Diagnosis of α-Mannosidosis by Measuring α-Mannosidase in Plasma

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α-Mannosidosis is a lysosomal storage disease resulting from a deficiency of lysosomal α-mannosidase activity. Diagnosis of α-mannosidosis has traditionally been accomplished by demonstrating reduced α-mannosidase activity in leukocytes. We describe a new assay of α-mannosidase in serum or plasma that allows specific detection of the enzyme deficiency in α-mannosidosis with small, easily obtained sample volumes. The assay utilizes 40 μL of serum or plasma and a fluorescent substrate, 4-methylumbelliferyl-α-D-mannopyranoside in sodium acetate buffer, pH 4.0. The mean activity of a control population was 194 (SD 67) mU/L, whereas the activities obtained for four α-mannosidosis patients were 0, 17, 17, and 33 mU/L. Comparison with the standard leukocyte α-mannosidase assay showed this serum or plasma assay to be equally effective in diagnosing α-mannosidosis.

Additional Keyphrases: lysosomes • heritable disorders

α-Mannosidosis is a lysosomal storage disorder caused by a deficiency of lysosomal α-mannosidase (EC 3.2.1.24) activity and characterized by coarse facial features, severe mental retardation, variable hepatosplenomegaly, hearing loss, and dysostosis multiplex (1). The lack of α-mannosidase activity results in storage of mannose-containing glycoconjugates (especially oligosaccharides) in tissues and their excretion in urine. Diagnosis of α-mannosidosis is accomplished by examination of urinary oligosaccharides and assay of acidic α-mannosidase activity. The tissues of choice for diagnostic determination of α-mannosidase activity have been peripheral blood leukocytes and cultured skin fibroblasts. However, it is often difficult to obtain adequate blood samples for leukocyte isolation, particularly from neonates, infants, and young children and from patients who have had large amounts of blood withdrawn for numerous diagnostic tests. Also, only a few patients have skin biopsies for diagnostic testing. A reliable serum or plasma assay for lysosomal α-mannosidase would make diagnosis of α-mannosidosis in infants and young children much easier.

Complicating the analysis of lysosomal α-mannosidase is the presence of at least one other genetically distinct α-mannosidase in serum. Other α-mannosidases, localized to other cellular compartments, have also been detected in various tissues (2–5). Lysosomal α-mannosidase, deficient in α-mannosidosis, is the topic of our investigation.

Reported determinations of plasma α-mannosidase (6–9) have not taken into account interfering factors that could lead to a misdiagnosis. Here we describe an assay of α-mannosidase in serum or plasma that eliminates interference by the nonlysosomal α-mannosidases and specifically detects the lysosomal form of the enzyme.

Materials and Methods

Materials. The substrate, 4-methylumbelliferyl-α-D-mannopyranoside, and the standard, 4-methylumbelliferyl-mannosidase, were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Fisher Scientific Co. (Pittsburgh, PA).

Samples. Serum, plasma, and leukocyte samples used in the α-mannosidase assay were obtained from patients with α-mannosidosis and from apparently healthy normal control subjects. α-Mannosidosis was diagnosed by assay of leukocyte α-mannosidase and urinary oligosaccharides. Samples were stored at –20 °C until use.

α-Mannosidase assay. We assayed α-mannosidase activity in serum and plasma samples by incubating 40 μL of serum or plasma, 10 μL of 4-methylumbelliferyl-α-D-mannopyranoside (10 mmol/L), and 50 μL of sodium acetate (0.25 mol/L, pH 4.0), for 30 min at 37 °C. (We used 0.25 mol/L sodium acetate buffer at various pH values to determine the effect of pH on plasma α-mannosidase activity, as indicated in Figure 1.) We assayed leukocyte α-mannosidase by using 50 μL of the substrate solution, 10 μL of sodium acetate buffer (1 mol/L, pH 4.0), and enough sample to yield 30–60 μg of protein per tube, with a final volume of 100 μL. After incubation,...

Fig. 1. Effect of pH on plasma α-mannosidase activity
Plasma samples from two apparently healthy control subjects (C, □) and three previously diagnosed α-mannosidosis patients (O, ●, △) were assayed for α-mannosidase activity at the indicated pH values

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tion, the samples were placed on ice and the reaction was stopped by adding 2 mL of glycine-KOH buffer (0.25 mol/L glycine, pH 10.3). Fluorescence was determined with a fluorometer at 365 (excitation) and 480 nm (emission). Plasma α-mannosidase activity was expressed as U/L and leukocyte α-mannosidase was expressed as U/g protein. Protein was determined by the method of Lowry et al. (9).

Results and Discussion

Figure 1 illustrates the effect of pH on plasma α-mannosidase activity and the need to perform this assay at a pH close to 4.0. Plasma from control subjects had quantifiable α-mannosidase activity at pH 3.75–4.25, whereas plasma from α-mannosidosis subjects had very little or no activity in this range. At pH ≥ 4.5, however, plasma from the α-mannosidosis patients had substantial α-mannosidase activity, with one patient reaching control concentrations at pH 4.75–5.0. Similar results were obtained by using citrate-phosphate buffer (data not shown). Other reported plasma α-mannosidase assays (6, 8) were done at pH 4.4, which, as shown here, could result in a missed diagnosis.

These data are consistent with previous reports that multiple isoenzymes of α-mannosidase exist in serum and that the form with activity at lower pH values is the enzyme deficient in α-mannosidosis (6, 7, 10, 11). As Figure 1 shows, the lysosomal isoenzyme is a minor contributor to total plasma α-mannosidase activity.

To further characterize this α-mannosidase assay, we examined several relevant indexes. With the use of two different control plasmas, the assay response varied linearly with respect to time (tested up to 60 min) and sample volume (tested up to 50 µL; data not shown). Intra-assay variability was assessed by assaying the same normal control sample in 10 different tubes in the same run. The mean α-mannosidase activity was 182 mU/L (SD 16.7). Likewise, interassay variability was examined by assaying plasma from the same control sample on six different occasions. The mean α-mannosidase activity was 180 mU/L (SD 20.0).

We determined a normal reference range by assaying α-mannosidase activity in plasma from 28 apparently healthy control subjects. The mean α-mannosidase activity of the control subjects was 194 mU/L (SD 67; range 100–367). All four unrelated patients with α-mannosidosis had values (0, 17, 17, and 33 mU/L) >2 SDs less than the mean of the control subjects with this assay.

α-Mannosidase activity in plasma of patients with other lysosomal storage diseases was increased two- to threefold above the normal mean. A similar increase in α-mannosidase activity was observed in plasma of pregnant women, consistent with previous findings (12). Reportedly, serum α-mannosidase is also increased in patients with alcoholic liver disease (13). The significance of these increased activities is unclear but may be related to hormonal effects on the regulation of lysosomal enzymes and the effects of alcoholic hepatopathology.

To date, >200 patients have been tested with this assay, and no false-positive or false-negative results have been obtained. That is, all patients who have had normal plasma α-mannosidase concentrations have not later been diagnosed as having α-mannosidosis. Also, all patients who exhibited below-normal α-mannosidase activity had the disease confirmed by assay of leukocyte or fibroblast α-mannosidase activity and by examination of urinary oligosaccharides.

We tested for possible interference by substances commonly found in excess in patients’ samples. Glucose at concentrations of 4 and 10 g/L and bilirubin at 0.025 and 0.1 g/L had no effect on α-mannosidase activity in this assay system (data not shown).

We found no significant difference between values obtained for serum (207 ± 77 mU/L) and plasma (199 ± 72 mU/L) α-mannosidase activity. Analysis of matched pairs yielded a confidence level of 99%, indicating that either type of sample is appropriate for this assay.

Finally, because the standard method of diagnosing α-mannosidosis has been the leukocyte assay, we compared the results obtained with our plasma assay and the leukocyte assay for four apparently healthy control subjects and two α-mannosidosis patients (Table 1). Both assays yielded results of 3–10% of the mean of the control-subject values for the α-mannosidosis patients. Therefore, the plasma assay can be used as a reliable replacement for the standard leukocyte assay.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Plasma, mU/L</th>
<th>Leukocytes, U/g protein</th>
</tr>
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<tbody>
<tr>
<td>Normal control 1</td>
<td>317</td>
<td>6.66</td>
</tr>
<tr>
<td>Normal control 2</td>
<td>150</td>
<td>5.48</td>
</tr>
<tr>
<td>Normal control 3</td>
<td>167</td>
<td>5.92</td>
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<tr>
<td>Normal control 4</td>
<td>250</td>
<td>6.93</td>
</tr>
<tr>
<td>Disease patient A</td>
<td>17</td>
<td>0.30</td>
</tr>
<tr>
<td>Disease patient B</td>
<td>17</td>
<td>0.20</td>
</tr>
</tbody>
</table>

In summary, we have described a specific assay for diagnosis of α-mannosidosis by using serum or plasma as the source of enzyme. Because of its reliability and because it will conserve leukocytes for other diagnostic tests, the assay will be useful in the evaluation of lysosomal storage disease in all patients for whom α-mannosidosis is a diagnostic consideration. It will be particularly useful for evaluating α-mannosidosis in young children and infants, in whom the amount of leukocytes is often limited, and will decrease the need for obtaining additional blood specimens. Finally, in contrast to the leukocyte assay, plasma and serum assays of lysosomal α-mannosidase activity are readily adaptable to automated analytical systems used in conventional clinical chemistry laboratories.

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References


