Transit of α-Mannosidase during Its Maturation in *Dictyostelium discoideum*

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ABSTRACT We proposed that *Dictyostelium discoideum* contains two linked pools of mature α-mannosidase (Wood, L., R. N. Pannell, and A. Kaplan, 1983, *J. Biol. Chem.*, 258:9426–9430). To obtain physical evidence for these pools, cells were pulse-labeled with [^{35}S]methionine, homogenized, and subjected to Percoll gradient centrifugation. After immune precipitation of α-mannosidase, its polypeptides were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and detected by fluorography. After a 30-min pulse with [^{35}S]methionine, the precursor and small amounts of cleaved enzyme were detected in a low density fraction (1.04 g/ml). Subsequently, cleaved enzyme was transferred to higher density fractions (1.05 and 1.07 g/ml) that were enriched in lysosomal enzymes. The half time for formation of the 1.07 g/ml pool was ~45 min, whereas formation of the 1.05 g/ml pool was not detected until 1.5 h after the pulse. The transfer of mature forms out of the 1.04 g/ml pool was inhibited by monensin (3.5 μM). Thus, α-mannosidase precursor appears to be cleaved in a prelysosomal organelle. The data also indicate that starving cells secrete precursor directly from this organelle to the extracellular space, whereas cleaved forms are first transferred into lysosomes before they are secreted.

Furthermore, 2 h after starvation, the secretion of mature forms ceases even though both transit of mature forms between the two pools and secretion of precursor continues. From this we inferred that the cessation of secretion of mature forms is due to a halt in fusion of lysosomes with the plasma membrane and that precursor follows a different route to the plasma membrane.

We are investigating the chemical maturation and intracellular transport of α-mannosidase, a lysosomal enzyme, in *Dictyostelium discoideum*. The biosynthesis and distribution of α-mannosidase are regulated by developmental and nutritional factors. For example, the synthesis of α-mannosidase dramatically increases at the beginning of cellular differentiation (1). Furthermore, during starvation, *D. discoideum* secrete up to 60% of their α-mannosidase activity (2). The molecular mechanisms responsible for these events are unknown. However, because the final destinations of the enzymes can be experimentally controlled, *D. discoideum* is an excellent system in which to study acid hydrolase compartmentation.

The maturation of acid hydrolases in *D. discoideum* is analogous but not identical to that in higher organisms. Initial chemical events in eucaryotic cells include glycosylation (3–8), phosphorylation (9–11), and carbohydrate processing (6–8), as well as specific proteolytic cleavage (12–15). In *D. discoideum*, α-mannosidase is also synthesized as a large molecular weight precursor (150,000 D) that is cleaved into a group of mature forms (46–58 × 10^3 D) (16, 17). Like mammalian enzymes these molecules contain high mannose-type oligosaccharides that are phosphorylated (18). However, unlike mammalian enzymes, the carbohydrates are also sulfated (19) and the mannosyl phosphates are O-methylated (20). Also *D. discoideum* contain no detectable phosphomannosyl receptor.

In an effort to understand the mechanisms of sorting of these enzymes, we have studied the starvation-induced secretion of α-mannosidase. We noticed that, under starvation conditions, newly processed α-mannosidase forms were secreted to a lesser extent than bulk enzyme activity (21).

Results that established a kinetic relationship between newly processed and efficiently secreted forms led us to conclude that mature α-mannosidase exists in linked pools.

In this paper, we present strong physical evidence for three pools of mature α-mannosidase. This was obtained by the separation of the pools on Percoll density gradients. The data indicate that proteolytic cleavage of the precursor occurs in a prelysosomal organelle. These cleaved forms are transferred to lysosomal fractions. Furthermore, in starving cells, transfer of mature forms to the lysosomes continues during secretion of mature forms and even after secretion ceases. A portion of the precursor escapes proteolysis and is secreted directly into the media. Thus, as in mammalian cells, two alternate routes through the cell are available to the α-mannosidase precursor.

MATERIALS AND METHODS

D. discoideum (strain Ax2) was maintained in log phase by daily dilution with HL-5 medium (22). The purification of α-mannosidase and preparation of antibody directed against this enzyme have been described (16). [^{35}S]-Methionine and [^{35}S]sulfate were purchased from Amersham Corp. (Arlington Heights, IL). Pansorbin was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Percoll and density marker beads were purchased from Pharmacia Inc. (Piscataway, NJ). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Subcellular Fractionation of Dictyostelium discoideum and Detection of Labeled α-Mannosidase Polypeptides

The following manipulations were performed at 0°C. Cells, radiolabeled as described in the figure legends, were resuspended and washed in 20 mM 2[*N*-morpholino]ethane sulfonic acid pH 6.5, 1 mM EDTA in 0.25 M sucrose. Cells were swollen in 1.5 ml of this buffer in a nitrogen-cavitation bomb (Parr Instrument Co., Moline, IL) for 5 min under 30 psi, and then lysed using a Teflon-glass homogenizer. This procedure disrupts 70–80% of the cells.

Nuclei and unbroken cells were pelleted by centrifugation at 200 *g* for 9 min. The supernatant (1.3 ml) was layered on 9 ml of 20% Percoll, 0.25 M sucrose, pH 6.5. The gradients were centrifuged with the brake off for 20 min at 31,000 rpm in a Ti50 rotor (Beckman Instruments, Palo Alto, CA).

Starting from the top of each gradient, twelve fractions (1.0 ml) were obtained using a Buchler Instruments, Inc. (Fort Lee, NJ) Auto Densi-Flow II C fractionator. Aliquots were removed for acid phosphatase, β-hexosaminidase, and α-mannosidase assays (21). Each fraction was centrifuged at 100,000 *g* for 90 min to pellet the Percoll. A portion of the membranes banded in the supernatant fluid. Another portion layered on top of the Percoll pellet. The layered membranes were scraped from the Percoll pellet and were combined with those in the supernatant fluid. The combined membranes were mixed with 1 vol of 2× lysis buffer (17). Before immune precipitation, samples were preadsorbed with 150 μl Pansorbin/ml and centrifuged at 100,000 *g* for 45 min. Then the supernatants were preadsorbed overnight with preimmune rabbit serum and again for 30 min with 150 μl/ml Pansorbin. Samples were then centrifuged, and supernatants were immunoprecipitated with anti-α-mannosidase antibody and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and autofluorography as described (17). In all cases, antibody and Pansorbin were added in excess. In the experiments where cells were labeled with [^{35}S]methionine, immunoprecipitates derived from identical amounts of trichloroacetic acid–precipitable protein radioactivity were loaded into each lane of the gel. Assuming a similar rate of incorporation of [^{35}S]methionine into most proteins, the fluorograms depict the specific activity of α-mannosidase in each gradient fraction.

Of the many gradients performed, only results replicated in triplicate are presented here. The gradients consistently separated vesicles with density differences of 0.03 g/ml or greater. Careful quantitation of the *d*1.05 pool, to be described below, was not possible since it was too closely adjoined to the *d*1.04 and *d*1.07 pools. Decreasing fraction sizes by half and thus doubling the number of fractions did not improve resolution.

The dashed density lines, drawn on Figs. 2, 4, and 5, were obtained from calibration gradients run at the same times as the experimental gradients. These were found to accurately mark densities in experimental gradients plus or minus one fraction. This explains the small amount of variability in the position of the peak fractions relative to the density markers.

With regard to all forms of α-mannosidase as well as the markers described in Fig. 3, 80–100% of the amount loaded on the gradient was recovered. A small but significant fraction (20 ± 5%) of the vesicles were disrupted during the transfer to gradient tubes and centrifugation. Disruption was revealed by the presence of soluble material at the top of the gradient. The degree of disruption was not correlated with the time of the chase period or the time of starvation of cells. Although concentrations in excess of 8 μM monensin caused destabilization of vesicles, no such effects were noted at 3.5 μM monensin.

The intense labeling procedure used tended to highlight the presence of minor contaminants in the immunoprecipitate. The main band that was evident, other than the α-mannosidase precursor and mature forms, is probably an intermediate that we described previously in some detail (17).

Marker Enzyme Analysis

Unlabeled cells were homogenized and subjected to differential and Percoll gradient centrifugation as described above. After removal of Percoll, fractions from the gradient were made 0.5% in Triton X-100 and assayed for glucose-6-phosphatase and 5′-nucleotidase. Protein was measured using Coomassie Blue G-250 as described by Sedmak and Grossberg (23).

Glucose-6-phosphatase was measured radiometrically using D[1-^{14}C]glucose-6-phosphate (New England Nuclear, Boston, MA). The 0.7-ml reaction mixture contained 22 mM sodium maleate, pH 6.0, 4 mM glucose-6-phosphate, 2 mM EDTA, 1 mM potassium fluoride, 0.01 μCi D[1-^{14}C]glucose-6-phosphate, and cellular sample (0.3–2.0 mg protein). After 60 min at 37°C, samples were boiled for 2 min. Reaction tubes were centrifuged for 2 min in a Beckman microfuge, and supernatants were added to 150 mg of the anion exchange resin Ag1-X8 (OH form 200–400 mesh, Bio-Rad Laboratories, Richmond, CA).

The resin was pelleted in the microfuge. Supernatants were mixed with 15 ml of Aquasol (New England Nuclear). Radioactivity was measured in a liquid scintillation counter to determine the free glucose released during the reaction. For assays performed at pH 4.5, 50 mM sodium acetate was substituted for sodium maleate in the reaction mixtures.

5′-Nucleotidase was assayed in a similar manner using [U-^{14}C]adenosine 5′-monophosphate (Amersham Corp.). The 0.7-ml reaction mixture contained 45 mM Tris-HCl, pH 7.5, 10 mM magnesium chloride, 1.5 mM adenosine monophosphate, cellular sample (0.2–0.6 mg protein), and 0.01 μCi [U-^{14}C]-adenosine 5′-monophosphate. After 90 min at 37°C, the reaction was stopped by boiling, and protein was pelleted in the microfuge. Supernatants were mixed with anion exchange resin and then prepared for liquid scintillation counting as described above.

Incorporation of [^{35}S]Sulfate into Acid-precipitable Material

Cells (7.5×10^6 cells/ml) were incubated for 2 h in 20 ml fresh HL-5, then centrifuged at 200 *g* for 9 min. Cells were then resuspended in 10 ml of a defined medium (24) minus sulfate. 3 mCi of [^{35}S]sulfate was added, and the incubation was continued for 7 min. Then 1 vol of fresh HL-5 was added, and cells were chilled and processed for subcellular fractionation as described above. After removal of the Percoll, supernatants were mixed on ice with 1 ml of 1% phosphotungstic acid in 0.5 N HCl. Proteins were pelleted in the microfuge and washed four times with this acid solution. 1 ml of 1 N NaOH was added to the final pellets, and samples were placed in scintillation vials containing 0.3 ml of 4 N HCl, 0.5 ml H_2O, and 18 ml Aquasol. Radioactivity was then measured.

RESULTS

Separation of α-Mannosidase-containing Vesicles

When *D. discoideum* are starved, newly processed α-mannosidase is secreted to a lesser extent than bulk forms of the enzyme (21). To explain this observation, we proposed that α-mannosidase resides in a presecretory pool before it enters an efficiently secreted pool. We tried to physically separate these pools. To accomplish this, growing cells were labeled with [^{35}S]methionine according to the pulse–chase protocol described in the legend of Fig. 1. Aliquots were removed, and cells were homogenized and subjected to subcellular fractionation. α-Mannosidase was detected in fractions obtained from the Percoll gradients by immunoprecipitation, sodium dodecylsulfate polyacrylamide gel electrophoresis, and fluorography.

Figs. 1 and 2 depict the distribution of α-mannosidase specific activity in the gradients at different times. At 0 h chase, the precursor and a major portion of the cleaved forms peaked in a bouyant region of the gradient (d = 1.04 g/ml). Mature forms of the enzyme were localized in the same gradient fractions as well as in high density fractions (d = 1.07 g/ml). After 1.5 h the precursor was completely converted to cleaved forms, and much of the cleaved α-mannosidase was localized in the high density fractions. After 3 h, all of the cleaved forms were detected in the high density fractions. However, a small peak of mature α-mannosidase was apparent in fractions at a density of 1.05 g/ml. This peak became more evident after 4 h of chase. The peak at 1.05 g/ml is visually difficult to separate from that at 1.07 g/ml. However, whenever chase periods were of sufficient duration (including four other gradients not shown), the d1.05 pool was detected by densitometry. The biphasic nature of the α-mannosidase activity pool is also supportive of this idea. Vesicles in this region of the gradient may represent a third pool of mature α-mannosidase. The half time for formation of the 1.07 g/ml pool was ~45 min, whereas formation of the 1.05 g/ml pool was not detectable until 1.5 h into the chase.

Newly cleaved α-mannosidase was first detected in the lower density organelles (d = 1.04 g/ml). To better identify the locale of the proteolytic event, we characterized the gradient fractions by marker enzyme analyses.

Characterization of Subcellular Fractions

As shown in Fig. 3, several enzyme activities could be separated by this fractionation procedure. When assayed at pH 6.5, glucose-6-phosphatase activity, an endoplasmic reticulum marker (25), was poorly localized in the gradient. This could be due to the presence of other phosphatases capable of hydrolyzing glucose-6-phosphate. To determine the amount of hydrolysis that could be attributed to acid phosphatase, we

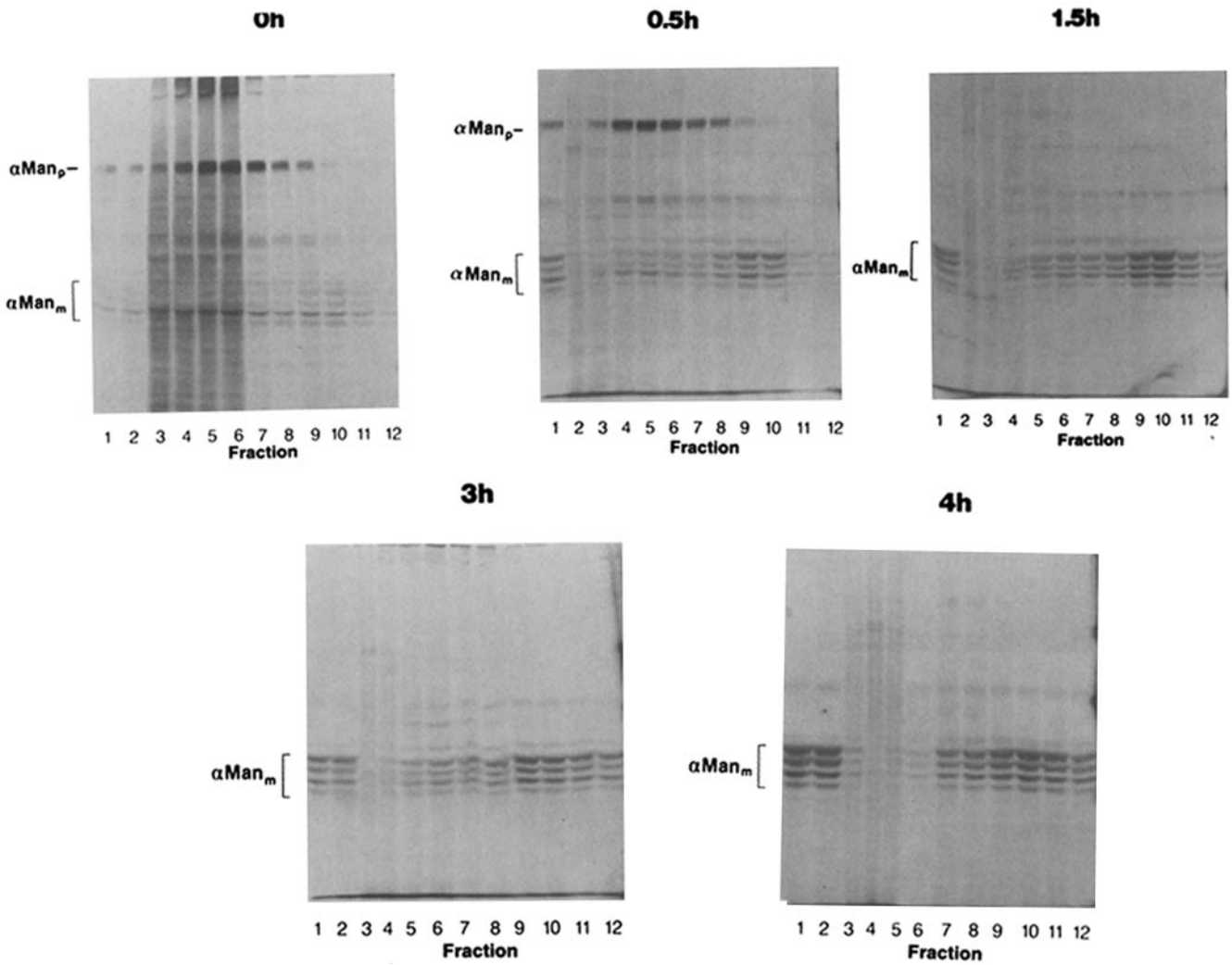

FIGURE 1 Effect of chase time on the distribution of specific activity of radiolabeled α-mannosidase in Percol gradients. For each time point, 1.5 × 10^8 cells (7.5 × 10^6 cells/ml) were incubated in HL-5 for 2 h, then transferred to a defined medium that lacked methionine. 2.5 mCi of [^{35}S]methionine were added, and the incubation was continued for 30 min. To begin the chase, the suspension was made 10 mM in methionine, and the cells were transferred to fresh HL-5. After the indicated period of time, cells were processed for subcellular fractionation. Labeled α-mannosidase forms were detected by immunoprecipitation, sodium dodecylsulfate polyacrylamide gel electrophoresis, and fluorography, as described in Materials and Methods. Fluorograms were exposed for 2–3 wk. For each gradient depicted, a constant number of cpm of trichloroacetic acid-precipitable radioactivity was loaded into each well of the gel. This number varied from gel to gel in the range 10,000–12,000 cpm/well. α-Man$_m$ is the group of mature α-mannosidase forms (46,000, 48,000, 54,000, and 58,000 D).

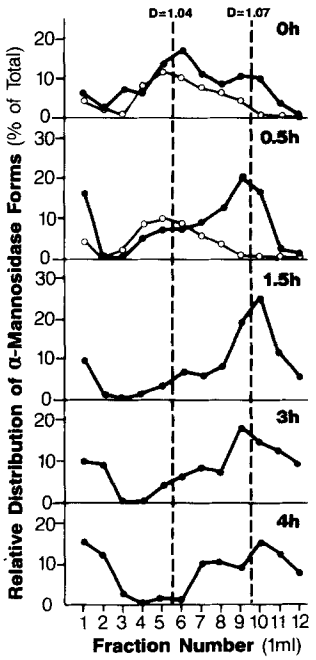

FIGURE 2 Densitometric analysis of fluorograms presented in Fig. 1. Fluorograms pictured in Fig. 1 were analyzed by densitometry using an LKB (Bromma, Sweden) Bromma Ultrascan laser densitometer. The graphs depict the percentages of total α-mannosidase precursor specific activity (○), or of total mature α-mannosidase specific activity (●) in each lane of the gel. 100% is the sum of all arbitrary densitometric units of either αMan_p or α-Man_m recovered from all lanes of the gel.

also performed glucose-6-phosphatase assays at pH 4.5. The ratio of enzyme activity at the two pH's was calculated. This ratio was maximal in fractions 3–5. Thus, these fractions appear to be rich in endoplasmic reticulum. A single peak of 5′-nucleotidase was detected at a density of 1.05 g/ml. By this criterion plasma membranes are localized in this region of the gradient (26). To determine the position of Golgi vesicles, we assayed sialyl-, galactosyl- (27), and fucosyltransferase activities (28). We could not detect the first two enzymes in crude membrane preparations from *D. discoideum*, or in gradient fractions. A small amount of fucosyltransferase activity was detected in membranes from the cells, but not in the gradients. As an alternative, we used the earliest site of incorporation of [^{35}S]sulfate into acid-precipitable material as a marker for the Golgi (29–38). Growing cells were resuspended in a defined medium (24) minus sulfate. The cells were pulse-labeled for 7 min with [^{35}S]sulfate and subjected to subcellular fractionation.

As shown in Fig. 3, a peak of acid-precipitable radioactivity was detected at a density range of 1.04–1.05 g/ml. By this criterion these fractions contain Golgi. We have detected a [^{35}S]sulfate-labeled α-mannosidase precursor in this same density range after a 30-min pulse–chase and gradient experiment performed as described in Fig. 1 (unpublished results). Although only α-mannosidase is shown, all lysosomal enzymes tested (α-mannosidase, β-hexosaminidase, and acid phosphatase) distributed bimodally with a major peak at 1.07 g/ml and a minor peak or shoulder at 1.05 g/ml. The soluble enzyme activities present at the top of the gradient can be attributed to leakage from damaged lysosomal vesicles.

Thus, pools of α-mannosidase were localized in three regions of the gradient. On the basis of marker enzyme analyses, the 1.07 g/ml pool co-migrated with lysosomes. The 1.05 g/ml pool co-migrated with lysosomes, plasma membranes, and Golgi. The 1.04 g/ml pool, which is the site of precursor cleavage, co-migrated with endoplasmic reticulum, plasma membranes, and Golgi. To further characterize the 1.04 g/ml pool of α-mannosidase, we monitored the transit of the enzyme in monensin-treated cells.

Monensin Slows Transit of Newly Cleaved α-Mannosidase

If the 1.04 g/ml pool resides in Golgi vesicles, then the transit of α-mannosidase might be inhibited in the presence of monensin (39). Although higher concentrations of monensin (5–10 μM) destabilized lysosomes and induced secretion of enzyme activity (data not shown), these complicating effects were not observed at 3.5 μM monensin. Therefore, cells in the presence of 3.5 μM monensin were labeled with [^{35}S]-methionine by the pulse–chase procedure described in Fig. 4. The addition of monensin caused an inhibition of transit of cleaved α-mannosidase. The distribution of α-mannosidase in the monensin-treated cells resembles that which was obtained after 1.5 h of chase in normal cells (compare Figs. 1 and 4). Monensin did not alter the distribution of α-mannosidase activity in the gradients. At the chase time depicted in Fig. 4, all precursor had been processed in both the presence and absence of monensin. Monensin never had a detectable effect on precursor processing at any chase time or at any concentration of monesin tested (even at concentrations above 5 μM where other forms of damage to the cells were noted).

Fate of α-Mannosidase Synthesized during Starvation

Next, we determined the effect of starvation on the transit of α-mannosidase forms. Transit of the enzyme from the 1.04 g/ml pool to the 1.07 and 1.05 g/ml pools continues during the first 2 h of starvation (data not shown). After 2 h of

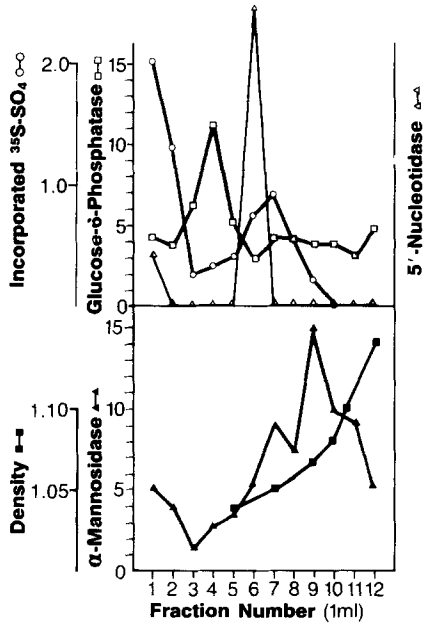

FIGURE 3 Distribution of marker enzyme activities in Percoll density gradients. For each activity measured, 1.5×10^6 cells were homogenized and subjected to subcellular fractionation, then fractions were assayed as described in Materials and Methods. The densities of the fractions were determined by the positions of density marker beads (Pharmacia Inc.). α-Mannosidase is expressed as U/μg protein × 10^2. 5′-Nucleotidase and incorporated [^{35}S]sulfate are expressed as cpm/μg protein. Glucose-6-phosphatase is expressed as the ratio of cpm/μg protein obtained at pH 6.5 to cpm/μg protein obtained at pH 4.5.

incubation in starvation buffer, cells stop secreting mature α-mannosidase (21). We wished to determine the site of the block in secretion. If the block occurs between the prelysosomal pool and the lysosomal pools, then we should not detect transport of mature forms out of the 1.04 g/ml pool. If the block occurs subsequent to transit of mature forms to the lysosomes, then we should detect levels of transit of enzyme similar to those in growing cells.

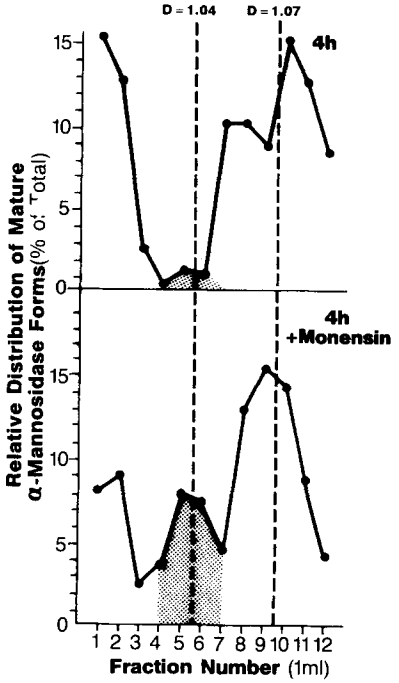

FIGURE 4 Effect of monensin on the distribution of specific activity of radiolabeled α-mannosidase in Percoll gradients. Cells were pulse-labeled and chased for 4 h in the absence or presence of 3.5 μM monensin, then processed, as described in Fig. 1. The fluorogram was analyzed by densitometry as described in Fig. 2. The graphs depict the percentages of total mature α-mannosidase specific activity in each lane of the gel.

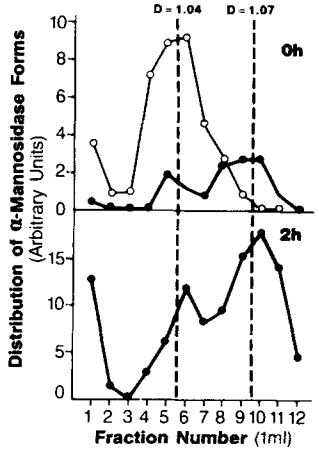

FIGURE 5 Effect of starvation on the distribution of specific activity of radiolabeled α-mannosidase in Percoll gradients. For each time point, 1.5×10^8 cells (7.5×10^6 cells/ml) were incubated in HL-5 for 2-h, then transferred to 5 mM sodium phosphate, pH 6.5 (starvation buffer). The incubation was continued for 1.5 h, then 2.5 mCi of [^{35}S]methionine was added. After 30 min, cells were transferred to fresh starvation buffer plus 10 mM methionine for chase. At 0 and 2 h, aliquots were removed, cells and media were separated, and cells were processed as described in Fig. 1. Media samples were subjected to immunoprecipitation and sodium dodecylsulfate polyacrylamide gel electrophoresis and fluorography as previously described (17). Fluorograms containing cellular samples were analyzed by densitometry as described in Fig. 2. The graphs depict the amounts of α-mannosidase precursor (○) or of mature α-mannosidase (●) in each lane of the gel.

Transit was measured under starvation conditions (Fig. 5). Cells that had been starved for 1.5 h were pulse-labeled with [^{35}S]methionine for 30 min in phosphate buffer. Aliquots were removed at 0- and 2-h chase times, the media was saved, and cells were processed for subcellular fractionation. α-Mannosidase was detected in the cells and media (17) as previously described. Fig. 5 clearly demonstrates that mature forms of α-mannosidase are transferred from the prelysosomal to lysosomal pools even hours after starvation began.

After 30 min the precursor and a small amount of cleaved forms were detected in the 1.04 g/ml pool. However, after 2 h in starvation buffer, a significant proportion of the precursor was extracellular (Fig. 6). Mature forms were not detected in the media at either time. Since the precursor continues to be secreted even up to 6 h (16) after starvation has begun, it probably follows an alternate route to the plasma membrane.

DISCUSSION

The main significance of the results presented here is that they contain strong physical evidence for the transit of α-

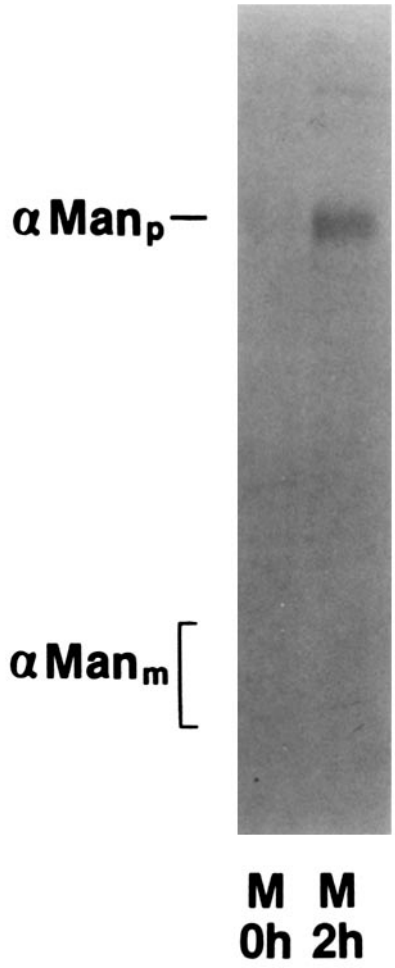

FIGURE 6 Secretion of α-mannosidase precursor by starved cells. Media samples from starved cells were obtained as described in Fig. 5. α-Man$_p$ is the 150,000-D α-mannosidase precursor. Man$_m$ is the group of mature α-mannosidase forms.

mannosidase through three linked pools. The prelysosomal pool (d = 1.04 g/ml) is the site of cleavage of the enzyme precursor. The higher density pools (d = 1.05 and 1.07 g/ml) also contain the steady state pools of α-mannosidase and other lysosomal enzyme activities. Transit of mature forms from the light pool to the heavy pools occurs in growth media as well as in starvation buffer and continues even when secretion of mature forms is complete. These results led us to develop a model for the maturation and distribution of α-mannosidase which is described below.

The physical evidence for the pools was obtained by their separation (Figs. 1 and 2). This was accomplished by gentle homogenization procedures, followed by differential and Percoll gradient centrifugation steps. Subcellular fractionation of *D. discoideum* with this resolution has not been previously described. This scheme consistently separates lysosomes from plasma membranes, Golgi, and endoplasmic reticulum, as determined by marker enzyme analysis (Fig. 3). We are recommending this scheme to others who wish to obtain lysosomal fractions from *D. discoideum*.

There seems little doubt that the 1.07 g/ml pool represents lysosomes, since it co-migrates with several lysosomal enzymes and has a relatively high density. The d1.05 pool also contains lysosomal enzymes but contains markers for other organelles as well. It was not possible to accurately measure the kinetics of formation of this pool. The significance of this pool is not yet clear. In human fibroblasts Rome et al. have detected two species of lysosomes on colloidal silica gradients (40). Their data suggest that the more buoyant species (d = 1.085) originates from the GERL, while the dense one originates from residual bodies. Müller has reported the bimodal distribution of *Tetrahymena pyriformis* acid hydrolases on sucrose gradients (41). These two species represent two distinct populations of lysosomes. It is not clear whether these pools are biosynthetically related.

The nature of the 1.04 g/ml pool of α-mannosidase was more difficult to resolve. Our data is consistent with the idea that the α-mannosidase precursor is cleaved in the Golgi (Figs. 2 and 3). Cleaved precursor was first detected in a region of the gradient that contained markers for endoplasmic reticulum and plasma membrane, as well as Golgi. Although we cannot rule out the possibility, it is unlikely that cleavage of the precursor takes place at the plasma membrane or in the endoplasmic reticulum. The results of the pulse–chase gradient analysis in the presence of monensin lend further support to the identification of the site of precursor cleavage as the Golgi (39). The addition of monensin caused an inhibition of transit of cleaved α-mannosidase from the 1.04 g/ml pool (Fig. 4). Interestingly, cleavage of the precursor occurs before the monensin block.

When these results are taken together with others (12–15), it is apparent that the locale of cleavage of a lysosomal enzyme precursor depends upon the cell type, the enzyme that is being cleaved, and even the cleavable sequence within a given enzyme. Thus Gieselmann et al. (14) reported that the first step in the cleavage of cathespin D in human fibroblasts begins in a prelysosomal compartment and continues after precursor is transported to lysosomes; while the second step in the proteolytic maturation of this enzyme appears to occur exclusively in lysosomes. This pattern is probably the same for alpha iduronidase in human fibroblasts (12). On the other hand, the cleavage of β-glucuronidase and β-galactosidase in mouse macrophages seems to occur solely in primary or secondary lysosomes (15). The idea of multiple potential sites for proteolytic processing is also supported by subcellular fractionation studies that indicate specific proteolysis can occur in lysosomal extracts (42) as well as either before or during the period hydrolases spend in coated vesicles (43). The earlier view that proteolytic processing is a marker for transport of acid hydrolases to lysosomes no longer seems tenable.

As a result of these and our previous studies (16, 17, 21), we have developed the following model for the transit of α-mannosidase. In growing cells the 150,000-D precursor is most likely synthesized and N-glycosylated in the rough endoplasmic reticulum. During its passage through the Golgi it undergoes sulfation (17), phosphorylation (18), and specific proteolytic cleavage. Cleaved forms are rapidly transferred into heavy lysosomes ($t_{1/2}$ = 45 min) and are subsequently localized in the d1.05 fraction (Fig. 2). The precursor of mature α-mannosidase in the d1.05 pool remains to be determined.

The transit of α-mannosidase between pools continues during starvation (Fig. 5). It appears that the secretion of mature enzyme stops after 2 h as a result of a halt in fusion of lysosomes with the plasma membrane. This fusion has been visualized by de Chastellier and Ryter by electron microscopic analysis (44). The continued secretion of endoglycosidase H–resistant precursor (17) into the media suggests that it follows a different route to the plasma membrane—most likely via the secretion of other Golgi-derived vesicles.

It may well be that the efficiently secreted forms of mature α-mannosidase originate from the 1.05 g/ml lysosomal pool. Müller has reported the preferential secretion of only the heavy class of lysosomes from starving *Tetrahymena pyriformis* (41). Experiments are currently underway to determine whether an analogous phenomenon occurs during starvation in *D. discoideum*. Also, further characterization of the light lysosomal pool may provide a clearer understanding of the heterogeneous nature of lysosomes. The techniques developed here can also be applied to the analysis of the problem of endocytosis in *Dictyostelium*.

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