Cell Type–specific Post-Golgi Apparatus Localization of a "Resident" Endoplasmic Reticulum Glycoprotein, Glucosidase II

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Abstract. Glucosidase II, an asparagine-linked oligosaccharide processing enzyme, is a resident glycoprotein of the endoplasmic reticulum. In kidney tubular cells, in contrast to previous findings on hepatocytes, we found by light and electron microscopy immunoreactivity for glucosidase II predominantly in post-Golgi apparatus structures. The majority of immunolabel was in endocytotic structures beneath the plasma membrane. Immunoprecipitation confirmed presence of the glucosidase II subunit in purified brush border preparations. Kidney glucosidase II contained species carrying endo H–sensitive, high mannose as well as endo H–resistant oligosaccharide chains. Some species of glucosidase II contained sialic acid. The sialylated species were enzymatically active. This study demonstrates than an enzyme presumed to be a resident of the endoplasmic reticulum may show alternative localizations in some cell types.

N-glycosylated glycoproteins acquire their oligosaccharide chains by en bloc transfer of the lipid-linked precursor $Glc_3Man_9GlcNAc_2$ to the nascent polypeptide. The oligosaccharide precursors are then modified by glycosidases and glycosyltransferases to generate high mannose-, hybrid-, or complex-type structures. The processing starts with the removal of glucose residues by glucosidases (Hirschberg and Snider, 1987). Glucosidase I removes rapidly ($t_{1/2}$ <1 min) the terminal α1,2-linked glucose residue (Hubbard and Robbins, 1979). Glucosidase II hydrolyzes the two inner α1,3-linked glucose residues (Grinna and Robbins, 1979; Burns and Touster, 1982). The outer α1,3-linked glucose residue is hydrolyzed more rapidly ($t_{½}$ ~5 min) than the inner one (after ~20–30 min) (Hubbard and Robbins, 1979). In hepatocytes, glucosidase II is a high mannose-type glycoprotein and was localized to the rough and smooth endoplasmic reticulum by biochemical fractionation procedures (Grinna and Robbins, 1979; Brada and Dubach, 1984) and by immunoelectron microscopy (Lucocq et al., 1986). Golgi apparatus cisternae were free of immunolabel for glucosidase II although subcellular fractions of Golgi apparatus contained small amounts of the enzyme which almost certainly represented contamination by the endoplasmic reticulum. The data on the biosynthesis of glucosidase II obtained in a rat hepatoma cell line are in agreement with the above results (Strous et al., 1987). The enzyme subunit was found to be an endo H–sensitive, 100-kD glycoprotein that remained unchanged over long chase periods. The half-life of glucosidase II was not altered by monensin treatment, further supporting the notion that glucosidase II does not enter the Golgi apparatus in hepatocytes. Collectively, these data demonstrated that, in hepatocytes, glucosidase II is a resident glycoprotein of the endoplasmic reticulum which is retained therein by an unknown mechanism.

In the present study we have investigated the distribution of glucosidase II in pig kidney. The examination of kidney tubular cells revealed unexpected findings. Our biochemical and immunocytochemical data demonstrate that glucosidase II is also present in the Golgi apparatus and predominantly post-Golgi apparatus compartments such as endocytotic elements and the plasma membrane. Furthermore, kidney glucosidase II contains enzymatically active, sialylated species. Thus, glucosidase II generally assumed to be a resident of the endoplasmic reticulum exhibits different intracellular distributions depending on the cell type.

Materials and Methods

Materials

Staphylococcal protein A, BSA, and fetuin were obtained from Sigma Chemical Co. (St. Louis, MO); tetrachloroauric acid ($HAuCl_4.4H_2O$), polyethylene glycol (20,000 mol wt), and Triton X-100 were from E. Merck (Darmstadt, Federal Republic of Germany). Concanavalin A–Sepharose 4B was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden) and Immunobead Second Antibody from Bio-Rad Laboratories (Richmond, CA). Methylumbelliferyl-α-D-glucoside was from Koch Light Laboratories (Slough, England). The *Limax flavus* lectin was from Calbiochem-Behring Corp. (San Diego, CA) and the *Sambucus nigra L.* lectin was kindly provided by Dr. I. J. Goldstein (University of Michigan, Ann Arbor, MI). Endoglycosidase H was from Miles Laboratories, Inc. (Naperville, IL). Deoxynojirimycin was a gift from Dr. E. Bause (University of Cologne, FRG).

Preparation and Purification of Anti–Glucosidase II Antibodies

The preparation and characterization of the antiserum against pig kidney

glucosidase II was described earlier (Brada and Dubach, 1984; Lucocq et al., 1986). The antiserum was freed from trace amounts of contaminating antibodies against gp 330, the pathogenic antigen of Heymann nephritis, as follows. Rabbit antiserum (200 μl) was diluted with PBS to 2 ml and circulated for 12 h over an affinity column containing 500 μg of purified gp 330 immobilized on Sepharose 4B (Kerjaschki and Farquhar, 1983). The purity of the antibody was confirmed by immunoprecipitation of purified kidney brush border preparation.

Preparation of Kidney Glucosidase II and Kidney Extract

Glucosidase II was purified from pig kidney and its activity measured as reported (Brada and Dubach, 1984).

A pig kidney extract was prepared as follows. Pig kidney was homogenized in 0.25 M sucrose, 1 mM EDTA, 1 mM $PhMeSO_2F$ (1:4 wt/vol). The homogenate was converted to an acetone powder as described earlier (Brada and Dubach, 1984). The acetone powder was extracted with 1% Triton X-100 in 10 mM NaP_i, pH 6.8, 1 mM $PhMeSO_2F$, 1 mM *N*-topsyl-L-lysylchloromethane hydrochloride, 1 mM *N*-topsyl-L-phenyl-alanylchlormethane, 0.2 mg/ml aprotinin at 4°C for 30 min (10 mg powder per 160 μl of buffer).

Preparation of Kidney Brush Borders

Kidney brush borders were prepared by calcium precipitation according to Malathi et al. (1979). The proteins were labeled with ^{125}I by the lactoperoxidase-glucose oxidase method (Kerjaschki and Farquhar, 1982).

Electrophoretic Procedures

Electrophoretic separation was carried out in 7.5% polyacrylamide SDS gel according to Laemmli (1970). For the detection of the immune complexes on nitrocellulose the "golden blot" procedure using protein A-gold (Brada and Roth, 1984) or Western blotting with ^{125}I-protein A were performed. Staining of an immune replica by an enzyme immunoassay was done as reported previously (Brada and Dubach, 1984).

Peptide mapping by limited proteolysis was according to Cleveland et al. (1977) as modified by Tijssen and Karstak (1983). In short, proteins from kidney and liver extracts were separated in a 7.5% SDS-polyacrylamide gel and gel pieces containing the subunits were cut out. They were then mounted perpendicularly in the spacer gel of a 10% SDS-polyacrylamide gel. α-Chymoptrypsin solution was layered onto it. When the bromophenol blue band approached the separating gel to a distance of 5 mm, the current was stopped for 30 min to digest the stacked proteins. The digest was then resolved in the 10% SDS polyacrylamide gel.

Quantitative Immunoprecipitation

10 μl of pig kidney extract (0.85 mg/ml protein) were mixed with 10 μl of either the anti-glucosidase II antibody, newborn calf serum (both 10-fold diluted with PBS), or 0.1% BSA in PBS and incubated at 37°C for 2 h. This was followed by incubation with goat anti-rabbit immunoglobulins coupled to Sepharose beads (Immunobead Second Antibody; Pharmacia Fine Chemicals) for 2 h at 37°C. The beads were washed by three centrifugation steps (2,000 *g*, 5 min) with 1% Triton X-100 in PBS. Glucosidase II activity was measured in the pellets and supernatants.

Glycoprotein Dot Assays

Glucosidase II, fetuin, and ovalbumin (1 μg of each in 0.5 μl PBS) were spotted on nitrocellulose strips which were then saturated with 0.5% BSA in PBS at 20°C for 1 h. This was followed by incubation with *Limax flavus* lectin (100 μg/ml) for 1 h and fetuin-gold (14 nm gold particles, diluted in 1% BSA, 0.1% Triton X-100, 0.1% Tween 20 in PBS to give an absorbance of 0.35 at 525 nm) at 20°C for 1 h. Other strips were incubated with *Sambucus nigra L.* lectin-gold complex (10 nm gold particles, diluted in PBS containing 1% BSA, 0.1% Triton X-100, 0.1% Tween 20 to an absorbtion of 0.7 at 525 nm) at 20°C for 1 h. This lectin-gold complex was prepared according to Taatjes et al. (1988*a*).

In control incubations, the *Limax flavus* lectin was preincubated with 5 mM sialic acid and the *Sambucus nigra L.* lectin-gold with 100 mM lactose before incubations of strips. Furthermore, strips were treated with neuraminidase (0.4–1 U/ml) at 37°C for 1–12 h before lectin labeling. Further controls included preincubation of the *Limax flavus* lectin or the *Sambucus nigra L.* lectin-gold with orosomucoid and asialo-orosomucoid (150 μg of each), respectively, for 30 min.

Glucosidase II Activity Measurement in Nitrocellulose-immobilized Lectin Assay

To determine whether or not the sialylated species of glucosidase II are enzymatically active, the following experiment was carried out. Nitrocellulose was pretreated with the sialic acid-specific *Limax flavus* lectin and cut into pieces of 0.16, 0.32, 0.48, and 0.64 mm^2 in size. The nitrocellulose pieces were incubated with a kidney extract (100 μg protein) at 4°C overnight. Glucosidase II activity bound to the immobilized *Limax flavus* lectin was measured with the fluorogenic substrate methylumbelliferyl-α-D-glucoside.

The specificity of this reaction was controlled by spotting BSA instead of *Limax flavus* lectin. Strips were also incubated with anti-glucosidase II antibody followed by protein A-gold to demonstrate the presence of enzyme polypeptide bound to the lectin. The sugar binding activity of the nitrocellulose-immobilized *Limax flavus* lectin was demonstrated by its positive interaction with fetuin-gold complex.

Tissue Processing for Light and Electron Microscopy

The kidney of a female pig (fasted 36 h) was fixed by perfusion through the renal artery. The kidney was flushed for ~1 min with a solution of 0.9% NaCl containing 5,000 U of heparin, 16% $CaCl_2$, and 70 mM $NaNO_2$ followed by perfusion for 10 min with 3% (para)formaldehyde-0.1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 4% polyvinylpyrrolidone, (30,000 mol wt). Pieces from kidney cortex and medulla were washed (three times for 5 min each time) in 0.1 M cacodylate buffer (pH 7.4) and placed in 100 mM NH_4Cl in cacodylate buffer for 45 min. The tissue pieces were then washed again in buffer. Tissue pieces were dehydrated in graded ethanol at progressively lowered temperatures (down to −35°C) and embedded in Lowicryl K4M (Carlemalm et al., 1982; Roth et al., 1981). Embedding in paraffin was performed according to standard protocols.

Immunocytochemistry

Immunostaining was performed with the protein A-gold technique (Roth et al., 1978).

For light microscopy, paraffin sections of semithin Lowicryl K4M sections were covered with 0.5% ovalbumin in PBS at 20°C for 5 min followed by incubation with the anti-glucosidase II antibody (diluted 1:10–1:50) at 20°C for 2 h and protein A-gold (14 nm gold particles, diluted in PBS containing 1% BSA, 0.1% Triton X-100, 0.1% Tween 20 to given an absorbance of 0.44 at 525 nm) for 1 h. The labeling was enhanced by photochemical silver reaction (Manigley and Roth, 1985).

For electron microscopy, Lowicryl K4M thin sections were floated on a drop of 0.1% BSA in PBS (0.5 M NaCl) at 20°C for 5 min. The grids were then transferred to drops (15 μl) of anti-glucosidase II antibody (diluted 1:10–1:50 in 0.1% BSA in PBS, 0.5 M NaCl) and incubated at 20°C for 2 h or at 4°C for 12 h. After two washes with PBS, the grids were transferred to protein A-gold (14 nm gold particles, diluted in 1% Triton X-100 in PBS to an absorbance of 0.35 at 525 nm) at 20°C for 45 min. Thin sections were stained with 2% uranyl acetate (5 min) and lead acetate (45 s) or with uranyl acetate-methylcellulose.

Cytochemical controls were as follows: (*a*) preincubation of the antibody with purified native glucosidase II (50 μg/15 μl of 1:10 diluted antibody) followed by protein A-gold; (*b*) replacement of the antibody by nonimmune rabbit serum followed by protein A-gold; (*c*) incubation with protein A-gold alone.

Preparation of Glucosidase II-Gold Complex and Application to Kidney Thin Sections

Purified glucosidase II was complexed to colloidal gold (14 nm) as follows. 1-ml aliquots of glucosidase II (5 μg/ml 10 mM phosphate buffer, pH 6.5) were rapidly mixed with 1-ml aliquots of colloidal gold followed by the addition of 100 μl 10% BSA in 10 mM phosphate buffer. The crude complexes were placed on a 20% glycerol cushion and centrifuged at 20,000 rpm (TFT 65.38 rotor; Kontron AG, Zürich, Switzerland) for 45 min. The sedimented glucosidase II-gold complexes were resuspended in PBS (pH 6.5) containing 0.5% BSA and 0.02% NaN_3 and stored at 4°C. Glucosidase II activity was measured with the fluorogenic substrate methylumbelliferyl-α-D-glu-

coside. After 10 d of storage, the enzyme activity of the complex was 92% of the initially measured activity, and after 4 wk 58%.

For binding studies, Lowicryl K4M thin sections from pig kidney were floated for 5 min on droplets of 1% BSA in PBS and then transferred onto droplets of glucosidase II–gold complexes (~100 μg/ml glucosidase II) for 1 h at 20°C. After two rinses with 1% BSA in PBS (2 min each) grids were placed on droplets of 1% glutaraldehyde in PBS for 20 min, rinsed with PBS and distilled water, and dried. Thin sections were counterstained as described above. In parallel, some grids were placed on glucosidase II–gold complexes that had been preincubated with 1 mM deoxynojirimycin for 30 min. Other grids were first placed on unlabeled glucosidase II (100 μg/ml) for 30 min followed by addition of glucosidase II–gold complex.

Results

Characterization of the Antibody and Antigen

The antiserum was prepared by immunizing rabbits with glucosidase II purified from pig kidney microsomes (Brada and Dubach, 1984) and shown to recognize specifically the enzyme subunit on Western blots prepared from pig liver homogenate (Lucocq et al., 1986). Since it can be often observed that antisera raised against kidney proteins contain contaminating antibodies reacting with the gp 330, the pathogenic antigen of the Heymann nephritis (Kerjaschki et al., 1984), we tested the immune serum by immunoprecipitation of isolated brush border preparations. We noticed in addition to a predominant band at 100 kD, representing the glucosidase II subunit, a weak band at 330 kD. The contaminating anti-gp 330 antibodies were completely removed by passing the antiserum over an gp 330 affinity column. The purified antibody was used in all experiments.

The purified antibody precipitated specifically glucosidase II activity from a pig kidney extract (Table I). For further demonstration of antibody specificity, proteins from pig kidney extract were separated on a 7.5% polyacrylamide SDS gel and analyzed by the golden blot. Fig. 1 *A* shows that the antibody recognizes specifically and exclusively the glucosidase II subunit. Sharing of antigenic determinants between the enzyme subunit and the active glucosidase II was evaluated by enzyme immunoassays. As can be seen from Fig. 1 *B*, the position of the peak of enzymatic activity corresponded exactly to the position of the band of glucosidase II subunit on the golden blot.

The molecular and enzymatic properties (including the cleavage of the oligosaccharide precursors $Glc_{1-2}Man_9GlcNAc_2$) of the kidney glucosidase II were characterized in detail previously (Brada and Dubach, 1984). To further confirm that the pig kidney enzyme recognized by the antibody is indeed the same molecule as the hepatocyte glucosidase II, we have directly compared both proteins by peptide mapping. Fig. 2 shows that liver and kidney glucosidase II share the same immunoreactive fragments. Similarly, upon prolonged storage at 4°C, liver and kidney extracts contained immunoreactive proteolytic degradation products of the same molecular weight. Thus, all the above results confirm that the antibody recognizes pig kidney glucosidase II specifically and quantitatively.

Table I. The Antibody Immunoprecipitates Specifically Glucosidase II Activity Present in a Pig Kidney Extract

	Glucosidase II activity*	
	Pellet	Supernatant
	%	%
Antibody	93	7
NBCS or 0.5% BSA in PBS	0	100

* Pig kidney extract was incubated with the antibody or NBCS or 0.5% BSA in PBS. Immune complexes were bound to goat anti-rabbit IgG antibody covalently coupled to polyacrylamide beads and separated by centrifugation. Glucosidase II activity was measured in the pellets and supernatants.

Glucosidase II Subunit and Activity Is Detectable in Purified Kidney Brush Border Preparations

Purified rat kidney brush border preparations were radioiodinated and the proteins immunoprecipitated with the anti-glucosidase II antibody followed by protein A–Sepharose. The immunoprecipitate was separated on a 3.6–8% gradient polyacrylamide SDS gel and autoradiographed. As demonstrated in Fig. 3 *A*, the kidney brush border fraction contains the glucosidase II subunit most probably represented by the upper band. The lower band is a breakdown product of the upper band. Similar results were sometimes obtained not only in kidney but also in liver extracts (Strous et al., 1987; Brada, D., unpublished observations). Western blot analysis

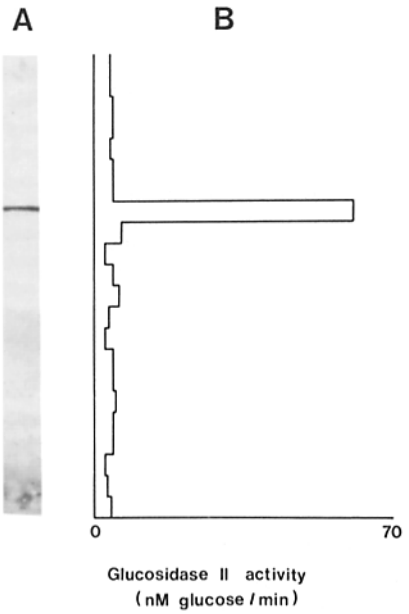

Figure 1. Anti-glucosidase II antibodies recognize specifically the enzyme subunit in a pig kidney extract. The glucosidase II subunit shares immunogenic determinants with the active enzyme. (*A*) Pig kidney extract (200 μg) was separated on a 7.5% polyacrylamide SDS gel. Proteins were transferred to nitrocellulose and decorated with the anti-glucosidase II antibodies followed by protein A-gold. A single band with an apparent molecular mass of 100 kD representing the glucosidase II subunit is visible. (*B*) Adjacent nitrocellulose strip obtained from the slab gel used in *A* was incubated with an excess of anti-glucosidase II antibodies. This was followed by incubation with purified, active glucosidase II which bound to free antigen binding sites. The nitrocellulose strip was cut into pieces (0.5 cm long) and glucosidase II actvitiy was measured with the fluorogenic substrate methylumbelliferyl-α-D-glucoside. The amount of enzyme activity is expressed as nanomolar solution of glucose released per minute per piece of nitrocellulose. With another enzyme immunoassay (exactly the same except that the entire nitrocellulose strip was incubated with the substrate soaked into a filter paper) the fluorescent enzyme reaction product, when viewed under ultraviolet light, showed the same single band as in *B*.

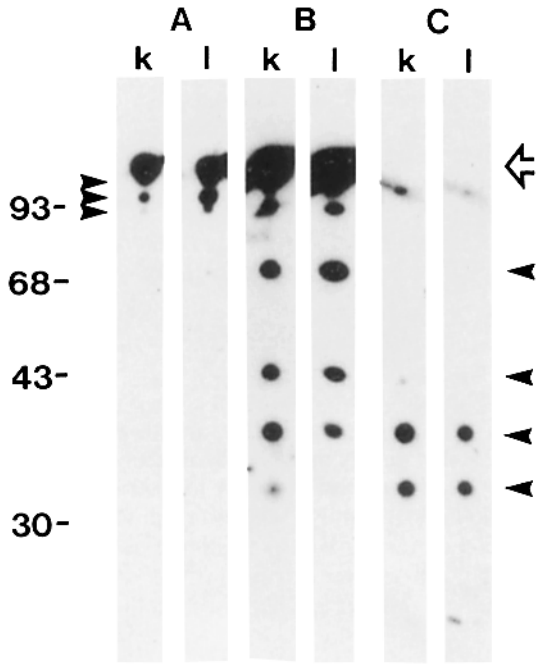

Figure 2. Kidney and liver glucosidase II subunits share immunoreactive fragments yielded by limited proteolysis. Proteins from pig kidney (*k*) and liver (*l*) extracts were separated in a 7.5% polyacrylamide SDS gel. The gel pieces containing the glucosidase II subunits were mounted perpendicularly in the spacer gel of a 10% polyacrylamide SDS gel. The limited proteolysis with α-chymotrypsin was carried out in the spacer gel. The resulting polypeptides were then electrophoretically resolved and visualized by Western blotting. *A*, *B*, and *C* differ in the extent of protease digestion increasing from *A* to *C*. The arrow indicates glucosidase II subunits and the arrowheads the shared breakdown products resulting from the limited proteolysis.

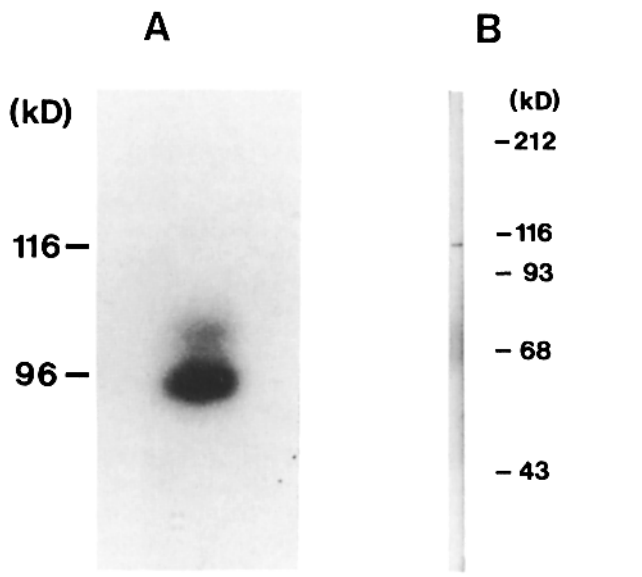

Figure 3. Glucosidase II is present in kidney brush border fraction. (*A*) A kidney brush border preparation was labeled with ^{125}I and glucosidase II immunoprecipitated. The immune complexes were bound to protein A-Sepharose and separated in a 3.6–8% gradient polyacrylamide SDS gel. Immunoprecipitated material was visualized by autoradiography. (*B*) Kidney brush borders were resolved in a 7.5% polyacrylamide SDS gel and transferred to nitrocellulose. The nitrocellulose was incubated with the antibody recognizing glucosidase II, ^{125}I-protein A, and autoradiographed.

of purified pig kidney brush borders with anti-glucosidase II antibodies revealed also the presence of immunoreactivity for the subunit of the enzyme (Fig. 3 *B*). The specific activity of glucosidase II in an extract of pig kidney brush borders was 1.5-fold enriched (1.5 nmol glucose released per minute per milligram protein) over an extract from whole kidney homogenate. Glucosidase II activity present in isolated pig kidney brush borders was immunoprecipitated with the antibody and an analysis of the immunoprecipitates by SDS-PAGE revealed the presence of the enzyme subunit. These results show that kidney brush borders contain indeed glucosidase II activity and subunit.

Kidney Glucosidase II Contains Species Carrying Endo H-sensitive and Sialylated Endo H-resistant Oligosaccharide Chains: The Sialylated Species Are Enzymatically Active

Purified kidney glucosidase II was digested with endo H, resolved on a 7.5% polyacrylamide SDS gel, and immunodetection performed by a golden blot. Fig. 4, lane *1* shows the occurrence of two bands with a difference in molecular mass of ~2–4 kD demonstrating that the purified glucosidase II subunit contains both endo H-sensitive and -resistant species. A similar analysis of extracts from whole pig kidney and isolated brush borders revealed also the presence of endo H-sensitive and -resistant oligosaccharides on glucosidase II subunit (Fig. 4, lanes *3–6*). Quantification of the amounts of the two different forms by counting the radioactivity showed that 23% of glucosidase II species in the whole kidney extract were endo H resistant and 45% in the brush border fraction. To demonstrate that the endo H resistance of glucosidase II is due to Golgi apparatus associated oligosaccharide processing (i.e., sialylation) the following experiments were performed. Purified glucosidase II as well as fetuin and ovalbumin were spotted on nitrocellulose strips which were then incubated with the sialic acid specific lectin from *Limax flavus* (Miller et al., 1982). The lectin binding to nitrocellulose-immobilized proteins was visualized by incubation with fetuin-gold complex (Roth et al., 1984). The lectin bound intensely to the sialoglycoprotein fetuin and also to glucosidase II, albeit less intensely (Fig. 5 *A*). No binding occurred to ovalbumin that contained high mannose- or hybrid-type oligosaccharides missing sialic acid residues. The lectin binding to glucosidase II and fetuin was due to the presence of sialic acid residues since it could be abolished or reduced by preabsorbtion of the lectin with free sialic acid (Fig. 5 *B*) or by neuraminidase pretreatment of the strips (Fig. 5 *C*). Further evidence for the presence of sialic acid in some species of glucosidase II was obtained with the recently characterized *Sambucus nigra L.* lectin, reported to have a marked preference for the NeuAc (α2-6) Gal/GalNAc disaccharide unit (Shibuya et al., 1987). This lectin also specifically bound glucosidase II and fetuin (Fig. 5 *D*). The binding was inhibited by lactose and neuraminidase pretreatment (Fig. 5, *E* and *F*). Collectively, these results indicate that kidney glucosidase II contains endo H-resistant oligosaccharides bearing sialic acid, part of which is α2-6-linked to Gal or GalNAc residues. However, it should be kept in

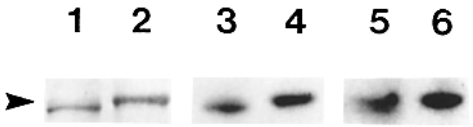

Figure 4. Kidney glucosidase II contains species with endo H-sensitive and -resistant oligosaccharides. Purified kidney glucosidase II (lanes *1* and *2*), an extract (100 μg protein) from whole kidney (lanes *3* and *4*), and an extract (100 μg protein) from a brush border fraction (lanes *5* and *6*) were treated with (lanes *1*, *3*, and *5*) or without (lanes *2*, *4*, and *6*) endo H. Samples were resolved on 7.5% polyacrylamide SDS gel, transferred to nitrocellulose and visualized with protein A-gold (lanes *1* and *2*) or ^{125}I-protein A (lanes *4–6*). The arrow indicates the glucosidase II subunit.

mind that processing to complex-type oligosaccharides does not absolutely result in endo H resistance. Oligosaccharides with only one antenna processed to the complex-type were found to be endo H sensitive (Varki and Kornfeld, 1983). And endo H-resistant oligosaccharides, on the other hand, do not necessarily contain sialic acid residues.

Next we performed experiments to elucidate whether or not the sialylated species of glucosidase II are enzymatically active. Nitrocellulose was saturated with the sialic acid-specific *Limax flavus* lectin and then incubated with active glucosidase II present in a kidney extract. Subsequently, the amount of enzyme activity bound to the lectin was measured with the fluorogenic substrate methylumbelliferyl-α-D-glucoside. The data in Fig. 6 show that the glucosidase II species carrying sialylated oligosaccharides are enzymatically active. Similar results were obtained when purified glucosidase II or a brush border extract were used.

Immunolocalization of Glucosidase II in Pig Kidney

For light microscopy, tissue sections of pig kidney embedded in paraffin (Fig. 7, *A* and *B*) and in Lowicryl K4M (Fig. 7, *C* and *D*) were processed by the protein A-gold technique

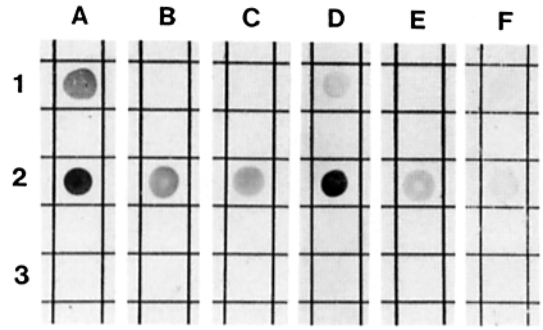

Figure 5. Purified kidney glucosidase II contains species carrying sialylated oligosaccharide chains. Kidney glucosidase II (*1*), fetuin (*2*), and ovalbumin (*3*) were spotted on nitrocelluose (1 μg in 0.5 μl of each). The strips were treated with (*A*) sialic acid specific lectin from *Limax flavus* followed by fetuin-gold; (*B*) *Limax flavus* lectin preabsorbed with 5 mM sialic acid followed by fetuin-gold; (*C*) neuraminidase followed by *Limax flavus* lectin and fetuin-gold; (*D*) sialic acid-specific *Sambucus nigra L.* lectin-gold complex; (*E*) *Sambucus nigra L.* lectin-gold complex preabsorbed with 100 mM lactose; (*F*) neuraminidase followed by *Sambucus nigra L.* lectin-gold complex.

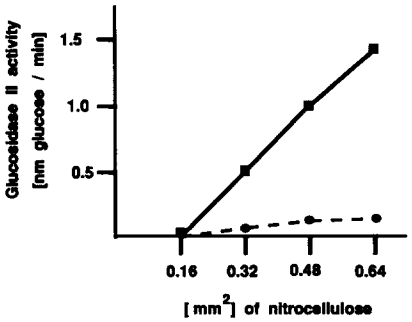

Figure 6. The sialic acid-containing species of glucosidase II are enzymatically active. Nitrocellulose was saturated with the sialic acid-specific *Limax flavus* lectin and cut into pieces of different sizes. Each piece of nitrocellulose was incubated with a kidney extract (100 μg protein). Glucosidase II bound to the lectin immobilized on the nitrocellulose was measured by incubation with the fluorogenic substrate methylumbelliferyl-α-D-glucoside (*solid line*). The enzyme activity is expressed as nanomolars of glucose released per minute. The amount of bound glucosidase II increases with increasing amount of immobilized lectin. In control experiments, BSA-saturated nitrocellulose was incubated with a kidney extract (*broken line*).

followed by photochemical silver amplification. Fig. 7 *A* shows at low magnification the kidney cortex and adjacent outer medulla. All tubular profiles exhibited immunoreactivity for glucosidase II, however, the glomeruli were not stained (Fig. 7, *A* and *B*). In proximal tubules that are lined by a single layer of cylindrical cells, immunolabeling was most intense in a zone below the brush border; the remaining cytoplasm was also labeled albeit less intensely (Fig. 7 *C*). In distal tubules a similar pattern of labeling was observed with an apical cell region being most intensely stained (Fig. 7 *C*). Labeling was also evident at the level of the striations formed by the plasma membrane of distal tubules and cortical collecting ducts (Fig. 7 *D*). The exact nature of the labeled structures in proximal and distal epithelial cells was determined by immunoelectron microscopy. In Lowicryl K4M thin sections, gold particle label indicative of glucosidase II immunoreactivity was detectable in the endoplasmic reticulum (Fig. 8 *C* and 9 *A*) and autophagosomes of all cell types (Fig. 8 *D*). However, in addition, the Golgi apparatus, vesicular and tubular profiles in the apical cytoplasm, of which the majority represents endocytic elements (Rodman et al., 1986), and the lower third of the brush border of proximal tubular cells including the intermicrovillar domains were labeled (Fig. 8, *A* and *B*). It should be emphasized that the endocytotic elements were the predominantly labeled cellular structures. The basolateral plasma membrane of the proximal tubular epithelia was not labeled. In distal tubules, immunolabel was found in apically located cytoplasmic vesicles as well as the apical and basolateral plasma membrane (Fig. 9, *A–C*).

A Ligand for Glucosidase II Is Present in Post-Golgi Apparatus Compartments

The detection of post-Golgi apparatus localized enzymatically active glucosidase II prompted us to determine if a ligand for this enzyme is present in these compartments. Purified glucosidase II was complexed to particles of colloi-

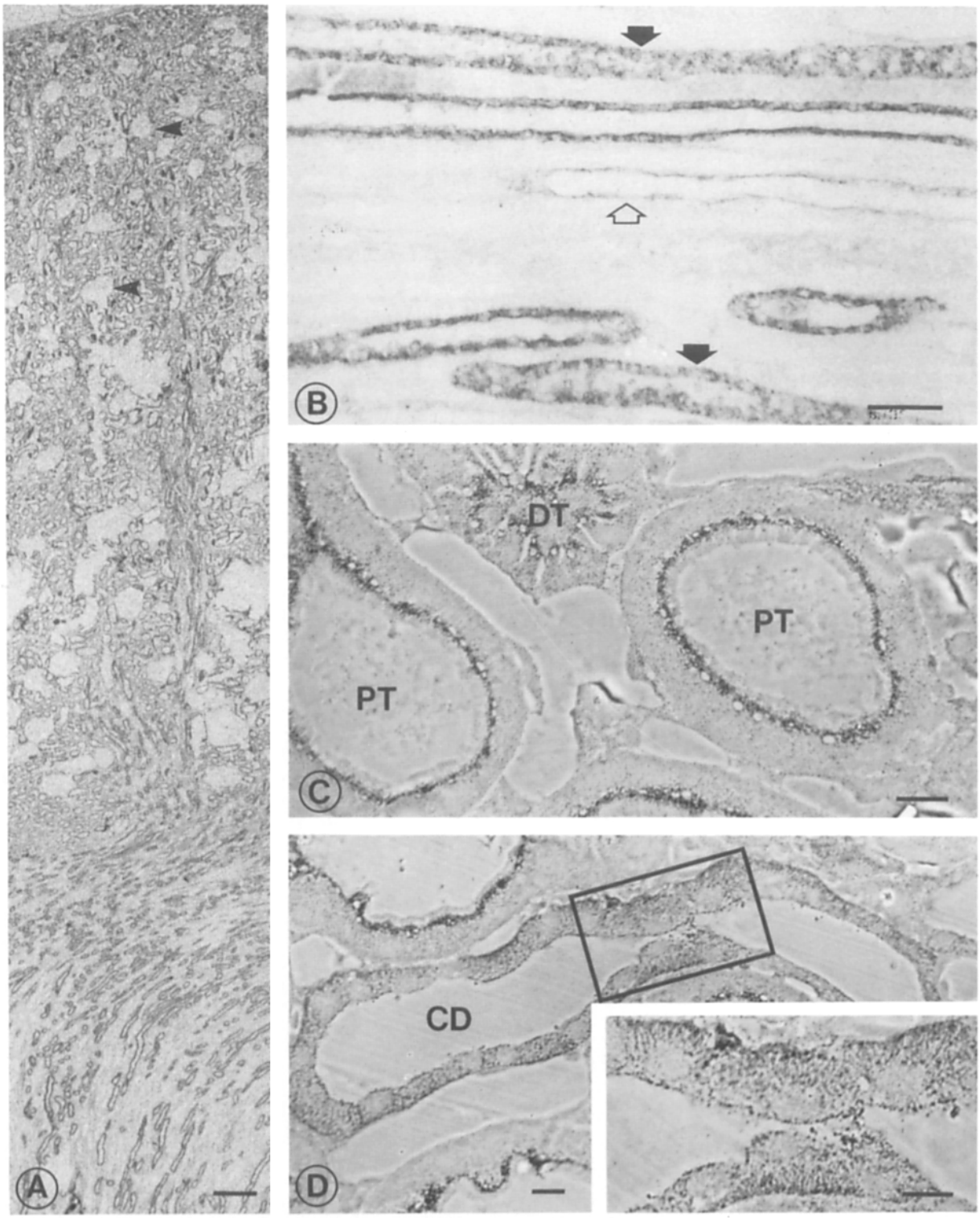

Figure 7. Light microscopic localization of glucosidase II immunoreactivity in pig kidney. Paraffin (*A* and *B*) or 1 μm Lowicryl K4M (*C* and *D*) sections from pig kidney. (*A*) All tubular profiles in kidney cortex and medulla are labeled; the glomeruli (*arrowheads*) are unstained. (*B*) Detail from inner stripe of outer medulla showing positive collecting ducts (*arrows*) and thin loop of Henle (*open arrow*). (*C*) In proximal tubules (*PT*), which are lined by a single layer of cylindrical cells, a distinct zone below the brush border is intensely stained whereas the remaining cytoplasm exhibits only faint immunolabel. In a distal convoluted tubule (*DT*), immunolabel is also concentrated in the apical cytoplasm. (*D*) Positive cortical collecting duct (*CD*). (*Inset*) Higher magnification of the region outlined in (*D*) showing the intensely stained basal membrane convolutions and apical cell surface. Bars: (*A* and *B*) 50 μm; (*C* and *D*) 10 μm.

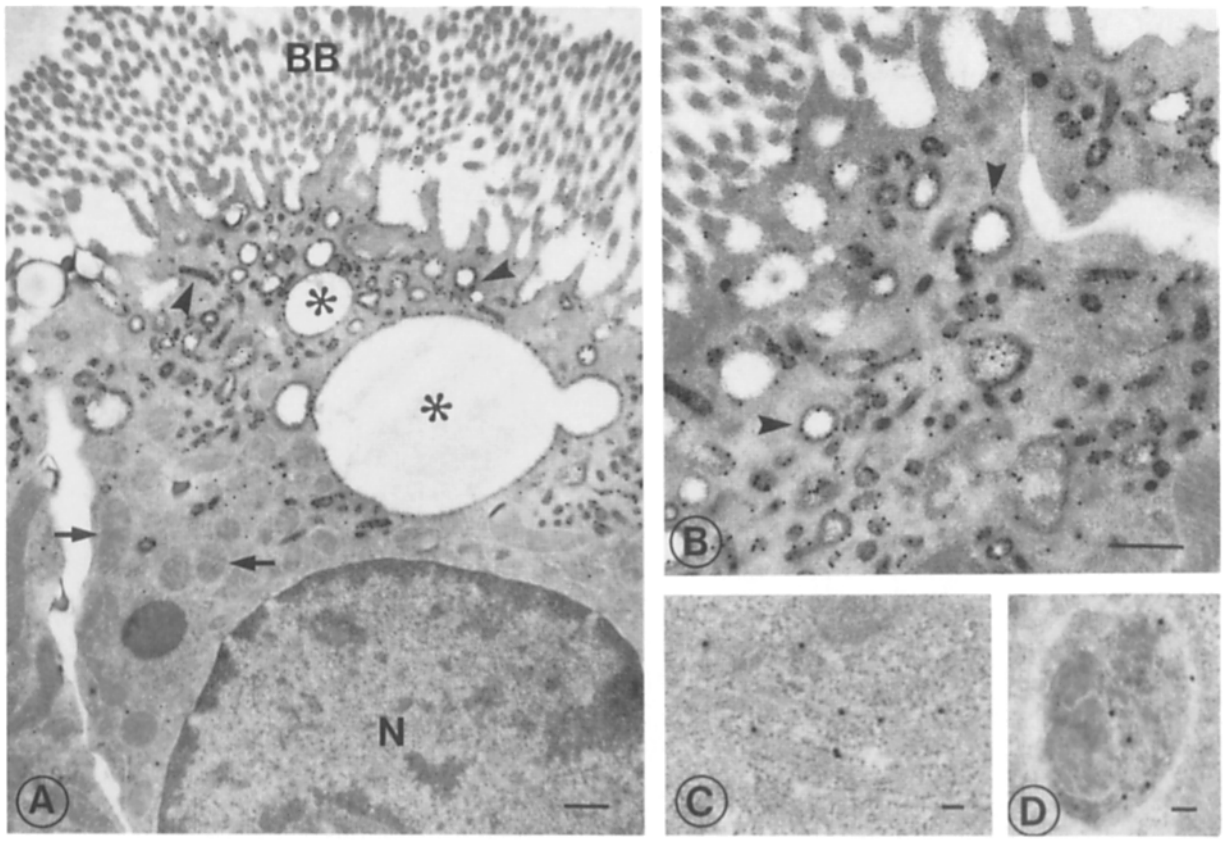

Figure 8. Electron microscopic localization of glucosidase II in proximal tubular cells. Lowicryl K4M thin sections stained with anti-glucosidase II antibody followed by protein A-gold. (*A*) Upper portion of proximal tubular cells with labeled brush border (*BB*), intensely labeled vesiculotubular profiles (*arrowheads*), and labeled vacuoles (*asterisks*). The lateral plasma membrane, nucleus (*N*), and mitochondria (*arrows*) are not labeled. (*B*) Detail from the apical cytoplasm showing the preferential membrane association of the gold particle label (*arrowheads*). (*C*) Rough endoplasmic reticulum is labeled for glucosidase II. (*D*) Labeling for glucosidase II over an autophagosome. Bars: (*A* and *B*) 0.5 μm; (*C* and *D*) 0.1 μm.

dal gold and found to exhibit catalytic enzyme activity when tested in an assay. Incubation of Lowicryl K4M thin sections with glucosidase II–gold complex resulted in its binding to the membranes of the tubulovesicular elements and the plasma membrane of proximal and distal tubules (Fig. 10). This binding could be abolished by preincubation of the enzyme–gold complex with 1 mM deoxynojirimycin, a specific inhibitor of glucosidase II (Fig. 10). Inhibition of binding also occurred in the presence of an excess of unlabeled glucosidase II.

Discussion

In the present study we have investigated the subcellular distribution of the "resident" ER glycoprotein glucosidase II in kidney epithelial cells. The striking finding that evolved is that this enzyme considered a resident ER glycoprotein leaves its compartment boundary and is found in the Golgi apparatus and post-Golgi apparatus structures. The processing of oligosaccharides on glucosidase II to sialylated species is direct evidence for transportation of the enzyme through the Golgi apparatus (Roth et al., 1985, 1986). Furthermore, the sialylated form of glucosidase II present outside the ER is enzymatically active. These data are in contrast to previous findings on various resident ER proteins (Brands et al., 1985; Yamamoto et al., 1985; Lucocq et al., 1986; Orci et al., 1984; Galteau et al., 1985). Specifically, in liver cells, glucosidase II was shown to be present throughout the ER and absent from the Golgi apparatus cisternae by immunoelectron microscopy (Lucocq et al., 1986). Biochemically, in hepatoma cells the enzyme exhibited all properties characteristic of a resident ER glycoprotein (Strous et al., 1987). There is a somewhat similar situation for the lysosomal enzyme β-glucoronidase. It is not only found in lysosomes but also in the ER where it is retained by complex formation with egasyn (Brown et al., 1987; Medda et al., 1987).

The mechanism by which glucosidase II is effectively retained within the ER in hepatocytes is not known. Some soluble proteins that are resident in the ER were shown to contain a retention signal: the carboxy-terminal tetrapeptide sequence, Lys-Asp-Glu-Leu (KDEL) (Munro and Pelham, 1987). Its deletion results in secretion of these ER proteins. On the other hand, addition of the KDEL sequence to a secretory protein, lysozyme, results in its accumulation in the ER.

The distal boundary of the compartment in which KDEL sequence–containing proteins are retained was recently defined (Pelham, 1988). The KDEL sequence was attached to the lysosomal enzyme cathepsin D and investigated as to whether or not this glycoprotein acquires the lysosomal

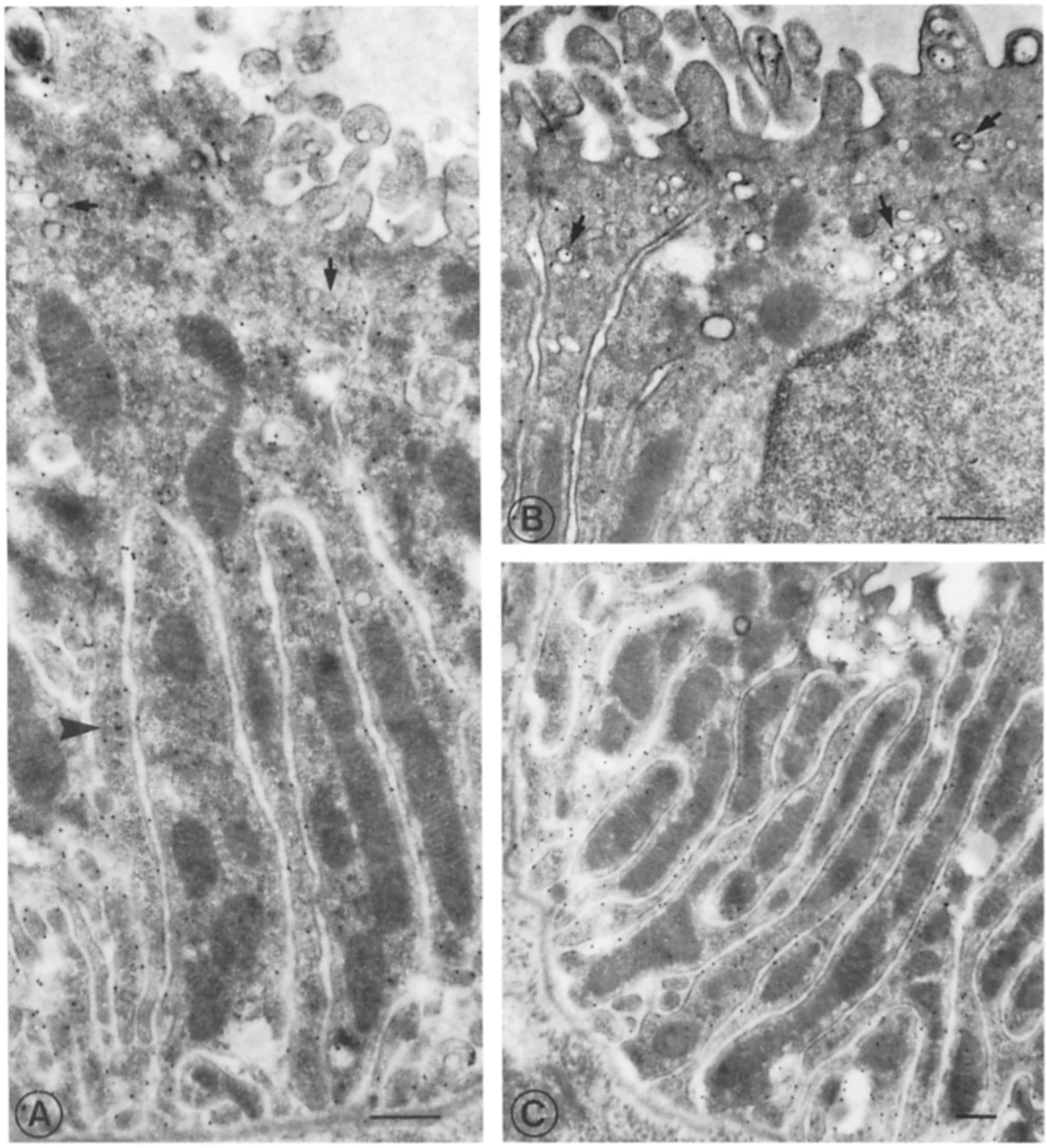

Figure 9. Distribution of glucosidase II immunoreactivity in distal tubular cells. (*A*) Distal tubular cell with gold particle label over rough endoplasmic reticulum (*arrowhead*) and cytoplasmic vesicles (*arrows*) which can be seen at higher magnification in *B*. (*A* and *C*) Immunoreactivity for glucosidase II is detectable in the apical and basolateral plasma membrane. Bars, 0.5 μm.

targeting signal mannose-6-phosphate. Cathepsin D accumulated indeed in the ER but was also modified by *N*-acetylglucosaminyl-1-phospho-transferase. This first acting enzyme in the formation of mannose-6-phosphate residues is believed to reside in *cis*-Golgi apparatus (Goldberg and Kornfeld, 1983). These results are in favor of a retrieval mechanism from a post-ER compartment, probably the *cis*-face of the Golgi apparatus. There are also recent data indicating that a membrane-bound receptor is not involved in this retention mechanism (Ceriotti and Colman, 1988). If soluble ER proteins are retrieved from the *cis*-Golgi apparatus, this then would imply the presence of five mannose residues on their oligosaccharides. However, the glucose-regulated protein GRP94 which contains the KDEL retention signal (Munro and Pelham, 1987) bears oligosaccharides with six and more mannose residues (Lewis et al., 1985). Therefore, the existence of a salvage compartment from which the ER soluble proteins would be recovered was proposed (Pelham, 1988; Warren, 1987). This salvage compartment would be an intermediate between the transitional elements of the ER and the Golgi apparatus.

It should be stressed that the situation with integral ER membrane proteins is different from soluble ones. Here, no KDEL sequence has been found and deletions in or close to the membrane-spanning region usually result in transport out of the ER (Poruchynsky et al., 1985; Pääbo et al., 1987;

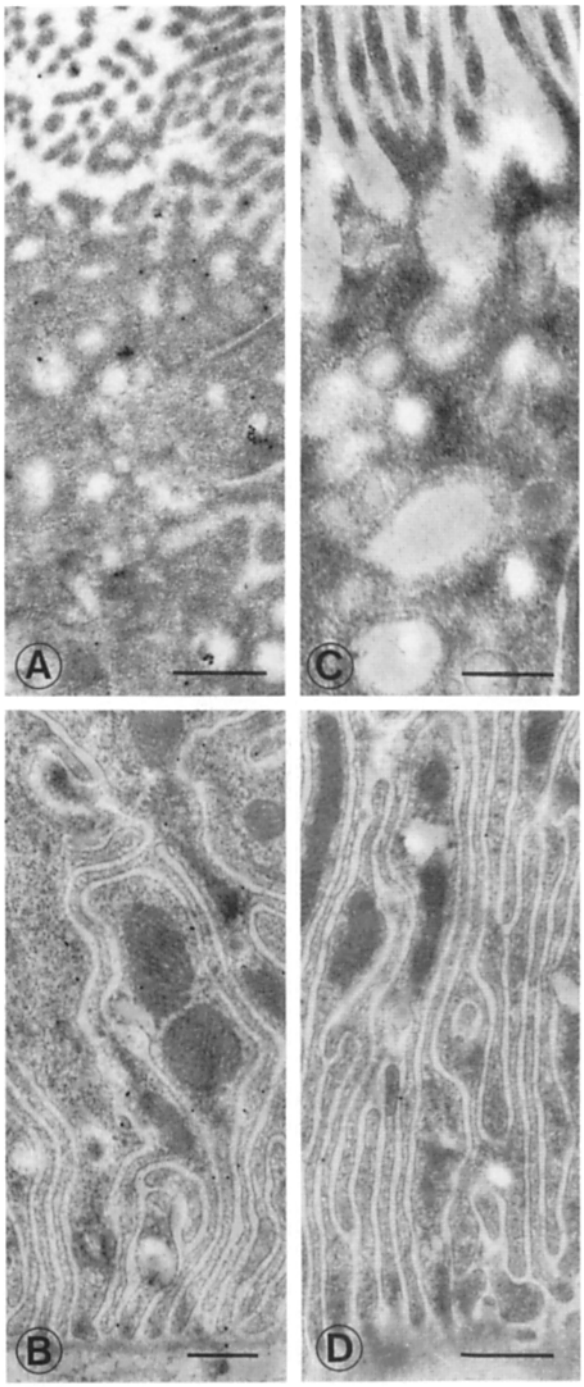

Figure 10. Presence of a ligand for glucosidase II–gold complex in tubular epithelial cells. Demonstration of binding of glucosidase II–gold complexes to tubulovesicular elements in proximal epithelial cells (*A*) and the plasma membrane of distal tubular cells (*B*). Binding of the enzyme–gold complex is inhibited by the specific inhibitor deoxynojirimycin (*C*, *D*). Bars, 0.5 mm.

Poruchynsky and Atkinson, 1988). Recently, the last six amino acid residues (DEKKMP) at the COOH terminus of the cytoplasmic tail of transmembrane ER proteins (adenoviral E3/19K glycoprotein, UDP-glucuronosyltransferase) were shown to represent a necessary and sufficient signal for ER retention (Nilsson et al., 1989).

All currently available data (reviewed by Rothman, 1987; Warren, 1987; Lodish, 1988) point to the existence of two distinct mechanisms for protein retention in the ER. Besides the retention signal mechanism for soluble ER proteins (Munro and Pelham, 1987), the conformation of a protein appears to play an important role in the targeting (Gething et al., 1986; Copeland et al., 1986; Kreis and Lodish, 1986). However, it is not known if the latter is also effective for resident ER proteins. In this context it needs to be emphasized that glucosidase II is neither a soluble nor an integral membrane protein but belongs to a third group of ER proteins that are only loosely associated with the luminal face of the ER membrane (Brada and Dubach, 1984; Strous et al., 1987). It remains to be established if one of the above mentioned or a different retention mechanism applies for glucosidase II. For such investigations, the kidney epithelial cells provide a natural model system since two species of glucosidase II exist, one that appears to be effectively retained in the ER and another that leaves this organelle. Work is in progress to determine if one or two genes code for different forms of glucosidase II and to disclose possible differences in the polypeptide structure that may be responsible for the differential distribution of the enzyme. It is equally possible that the sole difference is in the modification of the same polypeptide by glycosylation.

What is the possible role(s) of glucosidase II in post-Golgi apparatus compartments of kidney epithelia? We have shown that the sialylated form of the enzyme is catalytically active and, therefore, could be involved in glucose removal from glycoproteins present in these locations. There is certain evidence for transient addition of glucose residues to glycoproteins after they had left the ER (Parodi et al., 1983). Such glycoproteins could represent a substrate for glucosidase II, since glucose residues are not found on mature glycoproteins. Transient glucosylation and deglucosylation by glucosidase II may play a role in the intracellular targeting. Mutant G protein was posttranslationally glucosylated and accumulated in the ER at the restrictive temperature. Upon returning to the permissive temperature, G protein was deglucosylated and processed (Suh et al., 1989). Moreover, it was recently shown that the plant glucosidase II is able to catalyze a transglucosylation reaction (Kaushal et al., 1989). Thus, the kidney enzyme may be involved in glucosylation of certain acceptors. Alternatively, glucosidase II may play a role in the recognition and binding of glucose and/or mannose containing glycoconjugates (e.g., exhibit lectin-like properties) that could be important for endocytosis. At present we cannot attribute either function to glucosidase II present in post-Golgi apparatus compartments although it seems likely from our studies that a not yet characterized ligand is present there.

In conclusion, we have shown that a glycoprotein considered to exhibit an organelle-specific location may indeed be found in additional cellular organelles. This observation together with the previously reported differential subcompartmentation of the Golgi apparatus (Roth et al., 1986; Taatjes et al., 1988*b*) provides further evidence for diversity in functional organization of cellular organelles in relation to cell types.

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