Recombinant human iduronate-2-sulphatase: correction of mucopolysaccharidosis-type II fibroblasts and characterization of the purified enzyme

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Mucopolysaccharidosis type II (MPS II, Hunter syndrome) is an X-chromosome-linked recessive lysosomal storage disorder that results from a deficiency of iduronate-2-sulphatase (12S). Patients with MPS II store and excrete large amounts of partially degraded heparan sulphate and dermatan sulphate. In order to evaluate enzyme-replacement therapy for MPS II we have expressed a chimaeric 12S cDNA in CHO (Chinese-hamster ovary)-K1 cells utilizing a plasmid vector that places the cDNA under the transcriptional control of the human polypeptide-chain-elongation factor-1α gene promoter. A clonal cell line that accumulated recombinant 12S at greater than 10 mg/ml in conditioned medium was identified. Enzyme secreted from this cell line grown in the presence of NH₄Cl was shown to be endocytosed into MPS II fibroblasts via the mannose 6-phosphate receptor and localized to the lysosomal compartment, resulting in correction of the storage phenotype of these cells. Milligram quantities of the recombinant 12S were purified, and the enzyme was shown to have a pH optimum and kinetic parameters similar to those for the mature form of 12S purified from human liver. The recombinant 12S had a molecular mass of approx. 90 kDa; this was reduced to 60 kDa by endoglycosidase treatment.

INTRODUCTION

Iduronate-2-sulphatase (12S; EC 3.1.6.13) is one of the lysosomal enzymes involved in the degradation of the glycosaminoglycans heparan sulphate (HS) and dermatan sulphate (DS). A deficiency of this enzyme activity results in the lysosomal storage disorder mucopolysaccharidosis type II (MPS II), also known as Hunter syndrome (McKusick and Neufeld, 1983), and is inherited as an X-chromosome-linked recessive trait. The clinical presentation of this disorder is characterized by skeletal dysmorphism, coarse facies, hepatosplenomegaly and, in its severe form, mental retardation and death by the age of 15 years (McKusick and Neufeld, 1983). MPS II patients store and excrete excessive amounts of partially degraded HS and DS fragments. Although clinically severe MPS II involves central-nervous-system (CNS) pathology, enzyme-replacement therapy is likely to be valuable in at least treating the somatic symptoms of the disease. It is also possible that the blood/brain barrier will be able to transport mannos 6-phosphorylated or suitably engineered 12S carrying alternative transport signals. Enzyme-replacement therapy for MPS II and other lysosomal storage disorders (LSDs) that have CNS involvement is therefore worthy of investigation.

Human 12S has been purified from liver in small quantities (Biellicki et al., 1990). As a source of enzyme, however, liver and other tissues are limited by the extreme low abundance of 12S, which results in a low yield of purified enzyme, for example, less than 10 μg of 12S/kg of liver. Recombinant DNA technology has been used successfully to express full-length N-acetylgalactosamine-4-sulphatase (4S) cDNA in CHO (Chinese-hamster ovary)-K1 cells with yields of several mg of enzyme/litre of culture medium (Anson et al., 1992). The 'high uptake' precursor form of 4S was observed to be similar to endogenous 4S with respect to its catalytic properties in vitro and its biological properties in vivo. In order to evaluate enzyme-replacement therapy for MPS II and to investigate possible transport mechanisms for lysosomal enzymes across the blood/brain barrier, we have expressed a full-length 12S cDNA in CHO-K1 cells and purified the secreted form of the enzyme from conditioned medium. Although the recombinant 12S (r12S) has a larger molecular mass than that reported for 12S from human liver (Biellicki et al., 1990), both were shown to have similar pH optimum and catalytic properties. The r12S was efficiently endocytosed by MPS II fibroblasts and subsequently localized to lysosomes, resulting in correction of the enzymatic defect and initiation of the degradation of stored 35S-labelled glycosaminoglycans. The recombinant enzyme is therefore suitable for initial evaluation of enzyme-replacement therapy in MPS II as well as providing a source of material for production of antibodies and for biochemical and physical studies.

MATERIALS AND METHODS

All enzymes for DNA manipulations, DNAase, dithiothreitol, kanamycin and streptomycin were purchased from Boehringer Mannheim (Dulwich, South Australia, Australia). DNA oligonucleotides were synthesized with an Applied Biosystems 391 DNA synthesizer. Na₄²⁵³⁰₄ (516 mCi/mmol) was purchased from New England Nuclear (du Pont, North Ryde, N.S.W., Australia). PBE94 chromatofocusing medium, Polybuffer 74 and high- and low-molecular-mass standard kits for SDS/PAGE and gel chromatography were obtained from Pharmacia (North Ryde, N.S.W., Australia). TSK G3000SW Ultrapac was pur-

Abbreviations used: BME, basal medium (Eagles); CHO, Chinese-hamster ovary; DS, dermatan sulphate; FCS, fetal-calif serum; G418, G418 sulphate (Geneticin); HS, heparan sulphate; Idoa2S-amM6S, l-O-α-iduronic acid 2-sulphate)-(1→4)-l-O-2,5-anhydro[1-⁵⁷C]mannitol 6-sulphate; I2S, iduronate-2-sulphatase; LSD, lysosomal storage disorder; MPS, mucopolysaccharidosis; r12S, recombinant iduronate-2-sulphatase; 4S, N-acetylgalactosamine 4-sulphatase; DTE, dithioerythritol; RSV-LTR, Rous-sarcoma-virus long terminal repeat; EF-1α, elongation factor-1α.

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chased from LKB (Bromma, Sweden). Blue A matrix agarose gel, ultrafiltration stirred-cell (model 8200) and Diaflo ultrafiltration membrane YM10 were obtained from Amicon (Danvers, MA, U.S.A.). Dialysis membrane with a 10–12 kDa cut-off was obtained from Union Carbide Corp. (Chicago, IL, U.S.A.). Endoglycosidase F was purchased from Novagen (du Pont Co., Wilmington, DE, U.S.A.). Dulbecco's modified PBS was purchased from Commonwealth Serum Laboratories (Melbourne, Vic., Australia). Nonidet P40, mannose 6-phosphate and BSA were purchased from Sigma (St. Louis, MO, U.S.A.). Basal medium Eagle's (BME), penicillin and glutamine were obtained from Flow Laboratories (Sydney, N.S.W., Australia) and fetal-calf serum (FCS), Ham's F12 nutrient mixture, CHO-SFM medium and G418 (Geneticin) were from Gibco (Glen Waverley, Vic., Australia).

DNA manipulation and recombinant plasmids

All DNA preparation, modification and cloning procedures were done using standard techniques (Ausubel et al., 1989). The I2S cDNA clone pBl2Sc1 contains bp 107 (NotI restriction-enzyme site) to bp 1870 (BstXI restriction-enzyme site) of the I2S cDNA sequence presented in Figure 1 of Wilson et al. (1990), cloned between the NotI and EcoRV restriction-enzyme sites of pBlueScript (Stratagene, La Jolla, CA, U.S.A.). The expression vector pRSVN.08 was derived from pRSVN.07 (Anson et al., 1992) by the introduction of an EcoRV site into the polylinker such that the order of restriction sites is 5' HindIII, XbaI, BamHI, EcoRV, EcoRI, NotI 3'.

Culture and electroporation of CHO-K1 cells

CHO-K1 cells were cultured and electroporated as previously described (Anson et al., 1992), unless otherwise stated.

Culture of fibroblasts

Human diploid fibroblasts were established from skin biopsies submitted to this hospital for diagnosis (Hopwood et al., 1982). Cell lines were maintained according to established procedures in BME, 10% (v/v) FCS and antibiotics unless otherwise stated. The two MPS II skin fibroblast cell lines used in the present study (SF-635 and SF-1779) both have low residual I2S activity (J. Bielicki, unpublished work).

Determination of I2S expression

Media samples, or cell lysates prepared by six cycles of freeze–thaw in 0.5 M NaCl/20 mM Tris/HCl, pH 7.0, were clarified by microcentrifugation (12000 g, 4 °C, 5 min) and were either assayed directly or after dilution in assay buffer. Where possible cell lysates were dialysed in 5 mM sodium acetate, pH 4.0, before assaying, as this results in higher measured enzyme activity. I2S was assayed using the radiolabelled disaccharide substrate l-0-(α-iduronic acid 2-sulphate)-(1→4)-p-D-2,5-anhydro[1-3H]mannitol 6-sulphate (IdoA2S-α-2-M6S) (Bielicki et al., 1990). Protein was determined by the method of Lowry et al. (1951), with BSA as standard.

β-Hexosaminidase

The fluorogenic substrate 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside was used to measure β-hexosaminidase activity (Leaback and Walker, 1961).

Correction of MPS II fibroblasts

For these experiments r12S was obtained from CHOEFI2S-9 cells cultured in CHO-SFM medium supplemented with 10 mM NH₄Cl and antibiotics. The medium was concentrated 10-fold by ultrafiltration and was shown to contain r12S with activity of 2.75 × 10⁶ pmol/min per ml (133 μg of I2S/ml). Fibroblasts from a normal individual (SF-3409) and from two MPS II patients (SF-635 and SF-1779) were grown to confluency in 25 cm² flasks and radiolabelled with Na₂³⁵SO₄ as previously described (Anson et al., 1992). The labelled cells were then exposed to r12S (5 × 10⁴ pmol/min per ml) for 72 h. After harvesting the cells by trypsin treatment and washing by centrifugation/resuspension in PBS, the cell pellet was resuspended in 100 μl of 20 mM Tris/HCl (pH 7.0)/0.5 M NaCl, and the cell lysates were prepared as described under 'Determination of I2S expression' above. The cell extracts were analysed for I2S activity, total protein, β-hexosaminidase activity and radioactivity.

Endocytosis of r12S

SF-1779 fibroblasts were plated in 20 wells (3.83 cm²) and allowed to reach confluency. Wells 1–4 were untreated controls. To each of wells 5–12 and 13–20 was added 1.0 ml of medium containing r12S at 5 × 10⁴ and 5 × 10⁵ pmol/min per ml respectively. In addition the medium in wells 9–12 and 17–20 was made 5 mM mannose 6-phosphate. The cells were then incubated for 6 h, after which time they were rinsed with medium and fresh medium added. The cells were incubated overnight and then harvested, washed and lysed as described above. The cell lysates were dialysed against 5 mM sodium acetate, pH 4.0, for 16 h at 4 °C and then analysed for I2S activity and total protein.

Subcellular fractionation

SF-635 fibroblasts were grown to confluency in 75 cm² flasks and then exposed to medium supplemented with 5 × 10⁴ pmol of r12S/min per ml. The cells were incubated for 72 h, then harvested and fractionated on Percoll density gradients as described in Anson et al. (1992). The resulting gradient was collected in 1.0 ml fractions by bottom puncture and the fractions analysed for I2S and β-hexosaminidase activity.

Large-scale production of r12S

CHOEFI2S-9 cells were inoculated into two two-layer cell factories (Nunc; 1200 cm²) in Ham's F12, 10% (v/v) FCS and antibiotics. Cells were grown to confluency, the medium removed and the cells were then rinsed three times with PBS and re-fed with 200 ml of Ham's F12 without FCS, but supplemented with antibiotics and 10 mM NH₄Cl. After 4 days in culture, the medium was collected and replaced with Ham's F12, 10% (v/v) FCS and antibiotics, but without NH₄Cl for 3 days. This cycle was repeated several times. The conditioned serum-free Ham's F12 medium supplemented with NH₄Cl was collected, clarified by filtration (0.2 μm-pore-size filter; Millipore) and stored at 4 °C.

The r12S was purified from the collected medium by a three-step column procedure. The medium was dialysed overnight at 4 °C against 30 mM Tris/HCl (pH 7.0)/10% (v/v) glycerol/0.1 mM dithioerythritol (DTE)/3 mM NaN₃ (buffer A) and was applied to a PBE94 column (8 cm × 1.5 cm) equilibrated in buffer A (flow rate 1.0 ml/min) and then washed with 100 ml of buffer A. Bound proteins were eluted with Polybuffer 74 that had been diluted 1:18 with water, the pH adjusted to 4.0 with
HCl and the solution made 10% (v/v) in glycerol, 0.1 mM in DTE and 3 mM in NaN₄. The column was further eluted with 100 ml of 15 mM 3,3-dimethyl glutarate (pH 6.0)/0.5 M NaCl/10% (v/v) glycerol/0.1 mM DTE/3 mM NaN₄ (buffer B). The rI2S eluted in buffer B was applied at a flow rate of 1.0 ml/min to a Blue A–agarose column (6 cm x 0.7 cm), also equilibrated in buffer B. The rI2S activity from this step was applied in 1.0 ml volumes to an LKB Ultrachrom GTi f.p.l.c. system with a TSK G3000SW Ultrapac column (30 cm x 0.8 cm) equilibrated and eluted in buffer B at a flow rate of 0.5 ml/min and pressure of 150 kPa. Fractions containing I2S activity were pooled and analysed under denaturing and non-denaturing conditions by SDS/PAGE (10% acrylamide) to estimate apparent subunit size. Gels were stained with either Gradipure Coilloidal Electrophoresis Gel Stain (Gradipure, Pyrmont, N.S.W., Australia) or silver-stained by the method of Merril et al. (1981). Native molecular mass was determined using an f.p.l.c. system described elsewhere (Bielicki et al., 1990). Kinetic (Kᵥ, Vᵥmax, and pH optima) and inhibition data were obtained as described previously (Bielicki et al., 1990).

Endoglycosidase F treatment of rI2S

To two identical 60 µl samples, each containing 2.5 µg of rI2S, was added an equal volume of buffer containing 100 mM sodium phosphate (pH 6.1)/30 mM EDTA/1% (v/v) Nonidet P40/0.1% (v/v) SDS/1% (v/v) 2-mercaptoethanol. After boiling both samples for 5 min, to one was added 1 unit and to the other 5 units of endoglycosidase. Both samples were incubated for 17 h at 37 °C. A control sample was untreated but stored in similar buffer conditions at 4 °C. Bromophenol Blue was added to each sample before analysis by SDS/PAGE. Molecular-mass standards were subjected to SDS/PAGE in the same buffer as the enzyme samples.

RESULTS AND DISCUSSION

Construction of I2S expression vectors

An initial expression construct containing the I2S cDNA from pBI2Sc17 cloned into pRSVN.08 expressed I2S at very low levels when introduced into CHO-K1 cells. A chimaeric I2S cDNA was then made by replacing the 5′ non-coding sequence of the I2S cDNA with 45 bp of the rat preproinsulin leader sequence (Figure 1) as an analogous chimaeric N-acetylgalactosamine-4-sulphatase cDNA construct resulted in the expression of high levels of enzyme activity in the same system (Anson et al., 1992). Briefly the sequence shown in Figure 1 was synthesized as two complementary oligonucleotides which were then phosphorylated with T4 polynucleotide kinase and annealed. The resulting double-stranded fragment was then cloned between the dephosphorylated NotI and StuI sites of pBl2Sc17. The resulting construct was designated pBl2SCN.1. The I2S cDNA insert was then excised from pBl2SCN.1 with XbaI and Hincll and cloned into Xbal/EcoRV-restricted and dephosphorylated pRSVN.08, resulting in the construct pRSVN.2SCN. In order to increase further expression of rI2S, the chimaeric I2S cDNA was placed under the transcriptional control of the human elongation factor-1α (EF-1α) gene promoter. This was done by excising the Rous-sarcoma-virus long terminal repeat (RSV-LTR) from pRSVN.2SCN1 with SalI/XbaI digestion and inserting the HindIII/XbaI fragment from pEF-BOS (Mizushima and Nagata, 1990) after making the HindIII and SalI ends blunt by filling in with the Klenow fragment of DNA polymerase I. This construct was designated pEFN.2SCN1. Both pRSVN.2SCN1 and pEFN.2SCN1 were electrophorotised into CHO-K1 cells, and G418-resistant cell clones were isolated. Individual clones were assayed for secretion of I2S activity into the culture medium. Replacement of the RSV-LTR promoter with the human EF-1α promoter resulted in a 2-fold enhancement of I2S expression. A clonal cell line, CHOEI2S-9, was selected on the basis of maximum expression of I2S activity. This clone secreted I2S such that, after 5 days of culture, approx. 11 mg of I2S was collected in 1 litre of medium.

Large-scale production of rI2S

Conditioned serum-free Ham’s F12 medium containing NH₄Cl was collected as described in the Materials and methods section. Enzyme was collected in this manner to facilitate purification by minimizing total protein in medium. As prolonged exposure to this medium resulted in loss of cell viability, the cells were cycled in Ham’s F12 with 10% (v/v) FCS to allow recovery. A total of 1 litre of serum-free medium, containing approx. 11 mg of I2S, was collected in this manner.

The rI2S bound very tightly to PEB94 medium and was not eluted in significant amounts during Polybuffer elution (less than 10% of the total enzyme recovered from this column was eluted with polybuffer; pool A; Figure 2a). Most of the rI2S (pool B; Figure 2a) had a pI of <4.0 and required NaCl for elution. Enzyme was eluted in buffer B in concentrated form (essentially in one 10 ml fraction). This permitted direct application to Blue A–agarose. Although the rI2S did not bind to this matrix, it was a necessary step to remove some minor contaminating proteins which were observed after f.p.l.c. when the enzyme from the chromatofocusing step was applied directly to f.p.l.c. Recovery of activity from Blue A–agarose was 80%. The final step in the purification (f.p.l.c.) resulted in an overall recovery of greater than 15% activity. The estimated native molecular mass on f.p.l.c. was 90 kDa. A single diffuse protein band of 80–92 kDa was observed when a sample from the f.p.l.c. step was subjected to SDS/PAGE (Figure 3). This diffuse band was observed on SDS/PAGE run under reducing or non-reducing conditions, indicative of a single subunit species with no disulphide bonding. Correlation of the protein species observed as a diffuse band on SDS/PAGE with I2S activity was demonstrated by PAGE run under non-reducing conditions by the method of Laemmli (1970), but with the modification that SDS was omitted from all buffers. Identical amounts of enzyme were applied to two lanes of the gel. One lane was stained for protein and, as with SDS/PAGE, a single diffuse band was observed. The other was cut into 2 mm slices and each slice was incubated in four times its volume of assay mix at 37 °C overnight. When corrected for the swelling which occurred during the staining procedure, the position of the
diffuse band corresponded to that of I2S activity in the lane that was sliced and assayed (results not shown).

The molecular size of I2S (after cleavage of the signal peptide) estimated from cDNA sequence data indicated a maximum of 58 kDa with seven potential glycosylation sites (Wilson et al., 1990). The mature or processed forms of I2S had various molecular sizes depending on the column matrix used. The native molecular size varied from 42 to 65 kDa, while, on a denaturing SDS/PAGE, two polypeptide bands of 43 kDa and 14.4 kDa were consistently observed (Bielicki et al., 1990). The recombinant form of I2S had a markedly larger molecular size (80–90 kDa; Figure 3) than predicted. The diffuse nature of the Coomassie Blue-stained band on SDS/PAGE implied that the protein was highly and variably glycosylated. To test the hypothesis that the difference in the observed molecular size and the expected estimated value was due to carbohydrate, rI2S was treated with endoglycosidase F as outlined in the Materials and methods section. Treatment with 1 unit of endoglycosidase F resulted in a decrease in molecular size (70–80 kDa). However, the enzyme still migrated as a diffuse band on SDS/PAGE (Figure 4; lane 2). Lane 3, which shows the result of treatment with a fivefold greater concentration of endoglycosidase F demonstrates the presence of a tightly staining 60 kDa protein band with a diffuse band above it (62–68 kDa). Other bands are due to endoglycosidase F.

We suggest that the 60 kDa band is the end product of the deglycosylation of rI2S by endoglycosidase F and that the diffuse bands in both lanes are the result of incomplete digestion. Endoglycosidase F cleaves the glycosidic bond between N-acetylgalactosamine (GlcNAc) residues of the chitobiose core in the N-linked carbohydrate chains, resulting in one GlcNAc residue remaining linked to asparagine. This would account for approx. 1540 kDa due to carbohydrate if all seven of the glycosylation sites were utilized, and may therefore account for the molecular size of I2S after endoglycosidase F treatment as being 60 kDa rather than 58 kDa.
Table 1  Comparison of the catalytic properties of recombinant and liver I2S

<table>
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<th></th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (µmol/min per mg)</th>
<th>Specific activity (µmol/min per mg)</th>
<th>pH optimum</th>
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<tr>
<td>Liver I2S</td>
<td>4.0</td>
<td>80</td>
<td>11.9</td>
<td>4.5</td>
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<tr>
<td>rI2S</td>
<td>3.0</td>
<td>3.35</td>
<td>20.8</td>
<td>4.5</td>
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Table 2  Comparison of the effect of various inhibitors on recombinant and liver I2S

Values shown are inhibitor concentrations giving 50% inhibition of I2S activity. For details, see the Materials and methods section.

<table>
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<tr>
<th>Inhibitor</th>
<th>Liver I2S</th>
<th>rI2S</th>
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</thead>
<tbody>
<tr>
<td>NaCl (mM)</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td>Na$_2$SO$_4$ (µM)</td>
<td>50</td>
<td>115</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$ (µM)</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Cupric acetate (mM)</td>
<td>15</td>
<td>8</td>
</tr>
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Kinetics of rI2S

Although both the liver and rI2S show a similar $K_m$ towards the disaccharide substrate (IdoA2S-anM6S) in the standard assay [50 mM sodium acetate (pH 4.0)/500 µg/ml BSA], they have a substantially different $V_{max}$. This suggests that the recombinant form of the enzyme may be less efficient in turning over the substrate than the mature form. Alternatively this may reflect a difference between enzyme produced in CHO cells and in liver. Both the (CHO) recombinant and (liver) mature form of the enzyme have similar pH optima and specific activities (Table 1).

Inhibition studies showed that the rI2S was similar to the liver enzyme with regard to inhibition by sulphate, phosphate and copper ions. The rI2S appears to be less sensitive to salt inhibition than liver enzyme (Table 2).

Demonstration of correction of MPS II fibroblasts

Fibroblasts from patients with MPS II store degraded HS and DS fragments. This storage is reflected in the accumulation of labelled material when the cells are metabolically labelled with Na$_2$H$_3$SO$_4$. Supplementing culture medium with rI2S at 5 x 10$^6$ pmol/min per ml resulted in clearance of this stored product to levels comparable with those seen in control fibroblasts (Table 3) and to levels of I2S activity 40–80% above normal in SF-1779 and SF-635 respectively. The activity of a second lysosomal enzyme, $\beta$-hexosaminidase, was not affected by endocytosis of I2S (Table 3).

To test whether endocytosis of the rI2S occurs via the mannose 6-phosphate receptor, MPS II cells (SF-1779) were cultured in medium supplemented with rI2S at 5 x 10$^4$ and 5 x 10$^5$ pmol/min per ml in the presence or absence of 5 mM mannose 6-phosphate. Inhibition of the uptake of I2S activity by mannose 6-phosphate at both doses of enzyme confirmed that uptake is mediated via the mannose 6-phosphate receptor. The degree of inhibition was dependent on the concentration of rI2S. However, as the concentration of I2S in cell lysates was the same, regardless of the dose, this implied that the rate-limiting factor for uptake was the availability of mannose 6-phosphate receptor (Table 4).

Localization of endocytosed rI2S

Endocytosed rI2S was instrumental in correcting the lysosomal storage in MPS II skin fibroblasts, as demonstrated by the loss of activity.
of accumulated 35S-labelled material. Confirmation of the subcellular localization of the endocytosed enzyme was demonstrated by fractionating the postnuclear supernatant of corrected and control MPS II skin fibroblasts on Percoll gradients as described in the Materials and methods section. Analysis of these gradients showed that in the corrected MPS II cells, I2S activity fractionated with the lysosomal enzyme β-hexosaminidase in the dense fraction of the gradient (Figure 5). Control MPS II fibroblasts contained no detectable levels of I2S activity and a similar β-hexosaminidase activity profile (results not shown).

Conclusion
We have demonstrated the high-level expression of I2S in CHO-K1 cells using a recombinant expression construct and have successfully purified milligram amounts of the secreted precursor form of the enzyme to homogeneity. Although this form of the enzyme showed some differences in its 'in vitro' kinetic parameters from the mature form of I2S purified from human liver, it was both effective in correcting the 'in vivo' storage phenotype in MPS II fibroblasts after mannose 6-phosphate-receptor-dependent endocytosis and was efficiently localized to the correct subcellular compartment. The rI2S would therefore appear to be suitable for initial evaluation of enzyme-replacement therapy of MPS II. Of particular interest is whether the precursor form of lysosomal enzymes, which carries mannose 6-phosphate residues, can cross the blood/brain barrier. The large amounts of this form of I2S, and of N-acetylgalactosamine-4-sulphatase (Anson et al., 1992) should allow one to properly investigate this question. The cellular, biochemical and enzymic analysis of normal and MPS-II-patient-derived I2S have also been limited by the lack of large amounts of enzyme and of specific antibodies to I2S. The recombinant enzyme will permit one to develop such reagents and to study these questions in detail.

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