Communications to the Editor

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New γ-Glutamyl Peptides in Garlic

For the study of sulfur metabolism during hydroponic cultivation of garlic (*Allium sativum*), tracer technique using sulfate[^{35}S] was employed. Amino acid fraction was prepared by the usual method, developed by two-dimensional paper chromatography, and the ^{35}S-containing amino acids were detected in radioautogram and by color reaction with Ninhydrin and chloroplatinate reagents. After 24 hours' feeding, considerable amount of S-allylcysteine sulfoxide (alliin), S-methylcysteine sulfoxide, and 3-methyl-1,4-thiazane-5-carboxylic acid 1-oxide (cycloalliin), which are characteristic to the species of Allium, were observed.*1

Besides these ^{35}S-containing amino acids, at least 5 or 6 acidic ^{35}S-containing peptides (called A, B, C……F) were extracted from the root, bulb, and aerial part. When sulfate-[^{35}S] was fed to excised root or aerial part, the same kind of ^{35}S-containing amino acids and peptides were biosynthesized. These peptides, which were adsorbed on Amberlite IR-4B (Cl form) and eluted from it by *N* acetic acid, were hydrolyzed with 6*N* hydrochloric acid. By this treatment, free glutamic acid was always found to be formed. The results are shown in Table I.

Table I. Rf Values of the Peptides from Garlic and their Hydrolysis Products

Peptide	Solvent: PhOH–0.08% NH_4OH (8:2)	Solvent: BuOH–AcOH–H_2O (5:1:4)	Amino acids liberated on hydrolysis
A	0.61	0.47	Glutamic acid, phenylalanine
B	0.61	0.47	Glutamic acid, S-methylcysteine, unknown substance
C	0.44	0.29	Glutamic acid, S-methylcysteine
D	0.39	0.14	Glutamic acid, S-methylcysteine sulfoxide
E	0.30	0.16	Glutamic acid, glycine, unknown acidic S-containing amino acid
F	0.05	0.05	Glutamic acid, cystine

Peptide A—With paper chromatographic technique (solvent system as given in Table I), peptide A cannot be separated from peptide B. However, by the modified ion exchanger column chromatography of Thompson[1] using Dowex 50(X2), peptide B was eluted with 0.2*M* $HCOONH_4$ buffer (pH 3.36) while peptide A was eluted with 0.2*M* $AcONH_4$ buffer (pH 4.72). The isolated peptide A crystallized from hydr. Me_2CO as fine needles, decomposing at 194~197°. It agreed with the composition of $C_{14}H_{18}O_5N_2 \cdot H_2O$. Hydrolysis product was found to consist of 1 mole of glutamic acid and 1 mole of phenylalanine. Glutamic acid was identified by paper chromatography and by the enzymatic method with glutamic acid decarboxylase. Phenylalanine was identified by measuring the ultraviolet absorption curve and by paper chromatography. Acid hydrolysis of DNP derivative of this peptide showed that glutamic acid was bound at the N-terminal. Accordingly, peptide A was proved to be γ-glutamylphenylalanine. After this work was presented at the 7th Kinki Local Meeting of the Japanese Biochemical Society (May 1960), it was found, in a short communication,[2] that Virtanen, *et al.* had already isolated the same peptide from onion.

Peptide B—This peptide was separated from peptide A as described above and crystallized from hydr. Me_2CO as fine needles, which softened at 148~150° and darkened at 187~188° (*Anal.* Found: C, 43.95; H, 6.96; N, 11.99; O, 28.61; S, 9.49). The hydrolysis products consisted of 1 mole of glutamic acid and 1 mole of S-methylcysteine. These amino acids were identified by paper chromatography, and S-methylcysteine was converted to its sulfoxide, followed by enzymatic degradation with alliinase. By acid hydrolysis of DNP derivative of this peptide, it was shown that the glutamic acid was bound

*1 Reported at the 13th Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1960.

1) A. R. Thompson : Biochem. J., **61**, 253 (1955).
2) A.I. Virtanen, E.J. Matikkala : Suomen Kemistilehti, **B 33**, 83 (1960).

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at its N-terminal, but elemental analysis did not agree with the dipeptide of glutamic acid and S-methylcysteine. Rf value and infrared curve were not identical with those of authentic sample of γ-glutamyl-S-methylcysteine. These facts suggest that another constituent is involved in peptide B. The structure of this peptide has not been studied in detail.

Peptides C and D—Rf values of peptide C was quite identical with that of authentic sample of γ-glutamyl-S-methylcysteine.*2 By oxidation with H_2O_2, peptide C was easily converted to peptide D. Rf value of peptide D agreed with that of γ-glutamyl-S-methylcysteine sulfoxide. From these facts, it is certain that peptide C is γ-glutamyl-S-methylcysteine and peptide D is γ-glutamyl-S-methylcysteine sulfoxide.

Peptide E—On hydrolysis with 6*N* HCl, peptide E gave glutamic acid, glycine, and an unknown acidic S-containing amino acid as shown in Table I. By hydrolysis of DNP derivative of peptide E, it was found that glutamic acid was bound at its N-terminal. It was also shown that the glycine was at the C-terminal end-group by Akabori's hydrazinolysis method.[3] On the paper chromatogram, the above S-containing amino acid showed characteristic grayish blue spot with Ninhydrin at Rf 0.40 (PhOH-H_2O-NH_4OH) and 0.41 (BuOH-AcOH-H_2O). This substance was treated with Raney Ni, and alanine and an unknown fatty acid were found as the desulfurization products. After these experiments were finished, it was found in the previous communication by Virtanen, *et al.*,[2] that they obtained a new peptide from onion and its structure was given as γ-glutamyl-S-β-carboxy-β-methylethylcysteinylglycine. Refering to these facts, it was ascertained that the above unknown fatty acid is isobutyric acid. Thus, the unknown S-containing amino acid was proved to be S-β-carboxy-β-methylethylcysteine ($HOOC-CH(NH_2)-CH_2-S-CH_2-CH(CH_3)-COOH$). This amino acid was identical with an authentic sample prepared from cysteine and methacrylic acid. It is certain, therefore, that peptide E is identical with the above new peptide reported by Virtanen, *et al.*

$$HOOC-CH(NH_2)-CH_2-CH_2-CO-NH-\underset{\displaystyle CH_2-S-CH_2-CH(CH_3)-COOH}{\underset{|}{CH}}-CO-NH-CH_2-COOH$$

It is interesting that Mizuhara had isolated S-β-carboxy-β-methylethylcysteine from human urine.*3

Peptide F—By hydrolysis with 6*N* HCl, peptide F gave glutamic acid and cystine. The structure of this peptide has not been conclusively elucidated.

These glutamyl peptides described above have never been found in garlic before. The biosynthetic route of these S-containing substances is now under examination and further details of these experiments will be reported in the near future.

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*2 This material was kindly furnished by Dr. R.M. Zacharius.
*3 Reported at the 33rd General Meeting of the Japanese Biochemical Society, in Tokyo, October, 1960.

3) S. Akabori, K. Ohno, K. Narita : Bull. Chem. Soc. Japan, **25**, 214 (1952).

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