Immunolocalization of the Oligosaccharide Trimming Enzyme Glucosidase II

John M. Lucocq, Daniela Brada, and Jürgen Roth

Interdepartmental Electron Microscopy Biocenter, University of Basel, CH-4056 Basel, Switzerland. Dr. Lucocq's present address is Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland. Dr. Brada's present address is Department of Biochemistry, University of California, Berkeley, California 94720.

Abstract. We used immunoelectron microscopy to localize glucosidase II in pig hepatocytes. The enzyme trims the two inner α1,3-linked glucoses from N-linked oligosaccharide precursor chains of glycoproteins. Immunoreactive enzyme was concentrated in rough (RER) and smooth (SER) endoplasmic reticulum but not detectable in Golgi apparatus cisternae. Transitional elements of RER and smooth membraned structures close to Golgi apparatus cisternae contained labeling for glucosidase II. Specific labeling was also found in autophagosomes. These results indicate strongly that glucosidase II acts on glycoproteins before their transport to, and processing in Golgi apparatus cisternae, and suggest that an important transitional region for glucosidase II exists between RER and Golgi apparatus cisternae. Degradation in autophagolysosomes could form a normal catabolic pathway for glucosidase II.

N-GLYCOSIDICALLY linked oligosaccharides of glycoproteins are transferred from a lipid-linked precursor, dolichol pyrophosphate-(*N*-acetylglucosamine)$_2$-(mannose)$_9$-(glucose)$_3$, to specific asparagine residues on nascent polypeptides (22). This occurs in the lumen of the rough endoplasmic reticulum (RER).[1] Posttranslational modifications of the oligosaccharide precursor chain start with the enzymatic removal of all three glucose residues by two α-glucosidases (11, 21, 26, 54, 55). Glucosidase I cleaves off the terminal α1,2-linked glucose and glucosidase II the two inner α1,3-linked glucoses. Processing of the oligosaccharide to high mannose, hybrid, or complex forms continues by trimming 1,2-mannosidases. α1,2-Mannosidase (mannosidase I) activities have been identified in ER (2) and Golgi apparatus (16, 46, 47, 52). Therefore, at least one mannose residue is removed before glycoproteins enter the Golgi apparatus. Further conversion to complex type oligosaccharides occurs via the action of *N*-acetylglucosamine transferase I (46) and α-mannosidase II, both of which have been localized to Golgi apparatus by immunoelectron microscopy (10, 32).

Though current data indicate that the early steps of oligosaccharide processing are compartmentalized, the precise intracellular location of glucosidase II is not known. Cellular fractionation studies have shown enrichment of glucosidase II activity (14) and immunoreactivity (4) in rough and smooth microsomal fractions. All three glucoses can be trimmed from G protein of vesicular stomatitis virus still associated with ribosomes (1). Pulse chase studies show rapid removal of two glucoses from glycoprotein precursors ($t_{1/2}$ <2 min, by glucosidase I, and $t_{1/2}$ ~5 min by glucosidase II), although the last is removed significantly slower than the others (after ~20–30 min, by glucosidase II) (21).

It has been suggested that proteins of the ER enter parts of the Golgi apparatus (41). The ER proteins investigated so far do not contain posttranslational modifications attributable to passage through the Golgi apparatus (27, 29, 35). Significant amounts of ER proteins (3, 17, 20, 24), including glucosidase II (4), have been found in subcellular fractions that contain Golgi apparatus membranes. However, direct evidence by in situ localization using immunocytochemistry is lacking.

Given that glucosidase II plays an important role in oligosaccharide processing and also possibly the intracellular targeting of glycoproteins (9, 12, 30), we decided to examine more closely the intracellular distribution of glucosidase II using high resolution immunoelectron microscopy. We applied specific antibodies against native glucosidase II to ultrathin sections of pig liver. In hepatocytes glucosidase II was found concentrated in the lumen of RER and smooth endoplasmic reticulum (SER). Golgi apparatus cisternae were not specifically labeled though transitional elements and smooth membraned profiles close to the Golgi apparatus contained glucosidase II. Autophagosomes, probably derived from cytoplasmic fragments that contain ER, were labeled.

The immunolocalization of glucosidase II indicates that this enzyme is concentrated in RER and SER and that it does not enter the Golgi apparatus cisternae in detectable amounts.

1. *Abbreviations used in this paper:* RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum.

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Materials and Methods

Reagents

Staphylococcal protein A was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Tetrachloroauric acid ($HAuCl_4 \cdot 4H_2O$), polyethylene glycol (20,000 mol wt), ascorbic acid, Tween 20, and Triton X-100 were from Merck (Darmstadt, FRG). Newborn calf serum came from Gibco (Basel, Switzerland). Polyvinyl pyrrolidone (40,000 mol wt) was from Fluka (Buchs, Switzerland) and bovine serum albumin (BSA) as well as ovalbumin were from Sigma Chemical Co. (St. Louis, MO). Protein A was iodinated by the chloramine-T procedure (23). Methylumbelliferyl-α-D-glucoside was from Koch light laboratories (Slough, England).

Preparation of Samples and Antibodies

Glucosidase II from pig kidney was purified, its activity measured, and the antibodies against it produced as described earlier (4). Pig liver extract for electrophoretic procedures was prepared as follows: Pig liver was briefly perfused with 0.25 M sucrose, 1 mM EDTA, 1 mM $PhMeSo_2F$ in order to remove blood, cut into small pieces, and frozen in liquid nitrogen. The tissue was powdered in liquid nitrogen and mixed with 50% trichloroacetic acid. Precipitated proteins were immediately neutralized with 1 M NaOH and an acetone powder was prepared as previously reported (4). Proteins were then extracted with 1% Triton X-100 in 10 mM NaP_i, pH 6.5 (60 mg of powder/1 ml of buffer).

Quantitative Immunoprecipitation

Pig livers were homogenized in 0.25 M sucrose, 1 mM EDTA, 1 mM Ph $MeSo_2F$ (1:4 wt/vol), the homogenate mixed with the same volume of 1% Triton X-100 in 10 mM NaP_i, pH 6.5, and extracted for 30 min at 4°C. High speed supernatant (100,000 *g* for 1 h) was used for the immunoprecipitation. 10 μl of this extract (diluted 200× to 1,000× with 0.15 M NaCl-0.01 M phosphate-buffered saline [PBS]) were incubated with 10 μl of the immune or control serum (diluted 10× with PBS) or 0.5% BSA in PBS at 37°C for 2 h. Immune complexes were bound to protein A-Sepharose CL-4B beads (for 1 h at 37°C), the beads were sedimented by centrifugation at 1,000 *g* for 5 min, and washed three times with 1% Triton X-100 in PBS (0.5 M NaCl).

Electrophoretic Procedures

SDS polyacrylamide gel electrophoresis was done according to Laemmli (28) and immunoblotting according to Towbin and co-workers (51) as reported before (4).

Staining of an immune replica by an enzyme-immunoassay was reported previously (4). Peptide mapping by limited proteolysis was according to Cleveland and co-workers (8) as modified by Tijssen and Karstak (48). Briefly, proteins from pig liver extract were resolved in a first dimension in a 7.5% SDS polyacrylamide gel and a gel strip that contained the polypeptides of interest was incubated in stacking buffer for 30 min. Then the strip was embedded perpendicularly in the spacer gel of a second SDS polyacrylamide gel and α-chymotrypsin solution layered onto it (48). When the bromophenol blue band approached the separating gel to a distance of 4–5 mm, the current was stopped for 30 min to allow digestion of the stacked polypeptides. The polypeptides were then separated in the second dimension in the second (10%) SDS polyacrylamide gel.

Tissue Preparation for Electron Microscopy

The liver of a single female pig (26 kilos, fasted 36 h) was perfusion fixed in situ via the portal vein. A prewash (1 min) with 0.1 M cacodylate buffer, pH 7.4, that contained 4% polyvinyl pyrrolidone and 70 mM $NaNO_2$ was followed by fixation with 3% (para)formaldehyde-0.1% glutaraldehyde in the same solution (without $NaNO_2$) for 10 min. Blanched hard liver portions (right lobe) were washed (3 × 5 min) in 0.1 M cacodylate buffer, pH 7.4, and placed in 0.1 M NH_4Cl in cacodylate buffer for 45 min. The tissue was then washed again in buffer (3 × 5 min). Some tissue pieces (~0.5-mm side) were embedded in Lowicryl K4M at −35°C after dehydration in graded ethanols at progressively lowered temperatures as previously described (6, 39). Other tissue pieces for preparing ultrathin frozen sections were stored at 4°C in 2% formaldehyde in cacodylate buffer or in buffer alone.

Immunocytochemistry

Lowicryl K4M Thin Sections. Thin sections (60-nm thick) of pig liver were incubated at room temperature on a drop of 0.5% ovalbumin in PBS, pH 7.4, for 5 min followed by antiserum against native glucosidase II diluted 1/5–1/20 in PBS that contained 0.5% Tween 20, 0.1% Triton X-100, and 1% BSA for 2 h. Inclusion of detergents reduced background staining over mitochondria and nucleus. After a brief wash in PBS (5 min), grids were applied to protein A–gold (particle size 6–8 nm). This was prepared as previously described (37, 44, 45) and diluted in PBS to an absorbance of 0.06–0.07 at 525 nm. After a wash in PBS (5 min) followed by distilled water, the sections were dried. Finally the sections were stained with 2% uranyl acetate (4–5 min) and lead acetate (45 s).

Ultrathin Frozen Sections. Sections, ~100-nm thick, prepared according to Tokuyasu (49), were preincubated on 10% newborn calf serum in PBS for 10 min and placed on antiserum against native glucosidase II diluted in 10% newborn calf serum (1/10–1/160) in PBS for 20 min at room temperature. Protein A–gold (6–8 nm) was used diluted in PBS, to an absorbance of 0.045 at 525 nm, for 25 min. Sections were contrasted with uranyl acetate and embedded in methyl cellulose (13, 50).

Cytochemical Controls. (*a*) Protein A–gold alone; (*b*) Nonimmune rabbit serum instead of antiserum (undiluted and diluted 1/10) followed by protein A–gold; (*c*) Preincubation of anti–glucosidase II anti-serum with purified native glucosidase II followed by protein A–gold.

Labeling that was reduced or absent in all three control conditions was considered to be specific for glucosidase II.

Quantification of Gold Particle Labeling. Areal density of gold labeling was measured by projecting negatives (~80,000× or 160,000× final magnification) taken on a Zeiss EM 10 electron microscope. Organelle areas (in μm^2) were measured by point counting methods (7) and gold particle density expressed as number of particles per $\mu m^2 \pm$ SEM. This involves placing a grid over projected negatives and counting the proportion of regularly spaced points that fall on structures of interest. An attempt was made to include roughly equal organelle areas in each micrograph, which was from a randomly selected cell, and to reduce SEM values to within 10% of the mean value. To assess gold particle distribution over RER, gold particles over the cisternal lumen, membrane, and cytoplasm between RER cisternae were counted and expressed as a percentage of total.

CMP-ase Enzyme Cytochemistry. CMP-ase (acid phosphatase) activity was localized using cytidine-5′ monophosphate as substrate and cerium chloride as capture reagent (34). The reaction was done on vibratome sections of fixed pig liver (20-μm thick), which were then embedded in Lowicryl K4M as above.

Results

Characterization of the Antiserum

The antiserum against the native pig kidney glucosidase II precipitated the enzyme activity specifically and quantitatively from a pig liver extract (Table I). When the proteins from the pig liver extract were separated electrophoretically, an immune replica of the gel revealed four bands of which the slowest moving corresponds to the enzyme subunit of pig kidney (Fig. 1*A*). We noticed that the liver glucosidase II is degraded even faster than the kidney enzyme when cell integrity is destroyed by biochemical procedures. The smaller polypeptides were very likely degradation products of the ~100-kD putative subunit. Staining of the immune replica by

Table I. Antiserum against the Pig Kidney Glucosidase II Immunoprecipitates Specifically and Quantitatively the Pig Liver Enzyme

	Glucosidase II activity	
	Pellet	Supernatant
	%	%
Immune serum	100	0
Control serum or 0.5% BSA in PBS	0	100

Pig liver extract was incubated with immune or control serum or 0.5% BSA in PBS. Immune complexes were bound to protein A–Sepharose CL-4B beads which were sedimented by centrifugation. Glucosidase II activity was measured in the pellets and supernatants.

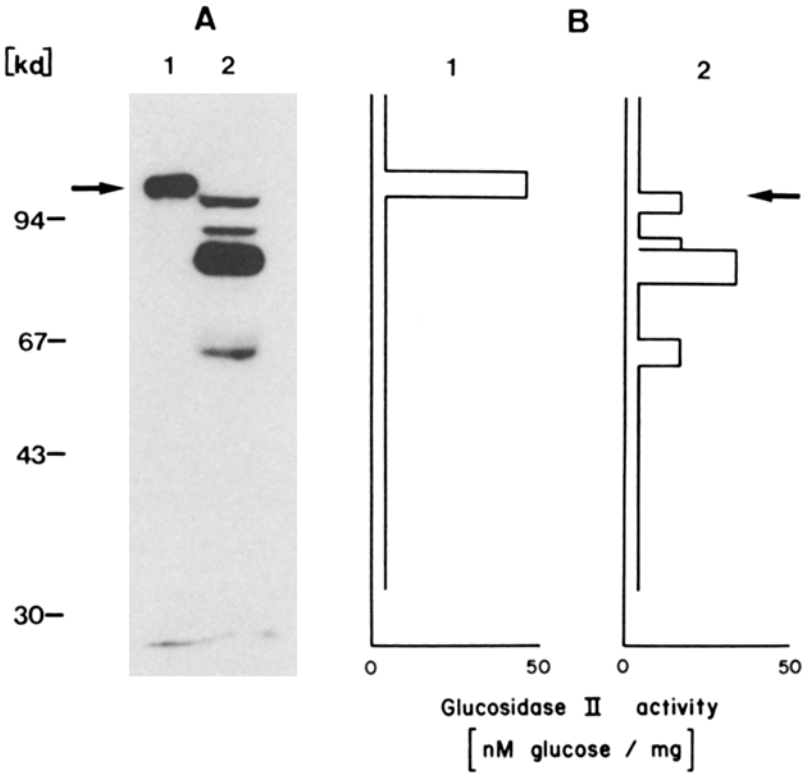


Figure 1. Pig liver glucosidase II subunit and all three smaller polypeptides present in an extract share immunogenic determinants with the active enzyme from pig kidney. (*A*) Proteins in pig liver extract were resolved in 7.5% SDS polyacrylamide gel, transferred to nitrocellulose, and an immunoblot prepared using the antiserum against native glucosidase II from pig kidney and ^{125}I-protein A. The arrow indicates the glucosidase II subunit. Lane *1*, Purified glucosidase II subunit from pig kidney. Lane *2*, Extract of pig liver. (*B*) Same as *A* except that an excess of antiserum was used and the ^{125}I-protein A step omitted. The nitrocellulose was then incubated with the active glucosidase II from pig kidney. The enzyme which bound to the free binding sites on immune complexes, immobilized on the nitrocellulose, was measured with the fluorogenic substrate methylumbelliferyl-α-D-glucoside. The amount of enzyme activity is expressed as nanomolar solution of glucose released per milligram weight of nitrocellulose pieces. When another enzyme-immunoassay (exactly the same except that the whole piece of nitrocellulose was incubated with the substrate soaked into a filter paper) was viewed under ultraviolet light, the same banding as in *A* appeared.

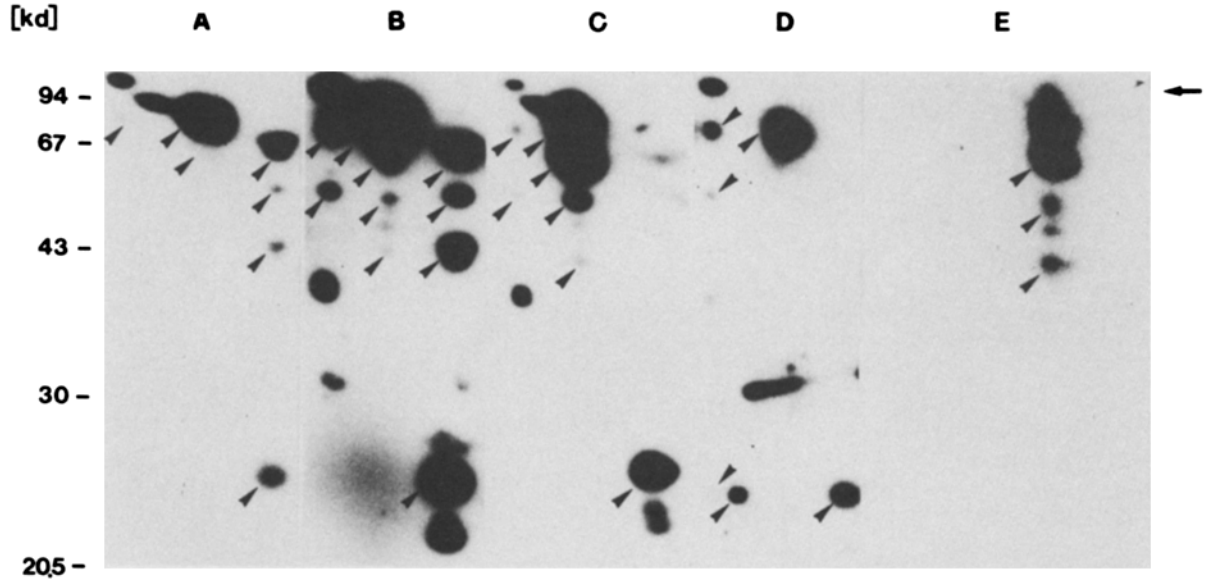


Figure 2. Limited proteolysis yields immunoreactive polypeptides shared by the glucosidase II subunit and also by the three faster migrating bands present in pig liver extract. Proteins in pig liver extract were separated in a first dimension by electrophoresis in 7.5% SDS polyacrylamide gel. In the second dimension a strip of this gel in which polypeptides were resolved was subjected to limited proteolysis and subsequent electrophoresis at right angles to the first separation in a 10% SDS polyacrylamide gel (see Materials and Methods). Resolved polypeptides were transferred to nitrocellulose. The gel replica was incubated with the antiserum against native glucosidase II from pig kidney and subsequently with ^{125}I-protein A. The immune complexes were visualized by autoradiography. *A*, *B*, *C*, *D*, and *E* differ only in the extent of digestion by the protease: it increases from *A* to *E*. In each photograph each individual column of spots represents the polypeptide fragments derived from one of the bands seen in Fig. 1 *A* (slowest moving band [subunit] to the left and fastest to the right), lane *2*. The arrow indicates the putative pig liver glucosidase II subunit. The arrowheads point to shared polypeptides that resulted from the limited proteolysis of separated polypeptides of Fig. 1 *A*, lane *2* by α-chymotrypsin.

an enzyme-immunoassay revealed indeed that not only the 100-kD form of pig liver glucosidase II, but also the three faster migrating bands, share the immunogenic determinants with the pig kidney glucosidase II subunit (Fig. 1 *B*). Moreover, immunoreactive degradation products of the same molecular weight have been noticed previously in pig kidney preparations (4) and rat liver extracts (Brada, D., unpublished observation).

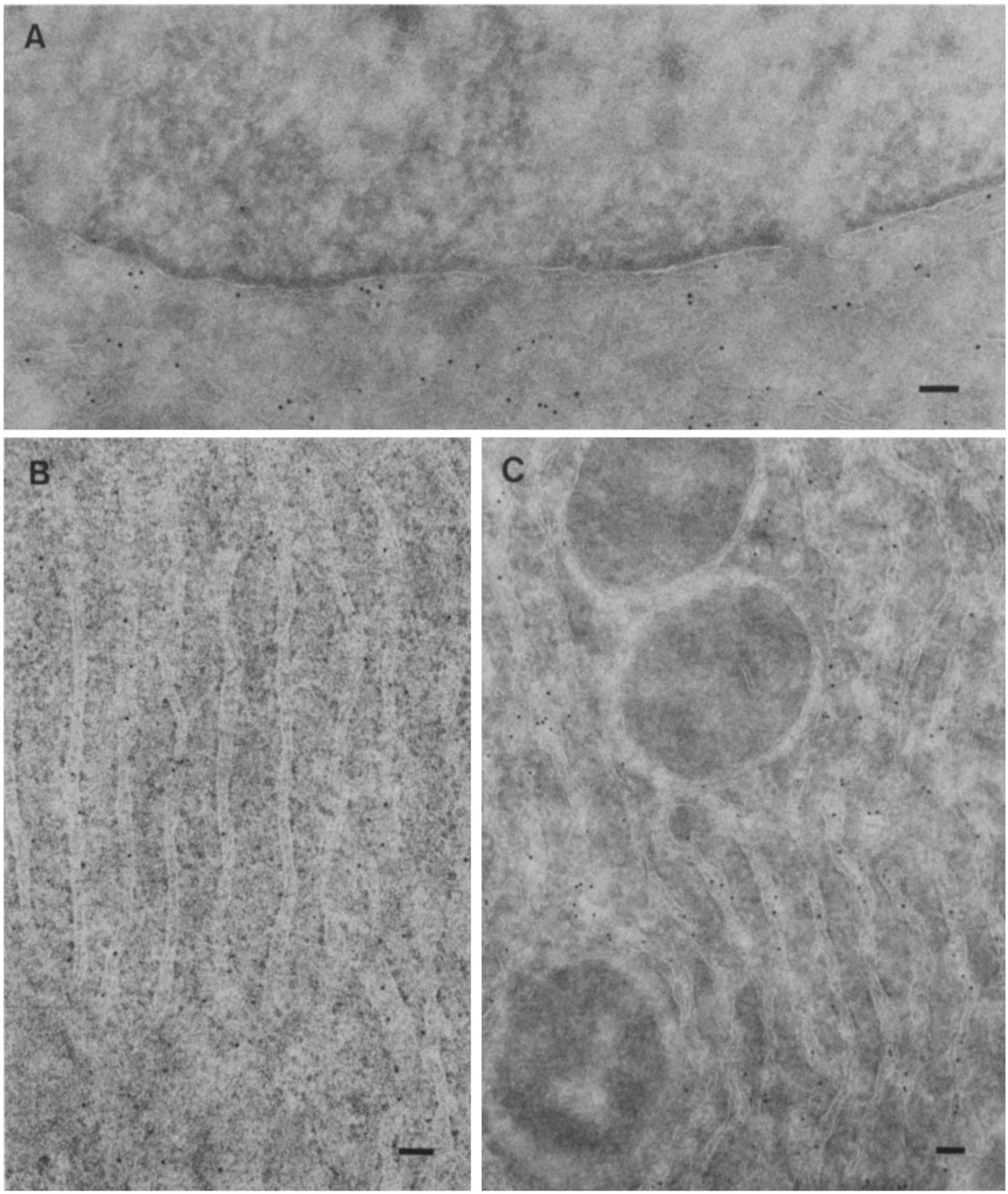


Figure 3. Glucosidase II localization in RER and nuclear envelope of pig hepatocytes. The luminal aspect of the nuclear cisterna is labeled and background over the nucleoplasm is low (*A*). The cisternal space of the RER is also labeled (*B* and *C*). (*A* and *C*) Ultrathin frozen sections; (*B*) a Lowicryl K4M thin section. Bars, 0.1 μm.

To further support the evidence that the smaller polypeptides are proteolytic degradation products of the 100-kD putative subunit of pig liver glucosidase II, limited proteolysis of the protein bands was done (Fig. 2). Limited proteolysis by α-chymotrypsin yields polypeptides of the same molecular weight from the 100-kD form and from the faster migrating bands in the pig liver extract.

In conclusion, the antiserum against the native pig kidney glucosidase II proved to recognize the pig liver enzyme specifically and quantitatively.

Immunocytochemistry

On ultrathin frozen sections and Lowicryl K4M thin sections, specific labeing for immunoreactive glucosidase II was observed principally over the nuclear envelope, RER, and SER (Figs. 3 and 4). In Lowicryl K4M sections, the distribution of

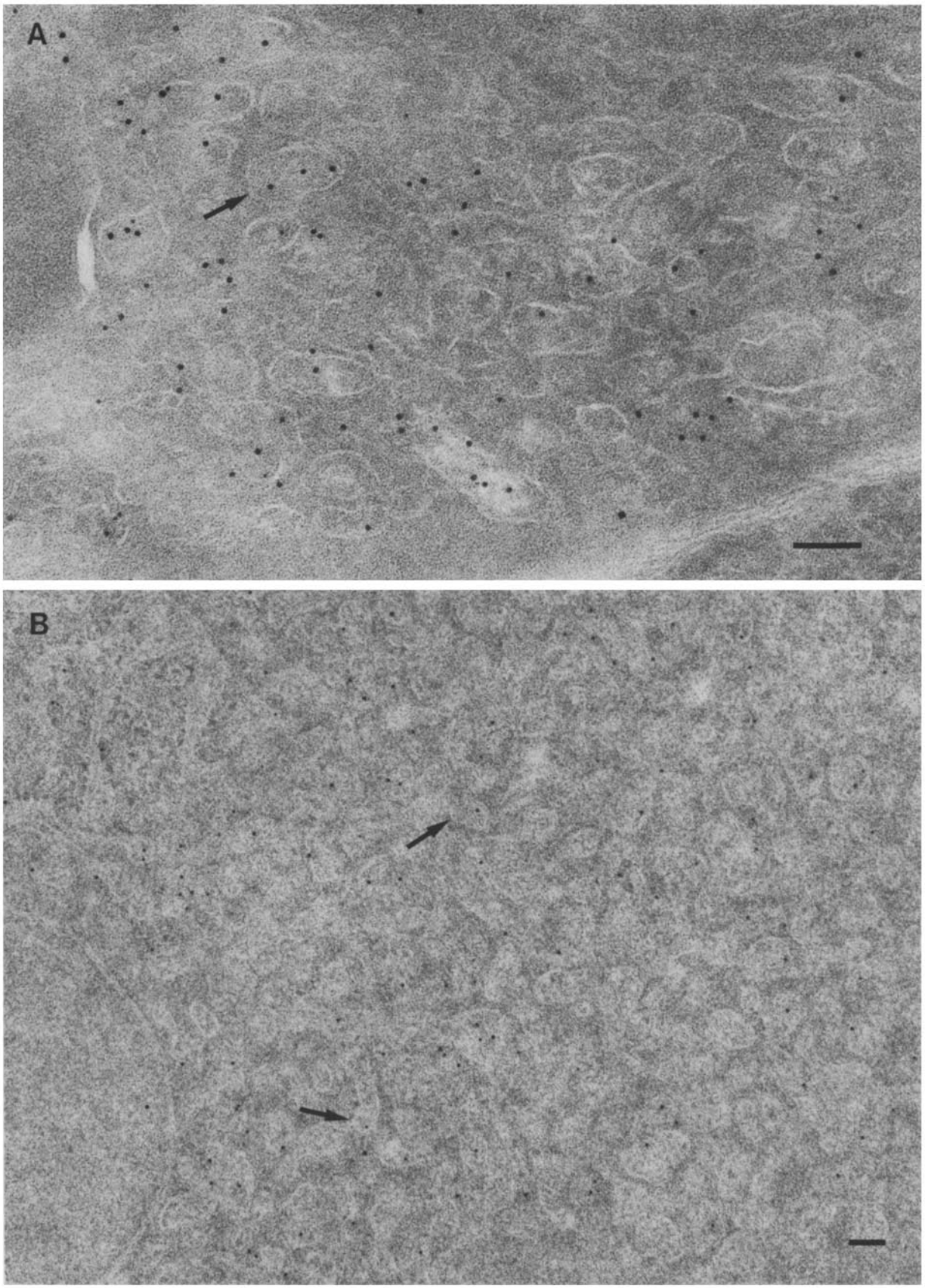


Figure 4. SER of pig hepatocytes. Gold particle labeling for glucosidase II is mainly situated over the lumen (*arrows*). (*A*) Ultrathin frozen section; (*B*) Lowicryl K4M thin section. Bars, 0.1 μm.

*Table II. Labeling Density of Gold Particles on Lowicryl K4M Thin Sections of Pig Hepatocytes**

	Glucosidase II antiserum		
	Exp. 1	Exp. 2	Control serum
RER	43.8 (± 2.4)	50.2 (± 1.4)	4.5 (± 1.8)
SER	45.5 (± 1.8)	42.1 (± 2.4)	ND
Nuclear envelope	26.4 (± 2.2)	27.1 (± 1.8)	ND
Golgi apparatus	4.8 (± 1.1)	6.0 (± 1.2)	4.8 (± 1.4)
Secondary lysosomes	3.7 (± 1.0)	1.7 (± 0.5)	ND
Autophagosomes	24.5 (± 3.6)	28.5 (± 2.6)	ND
Peroxisomes	11.7 (± 1.1)	15.2 (± 1.4)	16.5 (± 0.9)
Mitochondria	1.9 (± 0.3)	1.7 (± 0.3)	2.2 (± 0.5)
Nucleoplasm	1.4 (± 0.1)	1.4 (± 0.1)	ND

ND, not determined.

* Particles per μm^2 ± SEM (see Materials and Methods).

gold particles over the RER was the following: 68% over RER cisternal lumen; 11% over the RER membrane; and 21% between RER cisternae. This indicates that the antigenic sites of glucosidase II are situated mainly over the cisternal space of the RER on the luminal aspect of the RER membrane. The relationship of gold particles to RER membranes cannot be assessed with certainty in hepatocytes since cisternal membranes are closely apposed. However, in some sinusoidal cells, Ito's fat storing cells (Fig. 7), where the RER cisternae are wide, labeling was not intimately associated with the membranes. In hepatocytes the labeling density appeared similar in RER and SER but was about half these levels over the nuclear envelope (Table II).

The Golgi apparatus cisternae generally appeared unlabeled (Fig. 5). Occasional particles over lipoprotein-containing structures were also observed on incubation with nonimmune rabbit serum. Transitional elements of the RER and smooth surfaced profiles close to the *cis* and *trans* aspect of the Golgi apparatus, situated between RER and Golgi cisternae, appeared labeled (Fig. 5).

Autophagosomes labeled specifically for glucosidase II. Vesicular and tubular elements inside these structures were often labeled on their "luminal" aspect (Fig. 6, *A* and *B*). When glucosidase II and CMP-ase (acid phosphatase) were localized on the same section, both CMP-ase positive and negative autophagosomes were labeled for glucosidase II. However, some autophagosomes that contained CMP-ase reaction product were also negative for glucosidase II. Large lucent lysosomes that contained ferritin-like electron dense particles were labeled at background levels (Table II, Fig. 6*A*).

Sinusoidal and lateral plasma membrane domains showed no immunoreactive glucosidase II. Bile cannalicular membranes were consistently unlabeled on Lowicryl K4M thin sections. Weak labeling with concentrated antiserum on ultrathin frozen sections was considered to be nonspecific.

Nuclei and mitochondria showed low background labeling (Table II). Peroxisomes were not labeled on ultrathin frozen sections but showed nonspecific staining on Lowicryl K4M sections that could be reproduced with nonimmune rabbit serum (Table II).

Discussion

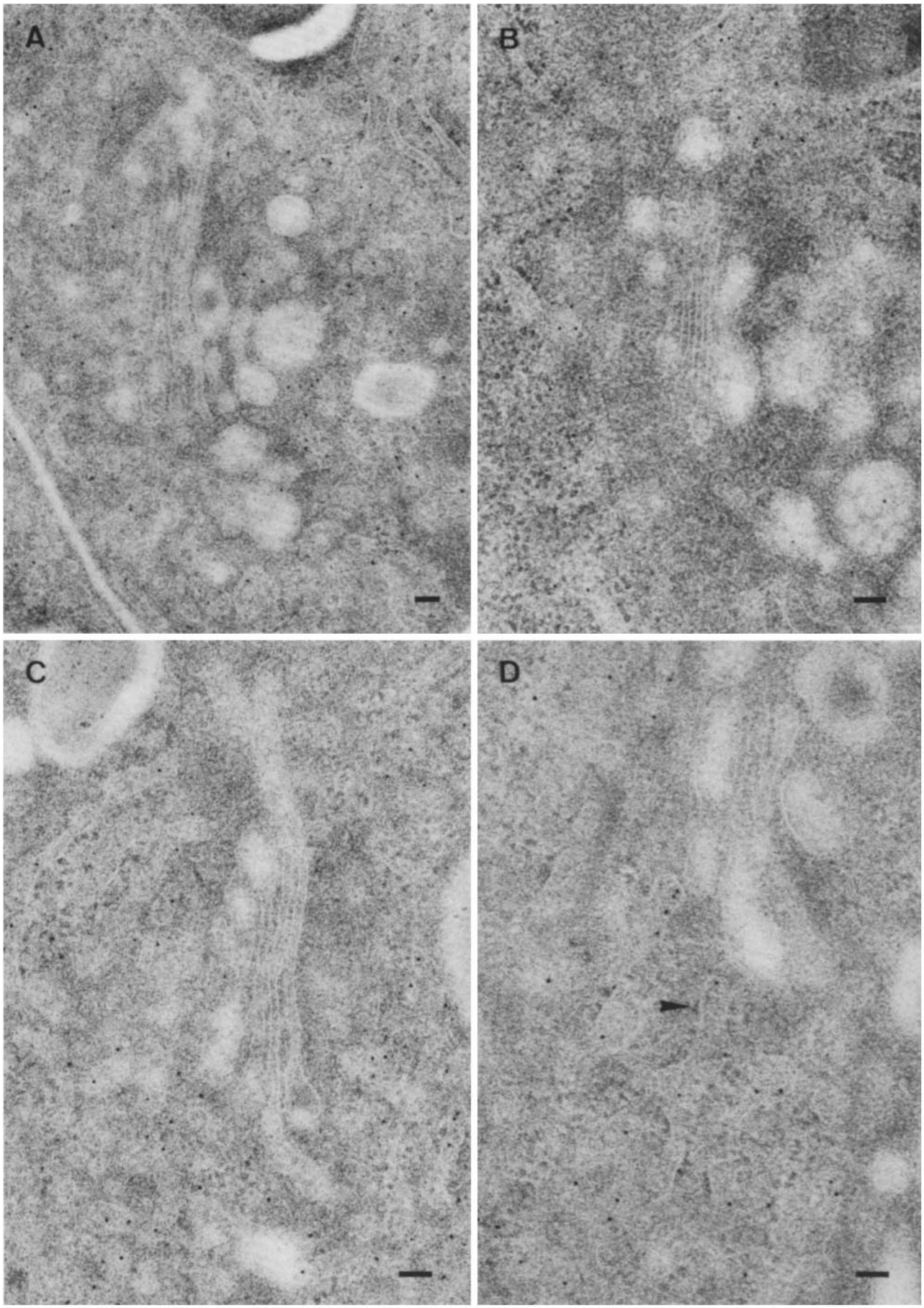
This work represents a first attempt to localize an endogenous ER protein in normal mammalian cells by post-embedding immunocytochemistry. Our in situ, high resolution study demonstrates that immunoreactive glucosidase II is concentrated in RER and SER of intact pig hepatocytes. Previous subcellular fractionation studies on rat liver have demonstrated that the enzyme activity and immunoreactive glucosidase II are enriched in rough and smooth microsomal fractions (4, 14). However due to the inherent limitations of the approach the precise intracellular distribution of the enzyme was difficult to reveal.

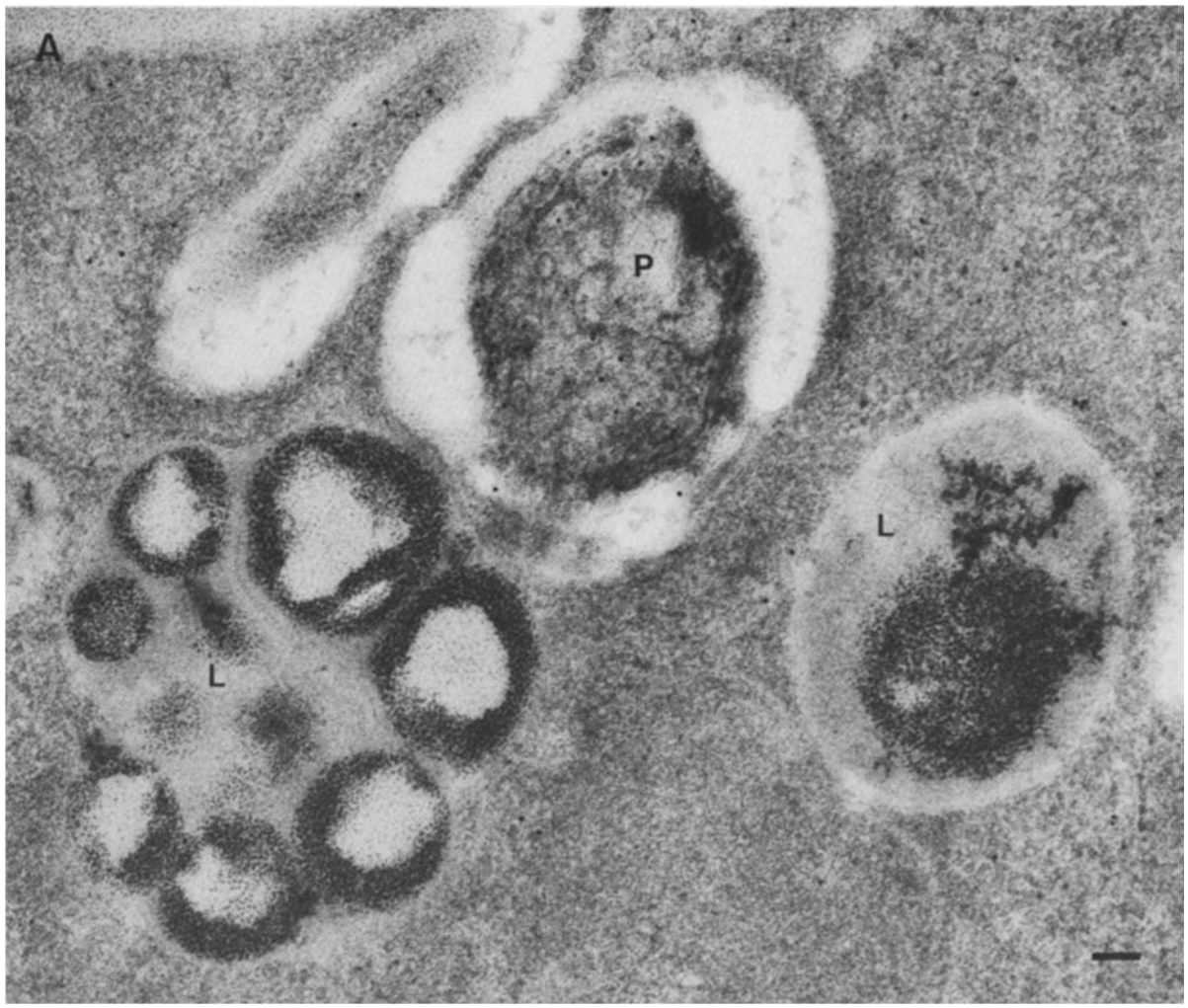
Studies on pulse labeled glycoproteins have demonstrated that the first glucose residue is rapidly removed ($t_{1/2}$ <2 min), the second one more slowly ($t_{1/2}$ ~5 min), and the third only after 20 or 30 min (21). G protein of vesicular stomatitis virus still has monoglucosylated oligosaccharides after 20–25 min of chase (26). In the present study the immunolocalization of glucosidase II to the ER is in accordance with the time course of glucose trimming. The late removal of the last glucose residue could be explained on the basis of compartmentalization of glucosidase II. However by in situ immunocytochemistry, we found the enzyme rather homogeneously distributed through the RER and SER. No intermediate compartment poor in glucosidase II labeling which would account for the lag in glucose removal could be identified. Therefore, more likely a difference in the rate of release of the second and third glucose residues could explain these biochemical results. In fact it has been reported that in vitro glucosidase II releases the second glucose two times faster than the third one (5).

The function of the glucose residues on the oligosaccharide precursor is not known. They are supposed to function as signal for the transfer to the nascent polypeptide chain (53) and also appear to protect the oligosaccharide precursor from degradation to a phosphooligosaccharide which starts the catabolic pathway (19). Moreover recent data suggest that the glucose residues and their trimming may be a prerequisite for the formation of complex-type oligosaccharide and proper phosphorylation of lysosomal enzymes (9, 12). The present finding that glucosidase II is concentrated in ER supports the notion that glucose removal from glycoproteins occurs before transport into the Golgi apparatus and therefore in separate cellular compartments to those in which conversion to complex-type oligosaccharides occurs, i.e., the Golgi apparatus (13, 22, 38).

Our quantitative and qualitative data suggest a mainly luminal location of glucosidase II in RER and SER. This is in accordance with in vitro experiments which showed that the enzyme activity and subunit were luminal in microsomal

Figure 5. Golgi apparatus of pig hepatocytes. The *cis*-Golgi region always lies to the left. Labeling for glucosidase II is absent from the cisternal stacks (*A–D*) but present in smooth-surfaced profiles (*A* and *C*) close to both sides of the Golgi apparatus. Transitional elements of RER (*arrowhead* in *D*) are also labeled. Lowicryl K4M thin sections. Bars, 0.1 μm.


A
B
C
D



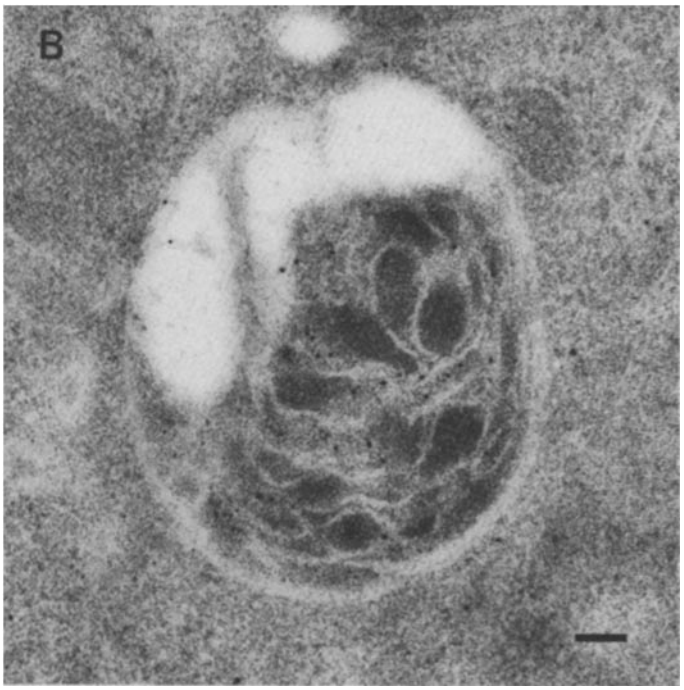


Figure 6. Glucosidase II labeling in autophagosomes and lysosomes of pig hepatocytes. (*A*) The content of autophagosome (*P*) is labeled. Lysosomes with ferritin-like content (*L*) are unlabeled. (*B*) The content of this autophagosome is positive for glucosidase II. Lowicryl K4M thin sections. Bars, 0.1 μm.

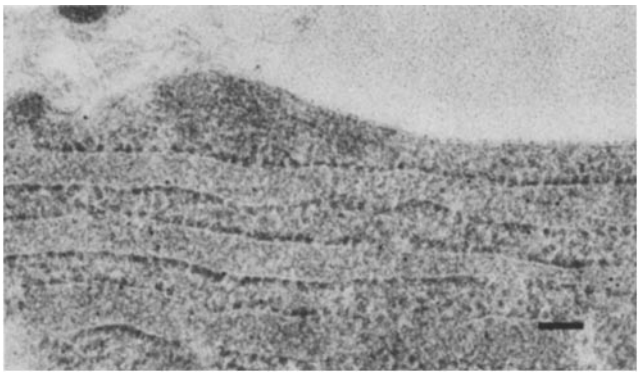


Figure 7. Fat storing cell of pig liver. The RER has characteristically wide cisternae. The labeling for glucosidase II is not intimately associated with membranes of the RER. Lowicryl K4M thin section. Bar, 0.1 μm.

membranes (4, 14). The gold particle labeling at the cytoplasmic aspect of RER membranes cannot be taken as evidence for antigenic sites in this location since the resolution of our technique is not better than the thickness of the RER membrane. In addition, the relationship of antigenic sites to RER membranes could not be precisely assessed in hepatocytes due to the narrow cisternal space. However in sinusoidal fat storing cells with wide RER cisternae (56), labeling for glucosidase II was not intimately associated with the membranes. In vitro the rat and pig liver enzymes could be released from detergent-treated microsomes in the presence of salt but not in its absence, conditions under which integral membrane proteins remained membrane bound (4). Altogether, these observations suggest that glucosidase II is probably only loosely associated with RER membranes.

Interestingly, nuclear envelope labeling was about half that in ER. This may reflect differences in turnover, or capacity of the membranes for glucosidase II. The specific activities of other ER enzymes is less in nuclear envelopes than in ER (15, 25, 42, 57). One might postulate that glucosidase II is specifically retained by (or in) RER (-like) membranes in amounts related to the quantity of these membranes. The lower labeling for glucosidase II could then reflect the fact that only one-half of the nuclear envelope, the outer membrane, is RER-like (31).

We did not find evidence for the presence of glucosidase II in Golgi apparatus cisternae. The lack of labeling probably reflects low concentrations within, or even absence from, this compartment. In either case a concentration difference for glucosidase II between ER and Golgi apparatus must somehow be generated and maintained. For example, glucosidase II might be excluded from Golgi apparatus cisternae either by active removal from them or simply by retention in ER membranes.

Immunoreactive glucosidase II was found in transitional elements of the RER and some smooth membraned structures close to, but not forming part of, the Golgi apparatus. These structures were often found situated between RER and Golgi apparatus cisternae. Saraste and Kuismanen (43) have found that at 15°C viral glycoproteins can be arrested in smooth membraned structures close to the Golgi apparatus before they enter the cisternae of this organelle. We do not know if the smooth membraned structures containing glucosidase II also contain proteins in transit from ER to Golgi apparatus, i.e., they form intermediates in the transport pathway. If they do then this compartment and/or transitional elements could be the sites where the apparent concentration difference of glucosidase II between RER and Golgi apparatus cisternae is generated. Finally, it is worth mentioning that some glycoproteins may be transiently monoglucosylated after all three glucose and up to two mannose residues have been removed (33). The authors suggested that transient glycosylation could serve as recognition signal in the targeting of the glycoprotein or to temporarily protect the oligosaccharide chain from improper processing. Trimming of such glucose residues late in the glycosylation pathway could be the main function of glucosidase II present in smooth membraned structures near the Golgi apparatus.

Is there any evidence that glucosidase II is processed in the Golgi complex? Other ER glycoproteins studied so far, HMG Co A reductase (29) and ribophorin I (35), do not contain complex oligosaccharide chains. Rat liver glucosidase II has been reported to contain at least one high-mannose oligosaccharide (18), but also to show a shift in its isoelectric point after neuraminidase treatment (5). However, on thin sections of Lowicryl K4M embedded pig liver we found that application of *Limax flavus* lectin (followed by fetuin–gold complexes) to visualize sialic acid residues (40), and *Ricinus communis* lectin I–gold complexes to demonstrate galactose residues (36), produced no specific labeling over ER. Also, a biochemical approach to examine pig liver glucosidase II oligosaccharides failed to support the presence of complex sugars in the molecule (unpublished observations).

Glucosidase II immunoreactivity could not be demonstrated in lucent secondary lysosomes (CMP-ase positive) that contained ferritin-like particles. However, specific labeling for the enzyme was found within bodies that contained segregated portions of cytoplasm surrounded by a single or double membrane. Some autophagosomes were positive and others negative for CMP-ase. However, both types could be shown to contain immunoreactive glucosidase II. At present the significance of these observations is not clear however we could speculate that segregation into autophagosomes and subsequent degradation of cytoplasm that contained glucosidase II may be a normal catabolic pathway for the enzyme in pig hepatocytes.

In summary our high resolution immunolabeling technique enabled us to determine precisely the intracellular distribution of glucosidase II. We found glucosidase II to be concentrated in the ER and to be undetectable in Golgi apparatus cisternae. These findings have important implications for the role of glucose trimming from oligosaccharide precursors in the processing and transport of glycoproteins between ER and Golgi apparatus.

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