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Note

Determination of N-monoacetylcystathionine in biological samples using isotachophoresis

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Cystathionine is an important intermediate of the transsulphuration pathway in mammalian tissues. It has been reported that cystathionine, N-acetylcystathionine (NAc-cysta) and other cystathionine metabolites are excreted into the urine of patients with cystathioninuria [1]. Recently, it has been reported that experimental cystathioninuria can be induced in rats by administration of DL- and L-propargylglycine, and that cystathionine and NAc-cysta are excreted into the urine and accumulated in several tissues [2,3]. The determination of NAc-cysta has been achieved by using an amino acid analyser [4], but the analysis was time-consuming.

The determination of cystathionine in rat tissues using isotachophoresis has been reported in a previous paper [5]. The isotachophoretic method [6–9] presented here is the first description of the use of this technique for the quantitative determination of NAc-cysta in biological samples.

EXPERIMENTAL

Materials

L-Cystathionine (Fig. 1) and DL-propargylglycine were obtained from Sigma (St. Louis, MO, U.S.A.). NAc-cysta (Fig. 1) was purified from the urine of a patient with cystathioninuria, as reported previously [11]. All other chemicals used were analytical grade.

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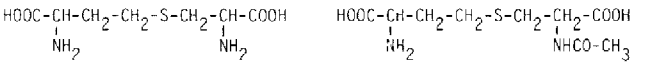

Fig. 1. Structures of L-cystathionine (left) and NAc-cysta (right).

Tissue samples

Male Wistar rats (average body weight 200 g) were used. Experimental cystathioninuria was induced in rats by the intraperitoneal administration of DL-propargylglycine (20 mg per 200 g), as reported previously [11].

Control rats and rats with cystathioninuria were killed by decapitation, and tissues were then removed, blotted, weighed and used. Each tissue was kept frozen at −80°C if not analysed immediately for measurement of NAc-cysta.

The urine of a patient with cystathioninuria [1] was used for the measurement of NAc-cysta in human urine.

Assay of cystathionine and NAc-cysta

Each tissue (0.5 g) was homogenized in three volumes of 2% sulphosalicylic acid, and the mixture was centrifuged at 2000 *g* for 15 min. The supernatant of each tissue and the urine (2 ml) of a patient with cystathioninuria were applied to columns containing 5 ml of Diaion SK-1 (H form). The columns were washed with deionized water and eluted with 2 *M* ammonium hydroxide. Each eluate was dried under reduced pressure. The residue was dissolved in 1 ml of deionized water. An aliquot of the solution was analysed by an amino acid analyser (Hitachi Model 835), liquid chromatography and an isotachophoretic analyser [6,7].

Instrumentation

The capillary apparatus was a Shimadzu IP-3A isotachophoretic analyser (Shimadzu Seisakusho, Kyoto, Japan). The separations were carried out in a capillary tube (200 mm×2 mm I.D.); the room temperature was maintained at 20°C. The detector cell was 0.05 mm×0.2 mm I.D. The migration current was 100 μA.

The leading electrolyte for the measurement of NAc-cysta consisted of 0.01 *M* hydrochloric acid and β-alanine (pH 3.1), and the terminating electrolyte was 0.01 *M* caproic acid. The analysis of NAc-cysta was performed in the anionic mode. The chart speed was 10 mm/min.

RESULTS AND DISCUSSION

The determination of NAc-cysta was carried out by measuring its zone length in isotachophoresis. Isotachophoretic runs of authentic NAc-cysta (A), normal human urine (B), the urine of a patient with cystathioninuria (C) and NAc-cysta added in the sample of C (D) are shown in Fig. 2. We ascertained that authentic NAc-cysta is easily separated under the conditions described in

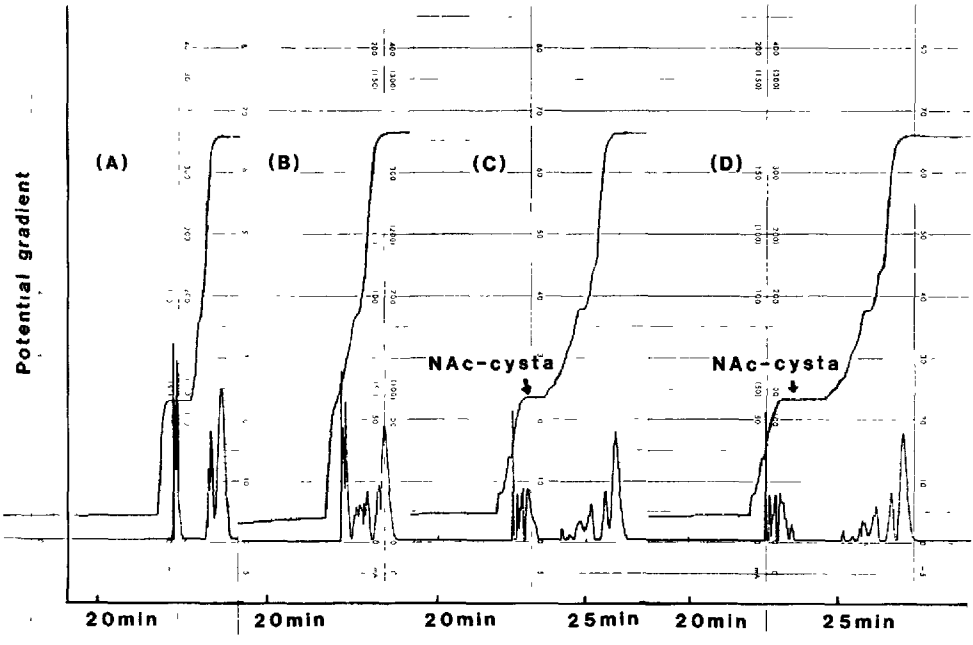

Fig 2. Isotachophoretic runs of (A) authentic NAc-cysta, (B) normal human urine, (C) urine of a patient with cystathioninuria and (D) sample C with added NAc-cysta. Analytical conditions are described in Experimental.

Experimental, as shown in Fig. 2A. The standard curve for authentic NAc-cysta was linear from 0 to 5 nmol and reproducible. The recovery of authentic NAc-cysta during the column chromatographic procedure described in Experimental was 93–97%. The detection limit was 1 nmol of NAc-cysta in biological samples when the isotachophoretic analyser was used under the analytical conditions described. The zone that has the same potential gradient as the zone of authentic NAc-cysta could not be detected in normal urine (Fig. 2B), but it was detectable in the urine of a patient with cystathioninuria (Fig. 2C). The zone was made to overlap by adding authentic NAc-cysta to the urine, resulting in an elongation of the zone in the urine as shown in Fig. 2D. The zone of NAc-cysta in the urine of a patient with cystathioninuria disappeared following hydrolysis with the hydrochloric acid for 24 h. These facts indicate that the elongated zone is the zone of NAc-cysta.

Isotachophoretic runs obtained with pancreas samples from normal rats with experimental cystathioninuria are shown in Fig. 3. The zone of NAc-cysta could not be found in normal samples (Fig. 3A), liver or kidney, but it was found in the pancreas from rats with experimental cystathioninuria (Fig. 3B), and it was made to overlap by adding authentic NAc-cysta to the sample of Fig. 3B (Fig. 3C).

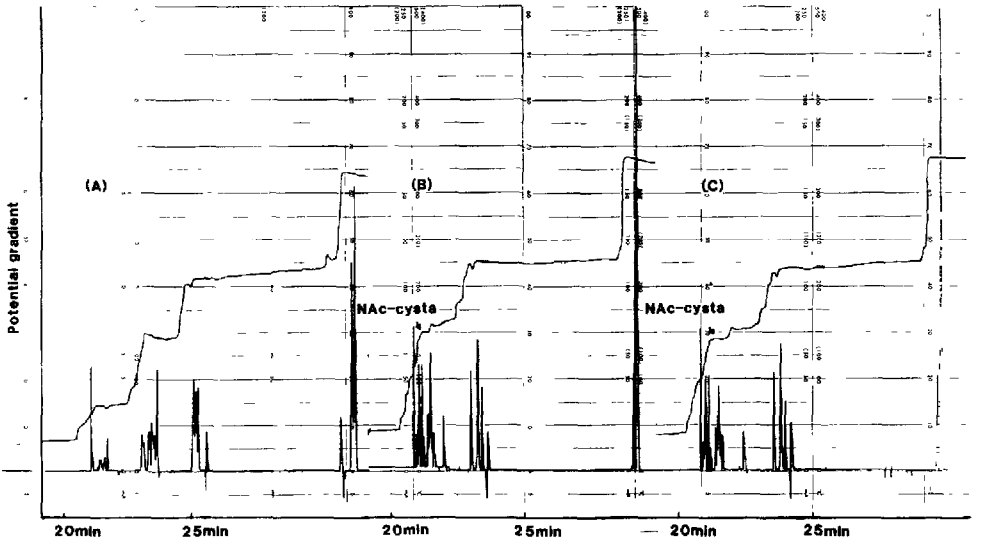

Fig. 3. Isotachophoretic runs of pancreas samples (A) of normal rat, (B) at 20 h after the administration of DL-propargylglycine and (C) sample B with added NAc-cysta. Analytical conditions are described in Experimental.

TABLE I

DETERMINATION OF CYSTATHIONINE AND NAc-CYSTA IN THE URINE OF A PATIENT WITH CYSTATHIONINURIA

Sample	NAc-cysta (μmol/ml)		Cystathionine (μmol/ml)	
	Isotachophoretic analyser	Amino acid analyser	Isotachophoretic analyser	Amino acid analyser
1	1.06	1.15	8.68	8.59
2	1.12	1.18	8.64	8 45
3	1.18	1.11	8.53	8.57
4	1 15	1.19	8.63	8.62
5	1.08	1.20	8.56	8.59
Mean ± S.D.	1.118 ± 0.044	1.166 ± 0.033	8.608 ± 0 055	8.564 ± 0.059

The determination of cystathionine using isotachophoresis was reported previously [5], but both cystathionine and NAc-cysta were determined in order to compare the contents of cystathionine and NAc-cysta in the urine of cystathioninuria and in the tissues of DL-propargylglycine-treated rats.

The determinations of cystathionine and NAc-cysta in the urine of patients with cystathioninuria using an isotachophoretic analyser and an amino acid analyser are compared in Table I. The two methods gave almost the same val-

TABLE II

DETERMINATION OF CYSTATHIONINE AND NAc-CYSTA IN THE PANCREAS OF DL-PROPARGYLGLYCINE-TREATED RATS

Time (h)	NAc-cysta (μmol/g)		Cystathionine (μmol/g)	
	Isotachophoretic analyser	Amino acid analyser	Isotachophoretic analyser	Amino acid analyser
5	N.D [a]	0.01	0.86	0.95
10	0.18	0.16	2 54	2.81
15	0.27	0 23	4.44	4.20
20	0.32	0.34	2.88	2.73
24	0.28	0.22	2.97	2.81

[a] N.D. = not detectable

ues for NAc-cysta. The amounts of cystathionine and NAc-cysta in the urine of a patient with cystathioninuria determined by this method were 8.68 ± 0.146 and 1.12 ± 0.119 μmol/ml, respectively. The content of NAc-cysta was ca. 12.5% of that of cystathionine.

The determinations of cystathionine and NAc-cysta in the pancreas of DL-propargylglycine-treated rats using an isotachophoretic analyser and an amino acid analyser are compared in Table II. The values for NAc-cysta obtained from the two analyses agreed well.

These results indicate that this method can be used for the measurement of NAc-cysta in the urine of patients with cystathioninuria and in various tissues of rats. The accumulation of cystathionine and NAc-cysta in the pancreas increased gradually after the administration of DL-propargylglycine and reached the maximum level at ca. 15 and 20 h, respectively, as reported previously [10,11].

The accumulation of cystathionine and NAc-cysta in the liver and kidneys was very similar to that in the pancreas. These results suggest that NAc-cysta is mainly synthesized from cystathionine accumulated in the liver, pancreas and kidneys of DL-propargylglycine-treated rats and excreted into the urine.

The isotachophoretic determination of NAc-cysta presented here is simpler than that with an amino acid analyser and should be very useful for the determination of NAc-cysta in various tissues of rats with experimental cystathioninuria and in the urine of patients with cystathioninuria.

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