Glycosylasparaginase (GA; EC 3.5.1.26) is a lysosomal enzyme that cleaves the N-glycosidic bond between asparagine and N-acetylglycosamine residues in the degradation of glycoproteins (1). Deficient enzyme activity leads to the lysosomal storage disease aspartylglycosaminuria (McKusick 208400). Human GA is synthesized as a single, enzymatically inactive precursor polypeptide. The single-chain precursor is thought to be activated on its way to lysosomes (2, 3) by cleavage into two N-glycosylated subunits of 24 kDa (α) and 18 kDa (β) and association of these subunits to form enzymatically active heterodimer or heterotetramer structures (4). GA is actively transported into human lysosomes via mannose-6-phosphate receptor-mediated endocytosis (5).

I-Cell disease (mucolipidosis II, McKusick 252500) and a clinically milder, form pseudo-Hurler polydystrophy (6) are autosomal, recessively inherited lysosomal storage diseases in which the transport of newly synthesized lysosomal enzymes into lysosomes is affected (6). The defect is in the enzyme, UDP-N-acetylglycosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (EC 2.7.8.17), which adds the phosphatase trafficking signal to lysosomal enzymes. The activity of phosphotransferase can be measured using radioactive UDP-N-acetylglycosamine as the donor substrate and endogenous lysosomal enzymes or artificial carbohydrate structures as the assay acceptors (7). The defective synthesis of mannose-6-phosphate recognition markers on the carbohydrate moieties of lysosomal enzymes leads to the leakage of newly synthesized enzymes out of cells instead of their transport into the lysosomes through mannose-6-phosphate receptor-mediated endocytosis (8). Almost normal activity of certain lysosomal enzymes such as β-glucocerebrosidase (EC 3.2.1.45) and lysosomal acid phosphatase (EC 3.1.3.2) remains in fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy (9). These enzymes continue to be transported to lysosomes as transmembrane proteins because this process does not involve mannose-6-phosphate receptors (10, 11). We now report our findings on GA activity in serum, urine, and fibroblasts from I-cell disease patients, which suggest that the measurement of GA activity can be used as a marker in the detection of I-cell disease.

The fibroblast cell lines were from two different children, both with phenotypes typical of I-cell disease (8). The cultured skin fibroblasts contained pleomorphic material in enlarged lysosomes and very low β-galactosidase (EC 3.2.1.23) and arylsulfatase (EC 3.1.6.1) activities. Highly increased β-galactosidase activity was detected in the serum of both patients. The GA activity was determined fluorometrically using L-aspartic acid β-(7-amino-4-methylcoumarin), from Bachem AG, as substrate (12). The GA activity in cultured fibroblasts and serum was assayed as described previously (12, 13). The GA activity in urine was determined by incubating 100 μL of sample with 20 μL of 10 mmol/L (in ethylene glycol) L-aspartic acid β-(7-amino-4-methylcoumarin) and 80 μL of Tris-HCl buffer (50 mmol/L, pH 7.5) for 2–3 h at 37 °C.

For β-galactosidase or arylsulfatase activity assays, harvested fibroblasts were resuspended in 1.0 mL of distilled water and sonicated for 1 min in ice. The fibroblast homogenate or serum was used to determine the activity of β-galactosidase and arylsulfatase, using 4-methylumbelliferyl β-D-galactopyranoside (9) and 4-methylumbelliferyl sulfate (14), respectively, as substrates (Sigma Chemical Co.). The protein concentration was determined with a protein assay kit (Protein Assay kit; Bio-Rad).

In fibroblasts from the patients with I-cell disease, the GA activity was 8–11%, the β-galactosidase activity was 0.1–4%, and the arylsulfatase activity was 5–12% of the mean activity of the corresponding enzymes in control fibroblasts (Table 1A). The GA activity in serum from the patients with I-cell disease was 9- to 12-fold higher than the mean value in controls. The serum activity of β-galactosidase in the patients with I-cell disease was 14- to 17-fold higher than in healthy controls (Table 1B). The

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**Table 1. GA activity in fibroblasts, serum, and urine in I-cell disease.**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity</th>
<th>I-Cell disease (n = 2)</th>
<th>Controls, mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol · min⁻¹ · mg protein⁻¹</td>
<td>pmol · min⁻¹ · mg protein⁻¹</td>
<td></td>
</tr>
<tr>
<td>A. Fibroblasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycosylasparaginase</td>
<td>5.5/7.5</td>
<td>70 (56–94); n = 5</td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>890/20</td>
<td>21 800 (12 500–33 700); n = 10</td>
<td></td>
</tr>
<tr>
<td>Arylsulfatase</td>
<td>90/190</td>
<td>1650 (960–2700); n = 10</td>
<td></td>
</tr>
<tr>
<td>B. Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycosylasparaginase</td>
<td>160/124</td>
<td>13.4 (9.7–15.4); n = 8</td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>833/1020</td>
<td>58.9 (13–300); n = 8</td>
<td></td>
</tr>
<tr>
<td>C. Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycosylasparaginase</td>
<td>17.8/33.3</td>
<td>2.0 (0.2–4.2); n = 10</td>
<td></td>
</tr>
</tbody>
</table>
activity of GA in urine of the patients with I-cell disease was 9- to 17-fold higher than the mean activity in control urine (Table 1C).

The highly increased GA activity in serum and urine from patients with I-cell disease shows that active GA is truly formed in this disease. Considerable residual GA activity in I-cell disease fibroblasts compared with that in GA-deficient aspartylglucosaminuria fibroblasts (12) indicates that the autoproteolytic cleavage of the precursor polypeptide of the enzyme into subunits and association of at least one of each subunit to form the active enzyme in humans (4,15) takes place inside the cells before leakage into extracellular fluids. The activation thus occurs before transfer of the enzyme into lysosomes, probably in the endoplasmic reticulum, as predicted experimentally (3).

The GA activity in I-cell fibroblasts was <11% of that in control cells, and its activity in serum from patients with I-cell disease was at least ninefold higher than in control serum. The magnitude of the changes in GA activity resembles those reported previously for β-galactosidase and arylsulfatase in patients with I-cell disease (8) and clearly differs from those reported for the membrane-bound enzymes β-glucocerebrosidase and lysosomal acid phosphatase, which continue to be transported to lysosomes in the disease (6,9). Both β-galactosidase and arylsulfatase are targeted into lysosomes predominantly via mannose-6-phosphate receptor-mediated endocytosis (8), which the available evidence suggests is the major transport route of GA into lysosomes in humans as well. This is also supported by in vitro experiments with purified recombinant human GA, in which the enzyme was actively transported from the culture medium into lysosomes of GA-deficient fibroblasts. Enzymatic removal of either the phosphate residues or the carbohydrate chains, however, abolished the uptake of the enzyme into the cells (5). The major increase in GA activity in serum and urine and the markedly reduced activity in cultured fibroblasts can be used as a diagnostic aid for detecting I-cell disease. These findings also suggest that newborn babies affected by I-cell disease could be detected on the basis of high GA values in neonatal screening of aspartylglucosaminuria (16). However, this assay, like others for increased lysosomal enzymes in serum, cannot distinguish between mucolipidosis II and mucolipidosis III, which usually must be differentiated on the basis of events seen in clinical progression.

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Human seminal transferrin, a 79-kDa β-glycoprotein synthesized mainly by Sertoli cells (1,2), is considered an index of gonadal function because its concentration in seminal fluid correlates with sperm count (3,4); it is therefore used as a reliable marker of testicular dysfunction (5,6). This analysis is useful for exploring and monitoring the treatment of idiopathic infertility (7,8). We considered the possibility of measuring seminal fluid transferrin by a simple peak-rate nephelometric method. Protocols used for measuring transferrin in serum or urine cannot be used, because the matrix of this fluid produces high-intensity nonspecific reactions, mainly as an

**References**


