Biochem. J. (1982) **204**, 557–563
Printed in Great Britain

A study of highly purified mucolipidosis III urinary *N*-acetyl-β-D-hexosaminidase B

Shirish HIRANI, Laureen LITTLE and Arnold L. MILLER
Department of Neurosciences, School of Medicine, University of California at San Diego, La Jolla, CA 92093, U.S.A.

(*Received 30 November 1981/Accepted 8 February 1982*)

Highly purified *N*-acetyl-β-D-hexosaminidase B from normal urine and urine of a patient with mucolipidosis III was used to determine whether it had undergone any of the alterations associated with this genetic defect. Examination by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis showed that both the enzyme preparations contained protein components with apparent M_r values of 55000 and 28000. No differences in the binding and apparent K_I (50%) to concanavalin A of the normal and mucolipidosis III enzymes were detected. However, the patient's *N*-acetyl-β-D-hexosaminidase B had a slightly greater affinity for the lectin from *Ricinus communis* than did the normal enzyme. Two-dimensional tryptic peptide maps of the corresponding normal and the patient's *N*-acetyl-β-D-hexosaminidase B subunits showed considerable homology. These results indicate that *N*-acetyl-β-D-hexosaminidase B does not undergo the significant carbohydrate alterations characteristic of other acid hydrolases in mucolipidosis III.

Like other lysosomal hydrolases, *N*-acetyl-β-D-hexosaminidase (EC 3.2.1.30) exists in several molecular forms. The two major forms present in human tissues have been designated *N*-acetyl-β-D-hexosaminidase A and B and were originally observed in human spleen (Robinson & Stirling, 1968). There is general agreement that the structural differences between *N*-acetyl-β-D-hexosaminidase A and B reside in their subunit compositions (Srivastava & Beutler, 1974; Beutler *et al.*, 1976; Geiger & Arnon, 1976). *N*-Acetyl-β-D-hexosaminidase A is thought to be composed of two polypeptide chains, α and β, and *N*-acetyl-β-D-hexosaminidase B of only the β chain. In man the α and β chains are coded by genes on chromosome 15 and 5 respectively (Gilbert *et al.*, 1975; Solomon *et al.*, 1976).

In the two related lysosomal storage diseases, mucolipidosis II (I-cell disease) and mucolipidosis III (pseudo-Hurler polydystrophy) a number of acid hydrolases, including *N*-acetyl-β-D-hexosaminidase, are affected (Neufeld *et al.*, 1975). Cultured fibroblasts from patients with mucolipidosis II and mucolipidosis III exhibit multiple lysosomal enzyme activity deficiencies (Leroy *et al.*, 1972; Thomas *et al.*, 1973) with a concomitant increase in the activities of several of these enzymes in the culture medium (Wiesmann *et al.*, 1971*a*) and in extracellular fluids (Wiesmann *et al.*, 1971*b*). The analogous clinical and biochemical characteristics of these disorders indicate a similar molecular aetiology of each disease, mucolipidosis III being the milder form. Genetic studies reveal the existence of several complementation groups within and between each disorder (Honey *et al.*, 1981; Mueller *et al.*, 1981; Shows *et al.*, 1982) which implies that different mutations may occur to either the same protein or to other proteins in the biosynthetic pathway shared by the lysosomal enzymes.

Recent evidence suggests that the primary mutation in mucolipidosis II and mucolipidosis III is due to a deficiency of *N*-acetylglucosaminylphosphotransferase activity (Hasilik *et al.*, 1981; Reitman *et al.*, 1981), an enzyme responsible for the transfer of *N*-acetylglucosamine 1-phosphate from 5′-diphosphate (UDP)-*N*-acetylglucosamine to the high-mannose-type oligosaccharide unit(s) of the acid hydrolases. The failure to attach the correct recognition marker on the mucolipidosis II and mucolipidosis III hydrolases subsequently leads to a conversion of their high mannose-type chains to complex type units prior to secretion from the cell (Sly *et al.*, 1979; Kress *et al.*, 1980*a*; Miller *et al.*, 1981*a*,*b*). In contrast to these reports the evidence

Abbreviations used: SDS, sodium dodecyl sulphate.

0306-3283/82/050557-07$01.50/1

we have obtained here suggests that *N*-acetyl-β-D-hexosaminidase B is not significantly altered in mucolipidosis III.

Materials and methods

Assay of N-acetyl-β-D-hexosaminidase

N-Acetyl-β-D-hexosaminidase was assayed by using the fluorogenic substrate 4-methylumbelliferyl-1-acetamido-2-deoxy-β-D-glucopyranoside (Koch–Light, Colnbrook, Bucks., U.K.) as described previously (Miller *et al.*, 1981*b*). One unit of activity is that amount of enzyme that transforms 1 nmol of substrate/min under these conditions.

Protein determination

Protein was measured by using the Coomassie Blue dye-binding method of Bradford (1976) with bovine serum albumin (Sigma) as a standard.

Purification of N-acetyl-β-D-hexosaminidase B

(*1*) *Initial fractionation.* Normal urine was collected from healthy male adults. The mucolipidosis III urine was collected from a single female patient whose clinical and biochemical background have been described elsewhere (Herd *et al.*, 1978). The urine samples were stored at 4°C with 0.02% NaN_3 as a preservative. The material precipitating from the urine samples between 0% and 60% saturation with $(NH_4)_2SO_4$ was recovered by centrifugation in the 6 × 250 ml angle head rotor of a Sorvall RC-5B centrifuge for 30 min at 4°C and 17000 **g**. The precipitate was redissolved in 10 mM-sodium phosphate buffer, pH 6.0, containing 0.02% NaN_3 and was dialysed against 10 litres of the same buffer, with three changes, for 4 days at 4°C.

(*2*) *Affinity chromatography.* The dialysed preparations were applied separately to a column (6 cm × 1.5 cm diam.) of Sepharose-2-acetamido-*N*-(ε-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine (kindly provided by Dr. B. Geiger, Weizmann Institute of Science, Rehovot, Israel) which had been previously equilibrated in the above buffer. The column was washed with this buffer until the A_{280} of the effluent became zero, when it was re-equilibrated with 10 mM-sodium citrate buffer, pH 4.4, containing 0.02% NaN_3. The material that had bound to the column was then eluted with this buffer containing 0.1 mM-2-acetamido-2-deoxy-β-D-gluconolactone (Koch–Light). Fractions containing *N*-acetyl-β-D-hexosaminidase activity were pooled and concentrated with an Amicon concentrator (Amicon, Danvers, MA, U.S.A.) fitted with a PM-10 Diaflo membrane. The concentrated material was then dialysed overnight against 10 mM-sodium phosphate buffer, pH 6.0.

(*3*) *Ion-exchange chromatography on DEAE-cellulose.* The *N*-acetyl-β-D-hexosaminidase activity was separated into forms A and B by ion-exchange chromatography on a column (6 cm × 1.5 cm diam.) of DEAE-cellulose (Whatman DE52; Whatman, Maidstone, Kent, U.K.), equilibrated in 10 mM-sodium phosphate buffer, pH 6.0. Elution was started with the equilibration buffer and continued with a linear NaCl gradient (0–0.3 M) in 150 ml of the same buffer. Fractions containing *N*-acetyl-β-D-hexosaminidase A (adsorbed peak) and *N*-acetyl-β-D-hexosaminidase B activities (unadsorbed peak) were pooled separately and concentrated as described above.

Polyacrylamide-gel electrophoresis

Electrophoresis of the purified samples was carried out in 15% (w/v) polyacrylamide-slab gels with a 4.5% (w/v) stacking gel in the presence of SDS according to the procedure of Laemmli (1970). Protein bands were detected by staining the gels with a 0.05% solution of Coomassie Blue R250 (Bio-Rad, Richmond, CA, U.S.A.) in propan-2-ol/acetic acid/water (5:2:13, by vol.). The gels were destained in 10% (v/v) acetic acid. A low M_r protein standards kit (M_r range 14300–94000) (Bio-Rad) was used to determine the molecular weights.

Electrophoretic blotting

Electrophoretic transfer of the enzyme components from SDS/polyacrylamide gels to nitrocellulose sheets was carried out according to the previously described procedure of Towbin *et al.* (1979). For this 'western' blotting technique radioiodinated *Staphylococcus aureus* protein A was used as the second antibody. Protein A (Pharmacia) was iodinated with ^{125}I (Amersham, Arlington Heights, IL, U.S.A.; sp. radioactivity ≃ 17 mCi/mmol) by a modification of the chloramine-T method (Hunter & Greenwood, 1962). The protein A solution (6.25 mg/ml in water) was diluted four-fold with 0.5 M-sodium phosphate buffer, pH 7.2. To 40 μl of this solution, 7 μl of ^{125}I (≃50 μCi/μl) and 1 μl of choramine-T (2.25 mg/ml) were added. The reaction was allowed to proceed at room temperature for 30 s, after which 5 μl of sodium metabisulphite (10 mg/ml) was added. The labelled protein A was recovered by passing the reaction mixture through a Bio-Gel P-2 column (Bio-Rad) packed in a 10 ml disposable plastic pipette, equilibrated in phosphate-buffered saline with bovine serum albumin (1 mg/ml) and glycerol (5% v/v).

Peptide maps

Tryptic peptide analysis of the *N*-acetyl-β-D-hexosaminidase B components was carried out according to the procedure of Elder *et al.* (1977).

Incubation with endo-β-N-acetylglucosaminidase H

Enzyme samples (approx. 3 μg of protein) were mixed with 5 μl of 1% (w/v) SDS. After boiling for

2 min the samples were diluted sufficiently with 50 mM-sodium citrate buffer, pH 5.5, in order to reduce the SDS concentration to 0.1% or less, and were then incubated with 5 munits of endo-β-*N*-acetylglucosaminidase H (gift from Dr. R. Trimble and Dr. F. Maley, Department of Health, Albany, NY, U.S.A.) at 37°C for 18 h. The resultant changes in the M_r were detected by polyacrylamide-gel electrophoresis as described previously. Ovalbumin was used as a test for this experimental procedure.

Concanavalin A binding

The binding assay using concanavalin A (Miles) was carried out in 0.1 M-sodium phosphate buffer, pH 5.5, containing human serum albumin (1 mg/ml) (Sigma) and 0.1 mM-$CaCl_2$, -$MgCl_2$ and -$MnCl_2$ using the conditions described previously (Miller *et al.*, 1981*b*).

Ricinus communis lectin column chromatography

Preparations of normal and mucolipidosis III *N*-acetyl-β-D-hexosaminidase B were applied separately to a column (3 cm × 1 cm diam.) of agarose-bound *Ricinus communis* (Vector Labs, Burlingame, CA, U.S.A.) equilibrated in 15 mM-sodium phosphate buffer, pH 6.0, containing 0.15 M-NaCl, human serum albumin (1 mg/ml) and 0.02% NaN_3. After sample application, the column was washed with this buffer and the material that had bound to the lectin was eluted with 15 ml of 0.1 M-galactose (Sigma) in the equilibration buffer.

For the neuraminidase-treated samples, 0.5 units of *Clostridium perfringens* neuraminidase, type IX (Sigma) (1 unit will liberate 1.0 nmol of *N*-acetylneuraminic acid/min), was incubated with approx. 15 units of *N*-acetyl-β-D-hexosaminidase B and 0.1 M-citrate/phosphate buffer, pH 5.0, containing human serum albumin (1 mg/ml) and 0.02% NaN_3 (final vol. 110 μl) at 37°C for 5 h. The treated samples were then analysed on *Ricinus communis* lectin as described above.

Results

Purification of human urinary N-acetyl-β-D-hexosaminidase B

N-Acetyl-β-D-hexosaminidase B was purified from normal and mucolipidosis III urines by the procedure shown in Table 1. The A form of the enzyme was separated from *N*-acetyl-β-D-hexosaminidase B at the final stage in the purification by column chromatography on DEAE-cellulose. Analysis of the enzyme preparations at this stage by SDS/polyacrylamide slab gel electrophoresis (Fig. 1*a*) showed that the normal and mucolipidosis III preparations contained protein bands with apparent M_r values of 55 000 and 28 000. The mucolipidosis III preparation also contained an additional minor band with an apparent M_r of 29 000.

The 'western' blot (Towbin *et al.*, 1979; Burnette, 1981) using rabbit antiserum raised against purified human placental *N*-acetyl-β-D-hexosaminidase B (a gift from Dr. B. Geiger) demonstrated the presence of cross-reacting material in both the enzyme preparations (Fig. 1*b*). Both the 55 000-M_r and the 28 000-M_r bands were detected, indicating that the two bands were part of the *N*-acetyl-β-D-hexosaminidase B holoenzyme.

Concanavalin A binding

Decreases in the affinity of concanavalin A have been reported for several lysosomal hydrolases from patients with mucolipidosis II and mucolipidosis III (Kress & Miller, 1979; Rousson *et al.*, 1979; Hirani & Winchester, 1980; Miller *et al.*, 1981*b*). The identical K_i (50%) values (normal 5 mM; mucolipodosis III 4.5 mM) (Fig. 2) and the proportion

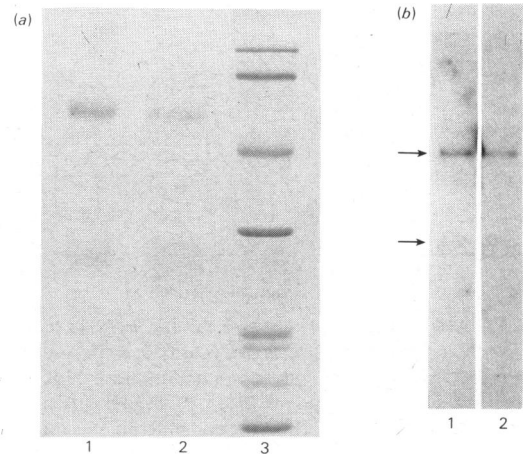

Fig. 1. (*a*) *SDS/polyacrylamide-gel electrophoresis and* (*b*) *electrophoretic transfer of N-acetyl-β-D-hexosaminidase B*

(*a*) SDS/polyacrylamide gel electrophoresis was carried out as described in the Materials and methods section. Lanes 1 and 2 are normal and mucolipidosis III *N*-acetyl-β-D-hexosaminidase B respectively, and lane 3 is protein standards (M_r values: lysozyme, 14 300; soya bean trypsin inhibitor, 21 000; carbonic anhydrase, 30 000; ovalbumin, 43 000; bovine serum albumin, 68 000; phosphorylase B, 94 000). (*b*) Following SDS/polyacrylamide-gel electrophoresis the protein components were transferred onto a nitrocellulose sheet. The blot was soaked in 10 mM-Tris/HCl buffer, pH 7.2, containing 3% (w/v) ovalbumin, 0.1% (v/v) Triton X-100 and 0.9% (w/v) NaCl at 40°C for 1 h, after which 5 μl of the antiserum was added to the above and incubated overnight at room temperature. The blot was washed and incubated with ^{125}I-labelled protein A (approx. 1 μCi) for 6 h. After re-washing, the blot was dried and analysed by autoradiography.

Table 1. *Purification of human urinary N-acetyl-β-D-hexosaminidase B activity*
The affinity column was Sepharose-2-acetamido-*N*-(ε-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine

Normal Step	Specific activity (units/mg of protein)	Purification	Yield (%)
Urine	0.53	1	100
0–60% satd. $(NH_4)_2SO_4$ precipitate	413	779	83
Chromatography on affinity column	4549	8583	61
Chromatography on DEAE-cellulose	24390	45017	3.7
I-cell			
Urine	6.2	1	100
0–60% satd. $(NH_4)_2SO_4$ precipitate	142	23	43
Chromatography on affinity column	10655	1718	22
Chromatography on DEAE-cellulose	23204	3743	3.1

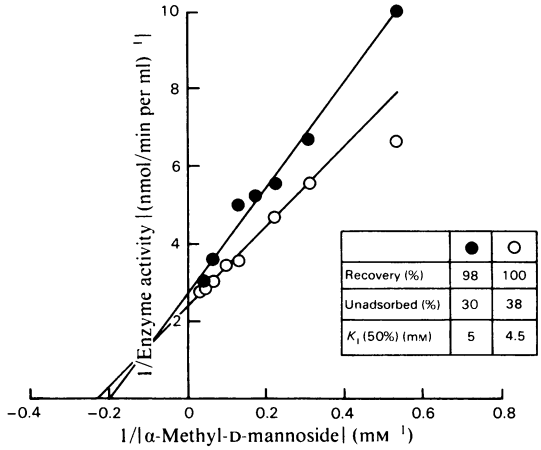

Fig. 2. *Concanavalin A binding of normal and mucolipidosis III urinary N-acetyl-β-D-hexosaminidase B*
To each tube the following components were added: concanavalin A (0.6 mg), 0.16 M-NaCl (final concn.), approx. 0.5 unit of normal (●) or mucolipidosis III (○) *N*-acetyl-β-D-hexosaminidase B, α-methyl-D-mannoside to give a final concentration range of 0–0.029 M and 0.1 M-sodium phosphate buffer, pH 5.5, containing human serum albumin (1 mg/ml), 0.1 mM-$CaCl_2$, 0.1 mM-$MgCl_2$ and 0.1 mM-$MnCl_2$ to bring the final volume to 150 μl. All solutions were prepared in the above buffer. The K_i (50%) is defined as the concentration of α-methyl-D-mannoside needed to prevent 50% of the added enzyme activity from binding to the lectin.

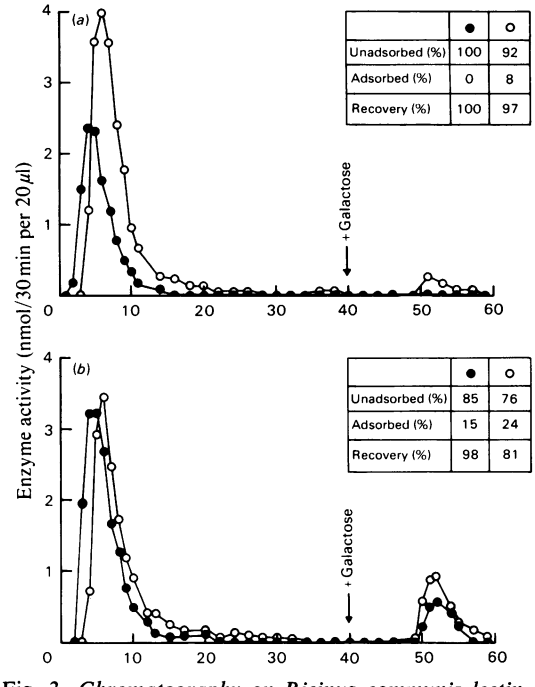

Fig. 3. *Chromatography on Ricinus communis lectin of (a) normal and (b) mucolipidosis III N-acetyl-β-D-hexosaminidase B*
The details of chromatography before (●) and after (○) neuraminidase treatment are given in the Materials and methods section.

of *N*-acetyl-β-D-hexosaminidase B activity that did not bind to the lectin (normal 30%; mucolipidosis III 38%) suggests that there was no significant change in the affinity of concanavalin A for the mucolipodosis III enzyme.

Chromatography on Ricinus communis lectin

Our previous studies with normal and the patient's urinary *N*-acetyl-β-D-hexosaminidase A showed that the mucolipidosis III enzyme has an increased affinity for *Ricinus communis* lectin (Hirani *et al.*, 1981). No appreciable binding was observed when normal *N*-acetyl-β-D-hexosaminidase B was chromatographed on the *Ricinus communis* lectin column (Fig. 3*a*). However, a small percentage of the activity (8%) did bind to the column following neuraminidase treatment (Fig. 3*a*). When the activity in the unadsorbed fraction was rechromatographed on

the column no additional binding to the lectin was observed. Approx. 15% of the mucolipidosis III enzyme bound to the column (Fig. 3*b*) which increased to 24% following neuraminidase treatment. These results suggest that there is a small increase in the number of galactose residues on the mucolipidosis III enzyme.

Treatment with endo-β-N-acetylglucosaminidase H

The nature of the carbohydrate moiety of the B form of the enzyme was examined further by treatment with endo-β-*N*-acetylglucosaminidase H. The results show that *N*-acetyl-β-D-hexosaminidase B is insensitive to endo-β-*N*-acetylglucosaminidase H (Fig. 4, lanes 1–4). The increase in the mobility of the 54000-M_r band of normal urinary *N*-acetyl-β-D-hexosaminidase A (Fig. 4, lane 7) clearly demonstrates that endo-β-*N*-acetylglucosaminidase H is active under these conditions.

Peptide maps

To determine whether the mutation causes an alteration to the protein structure, the 55000 and 28000-M_r chains were analysed by two-dimensional

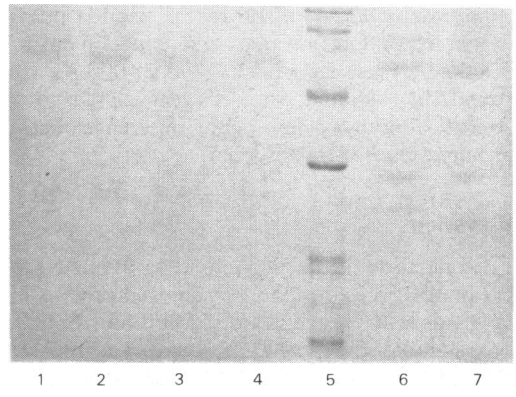

Fig. 4. *SDS/polyacrylamide-gel electrophoresis following incubation with endoβ-N-acetylglucosaminidase H*
The details of the experimental procedure are given in the Materials and methods section. Lane 1, mucolipidosis III *N*-acetyl-β-D-hexosaminidase B; lane 2, treated mucolipidosis III enzyme; lane 3, treated normal enzyme; lane 4, normal enzyme; lane 5, protein standards (same as in Fig. 1); lane 6, normal *N*-acetyl-β-D-hexosaminidase A; lane 7, treated *N*-acetyl-β-D-hexosaminidase A.

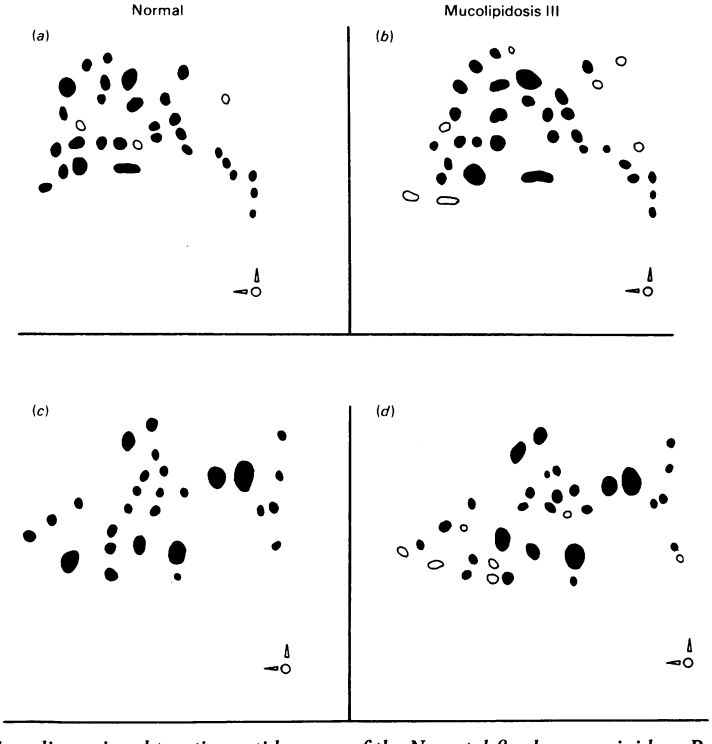

Fig. 5. *Two-dimensional tryptic peptide maps of the N-acetyl-β-D-hexosaminidase B subunits*
Trace drawings of the peptide maps were made from the autoradiographs. The M_r 55000 (*a* and *b*) and M_r 28000 (*c* and *d*) subunits from normal and mucolipidosis III *N*-acetyl-β-D-hexosaminidase B were compared. Identical spots on the maps are represented by the shaded areas.

peptide maps. Comparison of the peptide maps of the corresponding normal and mucolipidosis III 55000-M_r (Figs. 5*a* and 5*b*) and 28000-M_r (Figs. 5*c* and 5*d*) subunits showed very similar overall patterns, although a few minor differences between the paired maps were observed.

Discussion

Examination of the enzyme preparations by polyacrylamide-gel electrophoresis showed protein staining bands with M_r values of 55000 and 28000. In both samples the 55000-M_r band was the major component. Previous studies have reported M_r values of 50000 and 25000 for this isoenzyme when purified from placenta (Geiger & Arnon, 1976). However, under non-reducing conditions the placental enzyme is only present as the larger subunit, indicating that the larger subunit is composed of two β chains (25000 M_r) held together by disulphide bonds (S. Hirani, unpublished work). The presence of the larger (M_r 55000) component in our enzyme preparations, under reducing conditions, indicates that this component may be a precursor of the smaller subunit. A molecular relationship between these two subunits was clearly demonstrated following the 'western' blot (Fig. 1*b*). Larger M_r subunits (63000 and 52000 daltons) for human fibroblast *N*-acetyl-β-D-hexosaminidase B have been reported previously by Hasilik & Neufeld (1980), where the M_r-63000 subunit was shown to be the precursor of the processed M_r-29000 (β) subunit. Besides *N*-acetyl-β-D-hexosaminidase, precursor forms that have higher apparent M_r values than mature enzymes have also been reported for α-glucosidase and cathepsin D (Hasilik & Neufeld, 1980) from human fibroblasts and β-galactosidase and β-glucuronidase from mouse macrophages (Skudlarek & Swank, 1981).

The peptide maps of the M_r 55000 and M_r 28000 subunits revealed a large degree of similarity between the normal and mucolipodisis III components (Fig. 5). Although some differences between the corresponding maps were noted, the basis for these is presently unknown. Comparison of the maps of the M_r 55000 and M_r 28000 chains from either normal or mucolipidosis III suggested that the two chains have distinct protein structures.

The similar proportions of normal and the patient's *N*-acetyl-β-D-hexosaminidase B that bound to concanavalin A and the near identical values of K_I (50%) (normal, 5mM; mucolipidosis III, 4.5mM) indicate that the mutation has not significantly altered the mannose-containing portion of *N*-acetyl-β-D-hexosaminidase B. These results are in contrast with previous studies when we observed that concanavalin A had a much lower affinity for the patient's *N*-acetyl-β-D-hexosaminidase A than did the corresponding normal enzyme (Hirani *et al.*, 1981). Decreased binding by this lectin for a number of acid hydrolases from mucolipidosis II and mucolipidosis III patients has also been reported by others (Kress & Miller, 1979; Rousson *et al.*, 1979; Hirani & Winchester, 1980; Kress *et al.*, 1980*b*; Miller *et al.*, 1981*b*).

Chromatography on *Ricinus communis* lectin showed that whereas none of the normal *N*-acetyl-β-D-hexosaminidase B bound to the lectin, a small proportion, 15%, of the mucolipidosis III enzyme did bind. This is in sharp contrast to the significant binding (65%) by the patient's *N*-acetyl-β-D-hexosaminidase A to this lectin (Hirani *et al.*, 1981) and to the increased binding by other mucolipidosis II and mucolipidosis III lysosomal hydrolases (Sly *et al.*, 1979; Van Elsen & Leroy, 1979; Miller *et al.*, 1981*b*). This aberrant binding to *Ricinus communis* lectin by mucolipidosis II and mucolipidosis III acid hydrolases is indicative of a conversion of their high-mannose chains to the complex type. The lack of any significant binding to this lectin by the patient's *N*-acetyl-β-D-hexosaminidase B suggests that only a few additional galactose residues are present on the enzyme.

Incubation of normal and patient's *N*-acetyl-β-D-hexosaminidase B with endo-β-*N*-acetylglucosaminidase H was carried out to test for the presence of high mannose chains. This endoglycosidase hydrolyses di-*N*-acetylchitobiose linkages in oligosaccharides and glycoproteins (Tarentino & Maley, 1974) and requires a tetrasaccharide structure, Man(α1→3)Man(α1→6)Man(β1→4)GlcNAc as its specific glycon (Tai *et al.*, 1977). It does not act on complex oligosaccharide chains nor on aglycone moieties with Fuc(α1→6)GlcNAc and Fuc(α1→6)-GlcNAc→Asn (Tarentino & Maley, 1975). Both the normal and the patient's *N*-acetyl-β-D-hexosaminidase B appeared to be poor substrates for endo-β-*N*-acetylglucosaminidase H when compared with normal *N*-acetyl-β-D-hexosaminidase A. Thus the present observation indicates that different oligosaccharide structures are present on *N*-acetyl-β-D-hexosaminidase A and B.

It appears that the patient's urinary *N*-acetyl-β-D-hexosaminidase A undergoes changes whereby its carbohydrate chains are converted to complex type chains (Kress *et al.*, 1980*a*; Hirani *et al.*, 1981). These changes seem to have occurred as a result of the failure of the recognition marker being transferred to the I-cell acid hydrolases (Hasilik *et al.*, 1981; Reitman *et al.*, 1981). Thus as a consequence the oligosaccharide chains of the I-cell acid hydrolases are further processed from high mannose chains to complex type and they are then secreted from the cell in a similar manner to other serum glycoproteins. Why *N*-acetyl-β-D-hexosaminidase B should escape the mutation compared with *N*-acetyl-

β-D-hexosaminidase A is not clear. Differences in their carbohydrate structures as indicated by the data obtained here and by others (Lee & Yoshida, 1976; Freeze *et al.*, 1979) may be an explanation, since it is now acknowledged that carbohydrate moieties of acid hydrolases play a key role in the recognition of these glycoproteins by specific receptors and their subsequent endocytosis.

We are grateful to the Muscular Dystrophy Association of America for a postdoctoral fellowship to S. H. A. L. M. is the recipient of an N.I.H. grant NS12138.

References

Beutler, E., Yoshida, A., Kuhl, W. & Lee, J. E. S. (1976) *Biochem. J.* **159**, 541–543

Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254

Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203

Elder, J. H., Pickett II, R. A., Hampton, J. & Lerner, R. A. (1977) *J. Biol. Chem.* **252**, 6510–6515

Freeze, H., Geiger, B. & Miller, A. L. (1979) *Biochem. J.* **177**, 749–752

Geiger, B. & Arnon, R. (1976) *Biochemistry*, **15**, 3484–3494

Gilbert, F., Kucherlapati, R., Creagan, R. P., Murnane, M. J., Darlington, G. J. & Ruddle, F. H. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 263–267

Hasilik, A. & Neufeld, E. F. (1980) *J. Biol. Chem.* **255**, 4937–4945

Hasilik, A., Waheed, A. & von Figura, K. (1981) *Biochem Biophys. Res. Commun.* **98**, 761–767

Herd, J. K., Dvorak, A. D., Wiltse, H. E., Eisen, J. D., Kress, B. C. & Miller, A. L. (1978) *Am. J. Dis. Child.* **132**, 1181–1186

Hirani, S. & Winchester, B. (1980) *Clin. Chim. Acta* **101**, 251–256

Hirani, S., Little, L., Kress, B. & Miller, A. L. (1981) *Biochem. Soc. Trans.* **9**, 147P

Honey, N. K., Mueller, O. T., Miller, A. L. & Shows, T. B. (1981) *Am. J. Hum. Genet.* **33**, 146A

Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496

Kress, B. C. & Miller, A. L. (1979) *Biochem. J.* **177**, 409–415

Kress, B. C., Freeze, H., Vedvick, T. & Miller, A. L. (1980*a*) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2082

Kress, B. C., Freeze, H. H., Herd, K. J., Alhadeff, J. A. & Miller, A. L. (1980*b*) *J. Biol. Chem.* **255**, 955–961

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Lee, J.-E. S. & Yoshida, A. (1976) *Biochem. J.* **159**, 535–539

Leroy, J. F., Ho, M. W., MacBrinn, M. C., Zielke, K., Jacob, J. & O'Brien, J. S. (1972) *Pediatr. Res.* **6**, 752–757

Miller, A. L., Freeze, H. H. & Kress, B. C. (1981*a*) in *Lysosomes and Lysosomal Storage Disorders* (Lowden, J. A. & Callahan, J., eds.), pp. 271–287, Raven Press, New York

Miller, A. L., Kress, B. C., Stein, R., Kinnon, C., Kern, H., Schneider, A. & Harms, E. (1981*b*) *J. Biol. Chem.* **256**, 9352–9362

Mueller, O. T., Honey, N. K., Miller, A. L. & Shows, T. B (1981) *Am. J. Hum. Genet.* **33**, 50A

Neufeld, E. F., Lim, T. W. & Shapiro, J. L. (1975) *Annu. Rev. Biochem.* **44**, 357–376

Reitman, M. L., Varki, A. & Kornfeld, S. (1981) *J. Clin. Invest.* **67**, 1574–1579

Robinson, D. & Stirling, J. L. (1968) *Biochem. J.* **107**, 321–327

Rousson, R., Ben-Yoseph, Y., Fiddler, M. B. & Nadler, H. L. (1979) *Biochem. J.* **180**, 501–505

Shows, T. B., Mueller, O. T., Honey, N. K., Wright, C. E. & Miller, A. L. (1982) *Am. J. Med. Genet.*, in the press

Skudlarek, M. D. & Swank, R. T. (1981) *J. Biol. Chem.* **256**, 10137–10144

Sly, W. S., Gonzalez-Noriega, A., Natowicz, M., Fisher, H. D. & Chambers, J. P. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 1256

Solomon, E., Bobrow, M., Goodfellow, P. N., Bodmer, W. F., Swallow, D. M., Povey, S. & Noël, B. (1976) *Somatic Cell Genet.* **2**, 125–140

Srivastava, S. K. & Beutler, E. (1974) *J. Biol. Chem.* **249**, 2054–2057

Tai, T., Yamashita, K. & Kobata, A. (1977) *Biochem. Biophys. Res. Commun.* **78**, 434–441

Tarentino, A. L. & Maley, F. (1974) *J. Biol. Chem.* **249**, 811–817

Tarentino, A. L. & Maley, F. (1975) *Biochem. Biophys. Res. Commun.* **67**, 455–462

Thomas, G. H., Taylor, H. A., Reynolds, L. W. & Miller, C. S. (1973) *Pediatr. Res.* **7**, 751–756

Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354

Van Elsen, A. F. & Leroy, J. G. (1979) in *Models for the Study of Inborn Errors of Metabolism* (Hammes, F. A., ed.), pp. 329–332, Elsevier/North-Holland Biomedical Press, Amsterdam

Wiesmann, U. N., Lightbody, J., Vassella, F. & Herschkowitz, H. (1971*a*) *New Engl. J. Med.* **284**, 109–110

Wiesmann, U., Vassella, F. & Herschkowitz, H. (1971*b*) *New Engl. J. Med.* **285**, 1090–1091