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Mucolipidosis III β-*N*-acetyl-D-hexosaminidase A

Purification and properties

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Mucolipidosis III acid hydrolases possess an altered carbohydrate recognition marker needed for their lysosomal localization. As a result of this alteration, a portion of these enzymes is secreted from the cell to the extracellular spaces. The structural changes that may have occurred to one of these secreted enzymes, β-*N*-acetyl-D-hexosaminidase A (EC 3.2.1.52) were investigated. Normal and mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A were purified to apparent homogeneity by using affinity [Sepharose-2-acetamido-*N*-(ε-aminocaproyl)-2-deoxy-β-D-glucopyranosylamine] and ion-exchange (DEAE- and CM-cellulose) chromatography. Sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis showed that both enzymes had similar subunit patterns consisting of apparent mol.wts. of 68000, 60000–58000, 55000 and 29000. Differences, however, were noted in the relative proportions of the protein bands where the normal urinary β-*N*-acetyl-D-hexosaminidase A contained predominantly the smaller subunits, whereas the mucolipidosis III enzyme had a predominance of the larger subunits. The binding of mucolipidosis III β-*N*-acetyl-D-hexosaminidase A to *Ricinus communis* lectin and concanavalin A with and without endo-β-*N*-acetyl-D-glucosaminidase H treatment indicated that the mutation leads to a modification of a portion of the normally occurring high-mannose-type oligosaccharide units to the complex-type. This was further supported by carbohydrate compositional analysis, which revealed a mannose/galactose ratio of 2.1 for the mucolipidosis III β-*N*-acetyl-D-hexosaminidase A compared with a ratio of 3.5 for the normal enzyme. Our results indicate that as a result of their inability to be properly localized to the lysosome the majority of the mucolipidosis III lysosomal hydrolase high-mannose oligosaccharide units are further processed to the complex-type before secretion of predominantly higher-molecular-weight subunits from the cell.

The lysosomal hydrolases are a class of cellular enzymes that function to degrade the cellular and extracellular substances that are transported to the lysosome. These enzymes exhibit diverse substrate specificities, are glycoproteins and have acidic pH optima. The carbohydrate moiety of these enzymes plays an essential role in their localization to the lysosome (Sly, 1980). The synthesis of this marker in cultured skin fibroblasts involves the transfer of α-*N*-acetylglucosamine 1-phosphate residue(s) to high-mannose-type oligosaccharide units of the lysosomal hydrolases (Tabas & Kornfeld, 1980; Varki & Kornfeld, 1980; Hasilik *et al.*, 1980). After this transfer the terminal α-*N*-acetylglucosamine residue(s) is removed by a phosphodiesterase, exposing a mannose 6-phosphate residue(s), which appears to be required for recognition of the acid hydrolases by a specific binding protein within the cell. This recognition is specific for lysosomal enzymes and is possibly one mechanism for segregating these glycoproteins to the lysosomes (Sly, 1980).

Mucolipidosis II (I-cell disease) and mucolipidosis III (Pseudo-Hurler polydystrophy) are childhood autosomal recessive disorders, characterized by the inability to assemble the correct recognition marker on lysosomal enzymes. Cultured skin fibroblasts obtained from patients with mucolipidosis II and mucolipidosis III demonstrate a wide range of

Abbreviations used: CNAG, Sepharose–2-acetamido-*N*-(ε-aminocaproyl)-2-deoxy-β-D-glucopyranosylamine; SDS, sodium dodecyl sulphate.

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decreased intracellular lysosomal enzyme activities with a concomitant extracellular increase of most of these activities (Leroy *et al.*, 1972; Wenger *et al.*, 1976; Hickman & Neufeld, 1972; Miller *et al.*, 1980). Serum and urine lysosomal hydrolase activities are also markedly elevated (Den Tandt *et al.*, 1974; Herd *et al.*, 1978). Apparently, as a result of their altered recognition marker, a portion of the acid hydrolases fails to be incorporated into the lysosome (Miller *et al.*, 1981) and is subsequently secreted from the cell. In the present paper we investigate the nature of the structural changes that have occurred to one secreted (urinary) mucolipidosis III acid hydrolase, *β-N*-acetyl-D-hexosaminidase A. Our results indicate that a portion of the normally occurring high-mannose-type oligosaccharide units on *β-N*-acetyl-D-hexosaminidase A is modified to the complex-type before its secretion from the cell. A preliminary report of this work has been published (Kress *et al.*, 1980*b*).

Materials and methods

Protein determination

Protein was determined by the Lowry method with bovine serum albumin as standard (Lowry *et al.*, 1951). All procedures were carried out at 0–4°C unless otherwise stated.

Assay of β-N-acetyl-D-hexosaminidase

β-N-Acetyl-D-hexosaminidase activity was assayed by using 6mM-4-methylumbelliferyl 2-acetamido-2-deoxy-*β*-D-glucopyranoside (Koch-Light, Colnbrook, Bucks., U.K.) as previously described by Miller *et al.* (1981). One unit of activity is defined as the amount of enzyme that will hydrolyse 1nmol of substrate/min at 37°C.

Enzyme purification

Normal urine (50 litres) from healthy male adults was collected over a period of approx. 1 month with the addition of 0.02% (w/v) NaN_3. The combined urine samples were stored at 0–4 °C until 50 litres was obtained. In addition, urine samples were collected from a single healthy male adult and no significant differences were obtained in the results reported for the pooled normal urine. The mucolipidosis III urine (13.5 litres) was collected from a single female patient whose clinical and biochemical background have been detailed elsewhere (Herd *et al.*, 1978).

The urine samples were made 60% saturated by the addition of solid ultra-pure $(NH_4)_2SO_4$ (Schwarz-Mann, Orangeburg, NY, U.S.A.) with stirring. The supernatant was decanted and the resultant settled precipitate was centrifuged for 30min at 17000*g* with the GSA rotor in a Sorvall RC2B centrifuge. The pellets from the normal and mucolipidosis III urines were separately resuspended in 0.01M-sodium phosphate buffer, pH6.0, containing 0.02% (w/v) NaN_3 and dialysed against this buffer over 4 days with three 10-litre buffer changes. The dialysed urine samples were used directly for affinity chromatography.

CNAG affinity chromatography

The dialysed mucolipidosis III and normal urine preparations were applied separately to a column (1cm × 6cm) of the CNAG affinity resin (a gift from Dr. Benjamin Geiger). The affinity column was washed extensively first with 30 column volumes of 0.01M-sodium phosphate buffer, pH6.0, containing 0.02% (w/v) NaN_3 and then with 10 column volumes of 0.01M-sodium citrate buffer, pH4.4, containing 0.02% (w/v) NaN_3. The enzyme was eluted with 5 column volumes of 0.01M-sodium citrate buffer, pH4.4, containing 0.1mM-2-acetamido-2-deoxy-*β*-D-gluconolactone (Koch–Light) and 0.02% (w/v) NaN_3 (Sandhoff *et al.*, 1977). Fractions containing the eluted peak of *β-N*-acetyl-D-hexosaminidase activity were pooled and concentrated by using an Amicon concentrator fitted with a PM-10-diaflo membrane (Amicon Corp., Lexington, MA, U.S.A.).

Ion-exchange chromatography

The concentrated normal and mucolipidosis III enzyme fractions eluted from the affinity column were dialysed overnight against 1000vol. of 0.01M-sodium phosphate buffer, pH6.0, containing 0.02% (w/v) NaN_3 using two changes of buffer and applied to separate columns of DEAE-cellulose (Whatman, Maidstone, Kent, U.K. (13ml bed volume) equilibrated in 0.01M-sodium phosphate buffer, pH6.0, containing 0.02% (w/v) NaN_3 at room temperature. The DEAE-cellulose columns were washed with 5 column volumes of 0.01M-sodium phosphate buffer, pH6.0, containing 0.02% (w/v) NaN_3 and then eluted with a linear NaCl gradient (0–0.3M) in 150ml of 0.01M-sodium phosphate buffer, pH6.0, containing 0.02% (w/v) NaN_3. The salt-eluted fractions containing *β-N*-acetyl-D-hexosaminidase A of highest specific activity were pooled and concentrated. The *β-N*-acetyl-D-hexosaminidase A from normal and mucolipidosis III urines was then dialysed overnight against 1000vol. of 0.02M-sodium citrate buffer, pH4.0, containing 0.02% (w/v) NaN_3 using two changes of buffer. The samples were applied separately to columns of CM-cellulose (50ml bed volume) equilibrated with 0.02M-sodium citrate buffer, pH4.0, containing 0.02% (w/v) NaN_3 at room temperature. The resin was washed with 5 column volumes of 0.02M-sodium citrate buffer at pH4.0 containing 0.02% (w/v) NaN_3 and *β-N*-acetyl-D-hexosaminidase A was eluted with a linear NaCl gradient (0–0.4M)

prepared in 200ml of 0.02M-sodium citrate buffer at pH4.0 containing 0.02% (w/v) NaN_3. The eluted fractions containing the highest specific activity of β-*N*-acetyl-D-hexosaminidase A were pooled and concentrated and used for the studies described below.

SDS/polyacrylamide-gel electrophoresis

Samples of purified β-*N*-acetyl-D-hexosaminidase A were subjected to slab-gel electrophoresis in the presence of SDS by the general procedure of Laemmli (1970). Gels were fixed, stained and destained as previously described (Fairbanks *et al.*, 1971). The following proteins were used as reference standards: phosphorylase *b* (mol.wt. 9.4×10^4); bovine serum albumin (6.8×10^4); ovalbumin (4.3×10^4); carbonic anhydrase (3.0×10^4); soya-bean trypsin inhibitor (2.1×10^4) and lysozyme (1.4×10^4).

Carbohydrate analysis

Samples of the purified preparations of β-*N*-acetyl-D-hexosaminidase A were dialysed against 1000vol. of 0.1M-NH_4HCO_3 and 0.002M-SDS for 36h employing two changes of the same buffer. The enzyme preparations containing 50–100μg of protein were mixed with 1–5μg of arabitol in screw-cap vials, freeze-dried, methanolysed and converted into trimethylsilyl ethers as previously described (Freeze *et al.*, 1979). Samples (2–5μl) of the derivatives containing the equivalent of 30–50μg of original enzyme protein were injected into the gas chromatograph and analysed with an oven temperature increase programmed at 2°C/min. To determine the reliability of the analyses of very small amounts of carbohydrate, samples containing 20–200μg of ovalbumin (1–10μg of total carbohydrate) and 0.5–5.0μg of arabitol were similarly analysed by using identical sample preparation and derivative preparation procedures.

Endo-β-N-acetylglucosaminidase H treatment

Normal and mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A preparations were separately incubated with 5m-units of endo-β-*N*-acetylglucosaminidase H (a gift from Dr. R. Trimble and Dr. F. Maley, Department of Health, Albany, NY, U.S.A.), by the procedure of Miller *et al.* (1981).

Concanavalin A binding

The binding properties of normal and mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A to the lectin concanavalin A (Sigma Chemical Co., St. Louis, MO, U.S.A.) with and without endo-β-*N*-acetyl-D-glucosaminidase H treatment were determined by a previously published procedure (Miller *et al.*, 1981). Briefly, 1 to 2 units of the purified enzymes were separately incubated with 0.6mg of concanavalin A and various concentrations of α-methyl D-mannoside for 60min at 37°C. The tubes were then centrifuged at 39000*g* for 30min at 4°C to precipitate the concanavalin A–enzyme complex. Portions of the resulting supernatant fluids were assayed in duplicate for β-*N*-acetyl-D-hexosaminidase activity that remained unadsorbed to the lectin. The K_i is defined as the concentration of α-methyl D-mannoside needed to inhibit the binding of 50% of the added enzyme activity to the lectin. This was determined by using Lineweaver–Burk kinetic analysis of the data. The initial percentage enzyme activity that remained unadsorbed to the concanavalin A was determined when all components except α-methyl D-mannoside were added to the tube. Recoveries of β-*N*-acetyl-D-hexosaminidase activity ranged from 95 to 100%.

Ricinis communis lectin column chromatography

Purified normal and mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A preparations (0.5ml) were applied separately to columns (2ml bed volume) of agarose–*Ricinus communis* agglutinin I (RCA_{120}) (Vector Laboratories, Burlingame, CA, U.S.A.) equilibrated in 0.1M-sodium phosphate buffer, pH6.0, containing 0.15M-NaCl and 0.02% (w/v) NaN_3. After sample application, the column was washed with 10vol. of this buffer followed by elution with 0.1M-D-galactose prepared in the equilibration buffer. Fractions (1ml) were collected and assayed for β-*N*-acetyl-D-hexosaminidase A activity. For the neuraminidase-treated samples, 1 unit of *Clostridium perfringens* neuraminidase type IX (Sigma) (1 unit will liberate 1.0μmol of *N*-acetylneuraminic acid/min at pH5.0 and 37°C) was incubated with the β-*N*-acetyl-D-hexosaminidase A preparations at 37°C for 5h. Control samples were treated identically except that buffer was substituted for neuraminidase. Recoveries of β-*N*-acetyl-D-hexosaminidase A activities were 90–100% after the neuraminidase treatment.

Results

Purification

β-*N*-Acetyl-D-hexosaminidase A was purified from normal and mucolipidosis III urines by $(NH_4)_2SO_4$ fractionation, affinity and ion-exchange column chromatography (Table 1). β-*N*-Acetyl-D-hexosaminidase A was separated from the B-isoenzyme on DEAE-cellulose column chromatography and subsequently purified to apparent homogeneity after CM-cellulose chromatography. The final yield and purification for β-*N*-acetyl-D-hexosaminidase A listed in Table 1 are based on total β-*N*-acetyl-D-hexosaminidase activity (A plus B) in the crude urine. Thus, the final yield and purification are somewhat higher than reported in

Table 1. *Purification of urinary β-N-acetyl-D-hexosaminidase A*

Units of enzyme activity are defined as nmol/min. DEAE-cellulose column chromatography separates β-*N*-acetyl-D-hexosaminidase isoenzymes A and B. Therefore values obtained after this purification step are for the A-isoenzyme.

Step	Volume (ml)		Units/ml		Total units		Protein (mg/ml)		Units per mg of protein		Yield (%)		Purification (fold)	
	Normal	I-cell	Normal	I-cell	Normal	I-cell	Normal	I-cell	Normal	I-cell	Normal	I-cell	Normal	I-cell
Crude urine	75000	5500	2.6	32	195000	176000	2.19	7.00	1.2	4.6	100	100	1	1
60%-satd.-$(NH_4)_2SO_4$ fraction	1940	530	77	346	149380	183380	1.35	3.40	57	101	77	104	48	22
β-*N*-Acetyl-D-hexosaminidase affinity column chromatography	31	16	1418	5806	43958	92896	0.95	0.98	1493	5924	23	53	1244	1316
DEAE-cellulose column chromatography	4.7	3.5	2996	6259	14081	21907	0.30	0.28	9986	22354	7	12	8322	4968
CM-cellulose column chromatography	0.25	0.7	21794	13595	5449	9517	0.28	0.15	77836	90633	3	5	64863	20140

Fig. 1. *SDS/polyacrylamide-gel electrophoresis of* (*a*) *normal and* (*b*) *mucolipidosis III* β-*N-acetyl-*D*-hexosaminidase A*

SDS/polyacrylamide-gel electrophoresis was carried out as described in the Materials and methods section. (*a*) Lane (1) contains protein standards, lane (2) contains normal placental β-*N*-acetyl-D-hexosaminidase A and lane (3) contains urinary β-*N*-acetyl-D-hexosaminidase A. (*b*) Lane (1) contains mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A and lane (2) contains protein standards.

Table 1. A similar purification scheme was obtained when the β-*N*-acetyl-D-hexosaminidase A was purified from urine collected from a single normal male adult. The final specific activities for the normal and mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A using the fluorogenic substrate are comparable with those values reported for β-*N*-acetyl-D-hexosaminidase A purified from human placenta (Srivastava *et al.*, 1974; Mahuran & Lowden, 1980) and human liver (Sandhoff *et al.*, 1977) and severalfold higher than other purified urinary preparations (Banerjee & Basu, 1975; Marinkovic & Marinkovic, 1978).

SDS/polyacrylamide-gel electrophoresis

The presence of subunits was determined by gel electrophoresis in a 12.5% polyacrylamide slab gel containing 1% SDS. The purified normal urinary β-*N*-acetyl-D-hexosaminidase A (Fig. 1*a*, lane 3) migrated as three bands with apparent molecular weights of 58000, 55000 and 29000. The mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A migrated as two major bands and two minor bands with apparent mol.wts. 68000, 59600, 55600 and 29500 respectively (Fig. 1*b*, lane 1). Highly purified normal placental β-*N*-acetyl-D-hexosaminidase A (a gift from Dr. B. Geiger) migrated as two major bands of mol.wt. 54000 and 26000 (Fig. 1*a*, lane 2).

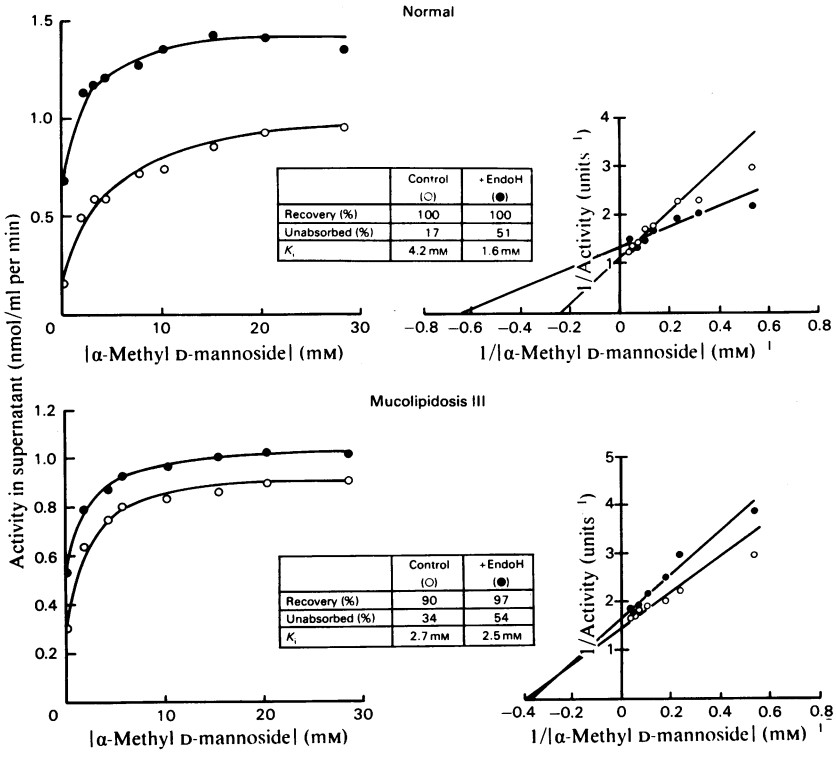

Fig. 2. *Concanavalin A binding of normal and mucolipidosis III urinary β-N-acetyl-D-hexosaminidase A before* (○) *and after endo-β-N-acetyl-D-glucosaminidase treatment* (●; *EndoH*)
The details of the experimental procedure are given in the Materials and methods section.

Concanavalin A binding

The affinity of concanavalin A for mucolipidosis III β-*N*-acetyl-D-hexosaminidase A (K_i) was less (2.7 mM) than the normal enzyme (4.2 mM; Fig. 2). In addition, more of the mucolipidosis III enzyme remained unadsorbed to concanavalin A (34.0%) in the absence of α-methyl D-mannoside when compared with the normal enzyme (17.0%). The concanavalin A affinity for the enzyme was then used as an index to investigate the endo-β-*N*-acetylglucosaminidase H-susceptibility of the oligosaccharide chains on these enzymes. As expected for high-mannose-type carbohydrate units, the concanavalin A affinity for the normal urinary β-*N*-acetyl-D-hexosaminidase A was significantly less after endo-β-*N*-acetylglucosaminidase H treatment. This is reflected in the lowered K_i (4.2 mM to 1.6 mM) and the 3-fold increase in the normal enzyme activity that was unadsorbed to concanavalin A in the absence of α-methyl D-mannoside. In contrast, the K_i of the mucolipidosis III β-*N*-acetyl-D-hexosaminidase A was unchanged after endo-β-*N*-acetylglucosaminidase H treatment and only a slight increase (1.6-fold) in the unadsorbed enzyme activity without α-methyl D-mannoside addition was observed. These results indicate relatively fewer endo-β-*N*-acetylglucosaminidase H-susceptible (high-mannose-type) oligosaccharide units on the mucolipidosis III β-*N*-acetyl-D-hexosaminidase A when compared with the corresponding normal enzyme.

R. communis lectin binding

Normal urinary β-*N*-acetyl-D-hexosaminidase A interacted poorly with *R. communis* lectin as 74.7% of the applied enzyme units remained unadsorbed to this galactose-specific lectin (Fig. 3). No significant changes were seen in the elution profile after neuraminidase or endo-β-*N*-acetyl-D-glucosaminidase H treatment. In contrast with the normal enzyme, 64.7% of the mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A activity applied to *R. communis* was adsorbed and specifically eluted with 0.1 M-D-galactose. Neuraminidase treatment of the mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A had little effect on the elution profile. Treatment of the mucolipidosis III enzyme with endo-β-*N*-acetylglucosaminidase H resulted in a

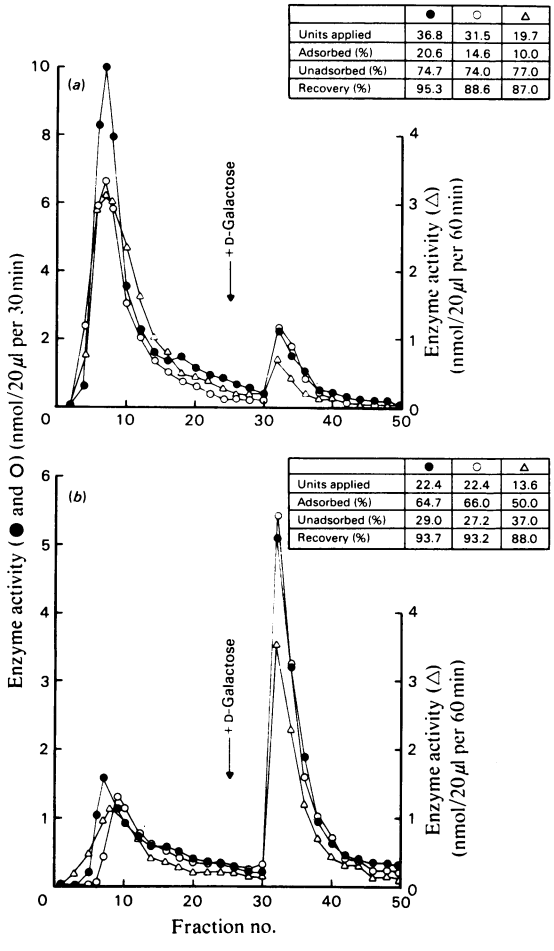

Fig. 3. *Chromatography on R. communis lectin of normal and mucolipidosis III urinary β-N-acetyl-D-hexosaminidase A*

The details of chromatography before (●) and after (○) neuraminidase and (△) endo-β-*N*-acetyl-D-glucosaminidase H treatments are given in the Materials and methods section. (*a*), Normal urinary β-*N*-acetyl-D-hexosaminidase A; (*b*), mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A.

slight decrease in the adsorbed activity (from 64.7% to 50.0%) and a corresponding increase in the unadsorbed activity (from 29.0% to 37.0%).

Carbohydrate composition

Gas-chromatographic analyses were carried out to ascertain if the differences in lectin binding (Figs. 2 and 3) reflected alterations in the carbohydrate composition of the mucolipidosis III β-*N*-acetyl-D-hexosaminidase A. The overall amount of carbohydrate on the mucolipidosis III enzyme was decreased when compared with the normal enzyme. This decrease was true of most sugars, including mannose, which decreased to 2.71 μg of mannose/100 μg of protein on the mucolipidosis III enzyme from 3.47 μg of mannose/100 μg of protein on the normal enzyme. The only exception was an increase in the amount of galactose present on the mucolipidosis III enzyme (1.3 μg of galactose/100 μg of protein) compared with the normal β-*N*-acetyl-D-hexosaminidase A (1.0 μg/100 μg of protein). The change in carbohydrate composition was clearly seen when the mannose/galactose ratio for the normal β-*N*-acetyl-D-hexosaminidase A (3.5) was compared with the ratio for the mucolipidosis III enzyme (2.1).

Discussion

Normal and mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A have been purified to apparent homogeneity. Significant differences were noted in the relative proportions of the protein bands where normal urinary β-*N*-acetyl-D-hexosaminidase A contained predominantly the smaller subunits, whereas the mucolipidosis III enzyme showed a predominance of the larger subunits (Figs. 1*a* and 1*b*). Although the normal and mucolipidosis III enzyme preparations had similar subunit patterns consisting of apparent M_r values of 68000 (α-chain), 59000 or 58000 (β-chain), 55000 (α-chain) and 29000 (β-chain), the 68000-M_r band and the 29000-M_r band of the normal and mucolipidosis III enzyme preparations respectively were seen to be present only as minor components. The β-chain designations were made by comparing the SDS/polyacrylamide-gel electrophoresis migration pattern with that of purified normal and mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase B, which contains only the β-chains (Hirani *et al.*, 1982). The presence of extracellular forms of urinary β-*N*-acetyl-D-hexosaminidase A with considerably larger chains than are found in the corresponding mature subunits is consistent with the results of Hasilik & Neufeld (1980*a*) using cultured skin fibroblasts and extracellular medium. These authors reported precursor forms of several acid hydrolases including β-*N*-acetyl-D-hexosaminidase A in the extracellular medium in which the cultured fibroblasts were grown, whereas the corresponding mature forms were found only within the cell. The precursor and mature β-*N*-acetyl-D-hexosaminidase subunits in cultured skin fibroblasts had apparent mol.wts. of 67000 and 54000 respectively for the α-chain and 63000 and 29000 respectively for the β-chain.

The localization of the proteinase(s) responsible for the proteolytic processing (i.e. maturation) of the

acid hydrolases in the lysosomes (Frisch & Neufeld, 1981) indicates that the mature β-*N*-acetyl-D-hexosaminidase is secreted through a lysosomal-related pathway. In contrast, the larger forms of the enzyme are apparently secreted via an alternative pathway. The presence of both of the larger and smaller subunits of β-*N*-acetyl-D-hexosaminidase A in normal and mucolipidosis III urines indicates both secretory pathways are involved. In mucolipidosis III the non-lysosomal pathway predominates, whereas the lysosomal pathway is the major secretory route for normal urinary β-*N*-acetyl-D-hexosaminidase A. The suggestion of two avenues of secretion for the normal acid hydrolases has previously been reported by us using cycloheximide-treated normal and mucolipidosis III cultured skin fibroblasts (Miller *et al.*, 1980) and by Vladutiu & Rattazzi (1980) using monensin-treated cultured skin fibroblasts. The implication that a portion of the mucolipidosis III β-*N*-acetyl-D-hexosaminidase enters the lysosome (and thus is processed to the mature enzyme) is supported by our recent findings with cultured skin fibroblasts showing that the residual cellular activity of mucolipidosis III β-*N*-acetyl-D-hexosaminidase is located within the lysosome (Miller *et al.*, 1981). Similar results were obtained by Robey & Neufeld (1982), who showed low but detectable amounts of cellular (mature) subunits of β-*N*-acetyl-D-hexosaminidase in mucolipidosis III skin fibroblasts. However, these authors were unable to detect mature subunits of β-*N*-acetyl-D-hexosaminidase in the extracellular medium of cultured mucolipidosis III skin fibroblasts.

The results reported here for normal urinary β-*N*-acetyl-D-hexosaminidase A are consistent with previous findings indicating that normal acid hydrolases contain predominantly high-mannose-type oligosaccharide units (Von Figura & Klein, 1979; Tabas & Kornfeld, 1980; Varki & Kornfeld, 1980). Our data include: (1) the significant interaction of β-*N*-acetyl-D-hexosaminidase A with concanavalin A and sensitivity to endo-β-*N*-acetyl-D-glucosaminidase H treatment (Fig. 2); (2) the poor interaction of the normal enzyme with *R. communis* lectin (Fig. 3) and (3) a mannose/galactose ratio of 3.5. Our results with the mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A suggest that the mutation in this disorder leads to a modification of a portion of the normally occurring high-mannose-type oligosaccharide units to the complex-type. Data supporting this contention include: (1) the poor interaction with concanavalin A and the relative insensitivity to endo-β-*N*-acetyl-D-glucosaminidase H treatment (Fig. 2); (2) the significant interaction with *R. communis* lectin (Fig. 3) and (3) the lower mannose/galactose ratio of 2.1 for β-*N*-acetyl-D-hexosaminidase A. Similar results were observed by us when the mannose/galactose ratios of the mucolipidosis III urinary (1.7) and mucolipidosis II liver (2.1) α-L-fucosidase were compared with a ratio of 10.4 for the corresponding normal liver enzyme (Kress *et al.*, 1980*a*). Furthermore, Hasilik & Von Figura (1981) reported that the secreted cathepsin D and β-*N*-acetyl-D-hexosaminidase from cultured mucolipidosis II skin fibroblasts contain predominantly endo-β-*N*-acetylhexosaminidase H resistant (complex) oligosaccharide units.

We emphasize, however, that our results suggest that complex oligosaccharide units only predominate on the mucolipidosis III β-*N*-acetyl-D-hexosaminidase A since this enzyme also displays characteristics consistent with the presence of some high-mannose and/or hybrid carbohydrate chain(s). This suggestion is supported by the susceptibility of mucolipidosis III urinary β-*N*-acetyl-D-hexosaminidase A to endo-β-*N*-acetyl-D-glucosaminidase H treatment, where a slight increase (1.6-fold) in the enzyme activity initially unadsorbed to concanavalin A was observed. Endo-β-*N*-acetyl-D-glucosaminidase H treatment also resulted in a decrease in the percentage of mucolipidosis III β-*N*-acetyl-D-hexosaminidase A adsorbed to *R. communis* lectin. In addition, recent studies indicate that partially purified mucolipidosis III lysosomal β-*N*-acetyl-D-hexosaminidase exhibited a low level of uptake into cultured skin fibroblasts, which was abolished either by the addition of mannose 6-phosphate or by previous incubation of the enzyme with endo-β-*N*-acetyl-D-glucosaminidase H (Miller *et al.*, 1981). Furthermore, Robey & Neufeld (1982) and Reitman *et al.* (1981) reported residual phosphorylation of mucolipidosis III acid hydrolases apparently allowing a portion of the newly synthesized enzymes to reach the lysosomes. We speculate that in mucolipidosis III most, but not all, of the acid hydrolases are unable to be properly localized within the lysosome, resulting in further modification of the oligosaccharide units to the complex-type, which occurs before their secretion from the cell.

Recent reports indicate an almost total deficiency of the *N*-acetylglucosamine 1-phosphate transferase activity in mucolipidosis II cultured skin fibroblasts and significant reduction in this same enzyme activity in mucolipidosis III cultured skin fibroblasts (Reitman *et al.*, 1981; Hasilik *et al.*, 1981; Robey & Neufeld, 1982). This enzyme deficiency has been proposed to be the primary defect in mucolipidosis III disease. Our data reported here indicate that the failure to attach the correct recognition marker to the oligosaccharide units of the mucolipidosis III acid hydrolases results in (1) further processing of the high-mannose chains to the complex-type before secretion from the cell and (2) secretion of predominantly the apparent higher-molecular-weight subunits of the acid hydrolases.

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