INTERMEDIATE GOLGI α -D-MANNOSIDASE ACTIVITY IN FIBROBLASTS FROM PATIENTS WITH MANNOSIDOSIS AND MUCOLIPIDOSIS II AND III.

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SUMMARY

We have examined extracts of fibroblasts from patients with mannosidosis, mucopolipidosis (ML) II, ML III, and normal controls for α -D-mannosidase activity against 4-methylumbelliferyl- α -D-mannopyranoside to test for the presence of the "intermediate" pH 5.5 enzyme activity that has been called the "Golgi mannosidase." Fibroblast extracts were prepared by sonication and sedimented to separate membrane-associated activities from cytosolic and lysosomal α -D-mannosidases. Membranes were extracted by salt washes (0.4 M NaCl) to desorb the lysosomal enzymes that sedimented with membranes. The α -D-mannosidase activity remaining with membranes showed many properties described for the Golgi mannosidase including: 1) an "intermediate" pH optimum (pH 5.5-6.0), 2) activity with the synthetic 4-methylumbelliferyl substrate, 3) lack of inhibibility by 200 mM methyl- α -D-mannopyranoside, and 4) partial resistance to solubilization by salt washing, and to a single extraction with the non-ionic detergent Triton X-100.

This intermediate activity was the major α -D-mannosidase activity remaining in membranes from normal fibroblasts following sedimentation and salt washing, and was the major component of the α -D-mannosidase activity in extracts of fibroblasts from patients with mannosidosis, ML II, and ML III, in all of which, the lysosomal "acid" α -D-mannosidase was greatly reduced.

The specific activity of intermediate α -D-mannosidase in membranes from fibroblasts from patients with mannosidosis, ML II, and ML III was not reduced compared to that of fibroblasts from controls. These studies provided no evidence to support the prior suggestion of a deficiency of "Golgi mannosidase" in ML II or ML III.

SPECULATION

Although these studies argue against a deficiency of "Golgi mannosidase" in mucopolipidosis (ML) II and ML III, other evidence suggests that there is an oligosaccharide processing abnormality affecting the oligosaccharides on acid hydrolases secreted by ML II fibroblasts. This abnormality is inferred from the presence of complex-type oligosaccharide chains rather than high-mannose type chains on l-cell secretion enzymes. We suggest that this abnormality can be explained by the primary defect in l-cell disease, failure to phosphorylate the high-mannose oligosaccharide chains of newly synthesized ML II acid hydrolases. The mannose-6-phosphate recognition markers normally bind receptors and effect segregation of acid hydrolases from secretory products of the endoplasmic reticulum. We suggest that the phosphate also prevents processing of the protected high-mannose oligosaccharide chains by mannosidases, which participate in the conversion of high-mannose type to complex type oligosaccharides that are typical of secretory glycoproteins. Absence of the phosphate would allow the unprotected high-mannose chains on l-cell enzymes to be processed to complex-type oligosaccharides in the Golgi apparatus before the enzymes are secreted, which would explain the properties of l-cell secretion enzymes.

INTRODUCTION

Animal tissues contain multiple α -D-mannosidases (α -D-mannoside mannohydrolase EC 3.2.1.24) which have distinct properties, subcellular localizations, and functions. Lysosomal α -D-mannosidase, also called "acid" α -D-mannosidase because its pH optimum is below pH 5.0, is important in the catabolism of macromolecules containing α -D-mannosyl residues (glycoproteins and some forms of keratan sulfate). An inherited deficiency for this enzyme produces a lysosomal storage disease called mannosidosis which has been recognized in man (15,21) and cattle (23). Only the lysosomal enzyme form is affected by the mutation producing mannosidosis (2,23).

A "neutral" α -D-mannosidase has been clearly distinguished from the lysosomal form by its pH optimum (pH 6.0-6.5), its cytosolic localization, and by several other properties (22,24). Although genetic (3,4), immunologic (22), and biochemical (22) evidence all indicate that this enzyme is unrelated to the lysosomal enzyme, the function of neutral α -D-mannosidase is still uncertain.

The third distinct α -D-mannosidase to be identified in mammalian tissues is the "Golgi" mannosidase which was discovered, purified, and characterized by Touster and co-workers (5,22,30). This enzyme was distinguished from the previously recognized α -D-mannosidases primarily by its "intermediate" pH optimum (pH 5.5), its localization in Golgi membranes in subcellular fractions from rat liver, and its strong association with these membranes from which it can be solubilized only with detergents. The recent work of Tabas and Kornfeld (28,29) and of Harpaz and Shachter (11) suggested that there may be two additional α -mannosidases associated with the Golgi apparatus that play important roles in removing mannose residues during the processing of oligosaccharide chains on newly synthesized glycoproteins in the Golgi apparatus.

It has been suggested that a deficiency or abnormality of the Golgi mannosidase described by Dewald and Touster (6), which has been assumed to comprise the main component of α -D-mannosidase present in normal human and bovine serum (14,19,34) may be important in the pathogenesis of mucopolipidosis II (ML II) and mucopolipidosis III (ML III) (17). ML II, also called l-cell disease, and ML III are severe and mild forms, respectively, of a lysosomal storage disease that results, not from a defect in a structural gene for a lysosomal enzyme, but rather from a defect in addition of the "common recognition marker" to acid hydrolases that allow them to be segregated into lysosomes rather than to be secreted (1,12,25). Fibroblasts from these patients show deficiencies for nearly all acid hydrolases intracellularly, but also exaggerated secretion of these hydrolases into the medium (13,32,33). Sera from affected patients show greatly elevated levels of most acid hydrolases, including α -D-mannosidase (5,8). On the basis of studies of the properties of the elevated α -D-mannosidase in sera from patients with ML II and ML III,

Kress and Miller (17) suggested that "absent or altered intermediate mannosidase may be responsible for the aberrant biochemical properties reported for other glycosidases in Mucopolipidosis II and Mucopolipidosis III." We have examined extracts of normal and mutant human fibroblasts directly for a membrane-associated enzyme with the properties reported for the Golgi mannosidase described by Touster's group (6,22,30). The purpose of this report is to present studies which show that such an activity could be demonstrated in extracts from normal human fibroblasts and that this activity was undiminished in fibroblasts from patients with lysosomal α -D-mannosidase deficiency, patients with isolated neuraminidase deficiency, also called mucopolipidosis I (ML I), and also in fibroblasts from patients with ML II and ML III. These studies provide no evidence to support the suggestion that a deficiency of the "intermediate" α -D-mannosidase (i.e., the Golgi mannosidase described by Touster and co-workers) (5,22,30) contributes to the abnormal localization (secretion rather than retention in lysosomes) or the abnormal processing (1, 12, 16, 25, 26, 27) of acid hydrolases in ML II and ML III.

MATERIALS AND METHODS

Materials

Most reagents were purchased from Sigma Chemical Co., St. Louis, Mo. 4-methylumbelliferyl substrates were obtained from Research Products International Corp., Elk Grove Village, Ill. Skin biopsies to establish fibroblasts were performed with informed parental consent. Cultures from four individuals without demonstrated biochemical abnormalities were used as controls. Cell lines from three patients with mannosidosis (GM-654, GM-2817, GM-2025), and three patients with sialidase deficiency (ML I) (GM-292, GM-1718 and GM-2837) were obtained from the Human Mutant Cell Repository, Camden, N.J.. The three patients with sialidase deficiency had three different clinical phenotypes. Fibroblasts from patient L.T. with ML II were kindly provided by Dr. T.E. Kelly, Charlottesville, Virginia. Fibroblasts of another patient (T.M.) with ML II were obtained from the Division of Medical Genetics, University of Texas Health Science Center, Dallas, Texas. Cultured fibroblasts were established from skin biopsy obtained from patient D.F. with ML III, also available as line GM-2559, from the Human Mutant Cell Repository Camden, N.J.

Fibroblast culture and subcellular fractionation

Fibroblasts were cultured as previously described (10). Cell cultures were harvested between 2 and 3 weeks after subculture. After removing the media, roller bottles were washed twice either with phosphate buffered saline (PBS) or normal saline. Cells were scraped from the roller bottle with a rubber policeman into either PBS or normal saline and washed twice by resedimentation in the same solution at 1000 rpm for 5 min in a clinical centrifuge. The pellet was resuspended in 6 volumes of either 0.005 M Hepes buffer, pH 7.6 or distilled water and sonicated on ice with a miniprobe for two periods of 12 seconds each at setting 30 (Biosonik III apparatus, Bronville Sci., Rochester, N.Y.). Part of this material, called crude sonicate (CS), was centrifuged at 30,000 x g for 15 min in a Sorvall refrigerated centrifuge, model RC-3. The supernatant (S₁) and part of the pellet (P₁) were saved for enzymatic assays and protein determination. The rest of the pellet was washed twice with 0.4 M NaCl by resedimentation at 30,000 x g for 15 min. The final pellet (P₃) was resuspended in either 0.005 M Hepes buffer pH 7.6 or in distilled water. All operations were carried out at 4 °C.

Enzymatic assays

Enzyme activities were determined fluorometrically as previously described (9). Synthetic substrates were 4-methylumbelliferyl- α -D-mannopyranoside, 8 mM in 0.1 M sodium acetate buffer, pH 4.0 or 5.5, for α -D-mannosidase activity, and 4-methylumbelliferyl- β -D-N-acetylglucosaminide, 1 mM in 0.02 M sodium phosphate-citrate buffer, pH 4.4, for β -D-hexosaminidase activity.

Assays were carried out by incubating 25 μ l of enzyme solution and 100 μ l of substrate at 37 °C for 30-60 min. Reactions were stopped by adding 1.8 ml of glycine-carbonate buffer, pH 10.5. All assays were performed in duplicate. When necessary, enzyme preparations were diluted with the assay buffer solution containing 0.075 M NaCl, 1 mg/ml human serum albumin and 0.001% Triton X-100. One unit is defined as the amount of enzyme that releases 1 nanomole of 4-methylumbelliferone per hour.

Protein determination

Protein concentration was estimated by the method of Lowry et al. (18) with bovine serum albumin as the standard

Triton solubilization

Triton solubilization of the enzymatic activities from membrane preparations (P₃) was carried out according to Tulsiani et al. (39). The P₃ fraction was suspended in 50 mM Tris-HCl buffer, pH 7.2, containing 5 mM MgCl₂, 0.1 to 0.5% Triton X-100 and 0.9 mg/ml protein. The suspension was vigorously stirred on a Vortex every 5 min for 30 sec and kept on ice when not stirred. After 20 min, the suspension was centrifuged at 105,000 x g for 1 h in a Beckman L2-65B ultracentrifuge and the sediment used for assays.

RESULTS

Several α -D-mannosidase activities in crude mammalian cell extracts contribute to the hydrolysis of 4-methylumbelliferyl- α -D-mannopyranoside at pH 4.0 and at pH 5.5, the pH optima of the "acid" lysosomal α -D-mannosidase and the "intermediate" one which has been found to be associated with membranes from the Golgi complex. Dewald and Touster (6) devised a fractionation scheme for rat liver to demonstrate the different subcellular localizations and properties of the pH 4.0 lysosomal α -D-mannosidase and the pH 5.5 "intermediate" Golgi mannosidase, and showed that the small amount of lysosomal enzyme that sedimented with Golgi membranes could be desorbed by salt washing with 0.4 M NaCl. We have used a simple cell disruption method (sonication) to release lysosomal enzymes from cultured fibroblasts and to release the sedimentable membranes similar to that described by Dewald and Touster (6) to evaluate the membrane associated α -D-mannosidase that was sedimented from sonic extracts of normal and

mutant fibroblasts. The cell disruption, fractionation, and salt washing procedures used are described in Methods.

Recoveries of acid hydrolases and pH 5.5 α -D-mannosidase in fractions from sonic extracts.

Table I reports the percentage of activities present in the crude sonicates for the lysosomal marker enzyme β -D-hexosaminidase, and the α -D-mannosidase activities measured at both pH 4.0 and 5.5 that were recovered in subcellular fractions from the fibroblast lines studied. Approximately 85% of the lysosomal marker enzyme was released from control fibroblasts into the first supernatant (S_1) following sonication. A further fraction of lysosomal enzymes adsorbed to membranes was released by washing the membranes (P_1) with 0.4 M NaCl. The salt washing reduced the β -hexosaminidase activity associated with membranes from 18.5% $\pm$ 7.7 in P_1 to 2.0 $\pm$ 0.4% in P_3 . The distribution of β -D-hexosaminidase recovered in fractions from sonic extracts of fibroblasts from patients with sialidase deficiency (ML I) and "acid" α -D-mannosidase deficiency was comparable to that seen in fractions from control fibroblasts. A slightly smaller fraction of β -D-hexosaminidase from ML II and ML III fibroblasts was released into the supernatant by sonication, and a correspondingly smaller amount of membrane-associated β -D-hexosaminidase was released by salt washes from the membrane fractions. These extracts had much lower levels of total β -D-hexosaminidase, pH 4.0 α -D-mannosidase and other acid hydrolases than control fibroblasts (see Fig. 1) but we have no explanation for the apparent slight difference in distribution of β -D-hexosaminidase in fractions from ML II and ML III fibroblasts from that seen in control fibroblasts.

The α -D-mannosidase measured at pH 4.0 showed the same distribution of enzyme recovered in fractions from control, ML I, ML II, and ML III fibroblasts as that shown by β -D-hexosaminidase, which was described above. A slightly larger fraction of the genetically reduced amount of pH 4.0 α -D-mannosidase activity in mannosidosis fibroblasts, which represents the sum of the residual "acid" α -D-mannosidase and a contribution to the pH 4.0 activity by the "intermediate" α -D-mannosidase, was associated with the sedimentable membrane fractions.

By contrast, the pH 5.5 α -D-mannosidase shows a different distribution than the lysosomal marker enzyme β -D-hexosaminidase and the pH 4.0 α -D-mannosidase. In all three cell lines with deficiencies of the lysosomal (pH 4.0) α -D-mannosidase (mannosidosis, ML II and ML III fibroblasts) a much larger fraction of the pH 5.5 activity that was present in the crude sonicates, was associated with membrane fractions P_3 . This result suggests that much of the pH 5.5 α -D-mannosidase activity measurable in sonicates of control and ML I fibroblasts is actually due to the activity of the "acid" lysosomal enzyme at pH 5.5.

Specific activities of β -D-hexosaminidase and α -D-mannosidase in fractions from sonic extracts of fibroblasts.

Figure 1A shows the specific activities of β -D-hexosaminidase in crude sonicates and subcellular fractions from normal and mutant fibroblasts. As expected for a lysosomal marker enzyme, the specific activity was increased in the supernatant fractions S_1 , and greatly reduced in the membrane fractions P_1 , from all cell lines. The specific activity in the membrane fractions was further reduced by salt washes (P_3). The reduced activity in all fractions from ML II and ML III fibroblasts is also clear from Figure 1A.

Figure 1B shows essentially the same profile of specific enzyme activity for the pH 4.0 α -D-mannosidase in fractions from control and ML I fibroblasts. However, in the ML II, ML III, and mannosidosis fibroblasts, the pH 4.0 α -mannosidase activity was greatly reduced, especially in the supernatant.

Figure 1C shows the specific activity profile for pH 5.5 α -D-mannosidase activity. Three points should be made from this figure. First, the specific activity was reduced to about 50% in P_3 for control and ML I fibroblasts, compared to that in the crude sonicate, reflecting removal of the contribution which the acid α -D-mannosidase makes to the pH 5.5 activity of the crude sonicate. Secondly, the specific activity of the pH 5.5 activity in the membrane fractions from ML II, ML III, and mannosidosis fibroblasts increased compared to that of the crude sonicate, indicating partial purification of this activity in membrane fractions. Third, in contrast to the specific activities of the acid α -D-mannosidase in S_1 , the specific activities of the pH 5.5 α -D-mannosidases in the P_3 fractions were not significantly different among the cell lines studied. This result indicates that the pH 5.5 activity associated with membranes in the P_3 fraction was not deficient in fibroblasts from mannosidosis, ML II, or ML III patients which were very deficient for the acid lysosomal α -D-mannosidase.

Enzyme release from membrane by single extraction with Triton X-100.

Tulsiani et al. (30) showed that the "intermediate" Golgi mannosidase was not easily solubilized from membranes by non-ionic detergents, although successive extractions with Triton X-100 released a large fraction of the enzyme and provided a means to solubilize the enzyme for purification from Golgi membranes. Figure 2 compares the release of P_3 -associated β -D-hexosaminidase and pH 5.5 α -D-mannosidase from membranes from control and ML II fibroblasts by a single extraction with increasing concentrations of Triton X-100. A single extraction with Triton concentrations above 0.1% released 120% and 160%, respectively of β -D-hexosaminidase from P_3 from control and ML II fibroblasts. This stimulation above 100% by Triton X-100 presumably reflects release of both adsorbed hexosaminidase and "latent" hexosaminidase that was vesicle enclosed prior to addition of detergent. The same concentrations of Triton released only 40-60% of the membrane associated pH 5.5 α -D-mannosidase from P_3 . The fraction of α -mannosidase which is resistant to release from membranes by a single extraction with Triton X-100 is considered analogous to the Golgi mannosidase described by Tulsiani et al. (30) and there was no evidence that this membrane-associated α -mannosidase activity was reduced in extracts of ML II fibroblasts.

pH-activity profiles of α -D-mannosidase in the crude sonicate and in Triton-extracted P_3 .

Figure 3 shows the pH activity profiles of α -D-mannosidase activity in the crude sonicate of control, ML II, and mannosidosis fibroblasts. The sonicate from control fibroblasts, which has 12 and 10 times as much pH 4.0 activity as the ML II, or mannosidosis fibroblasts respectively, under these assay conditions, shows peak activity at this pH and no obvious additional peaks at higher pH's. The pH-activity profile of the 30,000 x g supernatant (S_1) from control fibroblast extracts (not shown) was no different than that shown for the crude sonicate. Both the ML II and

mannosidosis fibroblast extracts, which have greatly reduced levels of the pH 4.0 lysosomal α -D-mannosidase, show two pH optima, one corresponding to that expected for the lysosomal enzyme, and an even larger peak corresponding to the intermediate α -D-mannosidase activity. Figure 3 also shows the pH-activity profiles of the salt washed pellet P_3 which was extracted further by treatment with 0.3% Triton. A small amount of residual pH 4.0 activity is seen in the membranes from control fibroblasts but the major activity is found in the pH range 5.5-6.0. This is the only activity present in Triton-extracted P_3 from ML II and mannosidosis fibroblasts. Figure 3 shows 1) that the intermediate pH 5.5 activity, which is obscured by the much larger amount of pH 4.0 activity in crude sonicates from control fibroblasts, is actually the major activity present in crude sonicates of ML II and mannosidosis fibroblasts, and 2) that this activity from both mutant and control fibroblasts sediments with membranes and remains sedimentable following treatment of the membranes with Triton X-100.

Effects of methyl- α -D-mannopyranoside on α -D-mannosidase activities.

Dewald and Touster (6) reported that both the lysosomal and cytosolic α -D-mannosidases in fractions from rat liver were inhibited by methyl- α -D-mannopyranoside with K_i values of 125 mM and 4.5 mM respectively. However, even 250 mM methyl- α -D-mannoside produced less than 5% inhibition of the Golgi mannosidase activity toward p-nitrophenyl derivatives. Table II shows the effects of 200 mM methyl- α -D-mannopyranoside on the α -D-mannosidase activities measured at pH 5.5 in the crude sonicate and fractions from controls, ML II, and mannosidosis fibroblasts. As expected from the results from the pH-activity profiles, much of the pH 5.5 activity in crude sonicates and in the 30,000 x g supernatants from control fibroblasts is due to activity of the lysosomal acid α -D-mannosidase, and is inhibitable by methyl- α -D-mannopyranoside. The pH 5.5 activity in P_3 from control or mutant fibroblasts is not significantly inhibited, as one would predict if this activity corresponds to the "intermediate" α -D-mannosidase described by Dewald and Touster (6).

DISCUSSION

From the data presented here it is clear that human fibroblasts have a membrane-associated α -D-mannosidase activity that shares many of the properties described for Golgi mannosidase by Touster and co-workers (6,22,30). These properties include an "intermediate" pH optimum (5.5-6.0), activity with synthetic substrates, association with sedimentable membranes, resistance to solubilization by salt extraction, and single extractions with non-ionic detergent Triton X-100, and also lack of inhibition by methyl- α -D-mannopyranoside. This activity is the major 4-MU- α -D-mannopyranoside hydrolytic activity remaining in membranes from normal fibroblasts which have been separated from the cytosolic activity in sonic extracts by sedimentation, and from the much larger amount of lysosomal "acid" enzyme by salt washing and detergent treatment.

The specific activity of the pH 5.5 α -D-mannosidase activity of washed membranes from fibroblasts from patients with mannosidosis was not reduced, compared to that of membranes from control fibroblasts. There was also no reduction in the specific activity of this enzyme in membranes from patients with ML I, ML II, or ML III. Thus, although these studies did not examine the activity of Golgi mannosidase on natural substrates (indeed, the natural substrate for Golgi mannosidase and its role in normal glycoprotein processing is not yet clear) the studies with the synthetic substrate provided no evidence to support the prior suggestion of a deficiency of Golgi mannosidase in ML II and ML III (17).

Since the original description of the Golgi mannosidase by Touster and co-workers (6,22,30), Tabas and Kornfeld (28,29), and Harpaz and Schachter (11) have provided evidence for at least two additional α -D-mannosidases that are associated with Golgi membranes, and that appear to function in the processing of oligosaccharide chains of newly synthesized glycoproteins. Asparagine-linked oligosaccharides on secretory glycoproteins are normally trimmed by mannosidases from high-mannose (Man₅) chains to Man₅ intermediates before glycosyltransferases add the sugars (N-acetyl-D-glucosamine, galactose, sialic acid) that are typical of complex chain oligosaccharides (28,29).

Although these studies do not exclude the possibility that one of these newly discovered mannosidases is deficient in ML II or ML III, there is really no good rationale for proposing a mannosidase deficiency to explain the findings in ML II. In this disease there is an oligosaccharide processing abnormality (16,25,26,27) but the abnormality appears to involve conversion of the high-mannose oligosaccharide chains that are normally found in acid hydrolases to complex-type oligosaccharide chains (20,21). The defect in ML II is now known to involve absence of the mannose-6-phosphate recognition marker on high-mannose chains of newly synthesized acid hydrolases (1,12). In the absence of the mannose-6-phosphate the acid hydrolases fail to bind to the receptors that normally segregate them from secretory products of the endoplasmic reticulum (7) and are secreted. We propose that there is an additional consequence of the absence of Man 6-P on the high-mannose chains of newly synthesized enzymes in ML II, namely that the high mannose oligosaccharide chains which are unprotected by Man 6-P are processed to complex-type oligosaccharide chains as the enzymes pass through the Golgi apparatus. This could explain why ML II enzymes are secreted with oligosaccharides that look like those of secretory glycoproteins instead of those of typical lysosomal hydrolases (25). Thus, we propose that the abnormal oligosaccharides on ML II enzymes result, not from a failure of the enzymes to be processed by mannosidases, but rather from just the reverse, from failure to be protected by the Man 6-P recognition marker (31) from processing by mannosidases. Consistent with this proposal is the observation by von Figura and Klein (31) that oligosaccharides released from acid hydrolases by endoglycosidase H contained phosphate-protected mannose groups that became sensitive to α -mannosidase only after the phosphate was removed by treatment of the oligosaccharides with phosphatase.

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 39. This research was supported in part by grants from the USPHS (GM 21096) and from the Ranken Jordan Trust for the Crippling Diseases of Children. Skin biopsies from some patients who participated in these studies were taken with informed parental consent while they were under study on the USPHS supported Clinical Research Unit of Washington University (RR 0036).

Table I
Percentage of enzymatic activities in the total homogenate recovered in each subcellular fraction.

Enzymatic Activity	Fraction ⁴⁾	Cell Line				
		Controls ¹⁾ (N=4)	ML I ¹⁾ (N=5) ³⁾	Mannosidosis ¹⁾ (N=3)	ML II ²⁾ (N=2)	ML III (N=1)
β -D-Hexosaminidase	S ₁	88.0±8.8	86.5±8.6	83.2±5.1	73.6	80.2
	P ₁	18.5±7.7	17.1±4.9	16.7±2.8	29.5	34.1
	P ₃	2.0±0.4	1.1±0.2	2.5±0.2	8.4	4.5
α -D-Mannosidase (pH 4.0)	S ₁	92.0±8.0	90.3±10.0	83.0±11.1	70.1	89.6
	P ₁	11.6±4.2	9.2±2.9	14.7±0.8	33.8	22.8
	P ₃	3.3±0.4	1.6±0.3	5.1±1.1	14.5	6.2
α -D-Mannosidase (pH 5.5)	S ₁	65.0±9.8	74.4±9.3	55.7±11.6	45.2	62.5
	P ₁	21.6±7.0	24.3±5.2	43.4±6.9	67.3	45.2
	P ₃	10.3±1.4	10.1±1.4	26.3±4.0	46.7	27.1

1) Mean ± S. D.

2) Mean Values

3) Two lines were used for two different preparations each. Values in P₃ are calculated on three experiments.

4) S₁ = first 30,000 x g supernatant; P₁ = first 30,000 x g pellet; P₃ = final pellet.

Table II
Effect of 200 mM methyl- α -D-mannopyranoside on α -D-mannosidase activity at pH 5.5

Cell Line	% Residual Activity		
	Crude Sonicate (CS)	First Supernatant (S ₁)	Final Pellet (P ₃)
Controls	61.7 67.9	59.8 74.7	99.1 97.1
ML II	87.2	83.9	101.7
Mannosidosis	n.t. ¹⁾	n.t. ¹⁾	100.0

1) Not tested.

Figure 1. Specific activities of β -D-hexosaminidase (A), pH 4.0 α -D-mannosidase (B) and pH 5.5 α -D-mannosidase (C) in crude sonicates (CS) and subcellular fractions from normal and mutant fibroblasts. Bars correspond to mean values whereas one standard deviation of the mean is indicated by the thin line. C-control fibroblasts; I-mucopolipidosis I; M-mannosidosis; II and III-mucopolipidosis II and III, respectively. Other experimental details are as in Table I.

Figure 2. Release of membrane-associated enzymes by single extraction with various concentration of Triton X-100 from membrane fraction P₃ obtained from control and ML II fibroblasts.

Figure 3. pH-activity profiles of α -D-mannosidase in the crude sonicate (left) and in Triton-extracted P₃ (right). Activity was assayed as described in Methods using 0.1 M sodium acetate buffer at the indicated pH.