Proc. Nat. Acad. Sci. USA
Vol. 69, No. 8, pp. 2048–2051, August 1972

The Defect in the Hurler and Scheie Syndromes: Deficiency of α-L-Iduronidase

(skin fibroblasts/mucopolysaccharidosis)

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Communicated by C. B. Anfinsen, May 19, 1972

ABSTRACT **Skin fibroblasts cultured from patients affected with the Hurler or Scheie syndromes (mucopolysaccharidoses I or V, respectively) have a functional deficiency of a protein required for catabolism of sulfated mucopolysaccharide that has been designated the "Hurler corrective factor." We now show Hurler factor purified from normal human urine to be associated with α-L-iduronidase activity. Cell lines deficient in Hurler corrective factor have no detectable activity of α-L-iduronidase (less than 3% of that found in cells from individuals of other genotypes). Such correspondence indicates that Hurler corrective factor and α-L-iduronidase are the same entity. Correction of deficient cells is accompanied by an efficient uptake of α-L-iduronidase from the medium.**

The Hurler syndrome (mucropolysaccharidosis I) is the most striking and best known of the inborn errors of mucopolysaccharide metabolism. Lysosomal deposits of mucopolysaccharide, found in nearly all cells, are no doubt responsible for the severe clinical manifestations that include skeletal deformities, hepatosplenomegaly, cloudy corneas, stunting of physical and mental growth, and cardiovascular pathology (1, 2). The mucopolysaccharides stored in the lysosomes and excreted in the urine are fragments of dermatan sulfate and heparan sulfate (2).

Fibroblasts cultured from the skin of Hurler patients likewise accumulate excessive mucopolysaccharide (3–5) because of the deficiency of a specific protein required for degradation (6). When this protein (designated "Hurler corrective factor") is supplied exogenously, mucopolysaccharide catabolism of Hurler fibroblasts is normalized.

Hurler corrective factor, purified 1000-fold from normal human urine, has no effect on the mucopolysaccharide metabolism of fibroblasts cultured from normal individuals, nor from patients with several other mucopolysaccharidoses, namely, the Hunter, Sanfilippo, and Maroteaux–Lamy syndromes (7). However, cells from individuals affected with the Scheie syndrome (mucopolysaccharidosis V) and from some individuals with a phenotype intermediate between Hurler and Scheie, are likewise deficient in, and correctible by, the Hurler factor (7–9).

Barton and Neufeld (7) reported that the Hurler factor assisted Hurler and Scheie cells in degrading intracellular mucopolysaccharide (primarily dermatan sulfate), but was distinct from the common lysosomal hydrolases such as β-D-galactosidase or β-D-glucuronidase. The recent chemical synthesis of phenyl α-L-iduronide has enabled us to test the factor for α-L-iduronidase activity. α-L-Iduronidase (α-L-iduronide iduronohydrolase) activity has been detected in homogenates of human liver and cultured skin fibroblasts (10), and a preliminary characterization has been presented for the enzyme extracted from rat-liver lysosomes (11). A deficit of α-L-iduronidase in cells from individuals with the Hurler syndrome has been reported by Matalon *et al.* (10).

We now present evidence for the identity of Hurler corrective factor and α-L-iduronidase. The Hurler and Scheie syndromes, previously shown due to a deficiency of Hurler corrective factor, may therefore be classified as α-L-iduronidase deficiency diseases.

MATERIALS AND METHODS

Methods for culture of skin fibroblasts, as well as for the preparation and assay of Hurler corrective factor (7), and for the preparation of phenyl α- and β-L-iduronides (10a), have been described elsewhere. Purified Hurler factor had been stored at 4° in 80% ammonium sulfate; immediately before use, bovine-serum albumin was added to a final concentration of 1 mg/ml, and the mixture was exhaustively dialyzed against 0.01 M sodium phosphate buffer (pH 6.0)–0.9% NaCl.

Cell-free extracts of fibroblasts were prepared from cells grown to confluence in a 250-ml Falcon flask. Cells were detached from the plate by trypsinization, centrifuged, rinsed twice with 5 ml of 0.9% NaCl, suspended in 0.25 ml of 0.9% NaCl, and subjected to three cycles of freezing and thawing. The resultant suspension was used for enzyme assay after protein determination (12). Acetone powders were prepared from cells grown in 1410-cm^2 Bellco roller bottles for at least 1 month, to ensure confluence, as will be described elsewhere, and stored at −18° (Hall, Cantz, and Neufeld, in preparation). The powders were extracted at 0° immediately before use with 0.9% NaCl (0.1 ml/mg powder) and centrifuged at 10,000 × *g*. The supernatant fluid was used for enzyme assay after protein determination.

α-L-Iduronidase was assayed by a modification of the method of Weissmann and Santiago (11). The conditions selected—pH, time, substrate, and salt concentrations—are based on data obtained for the liver enzyme (11); because of the limited amount of substrate available, no attempt was made to optimize conditions for the urinary or fibroblast preparations. 50 μl of extract in 0.9% NaCl or Hurler corrective factor in 0.9% NaCl buffered with 0.01 M sodium phosphate (pH 6.0) were incubated with 40 μl of 0.01 M

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phenyl α-L-iduronide and 250 μl of 0.1 M sodium formate buffer at pH 3.5 (when unbuffered extracts were used) or at pH 3.2 (when Hurler corrective factor in buffered saline was to be tested). The formate buffers contained 0.2 M NaCl and 0.04% NaN_3. After 17 hr at ambient temperature (24°), the reaction was stopped with 1.0 ml of Folin—Ciocalteu phenol reagent (Fisher Scientific Co., diluted before use with two volumes of water) and precipitated protein was removed by centrifugation. 1 ml of the supernatant fluid was withdrawn and made alkaline with 1.5 ml of 12% Na_2CO_3; the color was developed for 20 min at 37°. Release of 0.1 μmol of phenol by the enzyme results in an absorbance of 0.60 at 660 nm.

Gas chromatographic analysis of uronic acids was performed by the procedure of Eisenberg (13), which involves reduction to aldonic acids followed by the formation of a butaneboronate derivative.

RESULTS AND DISCUSSION

α-L-Iduronidase activity of purified Hurler corrective factor

Purification of urinary Hurler factor (7) involved ammonium sulfate precipitation, gel filtration on Sephadex G-200, and chromatography on carboxymethylcellulose and, finally, on hydroxyapatite. In the last step, there occurred a resolution of factor activity into two discrete peaks, the most active fractions of which were purified 1000-fold over the starting material.

Selected fractions from the hydroxyapatite separation previously described, as well as those from a repetition of the purification procedure, were simultaneously tested for α-L-iduronidase and retested for activity of Hurler corrective factor. In both cases, two isozyme peaks of α-L-iduronidase activity were found, matching precisely the two peaks of "isofactors" in elution position (Fig. 1).

The ratio of α-L-iduronidase activity to corrective factor activity in the first peak was nearly twice that in the second peak. Presumably, some structural difference between the two isozymes may be the cause of more efficient entry of the second isozyme into Hurler cells, which in turn would result in a better correction. A freshly prepared ammonium sulfate concentrate of urinary proteins [step 1 of the purification (7)], tested at the same time, had a ratio of enzymatic to corrective factor activity similar to that of the second peak. The nature of the difference between the isozymes clearly requires further study.

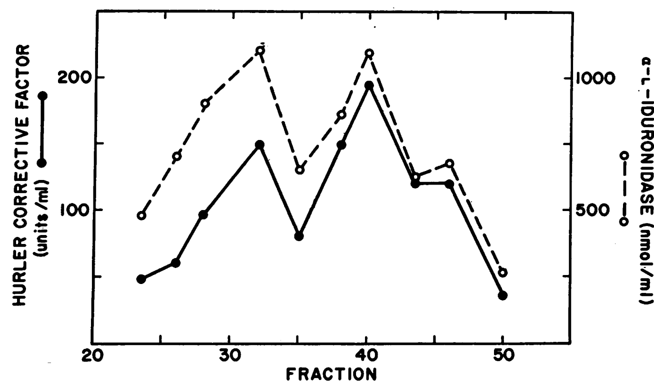

FIG. 1. Correspondence of activities of Hurler corrective factor and α-L-iduronidase in fractions eluted from hydroxyapatite (7). The α-L-iduronidase activity was measured as nmol of phenol liberated in the standard assay, and is expressed on the basis of volume of column fraction used. Activity of Hurler corrective factor is expressed on the same basis.

TABLE 1. *α-L-Iduronidase activity in cell-free extracts of cultured fibroblasts*

Extract	Fibroblasts	Enzyme activity, nmol phenol liberated per 17 hr per mg of protein*
Acetone powder		
	Hurler (J.F.)	0
	Normal	550
	Normal	1000
	Hunter	920
	Sanfilippo A	680
	Sanfilippo B	840
	Maroteaux-Lamy	660
	Morquio	1100
	β-Glucuronidase deficiency	830
	"Gal +"	430
	I-cell	90
Frozen-thawed		
	Hurler (M.B.)	17
	Hurler (C.P.)	9
	Hurler (M.W.)	0
	Scheie (M.Mc.)	11
	Hurler/Scheie (R.S.)	0
	Normal	800
	Hunter	1600

* Values corrected for enzyme blank. Apparent activity of 20 nmol of phenol liberated in the assay per mg of protein is within experimental error of zero activity.

The second product of hydrolysis of phenyl α-L-iduronide was verified to be iduronic acid by gas-liquid chromatography of the butane boronate derivative of idonic acid. Phenyl β-L-iduronide was not hydrolyzed by Hurler corrective factor. Purified Hunter corrective factor (13a) had no α-L-iduronidase activity, nor did it affect the α-L-iduronidase activity of Hurler corrective factor when mixed with it.

Absence of intracellular α-L-iduronidase activity in fibroblasts of patients with Hurler and Scheie Syndromes

Extracts prepared from acetone powders of fibroblasts, or from freshly harvested fibroblasts, were examined for α-L-iduronidase activity. As summarized in Table 1, cells from patients with the Hunter, Sanfilippo, Maroteaux-Lamy, or Morquio syndromes (1), or from patients with atypical mucopolysaccharidoses, such as β-glucuronidase deficiency (14) and "gal +" mucopolysaccharidosis (15, 16), have normal α-L-iduronidase activity. Extracts devoid of α-L-iduronidase activity were obtained from cells of four Hurler patients, one Scheie patient, and one patient reported to have a novel mucopolysaccharidosis (17), but probably fitting the clinical picture of a patient who might have one Hurler and one Scheie gene (a genetic compound, ref. 9). These six lines of

fibroblasts had been previously found deficient in Hurler corrective factor. In addition, α-L-iduronidase activity was low (though detectable) in fibroblasts from a patient with I-cell disease—an unusual genetic disorder characterized by the deficiency of several lysosomal enzymes in fibroblasts (18–20), as well as of Hurler and Hunter corrective factors in fibroblast secretions (21). A profound deficiency of α-L-iduronidase in fibroblasts of Hurler and I-cell patients, as well as in liver and urine of Hurler patients, has also been reported by Matalon and Dorfman (22).

Absence of α-L-iduronidase is not a function of storage time of acetone powders, nor of time between transplantation and harvest of fibroblasts, since the iduronidase-positive and iduronidase-negative cultures were matched with respect to these variables. Mixing the acetone-powder extract of cells of J.F. with that of normal cells gave the calculated average activity; thus, absence of α-L-iduronidase activity in Hurler cells cannot be attributed to the presence of a soluble inhibitor.

α-L-Iduronidase is the only lysosomal enzyme known to be deficient in fibroblasts from patients with the Hurler syndrome. The acetone powder of J.F. cells had normal levels of β-D-galactosidase, α-D-mannosidase, α-L-fucosidase, and arylsulfatase A activities, and elevated β-*N*-acetyl-D-glucosaminidase, β-*N*-acetyl-D-galactosaminidase, and β-D-glucuronidase activities.

It is clear that deficiency of α-L-iduronidase activity can be used for the diagnosis of the Hurler and Scheie syndromes. The enzymatic test is as specific as the bioassay for Hurler corrective factor, and is simpler to perform. The sensitivity of the two tests is similar, requiring about 1–2 mg of cell protein (about $2\text{–}4 \times 10^6$ cells). The α-L-iduronidase assay will therefore not materially reduce the time (5 weeks or even longer) now required for antenatal diagnosis of the Hurler syndrome by assay of the Hurler corrective factor in cultured amniotic fluid cells (23).

Uptake of α-L-iduronidase by Hurler cells

For determination of the relationship between uptake of α-L-iduronidase and correction an unpurified concentrate of urinary proteins, of measured enzymatic and corrective activity, was applied to cells of a Hurler patient (M.W.) under conditions standardized for assay of corrective factor (7). After 48 hr, cells were harvested and intracellular α-L-iduronidase activity was measured (Table 2).

Several points emerge from the results of this experiment. First, maximal correction is not a function of saturation of some transport system for the α-L-iduronidase, since the uptake of the enzyme increases substantially beyond the point of 90% correction. As previously suggested (7), maximal correction is probably reached when the concentration of the product of the added α-L-iduronidase (i.e., dermatan sulfate chains from which the terminal iduronic acid has been removed) becomes saturating for subsequent enzymes in the catabolic chain.

Second, the uptake of α-L-iduronidase from the medium is remarkably efficient, approaching 40% at the lowest level used. This should be compared to a measured uptake of less than 1% for ^{125}I-labeled albumin under similar conditions. The uptake of α-L-iduronidase is clearly attributable to a selective mechanism, rather than to nonspecific pinocytosis.

Finally, the amount of intracellular α-L-iduronidase required to give 90% or higher correction is but a fraction of the normal level of that enzyme (compare with Table 1). Normal cells are apparently supplied with a large excess of α-L-iduronidase, a finding consistent with the normal phenotype of Hurler heterozygotes. One might predict, accordingly, that therapeutic attempts at activation or replacement of the deficient enzyme would be successful if the Hurler patient were provided with only a low level of α-L-iduronidase activity. Indeed, the modest requirement of cells for α-L-iduronidase may explain, in part, the surprising effects of infusing Hurler patients with normal plasma, which has very little activity of Hurler factor (24).

TABLE 2. *Relationship of correction to uptake of α-L-iduronidase*

Applied in medium		Recovered in cells		
Corrective factor (units/plate)	Iduronidase (μunits†/plate)	Iduronidase (μunits†/plate)	Uptake of iduronidase‡	Correction* (% of maximum)
10	175	65	0.37	90
30	525	84	0.16	97
100	1750	160	0.09	99

* Theoretical value, calculated from the number of corrective factor units applied, as described in (7).

† 1 μunit corresponds to the release of 1 nmol of phenol in 1000 min.

‡ Ratio of iduronidase activity recovered in cells to that applied in medium.

We thank Dr. Frank Eisenberg for the gas chromatographic analyses. This work was supported in part by Grant AM 02479 from the National Institutes of Health.

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