Processing of iduronate 2-sulphatase in human fibroblasts

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Iduronate 2-sulphatase (IDS) is a lysosomal enzyme involved in degradation of dermatan sulphate and heparan sulphate. Antigenic material was obtained either by purification of placental IDS (A and B forms) or by expression of three different fusion peptides in *Escherichia coli* allowing the production of five specific antibodies. Pulse-chase-labelling experiments in over-expressing fibroblasts showed poor IDS processing but large amounts of precursors were secreted into the medium. The endocytosis of the ³⁵S- or ³³P-labelled precursors by deleted fibroblasts together with glycosylation studies and proteolysis

inhibition by leupeptin allowed better elucidation of IDS maturation. The initial 73–78 kDa form is converted into a phosphorylated 90 kDa precursor after modification of its oligosaccharide chains in the Golgi apparatus. This precursor is processed by proteolytic cleavage through various intermediates to a major 55 kDa intermediate, with the release of an 18 kDa polypeptide. Further proteolytic cleavage by a thiol protease gives the 45 kDa mature form containing hybrid and complex-type oligosaccharide chains.

INTRODUCTION

Iduronate 2-sulphatase (IDS; EC 3.1.6.13; L-iduronate-2-sulphate 2-sulphohydrolase) is a lysosomal enzyme which hydrolyses sulphate ester bonds of iduronate 2-sulphate in dermatan sulphate, heparan sulphate and heparin. IDS deficiency is responsible for mucopolysaccharidosis type II or Hunter syndrome, a rare X-linked lysosomal storage disease [1]. Human IDS has been purified from human liver [2], and a cDNA clone has been isolated that contains the complete coding region of the enzyme [3]. The organization of the IDS gene has recently been reported [4,5]. The present paper reports IDS polyclonal antibody production and maturation studies of IDS in human fibroblasts.

MATERIALS AND METHODS

Materials

Tritiated disaccharide substrate, *O*-(α -L-idopyranosyluronic acid 2-sulphate)-(1 $\rightarrow$ 4)-2,5-anhydro-D-[³H]mannitol 6-sulphate (450 mCi/mmol) and Cellex E were purchased from the Hospital for Sick Children (Toronto, Ont., Canada). Imedex (Chaponost, France) provided 5 kg of a 29% alcoholic precipitate from human placentas. An IDS cDNA clone (pc2S15) containing 90% of the coding sequence and a full-length IDS cDNA clone (pB2Sc17) were kindly provided by J. J. Hopwood (Adelaide Children's Hospital, North Adelaide, South Australia, 5006 Australia). Concanavalin A-Sepharose, phenyl-Sepharose CL-4B, Superdex 75 HR 10/30, pGEX2T vector, isopropyl β -D-thiogalactoside (IPTG), U.S.E. mutagenesis kit, Protein A-Sepharose CL-4B and FPLC system were from Pharmacia. Ham's F10 medium, PMSF, PBS and leupeptin were from Sigma. Reagents used for SDS/PAGE and the Gene Pulser electroporation unit were from Bio-Rad. Carrier-free [³³P]P_i, Western-blotting analysis system enhanced chemiluminescence (ECL), horseradish peroxidase-labelled rabbit antibody, Amplify and Rainbow molecular-mass markers were from Amersham. Tran³⁵S-label and Eagle's modified minimum essential medium deficient in methionine and cysteine or phosphate were from ICN. Endoglycosidase H (endo H) and *N*-glycosidase F were from Boehringer.

Enzyme assay [6,7]

Samples were dialysed overnight at 4 °C against deionized water, and then 25 μ l containing about 50 μ g of protein was incubated with 15 μ l (0.075 nmol) of tritiated disulphated disaccharide substrate in 0.33 M sodium acetate buffer (pH 4.5) at 37 °C for an appropriate period of time. Reaction was stopped with 1 ml of 1 mM Na₂HPO₄. Reaction products were eluted from 0.6 ml columns of anion-exchange resin (Cellex E) with 0.07 M sodium formate and counted in a liquid-scintillation counter. Protein concentrations were measured by the method of Lowry et al. [8].

IDS purification

IDS was purified from human placenta by the procedure described by Bielicki et al. [2] with the following modifications. First 5 kg of a 29% alcoholic precipitate from about 2000 frozen placentas was redissolved in 200 litres of 15 mM sodium dimethylglutarate buffer (pH 6.0) containing 0.5 M NaCl and 0.1 mM dithioerythritol. The supernatant was concentrated to a final volume of 14 litres and further purified by the six-step column procedure previously described [2], except that the final gel-filtration step was performed on a Superdex 75 column, using the FPLC system.

IDS from 12 mg of fibroblast proteins was semi-purified by affinity chromatography (Concanavalin A-Sepharose) and hydrophobic chromatography (phenyl-Sepharose CL-4B) as described for the purification from placenta, and used for immunoblotting studies.

Expression of IDS peptides in *Escherichia coli*

cDNA amplification

Three different fragments were obtained by PCR from IDS cDNA (pc2S15) using the following primer sets containing the *Eco*RI restriction site. Fragment 1: from nt 222 to 929.

I1: 5'-GCCAGAATTTCCACAGATGCTCTGAACG-3'

I2: 5'-TGCCGAATTTCCCATCCAGGGGTTGTAGG-3'

Fragment 2: from nt 846 to 1538.

I3: 5'-TTTCGAATTCTGTATCCCTTGGAGAACATC-3'

I4: 5'-CACTGAATTCTGTCTGAAGGCCGGGGATAC-3'

Fragment 3: from nt 222 to 1538 using I1 and I4 primers.

Cloning

PCR products were inserted in-frame from the glutathione S-transferase (GST) gene in the *Eco*RI site of the bacterial expression vector pGEX2T [9]. Restriction-enzyme digestions, ligations and transformations were performed using standard procedures [10].

Expression and purification of recombinant fusion proteins

E. coli clones were grown overnight at 37 °C, then diluted in fresh medium (500 ml) and grown for 1 h at 37 °C before the addition of IPTG to a final concentration of 1 mM. After 2 h at 37 °C, the bacteria were harvested by centrifugation (10000 *g* for 15 min at 4 °C). Cells were lysed on ice by mild sonication in lysis buffer [PBS containing 1% (v/v) Triton X-100, 1 mM PMSF and 10 mM EDTA]. After centrifugation (12000 *g* for 5 min at 4 °C), pellets and supernatants were analysed by SDS/PAGE and fusion proteins were found in the insoluble fraction. The pellets were rewashed with lysis buffer and subjected to SDS/PAGE; fusion protein bands were cut out of the gels. The gel slices were mashed and proteins were eluted overnight in PBS with constant agitation. Polyacrylamide was removed by centrifugation.

SDS/PAGE

This was performed under reducing conditions [5% (v/v) 2-mercaptoethanol] on slab gels as described by Laemmli [11].

Rabbit immunization

The purified IDS (A and B forms), the three fusion proteins and the GST alone were used for immunization. Proteins were emulsified in Freund's complete adjuvant and injected subcutaneously (50 µg for purified IDS or 200 µg for fusion proteins) into rabbits. Three booster injections (20 µg for purified IDS or 100 µg for fusion proteins in Freund's incomplete adjuvant) were performed at 2-week intervals. IgG was purified from immune and non-immune rabbit sera on Protein A-Sepharose columns. The final IgG concentration was 6–8 mg/ml.

Cell culture and transfection procedure

Human skin fibroblasts were grown in Ham's F10 medium containing 12% (v/v) fetal calf serum and supplemented with glutamine (1 mM) and antibiotics. Fibroblasts from a patient with a complete deletion of the IDS gene [12] (deleted fibroblasts) were transfected with a eukaryotic expression vector [13] containing the full-length human IDS cDNA (pCMV-IDS). DNA was introduced into fibroblasts by electroporation using the Gene Pulser electroporation unit [14]. Transfection efficiency was measured with the same vector containing the bacterial β -galactosidase gene. Conditions used included: 18×10^6 cells, 150 µg of plasmid DNA (pCMV-IDS) and a voltage pulse of 260 V (capacitance 960 µF). These conditions allowed transfection of about 60% of the cells. A transfection with the same vector containing the IDS cDNA in antisense orientation was performed as a negative control. After electroporation, the cells were placed in 75 cm² flasks.

Construction of the mutant IDS cDNA (mutation R443X)

A single C1327 to T nucleotide transition on the plasmid pCMV-IDS was made by oligonucleotide-directed mutagenesis using the U.S.E. mutagenesis kit. This mutation introduces a termination codon instead of an arginine at position 443 of the IDS polypeptide [15].

Immunoblotting

Fibroblast proteins were separated by SDS/PAGE and blotted on to nitrocellulose filters by transverse electrophoresis at 50 V, overnight at 4 °C [16]. Further steps were performed at room temperature. The membrane was blocked for 8 h in 3% (w/v) gelatin in Tris-buffered saline (20 mM Tris/HCl, pH 7.6, 137 mM NaCl) containing 0.05% (v/v) Tween 20, then incubated overnight with rabbit IgG (1:5000 dilution in blocking solution). Immune complexes were detected with horseradish peroxidase-labelled antibodies (1:5000 in blocking solution) using the ECL system.

Pulse-chase labelling

At 48 h after transfection, the cells were metabolically labelled. The cells were preincubated for 1 h in 4 ml of Eagle's modified minimum essential deficient medium containing 10% (v/v) dialysed fetal bovine serum (methionine- and cysteine-free medium for labelling with Tran³⁵S-label or phosphate-free medium for labelling with [³³P]P_i), then labelled in 2.5 ml of the deficient medium containing Tran³⁵S-label (200 µCi/75 cm² flask) for 1 h or [³³P]P_i (500 µCi/75 cm² flask) for 3 h. After removal of the pulse medium, the cells were washed twice with PBS and incubated for the chase periods indicated in the Figures, with 3 ml of growth medium supplemented with unlabelled methionine (2 mM) and cysteine (5 mM) for ³⁵S labelling or with 5 mM P_i for ³³P labelling. For ³³P labelling, 10 mM phosphate was included in buffers used to wash the immunoprecipitates. Cells and media were treated for immunoprecipitation.

Endocytosis of IDS precursors

IDS activity

Growth medium of electroporated fibroblasts (48 h after transfection) was added to deleted cells for 4 h then replaced by normal medium. IDS activity was measured at 4, 24 and 48 h.

Maturation study

At 48 h after transfection, fibroblasts were pulse-labelled with Tran³⁵S-label or [³³P]P_i as described above. The labelling medium was removed and the cells were incubated with chase medium (3 ml) for 4 h. This medium was added to confluent deleted fibroblasts (in 75 cm² flasks) for 4 h, then replaced by normal medium. Cells were harvested after 4, 24 or 48 h or 6 days and treated for immunoprecipitation.

Immunoprecipitation

Media were adjusted to a final concentration of 1 mM EDTA and 0.2 mM PMSF and treated directly. Fibroblast pellets were lysed on ice by sonication in buffer A [50 mM Tris/HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.2 mM PMSF] and centrifuged for 30 min at 14000 *g* at 4 °C. Radiolabelled supernatants and media were pretreated for 1 h at 4 °C with 15 µl of Protein A-Sepharose [50:50 (v/v) in buffer A containing 1% (v/v) Triton X-100] to minimize background. Samples were adjusted

to 1 % (v/v) Triton X-100 and 0.15 % (w/v) gelatin and incubated for 1.5 h at 4 °C with 3 μ l of anti-IDS IgG (against purified form B) pretreated with deleted cells. Immune complexes were adsorbed to 20 μ l of Protein A–Sepharose beads (pretreated with deleted cells) for 1 h at 4 °C, then collected by centrifugation. The beads were washed four times with buffer A containing 1 % (v/v) Triton X-100, 0.5 M NaCl and 0.1 % (w/v) SDS and once with 10 mM Tris/HCl, pH 7.5, containing 0.15 M NaCl. Immuno-precipitated proteins were separated from the beads by 5 min heating at 100 °C in electrophoresis sample buffer and analysed by SDS/PAGE. Gels were fixed in 10 % (v/v) acetic acid and 25 % (v/v) methanol for 30 min, incubated in Amplify for 30 min, dried and exposed to Hyperfilm-MP (Amersham) at –80 °C.

Glycosidase digestion

Protein A–Sepharose beads of immunoprecipitated proteins were boiled for 5 min in 40 μ l of 50 mM Tris/HCl, pH 6.8, containing 0.8 % (w/v) SDS and 1 % (v/v) 2-mercaptoethanol for endo H digestion and 40 μ l of 100 mM sodium phosphate buffer, pH 7.4, containing 0.5 % (w/v) SDS and 1 % (v/v) 2-mercaptoethanol for *N*-glycosidase F digestion. After centrifugation, 10 μ l samples were used for each digestion. The reaction mixture was 80 mM sodium citrate buffer (pH 5.5), 1 mM PMSF and 10 m-units of endo H (final volume 100 μ l) or 80 mM sodium phosphate buffer, pH 7.4, containing 1.2 % (v/v) Triton X-100, 7 mM EDTA, 1 mM PMSF and 100 m-units *N*-glycosidase F (final volume 40 μ l). After incubation for 18 h at 37 °C, BSA was added to a final concentration of 0.5 g/l, and proteins were precipitated with acetone for 1 h on ice. The precipitates were dried, dissolved in SDS/PAGE sample buffer and analysed by electrophoresis.

Protease inhibition

³⁵S-labelled precursors were obtained after a 1 h pulse and a 4 h chase as described above. The collected medium was incubated with fibroblasts in the presence of leupeptin at a final concentration of 0.1 mM. This inhibitor was added to the culture medium 1 h before endocytosis for 24 h.

RESULTS

Purified IDS and IDS fusion peptides

Placental IDS forms A and B, which differed by their pI values [2], were eluted in different fractions by chromatofocusing and subsequently purified. IDS purity was checked by SDS/PAGE with silver staining: two major bands of about 53 and 42–44 kDa and two faint bands of 30 and 18 kDa were observed, and an additional band of 66 kDa was present in the B form.

High amounts of the expected-size fusion proteins (FP) were observed: 57 kDa for FP1, 56 kDa for FP2 and 83 kDa for FP3 (size of GST alone 26 kDa). These overexpressed IDS peptides form inclusion bodies, which are difficult to purify from the bacterial lysate by one-step affinity chromatography on immobilized glutathione. We could partially solubilize the inclusion bodies with 6 M urea or alkaline pH but this treatment damaged the binding site to glutathione. Our attempts to minimize the formation of inclusion bodies by decreasing the temperature of growth to 30 °C or modifying IPTG concentration were not successful. As these peptides were only used for rabbit immunization, we finally decided to elute the fusion proteins from polyacrylamide-gel slices.

Figure 1 Immunoblot of IDS from transfected fibroblasts

Deleted fibroblasts were transfected with the normal IDS cDNA (lanes 1) or the nonsense mutant R443X IDS cDNA (lanes 2). At 48 h transfection, 15 μ g of protein extract was immunoblotted and visualized with IgG from rabbits immunized with the three fusion proteins (FP1, FP2 or FP3) or the purified IDS (A or B). Detection was carried using the ECL system (10 s exposure).

Figure 2 Pulse–chase labelling of IDS in fibroblasts

Cultured fibroblasts transiently transfected with the normal IDS cDNA were labelled with Tran-³⁵S-label for 1 h and harvested immediately or after the indicated chase period in unlabelled medium. Cells and medium extracts were immunoprecipitated with anti-(placental IDS form B) and analysed by SDS/PAGE and fluorography. Exposure time was 24 h, except for cell extracts after 24 and 48 h chase (6 days).

Immunoblotting of IDS from fibroblasts

In normal fibroblasts, IDS could not be detected directly using 100 μ g of cellular protein. The semi-purification of IDS from 12 mg of fibroblast proteins allowed the detection of a single band of 45 kDa.

IDS overexpressed in deleted fibroblasts is observed as two major bands of 73–76 and 45 kDa, and two fainter bands of 64 and 55 kDa whatever the antibody used (Figure 1). A single band of 60 kDa is detected in fibroblasts transfected with the nonsense mutant cDNA by the five antibodies (Figure 1).

Pulse–chase labelling of transfected fibroblasts

The results of pulse–chase labelling with Tran-³⁵S-label are shown in Figure 2. After a 1 h labelling period, a polypeptide of about

Figure 3 Endocytosis of ^{35}S -labelled precursors

Radiolabelled precursors were obtained from the medium of transfected fibroblasts after a 1 h pulse with Tran^{35}S -label and a 4 h chase in unlabelled medium. The medium collected was incubated with deleted fibroblasts for the times indicated (the radiolabelled medium was changed to fresh medium after 4 h). Cells and medium extracts were immunoprecipitated with anti-(placental IDS form B) and analysed by SDS/PAGE and fluorography.

76–78 kDa is detected intracellularly. After a 4 h chase, this polypeptide becomes a broader band ranging from 73 to 78 kDa, and a very faint 90 kDa band and additional peptides of 64, 55 and 45 kDa appear. The 73–78, 55 and 45 kDa polypeptides and an 18 kDa band can be visualized after a 24 or 48 h chase whereas the 90 kDa is no longer detectable. Under these conditions, the amounts of the 55 and 45 kDa forms do not increase and the need for a longer exposure shows that most of the unprocessed 73–78 kDa precursor is degraded.

In the medium, different polypeptides are readily observed. After a 4 h chase, large amounts of precursor forms consisting of smeary bands of 73–78 and 90 kDa are excreted. They progressively disappear after a 24 and 48 h chase. Labelling experiments with ^{33}P show that, in the medium, only the 90 kDa band is phosphorylated (see Figure 4).

Endocytosis

After 4 h of incubation with the ^{35}S -labelled medium, IDS precursors are processed to an 18 kDa polypeptide and through several intermediate forms to a 55 kDa major intermediate. A 45–46 kDa doublet is faintly visible at the end of endocytosis (4 h), while the 45 kDa band becomes the major one 48 h after endocytosis and the 55 kDa polypeptide slowly disappears. After 6 days, only the 45 and 18 kDa polypeptides can be detected in the cells (Figure 3). Similar experiments with ^{33}P -labelled precursors show that these species, except the 18 kDa polypeptide, are phosphorylated (Figure 4).

After 4 h endocytosis of unlabelled precursor, IDS activity could be measured in fibroblasts and found to be about 1.5-fold higher than normal (Table 1). Activity slowly decreased to normal 48 h after endocytosis.

Figure 4 Endocytosis of ^{33}P -labelled precursors

Radiolabelled precursors were obtained from the medium of transfected fibroblasts after a 3 h pulse with ^{33}P and a 4 h chase in unlabelled medium. The medium collected was incubated with deleted fibroblasts for the times indicated (the radiolabelled medium was changed to fresh medium after 4 h). Cells and medium extracts were immunoprecipitated with anti-(placental IDS form B) and analysed by SDS/PAGE and fluorography. Exposure time was 10 days.

Table 1 Endocytosis of IDS precursors by deleted fibroblasts

Medium from transfected fibroblasts was incubated with deleted fibroblasts for 4 h and replaced by normal medium for a chase period as indicated. The IDS activity for three control fibroblasts is shown.

Fibroblasts	Incubation	IDS activity (pmol/h per mg)
Deleted	0 h	0
	4 h	135
	4 h and 24 h chase	74
	4 h and 48 h chase	59
Control		72, 94, 118

Glycosylation studies

Figure 5 shows the electrophoretic mobility of medium IDS precursors from transfected fibroblasts before and after treatment with endo H or *N*-glycosidase F. After digestion with endo H, the 73–78 kDa band is converted into a unique band of 60 kDa and the smeary 90 kDa band is converted into a smeary band of intermediate mobility. Treatment of both precursor forms with *N*-glycosidase F gives the 60 kDa band. Thus the 73–78 kDa forms contain only hybrid and/or high-mannose-type oligosaccharide chains whereas the 90 kDa secreted precursor contains some hybrid and/or high-mannose glycans but more complex glycans.

Figure 5 Digestion of radiolabelled precursors with endo H and *N*-glycosidase F

Radiolabelled precursors were obtained from the medium of transfected fibroblasts after a 1 h pulse with Tran^{35}S -label and a 4 h chase in unlabelled medium. Immunoprecipitated enzyme was incubated with either endo H or *N*-glycosidase F. Control samples without endo H or *N*-glycosidase F were processed in parallel. Samples were analysed by SDS/PAGE and fluorography.

Figure 6 Effects of leupeptin on the proteolytic processing of IDS

^{35}S -labelled precursors (obtained as in the legend of Figure 3) were incubated with deleted fibroblasts in the absence (lane 1) or presence (lane 2) of 0.1 mM leupeptin for 24 h. Cell extracts were immunoprecipitated with anti-(placental IDS form B) and analysed by SDS/PAGE and fluorography.

Glycosidase digestion of the cellular extracts after 4 h endocytosis of labelled precursors and 24 and 48 h maturation (results not shown) indicates that the 55 and 45 kDa polypeptides are sensitive to *N*-glycosidase F and are therefore glycosylated (the 45 kDa form is converted into a 39 kDa polypeptide). Each gives a band of intermediate mobility when treated with endo H and contains hybrid and complex-type oligosaccharide chains. The 18 kDa polypeptide is sensitive to *N*-glycosidase F and resistant to endo H and contains only complex-type oligosaccharides.

Proteolysis study

IDS polypeptides obtained 24 and 48 h after 4 h endocytosis of the precursors were subjected to SDS/PAGE under reducing or non-reducing conditions. The absence of 2-mercaptoethanol does not modify the pattern observed. The 18 and 45 kDa polypeptides do not result from subunit dissociation but from proteolytic cleavage.

A longer endocytosis period (24 h) of the medium-labelled precursors was performed in the absence or presence of leupeptin (Figure 6). In the presence of leupeptin, a short-lived polypeptide

of about 70 kDa appears while the 55 kDa peptide accumulates, the 45 kDa peptide is absent and the 18 kDa peptide is present normally. Leupeptin added to the cells after a 24 h period of endocytosis inhibits the cleavage of the 55 kDa to the 45 kDa form (not shown).

DISCUSSION

Preparation of IDS antibodies

The low abundance of IDS makes its purification very tedious. Thus we chose two different approaches for the preparation of purified antigenic material: purification of placental IDS from an industrial product and preparation of recombinant IDS peptides as fusion proteins with GST in *E. coli*. The latter procedure is faster and allows the easy preparation of large amounts of peptide but does not necessarily give high-quality antibodies. The five antibodies obtained were tested by immunoblotting. The very low level of IDS in normal fibroblasts does not allow its direct visualization by immunoblotting without a semi-purification step. Thus the relative amount of IDS activity was increased about 500-fold by transfection with a eukaryotic expression vector in which the normal IDS cDNA is under the control of a strong promoter (cytomegalovirus). This expression system was used to evaluate the five antibodies that react similarly by immunoblotting. The expected difference in size of the truncated precursor obtained by transfection of the nonsense mutant R443X and the absence of any band after transfection with an antisense IDS cDNA give additional evidence for IDS specificity of our five antibodies (Figure 1).

IDS processing in fibroblasts

Transfected fibroblasts

In an attempt to understand IDS processing, pulse-chase experiments were performed in transiently transfected fibroblasts. In these overexpressing conditions, only a small fraction of the 73–78 kDa initial precursor is processed. Most of it is either intracellularly degraded or excreted into the medium as a hybrid/high-mannose unphosphorylated form. This initial precursor does not bear the mannose-6-phosphate recognition marker and therefore cannot be endocytosed by the fibroblasts. It is completely deglycosylated to a 60 kDa polypeptide, in agreement with the estimation of the molecular mass of the polypeptide chain deduced from the cDNA sequence [3]. The presence in the medium of this unphosphorylated 73–78 kDa form that accumulates in the cells is due to high levels of IDS overexpression by the strong cytomegalovirus promoter. There may be several reasons for the poor processing observed in transfected cells: aggregation in the endoplasmic reticulum of high amounts of newly synthesized 73–78 kDa glycopeptides and/or saturation of the mannose-6-phosphate pathway. Under similar conditions of high level of overexpression, Anson et al. [17] demonstrated that the overexpression of α -L-iduronidase in fibroblasts induces decreased activity of other mannose-6-phosphate-dependent enzymes.

The extracellular 90 kDa precursor is phosphorylated and is therefore able to be endocytosed by the mannose-6-phosphate pathway in fibroblasts [18]. After electroporation, as no selection was applied, the cell content is a mixture of about 60 % of cells overexpressing IDS and 40 % of non-transfected cells that are able to internalize the medium phosphorylated precursor and to process it to the 45 kDa mature form. The small amount of the mature 45 kDa form detected in these non-transfected cells can be explained by rapid degradation of the endocytosed precursor. It has been shown that overexpression of *N*-acetylgalactosamine

4-sulphatase induces multiple sulphatase deficiency (including the non-lysosomal steroid sulphatase) [19]. The decreased half-life of the endocytosed IDS may be due to an excess of IDS precursor and a limited availability of a sulphatase-specific-pathway factor.

Endocytosis of IDS precursors by deleted fibroblasts

As unlabelled precursors produced in the medium of transfected fibroblasts and added to deleted fibroblasts restore normal activity to these cells within a few hours, they must be internalized and processed to the mature form. Maturation of IDS was studied after endocytosis of a limited amount of precursor by deleted fibroblasts. The labelled phosphorylated 90 kDa precursor is processed in fibroblasts through various intermediates to the major 55 kDa intermediate then to the 45 kDa mature form. The 45 kDa band corresponds to the mature form of IDS as the mature form of lysosomal enzymes is the major one in most tissues. It is the only form that could be visualized after semi-purification of IDS from normal non-transfected fibroblasts (the present results), and the main band obtained after purification of IDS from liver and placenta [2]. This 45 kDa band arises from the 55 kDa polypeptide by proteolytic cleavage by a thiol protease since it is inhibited by leupeptin.

The 18 kDa polypeptide bears complex-type oligosaccharide chains and is the only unphosphorylated form. It results from proteolytic cleavage that is not inhibited by leupeptin and occurs early in the maturation process of endocytosed precursor as it could be detected as early as 1.5 h after endocytosis when the 45 kDa polypeptide is hardly detectable. Its cleavage may be involved in IDS folding, influencing its processing, activity and/or stability.

IDS has also been expressed in lymphoblasts from patients with mucopolysaccharidosis type II [20] and has been shown to be able to correct storage in these cells; however, no study of the IDS maturation in these cells has been reported.

A knowledge of normal IDS maturation is the first step in the functional study of mild mutants affecting the processing or stability of the enzyme.

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