Lysosomal Enzymes in *Dictyostelium discoideum* Are Transported to Lysosomes at Distinctly Different Rates

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Abstract. We are investigating the molecular mechanisms involved in the localization of lysosomal enzymes in *Dictyostelium discoideum*, an organism that lacks any detectable mannose-6-phosphate receptors. The lysosomal enzymes α-mannosidase and β-glucosidase are both initially synthesized as precursor polypeptides that are proteolytically processed to mature forms and deposited in lysosomes. Time course experiments revealed that 20 min into the chase period, the pulse-labeled α-mannosidase precursor (140 kD) begins to be processed, and 35 min into the chase 50% of the polypeptides are cleaved to mature 60 and 58-kD forms. In contrast, the pulse-labeled β-glucosidase precursor (105 kD) begins to be processed 10 min into the chase period, and by 30 min of the chase all of the precursor has been converted into mature 100-kD subunits. Between 5 and 10% of both precursors escape processing and are rapidly secreted from cells. Endoglycosidase H treatment of immunopurified radioactively labeled α-mannosidase and β-glucosidase precursor polypeptides demonstrated that the β-glucosidase precursor becomes resistant to enzyme digestion 10 min sooner than the α-mannosidase precursor. Moreover, subcellular fractionation studies have revealed that 70–75% of the pulse-labeled β-glucosidase molecules move from the rough endoplasmic reticulum (RER) to the Golgi complex less than 10 min into the chase. In contrast, 20 min of chase are required before 50% of the pulse-labeled α-mannosidase precursor exits the RER. The β-glucosidase and α-mannosidase precursor polypeptides are both membrane associated along the entire transport pathway. After proteolytic cleavage, the mature forms of both enzymes are released into the lumen of lysosomes. These results suggest that β-glucosidase is transported from the RER to the Golgi complex and ultimately lysosomes at a distinctly faster rate than the α-mannosidase precursor. Thus, our results are consistent with the presence of a receptor that recognizes the β-glucosidase precursor more readily than the α-mannosidase precursor and therefore more quickly directs these polypeptides to the Golgi complex.

THE mechanisms by which cells direct proteins to their proper subcellular location remain largely unknown (17). Membrane-bound, secretory, and lysosomal enzymes are all synthesized on polysomes attached to the endoplasmic reticulum (ER).[1] After translocation into the lumen of the ER, many of these proteins are glycosylated and move to the Golgi complex where additional posttranslational modifications are thought to occur (4). Sorting of the proteins into classes destined for different subcellular locations may also occur in the Golgi complex. Little is known, however, about the molecular nature of the "sorting" signals on membrane and secretory proteins or the mechanisms by which these proteins are localized. Proteins destined for lysosomes are modified in the Golgi complex by the addition of phosphate groups to the mannose-rich oligosaccharide side chains (18). At least two classes of membrane-bound receptors exist which recognize and bind the mannose-6-phosphate groups and direct the proteins to lysosomes (9). However, mechanisms exist for the localization of lysosomal enzymes that are independent of the phosphomannosyl recognition system. For instance, lysosomal enzymes in cells of patients with I-cell disease lack the mannose-6-phosphate recognition marker. As a consequence, the enzymes do not bind to receptors and are instead secreted. However, in some cells such as leukocytes, hepatocytes, and Kupffer cells, there are nearly normal levels of lysosomal enzymes (14).

We are studying the molecular mechanisms used by the cellular slime mold, *Dictyostelium discoideum*, to direct lysosomal enzymes to lysosomes (reviewed in reference 1). Because this organism lacks any detectable phosphomannosyl receptors (Ebert, D. L., unpublished results), it represents an ideal system in which to investigate the nature of alternative localization mechanism(s). Previous reports have demonstrated that the lysosomal enzyme α-mannosidase is synthesized as a 140-kD precursor polypeptide that is proteolytically processed to mature forms of 60 and 58 kD (12, 15). The

[1] *Abbreviations used in this paper:* endo H, endoglycosidase H; ER, endoplasmic reticulum; RER, rough endoplasmic reticulum.

precursor, synthesized on membrane-bound polysomes, is co-translationally N-glycosylated in the lumen of the ER (2) and rapidly transported to the Golgi complex (13). After additional modifications, which occur in the Golgi complex, 10% of the precursor is secreted from cells, while the remainder is directed to lysosomes and proteolytically processed. The precursor form of the enzyme is tightly associated with membranes along the entire cellular pathway until it is proteolytically processed to the mature forms.

In this paper, we compare the biosynthesis, transport, and processing of the lysosomal enzymes β-glucosidase and α-mannosidase. β-Glucosidase, like α-mannosidase, is synthesized on membrane-bound polysomes as a precursor polypeptide that is membrane-associated during intracellular transport. However, β-glucosidase is transported and processed at a much faster rate than is α-mannosidase. These results suggest that transport of lysosomal enzymes in *Dictyostelium discoideum* is not due to bulk-phase movement of vesicular contents but instead may be dependent on a receptor-mediated mechanism.

Materials and Methods

Organism

Dictyostelium discoideum strains AX3 (wild-type) and GM1 (carrying mutations in the structural genes for α-mannosidase and β-glucosidase) were grown in axenic culture in TM medium (7) at 21°C on a rotary shaker.

Radioactive Labeling

Exponentially growing cells were collected by centrifugation (1,000 *g* for 3 min) and resuspended to a titer of 10^7 cells/ml in TM medium in the presence of 500 μCi/ml of [^{35}S]methionine (Amersham Corp., Arlington Heights, IL: 1,200 Ci/mmol). After a pulse period of 10–15 min, cells were collected by centrifugation and resuspended to 10^7 cells/ml in fresh TM medium (chase).

Immunoprecipitations

[^{35}S]Methionine-labeled α-mannosidase and β-glucosidase polypeptides were immunoprecipitated from cellular, subcellular, and extracellular fractions using monoclonal antibodies specific for α-mannosidase (2H9) and β-glucosidase (mBG11; Golumbeski, G., and R. Dimond, manuscript submitted for publication). Complete methods have been previously described (12, 13). The immunoprecipitated proteins were subjected to SDS PAGE as described below.

Subcellular Fractionations

Pulse-labeled Ax3 cells (1–5 × 10^7 cells) were mixed with a 50–100-fold excess of cells of strain GM1 and broken in TKM buffer (50 mM Tris-HCl, pH 7.6, 25 mM KCl, and 5 mM $MgCl_2$) plus 0.25 M sucrose by 10–15 strokes using a Dounce homogenizer. GM1 is an α-mannosidase and β-glucosidase structural gene mutant that lacks any detectable α-mannosidase or β-glucosidase polypeptides. Thus, the strain is used to facilitate breakage and handling of cells. Postnuclear supernatants were separated into different organelle fractions by centrifugation (SW 40, 38,000 rpm, 2.5 h, 4°C) on discontinuous sucrose gradients (1, 13). After centrifugation, fractions that contained the Golgi membranes (1.1–1.3 M sucrose), lysosomes (1.3–1.5 M sucrose), and rough ER (RER) vesicles (1.5–1.8 M sucrose) were collected from the top, diluted with TKM, and centrifuged (150,000 *g* for 40 min) to pellet membranes. Membrane pellets were suspended in 1% Triton X-100 and the labeled α-mannosidase and β-glucosidase polypeptides immunoprecipitated.

Gel Electrophoresis

Immunoprecipitates were resuspended in 50 μl of a solution (gel sample buffer) that contained 0.125 M Tris-HCl, pH 6.8, 2% SDS, 15% sucrose, 5% β-mercaptoethanol, and 0.001% bromophenol blue. The solution was incubated at 80°C for 5 min, centrifuged, and the supernatants loaded onto 7.5% polyacrylamide slab gels (10). After electrophoresis, gels were prepared for fluorography using Enhance, dried, and exposed using Kodak X-Omat film at −70°C. The amount of radioactively labeled protein resolved by electrophoresis was quantitated by scanning fluorographs in an LKB 2202 Ultrascan Laser Densitometer (LKB Instruments, Inc., Gaithersburg, MD). The data were recorded using an Apple II+ computer (Apple Computer Inc., Cupertino, CA) and peak calculations were performed using the Vidi chart integration program (P. K. Warme, Interactive Microwave, Inc., State College, PA).

Endoglycosidase H (Endo H) Digestions

Immunoprecipitates were suspended in 25 μl of a solution that contained 2% SDS, 10% β-mercaptoethanol. Samples were heated at 80°C for 3 min and centrifuged at 10,000 *g* for 3 min. An equal volume of 100 mM Na citrate, pH 5.5 was added to the supernatant followed by the addition of 1.5 μl of endo H (1 U/ml in 50 mM phosphate buffer, pH 5.5). Samples were incubated at 37°C for 16 h and then mixed with an equal volume of 2× gel sample buffer.

Results

β-Glucosidase Is Proteolytically Processed Faster Than α-Mannosidase

Both α-mannosidase and β-glucosidase are synthesized as precursor polypeptides that are proteolytically processed to mature forms just before or concomitant with localization in lysosomes (2, 12, 13, 16).[2] To compare the rates of processing of the precursor polypeptides, growing cells were pulse-labeled for 15 min with [^{35}S]methionine, collected by centrifugation, and resuspended in fresh growth medium to initiate the chase period. At various times during the chase, cells were harvested by centrifugation, resuspended in 1% Triton X-100, and the labeled α-mannosidase and β-glucosidase polypeptides immunoprecipitated with monoclonal antibodies. The immunoprecipitates were subjected to SDS PAGE followed by fluorography. The results of this experiment are shown in Fig. 1. β-Glucosidase is initially synthesized as a 105-kD precursor polypeptide[2] that is processed first to a 103-kD intermediate form and finally to a 100-kD form which resides in the lysosome. As indicated in Fig. 1, 10 min into the chase, 50% of the 105-kD precursor has been processed into the 103-kD intermediate form. After 25 min of chase, all of the precursor polypeptides have been converted to equal amounts of the 103- and 100-kD forms of the enzyme. Finally, after 35 min, ~90% of the newly synthesized β-glucosidase has been converted to the 100-kD mature form. In contrast, after 30 min of chase only 50% of the newly synthesized 140-kD α-mannosidase precursor is processed into the mature 60 and 58-kD forms (Fig. 1). In fact, even after a 60-min chase, a small percentage (~5%) of the α-mannosidase precursor remains unprocessed.

β-Glucosidase Is Transported from the RER to the Golgi Faster Than α-Mannosidase

In *Dictyostelium discoideum*, the precursors to lysosomal enzymes are most likely proteolytically processed in a prelysosomal or lysosomal compartment (13). The faster rate of processing of the β-glucosidase precursor relative to α-mannosidase is consistent with β-glucosidase being more sensitive to a processing enzyme or with the β-glucosidase precursor reaching the proteolytic compartment sooner than α-mannosidase. If the latter possibility is true, then the β-glucosidase precursor may also reach the Golgi complex faster than the α-mannosidase precursor. To examine this, cells were labeled as described above and the α-mannosidase and β-glucosidase polypeptides immunoprecipitated at different times during the chase. The antibodies were added simultaneously to the

[2] Golumbeski, G., and R. Dimond. Manuscript in preparation.

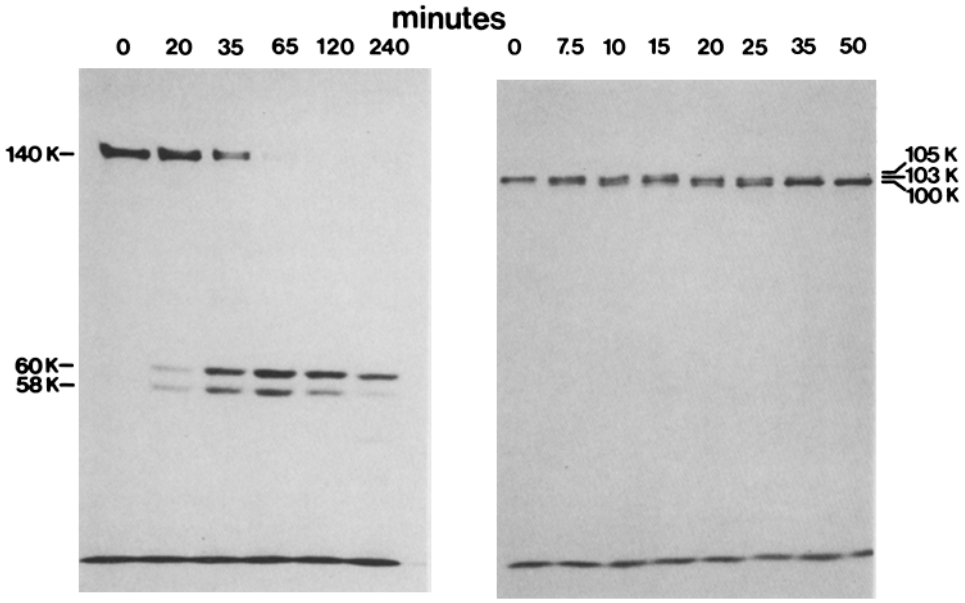

Figure 1. Rate of proteolytic processing of newly synthesized α-mannosidase and β-glucosidase precursor polypeptides. Logarithmically growing cells were pulse-labeled with [^{35}S]methionine for 15 min, washed by centrifugation, and resuspended in fresh grown medium to initiate the chase. At the indicated times, 10^7 cells were collected by centrifugation, resuspended in 0.5% Triton X-100, and incubated with saturating amounts of α-mannosidase (*left*) or β-glucosidase (*right*) monoclonal antibodies. Immunoprecipitates were washed, resuspended in gel sample buffer, and subjected to SDS PAGE followed by fluorography. *K*, kilodalton.

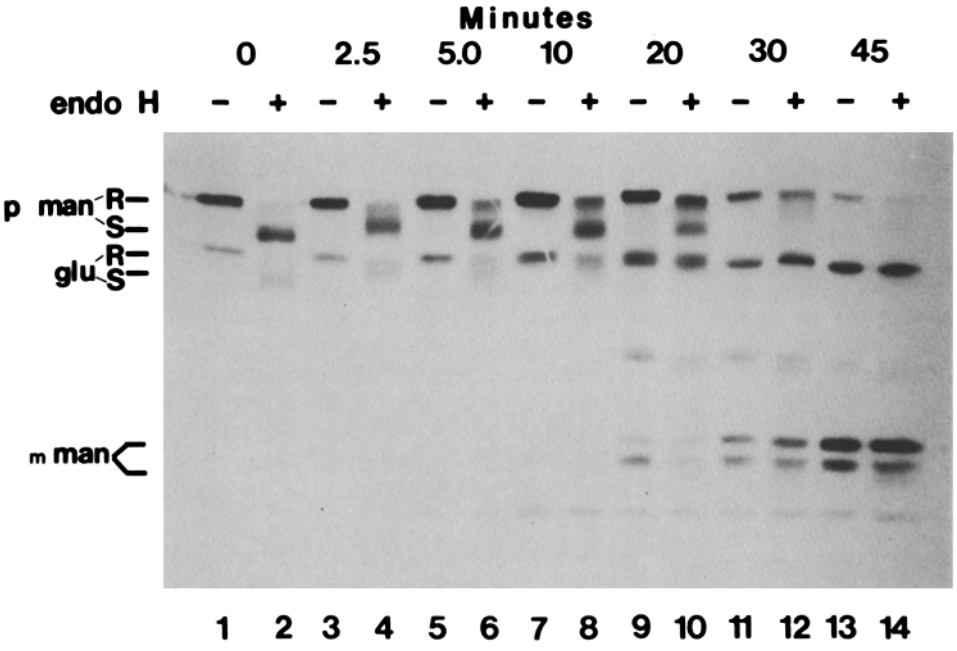

Figure 2. Rate at which newly synthesized α-mannosidase and β-glucosidase precursors become resistant to endo H. Logarithmically growing cells were labeled and chased as described in Fig. 1. At the indicated times, cells were collected by centrifugation and the labeled cellular α-mannosidase and β-glucosidase precursor (*p*) and mature (*m*) polypeptides were immunoprecipitated with a combination of excess α-mannosidase and β-glucosidase specific antibodies. Samples were resuspended in 2% SDS, 10% β-mercaptoethanol and incubated with endo H as described in the Materials and Methods section. After digestions, samples were subjected to SDS PAGE followed by fluorography. The letters *R* and *S* denote the position of the endo H resistant and sensitive forms of the α-mannosidase and β-glucosidase precursor polypeptides.

labeled extracts so that both α-mannosidase and β-glucosidase polypeptides appear in each lane. One half of each sample was digested with endo H before SDS PAGE. Endo H removes N-linked mannose rich oligosaccharide side chains by cleaving between the N-acetylglucosamine residues making up the chitobiose core. β-Glucosidase and α-mannosidase are extensively modified glycoproteins (8) that become resistant to this enzyme in the Golgi complex (13). Therefore, the rate at which these enzymes become resistant to endo H should be proportional to the rate at which they move from the RER to the Golgi complex. As indicated in lanes *1* and *2* of Fig. 2, endo H treatment of immunopurified pulse-labeled α-mannosidase converts the 140-kD form into a new polypeptide of ~120 kD (13). The 120-kD polypeptide corresponds in molecular weight to the primary translation product of the α-mannosidase mRNA (2). Endo H treatment of the immunopurified pulse-labeled β-glucosidase precursor (lanes *1* and *2* of Fig. 2) converts the 105-kD form into a 94-kD form which also corresponds in molecular weight to the β-glucosidase mRNA primary translation product.[2] As summarized in Fig. 3, within 20 min of the chase, 50% of the pulse-labeled 140-kD α-mannosidase precursor becomes resistant to endo H digestion (Fig. 2, lanes *7–10*). In contrast, 50% of the β-glucosidase precursor polypeptides become resistant to endo H as early as 5 min into the chase (Fig. 2, lanes *5* and *6*; Fig. 3). Moreover, at 20 min, when 100% of the β-glucosidase polypeptides are resistant to endo H, at least 30% of the α-mannosidase precursor remains sensitive to enzyme treatment (Fig. 3, lanes *9* and *10* of Fig. 2).

These results suggest that the β-glucosidase precursor exits the RER and reaches the Golgi complex at a faster rate than does the α-mannosidase precursor. To more directly examine this, cells were pulse-labeled with [^{35}S]methionine, broken in 0.25 M sucrose using a Dounce homogenizer, and fractionated on discontinuous sucrose gradients. This subcellular fractionation procedure separates crude membranes in RER,

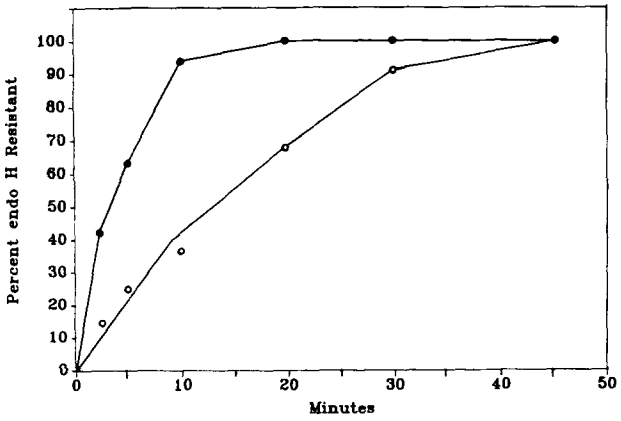

Figure 3. Newly synthesized α-mannosidase and β-glucosidase precursors become resistant to endo H at different rates. The fluorograms shown in Fig. 2 were scanned by laser densitometry (see Materials and Methods section) to determine the relative percentage of endo H sensitive and resistant α-mannosidase and β-glucosidase polypeptides.

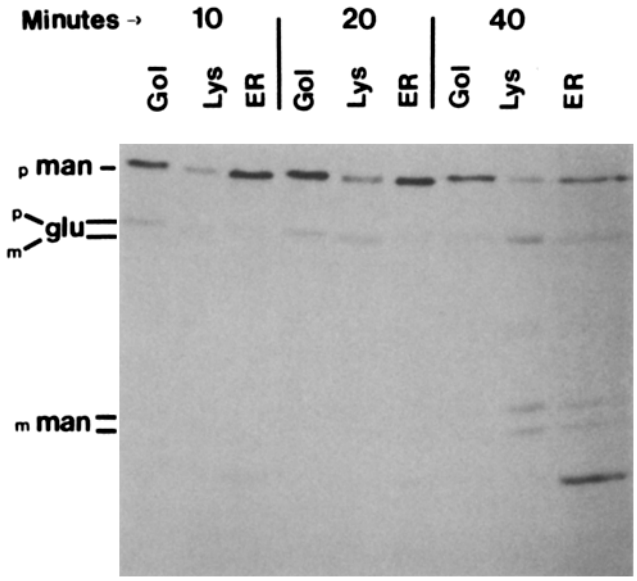

Figure 4. Intracellular transport rates for α-mannosidase and β-glucosidase precursor polypeptides. Logarithmically growing cells were pulse-labeled and chased as described in Fig. 1. Labeled cells were collected by centrifugation and mixed with a 50-fold excess of the mutant strain GM1. The mutant strain lacks any detectable β-glucosidase or α-mannosidase and is used to facilitate breakage and handling of labeled cells. After homogenization, crude membranes were prepared by centrifugation and fractionated on discontinuous sucrose gradients as described in the Materials and Methods section. Regions of the gradient corresponding to Golgi apparatus, lysosomes, and ER membranes were collected, concentrated by centrifugation, and incubated with α-mannosidase and β-glucosidase monoclonal antibodies. The immunoprecipitated labeled α-mannosidase and β-glucosidase precursor (*p*) and mature (*m*) polypeptides were subjected to SDS PAGE followed by fluorography.

Golgi, and lysosomal fractions (1, 13). Thus, using this technique, one can measure the rate of transport of *Dictyostelium* proteins from one cellular compartment to another. As indicated in Fig. 4, after 10 min of chase, at least 75% of the newly synthesized 105-kD β-glucosidase precursor polypeptides have exited the RER and reached the Golgi complex. In fact, during this 10-min period, 10–15% of the β-glucosidase precursor has been converted to the 100-kD mature form and deposited in lysosomes. After 20 min, <10% of the β-glucosidase precursor remains in the RER. Instead, the majority of the β-glucosidase polypeptides are found in either the 105-kD precursor form in the Golgi membrane or in 100-kD mature form in the lysosomes (Fig. 4). However, by this time only 50% of the α-mannosidase precursor has moved from the RER to the Golgi and <5% of these polypeptides have been converted into the 60 and 58-kD mature lysosomal forms. After 40 min of chase, all of the β-glucosidase precursor has been processed to the 100-kD mature forms and deposited in lysosomes. In contrast, at this time 20–30% of the α-mannosidase precursor remains unprocessed and is found distributed predominately in RER and Golgi vesicles. These results indicate that the β-glucosidase precursor moves from the RER to the Golgi complex significantly faster than does the α-mannosidase precursor.

As observed in Fig. 4, 5–10% of the immunoprecipitated α-mannosidase precursor polypeptides co-fractionate with lysosomes on sucrose gradients. This suggests that a certain percentage of the precursors can accumulate in these organelles before proteolytic cleavage. However, these polypeptides are completely sensitive to endo H digestion, which suggests the precursors have not yet passed through the Golgi complex and therefore have not reached lysosomes (results not shown). Moreover, in the experiment described in Fig. 4, ~10% of the recovered ER marker enzyme α-glucosidase-2 co-fractionated with lysosomes. Thus, these results suggest that the α-mannosidase precursors observed sedimenting in the middle region of the sucrose gradient represent polypeptides that reside inside ER vesicles that contaminate the lysosomal fraction.

Both β-Glucosidase and α-Mannosidase Precursors Are Membrane Bound during Intracellular Transport

We have previously demonstrated that until the α-mannosidase precursor polypeptides are proteolytically cleaved they remain tightly associated with intracellular membranes (1, 13). Proteolytic cleavage of the precursor occurs just before or concomitant with release of the mature subunits into the lumen of the lysosome. Thus, the β-glucosidase precursor may be transported to the Golgi complex faster than the α-mannosidase precursor because it remains soluble in the lumen of the ER and is therefore more accessible to a transport-mediating receptor. To examine this possibility, cells were pulse-labeled with [^{35}S]methionine and broken in water using a Dounce homogenizer. These breakage conditions have previously been shown to disrupt membrane vesicles, thereby effecting a release of the luminal contents (13). After homogenization, membranes were separated from the soluble fraction by centrifugation. Radioactively labeled β-glucosidase and α-mannosidase polypeptides were immunoprecipitated from both fractions and subjected to SDS PAGE followed by fluorography. Fig. 5 indicates that >90% of the labeled α-mannosidase and β-glucosidase precursor polypeptides remain membrane-associated. In contrast, >80% of the mature 100-kD β-glucosidase and the 60 and 58-kD α-mannosidase subunits remain in the soluble fraction after the pelleting of membranes.

The following lines of evidence suggest that the lysosomal enzyme precursor-membrane association is not mediated by mannose-6-phosphate receptors. First, the precursor polypeptides remain bound to membranes after incubation of isolated

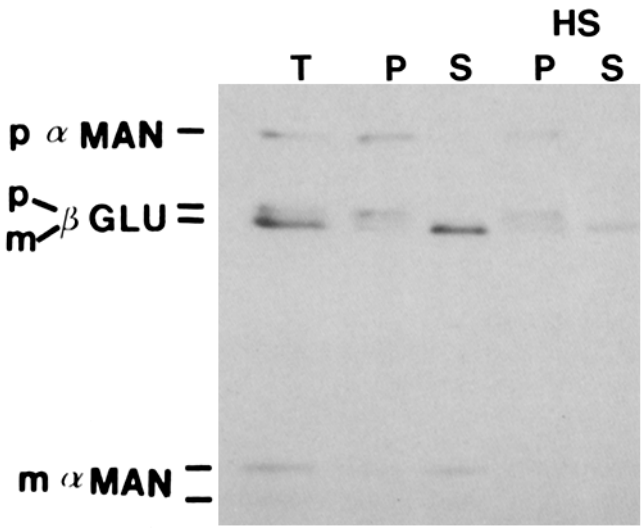

Figure 5. α-Mannosidase and β-glucosidase precursor polypeptides are membrane associated. Growing cells were pulse-labeled for 60 min with [^{35}S]methionine and collected by centrifugation. The recovered cells were mixed with excess GM1 cells (see legend to Fig. 4) and homogenized in water to disrupt membrane vesicles. One sample of the postnuclear supernatants that contained total (*T*) membranes was incubated with α-mannosidase and β-glucosidase antibodies. The remaining sample was divided in two and one tube adjusted to 0.5 M NaCl (*HS*). Samples were centrifuged and the supernatant (*S*) and resuspended membrane pellets (*P*) incubated with antibodies. Immunoprecipitates were subjected to SDS PAGE followed by fluorography.

crude membranes with 10 mM mannose-6-phosphate (1). Incubation of membranes isolated from mammalian cells with mannose-6-phosphate leads to the release of receptor-bound lysosomal enzymes. Second, we have been unsuccessful in detecting any phosphomannosyl receptors in *Dictyostelium* (Ebert, D. L., unpublished results) using procedures identical in design to the direct binding assays used by others to identify both the cation dependent and cation independent phosphomannosyl receptor in fibroblasts and macrophages (8). For these experiments, crude membranes isolated from an α-mannosidase structural gene mutant were incubated under low pH conditions or in the presence of mannose-6-phosphate to remove any phosphomannosyl receptor bound enzyme. Membranes were recovered by centrifugation and incubated with purified α-mannosidase in the presence and absence of 10 mM $MnCl_2$ and $MgCl_2$. After incubation, samples were centrifuged and the resuspended membrane pellets and supernatants assayed for α-mannosidase activity. Under these incubation conditions, no significant binding of α-mannosidase to membranes was detected.

Lysosomal Enzyme Precursor Polypeptides Can Follow More Than One Intracellular Pathway

After synthesis, the α-mannosidase precursor can embark on one or two intracellular transport pathways. One pathway, followed by ~90% of the precursor molecules, ends in the lysosome with the formation of mature enzyme (1, 12). The other pathway followed by ~10% of the molecules leads to the rapid secretion of the α-mannosidase precursor (1, 13). To determine if the β-glucosidase precursor also follows both pathways, cells were pulse-labeled and separated by centrifugation into cellular and extracellular fractions. Labeled proteins were immunoprecipitated and subjected to SDS PAGE. As indicated in Fig. 6, a small percentage (<10%) of both β-glucosidase and α-mannosidase precursors are secreted from cells within 30 min after synthesis. The amount of extracellular precursor secreted for both enzymes remains constant over the next 8 h. Furthermore, after 2–3 h, the mature forms of both enzymes begin to be secreted presumably as a result of exocytosis of lysosomal contents. The secretion of the labeled mature forms continues over the next 8–10 h. These data suggest that newly synthesized β-glucosidase and α-mannosidase follow one of two intracellular pathways. One pathway ends in the rapid secretion of 5–10% of the precursors, while the other pathway ends with the formation of mature enzymes which are deposited in lysosomes.

Discussion

In this report, we provide compelling evidence that indicates that precursors to two *Dictyostelium* lysosomal enzymes, α-mannosidase and β-glucosidase, are proteolytically processed and deposited in lysosomes at different rates after their synthesis. Our experiments, which involve a radioactive pulse-chase protocol coupled with endo H digestions and subcellular fractionation, demonstrate that 50% of the pulse-labeled 105-kD β-glucosidase precursor is transported from the RER to the Golgi complex in ~5 min. In contrast, 20 min of chase are required before 50% of the pulse-labeled 140-kD α-mannosidase precursor reaches the Golgi complex from the RER. Thus, our data clearly demonstrate that α-mannosidase and β-glucosidase are transported from the RER to the Golgi complex at distinctly different rates. This difference in transport rate may account for the observed differences in the rate of processing and deposition in lysosomes for these two enzymes. However, we cannot directly determine whether the two enzymes are transported from the Golgi complex to lysosomes at different rates. In addition, once inside the lysosome, the α-mannosidase and β-glucosidase precursors may be proteolytically processed with different kinetics. Therefore, we cannot conclude that the rate determining step

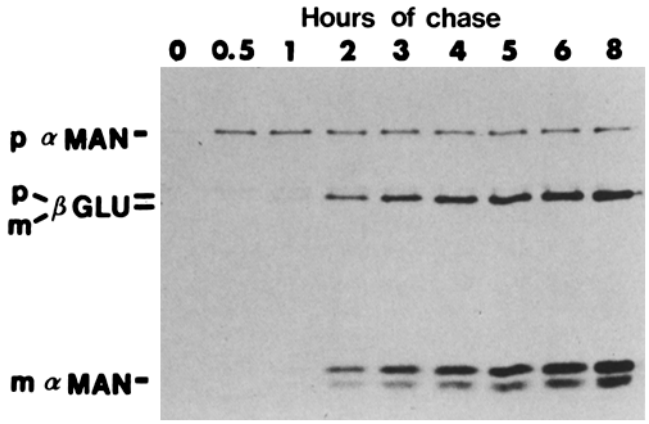

Figure 6. Secretion of α-mannosidase and β-glucosidase precursor and mature polypeptides. Logarithmically growing cells were pulse-labeled and chased as described in Fig. 1. At the indicated times, cells were removed by centrifugation and the supernatants incubated with α-mannosidase and β-glucosidase antibodies. The immunoprecipitated α-mannosidase and β-glucosidase precursor (*p*) and mature (*m*) polypeptides were subjected to SDS PAGE followed by fluorography.

in the transport and processing of these enzymes is the RER to Golgi complex transition.

Other than the differences in transport rates, however, the two enzymes show similar properties. For instance, both β-glucosidase and α-mannosidase are synthesized on membrane-bound polysomes as precursor polypeptides that are cotranslationally N-glycosylated and sequestered within the lumen of the ER (2).[2] The precursors pass through the Golgi complex where they are sulfated and possibly phosphorylated (1, 13). Both precursors are proteolytically processed to mature forms just before or concomitant with deposition in lysosomes (3, 13). Finally, along the entire transport pathway both precursors are tightly bound to membranes until proteolysis in the lysosomes.

Because of these similarities, it remains to be determined why β-glucosidase reaches the Golgi complex faster than does α-mannosidase. However, our results that concern the difference in transport rates of the two precursors are inconsistent with models that propose bulk or fluid phase movement of proteins from one intracellular compartment to another. If this were the case, one would expect α-mannosidase and β-glucosidase to be transported from the ER to the Golgi complex at similar rates. Instead, we propose that in *Dictyostelium*, the transport of enzymes targeted to lysosomes depends on these proteins that interact with transport-mediating molecules. This model has recently been proposed to account for the different rates of transport of mammalian secretory and membrane proteins from the RER to the Golgi complex (6, 11). The model argues that receptors with varying affinities recognize specific "signals" on secretory and membrane proteins. Thus, the proteins that are transported from the RER to the Golgi complex most efficiently and rapidly are those proteins that bind with the highest affinity to transport receptors. Therefore, we predict, that β-glucosidase is more efficiently or tightly bound than α-mannosidase by a receptor in the lumen of the ER that plays a role in the transport of the proteins to the Golgi complex.

Alternatively, our findings are consistent with differential binding of lysosomal enzymes to the ER membrane. A model based on this would predict that α-mannosidase resides in the ER longer than β-glucosidase because it is bound to some stationary component of the ER for a longer period of time. Thus, the transport "signal" in this case would not be something recognized by a transporting receptor but it would instead facilitate the release of the enzyme from the ER membrane. The protein, egasyn, is one example of an ER-localized macromolecule in kidney cells which selectively binds a significant proportion of newly synthesized β-glucoronidase and prevents the enzyme from reaching lysosomes (15).

ER-localized glycosyl-modifying enzymes (e.g., α-glucosidase) may also act to retard movement of glycosylated enzymes to other organelles. Thus in *Dictyostelium*, α-mannosidase being more extensively glycosylated may be transported out of the ER more slowly than β-glucosidase because it binds for a longer period of time to α-glucosidase. This model would predict that unglycosylated precursors might be transported from the ER to the Golgi complex at the same rate. To explore this possibility, we have tried to measure the transport rate of α-mannosidase and β-glucosidase precursors in tunicamycin-treated cells. However, all of our studies indicate that tunicamycin either inhibits lysosomal enzyme synthesis in *Dictyostelium discoideum* or the unglycosylated precursors are rapidly degraded (Cardelli, J., unpublished observations). Thus, we cannot directly test this second model.

Additional support exists, however, for the idea that transport of lysosomal enzymes in *Dictyostelium* is receptor mediated. A strain carrying a mutation in the α-mannosidase gene, HMW-437, synthesizes an α-mannosidase precursor polypeptide of normal molecular weight as determined by SDS PAGE. Endo H treatment of the mutant α-mannosidase precursor generates a polypeptide of 120-kD; thus, the mutant enzyme likely contains the normal quantity of "wild type" oligosaccharide side chains. However, HMW 437 cells do not transport the precursor from the RER to the Golgi complex, which suggests that the number of oligosaccharide side chains does not determine the transport rate of lysosomal enzymes in *Dictyostelium* (Woychik, N. A., J. A. Cardelli, and R. L. Dimond, manuscript submitted for publication). Nonetheless, α-mannosidase precursor in this mutant has a sedimentation rate on sucrose gradients different from the wild-type precursor, and the mutant precursor is more sensitive to protease digestion. These results suggest that the conformation of the mutant α-mannosidase precursor is altered. Thus, the mutant phenotype most likely results from the inability of the transport-mediating receptor to recognize the altered α-mannosidase polypeptide.

Note, however, that the molecular details of the model we have proposed remain unclear. Specifically, the nature of the precursor-specific membrane-association requires further study. While our current data are consistent with the precursors being membrane-associated because they are bound to a receptor, other models are consistent with our results. For example, the observed membrane association may reflect interaction with an ER membrane protein or anchoring of the precursors due to an uncleaved signal sequence. In fact, preliminary studies suggest that both α-mannosidase and β-glucosidase may retain their signal sequences after synthesis and translocational discharge (Cardelli, J., and G. Golumbeski, unpublished observations). Therefore, the proposed receptor-mediated transport and the observed precursor-specific membrane association may be separate entities.

The molecular nature of the "sorting" signals responsible for directing proteins to different subcellular locations remains to be determined. *Dictyostelium* lacks phosphomannosyl receptors, which suggests that mannose-6-phosphate residues do not play a role in the localization process. However, in this paper we present evidence consistent with a receptor-mediated delivery of lysosomal enzymes to lysosomes in *Dictyostelium*. We are presently using a genetic and biochemical approach to determine the composition of the sorting signal(s), the nature of the receptor(s), and the molecular mechanisms that operate to localize lysosomal enzymes to lysosomes in *Dictyostelium*.

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