Identification and Purification of the Rat Liver Golgi Membrane UDP-N-acetylgalactosamine Transporter*

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Glycosylation of glycoproteins, proteoglycans, and glycosphingolipids occurs mainly in the lumen of the endoplasmic reticulum and the Golgi apparatus. Nucleotide sugars, donors of all the sugars involved in Golgi glycosylation reactions, are synthesized in the cytoplasm and require specialized transporters to be translocated into the lumen of the Golgi apparatus. By controlling the supply of sugar nucleotides in the lumen of the Golgi apparatus, these transporters directly regulate the glycosylation of macromolecules transiting the Golgi. We have identified and purified the rat liver Golgi membrane UDP-N-acetylgalactosamine transporter. The transporter was purified to apparent homogeneity by a combination of conventional and dye color chromatography. An ~63,000-fold purification (6% yield) was achieved starting from crude rat liver Golgi membranes and resulting in a protein with an apparent molecular mass of 43 kDa. The transporter was active when reconstituted into phosphatidylcholine vesicles and could be specifically photolabeled with P32-(4-azidoanilido)-uridine-5'-[P32P]triphosphate, an analog of UDP-N-acetylgalactosamine. Native functional size determination on a glycerol gradient suggested that the transporter exists as a homodimer within the Golgi membrane.

Glycoproteins, proteoglycans, and glycosphingolipids play fundamental roles in physiologic and pathologic processes, including cell growth, development, modulation of growth factors and transmembrane signals, oncogenesis, immune response, control of permeability in kidney basement membrane, and coagulation. These glycoconjugates also act as receptors for hormones, viruses, and bacterial toxins (reviewed in Refs. 1–7). The common characteristic of these complex cellular constituents is the presence of a carbohydrate chain attached to a protein or lipid core, which often confers a specific physiological or pathological role.

The glycosylation reactions involved in the assembly of the above macromolecules occur mainly in the lumen of the endoplasmic reticulum and the Golgi apparatus (reviewed in Refs. 8 and 9). Nucleotide sugars, donors of all of the sugars involved in Golgi glycosylation reactions, are synthesized in the cytoplasm and require specialized transporters to be translocated into the Golgi apparatus to participate in these biosynthetic events (reviewed in Refs. 8 and 9). These transporters are antiproters; they translocate the nucleotide sugar to the inside of the Golgi apparatus while the corresponding nucleoside monophosphate, generated in the lumen of the Golgi apparatus through the action of a glycosyltransferase and a nucleoside diphosphatase, exits (reviewed in Refs. 8 and 9).

Nucleotide sugar transporters are of physiologic importance because mutant mammalian, yeast, and Leishmania cells deficient in these transport activities have a defect in the biosynthesis of the corresponding macromolecules (9–12). This defect seems to be not only quantitative but also qualitative. A mutant MDCK cell line (10, 11) deficient by 98% in UDP-galactose transport into the Golgi lumen showed a marked reduction in galactosylation of glycoproteins, glycosphingolipids, and those proteoglycans containing galactose in their polymer, like kertan sulfate. Other proteoglycans, like chondroitin sulfate and heparan sulfate, which contain galactose solely in the linkage region between the sugar polymer and the protein backbone, were not reduced. Changes in the sulfation pattern of chondroitin sulfate, probably a secondary effect, were also observed. Simililar results were also observed in Saccharomyces cerevisiae (12) where a defect in the entry of GDP-mannose into the lumen of the Golgi apparatus resulted in a significant decrease in the mannosylation of lipids and O-linked mannospolypeptides and in the elongation of N-linked carbohydrates of vacuolar proteins. A partial defect in the biosynthesis of N-linked mannan chains of secreted proteins was also observed. Together, the above results support the hypothesis that by limiting the supply of sugar nucleotides in the lumen of the Golgi apparatus, these transporters directly regulate the synthesis of glycoconjugates. Finally, in Leishmania donovani, a defect in the transport of GDP-mannose into the Golgi apparatus resulted in an absence of virulence and in transmission of the parasite, offering an attractive target for chemotherapy (13).

Considerable progress has been made in the past few years on the molecular cloning of genes encoding some of these Golgi membrane transporters. Genes encoding CMP-sialic acid (14, 15), UDP-galactose (16, 17), and UDP-N-acetylglucosamine (18) transporters were cloned from mammalian species, and a gene encoding a GDP-mannose transporter (13, 19) was identified in L. donovani. Also, genes encoding transporters for UDP-N-acetylglucosamine (20) and UDP-galactose (21) were cloned from yeast. All of these genes were identified by complementation cloning in glycosylation mutant cells. However, no cells have been identified that display a defect in the transport

* This work was supported by National Institutes of Health Grants GM34396 (to C. B. H.) and GM55427 (to A. K. M.) and the Italian Ministry of Health, Rome, Italy (to L. P. and A. K. M.).

§ Supported by a Human Frontiers Science Program Organization long term fellowship.

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4474 This paper is available on line at http://www.jbc.org
of UDP-N-acetylglucosamine into the Golgi apparatus, suggesting that approaches other than complementation cloning must be used to identify the Golgi membrane UDP-GalNAc transporter. We chose to identify the UDP-GalNAc transporter by classical protein purification and biochemical reconstitution techniques. We previously showed that detergent extraction of Golgi membranes followed by reconstitution of the extract into phosphatidylinositol liposomes resulted in proteoliposomes that were active in specific nucleotide sugar transport (22). Here we used this reconstituted proteoliposome system to monitor the purification of the UDP-GalNAc transport activity from a rat liver Golgi membrane preparation. Column chromatography and photoaffinity labeling were used to identify a 43-kDa protein as the UDP-GalNAc transporter. Proteoliposomes containing this protein were active in UDP-GalNAc but not in other uridine nucleotide sugar transport, and native functional size determination on a glycerol gradient suggested that the transporter exists as a homodimer in the membrane.

EXPERIMENTAL PROCEDURES

Materials

Frozen rat livers were purchased from Pel-Freez Biologicals. [3H]UDP-GalNAc (5–15 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc. and Na125I (350–600 μCi/ml) from Amersham Pharmacia Biotech. Extracti-Gel G was purchased from Pierce. All other chemicals were obtained from Sigma.

Methods

Purification of the Rat Liver Golgi Membrane UDP-GalNAc Transporter

All of the operations below were performed at 4 °C.

Step 1: Detergent Extraction—A crude Golgi fraction was prepared from 6 kg of frozen rat livers according to the procedure of Leelavathi et al. (23). This fraction was resuspended in 10 mM Tris-HCl, pH 7.2, 1 mM MgCl2, 1 mM dithiothreitol, 0.3 mM sucrose, 20% glycerol (v/v), and 0.3% Triton X-100 with protease inhibitors (0.5 mM 4-(2-aminoethyl)-benzene-sulfonyl fluoride, 0.1 mM benzamide, 0.1 mM leupeptin, and 1 μg/ml aprotinin). The suspension was stirred for 45 min at 4 °C and centrifuged at 100,000 × g for 45 min. The supernatant solution was discarded, and the pellet was resuspended in the above described buffer containing a final concentration of 1.1% Triton X-100. The mixture was stirred and centrifuged again as described above. The supernatant was decanted again, and a final Triton X-100 concentration of 0.5% NaCl up to a final concentration of 200 mM was added.

Step 2: First DEAE-Sephacel Column—The above described Triton X-100 extract was applied to three DEAE-Sephacel columns (Sigma; 23 × 3 cm each) equilibrated in Buffer A containing 100 mM NaCl. The UDP-GalNAc transport activity eluted in the flow-through.

Step 3: Second DEAE-Sephacel Column—The flow-through from the above described Triton X-100 column was diluted to 100 mM NaCl with Buffer A and applied to eight DEAE-Sephacel columns (Sigma; 23 × 2.7 cm each) equilibrated in Buffer A containing 100 mM NaCl. The UDP-GalNAc transport activity eluted in the flow-through.

Step 4: Third DEAE-Sephacel Column—The above described fraction was applied to 12 DEAE-Sephacel columns (Sigma; 23 × 3 cm each) equilibrated in Buffer A containing 20% glycerol and 1.5% Triton X-100 (Buffer B). Elution was with 4 column volumes of the equilibration buffer followed by 4 column volumes of Buffer B containing 300 mM NaCl and 3 column volumes of Buffer A containing 1.5 μM Na25I. This last elution fraction was recovered in fractions of 18.5 μl each, and the UDP-GalNAc transport activity was eluted in fractions 1–30, which were pooled, desalted, and concentrated up to a final concentration of 20% glycerol and 1.5% Triton X-100.

Step 5: Carboxymethylcellulose Column—The second DEAE-Sephacel column was applied to three DEAE-Sephacel columns (Sigma; 23 × 3 cm each) equilibrated in Buffer A containing 20% glycerol and 1.5% Triton X-100 (Buffer B). Elution was with 4 column volumes of the equilibration buffer followed by 4 column volumes of Buffer B containing 300 mM NaCl and 3 column volumes of Buffer A containing 1.5 μM NaCl. This last elution fraction was recovered in fractions of 18.5 μl each, and the UDP-GalNAc transport activity was eluted in fractions 1–30, which were pooled, desalted, and concentrated up to a final concentration of 20% glycerol and 1.5% Triton X-100.

Step 6: Third DEAE-Sephacel Column—The above described fraction was applied to three Fractogel-EMD columns (EM Science; 15 × 1.5 cm each) equilibrated in Buffer A containing 20% glycerol and 1.5% Triton X-100 (Buffer B). Elution was with 4 column volumes of the equilibration buffer followed by 4 column volumes of Buffer B containing 100 mM NaCl and 3 column volumes of Buffer A containing 1.5 μM NaCl. This last elution fraction was recovered in fractions of 18.5 μl each, and the UDP-GalNAc transport activity was eluted in fractions 1–30, which were pooled, desalted, and concentrated up to a final concentration of 20% glycerol and 1.5% Triton X-100.

Step 7: Fractogel-EMD Column—The above fraction was applied to three Fractogel-EMD columns (EM Science; 15 × 1.5 cm each) equilibrated in Buffer A containing 20% glycerol and 1.5% Triton X-100 (Buffer B). Elution was with 4 column volumes of the equilibration buffer followed by 4 column volumes of Buffer B containing 300 mM NaCl and 3 column volumes of Buffer A containing 1.5 μM NaCl. This last elution fraction was recovered in fractions of 18.5 μl each, and the UDP-GalNAc transport activity was eluted in fractions 1–30, which were pooled, desalted, and concentrated up to a final concentration of 20% glycerol and 1.5% Triton X-100.

Glycerol Gradient

The apparent functional weight of the UDP-GalNAc transporter was estimated by analytical ultracentrifugation using an 8–30% glycerol gradient in Buffer A. An active fraction obtained either from purification Step 4 or Step 7 was concentrated and exchanged to Buffer A in Centricon filters (Amicon) to obtain a final glycerol concentration of 8%. The 10-ml glycerol gradient was equilibrated at 4 °C for 17 h before loading the sample (0.5 ml) and then centrifuged in a SW 50 rotor at 46,000 rpm for 40 h at 4 °C. Fractions of 0.35 ml were collected. β-Amylase (200 KDa), alcohol dehydrogenase (150 KDa), β-galactosidase (120 KDa), phosphorylase b (100 KDa), tumor necrosis factor α-convertase (80 KDa), and bovine serum albumin (66 KDa) were used as internal molecular markers.

Isolation and Topography of Rat Liver Golgi Vesicles

For the photoaffinity radiolabeling studies, rat liver Golgi vesicles were isolated as described (23) and resuspended in cryoprotective buffer (24). Sialyltransferase activity was enriched ~50-fold over crude homogenate. Approximately 90% of the vesicles were sealed and were of the same membrane topographical orientation as those found in vitro (25).

Photoaffinity Radiolabeling with [32P]AAPUT

All of the following experiments were performed in a dark room in the presence of a filtered safe-light. The photoaffinity reagent, [32P]AAPUT, P3-(4-azidoanilido)-uridine-5′-P3-(2′-P3)triphosphate; AAPUT, P3-(4-azidoanilido)-uridine-5′-triphosphate; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; PAG, polyacrylamide gel electrophoresis.

### Table I

| Step                                      | Total Protein (mg) | Specific Activity (pmol/mg in 5 min) | Activity (pmol in 5 min) | Purification Over Crude Golgi | Yield (%) |
|-------------------------------------------|--------------------|--------------------------------------|--------------------------|-------------------------------|-----------|
| Triton extract                            | 25,400             | 7                                    | 184,000                  | 15                            | 100       |
| First DEAE-Sephacel                       | 2,040              | 70                                   | 143,500                  | 150                           | 78        |
| Blue-Sepharose                            | 540                | 223                                  | 120,600                  | 480                           | 66        |
| Second DEAE-Sephacel                      | 52                 | 2,110                                | 109,700                  | 4,560                         | 59        |
| Carboxymethylcellulose                    | 11                 | 8,100                                | 89,400                   | 17,560                        | 48        |
| Third DEAE-Sephacel                       | 2.2                | 16,200                               | 36,900                   | 35,100                        | 20        |
| Fractogel-EMD                             | 0.4                | 29,100                               | 11,600                   | 63,200                        | 6.3       |

1 The abbreviations used are: [32P]AAPUT, P3-(4-azidoanilido)-uridine-5′-P3-(2′-P3)triphosphate; AAPUT, P3-(4-azidoanilido)-uridine-5′-triphosphate; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; PAGE, polyacrylamide gel electrophoresis.
Purification of the UDP-GalNAc Transporter

RESULTS

Purification of the UDP-GalNAc Transporter—The rat liver Golgi membrane UDP-GalNAc transporter was purified ~63,000 over the crude Golgi preparation with a yield of 6.3% (Table I). We began the purification with a crude Golgi membrane preparation; the UDP-GalNAc transporter was purified from the third DEAE-Sephacel column. Elution was with 3 column volumes of Buffer A containing 1.5 M NaCl. 18.5-ml fractions were collected, and 100-μl aliquots were used to assay UDP-GalNAc transport activity, as described under “Experimental Procedures.”

The Triton X-100 extract (Fig. 1, lane 1) was loaded onto a first DEAE-Sephacel column followed by elution with 700 mM NaCl in Buffer A. 78% of the transport activity was recovered with a 150-fold purification over the crude Golgi preparation (Fig. 1, lane 2). In the next chromatographic step, we used a tandem sequence of three consecutive negative columns, respectively, Blue-Sepharose, DEAE-Sephacel, and carboxymethylcellulose. An important factor in this step was the Triton X-100 concentration. When these columns were run in the presence of 0.5% Triton X-100, the UDP-GalNAc transport activity was always found in the flow-through (negative chromatography), whereas when used in the presence of 3% Triton X-100, the activity always bound to the matrix (positive chromatography). These three negative columns combined together provided important and substantial purification (Fig. 1, lanes 3-6), resulting in the binding of most of the applied proteins but...
not the UDP-GalNAc transport activity. 62% of the activity found in the active fraction from the first DEAE-Sephacel column was recovered after these steps with a 17,560-fold overall purification. In the next step we again used a DEAE-Sephacel column but this time at a higher Triton X-100 concentration (positive chromatography). The active fraction obtained from the carboxymethylcellulose column was concentrated to obtain a 3% Triton X-100 concentration and applied to the third DEAE-Sephacel column. The transporter activity was eluted with 1.5 M NaCl in Buffer A and recovered in the first 30 fractions of the elution volume (Fig. 2) with a 35,000-fold overall purification. This represented an important concentration step because from the initial ~11 liters of the applied sample, the transporter activity was recovered in ~500 ml. Silver staining of the active fraction from the third DEAE-Sephacel column shows eight predominant bands (Fig. 1, lane 1). In the next chromatographic step we used a Fractogel-EMD column in the presence of 1.5% Triton X-100; this resulted in the binding of all of the transporter activity to the column. Although most of the proteins were eluted with 1 M NaCl, the transporter activity was found in the 1.5 M NaCl eluate. The Fractogel-EMD chromatography gave a high specific activity transporter fraction. In order not to use high volumes of this active fraction, small aliquots of the sample were subjected to radioiodination with chloramine T prior to electrophoresis and visualization with autoradiography. For comparison, the active sample from the third DEAE-Sephacel column (sample applied to the Fractogel-EMD) is shown after silver staining (Fig. 1, lane 7). The SDS-gel profile (Fig. 1, lane 10) of the active fraction from the Fractogel-EMD chromatography showed two bands of 98 and 43 kDa. Only the 43-kDa band was not visualized in fractions inactive (Fig. 1, lane 11) for the transport activity, suggesting that the UDP-GalNAc transport activity had a mobility of 43 kDa.

**Photoaffinity Radiolabeling with [3P]AAUTP**—Photoaffinity radiolabeling in the presence or absence of nonradioactive UDP-GalNAc was used as a different criterion to demonstrate that the transport activity is a 43-kDa protein. AAUTP is a membrane-impermeant photoaffinity reagent that has been shown to bind and label various glycosyltransferases and to show the proteins were eluted with 1 M NaCl, the transporter activity was found in the 1.5 M NaCl eluate. The Fractogel-EMD chromatography gave a high specific activity transporter fraction. In order not to use high volumes of this active fraction, small aliquots of the sample were subjected to radioiodination with chloramine T prior to electrophoresis and visualization with autoradiography. For comparison, the active sample from the third DEAE-Sephacel column (sample applied to the Fractogel-EMD) is shown after silver staining (Fig. 1, lane 7) and iodination (Fig. 1, lane 8). The SDS-gel profile (Fig. 1, lane 10) of the active fraction from the Fractogel-EMD chromatography showed two bands of 98 and 43 kDa. Only the 43-kDa band was not visualized in fractions inactive (Fig. 1, lane 11) for the transport activity, suggesting that the UDP-GalNAc transport activity had a mobility of 43 kDa.

**Photoaffinity Radiolabeling with [3P]AAUTP**—Photoaffinity radiolabeling in the presence or absence of nonradioactive UDP-GalNAc was used as a different criterion to demonstrate that the transport activity is a 43-kDa protein. AAUTP is a membrane-impermeant photoaffinity reagent that has been shown to bind and label various glycosyltransferases and to inhibit uridine nucleotide sugar transport into endoplasmic reticulum vesicles (26). When used at different concentrations in the absence of UV light, AAUTP inhibited transport of UDP-GalNAc into Golgi membrane vesicles (Table II) without affecting the integrity of the vesicles (results not shown). Under the same conditions AAUTP also inhibited other uridine nucleotide sugars but not CMP-sialic acid transport (results not shown). We then subjected Golgi membrane vesicles to photolabeling with [3P]AAUTP in the absence or presence of nonradioactive UDP-GalNAc. Although several bands could be visualized (Fig. 3, lane 1), the 43-kDa band was the only one to be protected after preincubation with nonradioactive UDP-GalNAc used at different concentrations (Fig. 3, lanes 2 and 3); 5 μM UDP-GalNAc was able to almost completely protect the 43-kDa band from photoaffinity labeling. Neither UV irradiation, used without the photoprobe, nor the photoprobe by itself, without UV irradiation, resulted in photolabeling of bands (results not shown). We finally reconstituted active and inactive fractions from the Fractogel-EMD column into proteoliposomes and subjected them to photolabeling. Only fractions that were active in the UDP-GalNAc transport showed a radiolabeled band of 43 kDa (Fig. 4, lane 4) whereas inactive fractions did not (Fig. 3, lane 5). When the highly purified active fraction from the Fractogel-EMD column was assayed for nucleotide sugar transport activities, no other uridine nucleotide sugars were active (Table III), indicating that the UDP-GalNAc transporter is highly substrate-specific.

**Glycerol Gradient**—A glycerol gradient was used to estimate the functional size of the UDP-GalNAc transporter. The rationale for using the glycerol gradient was based on the fact that Golgi nucleotide derivative transporters are homodimers in the membrane (9, 28), and when solubilized in the presence of 0.5% Triton X-100, they also appear to behave as dimers. 2 Separate

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**TABLE II**

| Condition          | Si       | t       | Total transport (Si + t) | Inhibition |
|--------------------|----------|---------|-------------------------|------------|
| UDP-GalNAc         | 182.3    | 45      | 227.3                   |            |
| UDP-GalNAc + 4 μM AAUTP | 193.3    | 49.9    | 243.2                   | 0          |
| UDP-GalNAc + 12 μM AAUTP | 148.3    | 36.4    | 184.7                   | 21         |
| UDP-GalNAc + 24 μM AAUTP | 79.3     | 20.9    | 100                     | 57         |

**FIG. 3.** SDS-PAGE and autoradiography of intact Golgi vesicles and proteoliposomes subjected to UV photolabeling with [3P]AAUTP. Rat liver Golgi membrane vesicles and reconstituted proteoliposomes were prepared as described under “Experimental Procedures.” [3P]AAUTP was always used at 0.2 μM final concentration, and photolabeling was performed at 0 °C for 1 min (5 cm, maximum energy). Lane 1, rat liver Golgi vesicles (8 μg of protein) photolabeled in the absence of nonradioactive UDP-GalNAc; lane 2, rat liver Golgi vesicles (8 μg of protein) preincubated for 1 min at 30 °C with 1 μM nonradioactive UDP-GalNAc prior to photolabeling; lane 3, rat liver Golgi vesicles (8 μg of protein) preincubated for 1 min at 30 °C with 5 μM nonradioactive UDP-GalNAc prior to photolabeling; lane 4, proteoliposomes from Fractogel-EMD active fraction (see Fig. 1, lane 10); lane 5, proteoliposomes from Fractogel-EMD inactive fraction (see Fig. 1, lane 11).
Glyceral gradient sedimentation profile of the UDP-GalNAc transport activity. An active fraction from either purification Step 4 or Step 7 was loaded onto an 8–30% glyceral gradient and centrifuged as described under “Experimental Procedures.” 0.35-ml fractions were collected and assayed for the UDP-GalNAc transport activity. Representative sedimentation profiles of four different gradients are shown. The bars indicate the sedimentation positions of β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), β-galactosidase (120 kDa), phosphorylase b (100 kDa), tumor necrosis factor α-converting enzyme (80 kDa), and bovine serum albumin (68 kDa).

 aliquots from active fractions of the second DEAE-Sephacel column or the Fractogel-EMD column were loaded on top of a 8–30% glyceral gradient and centrifuged for 40 h, as described under “Experimental Procedures.” Fig. 4 shows the profile of the transporter activity throughout the gradient. A peak in the 80–90-kDa area was observed consistent with the proposal that the 43-kDa band is recovered as a dimer in Triton extracts and may function as a dimer within the Golgi membrane.

**DISCUSSION**

We have identified and purified the UDP-GalNAc transporter activity from rat liver Golgi membranes. The transporter showed an apparent molecular mass of 43 kDa, and its identity was confirmed by functional reconstitution of purified material as well as photoaffinity labeling. To identify the UDP-GalNAc transporter by column chromatography, we had to obtain a ~63,000-fold purification. This was expected because a similar apparent fold of purification was required for other low abundance Golgi membrane proteins, such as the PAPS transporter (28), and the heparan sulfate N-deacetylase/N-sulfotransferase (31). A key element during the purification was the use of three different negative chromatographic columns. They were specifically designed to obtain a rapid and substantial purification of the transporter activity. The nature of the influence of the final Triton X-100 concentration on the chromatographic behavior of the transporter during these negative chromatographic steps is unclear. It is possible that the precise Triton X-100 concentration affects the physical environment of the protein in such a way so as to modulate the exposure of charged residues on the protein and the efficiency with which they interact with the various chromatographic resins (32). The UDP-GalNAc transporter, like other Golgi membrane transporters, is an integral membrane protein and would be expected to have a highly hydrophobic character. The hydrophobicity of the environment as caused by the Triton X-100 concentration can probably determine the number of charges exposed and ultimately the possible interaction between the protein and the active group of the resin (32).

The Fractogel-EMD column eluate (Fig. 1, lane 10) showed two bands of 98 and 43 kDa, respectively. However, the 43-kDa band was not present in the inactive fractions (Fig. 1, lane 11). Although not reported under “Results,” another column, Macro-Prep-Q-Aion (Bio-Rad), also gave similar results suggesting that the 43-kDa band was the only band always found in the active fractions and never in the inactive fractions. In conclusion, the results obtained by column chromatography suggest that the UDP-GalNAc transporter is a 43-kDa protein.

Additional independent evidence suggesting that the 43-kDa band is indeed the UDP-GalNAc transporter was obtained with photoaffinity radiolabeling using AAUTP, an azido-anilido derivative of UTP. When we photolabeled Golgi membrane vesicles with [32P]AAUTP, several bands were visualized, but only one band of 43 kDa was protected from labeling when nonradioactive UDP-GalNAc was included in the labeling reaction. This result is in strong agreement with previous studies showing that nucleotide sugar transporters are highly specific (reviewed in Refs. 8 and 9). A 43-kDa band could also be visualized from a fraction that was active for the UDP-GalNAc transport activity. The absence of radiolabeling of fractions that were inactive in UDP-GalNAc transport activity is consistent with these results. Nucleotide derivative transporters appear to be arranged in the Golgi membrane as homodimers. Using glyceral gradient ultracentrifugation (33), we showed that the UDP-GalNAc transporter has a size of 80–90 kDa, roughly twice its apparent molecular mass as determined by reducing gel electrophoresis. It is well known that the assembly state of a membrane protein in the presence of a detergent also depends on the concentration and physical characteristics of the detergent and the ionic strength of the medium (33). Although the results we obtained cannot by themselves demonstrate the assembly state of the transporter and therefore the subunit size, they are consistent with the results obtained after column chromatography, i.e. the UDP-GalNAc transporter, like other nucleotide derivative transporters, may be functional as a homodimer in Golgi membranes. Our interpretation is supported by the evidence that the PAPS transporter, a 75-kDa protein that has been shown to oligomerize as a homodimer (28), migrated in the 150-kDa area of a glyceral gradient performed

**TABLE III**

Uridine nucleotide sugar transport activities from the active fraction of the Fractogel-EMD column

An aliquot of the 1.5 ml elution fraction from the Fractogel-EMD column was reconstituted into phosphatidylcholine liposomes as described under “Experimental Procedures” and assayed for transport of different uridine nucleotide sugars.

|                       | cpm/100 μl | pmol/100 μl |
|-----------------------|-----------|------------|
| UDP-GalNAc            | 760       | 1.03       |
| UDP-Gal               | 0         | 0          |
| UDP-Glc               | 0         | 0          |
| UDP-GlcNAc            | 0         | 0          |
| UDP-GlcA              | 0         | 0          |

**FIG. 4.** Glyceral gradient sedimentation profile of the UDP-GalNAc transport activity. An active fraction from either purification Step 4 or Step 7 was loaded onto an 8–30% glyceral gradient and centrifuged as described under “Experimental Procedures.” 0.35-ml fractions were collected and assayed for the UDP-GalNAc transport activity. Representative sedimentation profiles of four different gradients are shown. The bars indicate the sedimentation positions of β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), β-galactosidase (120 kDa), phosphorylase b (100 kDa), tumor necrosis factor α-converting enzyme (80 kDa), and bovine serum albumin (68 kDa).
under exactly the same conditions.

Better understanding of how and to what extent the regulation of nucleotide sugar transport into the Golgi lumen can affect the glycosylation of macromolecules in the Golgi apparatus requires knowledge of the amino acid and nucleic acid sequences of such transporters. This can only be obtained by the identification of more mutants defective in transport and cloning of the respective genes or by the purification of such transporters via the approach outlined here. The purification of the UDP-GalNAc transporter constitutes an important step toward understanding the role of N-acetylgalactosamine in the above glycoconjugates and will enable us to obtain the peptide sequence of the transporter and then proceed toward its cloning. This cloning in turn will allow us to study in more detail how the transporter is arranged in the membrane, whether it is structurally related to other nucleotide sugar transporters, and most importantly, whether its expression can regulate the biosynthesis and modifications of Golgi luminal glycoproteins, proteoglycans, and glycosphingolipids containing N-acetylgalactosamine in their carbohydrate chains. The purification will also permit us to make specific antibodies and to determine the localization of the transporter within the Golgi apparatus and whether it forms a complex with the corresponding transporters via the approach outlined here. The purification of the UDP-GalNAc transporter will also permit us to make specific antibodies and to determine the localization of the transporter within the Golgi apparatus and then proceed toward its cloning. This cloning in turn will allow us to study in more detail how the transporter is arranged in the membrane, whether it is structurally related to other nucleotide sugar transporters, and most importantly, whether its expression can regulate the biosynthesis and modifications of Golgi luminal glycoproteins, proteoglycans, and glycosphingolipids containing N-acetylgalactosamine in their carbohydrate chains. The purification will also permit us to make specific antibodies and to determine the localization of the transporter within the Golgi apparatus and whether it forms a complex with the corresponding transferases and nucleotide diphosphatase in the Golgi membrane.

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J. Biol. Chem. 1999, 274:4474-4479.
doi: 10.1074/jbc.274.7.4474

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