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Excretion of α-*N*-acetylcitrulline in citrullinaemia

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SUMMARY

α-*N*-Acetylcitrulline has been identified in the urines of two unrelated patients with citrullinaemia. Identification was made on the basis of similarity to the synthetic compound prepared in this laboratory.

Excretion of α-*N*-acetylcitrulline during normal protein intake was 75–120 mg/24 h compared to citrulline excretion of 360–770 mg/24 h in the 10-week-old patient, and in the 30-year-old patient it was 610 mg/24 h compared to citrulline excretion of 2200 mg/24 h.

An amino acid derivative, not previously reported in nature, has been found in the urine of two unrelated patients with citrullinaemia[1], an inborn error of the urea cycle[2]. Identification of the compound as α-*N*-acetylcitrulline was made on the basis of its chemical properties and similarity to the synthetic compound prepared in this laboratory. The existence of this compound in urine was initially suspected by the presence of an unusually high peak in the position of *O*-phosphoethanolamine on the amino acid analyser, using standard sodium citrate buffers[3].

We were also alerted to the possibility of a new compound by the presence of an unidentified yellow spot after staining paper chromatograms of the urines with Ehrlich's reagent[4]. A sample of this unknown compound was eluted from a preparative paper chromatogram. On two-dimensional thin-layer chromatography[5], this eluate gave only one spot, which stained yellow with Ehrlich's reagent. Chromatography on the amino acid analyser gave a peak with the same elution time as *O*-phosphoethanolamine.

In order to isolate the unknown peak from the analyser, urine was chromatographed using a volatile buffer system. A 0.9 cm x 55 cm column of Beckman resin PA-28 was converted to the pyridine form and equilibrated with 0.1 N pyridine–formate buffer (pH 2.90). 3 ml of urine from one of the patients was placed on the column and eluted with the same buffer at 40° and at a pumping rate of 50 ml/h. The eluate was collected in 2 min fractions, freeze dried and reconstituted with small quantities of water. The compound was present in the 13th fraction.

An aliquot of this fraction was subjected to two-dimensional thin-layer chromatography. Some faint ninhydrin-positive spots were seen, along with one area which appeared white when stained with ninhydrin and brilliant yellow when overstained with Ehrlich's reagent. This compound had the same chromatographic characteristics as the one isolated from urine by preparative paper chromatography (Table I).

TABLE I

PROPERTIES OF ISOLATED AND SYNTHETIC α-*N*-ACETYLCITRULLINE

	α-N-Acetylcitrulline isolated from urine	*Synthetic α-N-acetyl-citrulline*
R_F in TLC, Solvent 1*	42	43
R_F in TLC, Solvent 2*	78	77
R_F on paper chromatography**	59	58
Ninhydrin stain on TLC	White	White
Ninhydrin stain on paper	None	None
Ehrlich's stain on TLC and paper	Yellow	Yellow
Elution position on amino acid analyser (ml after cysteic acid)	8	8

* Thin-layer chromatography (TLC) on Eastman cellulose plates[4]. Solvent 1, pyridine–acetone–NH_4OH (58%)–water (45:30:5:20, by vol.). Solvent 2, isopropanol–formic acid–water (150:25:25, by vol.).

** Descending paper chromatography on Whatman 3 MM paper with *n*-butanol–acetic acid–water (12:3:5, by vol.).

A second aliquot of the fraction containing the abnormal peak was subjected to 3 h of hydrolysis in 6 M HCl at 110°. Following freeze drying, the hydrolysed sample was reconstituted in 0.2 N sodium citrate buffer and chromatographed on the conventional amino acid analyser. The hydrolysate contained a large amount of citrulline, some glutamic acid and trace quantities of a few unidentified compounds. The possibility that α-*N*-acetyl-citrulline was present was then investigated.

Synthetic α-*N*-acetylcitrulline was prepared bydissolving L-citrulline in 4 M pyridine and adding a molar excess of acetic anhydride in five portions with vigorous shaking. The product mixture was freeze dried and dissolved in hot 95% ethanol. Unreacted citrulline and ureido-*N*-acetyl products of the reaction were separated by crystallization from hot benzene–ethanol (approximately 1:1, v/v). The α-*N*-acetylcitrulline, present in the clear filtrate, was obtained by crystallization from benzene–ethanol (approximately 3:1, v/v).

The synthetic α-*N*-acetylcitrulline chromatographed and stained identically with the unknown compound isolated from urine, as shown in Table I. It had a melting point of 147.2–147.7°. On the amino acid analyser, the product gave a peak with the same elution time as the unknown; it had a color yield of 1.0% of that of leucine and 50% of that of urea.

Our unknown compound, which was a conjugate of citrulline, had the same chromatographic and staining characteristics as the synthetic α-*N*-acetylcitrulline; thus we concluded that α-*N*-acetylcytrulline was present in the urine of these patients and was at least partly responsible for the abnormal peak on the amino acid analyser.

Since α-*N*-acetylcitrulline gave a low color yield with ninhydrin and since other compounds were present in urine which eluted in the same region on the amino acid

analyser, quantitation of α-*N*-acetylcitrulline was achieved as follows. The column eluent from urine samples run on the pyridine–formate buffer column was collected for the first 50 min. These fractions were freeze dried and then hydrolysed in 6 M HCl for 22 h at 110°. Citrulline was subsequently measured on the amino acid analyser and the values were taken to represent the amount of α-*N*-acetylcitrulline present originally. The results obtained for α-*N*-acetylcitrulline excretion in these patients are summarized in Table II, with the urinary citrulline values given for comparison.

TABLE II

URINARY EXCRETION OF α-*N*-ACETYLCITRULLINE AND CITRULLINE IN TWO PATIENTS WITH CITRULLINAEMIA

	Age	*Diet*	*Urinary excretion (mg/24 h)*	
			α-N-Acetylcitrulline	*Citrulline*
Patient 1	10 weeks	Normal	120	770
	10 weeks	Normal	75	360
	11 weeks	Low protein	4	60
Patient 2	30 years	Normal	610	2200

The different reaction characteristics of α-*N*-acetylcitrulline with ninhydrin on paper, thin-layer plates and the amino acid analyser (see Table I) appear to be paradoxical. They are, however, identical to the behavior of urea under the same conditions. This similarity is perhaps not surprising, since they both possess ureido groups.

α-*N*-Acetyl derivatives of several amino acids have been reported in other disorders of amino acid metabolism. These include *N*-acetylphenylalanine in phenylketonuria[6], *N*-acetyltyrosine in tyrosinaemia[7,8] and *N*-acetylcystathionine in cystathioninuria[9]. To this list we now add α-*N*-acetylcitrulline in citrullinaemia. From this information it appears likely that *N*-acetylation of many amino acids may occur when there is sufficient distortion of plasma or tissue amino acid levels.

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