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ISOLATION AND DETERMINATION OF TAURINE BY ION-EXCHANGE CHROMATOGRAPHY

Reiko Akagi and Toshihiko Ubuka*

Taurine, 2-aminoethanesulfonic acid, is one of the end products of cysteine metabolism and is widely distributed in the animal kingdom. But it is not found in the protein. Although taurine is a stable substance and is not metabolized in the mammarian cells, the correct determination of taurine is not very easy. Like most amino acids, taurine can be analyzed by amino acid analyzer, and is eluted in the fraction, in which strongly acidic amino acids are eluted. But the complete separation of taurine from other strongly acidic amino acids including hypotaurine is difficult when usual amino acid analyzing system with cation exchange resin is used. Hypotaurine, 2-aminoethanesulfinic acid, which is known to be the main precursor of taurine, is rarely detected in normal tissues, but it was reported that hypotaurine was found in high concentration in the regenerating rat liver after partial hepatectomy[1]. Taurine determination by the column consisted of cation and anion exchange resins has been reported [2][3][4].

The purpose of this study is to develop a convenient method of taurine determination separating taurine from other amino acids including sulfur amino acids which are also metabolites of cysteine.

Experimental

All chemicals were perchased from Sigma Chemical Company, St. Louis, Mo., USA.

Dowex 1-X 8 (acetate form, 200-400 mesh, 0.7×5cm) was packed in a Econo column (0.7×15 cm, Bio-Rad Lab., Richmond, California, USA) and Dowex 50W-X8 (H^+ form, 200-400 mesh, 0.5×6.5 cm) was packed in a Tuberculin plastic syringe. These columns were washed with water until the washings became nutral. The top of the latter column was connected with a minimal dead space to the bottom end of the former column using a silicon rubber packing. One-ml volume of an amino acid mixture (containing 4 μ moles of glycine, alanine, serine, threonine, glutamic acid and aspartic acid, 50 μ moles of hypotaurine, cysteinesulfinic acid and cysteic acid, and various concentrations of taurine) was placed on the top of the combinated column. The column was washed three times with 0.2 ml portions of water, and elution was carried out. The initial effluent and washings were discarded. Then elution was performed with 5 ml of water. Five tenth ml of the pooled effluent was used for taurine determination, which was performed using Moore's manual ninhydrin reagent[5].

High voltage paper electrophresis was performed in formic acid-acetic acid-water (6:24:170, pH 1.9) at 3000 V for 30 minutes[6]. Amino acids were detected with 1 % ninhydrin-2 % pyridine in acetone.

Results and Discussion

Fig. 1 shows the elution profile when the amino acid mixture was applied and elution

* Department of Biochemistry, Okayama University Medical School

was performed with water collecting fractions of one ml. It showed sharp single peak which was eluted in 5 ml of the effluent irrespective of the amount of taurine. The fraction 3, 4 and 5 were collected separately and concentrated by a flash evaporator and were subjected to high voltage paper electrophoresis. Fig. 2 shows the paper electrophoretic profile of these three fractions. All the fractions contained only the substance having the same movility and the same color in ninhydrin reaction as standard taurine. Amino acids other than taurine was not eluted under the present conditions.

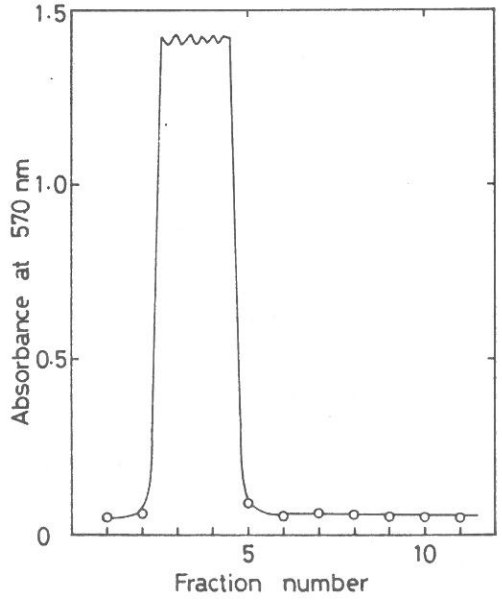

Fig. 1. Elution profile of taurine when an amino acid mixture was applied to the combined ion-exchange columns. Details are described under Experimental.

Table 1 shows the recovery of taurine in the present method. The recovery was over 98 % when sample solutions containing 5 to 50 μ moles per ml of taurine were applied.

The results in the present study indicate that taurine can be separated by the combined ion-exchange columns from other amino acids including acidic amino acids and cysteine metabolites such as hypotaurine and cysteine sulfinic acid, and that taurine in such amino acid mixture can be successfully determined by the present method.

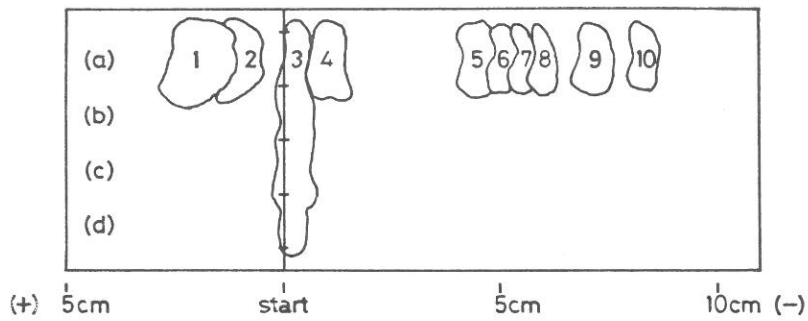

Fig. 2. Paper electophpresis of the eluates from the combined ion-exchange columns. Electrophoretic conditions were described under Experimental. Bands of lane (a) are : 1, cysteic acid; 2, cysteine sulfinic acid; 3, taurine; 4, hypotaurine; 5, aspartic acid; 6, glutamic acid; 7, threonine; 8, serine; 9, alanine and 10, glycine. Fraction 3, 4 and 5 of Fig. 1 were applied to lane (b), (c) and (d), respectively.

TABLE 1. RECOVERY OF TAURINE IN THE METHOD USING COMBINED COLMNS
Experimental detail was described under Experimental. Taurine concentrations were determined using Moore's manual ninhydrin reagent.
*Values are means ± SD (n=5).

Taurine concentration (μmoles/ml)	5	10	50
Recovery* (%)	104.3±1.4	102.8±1.8	97.9±0.6

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