Urinary N-Acetyl-β-d-glucosaminidase Isoenzyme Activity as Measured by Fast Protein Liquid Chromatography in Patients with Nephrotic Syndrome

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Exactly why N-acetyl-β-d-glucosaminidase (NAG) excretion is increased in patients with nephrotic syndrome with glomerular lesions is poorly understood. Glomeruli contain less NAG than do proximal tubules. In this study, we have tried to measure the NAG isoenzymes automatically by use of the recently developed fast protein liquid chromatography (FPLC) system, followed by column chromatography on DEAE cellulose (Mono Q). Three isoenzyme peaks—B, I + II, and A—were observed for urine from both healthy subjects and nephrotic patients. The B isoenzyme usually constituted about 10% of the total NAG in healthy controls, 30% in nephrotic patients. In contrast, the proportion of the A isoenzyme was inversely related to that of the B isoenzyme when healthy controls and nephrotic patients were compared. Our system for measuring NAG isoenzymes is reproducible and fast, and it should be useful in further studies.

More protein is excreted in urine in renal glomerular diseases, and this can be an index of renal damage. Those of low molecular mass, such as β₂-microglobulin (β₂-m), and enzymes such as alkaline phosphatase (EC 3.1.3.1), γ-glutamyltransferase (EC 2.3.2.2), and NAG (EC 3.2.1.30) are commonly increased in the urine in renal tubular acidosis (1), diabetes mellitus (2), and in tubular damage resulting from treatment with aminoglycoside (3). Of these indices of renal tubular function, β₂-m and NAG are thought to be the best.

Recently, measurement of NAG isoenzymes in urine was studied in relation to evaluation of the clinical grades of renal diseases (4), urinary tract infections (5), and renal-transplant rejection (6). Further, nephrotic syndrome usually induces massive urinary NAG excretion, which is paralleled by increased urinary protein excretion (7).

We undertook a study of the mechanisms involved in this increased urinary NAG excretion, using newly automated systems to measure the NAG isoenzyme.

Materials and Methods

We studied 25 nephrotic patients (15 men, 10 women) with proteinuria of 0.3 to 7.8 g per day, who have been attended in our department. The nephropathies were categorized by histological examination as minimal-change nephrotic syndrome, focal glomerulosclerosis, proliferative glomerulonephritis, or membranous glomerulonephritis.

Ten healthy persons (six men, four women) served as controls.

Twenty-four-hour urine specimens from the nephrotic patients and healthy persons were collected into ice-surrounded containers. A 5-mL aliquot of each specimen was centrifuged (3000 rpm, 10 min) through filter apparatus to concentrate the samples and exclude low-molecular-mass substances. The 0.5-mL effluent was then passed through a 5 × 0.5 cm column of diethylaminoethyl (DEAE)-cellulose (Mono Q; Pharmacia, Uppsala, Sweden) connected to the FPLC (LCC Model 500; Pharmacia).

The conditions used in the FPLC were as follows: linear gradient of NaCl in Tris buffer (pH 8.0), from 0 to 0.3 mmol/L; flow rate, 2 mL/min; and total elution volume, 32 mL. We obtained 16 fractions within 10 min after specimens were applied. Each fraction was assayed for NAG activity by measuring the conversion of sodium cresol-sulphophthaleinyl N-acetyl-β-d-glucosamide to p-mercapto- cresol (8).

To calculate analytical recovery of added NAG activity, we used placenta-derived NAG (Sigma Chemical Co., St. Louis, MO).

Results

Figure 1 illustrates a typical FPLC fractionation pattern of NAG isoenzyme. Most samples contained all three peaks illustrated. The chemically neutral isoenzyme B was the first eluted, followed successively by I + II and the acidic A isoenzyme as the concentration of NaCl in Tris buffer was increased.

Table 1 shows the urinary NAG isoenzyme pattern for the nephrotic patients and the control subjects. In the controls, the B isoenzyme constituted about 10% of the total, but 30% in the nephrotic patients, significantly more (P <0.05). In nephrotic patients, the proportion of I + II was also greater than in the controls. The proportion of B isoenzyme was increased (compared with the normal values) in patients with minimal-change nephrotic syndrome.

![Fig. 1. Elution pattern of NAG isoenzymes in the FPLC system](image-url)

The contents of tubes were analyzed for NAG activity as described in the text.

1 Nonstandard abbreviations: NAG, N-acetyl-β-d-glucosaminidase; β₂-m, β₂-microglobulin; FPLC, fast protein liquid chromatography; and DEAE, diethylaminoethyl.

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and focal glomerulosclerosis, but not in those with proliferative glomerulonephritis or membranous glomerulonephritis (Figure 2). There was no correlation between the NAG isoenzyme proportions obtained by FPLC and either the degree of impairment of renal function or the daily urinary protein excretion.

**Discussion**

The concentration of low-molecular-mass protein or enzyme activity is usually determined in urine in evaluating tubular diseases from various causes. Because NAG is localized to the proximal tubules, a high concentration being present in the kidney (9), it was easy to determine the site of damage to tubular segments by evaluating the total NAG in urine. NAG in the urine is frequently increased in clinical grades of tubular damage. Nonetheless, it is difficult to measure NAG in urine when there is total loss of renal function, as in chronic renal failure, because of the NAG depletion in damaged tubules.

We have now demonstrated that the nephrotic syndrome, as well as tubular damage, can result in increased urinary excretion of NAG. Although the existence of increased total NAG activity in rat glomeruli has been recently suggested, this alone cannot explain the mechanisms of increased urinary NAG excretion in nephrotic patients. A more convenient method is necessary, such as the analysis of NAG isoenzyme in urine. With our automated method of NAG isoenzyme separation and analysis, we observed increased urinary B isoenzyme excretion in membrano-proliferative glomerulonephritis, focal glomerulosclerosis, and minimal change nephrotic syndrome, but not in proliferative glomerulonephritis, or membranous glomerulonephritis.

These results suggest the possibility of determining the histological type by analysis by NAG isoenzymes; however, we found no relationship between clinical severity as defined by urinary protein excretion or the urinary B isoenzyme excretion.

Analysis for NAG isoenzyme has been performed manually with use of DEAE-cellulose column chromatography and fluorometric or electrophoretic quantification of each isoenzyme fraction (10). These earlier methods require large elution volumes, and they are complex and unrepeatable. Our method is both reproducible and rapid.

The B isoenzyme was increased in our patients with nephrotic syndrome. Evidently, in nephrotic syndrome the B isoenzyme partly originates from the glomeruli, as suggested from data for puromycin-induced nephrotic rats studied in our laboratory (11). Two forms of NAG A isoenzyme have been described. The isoenzyme Aβ form originates from blood vessels, and is eluted beside the neutral (compared to Aγ) form by DEAE-cellulose chromatography. This form was not increased in the urine. However, the Aγ form, of tubular origin, is increased in nephrotic patients (12). From these data, we consider the origin of urinary NAG in glomerular diseases to be partly glomerular, but not of blood-vessel origin. Although the molecular mass of NAG is about 140 000 Da, some NAG B isoenzyme may pass through the glomerular basement membrane into the urine in nephrotic syndrome. Although further examination is required to understand the mechanisms by which urinary NAG is excreted from glomeruli, our system may be useful in further characterization and diagnosis of renal diseases.

**References**


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**Table 1. NAG isoenzyme Pattern in Urine from Nephrotic Patients and Healthy Controls**

<table>
<thead>
<tr>
<th>NAG isoenzyme, % of total</th>
<th>Nephrotic patients</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>B</td>
</tr>
<tr>
<td>Nephrotic patients</td>
<td>35</td>
<td>22 ± 10</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>10</td>
<td>8 ± 4</td>
</tr>
</tbody>
</table>

**Fig. 2. Urinary NAG isoenzyme activity in various renal histological changes**

Column, left to right: MCNS, MN, PGN, FGS.