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Note

Isotachophoretic analysis of isovalerylglycine in urine of a patient with isovaleric acidemia

HIROYUKI KODAMA

Department of Chemistry, Kochi Medical School, Kochi (Japan)

and

SHIGEKI UASA

Department of Biochemistry, Okayama University Medical School, Okayama (Japan)

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Isovaleric acidemia is an inborn error of leucine metabolism; large amounts of isovalerylglycine (300—1200 mg/day) are known to be excreted in the urine as a detoxication product [11]. The determination of this compound has been achieved by the use of gas chromatography [1] or thin-layer chromatography [2]. However, these methods are time-consuming for the pretreatment of the sample.

Recently, we had an opportunity of examining a patient with isovaleric acidemia who was found in Okayama, and a new simple and rapid method for detecting urinary isovalerylglycine was devised. The isotachophoretic method [3—10] presented here has several advantages over previously described techniques.

EXPERIMENTAL

The capillary apparatus used was a Shimadzu IP-IB isotachophoretic analyser [12] (Shimadzu Seisakusho, Kyoto, Japan). The separations were carried out in a capillary tube 20 cm long with an I.D. of 0.5 mm, which was maintained at a constant temperature of 20°. The detector cell had an I.D. of 0.5 mm and length 0.05 mm. The migration currents were 50, 100 and 150 μA. The leading electrolyte consisted of 0.01 *M* hydrochloric acid and β-alanine (pH 3.1 and pH

4.5) and 0.01 *M* hydrochloric acid, β-alanine and 0.001 *M* cupric chloride (pH 3.1). The terminating electrolyte was 0.01 *M* caproic acid.

The chemicals used were analytical grade. Authentic isovalerylglycine was synthesized from the corresponding isovalerylchlorides and glycine.

The normal urine samples were from laboratory personnel. The samples from the patient with isovaleric acidemia were obtained from Okayama National Hospital. The samples were kept frozen if not analysed immediately.

RESULTS AND DISCUSSION

The patient with isovaleric acidemia was found in Okayama using gas chromatography, but the method is time-consuming on account of the pretreatment of the samples. Therefore, the purpose of our investigation was to develop a simple and rapid method for the determination of urinary isovalerylglycine in cases of isovaleric acidemia. Isotachophoresis is a method of high resolution for the separation of compounds according to their net mobility in a given electrolyte system.

We tried to determine isovalerylglycine in the urine of the isovaleric acidemic patient by isotachophoresis. Aliquots (0.1 μl) of normal human urine and of the urine of the isovaleric acidemic patient were subjected to isotachophoresis. No zone was detected in normal human urine, but a large zone was detected in the urine of the isovaleric acidemic patient. It was necessary to identify this

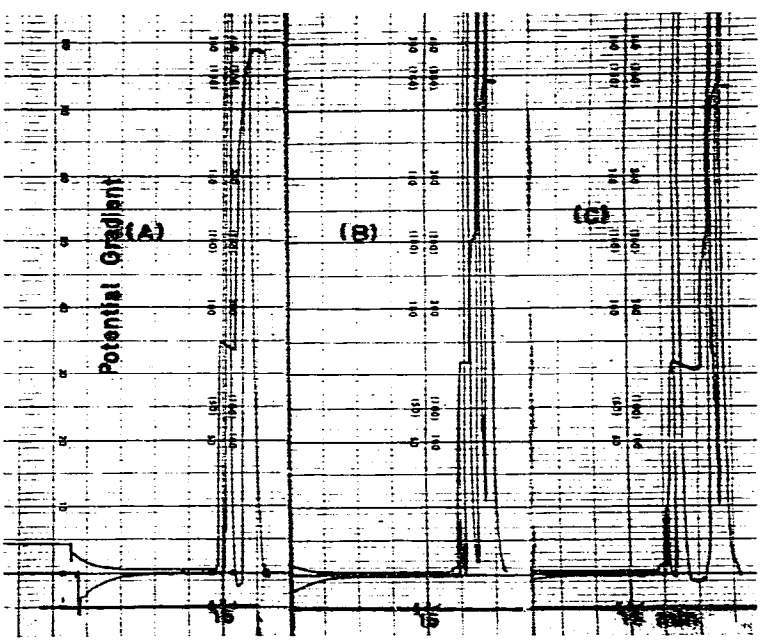

Fig. 1. Isotachophoretic runs of urine from a patient with isovaleric acidemia and of authentic isovalerylglycine. (A) Synthetic isovalerylglycine; (B) urine sample of patient with isovaleric acidemia; (C) mixture of synthetic isovalerylglycine and urine sample of patient. The leading electrolyte was 0.01 *M* HCl and β-alanine (pH 3.1) and the terminator was 0.01 *M* caproic acid. Migration current, 150—100 A; chart speed, 10 mm/min; temperature of electrolyte, 20°.

large zone as isovalerylglycine by the addition of authentic isovalerylglycine. Authentic isovalerylglycine was added to the urine of the isovaleric acidemic patient and the mixture was subjected to isotachophoresis. The zones were coincident (Fig. 1). However, when members of the tricarboxylic acid cycle and acidic amino acids were subjected to isotachophoresis, it was found impossible to distinguish the zones of aspartic acid and isovalerylglycine in the same electrolyte (pH 3.1). Isovalerylglycine and aspartic acid could be separated by ion exchange, but this method was as time-consuming as the gas chromatographic method for determining isovalerylglycine in the urine of the isovaleric acidemic patient. We then studied high-resolution methods for the separation of these compounds by changing the electrolyte system. When aspartic acid and isovalerylglycine were run separately (pH 4.5) good resolution was obtained, but their mixture did not give good separation in the same leading electrolyte.

Eventually, we obtained good results, as shown in Fig. 2, by adding 0.001 *M* cupric chloride to the leading electrolyte (pH 3.1).

The standard curves for aspartic acid and isovalerylglycine were linear. In a given electrolyte system, the separation pattern of isovalerylglycine was reproducible and its identification generally did not cause problems. The coefficient of variation for our technique was 2.15% ($n = 7$). riation of 2.15%

When there is any ambiguity, it is possible to add an authentic sample to an actual sample or change the leading electrolyte. The patient with isovaleric acidemia excreted 600--950 mg of isovalerylglycine per day.

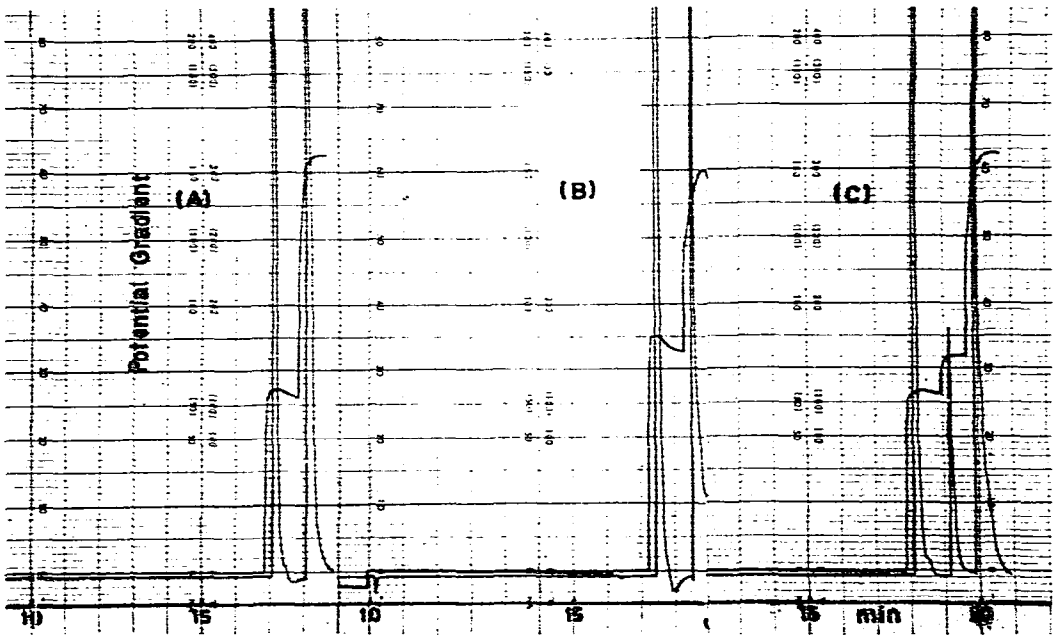

Fig. 2. Isotachophoretic runs of aspartic acid and isovalerylglycine. (A) Synthetic aspartic acid; (B) synthetic isovalerylglycine; (C) mixture of aspartic acid and isovalerylglycine. The leading electrolyte was 0.01 *M* HCl, β-alanine and 0.001 *M* $CuCl_2$ (pH 3.1) and the terminator was 0.01 *M* caproic acid. Conditions as in Fig. 1.

This method can measure isovalerylglycine by applying 0.1 μl of urine directly without any pretreatment. The method is very simple and rapid compared with gas chromatography or thin-layer chromatography and is very useful for screening inborn errors of metabolism such as isovaleric acidemia.

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