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Immunocytochemical demonstration of the lysosomal enzyme α-glucosidase in the brush border of human intestinal epithelial cells

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In investigations on the intracellular transport route(s) of lysosomal enzymes in polarized epithelial cells, we used immunocytochemical methods to localize lysosomal α-glucosidase in human small-intestinal epithelial cells.

Two monoclonal antibodies which can discriminate between different biosynthetic forms of this enzyme were used. One monoclonal antibody, 43D1, which recognizes all forms of the enzyme, showed labeling of the Golgi apparatus, the lysosomes and, unexpectedly, of the brush border of the cells. Multivesicular bodies were free of label. In contrast, monoclonal antibody 43G8, which recognizes all forms except the 110000 Da precursor of α-glucosidase, showed labeling of the lysosomes only. This leads us to conclude that the 110000 Da precursor form of α-glucosidase is present in the Golgi apparatus and the brush border of human small-intestinal epithelial cells. Moreover, biochemical experiments show that this precursor copurifies with sucrase, a typical brush-border marker, when a partially purified microvilli fraction is prepared.

Introduction

To study the transport routes for endogenous glycoproteins in polarized epithelial cells we used the absorptive columnar cell of the small intestine as an example [5, 11]. With the aid of electron microscopic autoradiographical methods, we were able to study the intracellular transport or radiolabeled glycoproteins in general [5, 16]. However, recent methodological developments offer unique perspectives to study the biosynthesis and intracellular transport of individual endogenous glycoproteins. The availability of monoclonal antibodies [11, 19, 21] and the application of immunocytochemical techniques at the ultrastructural level [11, 31, 35] allowed us to study the immunocytochemical localization of a brush-border enzyme, sucrase-isomaltase [11, 19]. From these and earlier investigations [5] we were able to demonstrate the intracellular transport route taken by this newly synthesized glycoprotein.

With the ultimate goal of studying the molecular mechanisms responsible for the sorting of newly synthesized glycoproteins with different destinations in the cell, we now studied the biosynthesis and intracellular transport route of acid α-glucosidase, a typical enzyme of the lysosomes [18].

Biosynthesis of lysosomal enzymes is thought to proceed along the same pathway as described for other glycoproteins [30, 32, 33]. However, the intracellular transport route of lysosomal enzymes is still not clear. Furthermore, the site at which sorting of lysosomal enzymes from secreting glycoproteins occurs is as yet unidentified (for a recent review see [42]).

We investigated the immunocytochemical localization of the lysosomal enzyme α-glucosidase, using monoclonal antibodies that can discriminate between different biosynthetic forms of α-glucosidase [26]. Our rationale was that a direct demonstration of the intracellular localization of precursor and mature forms of the enzyme should enable us to elucidate the intracellular transport route of this lysosomal enzyme. Using this approach we found unexpectedly that a precursor of lysosmal α-glucosidase is present in the microvilli of human intestinal epithelial cells.

Materials and methods

Tissue processing for immunocytochemistry

Human jejunal biopsy specimens were obtained from subjects being examined for gastrointestinal disorders. Only freshly obtained material showing no pathological alterations in light and electron microscopy and having normal levels of brush-border enzymes was used in this study.

Immediately after collection, the biopts were cut in half; one piece was fixed immediately for routine electron microscopy and the other half was fixed in a mixture of 2% formaldehyde, freshly prepared from paraformaldehyde, and 0.1% glutaraldehyde in 0.1 M phosphate buffer, *p*H 7.4 for 2 h at room temperature, and stored in 2% formaldehyde in the same phosphate buffer at 4 °C until further processing.

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Tissue processing for immunochemical procedures

A jejunal segment of approximately 20 cm was obtained from a 54-year-old kidney donor. The segment was taken at a distance of about 40 cm distal from the pylorus and stored at −20 °C. After thawing, mucosal scrapings were obtained and suspended in a solution containing 50 mM mannitol and 2 mM Tris, *p*H 7.1. Homogenization and partially purification of the microvillar membranes was performed as described previously [38]. Briefly, the mucosal scrapings were homogenized in the Tris/mannitol buffer, using an Ultra-Turrax mixer. The homogenate was filtered through a small-mesh nylon gauze, and solid $CaCl_2$ was added to a final concentration of 10 mM. The homogenate was placed on ice and stirred for 30 min, and centrifuged at 2000*g* for 20 min. The supernatant (fraction S1) was centrifuged at 25000*g* for 30 min. Both the soluble glycoproteins in the supernatant (fraction S2) and the partially purified microvilli in the pellet (fraction P2) were retained for immunoprecipitation experiments. Purification was monitored by the activity of sucrase, a typical brush-border marker, according to the method of Dahlqvist [9]. One unit of sucrase activity is defined as 1 µmol substrate hydrolyzed per min per mg protein. For immunoblotting experiments, glycoprotein fractions were prepared by incubating S1, S2, and P2 with Concanavalin A-Sepharose 4B and eluting the beads with 1 M methyl-α-D-glucopyranoside as described [25]. The eluates were dialyzed before use.

Antibodies

Monoclonal antibodies 43G8 and 43D1 were obtained using splenocytes from a mouse that has been immunized with lysosomal α-glucosidase from human placenta [21]. Preparations of purified lysosomal α-glucosidase from placenta contain exclusively intermediate (M_r 95000) and mature (M_r 76000 and 70000) forms of the enzyme [21, 25]. In contrast, such preparations from urine also contain a 110000 Da precursor form of the enzyme [25].

The monoclonal antibodies were partially purified from the culture medium of the hybridoma cell lines by 50% $(NH_4)_2SO_4$ precipitation at 4 °C. The precipitate was dissolved in water, dialyzed against phosphate buffered saline and stored in small aliquots at −20 °C. Polyclonal antibodies were obtained by immunization of a rabbit with lysosomal α-glucosidase purified from human placenta. The antiserum obtained was monospecific [25, 37].

Immunocytochemical procedures

Low temperature embedding in Lowicryl K4M was performed according to techniques described elsewhere [1, 31]. Briefly, fixed tissue fragments were dehydrated in a graded ethanol series, during which the temperature was lowered stepwise to −35 °C. Infiltration with Lowicryl K4M and embedding in Beem capsules took place at −35 °C. Polymerization by UV light was performed for 48 h at −40 °C, followed by 48 h at room temperature. Sections were preincubated for 5 min at room temperature on drops of 1% bovine serum albumin dissolved in phosphate buffered saline, *p*H 7.4 (PBS/BSA). Sections were incubated at room temperature successively with monoclonal anti-(α-glucosidase) antibody dissolved in PBS/BSA for 1 h, with rabbit anti-(mouse IgG) in PBS/BSA for 1 h, and finally with protein A complexed to 10 nm colloidal gold particles [35] in PBS/BSA for 1 h. In some experiments rabbit anti-(α-glucosidase) antibodies were used. The procedure was as described above, except that the incubation with the second antibody was omitted. The sections were washed thoroughly with PBS/BSA after each incubation. In control incubations the first antibody was omitted, which resulted in little to no background labeling. The optimal dilution of the first antibody was established in a dilution series, in which the labeling over the mitochondria and the nucleus was used as criterion for the background labeling of the first antibody. After washing with distilled water, the sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate.

Ultrathin cryosectioning and immunolabeling of the cryosections was performed according to techniques described previously [11]. The sections were examined in a Philips EM 201 or EM 410 electron microscope operating at 80 kV.

Immunochemical procedures

Immunoprecipitation of α-glucosidase from fibroblasts that had been metabolically labeled with [^{35}S]methionine was carried out as described previously [25]. Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) followed by immunoblotting with the different antibodies was carried out as described previously [28].

α-Glucosidase activity in Triton X-100 extracts of fractions S1, S2 and P2 of intestinal mucosa was determined after immunoprecipitation of the enzyme with Sepharose 4B to which monospecific rabbit anti-(placental α-glucosidase) antibodies had been covalently coupled. After centrifugation, the activity on the beads was measured as described previously [25]. One unit of α-glucosidase activity is defined as 1 µmol substrate hydrolyzed per min per mg protein.

Quantification of the amount of precursor form present in fractions S1, S2 and P2 was performed according to methods described previously [26]. Briefly, the α-glucosidase activity in Triton X-100 extracts of the fractions was titrated with monoclonal anti-(α-glucosidase) antibody 43G8 bound to Sepharose 4B beads. After centrifugation, the α-glucosidase activity remaining in the supernatant, which represents the precursor form of α-glucosidase [26], was precipitated with immobilized rabbit anti-(α-glucosidase) antibodies and measured as described above.

Results

Molecular forms of α-glucosidase and their reactivity with monoclonal antibodies

Previous studies using metabolically labeled fibroblasts [27], have shown that lysosomal α-glucosidase is synthesized as a precursor with an apparent M_r of 110000 which is processed via a 95000 Da intermediate form to a mature form of M_r 76000. Figure 1 illustrates this sequence of events. After longer chase periods a doublet of 70000 Da is also observed [27].

Since the half-life of the precursor form of α-glucosidase is very short compared with the half-life of the mature enzyme molecule, only a very small fraction of the α-glucosidase activity represents precursor molecules. Indeed, immunoblotting of α-glucosidase from placenta [18, 21] or fibroblasts [28] reveals only the intermediate and mature forms of the enzyme; apparently the amount of precursor α-glucosidase is too low to be detected by this method. On the other hand, about 50% of the α-glucosidase activity in urine represents the 110000 Da form of the enzyme [25].

We have produced a panel of monoclonal antibodies against human lysosomal α-glucosidase purified from placenta [21]. One of these antibodies, 43G8, exhibits a specific affinity for processed forms of the enzyme. As shown previously [26], in immunoprecipitation studies with native enzymes, monoclonal antibody 43G8 recognizes the 95000, 76000 and 70000 Da forms of α-glucosidase but not the 110000 Da precursor form. When the α-glucosidase mole-

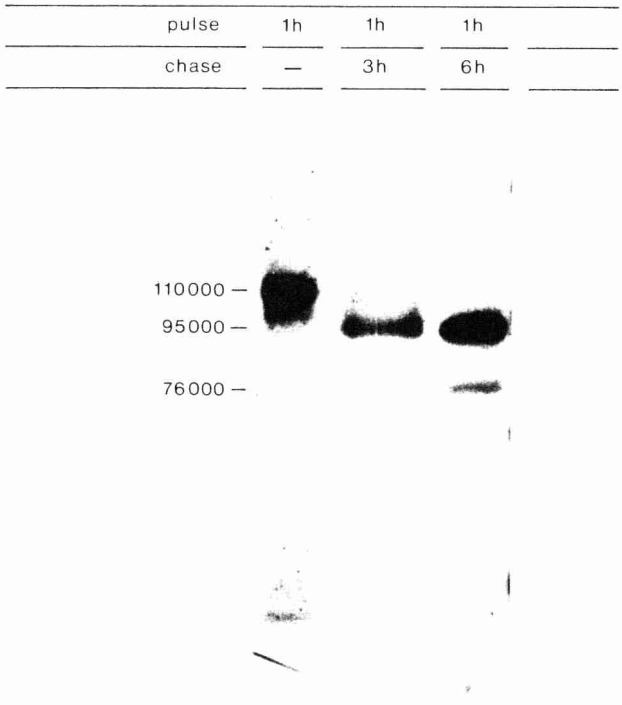


Fig. 1. Processing of α-glucosidase in human fibroblasts. Confluent human fibroblasts were pulse-labeled for 1 h with 0.1 mCi [^{35}S]methionine and either harvested immediately or chased with unlabeled medium for 3 or 6 h. The cells were lysed and α-glucosidase was immunoprecipitated with a monospecific polyclonal rabbit antiserum against the enzyme. The precipitated material was analyzed by SDS-PAGE followed by fluorography to visualize the radioactive proteins.

cule is completely denatured, as after SDS-PAGE followed by blotting onto nitrocellulose, monoclonal antibody 43G8 reacts only with the 95000 and 76000 Da forms. In addition, we used monoclonal antibody 43D1 which reacts with all forms of the enzyme.

Both monoclonal antibodies were tested for their specificities against glycoproteins in the partially purified microvilli fraction (P2) and against soluble glycoproteins remaining in the supernatant (S2). In an immunoblot monoclonal antibody 43D1 recognizes the 110000, 76000 and 70000 Da forms of α-glucosidase in the P2 fraction (Fig. 2); the reaction with the 110000 Da band was weak. In both the S1 and the S2 fractions this monoclonal antibody only recognized the 76000 and 70000 Da bands; the 110000 Da precursor form could not be detected.

Monoclonal antibody 43G8, known to react only with the intermediate and mature forms of α-glucosidase in fibroblasts and urine [26] shows the same specificities in intestinal tissue. In the P2 fraction it recognized the 76000 Da form of the enzyme (Fig. 2). No reaction was observed with the 110000 Da form in the P2 fraction or in the urinary enzyme preparations.

The different specificities of these two monoclonal antibodies thus allow one to study the immunocytochemical localization of precursor and mature forms of α-glucosidase in intestinal epithelial cells.

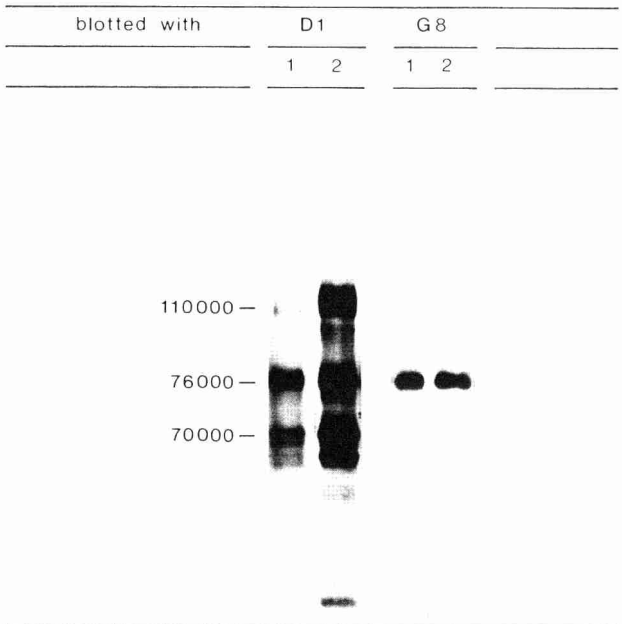


Fig. 2. Molecular forms of α-glucosidase present in the partially purified microvilli fraction. Partially purified microvilli from human intestine *(lanes 1)* and purified total α-glucosidase from human urine *(lanes 2)* were analyzed by SDS-PAGE followed by immunoblotting with monoclonal antibodies 43D1 (= D1) and 43G8 (= G8) as described in Materials and methods.

Immunocytochemistry

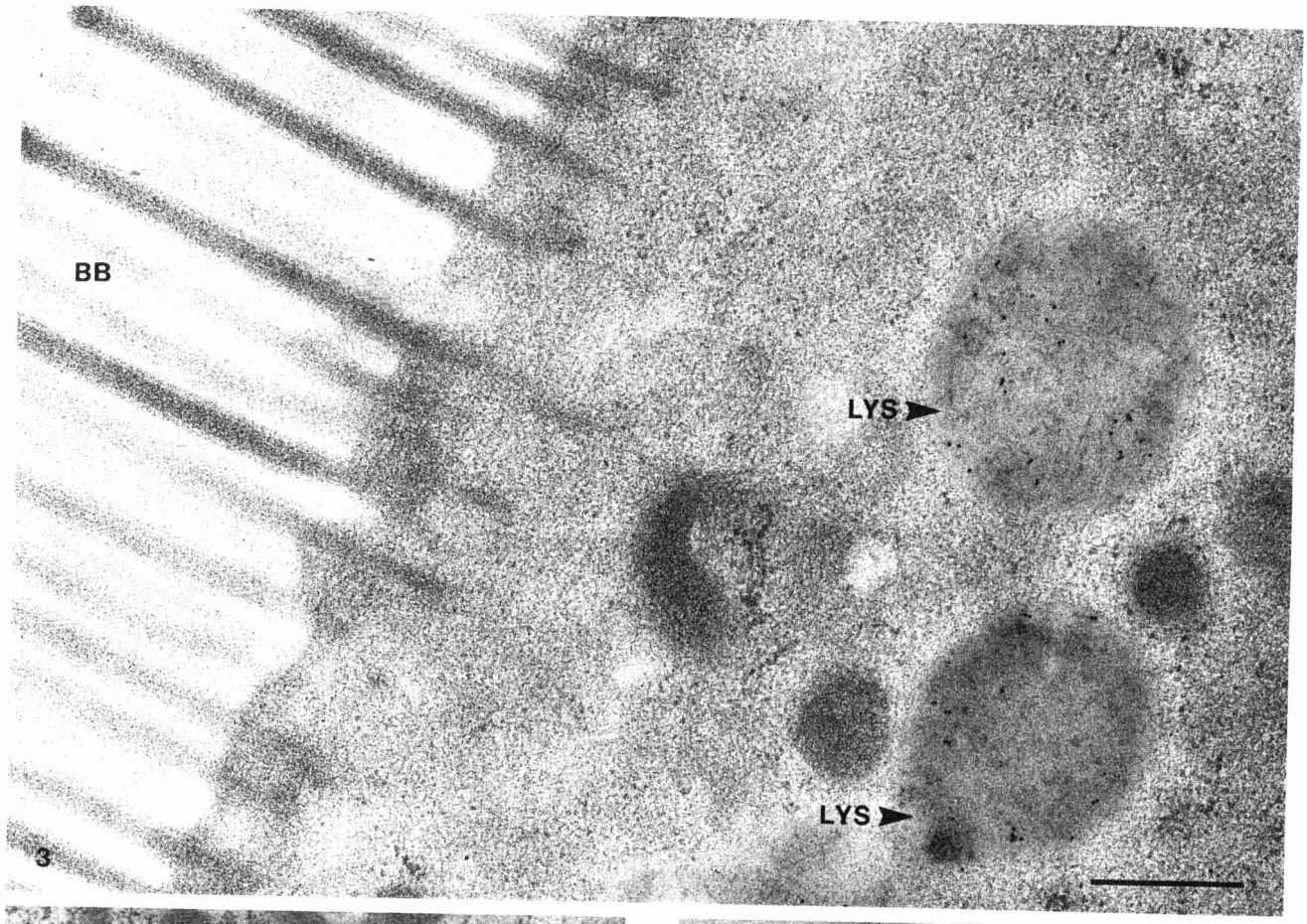
In the apical cytoplasm of the small-intestinal epithelial cells multivesicular bodies (mean diameter 0.36 μm), in which the matrix contains small vesicles, and electron-dense bodies, i.e., the lysosomes (mean diameter of 0.53 μm), have been described [15]. Both organelles were easily recognized in cryosections and sections of Lowicryl embedded tissue.

With a monospecific polyclonal antiserum, raised against placental α-glucosidase, gold labeling was observed over the Golgi apparatus, the electron-dense bodies and, unexpectedly, over the brush border of the intestinal epithelial cells; multivesicular bodies and the basolateral membrane were free of label. In view of the possibility that the polyclonal antiserum was not monospecific under the

Fig. 3. Immunolabeling of a section of Lowicryl K4M embedded human intestinal epithelium with monoclonal antibody 43G8. Only the lysosomes are labeled with this antibody. — BB Brush border. — LYS Lysosomes. — Bar 0.5 μm.

Fig. 4. Immunolabeling of a section of Lowicryl K4M embedded human intestinal epithelium with monoclonal antibody 43G8. Labeling is only observed over the lysosomes. The Golgi apparatus is free of label. — LYS Lysosomes. — GA Golgi apparatus. — Bar 1 μm.

Fig. 5. Immunolabeling of a section of Lowicryl K4M embedded human intestinal epithelium with monoclonal antibody 43D1. This antibody showed labeling over the brush border of these cells. — BB Brush border. — Bar 0.5 μm.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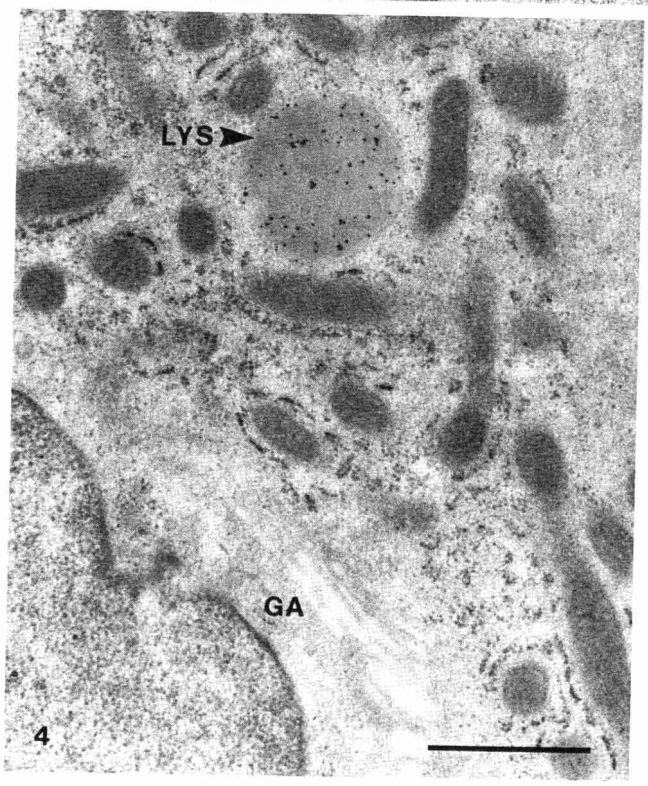
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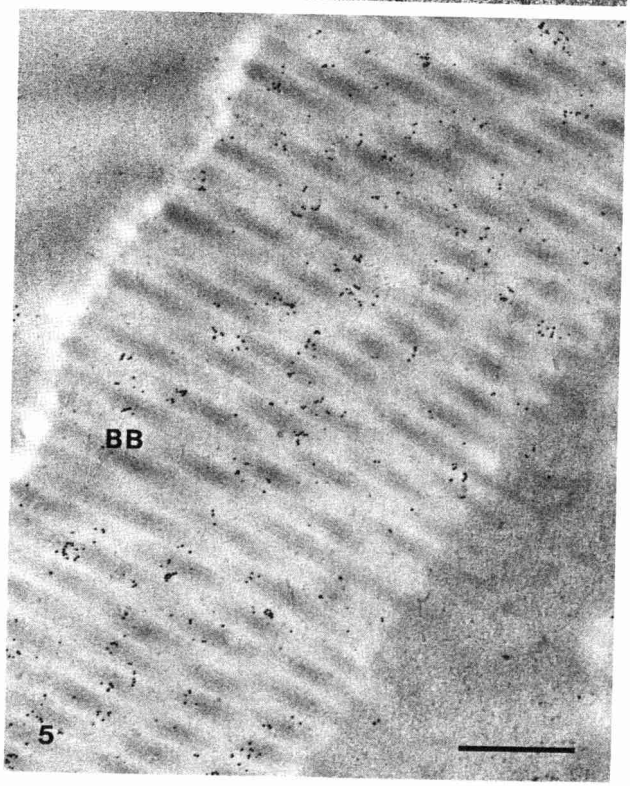
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5

Tab. I. Forms of α-glucosidase present in different fractions of human intestinal mucosa.

Fraction	Sucrase activity	α-Glucosidase activity			
		total	bound to 43G8	Precursor	
	mU/mg	mU/mg	mU/mg	mU/mg	(% of total)
S1	164.3	0.69	0.57	0.13	18
S2	37.7	0.73	0.68	0.05	7
P2	1194.4	2.58	1.57	1.02	39

A postnuclear supernatant (S1) was prepared from human intestinal mucosal scrapings, and a partially purified brush-border fraction (P2) was isolated as described in Materials and methods. Fraction S2 represents the supernatant obtained after centrifugation to obtain the P2 fraction. The immunoprecipitable α-glucosidase activity and the activity of sucrase, a brush-border marker enzyme, were determined in Triton X-100 extracts of the fractions as described in Materials and methods. Precursor α-glucosidase is defined as the activity that does not bind to monoclonal antibody 43G8 immobilized by coupling to Sepharose 4B beads (see text for details).

conditions of the immunocytochemical assay, we first repeated the experiments with a mixture of 11 monoclonal antibodies against α-glucosidase [21]. The same localization was observed.

Subsequently, the monoclonal antibodies were tested separately. Three out of the eleven monoclonal antibodies showed a positive reaction in the immunocytochemical assay. Two antibodies, i.e., 43G8 and 43D1, were used for further studies because of their specificities (see above).

Monoclonal antibody 43G8, which reacts with all native forms of α-glucosidase except the 110000 Da precursor form, labeled only the electron-dense bodies (Fig. 3). The brush border (Fig. 3) and the Golgi apparatus (Fig. 4) did not show any labeling. Multivesicular bodies were also negative. In contrast, monoclonal antibody 43D1 which reacts with all forms of α-glucosidase, labeled the microvilli and the electron-dense bodies of these cells (Figs. 5, 6a; Tab. II). The Golgi apparatus showed a very weak labeling, predominantly in the trans-Golgi region (Fig. 6b). The multivesicular bodies and the basolateral membrane were devoid of labeling (Fig. 6c).

These results indicate that the 110000 Da precursor of α-glucosidase is present in the microvilli and the Golgi apparatus of human small intestinal epithelial cells.

Quantification of the amount of precursor present

In an attempt to quantify the amount of precursor present, we measured the α-glucosidase activity in the different fractions using the specificity of monoclonal antibody 43G8.

As shown in Table I, in the postnuclear supernatant (fraction S1) of human intestinal mucosa, about 20% of the total immunoprecipitable α-glucosidase activity does not bind to monoclonal antibody 43G8 and represents the 110000 Da precursor form of the enzyme. In fibroblast homogenates less than 10% of the α-glucosidase activity is due to the precursor form of the enzyme (not shown; [28]).

The immunoprecipitable α-glucosidase activity that does not bind to monoclonal antibody 43G8 copurifies with sucrase when a partially purified brush-border fraction (P2) is made (Tab. I). In this P2 fraction about 40% of the total

Tab. II. Labeling patterns obtained with monoclonal antibodies 43D1 and 43G8.

Antibody	Specificity	Labeling of the				
		GA	MVB	LYS	MV	BLM
43D1	110000, 95000, 76000, 70000 Da	+	–	+	+	–
43G8	95000, 76000, 70000 Da	–	–	+	–	–

Labeling obtained over the different cell organelles in intestinal epithelial cells, after the immunocytochemical procedures described in Materials and methods. — Abbreviations: GA Golgi apparatus. — MVB Multivesicular bodies. — LYS Lysosomes. — MV Microvilli. — BLM Basolateral membrane. The absence of label with antibody 43G8 over the Golgi apparatus and the microvilli and its presence with antibody 43D1 indicate that the 110000 Da precursor is present in these organelles.

α-glucosidase activity does not bind to monoclonal antibody 43G8 and apparently represents the 110000 Da precursor form.

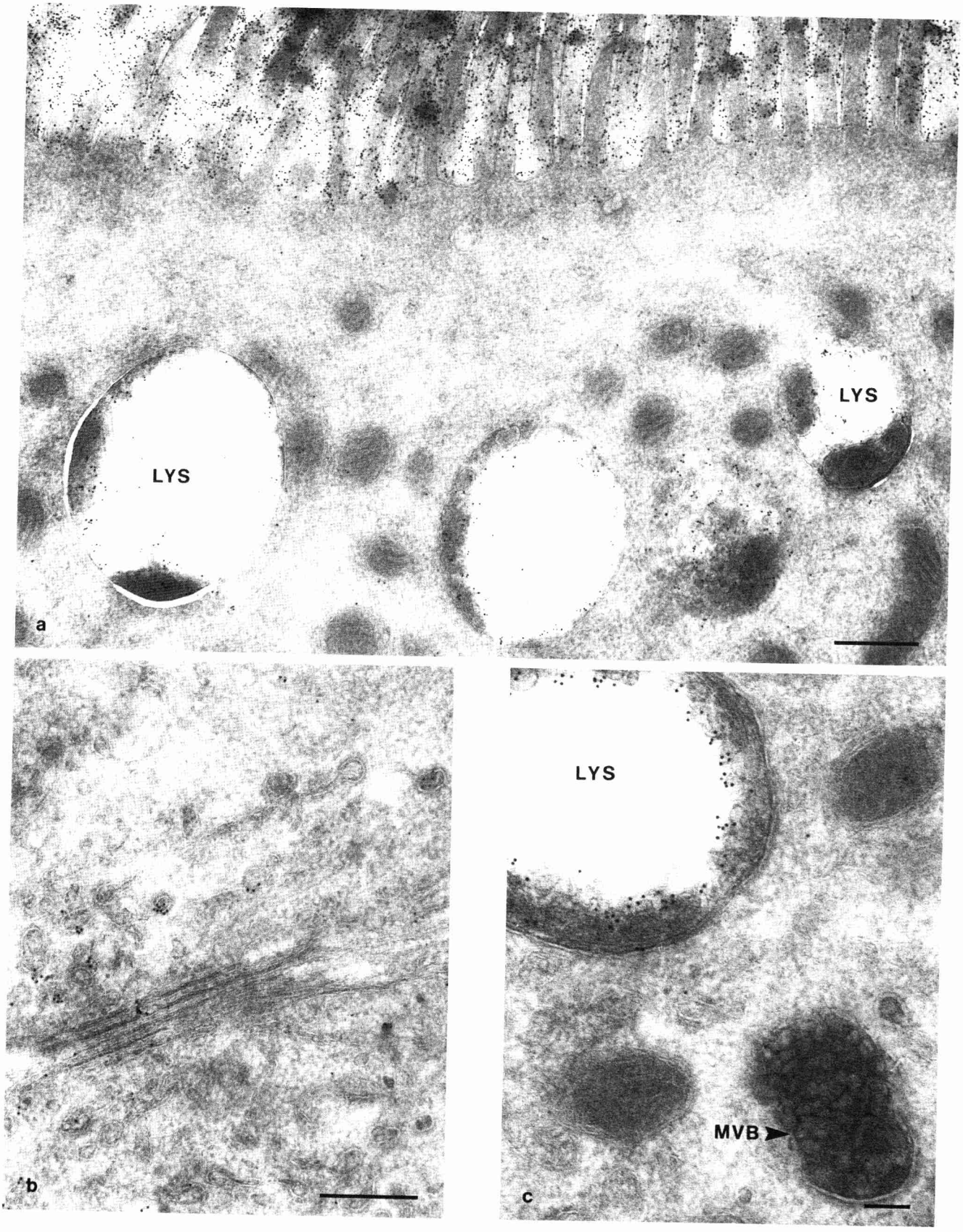
Discussion

We used immunocytochemical methods for the localization of lysosomal α-glucosidase in the human intestinal enterocyte to determine the intracellular transport route(s) of this enzyme. Using monoclonal antibodies which can discriminate between different biosynthetic forms of the enzyme, we came upon the unexpected finding that a precursor form of α-glucosidase appears to be present in the microvilli of human intestinal epithelial cells.

The labeling of the Golgi apparatus and the lysosomes was expected from previous immunocytochemical studies on the localization of lysosomal enzymes in different cell types [24, 39, 40, 43, 45, 46]. The weak labeling of the Golgi apparatus can be explained by the low steady-state concentration of the enzyme in the Golgi apparatus due to the relatively short sojourn of lysosomal enzymes in this compartment [14, 27]. The labeling of the Golgi apparatus seems to be concentrated at the trans face. No labeling of the multivesicular bodies was observed with any of the antibodies used in this study.

The specificities of the antibodies are an important prerequisite for immunocytochemical studies [34]. Because of the strong labeling of the microvilli we carefully checked the reactivity of the monoclonal antibodies 43D1 and 43G8 against human intestinal tissue. In both immunoblotting and immunoprecipitation experiments cross-reactivity was never observed with bands other than those specific for α-glucosidase. As shown, the specificities of these monoclonal antibodies also correspond to those observed in studies with fibroblasts [25, 26]. The 95000 Da interme-

Fig. 6. Immunolabeling of cryosections of human intestinal epithelium with monoclonal antibody 43D1. — **a.** Labeling of the lysosomes and the brush border. — **b.** Labeling of the Golgi apparatus, predominantly in coated vesicles at the trans face. — **c.** Labeling of a lysosome; the multivesicular bodies are free of label. — LYS Lysosomes. — MVB Multivesicular bodies. — Bars 0.5 μm (**a**, **b**), 0.1 μm (**c**).


LYS
LYS
a
b
LYS
MVB
c

diate form could not be detected in immunoblots. This is due to the low amount of the intermediate form present in the steady-state situation. The specificity of monoclonal antibody 43G8 was tested previously [26]. Under nondenaturing conditions, as in immunoprecipitation studies, it recognizes the 95000, 76000 and 70000 Da forms, but not the 110000 Da form. These conditions correspond to the mild conditions used for the immunocytochemical studies in which a low temperature embedding procedure that should prevent denaturation of proteins is used [2]. This is illustrated by the observation that eight of the monoclonal antibodies in our panel which react with α-glucosidase only under denaturing conditions, e.g., in an immunoblot or in an enzyme-linked immunosorbent assay, do not react in the EM immunocytochemical procedures (not shown). Furthermore, in ultrathin cryosections, where the conditions are even milder, the same observations were made. Thus, in our immunocytochemical studies, the only difference between the two monoclonal antibodies is their reaction with the 110000 Da precursor form; this form is not recognized by monoclonal antibody 43G8. It is only in immunoblots, i.e., under denaturing conditions, that monoclonal antibody 43G8 also does not react with the 70000 Da forms of α-glucosidase, probably because of the denaturation of this "aged" mature form, which in pulse-chase experiments is found only after long chase periods [26].

With immunocytochemistry one can only study differences in localization qualitatively. It is not possible to draw quantitative conclusions since labeling efficiencies vary considerably between different cell organelles [17]. This is illustrated by the observation that the labeling intensities of the lysosomes and the Golgi apparatus are similar in Lowicryl K4M- and cryosections, whereas the labeling intensity of the brush border varies considerably (Figs. 5, 6a). In addition, the 110000 Da band is not detectable in an immunoblot of the soluble glycoprotein fraction from intestinal mucosa. We were only able to label the 110000 Da precursor form in immunoblots of the P2 fraction after preparing glycoprotein fractions and subsequent immunoprecipitation. Therefore, we do not consider these immunoblots to give quantitative results. We believe that it is impossible to assess the quantitative distribution of the different forms of α-glucosidase by comparing the immunocytochemical and immunoblotting data. We attempted to quantify the amount of precursor present in the different fractions by immunoprecipitation studies with monoclonal antibody 43G8. As shown in Table I, the α-glucosidase activity which did not bind to 43G8 copurified with the brush-border marker sucrase (enrichment of the two activities 7-8-fold) and about 40% of the total α-glucosidase activity in the partially purified microvilli fraction represents the precursor form, in contrast with about 7% in the soluble glycoprotein fraction.

The combination of the immunocytochemical localizations observed with monoclonal antibodies 43D1 and 43G8 (Tab. II) and the biochemical findings leads us to conclude that the 110000 Da precursor of α-glucosidase is present in the microvilli and the Golgi apparatus of human intestinal epithelial cells, and that the other forms of the enzyme are present in the lysosomes.

We cannot exclude the possibility that the precursor of α-glucosidase present in the microvilli is not synthesized by the enterocytes, but is secreted by other cells in the digestive tract and sticks to the glycocalyx of the small intestinal enterocytes. However, in this case one would expect a gradient in the labeling from the glycocalyx to the base of the microvilli; this was never observed. In addition, the presence of a precursor of α-glucosidase in the microvilli is not confined to human small intestinal enterocytes. Preliminary experiments using monoclonal antibodies 43D1 and 43G8 with two polarized human colon carcinoma cell lines, Caco-2 and HT29 [23], and with human kidney (R. P. J. Oude Elferink, J. A. M. Fransen, unpublished observations) also showed the presence of the 110000 Da precursor of α-glucosidase in the microvilli. Moreover, biochemical data have shown that the precursor is synthesized and secreted by these cell lines [23].

Recent biochemical studies have shown that the processing of the 110000 Da form of α-glucosidase to forms of lower molecular mass takes place in the lysosomes [27]. The presence of the non-processed 110000 Da form of α-glucosidase in the brush border may indicate that transport of the enzyme to the brush border occurs directly from the Golgi apparatus in a polarized way, and not via the lysosomes. Transport via the lysosomes would imply the presence of processed forms in the brush border, which was never observed.

What are the implications of these findings for the views on the intracellular transport route(s) of lysosomal enzymes?

All soluble lysosomal enzymes studied so far are synthesized in the rough endoplasmic reticulum as precursors with a higher molecular mass than that of the mature forms and are transported to the lysosomes via the Golgi apparatus [8, 36, 42]. All soluble lysosomal enzymes bear a common recognition marker which binds to specific receptors located in the Golgi apparatus [6, 7, 10, 12]. It is now commonly accepted that two mannose 6-phosphate/mannose 6-phosphate receptor systems [22, 42] are the carriers for lysosomal enzymes from the Golgi apparatus to the lysosomes (reviewed [42]). There is, however, still no agreement on the exact pathway taken by lysosomal enzymes.

Two models of mannose-6-phosphate receptor dependent transport of lysosomal enzymes have been proposed. The first is a direct intracellular transfer from the Golgi apparatus to the lysosomes [6–8, 10, 36]. In this model, the mannose-6-phosphate receptor serves as an intracellular sorting mechanism separating lysosomal enzymes from proteins following the secretory and other routes. This segregation probably takes place at the level of the Golgi apparatus [6, 7, 10]. Although secretion of precursors of lysosomal enzymes occurs, secretion followed by endocytosis is not considered a quantitatively important pathway for transport of newly synthesized enzymes to the lysosomes (see [8] for a review).

The second model is an adaptation of the secretion-recapture hypothesis, originally proposed by Hickmann and Neufeld [20]. According to this model lysosomal enzymes bound to their receptors are transported via the nor-

mal secretory route to the plasma membrane where they remain bound to their receptors and are sorted out from secretory glycoproteins by receptor-mediated endocytosis [13, 41, 42]. It has been shown that the mannose-6-phosphate receptor and lysosomal enzymes follow the same route as observed for other receptor-ligand systems; endocytosis and transport to the lysosomes occur via coated pits, CURL (the compartment of uncoupling of receptor and ligand), and multivesicular bodies [13, 44]. However, the possibility of an intracellular route to CURL could not be excluded.

In view of our findings it is tempting to speculate that α-glucosidase can be transported according to the second model (i.e., via the secretory route) in intestinal epithelial cells. However, the absence of labeling of multivesicular bodies described here may indicate that secreted lysosomal α-glucosidase is not re-internalized in these intestinal epithelial cells. In previous studies we have shown that multivesicular bodies are part of a fluid-phase endocytosis route in human intestinal epithelial cells, but that brush-border associated glycoproteins are excluded from the endocytotic process [3, 4]. We therefore think that the majority of precursor molecules of α-glucosidase present in the microvilli are not re-internalized but instead are secreted into the lumen of the intestine.

Secretion of precursors of lysosomal enzymes by polarized epithelial cells is not unique to intestinal epithelial cells. Such a process is observed in kidney, where, for instance, large amounts of the 110000 Da precursor form of α-glucosidase are secreted into the urine [25, 29]. According to Paigen and Petersen [29] the lysosomal enzymes are actively secreted, possibly by the proximal tubule epithelial cells, and do not originate from exfoliated cells. Our preliminary observations indicating that the 110000 Da precursor is also present in the microvilli of renal proximal tubule epithelial cells (R. P. J. Oude Elferink, J. A. M. Fransen, unpublished observations) are in favor of our assumption that the precursor of α-glucosidase present in microvilli is secreted and not endocytosed. On the other hand, in fibroblasts, which do not normally secrete significant amounts of the precursor of α-glucosidase [26], no plasma membrane localization has been reported in immunocytochemical studies [39, 40].

Endocytosis of small amounts of precursor, not detectable by our methods, cannot be excluded. The question remains whether endocytosis of a small amount of the precursor present in the microvilli is the major pathway for lysosomal enzyme delivery. We believe that a direct intracellular transport from the Golgi apparatus to the lysosomes is likely, either directly or via a prelysosomal compartment. Moreover, such a pathway for glycoproteins exists in intestinal epithelial cells, as was shown in previous studies using metabolically labeled explants [16]. In these studies, a crinophagic pathway, in which transport vesicles fuse directly with the lysosomes, has been postulated for the regulation of brush-border associated glycoproteins [5]. Several investigations suggest the involvement of a prelysosomal compartment in the delivery of lysosomal enzymes into the lysosomes [13, 14, 42]. In our studies we never found labeling of intracellular organelles other than the Golgi apparatus and the lysosomes. If a prelysosomal compartment is involved, the amount of α-glucosidase present in this organelle may be too low and/or the transport too fast to be detected.

Investigations are in progress to study the biosynthesis and secretion of lysosomal α-glucosidase in two colon carcinoma cell lines. These cell lines synthesize and express the precursor form of α-glucosidase in the microvilli [23] and should provide an excellent tool for studying the intracellular transport routes of lysosomal enzymes in polarized epithelial cells.

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