

Developing *Dictyostelium* Cells Contain the Lysosomal Enzyme α -Mannosidase in a Secretory Granule

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Abstract. The prespore vesicle (PSV) is an organelle which secretes spore coat proteins and gal/galNAc polysaccharides from prespore cells of *Dictyostelium*. By combining the techniques of protein A-gold immunocytochemistry and ricin-gold affinity cytochemistry we have demonstrated colocalization of the lysosomal enzyme α -mannosidase with gal/galNAc polysaccharides in prespore vesicles and the spore coat. To determine the origin of prespore vesicles a series of pulse-chase experiments were performed. Cells were labeled with [35 S]methionine or [35 S]sulfate at different times during development and allowed to differentiate in the presence of unlabeled methionine or sulfate for various periods of time. The cells were homogenized and in-

tracellular organelles were separated using Percoll density gradient centrifugation. The distribution of [35 S]methionine-labeled α -mannosidase and [35 S]sulfate-labeled glycoproteins in the Percoll gradients was determined. It was found that prespore vesicles contained protein which was previously found in lysosomes. Newly labeled protein also entered these vesicles. The data suggest that developing *Dictyostelium* cells either restructure preexisting lysosomes into prespore vesicles or transport protein between these two organelles. We propose that secretory granules and lysosomes may have a common biosynthetic origin and may be evolutionarily related.

As a result of studies performed on a variety of cell types, it is now becoming evident that common events are involved in mediating secretion by lysosomes and secretory granules (for reviews on lysosomes and secretory granules see references 4, 25, and 42). Polymorphonuclear leukocytes and neutrophils secrete acid hydrolases from lysosomes as a consequence of increased phosphoinositide metabolism (8, 28, 41). Under these same conditions chromaffin cells secrete catecholamines from chromaffin granules (13, 50). Increases in intracellular inositol triphosphate, 1,2-diacylglycerol, calcium, and protein kinase C activity have been implicated to be important in mediating these secretion events. Such similarities between different organellar types suggests there may be a common evolutionary and biosynthetic origin for lysosomes and secretory granules.

The prespore vesicle (PSV)¹ is a secretory granule which arises as *Dictyostelium* cells differentiate into spores. It contains spore coat proteins (9, 10) and gal/galNAc polysaccharides (10, 24) which are exocytosed during the terminal stages of prespore cell differentiation. These vesicles were originally defined morphologically as having an electron-dense layer juxtaposed to the membrane and a fibrous matrix of variable appearance (21, 22, 32).

We recently identified two distinct lysosomal enzyme-

containing organelles which are present during starvation-induced differentiation of *Dictyostelium* (27). The two organelles can be easily separated by Percoll gradient centrifugation. The higher density acid hydrolase-containing vesicles arose as part of the *Dictyostelium* developmental program. These vesicles secreted their contents during the terminal stages of differentiation. Cytological studies revealed that prespore cells contain an acid phosphatase-staining organelle with a morphology similar to the PSV. Furthermore, PSVs were identified in the higher density fractions from Percoll gradients. We suggested that the higher density acid hydrolase-containing vesicles might be identical to PSVs (27). In this report we demonstrate by gold-labeled affinity cytochemistry that PSVs contain the lysosomal enzyme α -mannosidase. Once the contents of PSVs are exocytosed the α -mannosidase is found in the spore coat.

A great deal of information is available concerning the biosynthesis, processing, and transport of α -mannosidase (5, 7, 16, 17, 29, 34, 35, 39, 49). It is a tetramer composed of two α subunits (M_r 58,000) and two β subunits (M_r 60,000) (17). Both subunit types are derived from a single precursor polypeptide (M_r 140,000) (34, 39). Like a number of other lysosomal enzymes, α -mannosidase contains N-linked carbohydrates which are modified in the Golgi apparatus by addition of phosphate and sulfate residues at the 6-position of mannoses (17-19). To better characterize the relationship between PSVs and lysosomes, we performed [35 S]methionine and [35 S]sulfate labeling experiments to study the origin of

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1. Abbreviation used in this paper: PSV, prespore vesicle.

PSV-associated α -mannosidase and bulk glycoproteins, respectively. From our results we determined that PSVs contain protein which was previously found in lysosomes of vegetative cells. PSVs also received newly synthesized protein. Models are offered which exemplify the possible biosynthetic origin of PSVs.

Materials and Methods

Percoll, galactose, *N*-acetylgalactosamine, and protein A-gold were obtained from Sigma Chemical Co. (St. Louis, MO). Ricin-gold was purchased from Polysciences, Inc. (Warrington, PA). Lowicryl, glutaraldehyde, and formvar-coated nickel grids were obtained from Electron Microscopy Sciences (Ft. Washington, PA). Liquiscint and autofluor were purchased from National Diagnostics, Inc. (Sommerville, NJ). XAR-5 X-ray film was obtained from Eastman Kodak Co. (Rochester, NY). [³⁵S]Methionine (TRAN³⁵S label) and [³⁵S]sulfate were purchased from ICN Pharmaceuticals, Inc. (Irvine, CA).

Organism and Development

Dictyostelium discoideum Ax3 is a wild-type haploid strain capable of axenic growth. Ax3 cells were grown in broth medium (16). Cells were collected by centrifugation (2,000 g for 10 min), washed, and development was initiated by depositing cells on membrane filters (43). At various stages during development cells were harvested and washed before cytochemical or biochemical analysis.

Enzymatic Assays

α -Mannosidase (EC 3.2.1.24), β -glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), α -glucosidase II (EC 3.2.1.20), and alkaline phosphatase (EC 3.1.3.1) were assayed by following previously published procedures (1, 3, 30, 31, 48).

Electron Microscopy

Cells at 18 or 24 h development were dissociated by repeated pipetting in 10 mM Tris, pH 7.2, 0.25 M sucrose. Cells were fixed for 20–120 min at 21°C with 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The cells were gently agitated for 30 min at 4°C in 0.2 M ammonium chloride, 1 mg/ml sodium borohydride, 10 mM Tris, pH 7.2, and collected by centrifugation. This rinsing step was repeated four times to ensure that all free aldehydes generated by the fixation procedure were blocked. Samples for electron microscopy were dehydrated in a graded series of ethanol and embedded in Lowicryl K4M according to the supplier's specifications. Specimens were sectioned with a diamond knife, mounted on formvar-coated nickel grids, and incubated in 0.5% ovalbumin, 0.05% Tween 20, 150 mM NaCl, and 10 mM Tris, pH 7.2 (blocking buffer). Monoclonal antibody (2H9) directed against α -mannosidase was generously provided by Dr. R. L. Dimond, University of Wisconsin, Madison, WI (33, 34). Polyclonal antibody directed against spore coat proteins was a kind gift from Dr. W. F. Loomis, University of California, San Diego, La Jolla, CA (9). For α -mannosidase and spore coat protein-labeled grids, thin sections were incubated in blocking buffer, exposed to primary antibody diluted in blocking buffer, washed with blocking buffer, incubated in blocking buffer containing protein A-gold (5-nm particles), and washed again with blocking buffer. Controls included incubation of thin sections in the absence of sera or in the presence of control sera in place of immune sera.

Gal/galNAc polysaccharides were detected by incubating thin sections with ricin-gold conjugates (20-nm particles) as described by Erdos and West (10). Ricin is a lectin which binds galactose and *N*-acetylgalactosamine residues. Controls included incubation of thin sections with 200 mM galactose plus 200 mM *N*-acetylgalactosamine. Specimens were stained with uranyl acetate and lead citrate and observed with an electron microscope (H500; Hitachi Ltd., Tokyo) operating at 75 kV. When gal/galNAc polysaccharides and α -mannosidase were detected by double labeling, it was necessary to first incubate thin sections with ricin-gold and subsequently with immune sera and protein A-gold (10).

Radioactive Labeling and Cell Fractionation

To efficiently label cells we modified the growth medium described by Franke and Kessin (14). The labeling medium contained the components

Table I. Composition of Labeling Medium

Component	Concentration
	mM
Glucose	56
Amino acids	
L-Arginine	3
L-Aspartate	2
L-Cysteine	1
L-Glycine	12
L-Glutamate	3
L-Histidine	1
L-Isoleucine	5
L-Lysine	5
L-Phenylalanine	3
L-Leucine	7
L-Proline	7
L-Threonine	4
L-Tryptophan	1
L-Valine	6
Salts	
K ₂ HPO ₄	6
Na ₂ SO ₄ *	2
MgCl ₂	0.4
ZnCl ₂	0.1
CaCl ₂	0.02
FeCl ₃	0.01

The concentration of the minimal components needed for optimal labeling with [³⁵S]methionine are given. The recipe given for the labeling medium is a modification of the defined minimal growth medium described by Franke and Kessin (14). Addition of the appropriate vitamins are needed to sustain growth for more than two generations.

* Na₂SO₄ was omitted and 2 mM L-methionine was added to the medium when cells were radioactively labeled with [³⁵S]sulfate.

listed in Table I. Vegetative cells were resuspended in 50 ml of labeling medium and incubated in the presence of either 1 mCi of [³⁵S]methionine or 1 mCi of [³⁵S]sulfate for various periods of time. These cells were either immediately homogenized or they were placed on a nitrocellulose filter and allowed to proceed through 10 h of development in the presence of 10 mM nonradioactive methionine and then homogenized. Under these conditions reincorporation of label into proteins is minimized (29). Cells at 9 h of development were labeled for various periods of time with either 1 mCi of [³⁵S]methionine or 1 mCi of [³⁵S]sulfate and then homogenized.

Intracellular vesicles were then separated by using either 25 or 35% Percoll density gradient centrifugation (27). The gradients were fractionated and the Percoll were removed from each fraction by centrifugation at 42,000 rpm for 3 h in a rotor (50Ti; Beckman Instruments, Inc., Palo Alto, CA). Membranes were isolated from gradient fractions by a freeze-thaw procedure (27).

To identify the location of organelles within Percoll gradients, the gradients were assayed for enzymes that serve as markers for different organelles. β -Glucosidase, acid phosphatase, and α -mannosidase were used as markers for lysosomes (48), α -glucosidase II for the ER (3), and alkaline phosphatase for the plasma membrane (1). We assessed where the Golgi apparatus enzyme sulfate transferase was by looking to see where newly sulfated glycoproteins were found (6). 25% Percoll gradients were used to separate plasma membranes, lysosomes, Golgi apparatus, and ER. We found that 35% Percoll was better than 25% Percoll when attempting to separate lysosomes from PSVs.

TCA Precipitation

TCA precipitation of [³⁵S]sulfate-labeled glycoproteins was performed by mixing 0.5 ml of sample with 0.5 ml of 1 mg/ml BSA, adding an equal volume of cold 20% TCA, and incubating for 30 min on ice. TCA-insoluble material was collected on glass fiber filters (GF-B; Whatman Inc., Clifton, NJ). The filters were suspended in 10 ml of lyescent and TCA-precipitable counts were determined by liquid scintillation counting.

Immunoprecipitation and Gel Electrophoresis

α -Mannosidase was immunoprecipitated by following a modification of previously published procedures (35, 36). Briefly, 2H9 was added to a given amount of Triton X-100-soluble sample and incubated at 4°C for 1 h. Affigel protein A was then added to the sample and incubated for another hour. We found it necessary to dilute the samples with an equal volume of 10 mM Tris, 50 mM NaCl to efficiently immunoprecipitate α -mannosidase. The immunoprecipitate was collected by centrifugation and washed five times with 0.05% Triton X-100, 50 mM NaCl, 0.05% BSA, 10 mM Tris, pH 7.2. The immunoprecipitate was subjected to SDS-PAGE (26). The gel was fixed in 5% acetic acid for 15 min, rinsed with distilled water for 45 min, and submerged in autofluor for 2 h. The gel was dried onto filter paper and exposed to x-ray film at -70°C (XAR-5; Eastman Kodak Co.).

Results

Prespore Vesicles and the Spore Coat Contain the Lysosomal Enzyme α -Mannosidase

The intracellular location of α -mannosidase and spore coat proteins in *Dictyostelium* cells at 18 (preculmination) or 24 (postculmination) h of development was determined by immunocytochemistry (Fig. 1). Numerous α -mannosidase-containing vesicles were present in prespore cells (Fig. 1 a). These vesicles were found to be 0.5–1.0 μ m in diameter and contained a 350-Å-thick electron-dense layer juxtaposed to the membrane. The interior of these vesicles contained a fibrous matrix of variable appearance and electron density. The average cell was estimated to contain 30–40 of these vesicles. 18-h developed cells contained spore coat proteins within vesicles (Fig. 1 c) with a morphology similar to α -mannosidase-containing vesicles (Fig. 1 a). Spores at 24 h of development were found to contain α -mannosidase and spore coat proteins (Fig. 1, b and d) in the spore coat. These observations suggest that vesicles which contain spore coat proteins (i.e., PSVs) also contain α -mannosidase. These data are consistent with our previous suggestion that the PSV contains acid hydrolases (27).

To confirm that PSVs contained α -mannosidase, we looked for colocalization of α -mannosidase with other PSV components. Erdos and West (10) used ricin-gold affinity cytochemistry to demonstrate that gal/galNAc polysaccharide is present in PSVs and the spore coat. We combined the techniques of ricin-gold affinity cytochemistry and immunocytochemistry to investigate if PSVs contained α -mannosidase. The results revealed that α -mannosidase is colocalized with the gal/galNAc polysaccharide in PSVs (Fig. 2) and the spore coat (Fig. 3). Ricin binding was inhibited by incubating thin sections in the presence of 200 mM galactose and 200 mM *N*-acetylgalactosamine. Vegetative cells showed no labeling with the ricin-gold particles. Thin sections incubated with only protein A-gold or control sera plus protein A-gold did not contain label in PSVs or the spore coat. We conclude that PSVs contain the lysosomal enzyme α -mannosidase.

Prespore Vesicles Contain α -Mannosidase Previously Found in Lysosomes as well as Newly Synthesized α -Mannosidase

After establishing that PSVs contained acid hydrolases, we wanted to determine the biosynthetic origin of PSVs. PSVs could form from Golgi apparatus-derived vesicles, from preexisting lysosomal elements, or from both Golgi- and lysosomally derived elements. To study the formation of

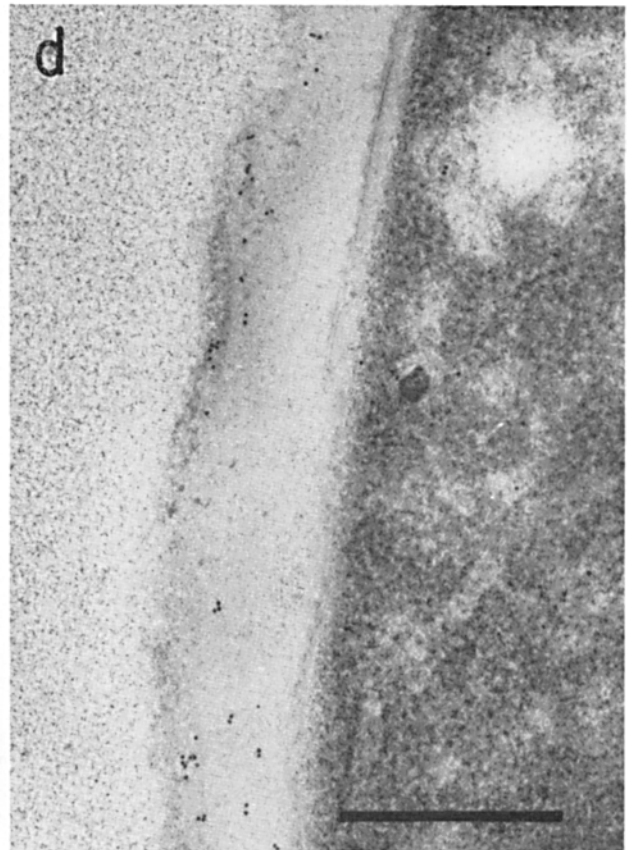
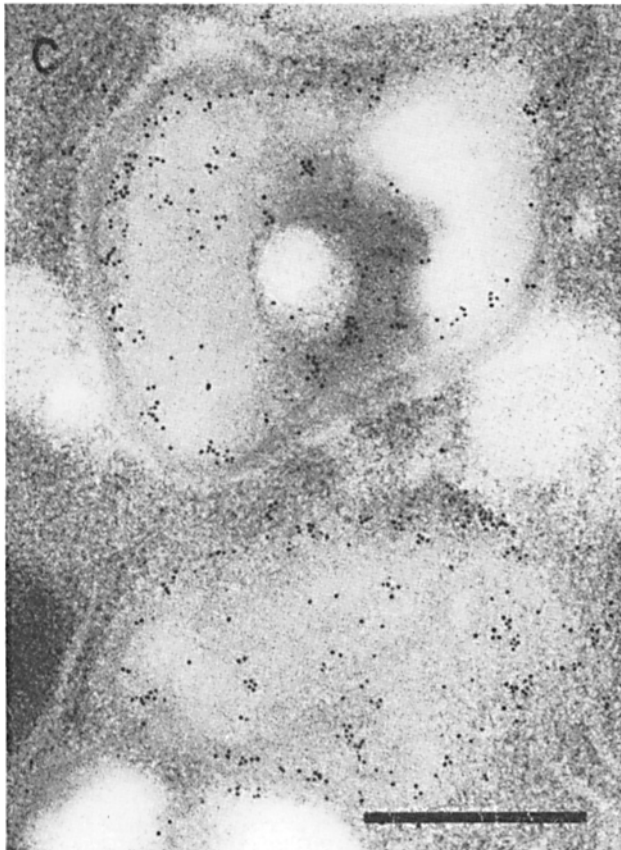
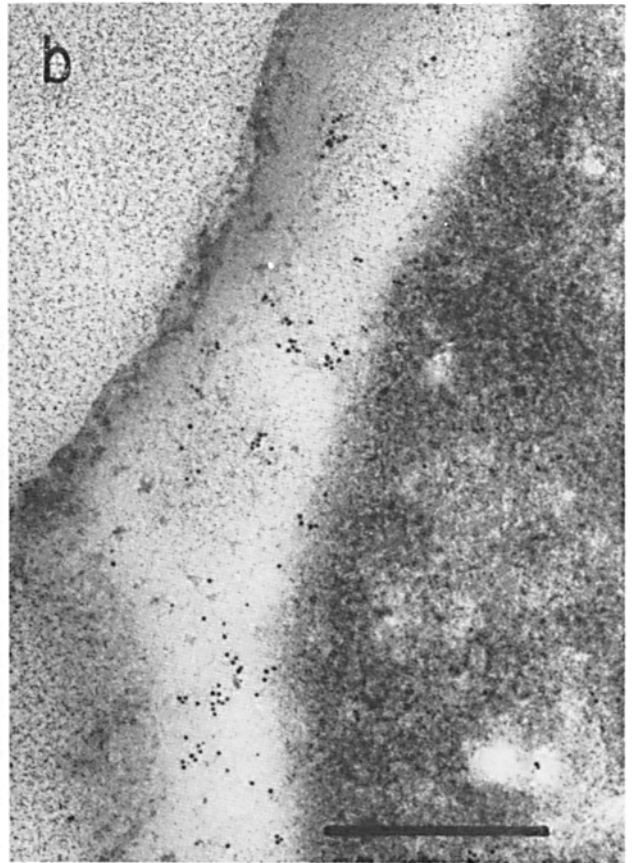
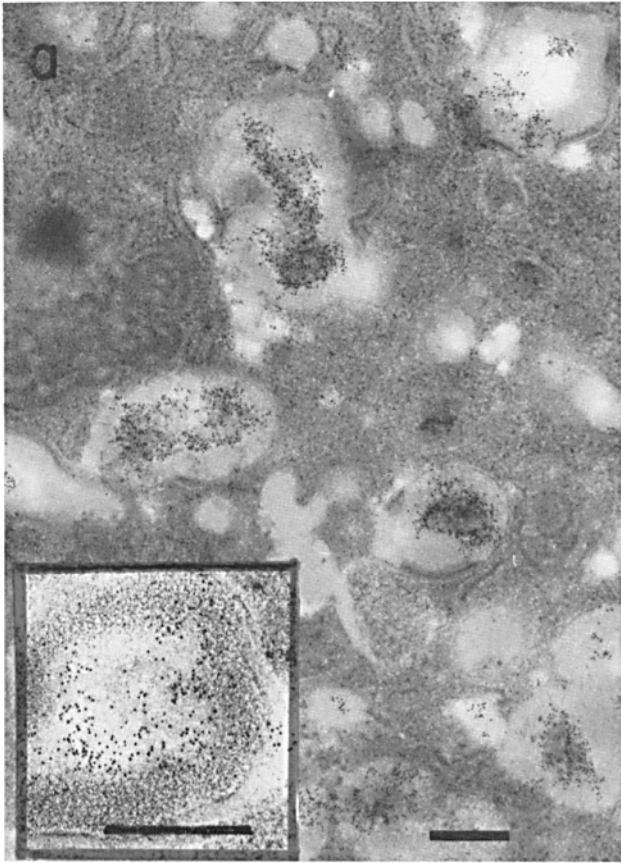
PSVs, experiments were performed to elucidate the source of α -mannosidase and sulfated glycoproteins associated with PSVs.

To test if PSVs contained α -mannosidase previously found within lysosomes, vegetative cells were labeled for 2 h with [³⁵S]methionine. The cells were then either immediately homogenized or they were allowed to undergo development for 10 h in the presence of excess nonradiative methionine and then homogenized. Intracellular vesicles were separated using 35% Percoll density gradients (Fig. 4). We have previously published evidence that the two populations of α -mannosidase-containing vesicles seen in 35% Percoll density gradients are lysosomes and PSVs (27). [³⁵S]Methionine-labeled α -mannosidase was immunoprecipitated from pooled gradient fractions (lysosomes and PSVs) and the precipitated complexes were subjected to SDS-PAGE and fluorography (Fig. 5). The results revealed that the lower density vesicles (lysosomes, 1.07 g/ml) from vegetative cells (lane 1) contained α -mannosidase as demonstrated by the presence of labeled 60- and 58-kD α -mannosidase subunits. As expected, vegetative cells were devoid of higher density vesicles (PSVs, 1.13 g/ml) and α -mannosidase could not be immunoprecipitated from that part of the gradient (lane 2). After 10 h of development both lysosomes (lane 3) and PSVs (lane 4) contained labeled α -mannosidase. The lower molecular mass bands (<58 kD) present within each lane represent degradation products of α -mannosidase. These results indicate that PSVs contain α -mannosidase which was previously found in lysosomes.

To test if PSVs receive newly synthesized proteins, cells at 10 h of development were labeled for 1 h with [³⁵S]methionine, homogenized, and intracellular vesicles were separated using 35% Percoll density gradients. [³⁵S]Methionine-labeled α -mannosidase was immunoprecipitated from pooled gradient fractions and the precipitated complexes were subjected to SDS-PAGE and autoradiography (Fig. 5). The data show that both lysosomes (lane 5) and PSVs (lane 6) contained newly made α -mannosidase. The above results indicate that PSVs receive newly made α -mannosidase from the Golgi apparatus as well as α -mannosidase which was previously found in lysosomes.

Sulfated Glycoproteins Previously Found in Lysosomes as well as Newly Synthesized Glycoproteins Enter Prespore Vesicles

Sulfation of *Dictyostelium* glycoproteins is known to occur in the Golgi apparatus (6). A number of lysosomal enzymes, including α -mannosidase, are known to be sulfated glycoproteins. To follow the routing of glycoproteins, we examined the fate of sulfated glycoproteins in vegetative and developing cells. Vegetative cells were labeled for 15 or 120 min with [³⁵S]sulfate, homogenized, and organelles were separated by using 25% Percoll gradients. 25% Percoll gradients allow for the separation of Golgi and lysosomes. Membranes from gradient fractions were isolated and the distribution of TCA-precipitable cpm associated with membrane-bound and soluble glycoproteins was determined. After a 15-min labeling, 70% of the radioactivity was membrane associated. Fig. 6 shows that after 15 min of labeling most of the sulfated glycoproteins were associated with membranes in organelles at a density of 1.04 g/ml (Fig. 6 A). We conclude that sulfation occurs on membrane-associated pro-



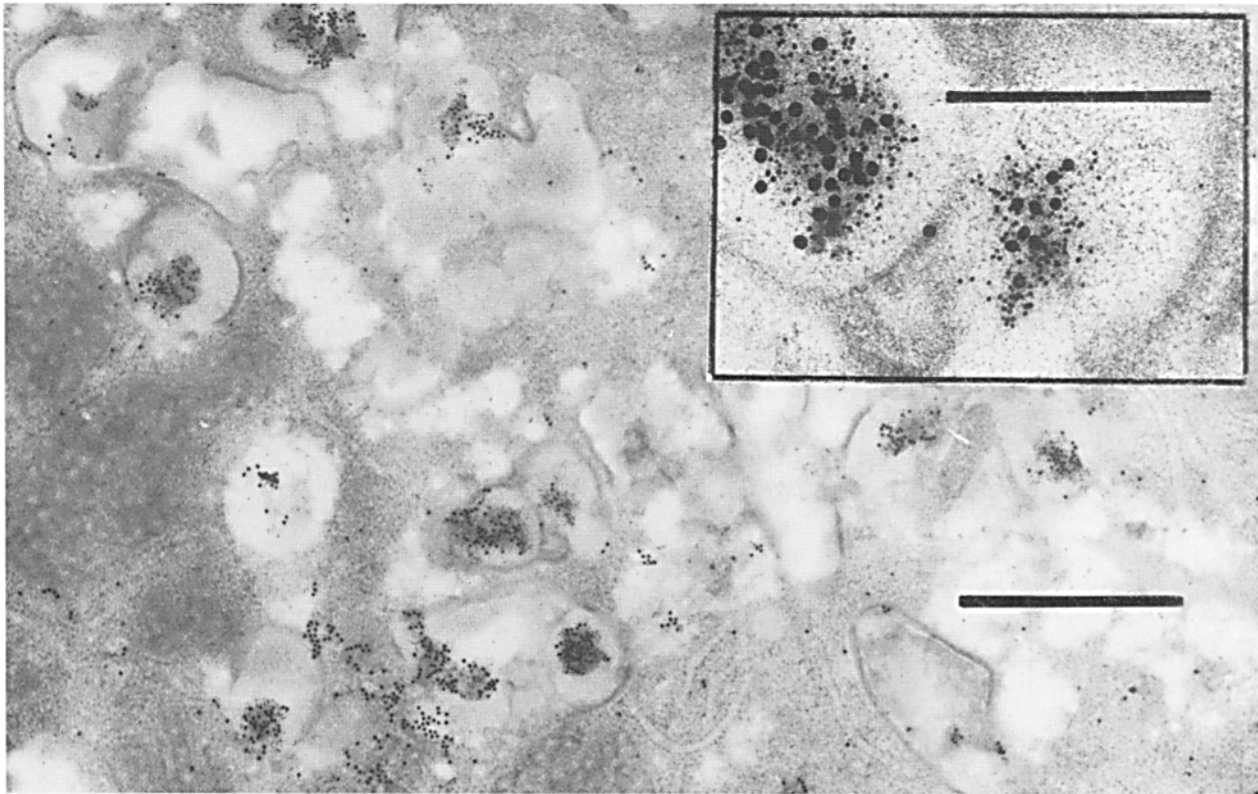


Figure 2. The intracellular location of α -mannosidase and gal/galNAc polysaccharides in prespore cells. The distribution of α -mannosidase and gal/galNAc polysaccharides in prespore cells was examined by immunocytochemistry and lectin affinity cytochemistry. The colocalization of α -mannosidase (5-nm gold particles) and gal/galNAc polysaccharide (20-nm gold particles) confirms that PSVs contain α -mannosidase. Bar, 1 μm (inset, 0.3 μm).

teins within the Golgi complex. If the cells were allowed to remain in the labeling medium for 120 min, 50% of the labeled material was found to be soluble. When cells were labeled for 120 min, the membrane-associated sulfated glycoproteins were predominately found in vesicles at a density of 1.04 g/ml (Fig. 6 B). Soluble sulfated glycoproteins were found predominately in vesicles at a density of 1.07 g/ml (Fig. 6 B). When we assayed for soluble β -glucosidase (Fig. 6 C), a lysosomal marker enzyme, we found the same gradient profile as shown for soluble sulfated glycoproteins (Fig. 6 B). Other lysosomal enzymes (α -mannosidase, acid phosphatase, *N*-acetylglucosaminidase, β -galactosidase I, and β -galactosidase II) were also found to have the same gradient profiles as shown for β -glucosidase. Membrane-associated acid hydrolases (Fig. 6 C), like the membrane-associated sulfated glycoprotein (Fig. 6, A and B), were found in vesicles at a density of 1.04 g/ml. We conclude that the vesicles containing membrane-associated sulfated glycoprotein at a density of 1.04 g/ml are Golgi vesicles and that the vesicles containing soluble glycoproteins at a density of 1.07 g/ml are lysosomes. Our data indicates that proteins are being sulfated

in the Golgi region while they are membrane associated and that some of these proteins are subsequently directed to the lysosome, where they are found as soluble glycoproteins. This conclusion is consistent with the results of Wood et al. (49) and Mierendorf et al. (35). They found that α -mannosidase precursor molecules are membrane associated in vesicles at a density of 1.04 g/ml (i.e., Golgi vesicles) and mature form α -mannosidase is soluble in vesicles at a density of 1.07 g/ml (i.e., lysosomes).

Marker enzymes were used to elucidate the presence of the ER and the plasma membrane in 25% Percoll gradients (Fig. 6 D). The profiles for these two organelles differed from that of lysosomes (Fig. 6, C with D). Within these gradients the ER and the plasma membrane were found in the same fractions as the Golgi apparatus (compare Fig. 6, A with D). When we compared the density of organelles from vegetative cells to those from developing cells we found that the density of the Golgi apparatus, the ER, and the plasma membrane remained the same (data not shown).

To determine if PSVs contain soluble glycoproteins previously found in lysosomes, vegetative cells were labeled for

Figure 1. The intracellular location of α -mannosidase and spore coat proteins in prespore and spore cells. Cells at 18 and 24 h of development were fixed and embedded in Lowicryl. Thin sections were incubated with anti- α -mannosidase or antispor coat protein IgG, washed, and incubated with protein A conjugated to 5-nm gold particles. Prespore cells can be seen to contain α -mannosidase (a) and spore coat proteins (b) within vesicles with similar morphologies. Spores were found to contain α -mannosidase (c) and spore coat proteins (d) within the spore coat. Bars, 0.3 μm .



Figure 3. The distribution of α -mannosidase and gal/galNAc polysaccharides in spores. The distribution of α -mannosidase and gal/galNAc polysaccharides in spores was examined by immunocytochemistry and lectin affinity cytochemistry. After exocytosis α -mannosidase (5-nm gold particles) and gal/galNAc polysaccharide (20-nm gold particles) are both located in the spore coat. Bar, 1 μ m.

2 h with [35 S]sulfate and either immediately homogenized or allowed to develop for 10 h in the presence of unlabeled sulfate and then homogenized. Intracellular vesicles were separated using 35% Percoll density gradients, which allow for the separation of lysosomes and PSVs. The gradient was fractionated and soluble organelle-associated protein was obtained. Soluble protein from each gradient fraction was

TCA precipitated and the percentage of the total recovered TCA-precipitable cpm found in each fraction was graphed as a function of the fraction number (Fig. 7). After the 2-h labeling the labeled soluble glycoprotein was present in lysosomes. If the cells were allowed to proceed through the first 10 h of development in the presence of unlabeled sulfate, labeled soluble glycoprotein was found in PSVs. These results

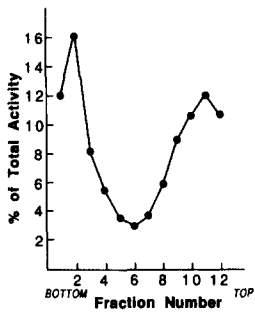


Figure 4. The separation of lysosomes and PSVs in a 35% Percoll gradient. Cells at 10 h of development were homogenized and the cellular organelles subjected to centrifugation in a 35% Percoll gradient (27). The gradient was fractionated and assayed for α -mannosidase activity.

suggest that PSVs contain sulfated glycoprotein which was previously found in vegetative lysosomes.

To determine if PSVs receive newly made glycoproteins, cells at 10 h of development were labeled for 10, 30, or 50 min with [35 S]sulfate, homogenized, and intracellular vesicles were separated using 35% Percoll density gradient. The amount of TCA-precipitable cpm and acid phosphatase activity in each vesicle population was determined. The results revealed that labeled glycoproteins began to accumulate in lysosomes (1.07 g/ml) and PSVs (1.13 g/ml) within 10 min of labeling (Table II). Longer periods of labeling resulted in the accumulation of labeled glycoproteins in the PSVs. We conclude that the PSV receives newly made proteins from the Golgi apparatus as well as proteins found previously in lysosomes.

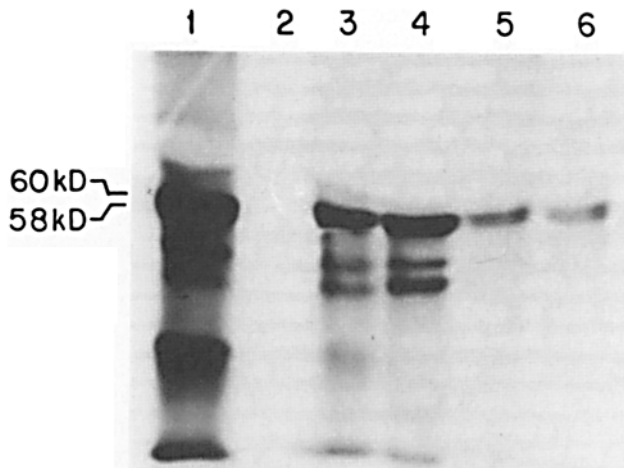


Figure 5. The origin of PSV-associated α -mannosidase. Cells were pulse-labeled with [35 S]methionine at various times during development and either harvested or allowed to differentiate in the presence of nonradioactive methionine. Intracellular vesicles were isolated and α -mannosidase was precipitated from an equivalent percentage of each pooled fraction (lysosomes and PSVs). The immunoprecipitates were subjected to SDS-PAGE followed by fluorography. (Lane 1) Lower density vesicles (lysosomes, 1.07 g/ml) from vegetatively labeled cells; (lane 2) higher density vesicles (PSVs, 1.13 g/ml) from vegetatively labeled cells; (lane 3) lysosomes from cells which were labeled during vegetative growth and allowed to proceed through 10 h of development; (lane 4) PSVs from cells which were labeled during vegetative growth and allowed to proceed through 10 h of development; (lane 5) lysosomes from cells labeled at 10 h of development; (lane 6) PSVs from cells labeled at 10 h of development.

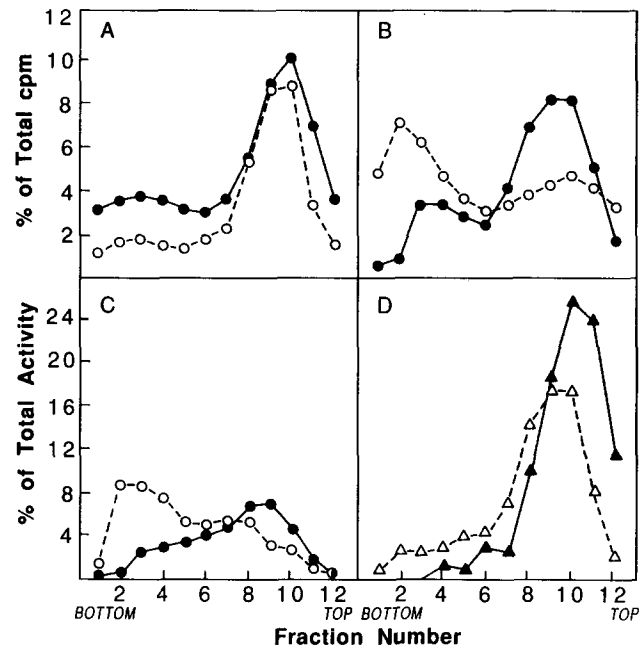


Figure 6. Marker assays for various intracellular organelles. Vegetative cells were labeled for 15 or 120 min with [35 S]sulfate, homogenized, and the intracellular organelles were separated by centrifugation in 25% Percoll density gradients. The gradients were fractionated and membrane-associated and soluble enzyme activities were separated following a freeze-thaw step (27). The percentage of total recovered membrane (\bullet) and soluble (\circ) organelle-associated activity present in each fraction is plotted versus the fraction number. (A) [35 S]Sulfate radioactivity, 15-min labeling (Golgi apparatus marker); (B) [35 S]sulfate radioactivity, 120-min labeling; (C) β -glucosidase activity (soluble enzyme serves as a lysosome marker), 120 min labeling; (D) α -glucosidase II (ER marker, \blacktriangle) and alkaline phosphatase (plasma membrane marker, \triangle) activity, 120-min labeling. Fraction 2, 1.07 g/ml (lysosomes); fraction 10, 1.04 g/ml (Golgi apparatus, plasma membrane, and ER).

Discussion

The results reported in this paper demonstrate that developing *Dictyostelium discoideum* cells contain the lysosomal enzyme α -mannosidase in PSVs (Fig. 2). Upon culmination,

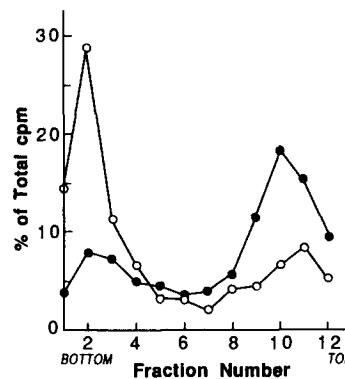


Figure 7. PSVs contain glycoproteins previously found in lysosomes. Vegetative cells were labeled for 120 min with [35 S]sulfate and either immediately homogenized or allowed to differentiate for 10 h in the presence of unlabeled sulfate before homogenization. Intracellular organelles were then separated by centrifugation in 35% Percoll density gradients. The gradient was fractionated, subjected

to rapid freeze-thawing, centrifuged, and soluble protein was collected from each fraction. The percentage of total recovered TCA-precipitable cpm present in each fraction is plotted versus the fraction number. (\bullet) Vegetative cells; (\circ) developing cells. Fraction 2, 1.13 g/ml (PSVs); fraction 10, 1.07 g/ml (lysosomes).

Table II. PSVs Receive Newly Made Glycoproteins

	10 min	30 min	50 min
% cpm found in PSVs	16.5	20.5	21.8
% AcPase found in PSVs	56.6	50.6	43
% cpm/% AcPase	0.29	0.41	0.51

Cells at 10 h of development were labeled for the indicated lengths of time with [³⁵S]sulfate, homogenized, and their intracellular organelles were separated by Percoll density gradient centrifugation. The percentage of total TCA-precipitable cpm and acid phosphatase activity recovered in PSVs was determined. To normalize for variations in recovery of PSVs between gradients, the percentage of total PSV-associated cpm was divided by the percentage of total PSV-associated acid phosphatase activity.

PSVs fuse with the plasma membrane and α -mannosidase can be found in the spore coat (Fig. 3). This is consistent with our earlier observations that a morphologically and biochemically distinct acid hydrolase-containing vesicle appears as part of the developmental program and secretes its contents during culmination (27).

Experiments were carried out to characterize the biosynthetic origin of PSV-associated protein. As part of these studies the sulfation of glycoproteins was examined in vegetative cells. Sulfation was found to occur on membrane-associated glycoproteins. Some of these proteins were subsequently directed to the vegetative lysosome, where they were found in a soluble form (Fig. 6). Our experiments suggest that PSVs received soluble sulfated glycoproteins from vegetative lysosomes (Fig. 7). PSVs were shown to receive α -mannosidase from vegetative lysosomes (Fig. 5). The data indicates that the lysosome serves as one source of PSV-associated protein.

Our data also indicates that PSVs are receiving newly synthesized α -mannosidase and sulfated glycoproteins (Fig. 5, Table II). This suggests that the Golgi apparatus serves as a second source of PSV-associated protein. This suggestion is supported by previous studies in which immunocytochemistry of spore antigens was used to demonstrate that PSV components are derived from the Golgi apparatus (37, 44).

Certain characteristics of the PSV satisfy criteria for calling it both a secretory granule and a lysosome. The PSV has been shown to act as a secretory granule which stores spore coat components destined to be exocytosed during the terminal stages of prespore cell differentiation (9, 10, 21, 22, 24, 32). Our data shows that the PSV is an organelle which concentrates a number of hydrolytic enzymes. Indeed, after 18 h of prespore cell development nearly all of the α -mannosidase can be found within PSVs. The finding that PSVs contain lysosomal enzymes suggests they may be modified lysosomes. Our observations that secretory granules contain acid hydrolases is not without precedence. Others have shown that renin-containing secretory granules of juxtaglomerular cells contain the lysosomal enzymes acid phosphatase (46), cathepsin B (45), and α -glucosidase (15). Although renin-containing granules are responsive to acidotropic amines and show some characteristics of autophagy (for review see reference 2) these features have not been demonstrated for PSVs. Further investigation on the lysosomal characteristics of PSVs is needed.

Lysosome heterogeneity has been a commonly observed phenomenon. Based on the kinetics with which α -mannosidase was secreted, Wood et al. (49) suggested that multiple

pools of lysosomal enzymes exist within the vegetative *Dictyostelium* cell. The PSV arises during the differentiation process and represents a pool of lysosomal enzymes in addition to those described by Wood et al. (49).

Cytological studies with prespore cells indicate that they contain both PSVs and lysosomes (20, 27, 40). We found that the ratio of lysosomes to PSVs decreases as the organism progresses through development. Just before culmination virtually all of the α -mannosidase is found in PSVs.

Three models for the origin of PSVs are consistent with our data. The first of these is that transport vesicles or connecting tubules may mediate transport from lysosomes to PSVs. This model is similar to the "stationary cisternae" model for Golgi transport which proposes that proteins pass from one Golgi stack to the next (for review see reference 4). In addition to receiving protein from lysosomes, the PSVs would also be receiving newly synthesized glycoprotein from the Golgi complex. A second model is that lysosomes may be maturing to become PSVs. In this model the maturation of lysosomes to PSVs may be facilitated by fusion of Golgi-derived transport vesicles with lysosomes. This model is similar to the "cisternal progression" model for Golgi transport which proposes that each Golgi stack matures into the next Golgi stack (for review see reference 11). A third related model is that Golgi-derived PSVs may be fusing directly with preexisting lysosomes. Precedence for this model comes from evidence that phagosomes, lysosomes, and secretory granules are capable of direct fusion with other preexisting lysosomes (12, 23, 38, 40).

Studies using *Dictyostelium* may be useful in elucidating the relationship between secretory granules and lysosomes. Based upon our results we propose that PSVs may be modified lysosomes which allow for the regulated secretion of spore coat materials. This suggestion indicates that secretory granules and lysosomes may be evolutionarily related. Future research on how proteins found previously in lysosomes enter PSVs may be helpful in elucidating the relationship between the two organelles.

It is difficult to assess what the functional significance of lysosomal enzyme secretion during the terminal differentiation of prespore cells is. One possibility is that the prespore cell may be using secretion to remove unneeded lysosomal enzymes from the spore. We feel that this is unlikely because it would cause an already starving cell to waste energy. Another possibility is that extracellular acid hydrolases may be playing a role in the processing of the extracellular matrix. Extracellular enzymes might serve in the formation of the spore coat during culmination or in the digestion of the spore coat during germination. Processing of the extracellular matrix by acid hydrolases has been proposed for tumor cells during metastasis (47). The localization of acid hydrolases (e.g., acid phosphatase and invertase) in the cell wall of fungi suggests the fungal cell wall plays an important role in retaining enzymes needed for extracellular digestion. In a similar manner the spore coat of *Dictyostelium* might retain acid hydrolases which serve in the digestion of exogenous food stuff. Yet another possibility is that these enzymes might serve as a protectant against other microbes.

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