Target Sizes of Galactosyltransferase, Sialyltransferase, and Uridine Diphosphatase in Golgi Apparatus of Rat Liver†

Becca Fleischer,*,‡ J. Oliver McIntyre,‡ and Ellis S. Kempner§

Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235, and Laboratory of Physical Biology, National Institutes of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland 20892

Received August 14, 1992; Revised Manuscript Received December 3, 1992

ABSTRACT: Target inactivation analysis was used to measure the functional size of uridine diphosphogalactose: *N*-acetylglucosamine $\beta(1,4)$galactosyltransferase (galactosyltransferase), cytidine monophospho-*N*-acetylneuraminic acid:β-galactoside $\alpha(2,6)$sialyltransferase (sialyltransferase), and uridine diphosphatase (UDPase) in Golgi membranes isolated from rat liver. The size of nucleoside diphosphatase (NDPase), an enzyme similar to UDPase but localized in rat liver endoplasmic reticulum, was also estimated by target inactivation analysis. The related enzymes, UDPase and NDPase, have target sizes of 96 ± 4 and 77 ± 3 kDa, while galactosyltransferase and sialyltransferase have target sizes of 97 ± 10 and 130 ± 20 kDa, respectively. The target inactivation sizes of galactosyltransferase and of sialyltransferase are about twice the monomer molecular weights of these enzymes obtained from sedimentation studies of the solubilized membranes as well as those predicted from previously reported cDNA sequences. We conclude from our studies that galactosyltransferase and sialyltransferase probably function as dimers in the Golgi membrane.

Terminal glycosylation of secreted proteins is a major function of the Golgi apparatus of liver. Uridine diphosphogalactose:*N*-acetylglucosamine $\beta(1,4)$galactosyltransferase (galactosyltransferase)[1] and cytidine monophospho-*N*-acetylneuraminic acid:β-galactoside $\alpha(2,6)$sialyltransferase (sialyltransferase) are membrane-bound, luminally-oriented enzymes characteristic of this organelle (Fleischer & Smigel, 1978; Fleischer, 1981). They act sequentially to add the penultimate galactose and terminal sialic acid groups of *N*-asparagine-linked glycoproteins (Kornfeld & Kornfeld, 1985). In hepatocytes, their localization within the Golgi reflects their function in a late stage of maturation of this type of secretory product. The galactosyltransferase is in the trans compartment of the Golgi (Roth & Berger, 1982), while the sialyltransferase is both in the trans cisternae and in the later trans Golgi network (Roth et al., 1985). The enzymes have been purified and cloned and their topology deduced from their primary sequence [reviewed in Paulson and Colley (1989)]. To date, however, there is no information as to the functional size of these enzymes in the Golgi membrane.

Uridine diphosphatase, like the transferases, is membrane-bound and luminally-oriented (Brandan & Fleischer, 1982). It is colocalized with galactosyltransferase in the trans cisternae of the Golgi (Roth et al., 1985), where it functions to convert UDP, the highly inhibitory product of the galactosyltransferase, to the less inhibitory UMP (Brandan & Fleischer, 1982). The enzyme has not been isolated as yet, so that little is known about its molecular weight or its relationship to a similar but distinct diphosphatase which has been isolated from rat liver microsomes (Ohkubo et al., 1980).

Radiation inactivation can be used to estimate the molecular size associated with a function (Kempner, 1988). It is particularly useful when applied to membranes since purified enzymes are not required, a number of enzymes can be studied simultaneously, and the size of the functional unit can be determined in situ because phospholipids do not contribute to the target size (McIntyre et al., 1983). The method has been successful in obtaining sizes of many membrane-bound proteins (Kempner & Fleischer, 1989). In this study, we have used target inactivation analysis to estimate the functional size of both galactosyl- and sialyltransferase in the Golgi membrane. We have also investigated the size of UDPase and compared it to the related nucleoside diphosphatase present in endoplasmic reticulum.

MATERIALS AND METHODS

Materials. Male Harlen rats, 300–350 g, fed ad libitum were used. They were killed by decapitation and exsanguinated, and the livers were removed and placed in cold 0.25 M sucrose before fractionation was carried out. Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* was obtained from Sigma. Bovine serum albumin was from Armour Pharmaceutical Co., and human serum transferrin was from Sigma Chemical Co. Ultrapure sucrose was used throughout and was obtained from Schwartz/Mann Bioteck. D_2O (99.9%) was from Aldrich Chemical Co., Inc. All other chemicals were of reagent grade, and all solutions were prepared in deionized water. Ampules used for irradiation of samples were Kimble, no. 12012-LAB. They were washed twice in deionized water and oven-dried before use.

Preparation of Subcellular Fractions. Golgi vesicles were prepared from rat livers using D_2O–sucrose gradients as described previously (Fleischer & Smigel, 1978). Golgi fractions were finally suspended in 0.25 M sucrose at protein

† These studies were supported by Grant NSF DCB-8908128 from the National Science Foundation.

* Author to whom correspondence should be addressed.

‡ Vanderbilt University.

§ National Institutes of Health.

[1] Abbreviations: galactosyltransferase, uridine diphosphogalactose: *N*-acetylglucosamine $\beta(1,4)$galactosyltransferase; sialyltransferase, cytidine monophospho-*N*-acetylneuraminic acid:β-galactoside $\alpha(2,6)$-sialyltransferase; UDPase, uridine diphosphatase; NDPase, nucleoside diphosphatase; UDP, uridine diphosphate; UMP, uridine monophosphate; DTT, dithiothreitol; GlcNAc, *N*-acetylglucosamine; UDPGal, uridine diphosphogalactose; CMPNeuAc, cytidine monophospho-*N*-acetylneuraminic acid; Gal, galactose; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; RER, rough endoplasmic reticulum; ER, endoplasmic reticulum; SDS, sodium dodecyl sulfate.

0006-2960/93/0432-2076$04.00/0

concentrations of 9–13 mg/mL and frozen in liquid nitrogen. They were stored in a liquid nitrogen freezer for 1–14 days before they were thawed and diluted as described below for irradiation. Rough endoplasmic reticulum fractions were prepared from rat livers as described by Fleischer and Kervina (1974) and were suspended in 0.25 M sucrose at 10–15 mg/mL protein, frozen, and stored as described above before use.

Irradiation of Samples. For irradiation, samples were diluted to 2 mg of protein/mL in 0.25 M sucrose containing 10 mM imidazole hydrochloride, pH 6.5, 2 mM DTT, and 16.7 μg/mL glucose-6-phosphate dehydrogenase added as an internal standard (McIntyre & Churchill, 1985). Before use, the dilution buffer was degassed and bubbled with argon. Aliquots (usually 0.4 mL) of the dilute samples were transferred into 2-mL glass ampules, gassed briefly with argon, frozen using liquid nitrogen, and flame-sealed with the contents still frozen. The samples were stored at −70 °C and shipped in dry ice, irradiated at −135 °C with 10 MeV electrons from a linear accelerator (Harmon et al., 1985), shipped back in dry ice, and stored at −70 °C until thawed for assays. The radiation dose was measured using thermoluminescent detectors as described previously (Harmon et al., 1985). In all cases, an unirradiated control sample was kept at −70 °C until assayed, and two additional control samples were shipped with the irradiated samples. The enzymic activities of shipped, unirradiated control samples did not differ significantly from control samples that were not shipped.

Assays. Protein was measured by the procedure of Lowry et al. (1951) using crystalline bovine serum albumin as a standard. Galactosyltransferase (Fleischer & Smigel, 1978), sialyltransferase (Fleischer & Smigel, 1978), UDPase (Brandan & Fleischer, 1982), and glucose-6-phosphate dehydrogenase (McIntyre & Churchill, 1985) assays have been described previously. Free GlcNAc was used as the acceptor for the galactosyltransferase assays while human asialotransferrin, prepared as described (Fleischer & Smigel, 1978), was used as the acceptor for the sialyltransferase assays. Nucleoside diphosphatase activities in the rough endoplasmic reticulum fractions were determined as described (Brandan & Fleischer, 1982) using inosine diphosphate as substrate. Each sample was assayed for enzymic activity in duplicate except for sialyltransferase assays, which were carried out in triplicate. In all cases, the samples were treated with detergent before enzymatic analysis in order to remove any permeability barriers to the substrates that remain in the Golgi vesicles. The type, amount, and manner of addition of the detergent in each of the assays are described in detail in the references given above for each assay.

Golgi membranes contain a number of distinct enzymes which transfer galactose from UDPGal to different acceptors. Free GlcNAc, however, is selectively glycosylated only by UDPGal:GlcNAc β(1,4)galactosyltransferase so that the assay is predominantly due to this enzyme (Schachter & Roseman, 1980). Similarly, rat liver Golgi contains mainly CMPNeuAc:Gal α(2,6)sialyltransferase, but it also contains a low level of CMPNeuAc:Gal α(2,3)sialyltransferase activity (Weinstein et al., 1982). The latter, however, is only 3% as active as the former when asialotransferrin is used as acceptor (Weinstein et al., 1982) as in the present study. Thus, the sialyltransferase activity we measure is referable to CMPNeuAc:Gal α(2,6)-sialyltransferase. UDPase in rat liver Golgi is distinct from nucleoside diphosphatase in the endoplasmic reticulum in that it has an absolute requirement for Ca^{2+} for activity (Brandan & Fleischer, 1982). In our Golgi fractions, about 10% of the total UDPase activity is not dependent on added calcium ions and could be due to contamination from endoplasmic reticulum. This level will not affect our results since a 96-kDa component contributing 10% of the activity would raise the apparent target size of the other 77-kDa component by less than 2% (see Results).

Data Analysis. The radiation inactivation data were analyzed assuming a single-target, single-hit model (Kempner et al., 1986). Semilogarithmic plots of the fraction of enzyme activity remaining as a function of irradiation dose were fitted by least squares. Target masses were calculated using the relationship $M = (17.92 \times 10^{11})K$ (Kempner & Pestka, 1986) where K is the slope of the inactivation curve.

Studies on Solubilized Golgi. Golgi fractions were treated by alkaline hypotonic shock to remove vesicular contents and membrane-associated proteins and the membranes then solubilized using Triton X-100 as described previously (Fleischer & Smigel, 1978). Sedimentation studies were carried out in linear (10–34%) sucrose–H_2O or sucrose–D_2O gradients containing 0.15 M NaCl, 10 mM Hepes, pH 7.5, and 2 mg/mL Triton X-100 as described (Fleischer & Smigel, 1978). Other sedimentation conditions are given in the legends to the figures illustrating the results. From the sedimentation behavior of the enzyme in these gradients, the sedimentation constant ($s_{20,w}$) and the partial specific volume (v^*) of the enzyme–detergent complex were calculated as described by Smigel and Fleischer (1977). Stokes' radii were estimated by gel filtration of the solubilized Golgi membranes on Sepharose 6B columns calibrated using proteins of known Stokes's radii as described (Fleischer & Smigel, 1978). The eluting buffer in all cases was 0.15 M NaCl, 10 mM Hepes, pH 7.5, and 2 mg/mL Triton X-100.

RESULTS

Inactivation of both galactosyltransferase and sialyltransferase activities in Golgi membranes occurred as a simple exponential function of radiation dose (Figure 1). Glucose-6-phosphate dehydrogenase from *L. mesenteroides*, added to the membranes before irradiation as an internal control, yielded a simple exponential loss of activity with radiation dose (data not shown). The target sizes derived from the data are summarized in Table I. The target size for glucose-6-phosphate dehydrogenase is only slightly larger than the known molecular mass of this enzyme, 104 kDa (McIntyre & Churchill, 1985), validating the technical aspects of the experimental approach we have used.

The target size observed for galactosyltransferase is much larger than the molecular weight of the solubilized enzyme previously estimated from a combination of sedimentation and gel exclusion chromatography. In those studies, a single active component of molecular weight 46 500 was found (Fleischer & Smigel, 1978). However, similar hydrodynamic studies of sialyltransferase activity in Golgi reveal more complex behavior. Two active species were observed after sedimentation in H_2O–sucrose gradients which are well resolved after sedimentation in D_2O–sucrose gradients and have about equal activity (Figure 2). The two species are not resolved by gel exclusion chromatography (Figure 3). Estimates of the molecular weights of the two species are summarized in Table II. Peak I is less dense and contains more detergent than peak II. The sialyltransferase in this peak has a molecular weight of about 53 000 which corresponds to the monomer molecular weight calculated from the reported cDNA sequences. Peak II, on the other hand, contains much less detergent, indicating that the protein has much less hydrophobic surface exposed. The molecular weight of the

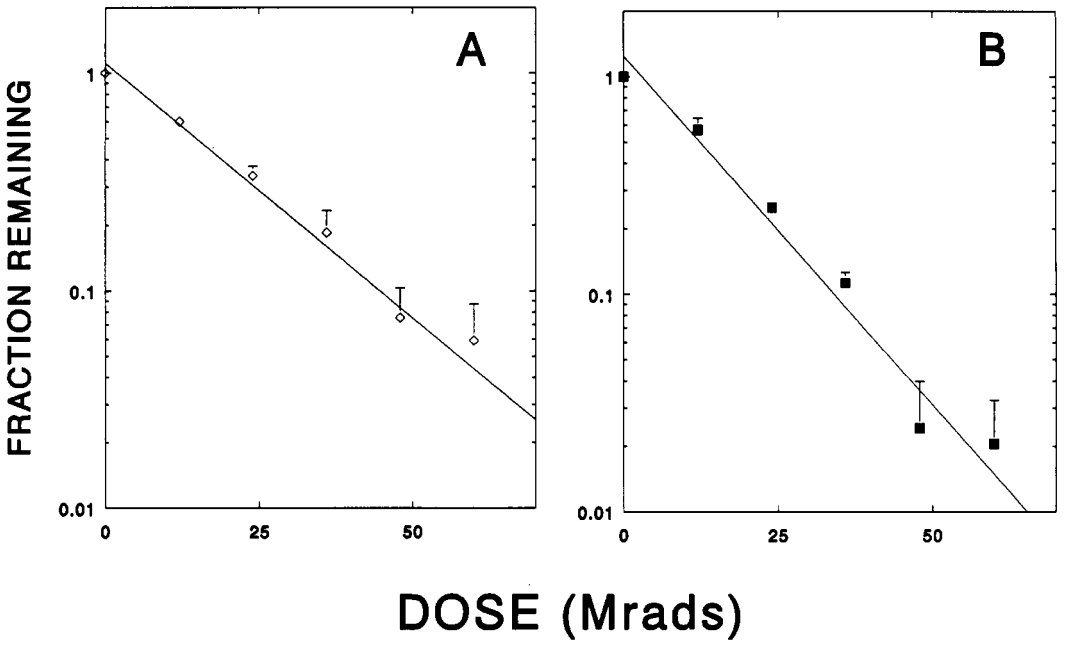

FIGURE 1: Radiation inactivation of glycosyltransferases in Golgi membranes from rat liver. Membrane samples were prepared, frozen in glass ampules, and irradiated in the frozen state as described under Materials and Methods. The fraction of activity remaining is shown as a function of radiation dose. The data points are the average of three individual experiments using different preparations of Golgi. Error bars indicate the standard deviation of the mean for each point, and are not shown for points in which the standard deviation is less than the diameter of the symbol. (A) Galactosyltransferase activity. (B) Sialyltransferase activity.

Table I: Molecular Masses (kDa) of Golgi Glycosyltransferases[a]

enzyme	radiation inactivation	DNA cloning
galactosyltransferase	97 ± 10	43[c]
sialyltransferase	130 ± 20	47[d]
glucose-6-phosphate dehydrogenase[b]	110 ± 3	

[a] Target sizes are averages ± standard error of the mean from independent determination on three different preparations. All values are for rat liver except as indicated. [b] Added as internal standard, M = 104 kDa (McIntyre & Churchill, 1985). [c] Calculated from the cDNA sequence obtained from studies on mouse mammary cells by Shaper et al. (1988). [d] Calculated from the cDNA sequence obtained from studies on rat liver by Weinstein et al. (1987).

sialyltransferase in this peak is about twice that calculated from cDNA data, and corresponds to a dimer within experimental error. We have shown previously (Fleischer & Smigel, 1978) that the bulk of the membrane phospholipids are dissociated from both glycosyltransferases after solubilization with the levels of Triton X-100 used in our hydrodynamic measurements. Our calculations, therefore, do not include possible contributions of any residual bound phospholipid in the protein–detergent complexes.

Like the transferases, uridine diphosphatase in Golgi membranes and nucleoside diphosphatase in endoplasmic reticulum also gave single-exponential inactivation curves as a function of radiation dose (Figure 4). Their calculated target sizes are summarized in Table III. Although the enzymes have similar target sizes, UDPase in Golgi is significantly larger than the nucleoside diphosphatase in RER ($p < 0.05$ by Student's two-tailed t test). Purification of the enzyme is currently in progress in our laboratory. After more than 1000-fold purification from isolated rat liver Golgi, the enzyme preparation exhibits one major band with a molecular weight of about 50 000 on polyacrylamide gel electrophoresis in SDS. Purified NDPase from ER, on the other hand, is reported to have a larger apparent subunit size ($M_r \sim 65\,000$, Table III).

DISCUSSION

In this study, we have used target inactivation analysis to estimate the functional size of galactosyltransferase, sialyltransferase, and uridine diphosphatase in Golgi membranes isolated from rat liver. We find that both transferases have target sizes about twice as large as the monomer molecular weights estimated from hydrodynamic data (Fleischer & Smigel, 1978; Table II). The latter are similar to those predicted from cloning data. The cDNA sequences obtained for galactosyltransferase from mouse mammary cells (Shaper et al., 1988) and for sialyltransferase from rat liver (Weinstein et al., 1987) predict molecular weights of 43 000 and 47 000, respectively. In addition, we find that the target size of the Golgi UDPase is about twice that of the partially purified enzyme estimated from SDS–polyacrylamide gel electrophoresis. Another related enzyme, a guanosine diphosphatase purified from Golgi membranes of *Saccharomyces cerevisiae*, has a molecular weight of 47 000 (Yanagisawa et al., 1990) as characterized by polyacrylamide gel electrophoresis after deglycosylation. This is similar to the value we observe for the nucleoside diphosphatase in rat liver Golgi. In contrast to the Golgi enzyme, the nucleoside diphosphatase in rat liver endoplasmic reticulum gives a target size which is about 20% greater than the monomer size ($M_r \sim 65\,000$) for the purified enzyme from rat liver microsomes estimated from polyacrylamide gel electrophoresis in SDS (Ohkubo et al., 1980). This enzyme is most likely a monomer in the endoplasmic reticulum membrane.

Each of the Golgi enzymes (galactosyltransferase, sialyltransferase, and UDPase) has a functional target size about twice its monomer molecular weight. The simplest explanation for these results is that, for each enzyme, the protomer is associated with another polypeptide in the membrane (either an identical peptide or a different one of about the same molecular weight). Either both of the polypeptides are required for function such that radiation damage to one results in loss of activity to both (homodimer model with a dimeric functional unit) or else a hit on either protomer results in

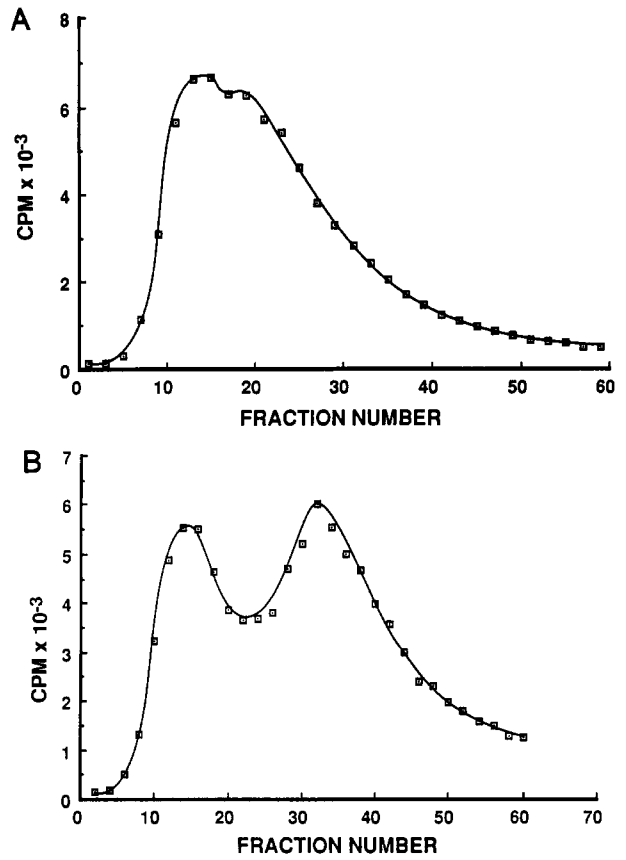

FIGURE 2: Sedimentation patterns obtained for sialyltransferase activity in sucrose–H_2O (A) or sucrose–D_2O (B) linear density gradients after solubilization of Golgi fractions in Triton X-100 as described under Materials and Methods. Gradients were centrifuged for 22 h at 45 000 rpm and 8 °C (H_2O) or for 48 h at 50 000 rpm and 8 °C (D_2O) using a Beckman SW 56 rotor. To calculate sedimentation constants, two different Golgi preparations were analyzed in singlicate (H_2) or in duplicate (D_2O) and averaged.

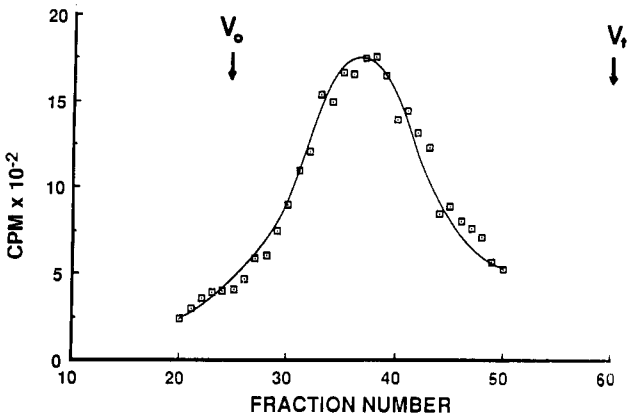

FIGURE 3: Sialyltransferase activities of fractions after gel filtration chromatography of solubilized Golgi membranes on Sepharose 6B. Membranes were solubilized in Triton X-100 and chromatographed as described under Materials and Methods. To estimate the Stokes' radius of sialyltransferase, two different preparations of Golgi membranes were each run in duplicate.

fragmentation of both (homodimer or heterodimer model with a functional monomer and intersubunit energy transfer). Such energy transfer has been documented in a number of target studies of membrane proteins (Kempner & Fleischer, 1989). These explanations do not preclude the possibility that the enzymes are components of larger structures than the functional target size reported here.

In order to avoid potential problems related to changes in substrate permeability of the membrane after irradiation,

Table II: Properties of Sialyltransferase Solubilized in Triton X-100

property	peak I	peak II
$s_{20,w}$ (S)	4.4	4.5
$\bar{\nu}^{*\,a}$ (mL/g)	0.86	0.79
R_s (Å)	54	54
$M^{*\,b}$ (g of complex/mol)	190000	132000
$X_p{}^c$ (g of protein/g of complex)	0.28	0.71
X_d (g of Triton X-100/g of complex)	0.72	0.29
$M_p{}^d$ (g of protein/mol)	53000	94000

^{a} $\bar{\nu}^* = 0.735X_p + 0.908X_d$. In deriving this expression, it is assumed that the partial specific volume of the complex ($\bar{\nu}^*$) is the sum of the partial specific volumes of the protein and of the detergent in the complex. It is further assumed that the density of the protein is 0.735 g/mL and that of Triton X-100 is 0.908 g/mL (Tanford et al., 1974). ^{b} $M^* = s_{20,w}6\pi\eta_{20,w}NR_s/(1 - \bar{\nu}\rho_{20,w})$. ^{c} $X_p + X_d = 1$. ^{d} $M_p = X_pM^*$.

activities were measured after solubilizing the membranes in detergent. Therefore, it is possible that, in detergent, two polypeptides are required for activity although they may not necessarily be associated in the membrane. This interpretation for the radiation effects is difficult to reconcile with the following observations: (1) both galactosyltransferase and sialyltransferase have been purified, cloned, and expressed, suggesting that a single type of polypeptide is sufficient for activity; (2) the catalytic activity and kinetic properties for all three Golgi enzymes in the membrane are comparable to the values obtained after solubilization, provided substrate permeability is maximized (Fleischer, 1981; Brandan & Fleischer, 1982; B. Fleischer, unpublished observations); and (3) the detergent-solubilized transferases behave hydrodynamically essentially as monomers.

In light of these observations, we believe the most likely interpretation of our target inactivation studies of these Golgi enzymes is that they exist as dimers in the membrane, either with themselves or with another polypeptide of similar molecular weight. Both glycosyltransferases show evidence of the ability to form tight associations with another polypeptide. Galactosyltransferase occurs in a soluble form in milk as a 1:1 complex with α-lactalbumin (Brodbeck et al., 1967). Complex formation between these proteins has also been demonstrated directly using purified protein components (Brew et al., 1974; Powell & Brew, 1975). α-Lactalbumin, a polypeptide of molecular weight 14 200, regulates the enzymic activity of the galactosyltransferase by changing the affinity of the enzyme for glucose (Khatra et al., 1974). This interaction also occurs when purified rat liver Golgi membranes solubilized in Triton X-100 are used as the source of galactosyltransferase (B. Fleischer, unpublished observations). α-Lactalbumin is not expressed in liver, however, so it cannot be contributing to the observed target size of the galactosyltransferase. Sialyltransferase also shows evidence of dimer formation as we find in our sedimentation studies after solubilization of Golgi membranes in Triton X-100 (Table II). Evidently, sialyltransferase is not completely dissociated under conditions which are sufficient to dissociate galactosyltransferase. We have not investigated whether complete dissociation of the sialyltransferase is possible under different conditions, such as higher detergent concentrations etc.

Radiation inactivation has been used previously to determine the molecular size of two other Golgi enzymes, *N*-acetylglucosaminyl phosphotransferase and α-*N*-acetylglucosaminyl phosphodiesterase, in lyophilized preparations (Ben-Youseph et al., 1986). Together, the enzymes are responsible for the synthesis of the mannose 6-phosphate recognition signal which targets soluble lysosomal proteins to the lysosome during their transport through the Golgi apparatus [reviewed in Kornfeld

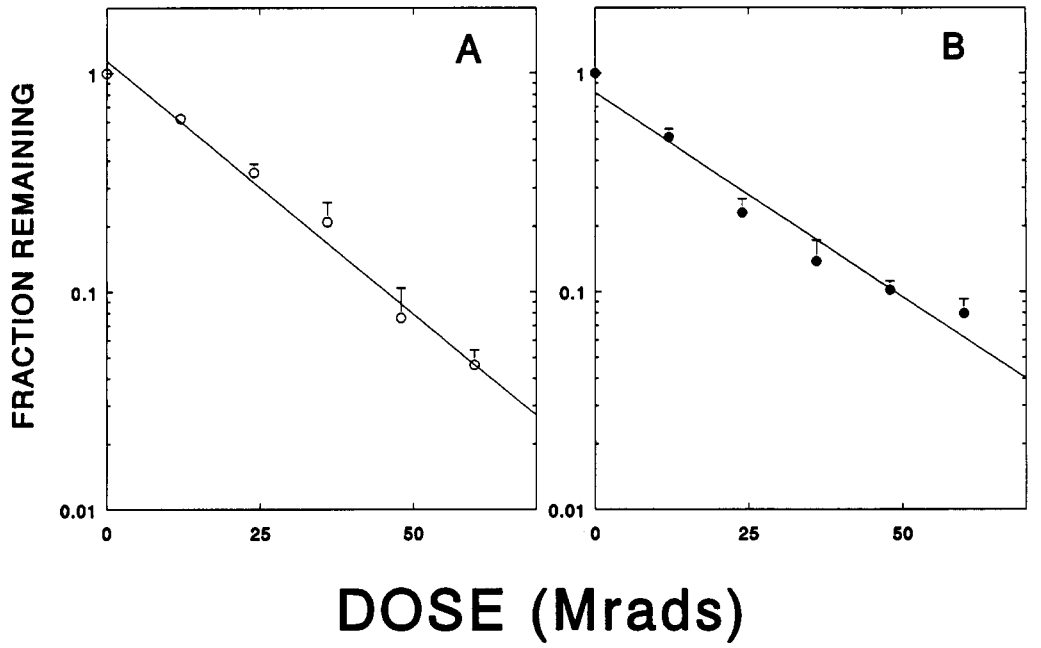

FIGURE 4: Radiation inactivation of nucleoside diphosphatase activities in membrane fractions of rat liver. Samples were prepared, irradiated, and assayed as described under Materials and Methods. Data are expressed as described in the legend to Figure 1. (A) Uridine diphosphatase activity of Golgi. (B) Inosine diphosphatase activity of rough endoplasmic reticulum.

Table III: Molecular Sizes of Nucleotide Diphosphatases of Golgi and Endoplasmic Reticulum[a]

enzyme	radiation inactivation (kDa)	SDS–PAGE ($M_r \times 10^{-3}$)
UDPase in Golgi	96 ± 4	~50[b]
NDPase in RER	77 ± 3	65[c]

[a] Target sizes are averages ± standard error of the mean from independent determinations on three different preparations. [b] J.-P. Xie and B. Fleischer, unpublished observations. [c] Data from Ohkubo et al. (1980).

and Mellman (1989)]. The enzymes are compartmentalized in the Golgi, with the transferase acting in a very early transitional compartment between the ER and the Golgi, while the phosphodiesterase acts somewhat later, in the cis Golgi. The phosphotransferase has two distinct functions, that of recognizing soluble lysosomal enzymes as well as the catalytic function of transferring *N*-acetylglucosamine 1-phosphate to terminal α(1,2)mannose units on *N*-asparagine-linked oligosaccharide chains of the lysosomal glycoproteins. The relatively large size of the phosphotransferase found by radiation inactivation (228–283 kDa depending on the source) may reflect the multimeric nature of this enzyme. The phosphodiesterase exhibited much smaller target size values of 129–156 kDa. Although lyophilized samples, especially of membrane-bound proteins, frequently yield artificially large target sizes (Kempner & Fleischer, 1989), recent studies on the purified phosphotransferase from *Acanthamoeba castellanii* have a shown the molecular mass of the enzyme to be about 250 kDa including a subunit with a molecular mass of about 93 kDa (Ketcham & Kornfeld, 1992).

The significance of the finding that all three Golgi enzymes, galactosyltransferase, sialyltransferase, and UDPase, appear to function as dimers in the membrane is not clear. If they all were heterodimers, association with another peptide in the Golgi membrane might suggest regulation of some sort. One type of regulation that could be common to all three enzymes is an association which results in retention of the enzymes in the trans compartment of the Golgi apparatus. At present, little is known of the mechanism which brings about this localization. Both glycosyltransferases have similar structural motifs consisting of a small cytoplasmic tail, a single transmembrane anchor, a stem region, and a large, luminally-oriented catalytic unit (Paulson & Colley, 1989). The signal for retention of the galactosyltransferase in the Golgi appears to reside in the transmembrane region (Nilsson et al., 1991; Wong et al., 1992). Retention of the sialyltransferase appears to depend both on the transmembrane segment and on the stem region (Munro, 1991; Colley & Paulson, 1992). Perhaps the topology of the UDPase is analogous to that of the transferases and its retention signal also resides in its transmembrane region.

ACKNOWLEDGMENT

We thank Kathryn Dewey for her technical assistance in carrying out this work.

REFERENCES

Ben-Youseph, Y., Potier, M., Pack, B. A., Mitchell, D. A., Melançon, S. B., & Nadler, H. L. (1986) *Biochem. J. 235*, 833–886.

Brandan, E., & Fleischer, B. (1982) *Biochemistry 21*, 4640–4645.

Brew, K., Shaper, J. H., Olsen, K. W., Trayer, I. P., & Hill, R. L. (1975) *J. Biol. Chem. 250*, 1434–1444.

Brodbeck, U., Denton, W. L., Tanahashi, N., & Ebner, K. E. (1967) *J. Biol. Chem. 242*, 1391–1397.

Colley, K. J., Lee, E. U., & Paulson, J. C. (1992) *J. Biol. Chem. 267*, 7784–7793.

Fleischer, B. (1981) *J. Cell Biol. 89*, 246–255.

Fleischer, B. (1983) *Methods Enzymol. 98*, 60–67.

Fleischer, S., & Kervina, M. (1974) *Methods Enzymol. 31*, 6–41.

Fleischer, B., & Smigel, M. (1978) *J. Biol. Chem. 263*, 1632–1638.

Harmon, J. T., Nielsen, T. B., & Kempner, E. S. (1985) *Methods Enzymol. 117*, 65–94.

Kempner, E. S. (1988) *Adv. Enzymol. 61*, 107–147.

Kempner, E. S., & Fleischer, S. (1989) *Methods Enzymol. 172*, 410–439.

Kempner, E. S., & Pestka, S. (1986) *Methods Enzymol. 119*, 255–260.

Kempner, E. S., Miller, J. H., & McCreery, M. J. (1986) *Anal. Biochem. 156*, 140–146.
Khatra, B. S., Herries, D. G., & Brew, K. (1974) *Eur. J. Biochem. 44*, 537–560.
Kornfeld, R., & Kornfeld, S. (1985) *Annu. Rev. Biochem. 34*, 631–664.
Kornfeld, S., & Mellman, I. (1989) *Annu. Rev. Cell Biol. 5*, 483–523.
Ketcham, C. M., & Kornfeld, S. (1992) *J. Biol. Chem. 267*, 11645–11653.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
McIntyre, J. O., & Churchill, P. (1985) *Anal. Biochem. 147*, 468–471.
McIntyre, J. O., Churchill, P., Maurer, A., Berenski, C. J., Jung, C. Y., & Fleischer, S. (1983) *J. Biol. Chem. 258*, 953–959.
Munro, S. (1991) *EMBO J. 10*, 3577–3588.
Nilsson, T., Lucocq, J. M., Mackay, D., & Warren, G. (1991) *EMBO J. 10*, 3567–3575.
Ohkubo, I., Ihbashi, T., Tanguchi, N., & Makita, A. (1980) *Eur. J. Biochem. 112*, 111–118.
Paulson, J. C., & Colley, K. J. (1989) *J. Biol. Chem. 264*, 17615–17618.
Powell, J. T., & Brew, K. (1975) *J. Biol. Chem. 250*, 6337–6343.
Roth, J., & Berger, E. G. (1982) *J. Cell Biol. 93*, 223–229.
Roth, J., Taatjes, D. J., Lucocq, J. M., Weinstein, J., & Paulson, J. C. (1985) *Cell 43*, 257–295.
Schachter, H., & Roseman, S. (1980) in *The Biochemistry of Glycoproteins and Glycolipids* (Lennarz, W. J., Ed.) pp 85–160, Plenum Press, New York.
Shaper, N., Hollis, G. F., Douglas, J. C., Kirsch, I. R., & Shaper, J. H. (1988) *J. Biol. Chem. 263*, 10420–10428.
Smigel, M., & Fleischer, S. (1977) *J. Biol. Chem. 252*, 3689–3696.
Tanford, C., Nozaki, Y., Reynolds, J. A., & Makino, S. (1974) *Biochemistry 13*, 2369–2376.
Weinstein, J., de Souza-e-Silva, U., & Paulson, J. C. (1982) *J. Biol. Chem. 257*, 13845–13853.
Weinstein, J., Lee, E. U., McEntee, K., Lai, P.-H., & Paulson, J. C. (1987) *J. Biol. Chem. 262*, 17735–17743.
Wong, S. H., Low, S. H., & Hong, W. (1992) *J. Cell Biol. 117*, 245–258.
Yanagisawa, K., Resnick, D., Abeijon, C., Robbins, P. W., & Hirschberg, C. B. (1990) *J. Biol. Chem. 265*, 19351–19355.