Am J Hum Genet 29:149–163, 1977

Electrophoretic Abnormalities of Lysosomal Enzymes in Mucolipidosis Fibroblast Lines

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INTRODUCTION

Inherited lysosomal storage diseases of man generally result from deficient activity of single lysosomal enzymes [1]. The human mucolipidoses, however, represent an important group of related disorders characterized by abnormal activity of several acid hydrolases. These disorders suggest a single genetic defect that is common to the final expression of several lysosomal enzymes. Several clinical forms of mucolipidosis have been described [2]. Mucolipidosis II (ML II, I-cell disease) is a severe neurodegenerative disorder showing Hurler-like clinical features, excess accumulations of mucopolysaccharides and glycolipids in tissues. It is fatal in early childhood and is inherited as an autosomal recessive defect [3, 4]. Mucolipidosis III (ML III, pseudo-Hurler polydystrophy), an autosomal recessive disorder, is characterized by milder clinical and pathological features, and affected individuals survive to adult life [5, 6].

The primary defect of these disorders is not yet known; however, evidence from cultured skin fibroblasts suggests a defect in the control or post-translational modification of multiple lysosomal enzymes [7]. Leakage of lysosomal enzymes from mucolipidosis cells was initially proposed since cultured ML II and ML III fibroblasts are severely deficient for seven or more acid hydrolases [8–11], and many of these enzymes are found in elevated levels in culture media and serum of patients [9, 10]. However, ML II fibroblasts have been shown to be capable of taking up and retaining normal acid hydrolases while hydrolases excreted by these cells are not as effectively accumulated [12]. These excreted enzymes appear to be deficient for a recognition marker necessary for normal cellular uptake [12]. Moreover, passage of Sindbis virus in ML II fibroblasts indicates that the mucolipidosis mutation not only alters lysosomal enzymes but also alters cell membranes [13].

It has been reported that ML II fibroblasts have increased sialic acid content and are deficient for an acid sialidase [15]. This finding is supported by lysosomal hydrolases *N*-acetyl-β-hexosaminidase, arylsulfatase, β-glucuronidase, and α-fucosidase that are excreted by ML II fibroblasts and show abnormal electrophoretic properties [14].

Received June 25, 1976; revised October 12, 1976.

This research was supported by grant HD 05196 from the U.S. Public Health Service and grant 1-485 from the National Foundation–March of Dimes.

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These abnormalities appear to result from excess sialic acid since these enzymes are corrected by neuraminidase treatment. Within ML II and ML III fibroblasts only the enzyme *N*-acetyl-β-hexosaminidase has been identified with an abnormal electrophoretic pattern [10, 16].

Since many enzymes have not been characterized electrophoretically within mucolipidosis fibroblasts, we have examined the electrophoretic patterns of eight lysosomal and 36 nonlysosomal enzymes in ML II and ML III fibroblast lines. Characterization of lysosomal and nonlysosomal enzymes in these cell lines is important to determine enzyme abnormalities that could illuminate the primary genetic defect common to the expression of many intracellular enzymes and help delineate different forms of these disorders. We report here abnormal electrophoretic patterns for lysosomal *N*-acetyl-β-hexosaminidase, acid phosphatase$_2$, esterase-A_4, and α-mannosidase and for the nonlysosomal enzyme adenosine deaminase-d in mucolipidosis cell lines. The abnormal enzyme patterns demonstrate electrophoretic heterogeneity between ML II cell lines and the possibility of distinguishing between ML II and ML III cell lines. The results of neuraminidase treatment of the altered enzymes is consistent with the hypothesis of a post-translational modification of enzymes in mucolipidosis fibroblasts.

Materials and Methods

*Mucolipidosis Fibroblast Cell Lines**

Cultured mucolipidosis cells consisted of skin fibroblast lines from four unrelated children with severe ML II symptoms and from a patient indicated to have the ML III disorder. Enzyme analysis of the cell line ChMk (designated C.M. [4]; C.McK. [17]) from a child with classic I-cell disease [2, 18] indicated severe deficiencies of acid hydrolases [4, 15] and abnormal *N*-acetyl-β-hexosaminidase [16]. Fibroblast line CyWm (designated C. W.) from a patient with typical ML II symptoms showed low levels of acid hydrolases (R. Matalon, unpublished report). Mucolipidosis lines TC-75-438 and GM 1006 were established from the same patient (L.T.) with typical ML II clinical symptoms and low lysosomal enzyme activities [19]. Cell line GM 1006 was passaged in three different laboratories prior to our analysis. Fibroblast line GM 521 was established from a child with typical ML II symptoms and severe hydrolase deficiencies (Murphy et al., unpublished observation). Heterozygous ML II lines GM 80 and GM 81 were obtained from the maternal and paternal parents of a ML II child (GM 521). Fibroblast line GM 113 from a child (T.H.) whose milder clinical symptoms [20] were typical of the ML III disorder [6] showed multiple acid hydrolase deficiencies ([20] and enzyme data from the Camden Repository). The clinical diagnosis is usually necessary to distinguish between ML II and ML III patients since the enzyme levels in ML III fibroblasts are often similar to those of ML II fibroblasts [6].

Fibroblast lines were maintained on Dulbecco's modified Eagle's medium with 15% fetal calf serum (same lot used throughout). Confluent monolayers of cells were harvested, washed twice with serum-free media, and homogenized in 0.05 M Tris buffer, pH 7.5, at 70×10^6 cells/ml. All cell lines were harvested at several different passages and demonstrated constant enzyme patterns between passages.

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* CmMk and CyWm cell lines were generously provided by Dr. Glyn Dawson (University of Chicago), and line TC-75-438 was kindly provided by Dr. George B. Thomas (Johns Hopkins University). Lines GM 521, GM 1006, GM 113, GM 80, and GM 81 were obtained from the Human Genetic Mutant Cell Repository (Camden, New Jersey). All cell lines had been stored frozen and recovered prior to being received.

Control Fibroblast Lines

Human control fibroblasts were karyotypically normal lung WI-38 fibroblasts (American Type Culture Collection, CCL 75); AnLy skin fibroblasts possessed an X/9 reciprocal translocation [21]; JoVa skin fibroblasts possessed a 1/2 translocation [22]; OnSer skin fibroblasts ($HPRT^-$) (ATCC, CRL 1112) was established from a 7-year-old male; and TS 408 and SH 421 skin fibroblasts were derived from males age 1–2 years [23]. Enzyme patterns in control skin fibroblasts corresponded to those of human tissues and of WI-38 lung fibroblasts included in the figures. Culture and harvest conditions were as described above.

Electrophoresis and Enzyme Staining

Cell homogenates were analyzed by vertical starch gel electrophoresis (Buchler Instruments Inc., Fort Lee, N.J.) at 4°C for 16 hr in 12% gels (Electrostarch Co., Madison, Wisconsin). Electrophoresis of β-D-N-acetylhexosaminidase (E.C.3.2.1.30) and cytoplasmic α-mannosidase (E.C.3.2.1.24) was accomplished in a Tris-citrate pH 7.0 buffer system [24]. Bands of β-hexosaminidase activity were produced employing the fluorescent 4-methylumbelliferyl-β-D-N-acetyl-glucosaminide substrate as reported [25]. Molecular forms of acid phosphatase (E.C.3.1.3.2) and α-mannosidase in figure 6 were separated in a bridge buffer of 0.2 M Na phosphate pH 6.0 with a gel buffer consisting of a 1/20 dilution of this. Lysosomal α-mannosidase in figure 3 was examined in this same buffer but at pH 5.0. Bands of acid phosphatase were stained as described [26], and fluorescent α-mannosidase activity was observed as reported [27]. Human esterase-A_4 (E.C.3.1.1.2) was resolved in a veronal pH 8.0 buffer and stained using α-naphthyl acetate as substrate and Fast Blue BB as dye coupler [28]. Adenosine deaminase (E.C.3.5.4.4) was determined in a Tris-EDTA-Borate, pH 8.6 system [28] with histochemical staining [29]. Cellulose acetate electrophoresis of β-hexosaminidase from mucolipidosis cells was run using a 0.025 M citric acid/sodium citrate buffer, pH 5.7, while α-mannosidase was examined with a 0.0045 M citric acid/0.058 M Na phosphate buffer, pH 7.4.

Nonlysosomal Enzymes Examined

Thirty-five additional nonlysosomal enzymes were examined by starch gel electrophoresis in all mucolipidosis cell lines. These enzymes consisted of glucose-6-phosphate dehydrogenase, lactate dehydrogenase-A and -B, malate dehydrogenase (cytoplasmic and mitochondrial forms), malic enzyme cytoplasmic form, isocitrate dehydrogenase (cytoplasmic and mitochondrial forms), 6-phosphogluconate dehydrogenase, superoxide dismutase (cytoplasmic and mitochondrial forms), hypoxanthine phosphoribosyl transferase, galactose-1-phosphate uridylyltransferase, glutamic oxaloacetic transaminase (cytoplasmic and mitochondrial forms), hexokinase-1, pyruvate kinase-3, adenine phosphoribosyl transferase, phosphoglycerate kinase, adenylate kinase-1 and -2, nucleoside phosphorylase, uridine monophosphate kinase, peptidase-A, -B, -C, and -S, mannosephosphate isomerase, phosphohexose isomerase, enolase, fumarate hydratase (cytoplasmic form), acid phosphatase-1 (red cell type), and esterase-D. References for the starch gel electrophoretic conditions and specific histochemical staining procedures for these enzymes have been reported [21, 25, 30].

Neuraminidase Treatment

Supernatant fractions of homogenates from cultured cells were treated with neuraminidase from *Vibrio Cholerae* (General Biochemicals, Chagrin Falls, Ohio; 500 U/ml) essentially as described [31]. Reaction mixtures consisted of 25 μl cell homogenate, 25 μl neuraminidase (12.5 U), and 10 μl 0.005 M citrate phosphate buffer, pH 4.8.

RESULTS

EXPRESSION OF LYSOSOMAL ENZYMES IN MUCOLIPIDOSIS CELL LINES

β-D-N-Acetylhexosaminidase

The electrophoretic pattern of β-D-*N*-acetylhexosaminidase (β-hexosaminidase) from mucolipidosis fibroblast lines is shown in figure 1. Two obligate heterozygous cell lines, GM 80 and GM 81 (fig. 1, channels 6 and 7), showed the normal pattern for HEX A and HEX B enzymes. This pattern was identical to other cultured human cells such as WI-38 fibroblasts [25]. The GM 80 and GM 81 cell lines were derived from parents of a ML II child whose cultured fibroblasts (GM 521, not shown here) demonstrated the same enzyme patterns as those expressed in the GM 1006 and TC-75-438 cell lines.

In fibroblasts from ML II children, β-hexosaminidase activity showed altered

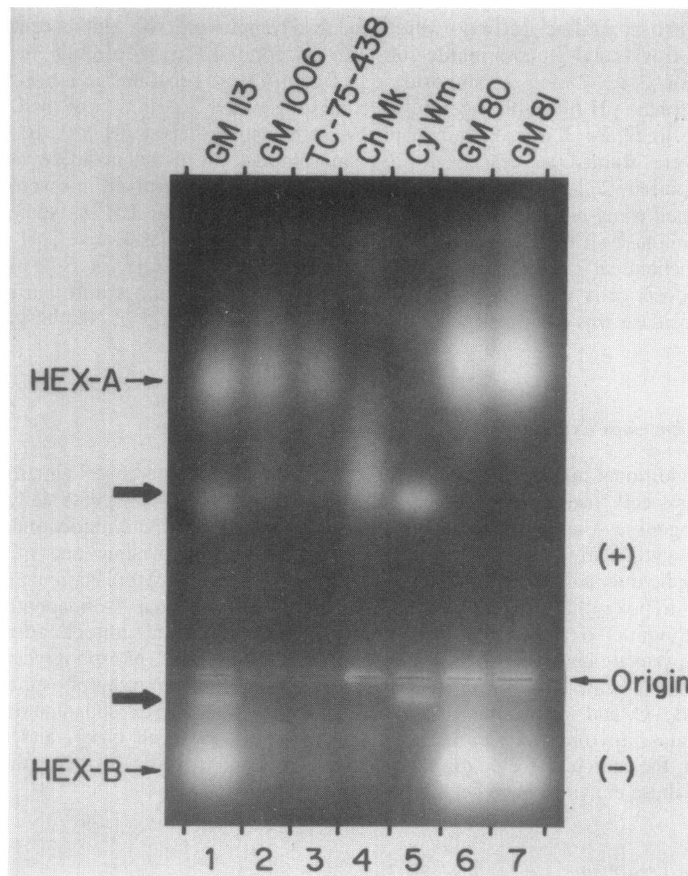

FIG. 1.—Electrophoretic pattern of β-hexosaminidase (HEX) observed in cell homogenates from cultured mucolipidosis fibroblasts. *Channel 1*, cultured fibroblasts from a child with the ML III disorder; *channels 2–5*, cultured fibroblast lines from ML II patients; *channels 6 and 7*, fibroblast lines from the maternal and paternal parents of a ML II child (GM 521) whose fibroblasts (not shown) showed a HEX pattern identical to GM 1006 and TC-75-438 cells. The heterozygous fibroblasts (*channels 6 and 7*) demonstrate the normal HEX pattern. *Arrows* indicate abnormal isozymes.

electrophoretic patterns on starch gels, confirming earlier evidence [16]. However, different enzyme patterns were observed in the ML II cell lines, demonstrating electrophoretic heterogeneity between lines. Cell lines ChMk and CyWm showed little or no activity in the HEX A and HEX B regions but revealed instead a prominent altered band (arrow) of activity cathodal to the HEX A region and for CyWm an altered band (arrow) cathodal to the origin. The GM 1006 and TC-75-438 fibroblasts, established from the same patient, as well as GM 521 cells revealed a less severely altered pattern with decreased activity for HEX A and HEX B bands and the presence of a single altered band seen in ChMk and CyWm cell lines migrating cathodal to the HEX A region. These electrophoretic patterns were also repeated on cellulose acetate gels, and the β-hexosaminidase pattern of the ChMk cell line was found to be the same as reported [16].

Mucolipidosis III fibroblasts, GM 113 (fig. 1, channel 1), also revealed an abnormal β-hexosaminidase phenotype consistent with a previous report [10]. These cells demonstrated the typical HEX B phenotype but decreased HEX A activity and two altered bands (indicated by arrows) as seen in the CyWm ML II fibroblasts. Thus, four distinct β-hexosaminidase electrophoretic patterns were observed in mucolipidosis fibroblasts: three separate patterns in ML II fibroblasts and a related but unique pattern in ML III fibroblasts.

Acid Phosphatase

Electrophoretic patterns of lysosomal acid phosphatase were significantly altered in different ML II cell lines (fig. 2). Variation was restricted to the lysosomal acid phosphatase (ACP_2) [26, 32], while the nonlysosomal "red cell" acid phosphatase (ACP_1, not shown) was unaltered. The normal electrophoretic pattern for ACP_2 in cultured human cells in our system is observed in the heterozygous cell lines GM 80 and GM 81 (fig. 2, channels 6 and 7). The ML II cell lines could be divided into two groups. Cell lines ChMk and CyWm (channels 4 and 5) revealed similar abnormal patterns showing virtual absence of the ACP_2 region but two distinct cathodal bands and a diffuse region of anodal activity (indicated by arrows). The GM 1006, TC-75-438, and GM 521 cells showed only minor normal activity and an altered diffuse anodal region. Previously reported enzyme assays indicated that acid phosphatase is normal in mucolipidosis II fibroblasts [8, 9]. However, evidence in figure 2 demonstrates that this normal activity is associated with altered electrophoretic mobility of the enzyme. In ML III fibroblasts, GM 113 (fig. 2, channel 1), ACP_2 was not altered, thus distinguishing these cells from the two ML II patterns.

α-Mannosidase

α-Mannosidase was resolved into two major zones of activity after starch gel electrophoresis (fig. 3; also see fig. 6) and on cellulose acetate gel electrophoresis (not shown). Similar bands of α-mannosidase activity have been demonstrated on cellulose acetate gels [27] showing the neutral cytoplasmic form (MAN A) and two acidic forms (MAN B) indicated to be localized in the lysosome [33]. Employing a Tris-citrate, pH 7.0, buffer system (fig. 3, top), the cytoplasmic MAN A band was observed in WI-38 fibroblasts and in homogenates from all mucolipidosis cell lines. Using a Na phosphate, pH 5.0, buffer system to best resolve the lysosome α-mannosidase (fig. 3,

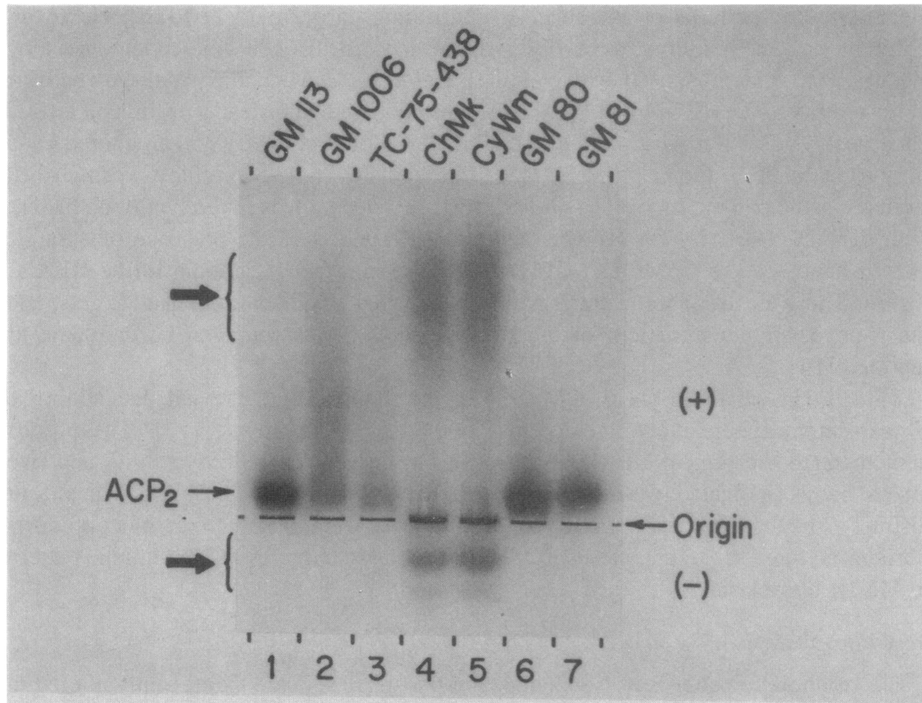

FIG. 2.—Lysosomal acid phosphatase (ACP_2) electrophoretic pattern in fibroblast homogenates. *Channel 1*, fibroblasts from a child with the ML III syndrome; *channels 2–5*, fibroblast lines established from ML II children; *channels 6 and 7*, fibroblasts derived from obligate heterozygous parents of a ML II child. The heterozygous fibroblasts (*channels 6 and 7*) demonstrate the normal ACP_2 pattern. *Arrows* indicate abnormal isozymes.

bottom), normal expression of the MAN B zone was observed in WI-38 fibroblasts (channel 1) and GM 113, GM 80, and GM 81 cell lines (channels 2, 7, 8). However, mucolipidosis II cell lines GM 1006 and TC-75-438 (GM 521 not tested) showed only faint MAN B expression (channels 3 and 4), while in homogenates from ChMk and CyWm cells this band was not detected (channels 5 and 6). In a Na phosphate, pH 6.0, gel system that resolves both MAN A and MAN B on the same gel (fig. 6), a further difference is demonstrated between ML II cell lines for α-mannosidase (channel 2). GM 1006 and TC-75-438 express considerable activity at the origin, whereas ChMk (channel 4) and CyWm express a normally migrating MAN A isozyme. These results are consistent with evidence of deficient activity of α-mannosidase in ML II fibroblasts [8, 9]. Deficient expression of acid α-mannosidase activity and the absence of the MAN B isozymes are also observed in fibroblasts from individuals with inherited mannosidosis [33, 34].

Esterase Isozymes

Examination of the electrophoretic pattern of nonspecific esterase activity in mucolipidosis cells revealed the deficient expression of the cathodal esterase-A_4 isozymes (ESA_4) in four homozygous ML II cell lines (fig. 4, channels 2–5). The ML

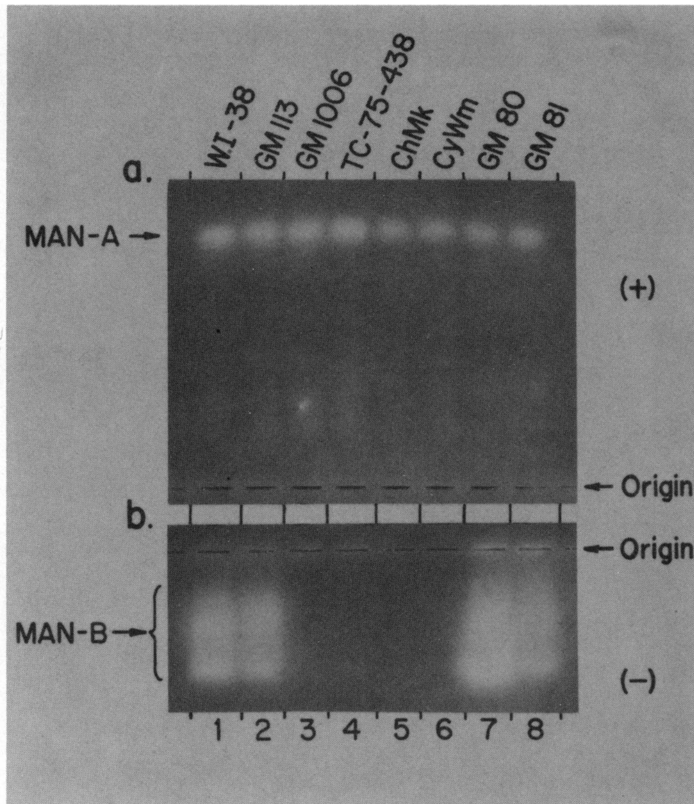

FIG. 3.—α-Mannosidase electrophoretic patterns in mucolipidosis fibroblast homogenates: *a*, cytoplasmic α-mannosidase (MAN A) electrophoresis in a Tris-citrate pH 7.0 buffer and stained at pH 7.0; *b*, lysosomal α-mannosidase (MAN B) after electrophoresis in a Na phosphate pH 5.0 buffer and stained at pH 4.5. Under these conditions, MAN A was not resolved in the anodal region. α-Mannosidase characteristics and nomenclature in human cultured fibroblasts have been described [27, 33].

II cell lines ChMk, CyWm, GM 1006, TC-75-438 and GM 521 showed greatly reduced activity in the ESA_4 region, while ChMk and CyWm cells showed elevated activity for the esterase-A_2 isozyme (ESA_2). Heterozygous ML II cell lines and the ML III cells demonstrated normal patterns of esterase isozymes similar to those reported for WI-38 fibroblasts and other human cell lines [28, 35]. The esterase-A_4 isozyme seen on starch gels possesses characteristics similar to the same primate specific esterase which has been localized to the lysosome [36]. This esterase band has also been shown to be virtually absent in SV40 transformed WI-38 fibroblasts [37].

Other Lysosomal Enzymes

Electrophoretic investigation of lysosomal enzymes qualitatively confirmed previous evidence of severe deficiencies of multiple acid hydrolases in ML II cell lines [8, 9] and in ML III fibroblasts [10, 11, 17]. Analysis of α-galactosidase, β-galactosidase, and β-glucuronidase demonstrated little or no activity on starch gels and could not be

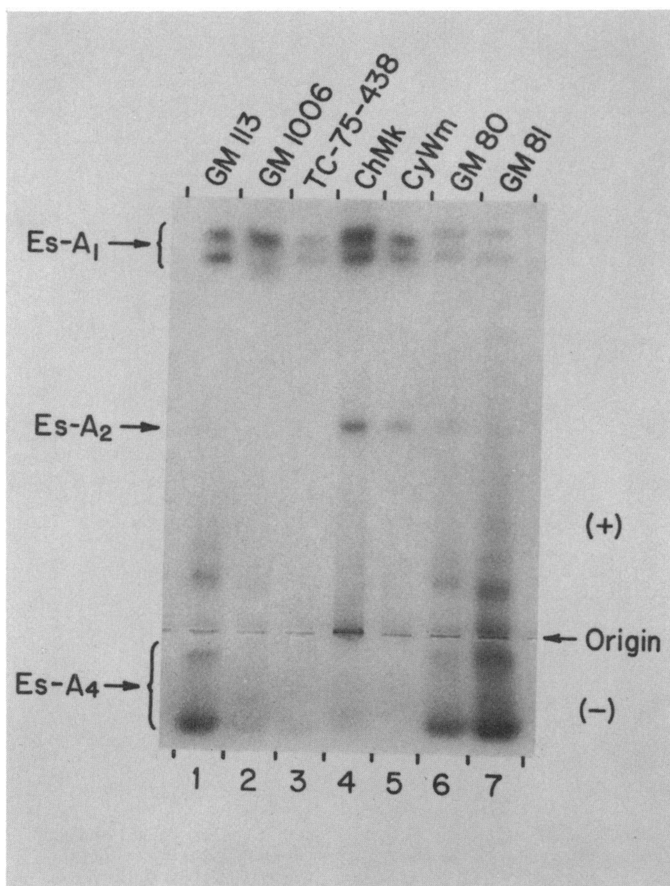

FIG. 4.—Nonspecific esterase electrophoretic pattern in cultured mucolipidosis fibroblast lines. *Channel 1*, ML III fibroblasts; *channels 2–5*, fibroblast lines established from ML II children; *channels 6 and 7*, fibroblast lines from maternal (GM 80) and paternal (GM 81) parents of a ML II child. The heterozygous fibroblasts (*channels 6 and 7*) demonstrate the normal esterase pattern. Characterization and nomenclature of esterases in cultured human cells have been reported [28].

evaluated for their electrophoretic properties. Reduced activity of α-glucosidase was observed in mucolipidosis fibroblasts but revealed a normal electrophoretic pattern.

EXPRESSION OF NONLYSOSOMAL ENZYMES IN MUCOLIPIDOSIS CELL LINES

Adenosine Deaminase

Three adenosine deaminase (ADA) phenotypes have been observed in normal cultured fibroblasts. One phenotype expresses ADA 1, the only form observed in red blood cells; one phenotype demonstrates the ADA-d form expressed in several tissues; and the third phenotype is composed of both molecular forms [29, 38, 39]. The ADA 1 and ADA-d forms in cultured human fibroblasts have been interconverted depending on different culture conditions [29] and an isolated factor [40].

Evidence suggests that the ADA 1 and ADA-d forms are controlled by a single genetic locus [41] and that the severe deficiency of these enzymes is associated with combined immunodeficiency disease [39, 42]. In most mucolipidosis lines the ADA 1 band was only faintly detected (fig. 5); however, three different phenotypes were observed in the ADA-d region in these cells. The usual form of ADA-d, observed in WI-38 control fibroblasts, was identified in the heterozygous cell lines GM 80 and GM 81 (fig. 5). However, a new ADA form was expressed in ML II cell lines ChMk and CyWm migrating between ADA 1 and ADA-d. The GM 1006, TC-75-438, and GM 521 (not shown) fibroblasts showed a composite pattern with both the new ADA form and ADA-d (fig. 5, channels 3 and 4). The ML III cell line demonstrated a normal ADA-d. These altered patterns in the ADA-d region are not similar to previous changes reported for the ADA isozymes [29, 38, 40, 41].

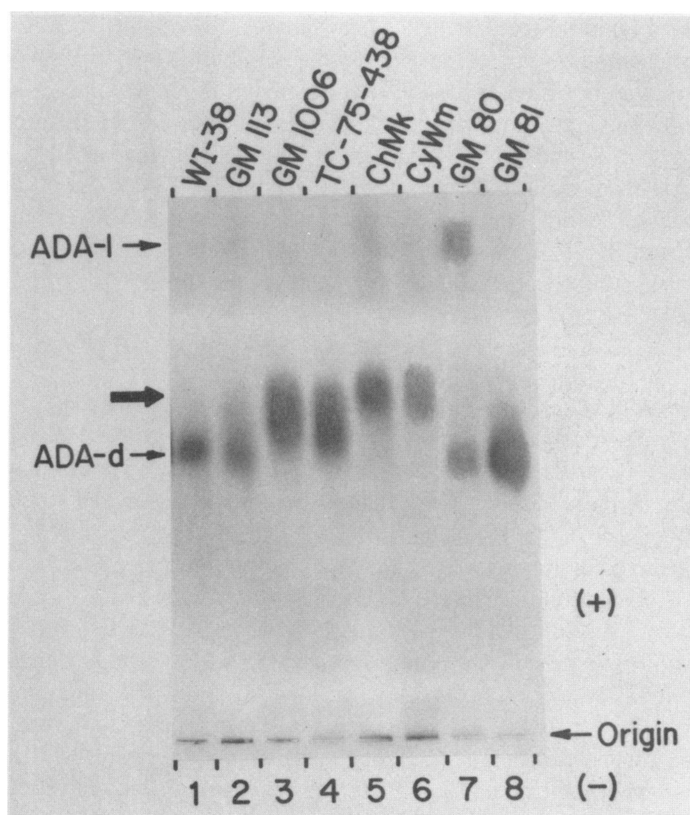

FIG. 5.—Adenosine deaminase electrophoretic pattern in mucolipidosis fibroblast homogenates. *Channel 1*, control WI-38 fibroblasts; *channel 2*, cultured ML III fibroblasts; *channels 3–6*, cultured skin fibroblasts from ML II patients; *channels 7 and 8*, fibroblasts from parents of a ML II child. Heterozygous GM 80 and GM 81 cells show variation in relative ADA1 and ADA-d expression seen in many normal human fibroblasts [21, 31]. *Arrow* indicates abnormal adenosine deaminase.

Other Enzymes

In addition to the lysosomal hydrolases and adenosine deaminase reported above, we also investigated in each mucolipidosis cell line 35 nonlysosomal enzymes (listed in Materials and Methods) routinely employed as biochemical markers in human gene mapping studies [30]. Differences in electrophoretic mobility or qualitative activity changes were not observed for these enzymes after gel electrophoresis.

ENZYME PATTERNS AFTER NEURAMINIDASE TREATMENT

Neuraminidase has been used to remove sialic acids and convert forms of lysosomal hydrolases [26, 43]. The action of neuraminidase was tested on the altered enzymes in mucolipidosis cell homogenates. Treatment of control WI-38 fibroblasts revealed that the lysosomal enzyme patterns being tested were not affected by neuraminidase treatment. However, neuraminidase treatment of ML II cells converted the altered anodal acid phosphatase activity to a region similar to the normal band (fig. 6*b*). Similarly, neuraminidase treatment of adenosine deaminase shifted the pattern to a more cathodal position (fig. 6*c*). The action of neuraminidase on α-mannosidase bands produced two different results (fig. 6*a*). A gel system different from figure 3 was now employed to demonstrate both MAN A and MAN B on the same gel. In one class of ML II cells, ChMk and CyWm, no effect on the α-mannosidase pattern was demonstrated. However, in cell lines GM 1006 and TC-75-438 incubation of cell homogenates with neuraminidase generated the previously undetected MAN B isozyme. Altered β-hexosaminidase bands were not changed after neuraminidase treatment nor were the α-galactosidase or β-galactosidase phenotypes.

DISCUSSION

Cultured fibroblasts from mucolipidosis patients demonstrate a complex array of lysosomal enzyme, biochemical, and cellular abnormalities. An understanding of the several anomalies expressed by mucolipidosis fibroblasts should aid in distinguishing between forms of these disorders and in determining the molecular defect for each syndrome.

We have observed in fibroblast lines established from four mucolipidosis II children, electrophoretic changes in lysosomal acid phosphatase (ACP_2) and α-mannosidase and deficient activity for lysosomal α-mannosidase (MAN B) and arylesterase-A_4 (ESA_4). Our results employing starch gel electrophoresis have confirmed and extended previous results with cellulose acetate gels showing altered β-hexosaminidase mobility in mucolipidosis cells [10, 16]. We have observed different β-hexosaminidase patterns in ML II fibroblast lines, and a related but distinct pattern was seen in ML III fibroblasts. The previous abnormalities all involved lysosomal enzymes but of considerable interest is the observation of an altered electrophoretic mobility for the nonlysosomal enzyme adenosine deaminase-d (ADA-d) in ML II fibroblasts. These results demonstrate that several intracellular electrophoretic enzyme patterns as well as previously reported extracellular enzymes [14] are altered in mucolipidosis II fibroblasts. These findings also provide important enzyme markers for characterizing the mucolipidosis mutation(s). We have additionally observed deficient activity qualita-

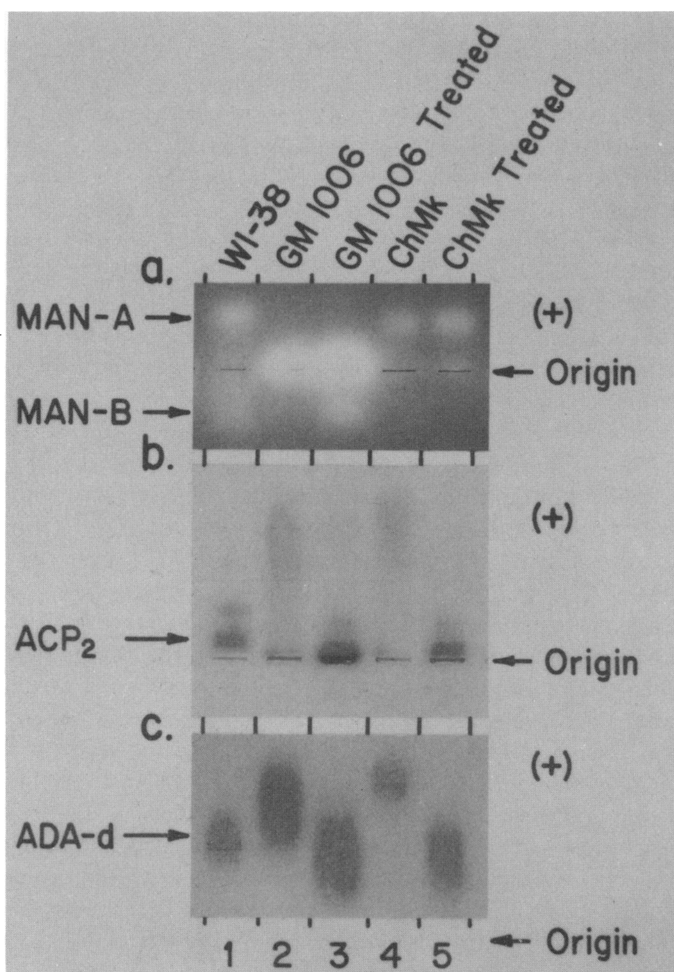

FIG. 6.—Electrophoretic patterns after neuraminidase treatment: *a*, α-mannosidase electrophoretic pattern in control and neuraminidase-treated cell homogenates shown in the Na phosphate pH 6.0 system; *b*, lysosomal acid phosphatase after incubation with neuraminidase. The cathodal acid phosphatase band in CyWm cells (*channels 4–5* and observed in fig. 2) showed much decreased activity after storage but was not electrophoretically altered by neuraminidase; *c*, adenosine deaminase pattern after neuraminidase treatment. Normal enzyme patterns observed in control WI-38 fibroblasts were not altered after neuraminidase treatment.

tively for several lysosomal enzymes in ML II and ML III fibroblasts by starch gel electrophoresis in agreement with previous evidence [8–11]. ML II and ML III fibroblasts have been shown to have similar deficiencies and overlapping levels of lysosomal enzymes [6, 10]; however, only β-hexosaminidase of the enzymes we tested was electrophoretically altered in the single ML III fibroblast line examined. If these electrophoretic differences are confirmed in additional mucolipidosis fibroblast lines, this provides a means of differentiating between ML II and ML III cultured cells and suggests different mutations for these disorders.

Heterogeneity of abnormal enzyme patterns was observed in ML II fibroblast lines which showed different electrophoretic patterns for the enzymes β-hexosaminidase, acid phosphatase, α-mannosidase, and adenosine deaminase-d (figs. 1, 2, 5, and 6). In addition, treatment of cell homogenates with neuraminidase generated the absent α-mannosidase MAN B band only in GM 1006 and TC-75-438 but not in CyWm and ChMk fibroblasts (fig. 6*a*). These data indicate that ML II fibroblasts can be differentiated by their enzyme patterns. Of the ML II fibroblast lines we tested, their electrophoretic characteristics appear to separate them generally into two major groups: one represented by cell lines GM 1006, TC-75-438, and GM 521; and the other group represented by CyWm and ChMk fibroblasts although additional heterogeneity for β-hexosaminidase was observed. Clinical and biochemical evidence has previously indicated genetic variability or heterogeneity within the ML II syndrome. Four cases of mucolipidosis II have been reported which showed variable clinical, pathological, and biochemical features, and which could be grouped into three types showing different severities [4]. The enzyme patterns observed in this report further suggest that different genetic forms of these disorders may be identifiable in cultured skin fibroblasts. Thus far, no clear relationship has been found between the observed electrophoretic patterns and the extent of lysosomal enzyme deficiencies reported for these cell lines. More extensive examinations will be necessary to try to correlate these electrophoretic patterns with enzyme levels and clinical heterogeneity within the ML II syndrome.

It is conceivable that these altered enzyme patterns could be associated with different culture conditions, cell passages, or viability following freezing. However, for several ML II fibroblast lines, stable enzyme patterns were observed after growth on two different culture media, at different passages, and following recovery from frozen storage. In addition, cell lines TC-75-438 and GM 1006, although established from the same patient, were obtained by us after passage in different laboratories and showed identical patterns indicating the stability of the enzyme patterns.

The molecular defect of the human mucolipidoses of several intra- and extracellular lysosomal enzymes has implicated a defect in sialylation, a post-translational modification or an alteration resulting from abnormal exocytosis or pinocytosis [7, 9, 12, 14]. It has been reported that ML II fibroblasts have increased sialic acid content and are deficient for a specific sialidase [15]. The cell membrane of ML II fibroblasts also appears to be implicated since these cells are sensitive to freezing and Sindbis virus passaged in ML II cells acquires defective viral envelopes [13]. Our results showing a cathodal shift in mobility for acid phosphatase and adenosine deaminase following neuraminidase treatment are consistent with a defect in enzyme processing associated with abnormal sialylation.These findings also agree with results showing abnormal sialylation of several extracellular lysosomal enzymes in ML II fibroblast media [14]. The recovery of the lysosomal α-mannosidase MAN B band in GM 1006 and TC-75-438 cells following neuraminidase treatment (fig. 6*a*) suggests that this enzyme is synthesized in ML II fibroblasts but exists in an abnormal form.

All of the lysosomal enzymes examined in ML II fibroblasts showed deficient or altered mobility, while all but one of 36 nonlysosomal enzymes were normal. This suggests that the ML II mutation predominantly affects enzymes associated with the lysosome and not enzymes associated with other organelles or metabolic pathways.

Adenosine deaminase-d was the only nonlysosomal enzyme examined which showed altered electrophoretic properties. This interesting observation suggests that the ADA-d enzyme may share a processing step or similar cellular relationship with lysosomal enzymes.

The heterozygous ML II fibroblast lines GM 80 and GM 81 (parents of GM 521) revealed normal electrophoretic patterns for all affected enzymes. This indicates that the multiple enzyme alterations seen in ML II fibroblasts probably do not represent mutations in the primary structure of individual enzymes and is consistent with this disorder resulting from a single recessive gene defect. In heterozygotes, function of the normal allele is apparently sufficient to prevent abnormal processing. Evidence from man-rodent somatic cell hybridization demonstrates that the enzymes affected by the ML II mutation are not closely linked within the human genome [30], and that in man-mouse cell hybrids formed with ML II fibroblasts, the ML II enzyme deficiencies and electrophoretic abnormalities are complemented by the mouse genome [44]. These results further indicate that the mucolipidosis mutation is a cellular processing or control defect which alters the expression of several unlinked gene products.

Summary

Electrophoretic properties of eight lysosomal hydrolases and 36 nonlysosomal enzymes were investigated in cultured fibroblasts from children with the inherited storage disease mucolipidosis II (ML II); fibroblasts from a child with a related disorder, mucolipidosis III (ML III); and two obligate heterozygous cell lines from parents of a ML II child. Cell homogenates of ML II fibroblast lines showed altered mobilities for lysosomal β-hexosaminidase, acid phosphatase$_2$, and α-mannosidase and deficient activity for the esterase-A_4 and lysosomal α-mannosidase-B electrophoretic phenotypes. Altered mobility was also detected for the nonlysosomal enzyme adenosine deaminase-d. Deficient activities of other lysosomal enzymes were observed as previously reported. In a single ML III fibroblast line, only β-hexosaminidase showed an abnormal electrophoretic pattern suggesting a difference between these cells and ML II fibroblasts. Thirty-five nonlysosomal enzymes associated with other cellular organelles and metabolic pathways were electrophoretically normal in all mucolipidosis cell lines. Heterozygous ML II cells showed normal expression for all enzymes. Two major patterns of altered lysosomal enzymes and adenosine deaminase were demonstrated in ML II cell lines, suggesting that at least two genetic forms of this disorder may exist. Neuraminidase treatment of ML II homogenates converted altered forms of acid phosphatase$_2$ and adenosine deaminase-d and in two ML II lines, recovered the previously undetected lysosomal α-mannosidase band. These results are consistent with the mucolipidosis defect(s) being associated with abnormal post-translational processing of multiple lysosomal enzymes and adenosine deaminase-d.

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