LABELLING OF NON C-TERMINAL GLUTAMIC ACID DURING C-TERMINAL ANALYSIS BY THE TRITIATION METHOD WHEN THE γ-GLUTAMYL-PEPTIDE LINKAGE IS PRESENT.°

G. Ramponi, G. Cappugi, P. Nassi
Department of Biochemistry, University of Florence, Florence, Italy.

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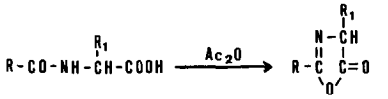
SUMMARY

When the tritiation method for C-terminal analysis is applied to glutathione the labelling of glutamic acid is evident in addition to that of the C-terminal amino acid. It is possible that glutathione, when treated with acetic anhydride in the pyridine medium, forms N-acetylglutathione. This product can cyclize to form the oxazolone ring which readily incorporates tritium in the presence of ^3H_2O. The labelling of non C-terminal glutamic acid may occur in proteins or peptides due to the presence of glutathione which can form mixed disulfides with proteins or when glutamic acid is in intrachain position with the γ-glutamyl-peptide linkage because this structure is very similar to acetylglutathione.

A novel method has been proposed by Matsuo et al.(1) for identifying the C-terminal amino acid in peptides or proteins by selective tritiation. It was shown that several N-acetylpeptides and C-terminal amino acids of polypeptides or proteins (I) undergo cyclization in the presence of acetic anhydride to form oxazolones (II).



I II

The oxazolones contain an active hydrogen and incorporate tritium when treated with ^3H_2O and pyridine. Holcomb et al.(2) have reported a critical evaluation of this method and have shown that selective tritiation is a good method for determining C-terminal amino acids. The method suffers from several limitations for proteins when the procedure is carried out in non-aqueous solvents, but most of the limitations are overcome by employing ^3H_2O as the solvent for the reaction.

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In the present paper we report a problem of interpretation regarding the labelling of glutamic acid.

In studies of the structure of horse muscle acylphosphatase we have found that glutamic acid readily incorporates tritium with the method of Matsuo, although it is not C-terminal as checked by hydrazinolysis and carboxypeptidase A (3).

The object of this paper is to provide a possible explanation for this result. It is demonstrated that non C-terminal glutamic acid may incorporate tritium when the γ-glutamyl-peptide linkage occurs in proteins or peptides.

MATERIALS AND METHODS

The hendecapeptide, eledoisin, from octopus was a generous gift from Farmitalia. RNAse and glutamyl-valyl-phenylalanine were purchased from Sigma Chem. Co.; glutathione from Merck and tritiated water from New England Nuclear Co.. All other reagents were pure chemicals.

Protein or peptide was dissolved in 0.1 ml (100 mC) of tritiated water; 0.2 ml of pyridine and 0.05 ml of acetic anhydride were added and the mixture was kept at room temperature for 5 hours. After evaporation in vacuo at 40° the residue was washed ten times with distilled water and subjected to hydrolysis in HCl 6 N at 110° for 24 or 72 hours. The identification of labelled amino acids was carried out by an improved procedure devised in our laboratory (4) consisting essentially in the use of the Unichrom amino acid analyzer for amino acid separation and in the direct measurement of radioactivity with a Nuclear Chicago liquid scintillation spectrometer in the fractions collected.

RESULTS AND DISCUSSION

Table I shows the results of the experiments performed on RNAse, glutamyl-valyl-phenylalanine, octopus eledoisin and glutathione. As can be seen, in RNAse, Glu-Val-Phe and octopus eledoisin only C-terminal amino acids are labelled i.e. valine,phenylalanine and methionine respectively, while in glutathione, both C-terminal (glycine) and N-terminal (glutamic acid) are labelled, Fig.1.

For this work we selected a protein and some peptides containing glutamic acid in various positions in the amino acid sequence or in different conformations. In RNAse glutamic acid is in intrachain position: the glutamic acid is not labelled, in agreement

TAB 1

Protein or peptide	RNAse	Glu-Val-Phe	Eledoisin	Glutathione
nmoles analyzed	35	210	136	385
amino acids		counts per minute		
Lys	0		0	
His	0			
Arg	0			
Asp	900		620	
Thr	0			
Ser	174		610	
Glu	200	350	0	156240
Pro	0		0	
Gly	724		0	97806
Ala	0		0	
Cys	0			200
Val	26625	0		
Met	0		2060	
Ileu	0		200	
Leu	0		0	
Tyr	0			
Phe	0	115115	0	

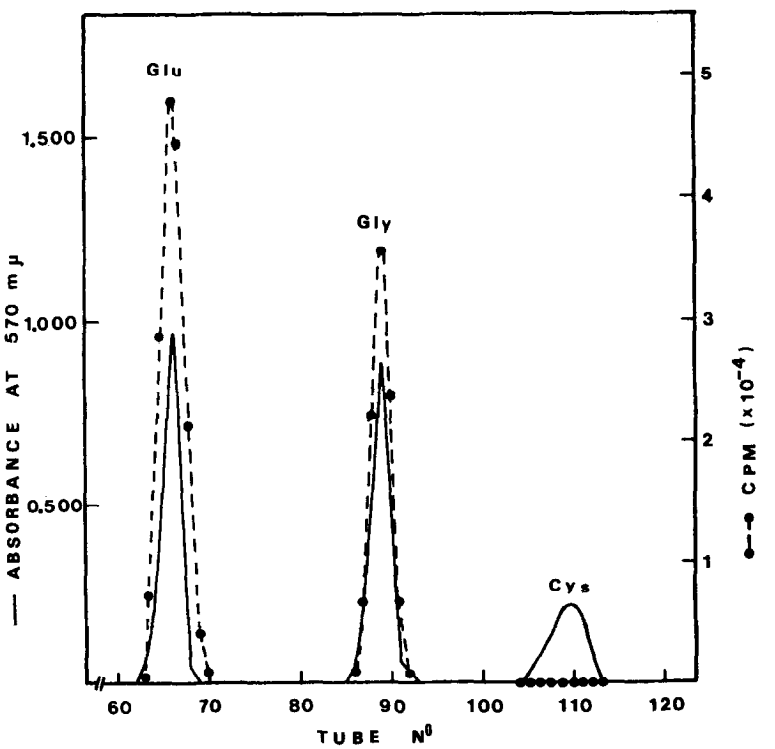
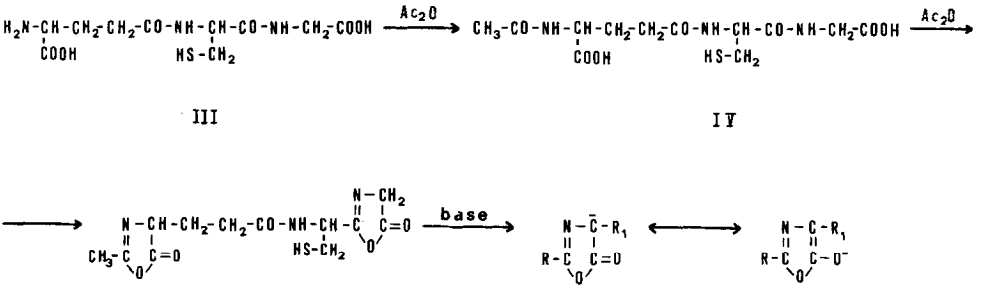


Fig. 1 : Determination of radioactivity in fractions collected during the amino acid analysis of tritiated glutathione.

with Holcomb et al.(2). In Glu-Val-Phe, where the glutamic acid is N-terminal and the α-carboxyl group is linked to valine, glutamic acid is not labelled. Octopus eledoisin contains N-terminal glutamic acid in the pyrrolidone conformation and in this instance only C-terminal methionine is labelled. As regards glutathione, in which the γ-glutamyl-peptide linkage occurs, the labelling of glutamic acid is evident in addition to that of C-terminal amino acid.

This fact may be explained by proposing that glutathione (III), when treated with acetic anhydride in the pyridine medium, forms N-acetylglutathione (IV). This product can then cyclize to form the oxazolone ring which readily incorporates tritium in the presence of $^{3}H_2O$ (V, VI).



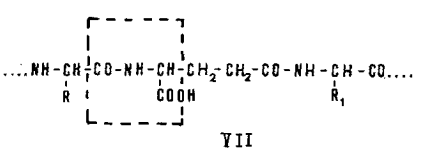
III IV

V VI

These results lead us to suggest that labelling of glutamic acid may occur in several proteins or peptides in which this amino acid is not C-terminal:

a) due to the presence of glutathione, which can form mixed disulfides with proteins; examples are human serum albumin (5) and hemoglobin A_3 which is a mixed disulfide from glutathione and hemoglobin A_1 (6).

b) when glutamic acid occurs in the intrachain position with the γ-glutamyl-peptide linkage, because this structure (VII) is very similar to acetylglutathione (IV).



VII

It has been recognized that glutamic acid, in the γ-glutamyl-peptide linkage, occurs in collagen, in a wide variety of natural peptides (glutathione, various folic acids, ophtalmic acid, agaritine etc.), in homopolymers such as the capsular peptide of Bacillus anthracis, in cell wall mucopeptides of bacteria (7), in various peptides of plants (8) and as substituent on the amino terminus of a protein of wheat (9).

We think that all the above considerations could be applied to aspartic acid when it undergoes an α-to β-rearrangement as proposed by Schroeder et al.(10) and Naughton et al.(11).

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