energy mechanism by which the above sugar nucleotides and PAPS are translocated across the Golgi vesicle membranes. We now present evidence suggesting that such a mechanism involves exchange with the corresponding nucleoside monophosphate via an antiport protein.

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MATERIALS AND METHODS

Radioactive Substrates. The following radioactive compounds were used: GDP-L-[1-^{14}C]fucose (264 Ci/mol; 1 Ci = 37 GBq), New England Nuclear; [*guanidine*-8-^{3}H]GDP-fucose (667 Ci/mol) synthesized as described (1); [*guanidine*-8-^{3}H]GMP (22 Ci/mmol) synthesized as described (1); [*adenine*-8-^{3}H]PAPS (870 Ci/mol) synthesized as described (4); [U-^{14}C]CMP (375 Ci/mol), Amersham; [U-^{14}C]UMP (484 Ci/mol), Amersham; CMP-[^{14}C]AcNeu (1.6 Ci/mol), New England Nuclear; [^{35}S]PAPS (1.3 Ci/mmol), New England Nuclear;

Isolation, Integrity, and Topography of Golgi Vesicles. Golgi vesicles were isolated from rat liver according to the procedure described by Leelavathi *et al.* (11). The vesicles were enriched, on average, ≈40-fold in sialyltransferase activity (compared to crude homogenate) (1, 4). At least 90% were sealed and of the same topographical orientation as *in vivo* (12).

Translocation Assay. The theoretical basis for the assays of translocation of the different nucleotide derivatives into Golgi vesicles has been described in detail (1, 2, 4). Briefly, it consists of (*i*) determining the total radioactive solutes associated with the Golgi pellet (S_t) after incubation with radiolabeled substrates and centrifugation of the Golgi vesicles (see below), and (*ii*) subtracting from this amount the total radioactive solutes trapped between the vesicles in the Golgi pellet (S_o). This latter value is obtained by multiplying the volume outside the vesicles in the Golgi pellet (V_o) by the concentration of radioactive solutes in the incubation medium ($[S_m]$). The volume outside (trapped) vesicles in the pellet was measured with a standard nonpenetrator such as [^{3}H]methoxyinulin.

RESULTS

In previous studies we had shown that sugar nucleotides and PAPS were translocated into Golgi vesicles *in vitro* (1, 5). This was done using sugar nucleotides and PAPS labeled with different radioisotopes in the nucleotide and sugar or sulfate. It was also shown that subsequent to translocation into the Golgi vesicle lumen, the sugars and sulfate were transferred to macromolecules facing the lumen of the vesi-

Abbreviations: PAPS, adenosine 3′-phosphate 5′-phosphosulfate; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; GlcNAc, *N*-acetylglucosamine; AcNeu, *N*-acetylneuraminic acid; Gal, galactose.

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cles, while nucleotides accumulated within the vesicles (relative to their concentration in the incubation medium) (1, 5). That these nucleotides were also leaving the Golgi lumen was suggested from experiments in which translocation of PAPS radiolabeled in the nucleotide and sulfate was measured (4).

Entry of GDP-Fucose into Golgi Vesicles Appears to Be Concomitant with Exit of GMP. The above studies led us to design an experiment to determine whether translocation of sugar nucleotides into the lumen of Golgi vesicles was coupled with exit of nucleotides from the Golgi lumen. For this purpose, Golgi vesicles were incubated with GDP-fucose ^{3}H-labeled in the guanidine ring; the total amount of radioactive solutes within the vesicles was determined after different incubation times. As can be seen in Fig. 1, there was a time-dependent accumulation of radiolabeled solutes within the Golgi vesicles that became constant after 10 min. No acid-insoluble radioactivity was detected (not shown).

In parallel experiments, GDP-[^{14}C]fucose was added (for 0.5–2 min) to Golgi vesicle suspensions that had been previously incubated with [^{3}H]GDP-fucose (for 1, 5, and 10 min). Fig. 1 shows that addition of GDP-[^{14}C]fucose resulted in (*i*) a concomitant decrease of the tritiated solutes within the vesicles (shown below to be [^{3}H]GMP) and (*ii*) a parallel increase in ^{14}C-containing species within the vesicles. This latter radioactivity was found to be, as previously determined, the sum of ^{14}C-containing solutes within vesicles and ^{14}C-containing radioactivity covalently bound to macromolecules facing the lumen of the vesicles (1). For example, when vesicles that had been incubated with [^{3}H]GDP-fucose for 10 min were then incubated with GDP-[^{14}C]fucose for 2 min, 50% of the [^{14}C]fucose within vesicles was acid-insoluble (not shown).

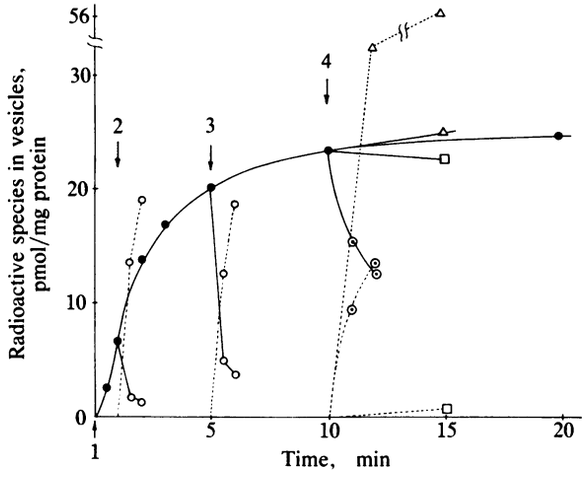

FIG. 1. Translocation of [^{3}H]GDP-fucose into Golgi vesicles and subsequent exchange of radiolabeled solutes from within the vesicles. Ultracentrifuge tubes, each containing Golgi vesicles (0.4 mg of protein), were incubated for different times at 25°C with [*guanidine*-8-^{3}H]GDP-L-fucose (0.27 μCi; final concentration, 0.4 μM; arrow 1) in 1.0 ml of buffer containing 10 mM Tris·HCl/0.25 M sucrose/1 mM $MgCl_2$/10 mM NaF/0.5 mM 2,3-dimercaptopropanol, final pH 7.5 (●). To samples that had been incubated for 1 (arrow 2) and 5 min (arrow 3), GDP-[^{14}C]fucose (5 μl, 0.12 μCi; 2 μM, final concentration) was added and the incubation was continued for 0.5 and 1 min (○). To samples that had been incubated for 10 min (arrow 4) with [*guanidine*-8-^{3}H]GDP-fucose, GDP-[^{14}C]fucose (5 μl; 0.12 μCi; final concentration, 0.45 μM was added and the mixture was incubated for 1 and 2 min (⊙). To another set of samples that had been incubated for 10 min (arrow 4) with [*guanidine*-8-^{3}H]GDP-fucose, [^{35}S]PAPS (5 μl; 0.44 μCi; final concentration, 2 μM) was added and the mixture was incubated for 2 and 5 min (△). To another group of samples incubated with [*guanidine*-8-^{3}H]GDP-fucose for 10 min (arrow 4), GDP-[^{14}C]mannose (5 μl; 0.31 μCi; final concentration, 1 μM) was added for 5 min (□). All reactions were stopped by placing tubes in a mixture of ice containing NaCl. Samples were then centrifuged, followed by determination of soluble (——; ^{3}H species) and total (soluble and insoluble) (------; ^{14}C or ^{35}S species) radioactivity within the Golgi pellet as described (1, 4).

The radioactive species within Golgi vesicles, after a 10-min incubation with [^{3}H]GDP-fucose were [^{3}H]GMP (65%–90%) and [^{3}H]guanosine (10%–35%). This result is in agreement with our previous studies (1). The soluble ^{14}C radioactive species within vesicles, after a 2-min incubation of the Golgi vesicle suspension described above with GDP-[^{14}C]fucose was mostly fucose, while the acid insoluble radioactivity was in fucoproteins. This result is also in agreement with our previous observations (1).

Exit of GMP from Golgi Vesicles Is Specifically Dependent on Entry of GDP-Fucose. Two types of experiment were done to determine that exit of GMP from Golgi vesicles was specifically dependent on entry of GDP-fucose into the vesicles: (*i*) Virtually no exit of ^{3}H-labeled solutes was observed from vesicles that had been incubated first with [^{3}H]GDP-fucose for 10 min and then with GDP-[^{14}C]mannose for 5 min (Fig. 1). We have obtained no evidence for entry of this latter sugar nucleotide into the Golgi lumen. These results strongly suggest that exit of [^{3}H]GMP of the previous experiment was dependent on entry of GDP-fucose into the vesicles. (*ii*) When [^{35}S]PAPS was added to vesicles that had been previously incubated with [^{3}H]GDP-fucose (for 10 min) there was no exit of [^{3}H]GMP from the vesicles (Fig. 1); however, the vesicles accumulated both soluble and acid-insoluble radioactive sulfur-containing species within their lumen (Fig. 1). We have recently shown that translocation of PAPS into Golgi vesicles is followed by transfer of sulfate into macromolecules facing the lumen of the vesicles (4, 5). The above experiment, therefore, strongly suggests that after a 10-min incubation with [^{3}H]GDP-fucose, the Golgi vesicles continue to be active in their ability to translocate other nucleotide derivatives and that exit of [^{3}H]GMP was specifically dependent on entry of GDP-fucose.

Additional evidence for the specificity of stimulation of exit of [^{3}H]GMP from Golgi vesicles is shown in Table 1. It can be seen that addition of 1–10 μM GDP-fucose to vesicles preloaded with [^{3}H]GDP-fucose resulted in exit of 68%–89% of [^{3}H]GMP from the vesicles. Considerably less exit was observed with UDP-Gal and with UDP-GlcNAc, both sugar nucleotides that are known to enter Golgi vesicles (3). Table

Table 1. Effect of sugar nucleotides, temperature, and inhibitors of anion transport on exit of [^{3}H]GMP from Golgi vesicles preincubated with [^{3}H]GDP-fucose for 10 min

Incubation		Exit
Substrate	Time, min	% [^{3}H]GMP remaining in vesicles
GDP-fucose (1 μM)	10	32
GDP-fucose (10 μM)	10	11
GDP-fucose/DIDS (1 μM)	10	68
GDP-fucose (2 μM) 4°C	10	80
UDP-GlcNAc (3 μM)	2	100
	5	100
UDP-Gal (25 μM)	10	89
PAPS (2 μM)	2	100
	5	100

Experimental conditions were the same as those described in the legend of Fig. 1. DIDS (5 μl; 100 μM, final concentration) was added after the preincubation; after 5 min, 1 μM GDP-fucose was added to the suspension. For studies on temperature dependence, the reaction was cooled to 4°C after the preincubation and continued thereafter at that temperature.

1 also shows that exit of [^{3}H]GMP was dependent on temperature. Addition of 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), a known inhibitor of GDP-fucose translocation (5), to preloaded vesicles resulted in inhibition of GDP-fucose-stimulated exit of [^{3}H]GMP from the vesicles (Table 1). This experiment provides additional evidence that exit of GMP from the vesicles is dependent on entry of GDP-fucose.

Stoichiometry Between Entry of GDP-Fucose to and Exit of GMP from Golgi Vesicles. An important assumption has to be made to measure the stoichiometry of entry of GDP-fucose into, and exit of GMP from, Golgi vesicles. The specific radioactivity of each radioactive species within Golgi vesicles cannot be accurately determined, because the size of the endogenous nonradioactive pool of GMP within the vesicles is not known. We have therefore made the assumption, for subsequent calculations, that the specific radioactivity of GMP within vesicles, after a 10-min incubation of vesicles with [^{3}H]GDP-fucose, is the same as that of the radioactive sugar nucleotide at the beginning of the incubation. This appears reasonable from the results seen in Fig. 1. These show that the amount of ^{3}H-labeled solutes within vesicles appeared to be constant after a 10-min incubation with [^{3}H]GDP-fucose; this suggests that equilibration between the external and internal pool of nucleotides has occurred at this time.

Table 2 shows that there was apparent stoichiometric exit of [^{3}H]GMP from the vesicles relative to entry of GDP-[^{14}C]fucose after 1 and 2 min. We hypothesize that the apparent somewhat lower amount of GMP that had exited in comparison to the amount of GDP-fucose that had entered is the result of assuming that the specific radioactivity of [^{3}H]GMP within vesicles is equal to that of the original [^{3}H]GDP-fucose of the incubation medium; the true former value is always less, with the highest value approaching that of the original [^{3}H]GDP-fucose, when both external and internal pools are fully equilibrated. This is almost achieved after a 10-min incubation with [^{3}H]GDP-fucose, (Fig. 1). This assumption predicts that at times prior to reaching equilibrium between the nucleotide pools, differences between exit and entry of nucleotides would be magnified if one used calculations of specific radioactivity values as those outlined above. That this is indeed the case can be seen when entry and exit of solutes are calculated after incubation of vesicles with [^{3}H]GDP-fucose for 1 min. As seen in Table 2, addition of GDP-[^{14}C]fucose for 1 min to such vesicles leads to an apparent larger entry of GDP-fucose than exit of GMP. After a 5-min incubation with [^{3}H]GDP-fucose, a time where the nucleotide pools are closer to reaching equilibrium (Fig. 1), the apparent difference between entry of sugar nucleotide and exit of GMP is similar to that observed at 10 min (Table 2).

Exit of GMP from Golgi Vesicles Preloaded with GMP Is Specific. The experiments described in the previous sections strongly suggest that entry of GDP-fucose into Golgi vesicles is coupled with a 1:1 stoichiometric exit of GMP from the vesicles. We had observed, in a preliminary experiment that Golgi vesicles transported GMP *in vitro* from an external compartment into a lumenal one. It was therefore of interest to determine whether one could measure GDP-fucose-dependent exit of GMP from Golgi vesicles that had been preloaded with GMP. For this, vesicles were incubated with [^{3}H]GMP for 20 min; at this time GDP-[^{14}C]fucose was added to the vesicle suspension and the amount of ^{3}H-labeled solutes that remained in the vesicles and the amount of ^{14}C radioactive species accumulating within the vesicles was measured at different times (up to 10 min). Fig. 2 shows that upon addition of GDP-[^{14}C]fucose to the Golgi vesicle suspension there was (*i*) a rapid decrease of ^{3}H-labeled solutes from the vesicles and (*ii*) a rapid increase of ^{14}C radioactive species within vesicles. Incubation of GDP-[^{14}C]fucose for 0.5 min resulted in exit of 2.6 pmol of [^{3}H]GMP and entry of 2.9 pmol of ^{14}C-containing radioactive species. This result suggests, similarly to those shown in Fig. 1 and Table 2, an equimolar exchange between GMP and GDP-fucose. Examination of Fig. 2 for the stoichiometry of exchange at times longer than 0.5 min suggests that more sugar nucleotides enter vesicles than those that exit (Table 2). The reason for this discrepancy is more apparent than real, because at these longer incubation times with GDP-[^{14}C]fucose, the initial assumption of the specific radioactivities of species within vesicles being equal to the specific radioactivity of the nucleotide derivatives of the incubation medium is no longer valid because nonradioactive GMP, derived from entry of GDP-[^{14}C]fucose, causes a decrease in the specific radioactivity of the [^{3}H]GMP pool within the Golgi vesicles.

Fig. 2 also shows that exit of GMP from Golgi vesicles was specific. Upon addition to the vesicles of CMP-AcNeu, no exit of radiolabeled GMP was detected, even though CMP-AcNeu is known to enter vesicles rather efficiently (1). Analyses by HPLC of the solutes within vesicles prelabeled with [^{3}H]GMP showed no exit of [^{3}H]guanosine after exchange with GDP-[^{14}C]fucose (not shown).

It was also of interest to determine whether addition of GTP or GDP to vesicles first incubated with [^{3}H]GMP resulted in exit of this latter nucleotide from the vesicles. Table 3 shows that both nucleotides stimulate exit of [^{3}H]GMP, although the effect was less than for the corresponding concentration of GMP. This suggests that the nucleoside tri- and diphosphates were first converted to the monophosphates (presumably by Golgi surface phosphatases) prior to entry of the monophosphate into the vesicles; however, the possibility that the translocator selectivity may not be absolute cannot be ruled out.

Table 2. Stoichiometry of entry and exit of guanidine nucleosides in Golgi vesicles

Preincubation		Incubation				
Substrate	Time, min	Substrate	Time, min	Exit of ^{3}H solutes, pmol	Entry of ^{14}C solutes, pmol	Entry/exit
[^{3}H]GDP-fucose	1	GDP-[^{14}C]fucose (2 μM)	1	4.8	19.0	4.0
	5		1	16.0	19.1	1.2
	10	GDP-[^{14}C]fucose (0.45 μM)	1	8.2	9.5	1.2
	10		2	10.8	13.3	1.2
[^{3}H]GMP	20	GDP-[^{14}C]fucose (2 μM)	0.5	2.6	2.9	1.1
	20		1	10.7	25.4	2.4
	20		10	14.9	47.1	3.2

Golgi vesicles were first incubated with [^{3}H]GDP-fucose or [^{3}H]GMP as described in the experiments shown in Figs. 1 and 2. At different times, GDP-[^{14}C]fucose was then added to the vesicle suspension for 0.5–10 min. Determination of amount of solutes entering and leaving the vesicles was done as described in the legend of Fig. 1.

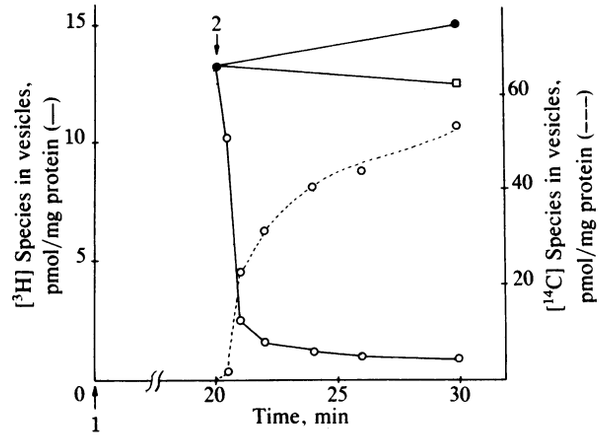

FIG. 2. Translocation of [^{3}H]GMP into Golgi vesicles and subsequent exchange of radioactive solutes from within the vesicles. Ultracentrifuge tubes, each containing Golgi vesicles as described in the legend of Fig. 1, were incubated for 20 min with [*guanidine*-8-^{3}H]GMP (0.3 μCi; final concentration, 0.3 μM; arrow 1, ●). At that time (arrow 2) GDP-[^{14}C]fucose (5 μl; 0.13 μCi; final concentration, 2 μM) was added to a group of tubes and the incubations were continued for 0.5, 1, 2, 4, 6, and 10 min (○). To another group of tubes that had been incubated for 20 min (arrow 2) with [*guanidine*-8-^{3}H]-GMP, CMP-AcNeu (5 μl; final concentration, 10 μM) was added and the mixtures were incubated for 10 min (□). Samples were then processed as described in the legend of Fig. 1 and *Materials and Methods*.

Preliminary Evidence for Other Coupled Sugar Nucleotide/Nucleoside Monophosphate and Nucleotide Sulfate/Nucleoside Monophosphate Exchange Reactions in the Golgi Apparatus Membrane. The above results strongly suggest that nucleotide sugars enter Golgi vesicles via a coupled equimolar exchange with nucleoside monophosphates. Previous studies from our and other laboratories have shown that Golgi vesicles can also translocate CMP-AcNeu, PAPS, UDP-GlcNAc, and UDP-Gal. We therefore hypothesized that these four nucleotide derivatives enter Golgi vesicles via a coupled exchange with the corresponding nucleoside monophosphate. To obtain preliminary evidence for such a mechanism, Golgi vesicles were first incubated for 20 min with [^{14}C]CMP, [^{3}H]UDP-GlcNAc, or [^{3}H]PAPS. Table 3 shows that exit of the nucleoside monophosphates from the vesicles was specifically stimulated by the corresponding nucleotide sugar and nucleotide sulfate. Quantitation of solutes entering and leaving the vesicles showed these to be occurring in ratios of close to 1. We know that part of the deviation from 1 is the result of assumptions on specific activity of solutes as previously discussed in detail for GDP-fucose/GMP exchange. Exact quantification of the intralumenal pools of nucleotide derivatives cannot be made; it is also possible that the equilibration time for uridine and cytidine pools is somewhat different from the guanidine pools. These results therefore suggest, in a preliminary manner, that the mechanism of exchange described in detail for GDP-fucose does also occur for other nucleotide sugars and PAPS.

Absence of Effect of Other Potential Perturbants on Translocation of Sugar Nucleotides and PAPS into Golgi Vesicles. The following compounds, when added to the incubation medium, had no effect on translocation *in vitro* of CMP-AcNeu into Golgi vesicles: ATP (200 μM), valinomycin (20 μg/ml), insulin (1 unit/ml), carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone (1–10 μM), cytochalasin B (2 μg/ml), nigericin (1–10 μg/ml), and monensin (1–20 μM). These same compounds as well as phospho*enol*pyruvate (100 μM) and oligomycin (10 μg/ml) had no effect on the translocation *in vitro* of PAPS. Together, these results therefore support our hypothesis that translocation of sugar nucleotides and PAPS into Golgi vesicles occurs via an antiport mechanism with the corresponding nucleoside monophosphates.

DISCUSSION

Evidence *in vitro* has been obtained showing that entry of GDP-fucose into the lumen of Golgi vesicles appears to be coupled with equimolar exit of GMP from the vesicles' lumen (Fig. 1). This phenomenon appears to be temperature dependent (Table 1), inhibited by DIDS, an inhibitor of GDP-fucose translocation, and specific for the type of sugar nucleotide. Thus, GDP-mannose, which does not enter Golgi vesicles, cannot stimulate exit of GMP (Fig. 1).

The Golgi vesicles used in this study have the same topographical orientation as *in vivo* (12). This, together with the fact that GDP-fucose appears to be synthesized in the cytosol (13) and previous evidence suggesting translocation of intact GDP-fucose into such vesicles (1), leads us to hypothesize that this translocation assay *in vitro* is of significance *in vivo* (1). We now postulate that translocation of GDP-fucose *in vivo* occurs via an antiport system such as shown in Fig. 3.

Table 3. Effect of nucleotide derivatives on exit of nucleoside monophosphates from Golgi vesicles preincubated with nucleoside monophosphates or sugar nucleotides

Preincubation (20 min)	Incubation					
			Exit			
Substrate	Substrate	Time, min	% radioactive solutes remaining in vesicles	pmol	Entry pmol	Entry/exit
[^{3}H]GMP (0.4 μM)	GTP (1 μM)	1	60			
	GDP (1 μM)		46			
	GMP (1 μM)		22			
	GDP-[^{14}C]fucose (1 μM)		29	19.3	22.9	1.2
	GDP-mannose (1 μM)		93			
[^{14}C]CMP (0.48 μM)	CMP-[^{3}H]AcNeu (1 μM)		43	70.7	124.8	1.8
	CMP (1 μM)		33			
	UDP-GlcNAc (1 μM)	5	85			
[^{3}H]PAPS (0.5 μM)	[^{35}S]PAPS (1 μM)	1	59	25.3	29.2	1.2
	GDP-fucose (1 μM)	5	96			
[^{3}H]UDP-GlcNAc (0.39 μM)	UDP-[^{14}C]GlcNAc (2.1 μM)	1	46	121.5	248.7	2.0
	[^{14}C]UMP (2 μM)	1	50	109.3	262.4	2.4

Golgi vesicles were first incubated for 20 min with [^{3}H]GMP, [^{14}C]CMP, [^{3}H]PAPS, and [^{3}H]UDP-GlcNAc. At that time, different radioactive and nonradioactive nucleotide derivatives were added to the vesicle suspension for 1–5 min. Determination of radioactive solutes entering and leaving the vesicle was done as described in the legend of Fig. 1.

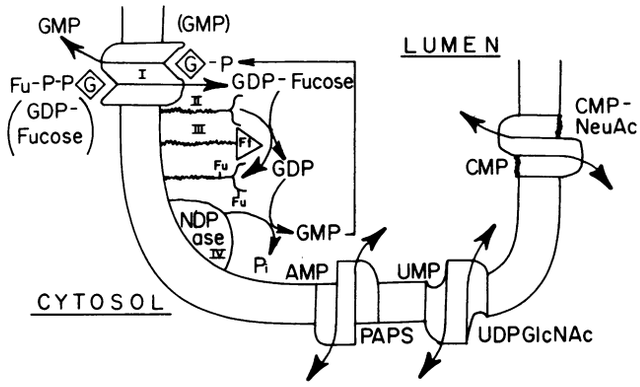

FIG. 3. Proposed mechanism of translocation of sugar nucleotides and PAPS across Golgi vesicle membranes. GDP-fucose binds through the guanidine to a specific antiport protein with a domain on the cytosolic side of the Golgi membrane (I). The sugar nucleotide is then translocated intact across the Golgi membrane into the lumen. Inside the Golgi lumen, GDP-fucose is a substrate, together with endogenous glycoproteins and glycolipids (II) for fucosylation reactions catalyzed by fucosyltransferases (III). GDP can then react with NDPase (IV) to yield GMP, which then binds the antiport protein through its lumenal domain. The nucleoside monophosphate can then exchange with cytosolic GDP-fucose in an equimolar stoichiometry. Similar specific antiport proteins are postulated to occur for PAPS, CMP-AcNeu, and UDP-GlcNAc (UDP-Gal).

Several lines of evidence support the scheme shown in Fig. 3: GDP-fucose is synthesized in the cytosol (13) and is translocated intact across Golgi vesicles via a carrier protein (1) now postulated to be an antiport protein. The sugar nucleotide appears to bind to the antiport protein through the nucleotide moiety (6). Once inside the Golgi lumen, the sugar nucleotide serves as substrate, together with endogenous acceptors (glycoproteins and glycolipids), for fucosylation reactions catalyzed by fucosyltransferases. These enzymes are known to occur in the Golgi (14, 15) and evidence consistent with their lumenal orientation as well as that of fucosylated products has also been obtained (1).

GDP is further degraded to GMP by nucleoside diphosphatase. This enzyme, which appears to be the same as thiamine pyrophosphatase (16), has been shown biochemically and cytochemically to have the active site toward the lumen of the Golgi (17–21). GMP has been detected in the lumen of Golgi vesicles (1, 22) and would then exit the vesicles via a coupled equimolar exchange with additional GDP-fucose.

Preliminary evidence has also been obtained suggesting that other sugar nucleotides and PAPS enter Golgi vesicles via specific antiports. It was shown that exit of radiolabeled nucleoside monophosphates (which had been allowed to enter vesicles during an initial incubation) could only occur if the corresponding nucleotide sugar entered the vesicles (Table 3). This coupled exchange also appeared to be equimolar, although more definitive studies on this have to be made. Coupled specific equimolar exchange was also observed with vesicles preloaded with UDP-GlcNAc or PAPS radiolabeled in the nucleotide. Previous studies from our and other laboratories (4, 8) strongly suggest that the radioactive species leaving the vesicles were UMP and 3′-AMP, respectively.

Kuhn and White (7) and Brandan and Fleischer (8) had previously shown that UMP (derived from UDP-galactose) was exiting the lumen of Golgi vesicles from mammary gland and rat liver. This was postulated as a mechanism for decreasing lumenal accumulation of UMP. Our results are in agreement with these observations and further suggest that both entry of sugar nucleotides and exit of nucleotide monophosphates are coupled in an equimolar stoichiometry. Isolation and characterization of these different antiport proteins should lead to a better understanding of their mechanism of action, including a possible role in regulation of glycosylation and sulfation reactions in the Golgi apparatus.

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