Inhibition of UDP-*N*-Acetylglucosamine Import into Golgi Membranes by Nucleoside Monophosphates†

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The specificity of the UDP-*N*-acetylglucosamine (UDP-GlcNAc) translocator for the binding of nucleoside monophosphates (NMPs) and nucleotide-sugars was examined in order to develop a quantitative understanding of how this enzyme recognizes its substrates and to provide a framework for development of novel drugs that target glycosylation. Competition studies reveal that tight binding requires a complete ribose ring and a 5′-phosphate. The enzyme is extremely tolerant to changes at the 3′-position, and the presence of 3′-F actually increases binding of the NMP to the enzyme. At the 2′-position, substitutions in the ribo configuration are well tolerated, although these same substitutions greatly diminish binding when present in the ara configuration. For the base, size appears to be the key feature for discrimination. The enzyme tolerates changing the C-4 oxygen of uridine to an amino group as well as substituting groups containing one or two carbons at C-5. However, substitution of groups containing three carbons at C-5, or exchange of the pyrimidine for a purine, greatly weakens binding to the translocator. Comparison of various UDP-sugars reveals that the UDP-GlcNAc translocator has lower affinity for UDP-*N*-acetylgalactosamine and UDP-glucose than for its cognate substrate and therefore indicates that this translocator requires both proper stereochemistry at C-4 and an aminoacetyl group at C-2. The impact of these observations on the design of more powerful nucleoside-based inhibitors of nucleotide-sugar import is discussed.

Introduction

Many different types of protein and lipid glycosylation reactions occur in the lumen of the Golgi complex and endoplasmic reticulum (ER).[1] Almost all of these reactions involve import of a nucleotide-sugar precursor from the cytoplasm, transfer of the sugar to the appropriate acceptor molecule, and export of the nucleotide byproduct. In the Golgi complex, this import of nucleotide-sugars and export of nucleotides (as NMPs) is accomplished by a series of translocators that are specific for individual nucleotide-sugars.[2,3] As illustrated in Figure 1, these transport reactions do not directly require energy but are thought instead to be driven by the NMP gradient created through the rapid rephosphorylation of NMPs by cytoplasmic kinases.[4] Conversely, when present at elevated concentrations on the cytosolic side of Golgi membranes, NMPs can compete with the corresponding nucleotide-sugars and potently inhibit their import into the Golgi complex.[5]

The ability of NMPs to inhibit nucleotide-sugar import suggests that it may be possible to develop novel nucleoside analogs that accumulate intracellularly as monophosphates and act as specific inhibitors of glycosylation. Whereas classical glycosylation inhibitors (tunicamycin, deoxynojirimycin, etc.) can be expected to affect most cells indiscriminately, nucleoside-based glycosylation inhibitors can be directed to specific cell types. For example, thymidine analogs can be targeted to rapidly growing cells since these cells contain particularly high levels of thymidine kinase.[6] Likewise, the unique specificity of Herpes thymidine kinase as compared to human nucleoside kinases should allow development of nucleosides that specifically inhibit glycosylation reactions in Herpes-infected cells.

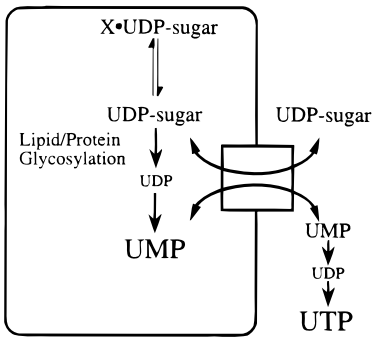

Figure 1. Diagram illustrating key reactions involved in Golgi-specific glycosylation reactions. The export of UMP produced in the Golgi lumen following sugar transfer drives import of nucleotide-sugars through a gradient resulting from rephosphorylation of UMP in the cytoplasm. X represents a putative lumenal binding protein responsible for accumulation of UDP-GlcNAc. The relative sizes of UMP, UDP, and UTP reflect their relative abundance.[4]

Recent work demonstrates the feasibility of this new chemotherapeutic approach. 3′-Azido-3′-deoxythymidine (AZT), the primary agent for treating HIV infection, accumulates in various human tissue culture cell lines almost entirely as the monophosphate, AZTMP, at concentrations often in excess of 1 mM.[7,8] As predicted, treatment of cells with AZT results in severe inhibition of lipid and protein glycosylation, an effect that may be the cause of some of the side effects associated with AZT therapy.[9] Furthermore, parallel *in vitro* studies with

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Table 1. Inhibition of UDP-GlcNAc Import by UMP Analogs

inhibitor abbreviation[a]	substituent at position 2′	3′	5	IC_{50} (μM) for inhibition of rate[b]	accumulation
ddTMP	H	H	CH_3	9 ± 2	nd
TMP	H	OH	CH_3	10 ± 2	11 ± 2
AZTMP	H	N_3	CH_3	7 ± 1	5 ± 1
3′-fluoro-ddTMP	H	F	CH_3	1.5 ± 0.3	nd
2′-dUMP	H	OH	H	8 ± 2	nd
UMP	OH	OH	H	8 ± 1	4 ± 1
2′-F(ara)-TMP	F(ara)	OH	CH_3	>200	nd
2′-F(ribo)-UMP	F(ribo)	OH	H	11 ± 2	nd
CMP	OH	OH	H	10 ± 2	nd
ara-CMP	OH(ara)	OH	H	>100	nd
acyclo-TMP	−	−	CH_3	>100	>100
L-ddTMP	H	H	CH_3	>200	nd
AZT phosphonate	H	N_3	CH_3	85 ± 15	>100
5-F-1′,3′-oxathiolate-2′-dUMP	H	−	F	18 ± 3	35 ± 5
5-ethyl-UMP	OH	OH	CH_2CH_3	3.5 ± 1	5 ± 1
BVdUMP	H	OH	CH=CHBr	5[c]	nd
5-propynyl-dUMP	H	OH	C≡CCH_3	>40	>40
5-propyl-dUMP	H	OH	$CH_2CH_2CH_3$	100[c]	nd
5-chloro-3′-F-ddUMP	H	F	Cl	9 ± 2	4 ± 1

[a] Unless otherwise noted, the sugars all had D configuration. The substituents at the 2′-, 3′-, and 5-positions are shown. The symbol (−) indicates the absence of any substituent. Abbreviations of nucleotides are listed in ref 1. [b] IC_{50}s for inhibition of the rate of import and extent of accumulation were determined as described under Experimental Procedures in assays containing 1 μM UDP-[^{3}H]GlcNAc. nd indicates not determined. [c] Data are from refs 10 and 11. The original assays contained 2 μM UDP-GlcNAc, whereas our assays with the other NMPs contained 1 μM UDP-GlcNAc. The values shown here are one-half of the IC_{50} measured with 2 μM UDP-GlcNAc.

Golgi-enriched membrane fractions verified that AZTMP potently inhibits nucleotide-sugar import and consequently blocks glycosylation reactions.[9] Likewise, 5-(bromovinyl)-2′-deoxyuridine (BVdU) and 5-propyl-2′-deoxyuridine, two nucleosides that have anti-Herpes activity, accumulate within Herpes-infected cells primarily as monophosphates and also inhibit protein glycosylation.[10,11]

A first step toward the rational design of more potent and specific inhibitors of nucleotide-sugar transport is to determine what features of the nucleotide and sugar residues are important for interaction with the translocator. Toward this goal, we have examined a series of pyrimidine nucleotides and UDP-sugars for their ability to inhibit the UDP-*N*-acetylglucosamine (UDP-GlcNAc) translocator in Golgi membranes. These studies reveal that whereas the translocator has an absolute requirement for an intact D-ribose ring and 5′-phosphate, it is quite tolerant to changes at the 2′- and 3′-positions of the ribose and base substituents of less than three carbons at C-5.

Results and Discussion

Import and accumulation of UDP-GlcNAc was used as a model system to investigate how nucleotide-sugar translocators interact with NMPs and nucleotide-sugars. As shown in Figure 2, Golgi membranes rapidly import UDP-GlcNAc, even in the absence of cytosolic proteins and an ATP regeneration system. Although the assays lack an obvious energy source, UDP-GlcNAc becomes concentrated within the Golgi membranes; at equilibrium, the concentration of UDP-GlcNAc inside of the membrane was 10−15-fold higher than the concentration outside of the membranes.[12] Such accumulation has been reported in similar studies from other laboratories[13,14] and appears to involve a saturable binding site on a lumenal protein.[15]

Effect of Sugar Substitutions on Inhibition of UDP-GlcNAc Import by NMPs. A series of nucleoside monophosphates were examined for their ability to reduce the rate of UDP-GlcNAc import into Golgi membranes and, in some cases, the extent of UDP-GlcNAc accumulation (Table 1). Rates were measured at early times (90 s), conditions when the amount of UDP-GlcNAc transported into the lumen of the Golgi membranes increased linearly with time (Figure 2). Only pyrimidine analogs were examined since previous studies have established that purine monophosphates do not inhibit the import of pyrimidine nucleotide-sugars.[5]

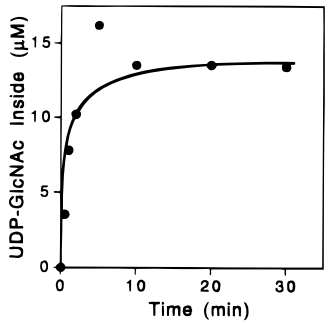

Figure 2. Import and accumulation of UDP-[^{3}H]GlcNAc in Golgi membranes. Golgi membranes were incubated with 1 μM UDP-[^{3}H]GlcNAc, and the amount of membrane-associated UDP-[^{3}H]GlcNAc was determined at various times using the filtration method, as described under Experimental Procedures. The lumenal concentration was calculated for each time point assuming an internal volume of 3 μL/mg of protein for the membranes.[14] The lumenal concentration of UDP-GlcNAc at equilibrium was 10−15-fold higher than that present outside the membranes.

The UDP-GlcNAc translocator is remarkably tolerant to changes at the 3′-position but appears very sensitive to the presence of an electronegative fluorine atom. Substitution of H, OH, and N_3 at the 3′-position resulted in compounds that bound equally well to the translocator, whereas the fluoro derivative bound approximately 6-fold tighter. Since F and H are of similar size, the tighter binding due to a 3′-F most likely arose from electronic effects of the F atom. The effects of the 3′-F cannot be easily explained by changes in sugar pucker (primarily C-2′-endo[16,17]) since, as shown below, an F at C-2′ in the ribo configuration has no effect on binding

(compare 2′-F(ribo)-UMP with either UMP or dUMP) and these two substitutions should have opposite effects on sugar pucker. This result does indicate, however, that it may be possible to design nucleoside analogs with increased potency toward glycosylation reactions.

At the 2′-position, replacing the OH of uridine with either an H or an F in the ribo configuration had only a minor effect on potency. In contrast, the presence of either an F or an OH in the ara configuration dramatically weakened binding of the NMP to the translocator, as revealed by comparison of TMP with 2′-F(ara)-TMP and of CMP with ara-CMP. NMR studies have shown that ara-C and cytidine have similar sugar pucker in solution (approximately 50% 2′-endo and 50% 3′-endo[18]). In combination with the results described above on 2′-(ribo)- and 3′-fluoro-NMPs, these data strongly suggest that changes in sugar pucker of the free NMP do not significantly affect binding to the translocator. The differential effects of substituents in the ribo and arabino configurations cannot be due to effects on the pK_a of the 3′-OH since the translocator does not require a 3′-OH group for tight binding (compare TMP with ddTMP). Rather, the lack of inhibition by arabino compounds suggests that the UDP-GlcNAc translocator makes a critical contact with the 2′2-H of the sugar. These results further demonstrate that modification of the 2′-position can generate nucleosides that should not inhibit glycosylation if present intracellularly as monophosphates.

Two different lines of evidence confirm the importance of the cyclic sugar moiety. First, addition of up to 100 μM acyclo-TMP resulted in no measurable inhibition of UDP-GlcNAc import, demonstrating that an intact cyclic sugar ring is essential for binding to the UDP-GlcNAc translocator. In addition, the translocator appears to distinguish between the D and L configurations of the sugar: Whereas D-ddTMP was a good inhibitor of import, L-ddTMP gave no detectable inhibition.

Effect of Phosphate and Base Substitutions on Inhibition of UDP-GlcNAc Import by NMPs. The UDP-GlcNAc translocator is very sensitive to modifications in the 5′-phosphate group of an NMP. Previous studies have demonstrated that nucleotide-sugar translocators do not bind nucleosides, indicating that a phosphate is required at the 5′-position.[5] Interestingly, we find that converting the 5′-phosphate into a 5′-phosphonate decreases potency by approximately 10-fold (AZT phosphonate, Table 1). Since these species have a similar charge at neutral pH, these data indicate that interaction with the translocator involves more than electrostatic interactions. Converting the monophosphate into a diphosphate also greatly weakens binding to the translocator (IC_{50} for UDP = 100 μM, Table 2).[19]

The weak binding of UDP most likely results from the additional negative charge present on UDP (−3) relative to either UMP (−2) or UDP-sugars (−2), since all of the atoms of UDP are present in UDP-GlcNAc, one of the substrates for this enzyme. Consistent with this critical role of charge, Waldman and Rudnick demonstrated that the dianionic form of UMP was a much better substrate for the UDP-GlcNAc translocator than the monoanionic form.[14] Interestingly, unlike many enzymes involved in nucleotide metabolism, the UDP-GlcNAc translocator does not require Mg^{2+} to interact with the negatively charged phosphate groups since addition of excess EDTA has no effect on translocator activity (E. T. Hall, unpublished results; also see ref 14). Together, these data suggest that the translocator interacts directly with the negative charges of the substrate(s) but does so productively only when exactly two charges are present. As expected from the strong discrimination against UDP, the translocator binds very poorly to nucleoside 5′-triphosphates.[5,20] *In vivo*, effective discrimination against NTPs is essential for import of nucleotide-sugars by the translocators since cells normally contain high concentration of NTPs (>1 mM).[4]

Table 2. Inhibition of UDP-GlcNAc Import by Other Nucleotide-Sugars

nucleotide-sugar	IC_{50} (μM)[a]
CMP-NeuAc	≫50
UDP	100 ± 20[b]
UDP-Gal	40 ± 5
UDP-Glc	7 ± 2
UDP-GalNAc	7 ± 2
UDP-GlcNAc	2 (K_M)[c]

[a] IC_{50}s were determined as described under Experimental Procedures. The assays contained 1 μM UDP-[^{3}H]GlcNAc. [b] The IC_{50} for UDP was measured in assays containing 2 mM EDTA. The half-life of UDP was approximately 3 min in the standard assay (±1 mM AMP-PNP) but ≫30 min if EDTA was included. Addition of EDTA had no measurable effect on either the rate or the extent of uptake of UDP-GlcNAc. [c] K_M value for UDP-GlcNAc is from ref 20.

The observation that translocators greatly prefer dianionic substrates may also explain why sialic acid and *N*-glycolylneuramic acid are conjugated to a nucleoside monophosphate (CMP) rather than to the standard nucleoside diphosphate used for all other sugars. Indeed, while the CMP-sugars have only one negative charge on the bridging phosphate, a second negative charge is provided by the carboxylate on the sugar which is in close proximity to the phosphate. Thus, both CMP-sugars and UDP-sugars will be dianionic, a recognition feature that may be critical for all of the translocators.

Comparison of NMPs containing various base modifications suggests that a key feature of binding of NMPs to the UDP-GlcNAc translocator is size. CMP, UMP, and nucleotides containing groups as large as bromovinyl at the 5-position were all good inhibitors of the translocator (Table 1). However, if the group at the 5-position is increased to three carbons (e.g., 5-propyl or 5-propynyl), or if the pyrimidine base is replaced by a purine, inhibition of the translocator is greatly weakened. Replacing the 5-methyl with a more electronegative 5-Cl group slightly weakens binding to the translocator, as evidenced by the lower inhibition of UDP-GlcNAc import by 5-Cl-3′-F-ddUMP as compared to 3′-F-ddTMP. These data demonstrate the feasibility of generating a variety of NMPs containing modified bases that will retain affinity for nucleotide-sugar translocators and thus potentially inhibit lipid and protein glycosylation reactions that occur in the lumen of the Golgi complex.

Inhibition of UDP-GlcNAc Accumulation by NMPs. The concentration of UDP-GlcNAc in the lumen of Golgi membranes at equilibrium was significantly greater than the concentration of UDP-GlcNAc on the outside of the membranes (Figure 2). We therefore tested the possibility that NMPs might differentially

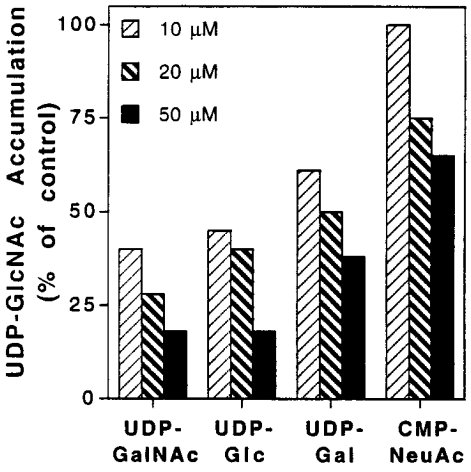

Figure 3. Inhibition of UDP-GlcNAc accumulation by nucleotide-sugars. Assays were performed as described under Experimental Procedures and contained 1 μM UDP-[^{3}H]-GlcNAc and the indicated concentrations of competing nucleotide-sugar. Results are expressed as percent of accumulation observed in absence of competitor. Similar results were obtained in three separate experiments.

inhibit transport and accumulation of UDP-GlcNAc in the lumen of the Golgi complex (Table 1). Accumulation of UDP-GlcNAc was measured after a 20−30 min incubation, a time sufficient for complete equilibration (see Figure 2). In all cases examined, the effects of NMPs on accumulation and the rate of import were very similar. Other studies suggest that accumulation of UDP-GlcNAc is mediated by binding of UDP-GlcNAc to some unidentified lumenal protein(s).[14,15] The inhibition studies described here therefore indicate that the nucleotide-binding properties of this putative 'accumulator protein' and of the translocator are very similar.

Inhibition of UDP-GlcNAc Import and Accumulation by Nucleotide-Sugars. Analysis of mutant CHO cell lines defective in glycosylation suggests the existence of specific translocators for each nucleotide-sugar.[21,22] Since many different nucleotide-sugars contain UDP as the nucleotide, this observation implies that translocators can recognize their substrates on the basis of the sugar moiety. However, little is currently known about how the sugar group modulates interactions with the translocator. Inhibition of both the import rate and accumulation of UDP-GlcNAc by other nucleotide-sugars was therefore examined to obtain further insights into the role of the sugar moiety for binding to the translocator (Table 2 and Figure 3).

Analysis of three UDP-GlcNAc analogs indicates that both the aminoacetyl moiety at C-2 and the stereochemistry at C-4 are important for binding to the UDP-GlcNAc translocator. For example, UDP-Gal, which relative to UDP-GlcNAc has opposite stereochemistry at C-4 and bears an OH at C-2 rather than an aminoacetyl group, binds very weakly to the UDP-GlcNAc translocator (Table 2). Changing either the stereochemistry at C-4 (UDP-Gal → UDP-Glc, UDP-GalNAc → UDP-GlcNAc) or the OH at C-2 to an aminoacetyl (UDP-Gal → UDP-GalNAc, UDP-Glc → UDP-GlcNAc) significantly improves binding.

CMP-*N*-acetylneuraminic acid (NeuAc) was an extremely weak inhibitor of the UDP-GlcNAc translocator (Table 2). Since CMP and UMP bind to the UDP-GlcNAc translocator equally well (Table 1), this result indicates that this translocator strongly discriminates against NeuAc. This result is not surprising, considering the significant impact of small changes in sugar structure described above. It should be noted that these studies only measure binding of the nucleotide-sugars to the translocator and provide no direct information on import of these alternate nucleotide-sugars by the UDP-GlcNAc translocator.

Nucleotide-sugars were also compared for their ability to interfere with accumulation of UDP-GlcNAc. As shown in Figure 3, the effects of these analogs on accumulation of UDP-GlcNAc are qualitatively similar to their effects on the rate of UDP-GlcNAc import. Whereas both CMP-NeuAc and UDP-Gal were poor inhibitors, UDP-GalNAc and UDP-Glc inhibited accumulation with IC_{50} values lower than 10 μM.

In agreement with the model that Golgi membranes contain distinct translocators for each nucleotide-sugar, we find that the UDP-GlcNAc translocator discriminates against UDP-sugars that lack either the proper stereochemistry at C-4 or an aminoacetyl moiety at C-2. The extent of discrimination, as measured by inhibition of UDP-GlcNAc import, was relatively modest at each position (7-fold or less[23]). A low level of discrimination by the UDP-GlcNAc translocator may be sufficient because UDP-GlcNAc is the nucleotide-sugar present at the highest concentration within cells,[24,25] thereby simplifying the discrimination problem for this translocator. In contrast, the UDP-Gal and UDP-GalNAc translocators would be expected to exhibit substantially greater discrimination against their noncognate UDP-sugars. Studies to examine this question are in progress.

While both the ER and Golgi membranes contain nucleotide-sugar translocators, our discrimination studies may only apply to those translocators that function in the Golgi complex. Inhibitor and affinity labeling studies suggest that there may be a single nucleotide-sugar translocator in ER membranes that binds and translocates several nucleotide-sugars.[26,27]

The UDP-GlcNAc translocator strongly discriminates against UDP, even though the normal substrate, UDP-GlcNAc, contains all atoms present in UDP. While consistent with the fact that hydrolysis of NDPs to NMPs in the Golgi lumen is essential for proper Golgi function,[28,29] the observation that the UDP-GlcNAc translocator evolved to discriminate so strongly against UDP remains surprising. Indeed, as illustrated in Figure 1, UDP export would provide a greater driving force for nucleotide-sugar import since the cellular concentration of UMP is substantially greater than that of UDP.[4] The lack of UDP export by the translocator may indicate that accumulation of UDP within the Golgi lumen serves an alternate, regulatory function: Under conditions of high rates of glycosylation, the UDP concentration could rise and provide an important feedback mechanism for the glycosylation and/or protein transport machinery. Alternatively, as discussed above, discrimination against UDP may simply indicate that the translocators evolved to interact only with dianionic substrates.

Conclusions

The competition studies described here establish that whereas some features of NMPs are essential for binding to the UDP-GlcNAc translocator, several posi-

tions can be modified extensively with minimal impact on inhibitory activity. Interaction of NMPs with the UDP-GlcNAc translocator requires an intact ribose ring with proper orientation (D). The 5′-phosphate also appears essential since it cannot be replaced by a 5′-phosphonate, -diphosphate, or -triphosphate. In contrast, all four substituents tested at the 3′-position and several others at C-5 were well tolerated by the enzyme. The 3′-azido group is of particular interest, since in at least three cases, AZT, 3′-azido-2′,3′-dideoxyuridine, and 5-iodo-3′-azido-2′,3′-dideoxyuridine, this replacement causes intracellular accumulation of the nucleoside as the monophosphate.[4,30−32] It should be possible to exploit this property of 3′-azido derivatives to generate a large number of thymidine analogs that accumulate as monophosphates and inhibit cellular glycosylation reactions in rapidly growing cells.

Experimental Procedures

Materials. All reagents were of the highest quality commercially available and, unless noted otherwise, were as previously described.[20] Golgi-enriched membranes were prepared from rabbit liver homogenates as described.[33,34] UMP, UDP, and TMP were from Sigma, and ddTTP was from US Biochemicals. 2′-F(ribo)-2′-dUTP was generously provided by NexStar Pharmaceuticals (Boulder, CO). ddTMP, ara-CMP, and 2′-F(ribo)-2′-dUMP were synthesized by treating either ddTTP, ara-CTP, or 2′-F(ribo)-2′-dUTP with phosphodiesterase,[35] while other NMPs were synthesized as described previously.[36] Tritiated UDP-*N*-acetylglucosamine (UDP-[^{3}H]-GlcNAc) was synthesized essentially as described using D-[6-^{3}H]glucosamine hydrochloride (NEN or American Radiolabeled Chemicals) as starting material.[37] [α-^{32}P]UDP was produced from [α-^{32}P]UTP by limited treatment with phosphodiesterase followed by purification over silica gel TLC (6:3:1.2 iPrOH:NH_4OH:H_2O). [α-^{32}P]UDP was eluted from the TLC plate with H_2O and stored at −20 °C.

Methods. Rates of nucleotide-sugar import were measured under initial velocity conditions using the filtration method.[14,20] Rates were determined from the amount of UDP-[^{3}H]GlcNAc imported into the lumen of the Golgi membranes measured after 90 s at 25 °C. IC_{50} values for each inhibitor were obtained from assays containing 1 μM UDP-[^{3}H]GlcNAc and various concentrations of inhibitor. IC_{50} values, and standard deviation, were calculated using the program Enzfitter (Biosoft, Ferguson, MO). The stability of UDP-[^{3}H]GlcNAc was determined by subjecting aliquots of a transport reaction to silica gel TLC (7:3 EtOH:1 M NH_4OAc) and quantifying the amount of ^{3}H remaining in UDP-[^{3}H]GlcNAc by scintillation counting of slices of the chromatogram. Less than 5% of the UDP-sugar was degraded under our assay conditions.

UDP-GlcNAc accumulation was measured in assays identical with those used to measure transport rates except that UDP-GlcNAc import was allowed to reach equilibrium (20−30 min) prior to measuring the amount present in the membranes.[20] Values shown are the average of at least three independent measurements. Under these conditions, less than 10% of the accumulated UDP-GlcNAc has been transferred to acid-precipitable material. Degradation of UDP-GlcNAc during these extended incubations was kept under 10% by addition of 1 mM AMP-PNP.[14]

The stability of UDP was determined by incubating 5 μM [α-^{32}P]UDP with Golgi membranes under the standard conditions used for measuring UDP-GlcNAc import. Aliquots were quenched with EDTA and subjected to silica gel TLC (6:3:1.2 iPrOH:NH_4OH:H_2O) to separate UTP, UDP, UMP, and P_i. The amount of ^{32}P in each species was quantified by Phosphorimager analysis (Molecular Dynamics).

Acknowledgment. We thank Leslie Boyd and Andy Freeman for the synthesis of L-ddTMP and 5′-AZT phosphonate, respectively.

References

(1) Abbreviations: acyclo-TMP, 1-[(2-phosphoethoxy)methyl]thymine; AZT, 3′-azido-3′-deoxythymidine; AZT phosphonate, 3′-azido-3′-deoxythymidine 5′-methylenephosphonate; BVdU, 5-(bromovinyl)-2′-deoxyuridine; EDTA, ethylenediaminetetraacetic acid, Na salt; Gal, galactose; Glc, glucose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid; ER, endoplasmic reticulum.

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