Liquid-Chromatographic Detection of Aspartylglycosaminuria

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We describe a specific, simple liquid-chromatographic method for detecting the lysosomal storage disease aspartylglycosaminuria. The method is based on identification and quantification of the major storage compound 2-acetamido-1-L-aspartamido-1,2-dideoxy-D-glucose in the urine of affected individuals. Sample preparation and chromatographic analysis requires 30 min. Within-day variation of the method was <4.4%, between-day variation <11.7% (n = 10 each).

Additional Keyphrases: heritable disorders • lysosomal storage disease • urine

Aspartylglycosaminuria (AGU) is due to an inherited defect in the catabolism of N-glycosidic glycoproteins, with decreased activity of the enzyme aspartylglycosaminidase, N\(^{\mathrm{as}}\)-(β-N-acetylglucosaminyl)-1-asparaginase (EC 3.5.1.26). First described in two mentally retarded English siblings in 1967 (1), the disease has subsequently been diagnosed in 100–200 patients, most of them in northern Finland and Norway (2). It is characterized by the accumulation of 2-acetamido-1-L-aspartamido-1,2-dideoxy-D-glucose (GlcNAc-Asn) and other neutral or acidic glycosaminoglycans in body fluids and tissues (3). The clinical findings relate to impairments in the central nervous system and connective tissues, and include psychomotor retardation and coarse features. The laboratory detection of AGU is usually based on the identification of GlcNAc-Asn in urine by chromatographic (4, 5) or enzymatic (6) methods. Specific analytical methods involving gas–liquid chromatography or gas–liquid chromatography linked to mass spectrometry have been described (7, 8), but because of tedious sample preparation they are of limited value in clinical laboratories. Here we describe a specific, simple liquid-chromatographic method for determination of GlcNAc-Asn in urine.

Materials and Methods

AGU-patients and control subjects. We studied five patients with confirmed diagnoses of AGU, four of them six to 15 years old and one 34 years old. The control subjects were 40 patients, ages one to 15 years, who did not have AGU, and two healthy adults, both 32 years old. We collected 24-h urine specimens (without dietary restrictions) and stored them frozen until processed.

Reference compound and reagents. GlcNAc-Asn was purchased from Vega-Fox Biochemicals, Tucson, AZ 85734, and “HPLC grade” acetanilide from E. Merck AG, Darmstadt, F.R.G. Sep-Pak C\(_{18}\) cartridges were purchased from Waters Associates, Mississauga, Ontario, Canada. Sodium phosphate buffer (2.5 mmol/L, pH 4.5) was prepared in “HPLC grade” water.

Sample preparation. An 2-mL aliquot from the 24-h urine specimen was passed through a Sep-Pak C\(_{18}\) cartridge that had been equilibrated with water. We then washed the cartridge with 2 mL of water, which was collected and combined with the sample. Usually we injected 20 μL of the combined eluate onto the chromatographic column.

“High-performance” liquid chromatography. We used a liquid chromatograph (Perkin-Elmer Corp., Norwalk, CT 06856; Series 4) connected to a variable-wavelength "UV-VIS" detector. For sample injection we used a Perkin-Elmer ISS-100 autosampler. Final results were automatically calculated by the technique of external standardization, with use of a Perkin-Elmer LCI-100 computing integrator. We used a 150 × 4.6 mm (i.d.) amino column (Spherisorb-NH\(_2\), 5-μm particles) with a mobile phase of phosphate buffer/acetanilide (30/70, by vol) at a flow rate of 1.5 mL/min. The detector wavelength was set to 205 nm; routine attenuation was about 0.05 A full-scale.

Calibration, analytical recovery, and reproducibility. The GlcNAc-Asn standard (300 μmol/L; 101 mg/L) was in aqueous solution. To establish the linearity of the detector response to GlcNAc-Asn, we measured standards in concentrations of 0 to 3 mmol/L, handling them the same way as the samples. Analytical recovery was assessed by using urine from a control subject, supplemented with 0.3 mmol of GlcNAc-Asn per liter. To test reproducibility, we used urine from an AGU patient that contained 0.28 mmol of GlcNAc-Asn per liter.

Results

The chromatograms of control and AGU urine analyzed for GlcNAc-Asn according to the present method are shown in Figure 1. Urine from the patient with AGU showed a peak for which the retention time (15 min) was identical to that of reference GlcNAc-Asn, whereas the control urine lacked that peak under the same conditions. We could use the same column for at least 400 analyses without repacking.

The detection limit with the standard compound in aqueous solution was about 0.003 mmol/L in urine samples, because of interfering material in the chromatograms, it was about 0.01 mmol/L. The absolute recovery of GlcNAc-Asn from normal urine supplemented with 0.3 mmol of this compound per liter was 92.4% (range 85.3–98.7%; n = 6) of that of the standard in aqueous solution. We evaluated analytical precision by repeated analysis of an AGU patient’s urine sample containing 0.28 mmol of GlcNAc-Asn per liter. The within-day variation (CV) was less than 4.4% (n = 10). The CV achieved from day to day with the same urine sample was less than 11.7% (n = 10). We analysed by the present method urine samples from five AGU patients. The mean value for GlcNAc-Asn excretion was 0.792 mmol/L (range 0.280–1.120 mmol/L). Figure 1 shows the chromatogram for the patient who secreted the least GlcNAc-Asn. The concentration of GlcNAc-Asn in 40

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1 Nonstandard abbreviations: AGU, aspartylglycosaminuria; GlcNAc-Asn, 2-acetamido-1-L-aspartamido-1,2-dideoxy-D-glucose; HPLC, "high-performance" liquid chromatography.

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involves between method control results and patient's N-acetyl-o-glucosamine (0.01 mmol/L), may of cultured ion-exchange has demonstrated that was made for enzymatic activity for aspartylglycosaminuria. The detection limit of the present method for GlcNAc-Asn in urine is about 0.01 mmol/L because of interfering material; therefore, the method does not allow quantitative analysis of GlcNAc-Asn in urine of normal individuals without further purification steps.

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References