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Chromatographic isolation of 2-acetamido-1-(β'-L-aspartamido)-1,2-dideoxy-β-D-glucose from urine

Since the original report[1] on the occurrence of 2-acetamido-1-(β'-L-aspartamido)-1,2-dideoxy-β-D-glucose (AADG) in the urine of two mentally retarded siblings, further cases have been diagnosed[2]. For the correct identification of this relatively rare disease, isolation of AADG from the urine had to be established. A detailed description of the isolation and identification procedure is presented in this paper.

Experimental

Large volumes of untreated or concentrated urine were pipetted onto Whatman No. 3 MM chromatography paper sheets. Descending development of the sheets in *n*-butanol–water–acetic acid (60:25:15) lasted at least 48 h before they were dried. Narrow strips were cut from the sheets and stained with SCRIVER's[3] solution*. The strong brown colour indicated the location of AADG, and corresponding areas were cut from the remaining unstained paper. These strips were eluted with water, and the eluates were evaporated to dryness. After adding 1 ml of water and two drops of acetic acid to the dry material, the mixture was brought onto the top of a column packed with Dowex 50 W resin. Before that, the resin had been treated with 2 *M* acetic acid–pyridine buffer (pH 5.8) and equilibrated with water. The column was then eluted with 0.1 *M* acetic acid–pyridine buffer (pH 3.8), and fractions each 0.5 to 1 ml were collected[4].

A small sample from each fraction was pipetted onto a Whatman No. 1 chromatography paper sheet, and the sheet was developed as above. The pure AADG appeared as single brown spots, thus proving that no other ninhydrin-positive compounds were present. These pure fractions were pooled, evaporated to dryness, and dissolved in 1 ml of 1 *N* or 6 *N* hydrochloric acid. The solution was transferred into a hydrolysis tube, flushed with nitrogen, and sealed under vacuum. The tube was placed in an oven with a temperature of 100° for 1–16 h. It was then opened, the hydrochloric acid was evaporated, and the remaining acid was removed by washing three times with water. The dry residue constituted the hydrolysate of the purified AADG.

Column chromatography was performed using the Unichrom (Beckman) automated amino acid analyser. Analysis of the unhydrolysed material confirmed the presence of only one peak, close to the taurine peak, in the area of acidic amino acids. Column chromatography of the hydrolysed material showed two peaks, one in the area of acidic amino acids and the other in the area of more basic amino acids. The first peak coincided with that of aspartic acid and the second with that of glucosamine. A positive Morgan–Elson reaction confirmed the presence of glucosamine in the isolated material.

Discussion

Urine contains a number of peptides, *e.g.* 17 different peptides were discovered in 83 l of normal urine[5]. Antibiotics are a well-known source of extra spots on amino

* SCRIVER's solution: ninhydrin 1.25 g, isatin 0.05 g, lutidine 5 ml *ad* 500 ml of acetone.

acid chromatograms. It is therefore necessary to isolate a compound suspected to be an abnormal urine constituent caused by an inborn error of metabolism.

Since the development of the present isolation procedure for AADG, another method involving a combination of exclusion chromatography on Sephadex G-15 and ion-exchange chromatography on Zeo-Karb 225 (sulphonic acid type) ion-exchange resin followed by recrystallisation was published[6]. Although no comparison between that method and the present system was performed, both procedures obviously yield equally well-purified material and can be utilised if chromatographically pure AADG is needed for biochemical studies. Synthesis of AADG[7] is, of course, also possible but may be beyond the capacity of most clinical research laboratories.

The only hitherto known condition in man in which AADG is excreted in large quantities (up to 20 mg/100 ml) in the urine is aspartylglucosaminuria (AGU). Altogether 17 patients have been recorded[1,2]. However, the disease may appear to be more common if more attention is paid to the unknown "peptide" spots occasionally detected on amino acid chromatograms. The isolation method described here will serve as tool for the correct diagnosis.

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