ELISA for Urinary Trehalase with Monoclonal Antibodies: A Technique for Assessment of Renal Tubular Damage

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Background: α,α-Trehalase, located on renal proximal tubules, is a glycoprotein that hydrolyses α,α-trehalose to two glucose molecules. Urinary trehalase reflects damage to renal proximal tubules, but its activity has not been measured routinely because measurement of catalytic activity is rather complicated and because conventional assays for enzyme activity might not reflect all of the trehalase protein because of enzyme inactivation in urinary samples.

Methods: We established novel monoclonal antibodies for human trehalase and a sandwich ELISA for quantification of urinary trehalase. We determined the urinary trehalase protein concentration with this ELISA and trehalase catalytic activity, and the results of these two methods were compared.

Results: The ELISA system was more sensitive than the detection of enzyme activity and could detect a subtle difference in the amount of trehalase present in renal diseases. The within- and between-assay CVs in the ELISA were 6.7–7.6% and 6.2–8.2%, respectively. Highly significant increases in both the quantity and activity were seen in patients with nephrotic syndrome (acute phase), Lowe syndrome, and Dent disease. The quantities were 70- to 200-fold greater, whereas enzyme activities were, at most, 10-fold higher than those of control subjects. In the detection of small amounts of trehalase in patients with chronic glomerulonephritis and renal anomalies, quantities were better than enzyme activities.

Conclusions: We have established an ELISA system for quantification of urinary trehalase that uses novel monoclonal antibodies. Our ELISA system is simpler and more sensitive than a conventional activity assay and reflects trehalase protein. This ELISA can be a useful as a common tool for clinical assessment of renal proximal tubular damage.

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α,α-Trehalase (EC 3.2.1.28) is an ectoenzyme located on the brush border membrane of mammalian kidney and small intestine. The enzyme catalyzes hydrolysis of α,α-trehalose (1-α-d-glucopyranosyl-α-d-glucopyranoside) to form two molecules of glucose, and substrate specificity of the enzyme is highly restricted. The glucose thus formed in the intestine probably is absorbed to be utilized as an energy source because patients deficient in intestinal trehalase have been reported to show diarrhea after ingestion of trehalose-containing mushrooms (1). The function of renal trehalase, however, is unknown. Increased trehalase activity in urine has been reported to be associated with damage to renal proximal tubules and some kinds of renal diseases (2–4).

Mammalian trehalase is linked to membrane by a glycosylphosphatidylinositol anchor, and phosphatidylinositol-specific phospholipase C is required for the trehalase to be completely liberated from the membrane (5). The enzyme has been purified from mammalian kidneys and shows a molecular mass of 75 kDa (6–8). Trehalase cDNA has been isolated from rabbit (6), human (7, 9), and rat (10) tissue. The complete sequence of the human trehalase cDNA showed that the enzyme consists of 583 amino acid residues and contains a putative leader peptide of 19 amino acids at the NH2-terminus, five putative glycosylation sites, and a hydrophobic region at the

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COOH terminus, corresponding to the site of the glycosylphosphatidylinositol anchor (9).

Trehalase activity in urine has been estimated by determination of formed glucose after the removal of endogenous glucose from urinary samples by complicated gel filtration (3,7). The enzyme activity is also measured spectrophotometrically with oxidation of glucose by glucose dehydrogenase (11). Quantification of trehalase is useful in the diagnosis of renal diseases, but the activity in human urine is quite low. Moreover, methods for determining the activity usually are time-consuming and complicated, and their application to routine clinical study is difficult. Measurement of the enzyme activity also has a disadvantage, because the activity of the enzyme present in urine can be different among diseases because of a membrane-anchored protein. Furthermore, it is unclear whether the urinary enzyme activity reflects the actual concentration.

Demonstration of functional expression of human trehalase in Escherichia coli (9) facilitates the isolation of monoclonal antibody for human trehalase that can be used to develop an ELISA method. In this study, therefore, we isolated the recombinant enzyme and obtained monoclonal antibodies against human trehalase; we then applied them in a rapid and highly sensitive ELISA method to evaluate urinary trehalase. In addition, we compared urinary trehalase concentrations and N-acetyl-β-d-glucosaminidase (NAG) activity in patients with renal diseases. We also performed histochemical analysis of renal trehalase and showed extremely high excretion of trehalase in the urine of patients with tubular damage.

**Materials and Methods**

**DESIGN AND SYNTHESIS OF PEPTIDE FOR IMMUNIZATION TO RAISE AN ANTIBODY**

Monoclonal antibodies against human trehalase were raised using recombinant trehalase and synthesized trehalase peptide. A sequence corresponding to amino acid residues 291–307 (SKDVELADTLPEGDREA) (9) was selected. A cysteine residue was introduced into the C-terminal region of the peptide for conjugation to hemocyanin. The above peptide was synthesized by the standard 9-fluorenlymethoxycarbonyl solid-phase synthesis method (12). Peptides were analyzed and purified by reverse-phase HPLC. Recombinant human trehalase (9) was also used as an immunogen after partial purification by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Eight-week-old female BALB/c mice were immunized by intraperitoneal injections of the recombinant protein or conjugated peptide with the adjuvant. Three or four consecutive injections of the immunogen were given at weekly intervals starting 2 weeks after the first immunization. The mice were sacrificed 3 or 4 days after the last injection. The spleen cells were fused with P3.X63/Ag8.U1 (P3.U1) by the modified method of Kohler and Milstein (13). The fused cells were cultured with 96-well culture plates (Nunc) in RPMI 1640 (Nissui) containing fetal bovine serum, hypoxanthine, aminopterin, and thymidine. After 8–10 days of culture, the antibody activity of the culture supernatant from each well of the hybridoma was tested by ELISA. Positive wells were subcloned twice by a limiting dilution method.

**SELECTION OF MONOCLONAL ANTIBODY BY ELISA**

A 96-well ELISA plate (Greiner) was coated with the recombinant trehalase purified by SDS-PAGE (9) or obtained conjugated peptide at 4 °C for 16 h. After the removal of the protein solutions, the plate was washed three times with phosphate-buffered saline (PBS) and blocked by PBS containing 10 g/L bovine serum albumin, followed by a 1-h incubation at room temperature. After the plate was washed with PBS, the supernatant from the hybridoma culture was added and incubated at room temperature for 2 h. The plate was washed four times with PBS containing 0.5 g/L Tween 20 (PBS-Tween 20), after which horseradish peroxidase (HRP)-conjugated rabbit anti-mouse immunoglobulin (Dako) was added, and the plate was incubated at room temperature for 1 h. After the plate was washed four times with PBS-Tween 20, it was incubated with HRP substrate, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and H2O2 at room temperature. The absorbance was measured with a microplate reader (Model 550; Bio-Rad) at 415 nm.

**SANDWICH ELISA FOR DETERMINATION OF URINARY TREHALASE**

The wells of 96-well microplates (Nunc) were coated overnight with 50 μL of monoclonal antibody for human trehalase KM2287 (10 mg/L in PBS) at 4 °C. After removal of the antibody solution, plates were washed three times with PBS. For blocking, PBS containing 10 g/L bovine serum albumin (100 μL) was added to each well, and the plates were incubated at 4 °C overnight. After removal of the blocking solution, urine samples diluted 10-fold with PBS containing 1 g/L SDS (50 μL) and then heated were added to the wells and incubated at 37 °C for 1 h by shaking at 130 rpm. The purified trehalase (50 μL) in a concentration range of 13–994 μg/L was also incubated and served as a calibrator. Plates were subsequently washed five times with PBS-Tween 20. A biotin-conjugated monoclonal antibody KM2275 solution (50 μL of a 1 mg/L solution in PBS containing 10 g/L bovine serum albumin) was added to the wells, and the plates were incubated at room temperature for 2 h. After the plates were washed five times with PBS-Tween 20, 50 μL of HRP-streptavidin (Dako) was added. The samples were left at room temperature for 1 h. The plates were washed five times with PBS-Tween 20, and then H2O2 and 50 μL.
of the substrate solution, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), were added to each well. After a 15-min incubation at room temperature, 50 μL of a 50 g/L SDS solution was added to stop the reaction. Absorbance was measured with a microplate reader at 415 nm. All experiments were performed in duplicate.

ASSAY OF TREHALASE ACTIVITY
The trehalase activity in urine was assayed as described previously (3,7). Briefly, a urine sample (0.5 mL) was passed through a Sephadex G-25M column (PD-10; Pharmacia, LKB Biotechnology) equilibrated with 5 mmol/L phosphate buffer, pH 6.2, to remove endogenous glucose. The fractions containing proteins were collected, and the volume was adjusted to 1.5 mL. To 0.9 mL of the eluted sample was added 0.1 mL of 0.25 mol/L trehalose; the mixture was then incubated at 37 °C for 120 min. The glucose thus formed was determined with a glucose determination kit (Kyowa Medics). Trehalase activity was expressed as micromoles of glucose liberated per hour per liter of urine.

NAG ENZYME ASSAY
Enzyme activity of NAG was assayed with the sodium-cresolsulphonphthaleinyl N-acetyl-β-D-glucosaminidase method using an NAG determination kit (Shionogi Co. Ltd.).

CREATININE ASSAY
Creatinine was assayed by the method of Jaffe with a creatinine determination kit (Wako Pure Chemicals).

The amount of trehalase (as estimated by ELISA), trehalase activity, and NAG activity were expressed as their ratios to creatinine.

IMMUNOBLOT ANALYSIS OF RENAL AND URINARY TREHALASE
A specimen of a human kidney cortex-mix obtained from a nephrectomized patient with renal carcinoma and sections of porcine and bovine kidneys were used for immunoblotting. The tissue was lysed with Laemmli sample buffer (14); the lysates were then sonicated and boiled for 1 min. The proteins were separated by SDS-PAGE. After an electrophoretic run, proteins on the gel were electroblotted onto a polyvinylidene difluoride membrane (Bio-Rad). The conditions of immunoblotting and detection of the cross-reactive antigen were as described previously (7). To assess the amount of urinary trehalase, urine samples were mixed with Laemmli sample buffer and boiled for 1 min, and urine samples (10 μL) were loaded in the slots of the gel. SDS-PAGE was carried out as described previously (7).

IMMUNOHISTOCHEMICAL STAINING
A piece of the human kidney described above was fixed with phosphate-buffered formalin, dehydrated, and embedded in paraffin wax. Tissue sections of 3 μm were cut and mounted onto glass slides and dried at 50 °C overnight. Sections were dewaxed in xylene, rehydrated in a gradual alcohol series, fixed in methanol, incubated for 10 min with 30 mL/L H2O2 to block the endogenous peroxidase activity, and subsequently washed with distilled water. For trehalase staining, sections were incubated for 15 min in a prewarmed solution of 1 g/L pepsin dissolved with HCl. After blocking with Blocking Solution 2 (Dako), sections were incubated with primary monoclonal antibody at 4 °C overnight. After sections were washed three times with Tris-buffered saline, they were incubated at room temperature for 90 min with HRP-conjugated antimouse immunoglobulin (EnVision System; Dako). After washing, sections were developed with diaminobenzidine reagent, rinsed in distilled water, counterstained with hematoxylin (Merck), dehydrated, and mounted with a Malinol (Muto Pure Chemicals).

URINE SAMPLES
Spot urine samples were obtained from 41 healthy children (16 males and 25 females; age range, 0–18 years) and 41 patients (23 males and 18 females; age range, 0–19 years) with renal diseases, including nephrotic syndrome (acute and remission phases), chronic glomerulonephritis such as IgA nephropathy and anaphylactoid purpura glomerulonephritis, renal anomaly, acute renal failure, and those with renal proximal tubular damage, including Lowe syndrome and Dent disease. Urine specimens were centrifuged at 900g at 4 °C, and supernatants were withdrawn and stored at −60 °C until analysis. All subjects gave their informed consent before participating in this study. The data were assessed by the Mann–Whitney U-test.

Results
CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR HUMAN TREHALASE
Cell fusion gave eight hybridoma clones that produced antibodies (KM2275, KM2276, and KM2285–KM2290). The monoclonal antibodies KM2275 and KM2276 were raised against recombinant trehalase, and others were against the constructed peptide. The antibodies KM2275 and KM2276 responded to the recombinant trehalase but not to the constructed peptide. We previously observed (9) that trehalase mRNA is expressed predominantly in the kidney and small intestine, a finding consistent with observations that trehalase is located on the brush border membrane of the proximal tubules, but other regions, such as glomeruli, distal tubules, and collecting ducts, were not stained (Fig. 1). Staining patterns similar to those above were observed with KM2287 (data not shown). Immunoblot analysis with KM2275 disclosed that a sharp band with molecular mass of 75
kDa was observed with the human kidney, but not with the porcine and bovine kidneys (Fig. 2A, lanes 1–3). Moreover, of all the antibodies we obtained, only KM2287 and 2275 exhibited a good response to both the recombinant trehalase and the urinary trehalase (Fig. 2A, lanes 4 and 5). The immunoblot analysis showed that the amount of 75-kDa protein from a patient with Lowe syndrome was high, which was consistent with the previous observation (7) that the urinary trehalase activity of this disorder was very high (248 μmol of glucose formed·h⁻¹·g creatinine⁻¹). We further examined the amount of trehalase in patients with other renal diseases. A band of 75 kDa was observed in patients with chronic glomerulonephritis and focal segmental glomerulosclerosis (FSGS; Fig. 2B, lanes 5 and 7). Pathological observations of a renal biopsy specimen of the FSGS patient showed progressively extended glomerulosclerosis and widespread destruction of proximal tubules (data not shown). Bands of ~30 kDa as well as 75 kDa were shown in urine of a patient with acute renal failure (Fig. 2B, lane 3), whereas trehalase was either absent or minimally present in urine specimens of healthy subjects (Fig. 2B, lanes 1, 2, 4, and 6).

**ASSAY CHARACTERISTICS**

We developed a sandwich ELISA to assess the amount of urinary trehalase, using KM2287 and biotinylated 2275 antibodies. As shown in Fig. 3A, the absorbance at 415 nm...
against calibrator exhibited a linear relation for trehalase concentrations of 0–1000 μg/L. To determine the optimal condition for the ELISA, we examined whether treatment of urine with SDS solution was suitable. Fig. 3B shows the effect of dilution of urinary samples with SDS. Compared with a native urine specimen from a healthy subject (Fig. 3B, panel a, column 1), when SDS was added at a final concentration of 1 g/L, the relative value increased (Fig. 3B, panel a, column 2). When the urine was diluted with 10 volumes of PBS containing 1 g/L SDS and heated at 95 °C for 1 min (column 3), or diluted with 10 volumes of PBS (column 4), Samples were from a healthy-10-year-old boy (a), a 15-year-old boy with chronic glomerulonephritis (b), a 1-year-old girl with hydronephrosis (c), a 12-year-old boy with nephrotic syndrome (acute phase; d), and a 10-year-old girl with FSGS (e). Relative values were expressed by calculations based on the absorbance values for the diluted samples. (C), effect of pH on the ELISA. The ELISA was carried out as described in Materials and Methods except that various pHs were used. (D), urine dilution curve. Urine samples were diluted with PBS containing 1 g/L SDS. Values were obtained by 10-fold dilution with PBS containing 1 g/L SDS.

We next examined the effect of pH on this ELISA system, and optimal values pH of 6.0–8.0 were obtained (Fig. 3C). A dilution curve showed that the values estimated by this ELISA were correlated with 10- to 20-fold diluted samples (Fig. 3D). When the urine was diluted with PBS, the pH of the samples adjusted to ~7.4, and thus a good response to this ELISA was obtained.

When urine values obtained with this ELISA for gel-filtered urines were compared with those obtained for unfiltered samples, the trehalase values obtained after gel filtration were 110–226 μg/L, whereas those obtained before gel filtration were 20–50 μg/L.

The precision of the ELISA was estimated with different urine samples containing trehalase enzyme. The within- and between-assay CVs were 6.7–7.6% (n = 8) and...
6.2–8.2% (n = 14), respectively (Table 1). Exogenously added trehalase was well recovered from urine samples containing different concentrations of endogenous trehalase (Table 2).

When urine samples were stored at −60 °C, −30 °C, or 4 °C for 30 days, the enzyme amount did not decrease. Possible interfering substances, such as ascorbic acid (10 g/L), glucose (100 g/L), albumin (10 g/L), or creatinine (1 g/L), did not affect this ELISA.

Table 1. Precision of this ELISA.

<table>
<thead>
<tr>
<th>Within-assay</th>
<th>n</th>
<th>Mean, µg/L</th>
<th>SD, µg/L</th>
<th>CV, %</th>
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<tr>
<td>Urine 1</td>
<td>8</td>
<td>251</td>
<td>18</td>
<td>7.0</td>
</tr>
<tr>
<td>Urine 2</td>
<td>8</td>
<td>364</td>
<td>28</td>
<td>7.6</td>
</tr>
<tr>
<td>Urine 3</td>
<td>8</td>
<td>447</td>
<td>30</td>
<td>6.7</td>
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<tr>
<td>Between-assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine 1</td>
<td>14</td>
<td>245</td>
<td>19</td>
<td>7.7</td>
</tr>
<tr>
<td>Urine 2</td>
<td>14</td>
<td>382</td>
<td>24</td>
<td>6.2</td>
</tr>
<tr>
<td>Urine 3</td>
<td>14</td>
<td>462</td>
<td>38</td>
<td>8.2</td>
</tr>
</tbody>
</table>

* Performed in duplicate over 14 days.

Table 2. Analytical recovery of calibrator added to human urine.a

<table>
<thead>
<tr>
<th>Trehalase added, µg/L</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45</td>
<td>242</td>
</tr>
<tr>
<td>50</td>
<td>90</td>
<td>279</td>
</tr>
<tr>
<td>100</td>
<td>136</td>
<td>326</td>
</tr>
<tr>
<td>200</td>
<td>240</td>
<td>425</td>
</tr>
<tr>
<td>400</td>
<td>437</td>
<td>611</td>
</tr>
</tbody>
</table>

a Urine samples prepared were analyzed after the addition of the indicated amount of purified human trehalase.

CONCENTRATION AND ACTIVITY OF TREHALASE IN HUMAN URINE SAMPLES

The urinary trehalase activity of the healthy group was 26.6 ± 14 µmol·h⁻¹·g creatinine⁻¹, and the trehalase concentration measured by the ELISA was 466 ± 343 ng/g creatinine. These values showed no sex- or age-related differences (data not shown). This new ELISA detected, as protein, a small amount of urinary trehalase whose activity was not detected.

There was a positive correlation in healthy subjects between the urinary trehalase activity and the trehalase concentration (P <0.01; r = 0.15; Fig. 4). The upper limits of trehalase activity and concentration were 56 µmol·h⁻¹·g creatinine⁻¹ and 906 ng/g creatinine, respectively. As shown in Fig. 5B, urinary trehalase concentrations in nephrotic syndrome patients (acute phase) were extremely high, i.e., ~200-fold higher than the control group (Fig. 5B, group B; P <0.05), but serum trehalase in patients was not detected. The concentrations in patients in remission from nephrotic syndrome were similar those in the controls (Fig. 5B, group C). The variability in activity in groups B and C was similar to that measured by the ELISA (Fig. 5A, groups B and C). The patients with Lowe syndrome and Dent disease showed very high concentrations, and they were 200- and 70-fold higher, respectively, than in the controls (Fig. 5B, a and b of group F). The trehalase activity of a patient with acute renal failure caused by Salmonella infection was low (15.7 µmol·h⁻¹·g creatinine⁻¹), whereas the trehalase concentrations estimated by the ELISA was markedly high (13 642 ng/g creatinine), and it was 27-fold higher than in the controls (Fig. 5, c of group F). Compared with the controls, patients with chronic glomerulonephritis (Fig. 5, group D) had higher trehalase activities (1.5-fold Fig. 4. Relationship between trehalase activity and trehalase concentration in healthy subjects.

Dotted lines show the upper limits (95th percentiles). Cr, creatinine.

Fig. 5. Urinary trehalase values in patients with renal diseases.

(A), urinary trehalase activity; (B), urinary trehalase concentration estimated by ELISA. Group A, controls (n = 41; M/F = 16/25); group B, patients with nephrotic syndrome (acute phase; n = 4; M/F = 2/2); group C, patients with nephrotic syndrome (remission phase; n = 7; M/F = 4/3); group D, patients with chronic glomerulonephritis (total, n = 16; M/F = 9/7; IgA nephropathy, n = 8; anaphylactoid purpura glomerulonephritis, n = 3; others, n = 5); group E, patients with renal anomaly (n = 11; M/F = 5/6); group F, others (a, 8-year-old boy with Lowe syndrome; b, 16-year-old boy with Dent disease; c, 7-year-old boy with acute renal failure attributable to Salmonella infection). Cr, creatinine.
higher) and concentrations (6.2-fold higher). The trehalase concentrations in patients with renal anomalies (Fig. 5, group E) were also very high compared with the controls \((P < 0.05)\), although the activities in these patients were not high compared with the controls \((P = 0.88)\). Patients with chronic glomerulonephritis and acute phase nephrotic syndrome exhibited 1.5- to 5-fold higher NAG activity (acute phase of nephrotic syndrome, 11.0–16.4 U/g creatinine; chronic glomerulonephritis, 4.5–10.4 U/g creatinine) than did healthy controls \((3.2 \pm 2.4 \text{ U/g creatinine})\). Thus, the extent to which the trehalase concentration was increased, as estimated by the ELISA, was much higher than the measured increases in trehalase activity and NAG activity.

**Discussion**

Using monoclonal antibodies, we developed an ELISA for determination of urinary trehalase. Urinary trehalase comes from the apical membranes of the renal proximal tubular cells: immunohistochemical staining of the renal cortex-mix shows the existence of this enzyme on the brush border of the renal proximal tubules (Fig. 1). Trehalase is an ectoenzyme that is linked to membrane by a glycosylphosphatidylinositol anchor, and excretion of the enzyme can be influenced by renal injuries. Other investigators have reported increased urinary trehalase activity in mercuric chloride-induced nephrotic rabbits as well as in patients with Itai-Itai disease and inhabitants of a cadmium-polluted area \((15, 16)\). Sasai-Takedatsu et al. \((3)\) also reported that the urinary trehalase activity is a better indicator of renal proximal tubular damage than NAG activity because of its early detection. However, in the measurement of the activity, the conditions needed to assess the urinary trehalase accurately are complicated \((3, 7)\) because gel filtration is necessary for each sample. Although analytical methods for urinary trehalase that use electrophoresis and immunoblots can provide interesting information on the underlying diseases, they are time-consuming and expensive for clinical examination. On the other hand, our ELISA system overcame these drawbacks as to its facility and cost.

In this ELISA, the treatment of urine with SDS solution led to an increase in binding of the antibodies. The obtained antibodies for the recombinant trehalase were raised by injection of trehalase purified by SDS-PAGE, indicating not only that the antibody preferably recognizes SDS-treated trehalase but also that SDS can solubilize trehalase protein in urine samples. Moreover, using the samples after gel filtration, we obtained trehalase values higher than those obtained with untreated samples, which indicated that low-molecular weight substances interfere with the binding of the antibody to the antigen. Thus, the dilution of urine samples with PBS was essential for obtaining reproducible values. This treatment contributes not only to neutralization of urine samples but also to dilution of interfering factors that may decrease the binding of antibodies to antigens. Although we could not identify the interfering substances, they may be present in some patients, as indicated by the increased binding of antibodies when samples were diluted 10-fold with PBS containing 1 g/L SDS.

The antibodies that we used for the ELISA exhibited a good response to human urine as well as human renal cortex-mix, and a 75-kDa band in urine may generally reflect the urinary trehalase activity (Fig. 2). There were apparent discrepancies in urinary trehalase values when compared between the ELISA and activity assay, especially in acute renal failure and renal anomalies. These discrepancies may be attributable to proteolytic degradation of trehalase in urine, although the stability of trehalase in the renal brush border membrane or urine is not clear. We found that the antibody reacted with the \(\sim 75\)-kDa and 30-kDa bands in urine from a patient with acute renal failure (Fig. 2B, lane 3). The 30-kDa band may be one of the proteolytic degradation products of intact trehalase. In this patient, trehalase activity was as same as in the control, although the 75-kDa band was obtained by urine SDS-PAGE. The reason for this is unclear, but it may be that the active center of trehalase is broken in fulminating infections. Because our designed ELISA can detect the truncated trehalase whose activity had been lost, it has the advantage of measuring the actual trehalase concentration even in urine samples that have been stored a long time. Actually, the enzyme concentrations in patients with acute phase nephrotic syndrome, chronic glomerulonephritis, and renal anomaly were much higher than the values estimated by the increased activities. These data suggest that our ELISA system can detect the whole trehalase enzyme better than the activity assay and can also detect renal tubular damage by reflecting the release of trehalase from apical membranes as a result of renal injury.

Glomerular disorders such as acute phase nephrotic syndrome show markedly increased trehalase concentrations, which indicates that the tubular cells are highly damaged. It has been reported that urinary NAG activity is increased in acute nephrotic syndrome \((17, 18)\). These authors concluded that patients with acute nephrotic syndrome might have tubular damage. Other investigators have suggested a relationship between increased NAG activity and the relapse of nephrotic syndrome \((19)\). Our study confirmed previous observations \((17, 18)\) and also described a new sensitive assay for determination of urinary trehalase to evaluate tubular damage. In this connection, although the urinary NAG activities of patients with renal damage was 1.5- to 5-fold higher than in controls, the magnitude of the increase in trehalase concentrations estimated by this ELISA was much higher (6.2- to 200-fold). Sasai-Takedatsu et al. \((3)\) reported that trehalase activity increased earlier than NAG activity in the patients with renal proximal tubular damage. These results suggest that measurement of trehalase in urine contributes to the identification of microdamage to proximal tubules.
In conclusion, this study describes for the first time a new sandwich ELISA for urinary trehalase that uses monoclonal antibodies. Our ELISA method is superior to conventional activity assays because of its ability to detect the whole enzyme. Although further studies are necessary to demonstrate the clinical validity of the present ELISA system, it may be a potent technique to assess damage to renal proximal tubules and may be useful in daily clinical assessment of renal diseases.

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