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α-L-Iduronidase in normal and mucopolysaccharidosis-type-I human skin fibroblasts

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α-L-Iduronidase synthesis and maturation were analysed in fibroblasts from normal controls and from α-L-iduronidase-deficient mucopolysaccharidosis-type-I (MPS-I) patients. Fibroblasts were radiolabelled with [^{3}H]leucine and α-L-iduronidase was isolated from cell lysates or culture medium by monoclonal-antibody affinity chromatography. Pulse–chase labelling of normal control fibroblasts showed that α-L-iduronidase was synthesized as an 81 kDa precursor and processed within 24 h via intermediates of 76 kDa and 70 kDa to a 69 kDa species. The incorporation of radiolabel into α-L-iduronidase in fibroblasts from three of four MPS-I patients was at levels that were either very low or undetectable. Fibroblasts from one MPS-I patient, however, exhibited levels of incorporation of radiolabelled amino acid into α-L-iduronidase similar to those shown by normal control fibroblasts, despite having undetectable α-L-iduronidase enzyme activity. The maturation of α-L-iduronidase in fibroblasts from this patient was delayed compared with normal controls and showed accumulation of the 76 kDa intermediate, as well as the major 69 kDa, form of the enzyme.

INTRODUCTION

Mucopolysaccharidosis type I (MPS-I) results from a deficiency of the enzyme α-L-iduronidase (α-L-iduronide iduronohydrolase, EC 3.2.1.76) and leads to excessive storage and excretion of the glycosaminoglycans heparan sulphate and dermatan sulphate. MPS-I patients can present with a wide range of clinical symptoms, with Hurler and Scheie syndromes representing opposite extremes of the clinical spectrum. The clinically severe form (Hurler syndrome) is characterized by severe skeletal deformities, stiff joints, coarse hirsute facies, corneal opacity, mental retardation and early death. By contrast the clinically mild affected individuals (Scheie syndrome) may have normal intellect and lifespan, with only mild skeletal deformities, stiff joints and corneal opacity (McKusick & Neufeld, 1983; Neufeld & Muenzer, 1989). It has been possible to predict the phenotype of the MPS-I patients from the kinetics of residual α-L-iduronidase activity (Hopwood & Muller, 1979, 1982) only at the clinical extremes. This method did not discriminate intermediate phenotypes.

α-L-Iduronidase has been purified from human liver, lung and kidney using monoclonal-antibody immunoaffinity chromatography and shown to contain seven polypeptides which may be derived by proteolytic or carbohydrate processing from a single α-L-iduronidase gene product (Clements *et al.*, 1989). Maturation of α-L-iduronidase in cultured human skin fibroblasts has previously been shown by immunoprecipitation of radiolabelled enzyme to be synthesized as a 75 kDa protein and subsequently trimmed to 72 kDa and 66 kDa species (Myerowitz & Neufeld, 1981). The maturation of α-L-iduronidase described was extremely slow compared with other lysosomal hydrolases (Myerowitz & Neufeld, 1981). Furthermore, even after a chase of 4–5 days, the α-L-iduronidase species isolated from fibroblasts incubated with radiolabelled leucine were of higher molecular mass than some of those extracted from kidney, lung or liver and purified by immunoaffinity chromatography by Clements *et al.* (1989). Another report (Schuchman & Desnick, 1988) described α-L-iduronidase protein levels in fibroblasts from 15 MPS-I patients of 38–100% of that seen in normal control fibroblasts and included fibroblast lines in which Myerowitz & Neufeld (1981) were unable to detect α-L-iduronidase synthesis. The present paper describes the maturation of α-L-iduronidase in fibroblasts from normal controls and from MPS-I patients using monoclonal-antibody immunopurification with the aim of more clearly defining the steps in α-L-iduronidase maturation and investigating the relationship between α-L-iduronidase synthesis or processing and the clinical phenotype of MPS-I patients.

MATERIALS AND METHODS

Materials

L-[3,4,5-^{3}H]Leucine (specific radioactivity 144–153 Ci/mmol), Hyperfilm-MP and a mixture of [^{14}C]methylated molecular-mass standards [myosin (200 kDa), phosphorylase *b* (92.5 kDa), BSA (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa) and lysozyme (14.3 kDa)] were purchased from Amersham International. Affi-Gel 10 was purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.) and used according to the manufacturers' recommendations. Dulbecco's modified phosphate-buffered saline was purchased from Commonwealth Serum Laboratories (Melbourne, Vic., Australia). Nonidet P40, Triton X-100 and proteinase inhibitors (Taylor *et al.*, 1990) were purchased from Sigma (St. Louis, MO, U.S.A.). DNAase and dithioerythritol were purchased from Boehringer-Mannheim (Sydney, N.S.W., Australia). Eagle's modified minimum essential medium, penicillin, streptomycin and glutamine were purchased from Flow Laboratories (Sydney, N.S.W., Australia) and foetal-calf serum was from Gibco (Glen Waverley, Vic., Australia).

Cell culture

Human diploid fibroblasts were established from skin biopsies submitted to this hospital for diagnosis (Hopwood *et al.*, 1982). Cell lines were maintained at 37 °C in air/CO_2 (19:1) in Eagle's modified essential medium, supplemented with antibiotics, non-essential amino acids, and 10% (v/v) foetal-calf serum as described previously by Taylor *et al.* (1990). Fibroblast cultures

Abbreviations used: MPS I and VI, mucopolysaccharidosis type I and type VI; α-L-iduronidase, α-L-iduronide iduronohydrolase.

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Table 1. Identifying data for MPS-I patients

Patient	Age at diagnosis	Relative clinical phenotype	Urinary sulphates (g/mol of creatinine)* DS	HS	CS	Residual α-L-iduronidase activity (pmol/min per mg of fibroblast protein)†	K_m (μM)	$V_{max.}$ (pmol/min per mg of fibroblast protein)†
728	1.5 years	Severe	100	16	27	2.0	227	1.9
GM 1898	6 years	Intermediate	na§	na	na	0.3	8.4	1.3
2662	18 months	Mild	3.7	1.8	5	6.3	19.6	1.4
2827	2 years	Severe	na	na	na	nd	nd	nd
Controls	2 months–2 years	Normal	< 2	< 16	< 22			
	2 months–6 years		< 1	< 8	< 11			
	> 6 years		< 0.7‡	< 6	< 8			
	All controls					96–900	18–55	488–800

* Urinary dermatan sulphate (DS), heparan sulphate (HS) and chondroitin sulphate (CS) were determined by the method of Hopwood & Harrison (1982).

† Residual α-L-iduronidase activity was determined using a radioactive disaccharide derived from heparin by the method of Hopwood & Muller (1979, 1982).

‡ Control urinary glycosaminoglycan ranges ($n > 1000$) determined in the Department of Chemical Pathology, Adelaide Children's Hospital.

§ Abbreviations: na, not available; *n*, number of individuals; nd, not determinable.

were not used beyond 15 passages. Four MPS-I-patient fibroblasts were selected from a panel of 23 for study on the basis of patients clinical severity, level of α-L-iduronidase protein (L. Ashton & D. Brooks, unpublished work) and residual enzyme kinetics (P. Clements & V. Muller, unpublished work). The MPS-I cultured skin fibroblast cell lines 2662, 728 and 2827 were deficient in α-L-iduronidase activity and derived from patients shown to have dermatan sulphate- and heparan sulphate-uria (Hopwood & Muller, 1982; Hopwood *et al.*, 1982). The MPS-I cell line GM 1898, used by both Myerowitz & Neufeld (1981) and Schuchman & Desnick (1988), was obtained from the Human Genetic Mutant Cell Repository (Camden, NJ, U.S.A.). The clinical and biochemical analysis of MPS-I patients is described in Table 1.

Incorporation of radiolabelled leucine into α-L-iduronidase

At confluency, cells were labelled for specified time periods in medium containing [^{3}H]leucine (0.4 mCi/75 cm^2 flask) as described by Taylor *et al.* (1990). Cells were either harvested immediately or chased for the times specified in 5.0 ml of cell-culture medium containing 10% (v/v) foetal-calf serum and 53 mM-leucine. In some instances, cells were cultured in medium supplemented with 10 mM-NH_4Cl.

$(NH_4)_2SO_4$ was added to the culture medium (5.0 ml), and precipitated material was isolated and dialysed as described by Hasilik & Neufeld (1980). Cultured fibroblasts were harvested by trypsin digestion as described by Conary *et al.* (1988). The cells were then suspended in 0.5 ml of buffer A, which contained 10 mM-Tris/HCl, pH 7.4, with 1% (v/v) Triton X-100, sodium deoxycholate (0.5 mg/ml), SDS (0.2 mg/ml), BSA (5 mg/ml), 1 mM-$MgCl_2$, proteinase inhibitors (Taylor *et al.*, 1990), DNAase (0.020 mg/ml) and 0.5 M-NaCl. Cell suspensions were then lysed by freeze-thawing six times, and the nuclear material was removed by precipitation with protamine sulphate as described by Hasilik & Neufeld (1980). A solution containing a mixture of proteinase inhibitors was added to cell and medium supernatants as described by Taylor *et al.* (1990), and supernatants used directly for immunoadsorption.

Monoclonal antibody

Monoclonal antibody designated 'Id1A' was raised against α-L-iduronidase and characterized as specific for this lysosomal enzyme (Clements *et al.*, 1985, 1989). Id1A (2 mg) was covalently linked to Affi-Gel 10 slurry (1.0 ml) and will hereafter be referred to as 'Id1A–Affi-Gel'. Affi-Gel 10 was blocked by incubation in 0.02 M-Tris/HCl buffer, pH 7.0, and was loaded and eluted as for Id1A–Affi-Gel as a control to monitor non-specific binding of radiolabelled polypeptides to the Affi-Gel 10 matrix, and will hereafter be referred to as 'Tris-blocked Affi-Gel'. Monoclonal antibody designated '4-S 4.1', raised against *N*-acetylgalactosamine 4-sulphatase, was characterized as specific for this lysosomal enzyme (Gibson *et al.*, 1987). The monoclonal antibody was linked to Affi-Gel 10 slurry as described previously (Gibson *et al.*, 1987) and referred to as '4-S 4.1–Affi-Gel'. This was used as an internal control to monitor incorporation of [^{3}H]leucine into a non-affected lysosomal enzyme.

Immunoadsorption

The monoclonal-antibody immunopurification protocol described by Clements *et al.* (1989) provided a method of isolating α-L-iduronidase. This protocol was modified to purify radiolabelled polypeptides from human skin fibroblasts using a similar technique to that described in Taylor *et al.* (1990). Purified carrier α-L-iduronidase isolated from human liver (Clements *et al.*, 1989) was added to cell extracts and cell-culture medium at a minimum concentration of 0.5 μg/ml before application to a 1.0 ml column of Id1A–Affi-Gel. The columns were incubated overnight at 4 °C and then subjected to an extensive washing regime as described previously by Taylor *et al.* (1990), but modified as outlined below with successive washes of 1.0 ml of: wash 1, buffer A; wash 2, buffer A without proteinase inhibitors, $MgCl_2$ and DNAase; wash 3, buffer A, substituting 2 M-KCl for SDS; wash 4, 0.6 M-NaCl/0.1% (w/v) SDS/0.05% (v/v) Nonidet P40/10 mM-Tris/HCl buffer, pH 8.6; wash 5, repeated washing with 14 mM-NaCl/1 mM-Tris/HCl buffer, pH 7.4, until radioactivity approached background levels (generally 80–100 1.0 ml volumes were required).

Radiolabelled and carrier enzyme were then eluted from the immunoaffinity columns with 2.0 M-NaCl/0.05 M-sodium citrate buffer, pH 4.0, as described previously (Taylor *et al.*, 1990), but with the modification that the collecting tubes contained 0.1 mM-dithioerythritol and 0.1% (w/v) Triton X-100 to prevent losses of enzyme activity during the immunopurification, and columns

were re-equilibrated with 0.5 M-NaCl/0.02 M-Tris/HCl buffer, pH 7.4.

The purified radiolabelled α-L-iduronidase and carrier α-L-iduronidase were then precipitated with trichloroacetic acid and subjected to SDS/PAGE on 7.5% (w/v) acrylamide (Laemmli, 1970) and fluorography as described by Bonner & Laskey (1974) and Laskey & Mills (1975). Radioactivity in specific bands was quantified from the radioactivity applied, together with determination of relative band intensity. Radioactivity was measured using an LKB Wallac 1216 Rackbeta II liquid-scintillation counter. Counting efficiency was 65% for [^{3}H]leucine.

Estimation of α-L-iduronidase activity

α-L-Iduronidase activities recovered from the various steps of the immunopurification process were determined with 4-methylumbelliferyl-α-L-iduronide substrate as described by Clements *et al.* (1985). α-L-Iduronidase activities (K_m and $V_{max.}$) in cultured fibroblasts documented in Table 1 were determined using a radiolabelled disaccharide substrate, α-L-iduronosyl-(1→4)2,5-anhydro-D-[1-^{3}H]mannitol 6-sulphate as described by Hopwood & Muller (1982).

RESULTS

Recovery of α-L-iduronidase activity after immunoaffinity chromatography

By using the monoclonal-antibody immunopurification procedure, approx. 89–100% of the carrier α-L-iduronidase activity bound to the Id1A–Affi-Gel, 0–11% of carrier applied was recovered in the flow-through fraction, and 25–30% was eluted during the wash procedure. The recovery of carrier α-L-iduronidase activity eluted with 2.0 M-NaCl/0.05 M-sodium citrate buffer, pH 4.0, ranged from 61 to 73% and of this 90–100% was precipitable with sodium deoxycholate/trichloroacetic acid. The rigorous washing procedures using wash solutions 1–5 (above), particularly the use of large volumes (approx. 100 ml) of 14 mM-NaCl/1 mM-Tris/HCl buffer, pH 7.4, at step 5, were essential to remove radioactive contaminants and decrease radioactivity to acceptable background levels (< 100 c.p.m. of [^{3}H]leucine) before elution of radiolabelled α-L-iduronidase.

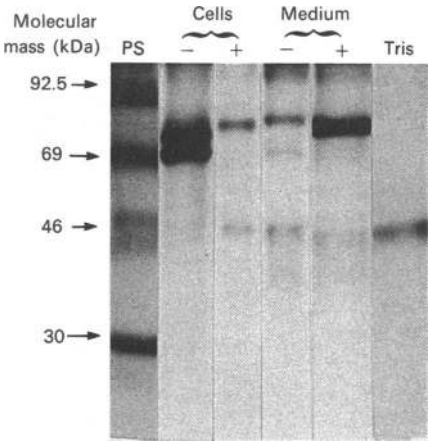

Fig. 1. SDS/PAGE and fluorography of α-L-iduronidase immunopurified from normal fibroblasts

Fibroblasts (one flask/lane) were incubated for 24 h in the presence of [^{3}H]leucine, with (+) or without (−) 10 mM-NH_4Cl, and α-L-iduronidase was isolated from the cells and cell-culture medium by immunoadsorption. 'Cells' and 'Medium' refer to α-L-iduronidase isolated from cell lysates and cell-culture medium respectively. 'Tris' refers to radiolabelled cell lysates bound to Tris-blocked Affi-Gel. The molecular masses of the protein standards (PS; first lane) are indicated.

Incorporation of [^{3}H]leucine into α-L-iduronidase by control fibroblasts

Fibroblast cell lines radiolabelled with [^{3}H]leucine for 24 h were used to prepare cell lysates from which α-L-iduronidase was purified by Id1A–Affi-Gel immunochromatography. Eluted α-L-iduronidase migrated as two major polypeptides of molecular mass 76±3.0 kDa and 69±3.0 kDa (average±S.D. for six different fluorograms) (Fig. 1) under both reducing and non-reducing SDS/PAGE conditions (Fig. 2*b*). The cells cultured in the presence of 10 mM-NH_4Cl incorporated [^{3}H]leucine into intracellular α-L-iduronidase, which migrated as a polypeptide of molecular mass 81±3.0 kDa (Fig. 1). Radiolabelled α-L-iduronidase in cell-culture medium migrated as two polypeptides of apparent molecular mass 82±3.0 and 69±3.0 kDa (Fig. 1). Addition of 10 mM-NH_4Cl to the culture produced an increase in radiolabelled α-L-iduronidase in the culture medium that migrated as a single polypeptide of molecular mass 81±3.0 kDa (Fig. 1). When lysates of radiolabelled cells or cell culture medium were applied to Tris-blocked Affi-Gel, only high-molecular-mass proteins in excess of 200 kDa bound on a regular basis, although occasionally bands at 44 kDa could be observed. These are believed to be unrelated to α-L-iduronidase.

Maturation of α-L-iduronidase in control fibroblasts

To examine the normal maturation sequence of α-L-iduronidase, fibroblasts were pulse-labelled for 3 h with [^{3}H]leucine and chased for up to 2 weeks. After a 3 h pulse, an 81 kDa polypeptide was the predominant radiolabelled intracellular species, whereas the medium contained a polypeptide of molecular mass 82 kDa (Fig. 2). During the 5 h chase, the enzyme matured intracellularly to two polypeptides of 76 kDa and 70 kDa, whereas 81 kDa and 70 kDa polypeptides were secreted into the medium. After a 24 h chase, only the 69 kDa polypeptide could be detected intracellularly (Fig. 2*a*) and this persisted after a 3-day (Fig. 3*b*) and 2-week chase (results not shown). Trace levels of the 69 kDa polypeptide could be detected in the medium after the 24 h chase, but no radiolabelled polypeptides were detected in the medium after a 5-day chase. All α-L-iduronidase polypeptides detected in cell lysates or culture medium were unaffected by reduction with dithioerythritol (Fig. 2*b*).

Incorporation of [^{3}H]leucine into α-L-iduronidase in MPS-I fibroblasts

Fibroblasts from three patients (one clinically severe, one intermediate and one mild; Table 1) showed extremely low levels of incorporation of [^{3}H]leucine into α-L-iduronidase. Incorporation of leucine into α-L-iduronidase by fibroblasts from two MPS-I patients, 728 and GM1898, was not detectable, whereas fibroblasts from patient 2662 showed very low levels of incorporation of radiolabel into α-L-iduronidase. This was detectable in both the cells and culture medium, but as shown in Fig. 3, frequently too low to be confidently distinguished from background.

Fibroblasts from patient 2827 showed incorporation of radiolabel into α-L-iduronidase at levels similar to that seen in normal control fibroblasts (Fig. 3*b*). The molecular species of α-L-iduronidase isolated from fibroblasts from patient 2827 after incubation with [^{3}H]leucine was similar to that seen with normal

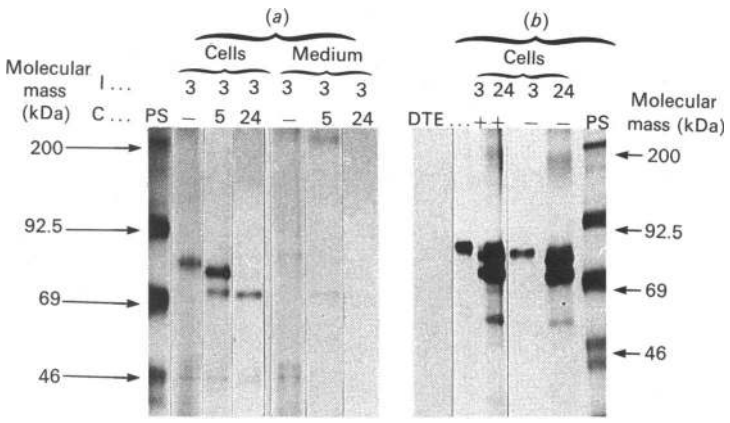

Fig. 2. SDS/PAGE and fluorography of α-L-iduronidase isolated from pulse–chase-radiolabelled fibroblasts

Normal control fibroblasts (one flask/lane) were incubated (I) for 3 or 24 h in the presence of [^{3}H]leucine and harvested after chase periods (C) of 5 and 24 h as indicated. α-L-Iduronidase was isolated from (*a*) the cell lysates or culture medium by immunopurification on Id1A–Affi-Gel, or (*b*) the cell lysates purified by Id1A–Affi-Gel chromatography run under reducing [dithioerythritol (DTE); +] or non-reducing (–) conditions. The molecular masses of protein standards (PS, first lane in *a*; last lane in *b*) are indicated.

control fibroblasts; however, the maturation of α-L-iduronidase was distinctly delayed (Fig. 3*b*). After a 22 h pulse, fibroblasts from patient 2827 incorporated radiolabel into a 76 kDa polypeptide (Fig. 3*b*); this was also seen with normal control fibroblasts after a 3 h pulse and 5 h chase (Fig. 2*b*). After a subsequent 3 day chase, incorporation of isotope into the 76 kDa polypeptide was still observed in addition to incorporation into the 69 kDa polypeptide (Fig. 3*b*). Normal control fibroblasts showed incorporation only into the 69 kDa polypeptide after the 3-day chase. In the presence of NH_4Cl, fibroblasts from patient 2827 incorporated radiolabel into an 81 kDa polypeptide in the culture medium which was indistinguishable in molecular mass and at similar levels to that seen with normal control medium.

MPS-I fibroblast cell lysates were subjected to 4-S 4.1–Affi-Gel chromatography (Taylor *et al.*, 1990) to investigate the incorporation of radiolabel into a non-affected lysosomal enzyme. The incorporation of radiolabel into *N*-acetylgalactosamine-4-sulphatase was demonstrated in MPS-I fibroblasts at levels indistinguishable from that seen with normal control fibroblasts (Fig. 3*c*).

DISCUSSION

The incorporation of radiolabel into α-L-iduronidase and its subsequent maturation is described for normal and MPS-I fibroblasts using an efficient and sensitive monoclonal-antibody immunopurification scheme. Pulse–chase radiolabelling showed that, by 24 h in normal fibroblasts, the enzyme is synthesized as a precursor with a molecular mass of 81 kDa which matures through intermediates of 76 and 70 kDa to a 69 kDa polypeptide. The 69 kDa form appears stable for at least 5 days in culture. Frequent, but inconsistent, contamination of radiolabelled immunopurified α-L-iduronidase with proteins of 44 kDa, and occasionally 59 kDa (Fig. 3*b*), prevented confident assessment of α-L-iduronidase maturation to species in this molecular-mass range. However, if present, low-molecular-mass forms of α-L-iduronidase were formed very slowly and at low levels after the chase periods studied. The protein of molecular mass 44 kDa was also detected on Tris-blocked Affi-Gel and could be eliminated if more stringent wash conditions, such as those employed previously (Taylor *et al.*, 1990), were used. The use of

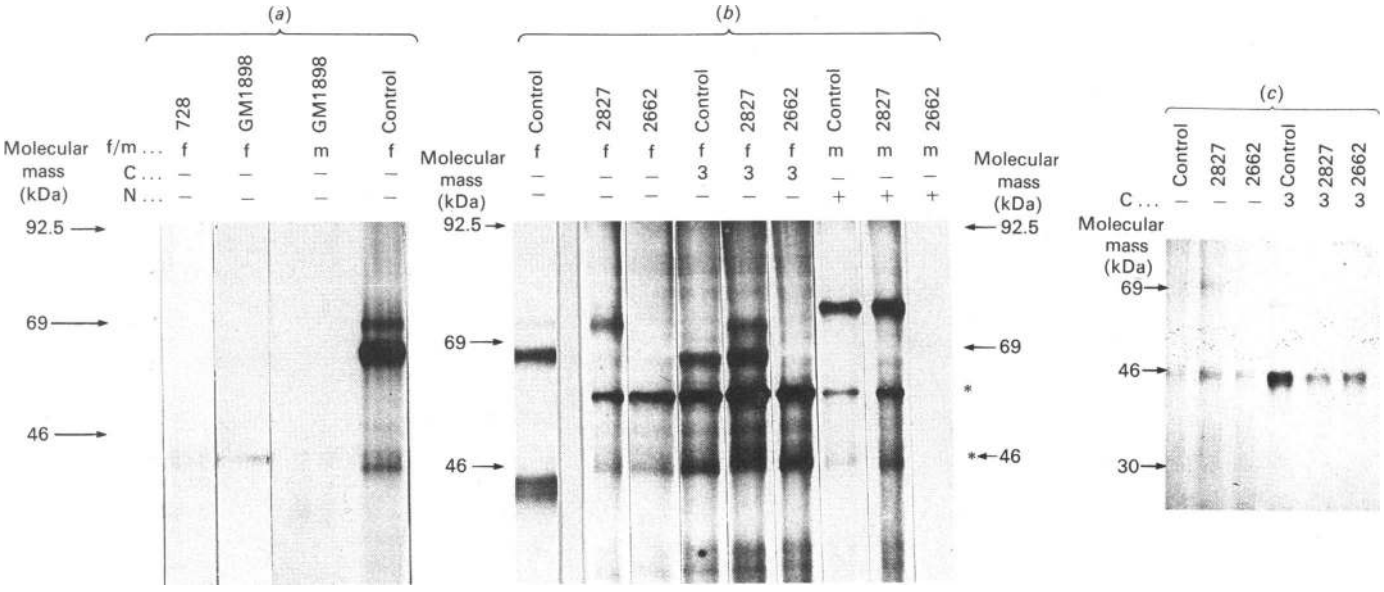

Fig. 3. SDS/PAGE and fluorography of α-L-iduronidase and *N*-acetylgalactosamine-4-sulphatase isolated from fibroblasts from normal control and MPS-I patients

Fibroblasts were incubated for 22 h in the presence of [^{3}H]leucine and harvested immediately (–) or after a chase (C) of 3 days (3) as indicated. *N*-Acetylgalactosamine-4-sulphatase from cells and cell-culture medium was immunopurified on 4-S 4.1–Affi-Gel and α-L-iduronidase immunopurified from the radiolabelled proteins in the flow-through fractions by subsequent application to Id1A–Affi-Gel. (*a*) and (*b*), Immunopurified α-L-iduronidase from fibroblast lysates (f) and from culture medium (m) after incubation with (+) or without (–) 10 mM-NH_4Cl (N); (*c*) immunopurified *N*-acetylgalactosamine-4-sulphatase from fibroblast lysates. Samples in (*b*) were subjected to modified wash buffer conditions of 0.5% Triton X-100 and 0.1% SDS. The migration positions of molecular-mass standards are marked on each individual SDS/polyacrylamide gel. Bands indicated by an asterisk (*) are believed not to be related to α-L-iduronidase.

the stringent wash conditions, however, damaged the Id1A–immunoaffinity columns, leading to reduced recoveries of α-L-iduronidase activity. In an attempt to minimize losses of α-L-iduronidase activity, the percentages of Triton X-100 and SDS in the wash buffers were reduced to 0.5% and 0.1% respectively. This resulted in the appearance of an additional 59 kDa polypeptide and a more intense polypeptide at 44 kDa (Fig. 3*b*).

A small proportion of α-L-iduronidase synthesized after 3 h was secreted into the culture medium. This had a molecular mass of 82 kDa, a value slightly higher than that of the protein found in fibroblasts at this time and may reflect reduced processing in the secretory pathway. Persistence of the 82 kDa species in the culture medium for somewhat longer than the 81 kDa species may reflect a protection of the secreted α-L-iduronidase from further maturation, owing to an absence of processing enzymes or the altered carbohydrate structure.

Myerowitz & Neufeld (1981) found a similar sequence of α-L-iduronidase maturation in fibroblasts in culture, although the molecular masses attributed to the α-L-iduronidase species differed slightly (75 kDa precursor, 72 kDa intermediate, 66 kDa mature intracellular species, and a 76 kDa medium species). These differences in molecular mass may be due to variation between analytical techniques. However, the molecular-mass changes involved in the processing of α-L-iduronidase from precursor to intermediate and intermediate to mature species and the processing times are directly comparable. α-L-Iduronidase isolated from human kidney, liver or lung was previously shown to contain proteins of molecular masses 74, 65, 60, 49, 44, 18 and 13 kDa (Clements *et al.*, 1989). The 65 kDa species was often the major protein species purified, and may reflect minor proteolytic or carbohydrate modification of the mature 69 kDa radiolabelled species found in fibroblasts. The presence of a high proportion of polypeptides of 60 kDa and less in α-L-iduronidase purified from tissues may reflect a continued slow proteolytic maturation, a maturation peculiar to the tissues and absent from, or exceedingly slow in, cultured fibroblasts, or proteolysis occurring during the purification procedure. The α-L-iduronidase identity of the polypeptides purified from tissues has been recently confirmed by immunoblotting and by *N*-terminal amino-acid-sequence analysis and comparison with gene sequences (Clements *et al.*, 1989; H. Scott, C. P. Morris & P. Clements, unpublished work). The presence of enzyme species (74 kDa) similar in mass to the prominent, though transient, intermediate form (76 kDa) observed in radiolabelled fibroblasts, together with evidence of lower-molecular-mass (less than 60 kDa) and apparently more highly processed species, may indicate either differential organellar or cellular compartmentation of α-L-iduronidase, particularly in tissues such as the lung, where the 74 kDa species was found in high concentration. The 'immaturity' of the 74 kDa species isolated from the lung was further suggested by its rapid mannose 6-phosphate-inhibitable uptake by α-L-iduronidase-deficient fibroblasts in culture (Clements *et al.*, 1989).

Three of the four fibroblast cultures obtained from MPS-I patients incorporated radiolabel into α-L-iduronidase at very low or undetectable levels. This is consistent with the observation of Myerowitz & Neufeld (1981), but in marked contrast with the immunoquantification data of Schuchman & Desnick (1988) and suggests, particularly since the same fibroblast line (GM1898) was used in all three studies, that the antibodies used by Schuchman & Desnick (1988) may not be specific for α-L-iduronidase. Fibroblasts from the fourth MPS-I patient (2827) showed levels of incorporation of [^{3}H]leucine into α-L-iduronidase similar to those of normal controls, but with a considerable delay in maturation. The delayed maturation did not appear to cause significant accumulation of the 81 kDa precursor, but persistent accumulation of the 76 kDa species. This may reflect a specific effect of the mutation on the structure of the enzyme such that the later processing step was more disrupted than the earlier, or, as suggested above, that the 76 and 69 kDa species are present in different subcellular compartments and traffic between these compartments has been impaired.

All four MPS-I-patient fibroblasts incorporated [^{3}H]leucine into a non-affected lysosomal enzyme, *N*-acetylgalactosamine-4-sulphatase, in a manner indistinguishable from normal controls. This demonstrates that the disruption of synthesis or transport is due to a mutation in the α-L-iduronidase gene and not to such non-specific events as the accumulation of substrate.

The low levels of incorporation of [^{3}H]leucine into α-L-iduronidase in MPS-I fibroblasts 728, GM1898 and 2662, together with the findings that all fibroblasts from 23 MPS-I patients with a wide range of clinical symptoms were shown by immunoquantification to contain little or no α-L-iduronidase protein (L. Ashton & D. Brooks, unpublished work), supports a proposal that many patients have MPS-I that results from gene defects which affect the protein structure of α-L-iduronidase. This may consequently impede intracellular transport and result in the rapid degradation of α-L-iduronidase (Goldberg & St. John, 1976; Rose & Doms, 1988). The high level of radiolabel incorporated into α-L-iduronidase in fibroblasts from patient 2827 has also been supported by immunoquantification, which showed accumulation of α-L-iduronidase protein at levels 7-fold higher than seen in normal control fibroblasts and is consistent with delayed turnover of α-L-iduronidase in these fibroblasts. Thus the MPS-I patient 2827 is unusual, as these fibroblasts accumulate high levels of inactive enzyme.

The data presented support the slow processing of α-L-iduronidase in human skin fibroblasts and suggest that compartmentation of α-L-iduronidase may differ from that of other lysosomal hydrolases. Further investigation of α-L-iduronidase maturation in fibroblasts from the MPS-I patient (2827) showing high levels of mutant α-L-iduronidase accumulation may help to delineate the transport and maturation of this lysosomal enzyme.

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