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Note

Automated ion-exchange chromatography in the detection of aspartylglucosaminuria

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Aspartylglucosaminuria (AGU) is an inherited neurovisceral storage disease associated with mental retardation and resulting from low activity of a lysosomal enzyme, 4-L-aspartylglycosylamine amidohydrolase (EC 3.5.1.26) [1—3]. Up to 14 different glycoasparagines have been found [4—7] in the urine of AGU patients. All glycoasparagines have a common structure of β-Gal-(1→4)-β-GlcNAc-Asn [8] and are homogeneous as judged by paper chromatography [9]. The main abnormal urinary metabolite in affected persons is aspartylglucosamine, i.e. 2-acetamido-1-N-(4′-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (GlcNAc-Asn) [9]. Screening for AGU in clinical laboratories takes place by demonstration of the presence of GlcNAc-Asn in urine either by gas chromatography—mass spectrometry [8, 10—13], gel chromatography [6, 9], paper chromatography and paper electrophoresis [4, 7, 9], thin-layer chromatography (TLC) [14, 15], or enzymatic determinations [16].

Since amino acid analysis is the most common method used for diagnosis of inborn errors of metabolism it is important that also AGU can be detected with an amino acid analyzer. It can not directly be used for the detection of AGU, though, because the glycoasparagines cannot be separated from other fast-eluting, highly acidic amino acids and peptides while using the lithium citrate buffer system for physiological fluids [17]. However, when the interfering compounds are removed by TLC before analysis, the suspected peaks become clearly detectable with slight modifications of the routine procedure [18]. The present paper describes a sensitive and relatively simple method for the determination of glycoasparagines in the urine of AGU patients.

EXPERIMENTAL

Samples

Urine samples from patients with AGU were obtained from Tampere Central

Hospital, Tampere, Finland (one male), and from Rinnekoti Institution for the Mentally Retarded, Majalampi, Finland (one female). The clinical findings included deepening mental retardation and structural abnormalities. The diagnosis was confirmed by the demonstration of aspartylglucosamine in the urine by various chromatographic and electrophoretic techniques.

Thin-layer chromatography

One hundred microlitres of urine were applied between the spots of a reference solution of 2-acetamido-1-N-(4'-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (Vega-Fox Biochemicals, Tucson, AZ, U.S.A.) on silica gel plates (Merck DC Alufolien Kieselgel 60; Merck, Darmstadt, G.F.R.). The plate was developed in *n*-butanol—acetic acid—water (50:25:25) for a distance of 10 cm. A glass plate was placed to cover the urine sample area and the TLC plate was sprayed with ninhydrin (Merck). The plate was heated as described by Palo and Savolainen [14, 15]. The area on the chromatogram of the urine sample corresponding to the reference substance was scraped off and collected into a conical centrifuge tube. The materials were extracted with 1 ml of distilled water. After centrifugation at 1000 *g* the supernatant was evaporated to dryness at 343°K with a rotary vacuum evaporator.

Amino acid analysis

The sample was dissolved in 100 μl of sample dilution buffer (lithium citrate, pH 2.2) [19]. Fifty microlitres of the sample were injected into a Beckman Multichrom M amino acid analyzer. Lithium citrate buffer (Durrum Pico-Buffer System IV, Pierce Eurochemie, Rotterdam, The Netherlands) was used according to the method of Perry et al. [17] with resin AA 20 from Beckman (Munich, G.F.R.) in a 0.4 cm × 24 cm water-jacketed glass column at a temperature of 305°K. The pH of the buffer A was lowered to 2.5 with concentrated HCl. The ninhydrin reagent [19] and buffer flow-rates were both 10 ml/h. One analysis lasted 90 min, including 25 min regeneration with lithium hydroxide and a 40-min equilibration period with the modified buffer A.

RESULTS AND DISCUSSION

In a laboratory concerned with the amino acid analysis of urine specimens it is customary to run either paper or thin-layer chromatograms of samples before the automated amino acid analysis. This reveals any qualitative or quantitative abnormalities in ninhydrin-staining properties of the spots. Aspartylglucosamines give a grayish-blue or grayish-green colour, and thus the absorbance at 440 nm is higher than at 570 nm. Most amino acids (with the exception of proline and hydroxyproline) have a higher absorbance at 570 than at 440 nm as do the other reference substances that elute in the region of very acidic amino acids (Fig. 1).

Fig. 2 illustrates an amino acid analysis with aspartylglucosamines from the urine of a patient with AGU. The glycoasparagines appear as two fairly broad peaks with retention times of 10.5 and 23.5 min. The latter peak includes 1-N-(4'-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine, as assessed by co-migration of the reference substance, but also other related glycoasparagines (Fig. 2C).

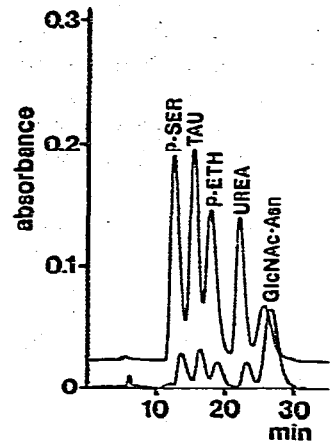

Fig. 1. Amino acid analysis of a reference mixture containing 100 μmol/l orthophosphoserine (P-Ser), taurine, ethanolamine phosphate (P-Eth) and 5 mmol/l urea, together with 0.1 g/l 2-acetamido-1-N-(4'-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (GlcNAc-Asn). The absorbances are measured at 570 nm (upper trace) and 440 nm (lower trace). The improved resolution is due to reduced buffer flow and pH.

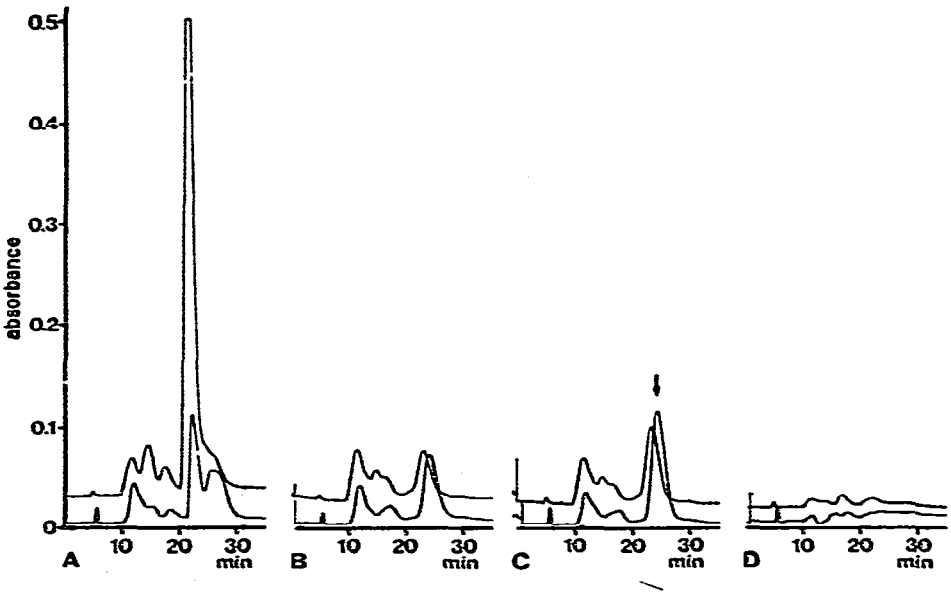

Fig. 2. Amino acid analysis of urine specimens. (A) Sample of a patient with aspartylglucosaminuria. (B) The same sample as in A after removal of interfering substances by TLC as described in the text. The aspartylglucosamines appear as two peaks with retention times of 10.5 and 23.5 min. (C) The same sample as in B with 5 μg of added GlcNAc-Asn (arrow). (D) A normal urine sample after the TLC procedure.

The structural identity of the first peak has not yet been established. Using Beckman Unichrom, Palo and Mattsson [20] detected only one peak in urine. It was close to the taurine peak, but it could not be detected in one brain biopsy specimen of a patient with AGU [21], although at the beginning of this amino acid chromatogram there are two unidentified peaks which could contain the first eluting glycoasparagine.

The procedure presented above is applicable to the analysis of glycoasparagines present in the urine of AGU patients. After co-migration with the reference compound in two different chromatographic systems the substances are most likely identical. The linear range of this procedure is from 0.01 to 1.6 g/l. If that amount is exceeded the sample must be further diluted with the sample dilution buffer (pH 2.2). The sensitivity of the amino acid analyzer or the smallest amount which can be visualized on TLC is 0.01 g/l, which is well exceeded by the GlcNAc-Asn content of urines determined by Maury [13] to range from 0.14 to 0.28 g/l. The recovery of the reference compound from thin-layer plates analysed by the described method was 73 ± 13% (S.D.; n = 10). Since many clinical laboratories do not have access to gas chromatograph—mass spectrometer systems for their definitive identification and quantitation, the method described above is easily applicable to determinations of glycoasparagines in biological specimens of patients with suspected aspartylglucosaminuria.

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