Journal of Chromatography, 172 (1979) 481–483

CHROM. 11,650

Note

Resolution of γ-glutamyl peptides

LEWIS B. JAMES

Department of Biochemistry, John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City 2601 (Australia)

(Received November 2nd, 1978)

The γ-glutamyl peptides Glu–Glu, Glu–Gln, Glu–Gly, Glu–Ala and Glu–Val have previously been detected in ox brain[1], and the γ-glutamyl tripeptide glutathione (GSH) has been found in most tissues of the central nervous system[2,3]. γ-Glutamyl peptides are also present in seeds; γ-Glu–Glu is found in *Acacia georginae*[4] and γ-Glu–Asp in soybean seedling extracts[4].

Detection and identification of these peptides in the past has been restricted to paper chromatography, and the use of Dowex-2 resin (formate form)[1]. Also Dowex-1 anion-exchange resin has been utilized for characterization of the peptides[4].

In the present work, a single column procedure, using an anion-exchange resin, has been developed to resolve γ-glutamyl peptides. The procedure allows rapid identification of the peptide, provides a quantitative estimation, and is simple to carry out.

A Technicon AutoAnalyzer Model AAA was modified in order to carry out the analytical determination. A single colorimeter module with a 15-mm pathlength cuvette and 570-nm interference filter was used. A reaction chamber and heat-exchanger described elsewhere[5,6] were included in the assembly.

The recorder chart paper speed was increased by replacing the existing gears with a 24-teeth (upper) gear and a 72-teeth (lower) gear. The channel selector was locked to the single colorimeter channel. A Technicon ball point pen was screwed into the locating mounting at the recorder printing head. The chromatogram produced by the recorder is now a single line trace in place of the usual dot printing. The recorder was operated at the zero to infinity range.

A conventional 23-cm column, used with Beckman 120B amino acid analyzers, was filled with Aminex A-28 (acetate form) resin to a height of 17 cm. The inside diameter of the column was 0.9 cm. A porous PTFE disc (tightly fitting) was positioned at the bottom of the resin and two discs were used at the top of the resin to protect the resin surface. A 0.9-cm diameter filter paper was also placed on top of the discs as an additional precaution against contamination of the resin. This filter paper was replaced after each analysis. The column was operated at a temperature of 42°.

The peptides were eluted with 0.3 *M* sodium acetate buffer at a pH of 4.62. The peptides were also dissolved in this buffer before being applied to the resin.

The ninhydrin reagent consisted of 3 l of methyl cellosolve, 600 ml of 4 *M* sodium acetate, 200 ml of deionized water, 60 g of ninhydrin and 10 ml of $TiCl_3$

(15%, w/v) solution. The reagent was protected from atmospheric oxygen as described elsewhere[6].

The ninhydrin reagent and bubbles of nitrogen were delivered by the proportioning pump. Tubing colour coded blue/yellow, solvaflex, (Technicon 116-0533-19) was used for ninhydrin and clear tubing, colour coded orange (Technicon 116-0532-08) was used in the delivery of nitrogen. The colorimeter exit flow line across the proportioning pump manifold was colour coded yellow, solvaflex (Technicon 116-0533-12).

The piston pump was adjusted to deliver 60 ml of buffer per h, and the exit flow line from the column was connected directly to the mixing coil, preceding the reaction chamber coil; thereby by-passing the proportioning pump manifold.

Gluthathione (reduced) was obtained from Fluka (Buchs, Switzerland). γ-Glutamylglycine was a gift from Dr. G. Johnston (A.N.U., Canberra, Australia) and all other peptides were from Calbiochem (Los Angeles, Calif., U.S.A.).

Solutions of the peptides were at a concentration of 1 m*M* except Glu–Gln which was at 2 m*M*. Aliquots of 0.1 ml of the peptide solutions were used in the analytical determination.

Fig. 1 shows a typical chromatogram obtained with the analytical method described above. The first peptide eluted from the resin being Glu–Gln and the last Glu–Glu. The elution pattern for the other dipeptides is in the order of increase in molecular weight of the peptide; elution beginning with Glu–Gly and followed by Glu–Val. Although Glu–Leu was not available for analysis its position on the chromatogram is expected to be near to the peak labelled glutathione.

The peak labelled unknown was suspected of being oxidised glutathione,

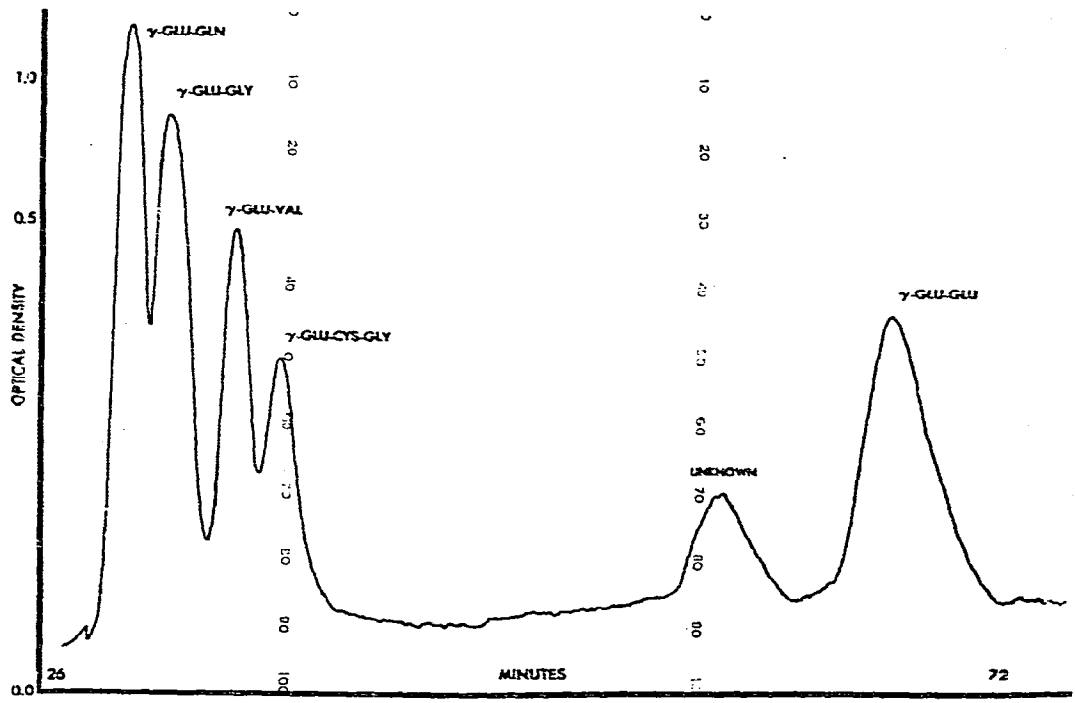

Fig. 1. Chromatogram of γ-glutamyl peptide analysis.

present either in the starting material or arising from contact with the elution buffer which does not contain an anti-oxidant.

Another peptide which would be desirable to locate in this analytical system would be Glu–Asp. The acidity of this compound should cause retention on the anion-exchange resin, and the peak should be located on the chromatogram immediately before Glu–Glu.

As would be expected the use of a cation-exchange resin in conjunction with an amino acid analyzer proved to be of no practical use in the resolution of γ-glutamyl peptides; all peptides being eluted either just before, along with, or just after aspartic acid when analyzed with a conventional amino acid analyzer. Glutathione (reduced), hydroxyproline, methionine sulphoxides and methionine sulphone are also chromatogrammed near the aspartic acid peak. This elution pattern is maintained when either a single or dual column analytical system is operated.

When using the anion-exchange analytical procedure it was found that Glu–Gly and Glu–Ala were not resolved. However when using cation-exchange resin (Beckman UR-30 or M72) in a 57-cm bed column and pH 3.25 sodium citrate buffer, Glu–Ala is eluted before aspartic acid and Glu–Gly is eluted after the aspartic acid peak.

Integration of peaks can be carried out using either a Technicon integrator/calculator (Model AAG) if available, or a Technicon chart reader comparator. The comparator method has the advantage of ease and speed once the calibration curve has been drawn on the plastic sheet.

For each determination, 100 nmol of peptide was applied to the resin. The exception being Glu–Gln in which case 200 nmol was taken. The larger concentration of the latter peptide was useful as a marker for identification purposes. As can be seen from Fig. 1, 200 nmol of Glu–Gln provides a peak with nearly a full scale deflection of the recorder, hence, the analytical procedure would be able to quantitate peptides at a concentration of 10 to 20 nmol.

The maintenance of uncontaminated resin surface was found to be critical; back pressure from the resin bed can increase very quickly if such contamination occurs, due to normal pumping of buffer. The pressure can increase from 250 to 800 p.s.i., resulting in either column breakage or failure of the column connector clamps to withstand the pressure. Two Whatman filter papers were placed on top of the porous PTFE discs that protect the resin surface from disturbance during sample application. The filter papers were not removed until termination of the analysis. If these simple precautions are dispensed with it will result in frequent repouring and settling of the resin.

REFERENCES

1 I. Sano, Y. Kakimoto, A. Kanazawa, T. Nakajimb and H. Shimuzu, *J. Neurochem.*, 13 (1966) 711.
2 K. L. Rechelt, *J. Neurochem.*, 17 (1970) 19.
3 K. L. Rechelt, *J. Biol. Chem.*, 238 (1963) 550.
4 K. Ito and L. Fowden, *Phytochemistry*, 11 (1972) 2541.
5 L. B. James, *J. Chromatogr.*, 136 (1977) 417.
6 L. B. James, *Lab. Pract.*, 21 (1972) 639.