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Note

Elution behavior of γ-L-glutamyl-L-aspartic acid during ion-exchange chromatography

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In this laboratory, many kinds of glutamyl peptides have been isolated and identified from such higher plants as soybean seed and seedling[1,2], green gram seed[3–5], ladino clover seed[6], azuki-bean seed[7], buckwheat seed[8] and broad-bean seed[9]. In previous papers[10,11], we reported that γ-glutamyl dipeptides could be easily distinguished from α-glutamyl dipeptides by using an NMR spectrometer and an amino acid analyzer.

During an analysis of glutamyl dipeptides with an amino acid analyzer, we noticed that γ-L-glutamyl-L-aspartic acid was eluted as two peaks under certain conditions. This paper describes the elution behavior of γ-L-glutamyl-L-aspartic acid during automated amino acid analysis; the elution times of several other α- and γ-glutamyl dipeptides are also reported and compared with those of a standard amino acid mixture.

EXPERIMENTAL

Preparation of α- and γ-glutamyl dipeptides

γ-Glutamylaspartic acid and γ-glutamyltyrosine were isolated from ladino clover seed[6]. γ-Glutamyl-leucine, γ-glutamylmethionine and its sulphoxide and γ-glutamyl-γ-glutamylmethionine were obtained from green gram seed[3–5].

The method used by Buchanan *et al.*[12] for synthesising α- and β-aspartyl peptides was used in the synthesis of α- and γ-glutamyl dipeptides, but with N-benzyloxycarbonylglutamic anhydride in place of N-benzyloxycarbonylaspartic anhydride. α-Glutamyl-β-alanine and α- and γ-glutamyl dipeptides containing asparagine, threonine, valine, proline, alanine, phenylalanine, glycine, α-aminoisobutyric acid and isopropylamine as C-terminal residue were prepared by this method, but γ-glutamyl-β-alanine could not be so obtained.

α-Glutamyl-leucine was prepared from the γ-benzyl ester of N-benzyloxycarbonylglutamic acid, and leucine benzyl ester *p*-tosylate by the mixed-anhydride method[13]. Isoglutamine was prepared from the γ-benzyl ester of N-benzyloxycarbonylglutamic acid and ammonia. α-Glutamylaspartic acid and γ-glutamyl-β-alanine were kindly donated by Dr. T. Shiba and Mrs. S. Kawamura, respectively.

The glutamyl and C-terminal residues of these peptides were all in the L-form. The IR spectra of these α- and γ-glutamyl peptides will be published elsewhere[14].

Preparation of sample solutions

Ten μmoles of γ-glutamylaspartic acid were dissolved in 10 ml of 0.2 *N* sodium citrate buffer of pH 2.2 (ref. 15); 0.2-ml portions of the solution were diluted with 0.2, 0.4, 0.6, 0.7, 0.8, 1.0, 1.2 and 1.4 ml of the same buffer solution, and half of each diluted solution was analyzed with the use of a Hitachi KLA-2 amino-acid analyzer. Each sample solution loaded onto the analyzer contained 0.1 μmole of γ-glutamylaspartic acid. The other α- and γ-glutamyldipeptides were also dissolved in the same 0.2 *N* sodium citrate buffer (pH 2.2) and analyzed.

Analytical conditions

The conditions used were as follows. The column (50 cm × 9 mm) contained Amberlite IR-120 resin (400 mesh) and was operated at 50°. The eluent was 0.2 *N* sodium citrate buffer solution of pH 3.25 for 90 min, then 0.2 *N* sodium citrate buffer solution of pH 4.25 (ref. 16). The flow-rate was 30 ml/h. Sample solution was loaded onto the column, then 1.0 ml of 0.01 *N* hydrochloric acid was added, and elution was carried out as described.

RESULTS AND DISCUSSION

As shown in Fig. 1, γ-L-glutamyl-L-aspartic acid was eluted as a single peak at 60 min when the volume of sample solution applied to the column was 0.3 ml or less,

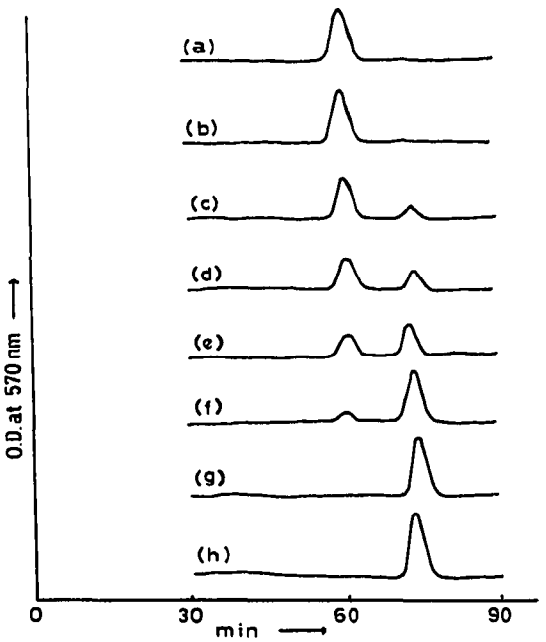

Fig. 1. Elution behavior of γ-L-glutamyl-L-aspartic acid on the amino acid analyzer; see text for analytical conditions. Volume of sample applied to column (a) 0.2 ml; (b) 0.3 ml; (c) 0.4 ml; (d) 0.45 ml; (e) 0.5 ml; (f) 0.6 ml; (g) 0.7 ml; (h) 0.8 ml.

and also as a single peak at 74 min when the volume applied was 0.7 ml or more, but the acid appeared as two peaks (at 60 and 74 min) when the volume of sample applied was in the range 0.4 to 0.6 ml.

The reason for this change in the elution behavior with alteration in the volume of solution applied to the column is not clear. Other α- and γ-glutamylpeptides so far examined were eluted as one peak when 0.5 ml of sample solution was applied. The elution patterns of α- and γ-glutamyl peptides are shown schematically in Fig. 2, together with that of a standard amino acid mixture.

γ-Glutamylaspartic acid has been isolated from soybean seedling[2], acacia seed[17], ladino clover seed[6] and broad-bean seed[9]. Attention should be drawn to the sampling volume when quantitative or qualitative analysis for this dipeptide is carried out with the use of an amino acid analyzer.

Zacharius and Talley[18] have reported the elution behavior of many kinds of ninhydrin-positive compounds, including several γ-glutamyl peptides, during ion-exchange chromatography. Arai *et al.*[19] have described the elution patterns of twelve α-glutamyl peptides in relation to their tastes.

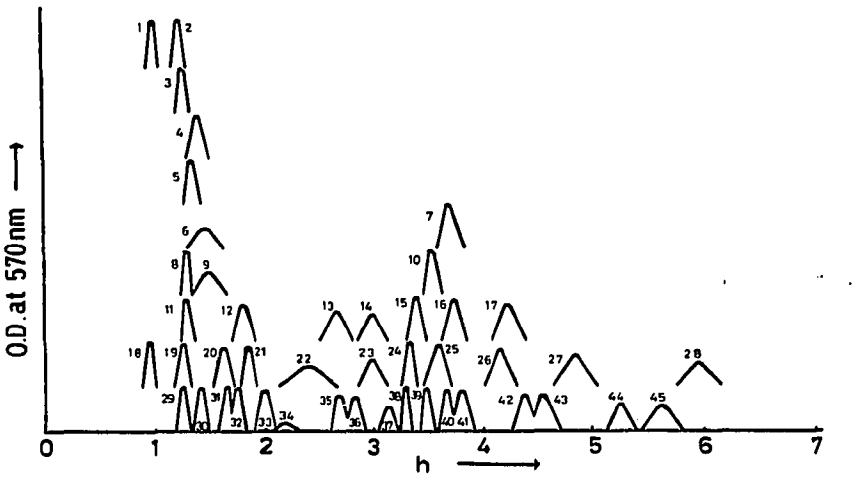

Fig. 2. Elution patterns of α- and γ-glutamyl peptides and free amino-acids; see text for analytical conditions. 1, γ-Glutamylaspartic acid (sample volume, 0.3 ml); 2, γ-glutamylaspartic acid (sample volume, 0.7 ml); 3, γ-glutamyl-γ-glutamylmethionine; 4, γ-glutamyl-α-aminoisobutyric acid; 5, γ-glutamylalanine; 6, γ-glutamylproline; 7, α-glutamyl-α-aminoisobutyric acid; 8, γ-glutamylglycine; 9, γ-glutamylvaline; 10, α-glutamylalanine; 11, γ-glutamylthreonine; 12, γ-glutamylmethionine; 13, α-glutamylaspartic acid; 14, α-glutamylasparagine; 15, α-glutamylglycine; 16, α-glutamylvaline; 17, α-glutamyl-β-alanine; 18, γ-glutamylmethionine sulphoxide; 19, γ-glutamylasparagine; 20, γ-glutamyl-β-alanine; 21, γ-glutamylisopropylmaide; 22, γ-glutamyl-leucine; 23, α-glutamylthreonine; 24, γ-glutamyltyrosine and γ-glutamylphenylalanine; 25, α-glutamylproline; 26, α-glutamyl-leucine; 27, α-glutamylphenylalanine; 28, α-glutamylisopropylamide; 29, methionine sulphoxide; 30, aspartic acid; 31, threonine; 32, serine, asparagine and glutamine; 33, glutamic acid; 34, proline; 35, glycine; 36, alanine; 37, α-aminoisobutyric acid; 38, valine; 39, methionine; 40, isoleucine; 41, leucine; 42, tyrosine; 43, phenylalanine; 44, β-alanine; 45, isoglutamine. All the peptides and free amino-acids were in the L-form.

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