Measurement of α-Glucosidase Activity in Serum from Patients with Cystic Fibrosis or Pancreatitis

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We measured the activity of a non-lysosomal α-glucosidase with pH optimum near 6.0 in serum from a wide variety of patients, using the fluorogenic substrate, 4-methylumbelliferyl-α-D-glucopyranoside. Acutely ill patients with cystic fibrosis (CF) demonstrated significant increases in α-glucosidase compared with CF outpatients. The former group of CF patients experienced far more severe chronic pulmonary disease than did the latter, whereas both groups had similar degrees of gastrointestinal impairment. Patients with pancreatitis associated with trauma or complicated by severe necrosis, hemorrhage, or abscess also displayed greater increases in α-glucosidase than did patients with uncomplicated (edematous) pancreatitis. For CF outpatients and patients with either edematous pancreatitis or pancreatic cancer, the α-glucosidase activity was similar to that for the general hospital-patient population. Corresponding changes were not observed for other measured serum glycosidases (α-fucosidase, α-mannosidase, β-galactosidase, β-N-acetylglucosaminidase). Measurement of serum α-glucosidase may be of value in assessing the clinical course in CF and in differentiating necrotizing from edematous pancreatitis.

Cystic fibrosis (CF), the most common lethal genetic disease among Caucasians, is characterized by abnormal exocrine secretions, resulting in chronic pulmonary disease, pancreatic insufficiency, failure to thrive, and the presence of abnormally high concentrations of sodium and chloride in sweat.

Speculation concerning possible abnormal glycoproteins or their metabolism in CF arises from the accumulation of glycoprotein-rich mucus secretions associated with much of the pathology of this disease (1, 2). Several investigators have studied lysosomal glycosidases, which are involved in glycoprotein catabolism (3, 4). Antonowics et al. (5) detected a marked increase in α-glucosidase (EC 3.2.1.20) activity but normal activities of six other hydrolases in the lysosomes of cultured lymphoid cells obtained from CF patients as compared with controls. Moreover, values for α-glucosidase activity were increased less in lysosomes from cultured CF heterozygote lymphoid cells. Increased α-glucosidase activity but normal activities for six other hydrolases was subsequently observed in cultured fibroblasts from some but not all cases of CF studied by Hosli et al. (6). Fibroblasts from the most severely affected CF patients expressed high α-glucosidase activity.

Other studies of lysosomal glycosidases have shown an increase in activity of β-N-acetylglucosaminidase, β-glucuronidase, β-glucosidase, and β-galactosidase but decreased activity of α-mannosidase and α-fucosidase in cultured fibroblasts from patients with CF as compared with controls (7); normal sialidase, α-mannosidase, and β-N-acetylglucosaminidase activities in CF liver tissue (8); or increased activity of α-fucosidase despite normal or decreased activities of nine other lysosomal hydrolases, including α-glucosidase, in CF fibroblasts (9). Differing tissue-culture conditions as well as disease or genetic heterogeneity may, in part, account for some of the inconsistencies noted.

CASOLA et al. (10) extended the study of lysosomal enzymes in CF by measuring the activity of eight glycosidases in serum. These investigators observed a significant increase in serum α-glucosidase, measured at pH 4.5, but normal values for other measured glycosidases for CF patients. On the other hand, Hultberg et al. (11) found, for sera from CF patients, a statistically significant increase not only in the activity of α-glucosidase but also of β-galactosidase, α- and β-glucuronidase, and β-N-acetylglucosaminidase. Both groups found that the degree of increase in serum α-glucosidase correlated with the severity of the disease.

No obvious or consistent pattern emerges from studies of lysosomal glycosidases that would suggest a possible biochemical defect in CF. Nevertheless, the consistent observation of increased serum α-glucosidase in CF patients prompted us to investigate further some kinetic properties of this enzyme and to define the clinical specificity and significance of its above-normal activity in serum.

Subjects, Materials, and Methods

Subjects

CF patients: We included 13 inpatients (nine male, four female; mean age, 10.6 ± 6.8 yr) and eight outpatients (four male, four female; mean age, 11.3 ± 7.0 yr) in the study. The severity of disease was judged, without prior knowledge of the serum α-glucosidase activity, according to the following criteria:

Pulmonary

Severe. The presence of one or more of the following conditions: severe roentgenographic picture such as marked increase in anterior–posterior diameter or advanced pulmonary parenchymal disease; three or more hospital admissions for pulmonary-related problems per year; severe clinical findings such as cyanosis, clubbing, hemoptysis, cor pulmonale, pulmonary hypertension, or chronic dyspnea; and (or) inability to perform regular activities, such as attending school.

Moderate. Mild pulmonary changes on chest roentgenogram; one or two hospital admissions per year; little or no clubbing, no cyanosis, no hemoptysis; attends school.

Mild. Minimal or no pulmonary abnormalities on chest roentgenogram or by auscultation; no hospital admissions within past year; good exercise tolerance, attends school.

Gastrointestinal

Severe. The presence of one or more of the following conditions: persistent fatty stools despite enzyme supple-

Reference:


mentation, meconium ileus equivalent, bowel obstruction or perforation, rectal prolapse, weight loss or failure to gain weight, hypoproteinemia with or without edema, severe liver disease (cirrhosis), diabetes mellitus.

Moderate. More than two stools per day, occasional fatty stool with enzyme supplementation, weight gain erratic and below 5th percentile, dietary restrictions required.

Mild. One or two stools per day, well formed, only rare fatty stool, consistent weight gain, easily maintained on enzyme supplementation, no dietary restrictions.

Pancreatitis patients

Complicated. Eight males, three females (mean age 39.6 ± 12 yr) with the following conditions surgically documented: pancreatic abscess (5), hemorrhagic pancreatitis (1), periampullary or retroperitoneal hematoma (2), periampullary or retroperitoneal hemorrhage with abscess (3).

Uncomplicated (edematous). Seven males, eleven females (mean age, 52 ± 18 yr) who were diagnosed as having pancreatitis based on clinical symptomology, ultrasound studies, elevated serum and/or urine amylase, elevated serum lipase, and an appropriate response to conservative therapy.

General hospital patients

Ninety-two males, 101 females (average age, 49 ± 21 yr) randomly selected from pediatric and adult medical, surgical, obstetrical, and psychiatric services.

Normal individuals

Twenty-six males, 16 females apparently healthy adults; average age 24 ± 3 yr.

Specimens

Blood samples were obtained by venipuncture, allowed to clot, and the sera separated by centrifugation and stored at −20 °C until time of assay.

Pancreatic extracts were prepared by homogenizing pancreatic tissue in a blender, then adding five times the volume of bovine serum albumin solution (50 g/L), mixing, and centrifuging at 1000 × g for 10 min. Aliquots were stored at −20 °C.

Reagents

4-Methylumbelliferyl-α-d-glucopyranoside (MUG), 4-methylumbelliferyl-α-L-fucopyranoside, 4-methylumbelliferyl-α-D-mannopyranoside, 4-methylumbelliferyl-β-D-glucuronide, 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside, and 4-methylumbelliferyl were from Research Products International Corp., Mt. Prospect, IL 60056; 2-methoxyethanol was from Aldrich Chemical Co., Milwaukee, WI 53233. All other reagents were analytical grade.

α-Glucosidase Assay

Substrate. A stock substrate solution (60 mmol/L) was prepared by dissolving 10.2 mg of MUG in 0.5 mL of 2-methoxyethanol. This requires intermittent vortex-mixing for approximately 30 min. Some heating (running hot water from tap) may also be required.

The working substrate (6.0 mmol/L) was prepared by diluting one part of stock substrate with nine parts of citric acid (0.1 mol/L), Na2HPO4 (0.2 mol/L) buffer, pH 6.0.

Because of limited aqueous solubility, it was necessary to first dissolve MUG in 2-methoxyethanol (12) before incorporating it into the assay buffer. The sensitivity of the assay is thereby greatly increased, because, in the absence of 2-methoxyethanol, MUG concentrations are limited to those well below the apparent Km of 1 mmol/L.

Enzyme activity determination. A 10-µL aliquot of serum was delivered to a 12 × 75 mm polystyrene test tube, followed by 100 µL of working substrate. The tubes were capped, their contents mixed, and the tubes were then placed in a 37 °C water bath for 60 min. The reaction was terminated by the addition of 2.0 mL of glycine buffer (0.2 mol/L, pH 10.5). The fluorescence of the released 4-methylumbelliflorone was measured with an Amino-Bowman spectrophotofluorometer with excitation and emission wavelengths at 365 and 445 nm, respectively, and compared to the fluorescence intensity of a standard solution of 4-methylumbelliflorone (0.01 mmol/L). A substrate blank was used to zero the instrument. The fluorescence produced by serum blanks was negligible and therefore such correction was not necessary. All assays were performed in duplicate.

One activity unit corresponds to 1 µmol of substrate hydrolyzed per hour under the assay conditions.

Under the assay conditions, the hydrolysis of MUG was linearly related to time over 60 min for enzyme activities up to at least 180 U/L.

pH optimum. Assays were performed at a constant MUG concentration of 6.0 mmol/L but with the composition of the citrate/phosphate buffer varied to provide pH values between 5.0 and 8.0.

Km determination. Assays were performed at pH 6.0 in citrate/phosphate buffer with MUG concentrations of 6.0, 3.6, 2.4, 1.2, 0.6, and 0.3 mmol/L. The Km was determined from a Lineweaver–Burk plot.

Assay of Other Glycosidases

Activities of four other serum glycosidases were determined in a similar manner to that for α-glucosidase. Substrate concentrations were as follows:

α-Fucosidase: 4-Methylumbelliferyl-α-L-fucopyranoside (0.65 mmol/L) in citric acid (0.1 mol/L), Na2HPO4 (0.2 mol/L) buffer, pH 5.5. Sera were pre-diluted 1:2 with NaCl solution (9 g/L), and samples were incubated for 30 min at 37 °C.

α-Mannosidase: 4-Methylumbelliferyl-α-D-mannopyranoside (10 mmol/L) in citric acid (0.1 mol/L), Na2HPO4 (0.2 mol/L) buffer, pH 5.5. Sera were pre-diluted 1:3 with NaCl solution, and samples were incubated as for α-glucosidase assay.

β-Glucuronidase: 4-Methylumbelliferyl-β-D-glucuronide (5 mmol/L) in sodium acetate (0.1 mol/L) buffer, pH 4.0. Sera were pre-diluted 1:3 with NaCl solution and samples incubated as for α-glucosidase assay.

β-N-Acetylglucosaminidase: 4-Methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside (6 mmol/L; prepared as for MUG) in citric acid (0.1 mol/L), Na2HPO4 (0.2 mol/L) buffer, pH 4.5. Sera were pre-diluted 1:21 with NaCl solution and samples incubated as for α-glucosidase assay.

Results

The mean activity of α-glucosidase was significantly higher for all groups studied when compared with normal controls (Table 1). Moreover, both CF inpatients and outpa-
Table 1. Activity of Five Glycosidases in Serum

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>CF patients</th>
<th>Pancreatitis</th>
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<tr>
<td></td>
<td>Normals</td>
<td>CF patients</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>α-Glucosidase&lt;sup&gt;4&lt;/sup&gt;</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>SD</td>
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<tr>
<td>n</td>
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<td>21</td>
</tr>
<tr>
<td>p</td>
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<tr>
<td>α-Fucosidase</td>
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<tr>
<td>SD</td>
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</tr>
<tr>
<td>n</td>
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<tr>
<td>p</td>
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<td>α-Mannosidase</td>
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<td>n</td>
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<tr>
<td>p</td>
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<td>β-Glucuronidase</td>
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<td>n</td>
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<td>p</td>
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<tr>
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</table>

<sup>a</sup> Mean activity (μmol/hr per liter), with SD, number of specimens (n), and significance value (p) for two-tailed Student's t-Test. <sup>b</sup> See also Figure 1. <sup>c</sup> p < 10<sup>-4</sup>. NS, not significant.

Patients had higher (p < 10<sup>-8</sup> and < 10<sup>-4</sup>, respectively) mean serum α-glucosidase when compared with the general hospital-patient population. However, α-glucosidase activity for CF outpatients and general hospital patients were within similar ranges (< 60 U/L), whereas CF inpatients all had values greater than 60 U/L (Figure 1). The difference between the mean α-glucosidase values for serum of CF inpatients and outpatients was statistically significant (p < 10<sup>-4</sup>).

The severity of disease in CF patients was evaluated according to the criteria presented under Subjects, without prior knowledge of the assay results, and was quantified by assigning a value of 3 for patients with severe, 2 for moderate, and 1 for mild pulmonary or gastrointestinal symptoms. The average scores for chronic gastrointestinal complications were similar for both CF inpatients and outpatients, i.e., 2.1 ± 0.5 and 2.1 ± 0.4, respectively. The associated scores for chronic respiratory disease were 2.6 ± 0.5 for the hospital group but only 1.4 ± 0.5 for the clinic patients (p < 10<sup>-4</sup>). Thus, CF patients with a greater degree of respiratory disease have higher α-glucosidase values than those CF patients with less severe pulmonary complications.

Of 222 non-CF patients, hospitalized for a variety of disorders, the only group with α-glucosidase increases in the same range as for hospitalized CF patients were those with severe pancreatic disease or trauma (Figure 1). Patients with uncomplicated acute pancreatitis generally had α-glucosidase activity in the same range as for the general hospital-patient population, as did 16 cancer patients, five of whom had pancreatic carcinoma.

α-Glucosidase in sera from patients with CF or pancreatitis displayed a broad pH activity profile, with a maximum between pH 5.5 and 6.0. Thus, the activity in serum of these patients is primarily due to the presence of increased "neutral" rather than lysosomal α-glucosidase. The latter has a pH optimum near pH 4.0 (14). In sera from some CF heterozygotes, normal individuals, and general hospital patients, the presence of additional α-glucosidase components with peak activity near pH 4.5 and pH 7.5 was observed. The apparent Km for α-glucosidase was found to be 1 mmol/L (1.1 ± 0.2 mmol/L; n = 19), with no significant differences between sera from normal persons, CF parents or patients, patients with pancreatitis or other diseases, or for pancreatic extracts. The similarities with regard to pH activity profiles and Km values suggest that different quantities of the same or a closely related enzyme are present in the serum of each of these individuals.
For other serum glycosidases measured (Table 1), no significant differences in the activities of either α-fucosidase, α-mannosidase, or β-glucuronidase were apparent when CF patients were compared with normal controls. Likewise, the activity of β-N-acetylgalactosaminidase was similar for CF patients, CF parents, and general hospital patients. Although sera from only a limited number of patients with complicated pancreatitis were examined, the activities of α-mannosidase and β-glucuronidase were similar to those for either normal controls or patients hospitalized for other causes, whereas α-fucosidase mean activity was significantly lower (p <0.05) than normal. Thus, of the five serum glycosidases measured, only α-glucosidase was significantly increased in severely affected CF patients and patients with traumatic/necrotic pancreatitis.

Discussion

α-Glucosidase in Cystic Fibrosis

Although α-glucosidase activity in serum may be increased in a wide variety of patients, the highest activity is observed in acutely ill CF patients and in patients with necrotic or traumatic pancreatitis (Figure 1). This observation, coupled with the similar pH optimum and K_m values for pancreatic tissue extract and serum α-glucosidase, suggests that enzyme release may occur after significant destruction of pancreatic tissue. However, clinical scores of the severity of pancreatic and pulmonary dysfunction for the group of hospitalized CF patients (α-glucosidase >60 U/L) compared with the CF clinic patients (α-glucosidase <60 U/L) appears not to support the concept of a direct relationship between pancreatic disease in CF and enzyme activity in serum. The average scores for chronic complications related to malabsorption were similar for clinic and hospitalized CF patients. On the other hand, the associated scores for chronic respiratory disease were significantly higher for the hospital group than for the clinic patients. Thus, the magnitude of the increase in serum α-glucosidase activity would appear to correlate more closely with the severity of pulmonary disease than with chronic pancreatic disease. In this regard, non-CF patients hospitalized for bacterial pneumonia, chronic obstructive pulmonary disease, pulmonary abcesses, synovial carcinoma metastatic to lung with bronchitis, or patients with asthma or generalized sepsis all had serum α-glucosidase activities below 50 U/L (Figure 1). Therefore, the magnitude of the enzyme elevations observed for the hospitalized CF group does not appear to be a general consequence of pulmonary disease or sepsis.

The division between CF patients on the basis of serum α-glucosidase activity and disease severity is further illustrated by follow-up evaluation of survival. In the group of 13 CF patients who were hospitalized at the time of our study (α-glucosidase >60 U/L), 10 subsequently died within one to four years. On the other hand, no deaths have occurred in the group of CF patients who were doing well when initially studied during a routine clinic visit (α-glucosidase <60 U/L).

Increased activity of α-glucosidase, but normal activities for six other hydrolases, has been observed in cultured lymphoid cells (5) and fibroblasts (6) from CF patients. Moreover, α-glucosidase activity was elevated in fibroblasts from some but not all CF patients (6). It was inferred that the more severely affected CF patients were those with increased α-glucosidase activity. This heterogeneity of α-glucosidase activity observed in cultured cells from CF patients and its implied association with clinical severity appear to extend to serum.

We can only speculate as to the mechanism of the increased serum α-glucosidase in severely affected CF patients. It is possible that these patients have an increased cellular content of α-glucosidase, perhaps a response to the accumulation of some abnormal mucoprotein product (6). General cell turnover, especially of leukocytes during an acute respiratory infection, may result in abnormally high α-glucosidase activity in serum. Non-CF patients with similar infections may also have some increase in serum α-glucosidase, but because of the normal cellular α-glucosidase activity not to the same degree as for this category of CF patients. However, high serum α-glucosidase is not limited to those CF patients with acute respiratory infections. Two CF patients had high serum α-glucosidase activity (118 and 154 U/L) when hospitalized for problems related to pancreatic disease, although each did experience severe chronic respiratory disease. Therefore, high serum α-glucosidase in CF may be a consequence of a more general release of enzyme from tissue. For instance, fundamental changes resulting in altered membrane release and/or uptake of α-glucosidase may occur in the more severely affected CF patients. The increase in α-glucosidase observed for acutely ill CF patients is selective in that similar elevations were not observed for α-fucosidase, α-mannosidase, β-glucuronidase, or β-N-acetylgalactosaminidase. Therefore, there apparently is no general defect in glycosidase transport.

Post-translational changes in α-glucosidase might also result in decreased clearance from the circulation by hepatocyte (14) or reticuloendothelial cell receptors (15, 16). Whatever the mechanism resulting in the increased serum α-glucosidase in CF patients, measurement of this enzyme activity may be of value for the early identification of those CF patients who are most severely affected.

α-Glucosidase in Pancreatitis

The development of pancreatic abscess is a complication of acute pancreatitis that requires surgical intervention, and the earlier this complication is recognized, the better the prognosis (17). The determination of serum methemalbumin has been used as an indicator of hemorrhagic pancreatitis but is generally unreliable (18). Measurement of serum ribonuclease as a marker for pancreatic necrosis is promising although preliminary (17). The increase in serum α-glucosidase that we observed in patients with necrotizing or traumatic pancreatitis suggests that measurement of this enzyme may be of value in assessing such complications in acute pancreatitis or abdominal trauma. We speculate that the increased α-glucosidase activity observed in the serum of these patients is derived directly from pancreatic tissue destruction. Increased serum α-glucosidase, measured at pH 6 with maltose as substrate, was previously reported for patients with acute pancreatitis (19). Moreover, the activity in serum continued to increase in one patient with hemorrhagic pancreatitis when the serum amylase was declining.

While further studies will be necessary to gain a more complete understanding of the significance of the increase in serum α-glucosidase observed in patients with CF or severe pancreatitis, the data reported here suggest that measurement of this enzyme activity may have clinical utility in each of these disease states. Moreover, fundamental studies related to the function of "neutral" α-glucosidase and to those factors that regulate its activity may lead to a better understanding of the basic defect in CF.
References