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Note

Use of 3-nitro-L-tyrosine as an internal standard for the lithium buffer, single-column amino acid analysis of complex amino acid mixtures

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Internal standards are used during amino acid analysis to monitor losses due to sample preparation and handling and to monitor variations due to chromatography column differences and changes[1]. The infrequent analysis of protein hydrolysates on a system dedicated to the analysis of physiological fluids requires an internal standard which is stable to acid hydrolysis. Although norleucine is most frequently used as an acid-hydrolysis-stable internal standard on both two-column and single-column sodium buffer systems[1–3] and on two-column lithium buffer systems[4], it cannot be used on some single-column lithium buffer systems in that it coelutes with tyrosine[5]. α-Amino-β-guanidinopropionic acid has been recommended as an internal standard for a single-column lithium buffer system[5], but it is not stable to acid hydrolysis[6].

3-Nitrotyrosine has been reported to be suitable as an acid-hydrolysis-stable internal standard on two-column, sodium buffer systems[7]. This communication deals with the suitability of 3-nitro-L-tyrosine as an internal standard for the infrequent analysis of protein hydrolysates on a single-column lithium buffer system.

EXPERIMENTAL

Analyses were conducted on a Beckman (Fullerton, Calif., U.S.A.) 119 CL amino acid analyzer utilizing a 22 × 0.6 cm bed of AA-10 resin and the lithium buffer system specified by the manufacturer[5].

3-Nitro-L-tyrosine was from Sigma (St. Louis, Mo., U.S.A.), ε-aminocaproic acid was from Aldrich (Milwaukee, Wisc., U.S.A.) and S-β-(4-pyridylethyl)-L-cysteine was from Pierce (Rockford, Ill., U.S.A.). A standard mixture containing nitrotyrosine was obtained by mixing 0.50 ml of the Beckman protein hydrolysate amino acid calibration standard (No. 312220), 2.50 ml of the Pierce physiological basic amino acid standard (No. 20076), 1.25 ml of 1.0 m*M* nitrotyrosine in 10 m*M* HCl, and 0.75ml of the Beckman lithium citrate dilution buffer (No. 339594) to give a final concentration of 0.25 μ*M* for each amino acid. Aliquots of 100 μl volume applied to the column gave peaks for 25.0 nmoles of each amino acid.

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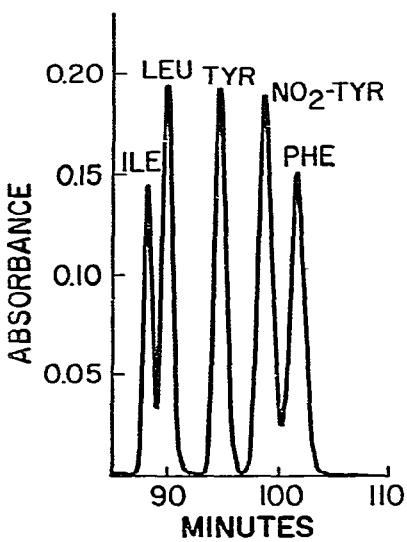
RESULTS AND DISCUSSION

ε-Aminocaproic acid has been reported to be suitable as an acid-hydrolysis-stable internal standard[8]. It eluted just before lysine on the basic column of a Beckman 120C two-column amino acid analyzer[8]. However, we have found here that ε-aminocaproic acid coelutes with histidine on the Beckman 119 CL single-column lithium buffer system.

S-β-(4-Pyridylethyl)-L-cysteine has also been reported to be suitable as an acid-hydrolysis-stable internal standard[9]. It eluted just before arginine on the basic column of a Beckman Spinco 120 two-column amino acid analyzer[9]. However, we have found here that S-β-(4-pyridylethyl)-L-cysteine elutes after arginine on the Beckman 119 CL single-column lithium buffer system. This has also been found to be the situation on the long basic (physiological) column of a two-column, sodium buffer amino acid analysis system[10]. Extension of the time of analysis, therefore, makes S-β-(4-pyridylethyl)-L-cysteine undesirable as an internal standard.

S-β-(4-Pyridylethyl)-D,L-penicillamine has been reported as an acid-hydrolysis-stable internal standard but also suffers from the disadvantage of late elution causing an extension of the time of analysis on both the short basic and the long basic (physiological) columns of a two-column sodium buffer amino acid analysis system[10].

In view of the problems involving the elution positions of these internal standards, the suitability of nitrotyrosine[7] was investigated. Whereas it eluted after phenylalanine on a Spinco 120 two-column sodium buffer amino acid analyzer system[7], we found that it elutes just before phenylalanine on the Beckman 119 CL single-column lithium buffer system as shown in Fig. 1. 3-Nitrotyrosine eluted sharply with a peak height-to-valley relationship to phenylalanine which indicated a resolution greater than that of the isoleucine–leucine doublet. The use of 3-nitro-



ig. 1. A typical elution pattern of an amino acid standard mixture containing 3-nitrotyrosine. The ortion of the chromatogram prior to the elution of isoleucine and after the elution of phenylalanine as been omitted to facilitate presentation. The buffers for development of the column and the ming sequence were as specified[5]. Each peak represents 25 nmoles.

tyrosine as an internal standard on the Beckman 119 CL single-column lithium buffer system enables the convenient analysis of infrequent protein acid hydrolysates on a system dedicated to the analysis of physiological fluids.

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