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Note

Separation of taurine and glycerophosphoryl ethanolamine on amino acid analyzers

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Taurine is present in abundant levels in vertebrate brain and much evidence suggests it plays a neurotransmitter role [1]. The usual method for monitoring taurine levels is separation and analysis on an amino acid analyzer, where taurine elutes near the void volume of the commonly used buffer systems. Accurate analysis does depend upon resolution of taurine from all other ninhydrin-positive components; this separation has been assumed in all such analyses reported in the literature.

Recently, however, Tachiki and Baxter [2] reported that another compound, glycerophosphoryl ethanolamine (GPEA), co-elutes with taurine on a Durrum amino acid analyzer system containing the DC-4A resin and Pico Buffer IV mixture, a lithium citrate system for analyzing physiological fluid samples. Since GPEA is found in various levels in vertebrate brain samples, the co-elution of GPEA with taurine would introduce an element of uncertainty in most previous studies of taurine levels in brain tissue.

We have been analyzing the levels of several brain amino acids, including taurine, using two amino acid analyzers from Beckman Instruments. The report of Tachiki and Baxter [2] stimulated us to determine whether GPEA co-elutes with taurine in the analyzer procedures that we use. With pure preparations of GPEA and taurine, we found that there is a clear separation of these two compounds in three out of four systems checked by us.

MATERIALS AND METHODS

Glycerophosphoryl ethanolamine and taurine were obtained from Sigma (St. Louis, MO, U.S.A.). The four systems for separating and analyzing amino acids were all from Beckman Instruments; the first two are the newer

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accelerated systems. System A: Beckman 121MB, 200 × 2.8 mm column of AA-10 resin; flow-rate 10 ml/h; buffer 0.20 *N* Na^+, pH 2.8; column temperature 50°C. System B: same as System A except the eluting buffer was 0.2 *N* Li^+, pH 2.83 at 40°C column temperature. System C: Beckman 120C, 560 × 9 mm column of AA-15 resin; flow-rate 70 ml/h; buffer 0.2 *N* Na^+, pH 3.488; column temperature 55°C. System D: Beckman 120C, 550 × 9 mm column of Aminex A-6 resin; flow-rate 70 ml/h; buffer 0.3 *N* Li^+, 0.16 *N* citrate, pH 2.80; column temperature 40°C.

RESULTS AND DISCUSSION

Fig. 1 shows the elution profiles for GPEA and taurine. GPEA eluted significantly prior to taurine in three out of the four methods tested: both of the Na^+ systems (usually employed for analyzing protein hydrolysates) and the slower of the physiological fluid (Li^+) systems. Only the accelerated physiological fluid System B failed to resolve the two compounds. Thus neither the Durrum [2] nor the Beckman standard accelerated lithium citrate system separated GPEA from taurine.

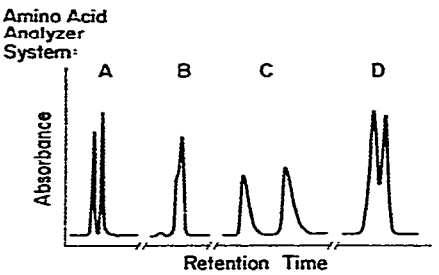

Fig. 1. Amino acid analyzer profiles for glycerophosphoryl ethanolamine (GPEA) and taurine. The four amino acid analyzer systems are described in Materials and methods. The first peak (or shoulder in system B) in each case is GPEA. Twelve nmol of GPEA and 8 nmol of taurine were applied in A and B, 72 and 50 nmol in C and D. The retention times (in minutes) for GPEA, taurine, and aspartic acid (the latter peak not shown in the figure) are in order: (A) 6.0, 7.6, 11.8; (B) 7.5 (GPEA + taurine), 20.6; (C) 26.6, 34.4, 42.6; (D) 28.3, 30.1, 54.0.

Our studies demonstrated that some, but not all, commercially available systems routinely used for the separation and analysis of amino acids do adequately separate GPEA from taurine. If GPEA is present in samples whose taurine content is to be monitored, care must be taken to choose an amino acid analysis technique that resolves these two compounds. It might be most advantageous to combine use of a Na^+ system for analysis of taurine in brain tissues and an accelerated Li^+ system for analysis of other ninhydrin-positive components in the samples. It is of course possible that, for a given amino acid analyzer system, other ninhydrin-positive compounds may overlap with GPEA or taurine; this was not tested in the present study.

An evaluation of previous reports on taurine levels must include consideration of the method used for resolution of taurine on an ion-exchange resin.

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