Amino Acid Composition of Green Gram (*Phaseolus radiatus* L. var. *typicus* Prain)

Part II. Contents of Free Amino Acids, γ -Glutamyl Peptides and Protein Amino Acids in Green Gram Seeds and Seedlings

By Takanori KASAI, Sadao SAKAMURA and Ryuji SAKAMOTO*

Department of Agricultural Chemistry, Faculty of Agriculture,
Hokkaido University

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Contents of free amino acids, γ -glutamyl peptides and protein amino acids of green gram seeds and seedlings were determined. γ -Glutamyl peptides, which were contained in seeds at high concentration, were not detected in seedlings. No significant relationship was found between the amino acid composition of protein and the free amino acid composition in both seeds and seedlings.

The authors reported in the previous papers¹⁻³⁾ the identification of ninhydrin positive substances, especially of γ -glutamyl peptides, and their changes during germination and maturation of soybean. Identification of four kinds of γ -glutamyl peptides and N-carboxymethyl- β -alanine from green gram seeds was also described in Part I of this series. In this report, the contents of those acidic ninhydrin positive compounds, free amino acids and protein amino acids of green gram seeds and seedlings are described.

MATERIALS AND METHODS

Analysis of free amino acids. Green gram seeds (*Phaseolus radiatus* L. var. *typicus* Prain) (0.4 g/10 seeds)

were pulverized and extracted with 70% ethanol. Etiolated seedlings were obtained commercially, which were prepared from green gram seeds as following procedure: Green gram seeds are immersed in a water bath maintained at 45~50°C for 3 hr. Seeds are transferred into a large sieve and poured with water three times a day for 5~6 days. The length and weight of seedlings at this stage were: 180~210 mm; 0.6 g/10 cotyledons+first leaves; and 4.2 g/10 hypocotyls+roots. They were cut into two parts, i.e., cotyledons plus first leaves and hypocotyls plus roots, and each part was homogenized in 70% ethanol. An aliquot of these extracts was analyzed with an amino acid analyzer.

Analysis of γ -glutamyl peptides. Ethanol extract prepared as above was treated with Amberlite IR-120 (H⁺) and Dowex 1 $\times$ 4 (AcO⁻) to obtain the acidic amino acids fractions. These fractions were subjected to amino acid analysis. γ -Glutamylmethionine was separated from other peaks but γ -glutamylleucine was overlapped with glutamic acid, so the acidic amino acids fractions were hydrolyzed under mild conditions (3 N HCl, 120°C, 3 hr) and the hydrolyzates were analyzed. The amount of leucine which appeared after the hydrolysis was regarded as the amount of γ -glutamylleucine.²⁾ Contents of γ -glutamylphenyl-

* Present address: Hokkaido Can Company Ltd.

1) T. Kasai, Y. Ishikawa, Y. Obata and T. Tsukamoto, *Agr. Biol. Chem.*, **30**, 973 (1966).

2) T. Kasai, Y. Ishikawa and Y. Obata, *ibid.*, **30**, 979 (1966).

3) T. Kasai, S. Sakamura, S. Ohashi and H. Kumagai, *ibid.*, **34**, 1848 (1970).

alanine and γ -glutamyltyrosine were too small to be determined.

Analysis of amide-ammonia. Ethanol extracts described above were concentrated and treated with 2 N HCl for 3 hr at 100°C, and ammonia in the hydrolyzates was analyzed. Amide-ammonia was estimated by subtracting the amount of free ammonia in the extract from that of ammonia in the hydrolyzate.

Analysis of protein amino acids. Green gram seeds were pulverized and extracted with hot water. Etiolated seedlings commercially obtained, which were

prepared from green gram seeds as mentioned above, were separated into two parts as in the case of free amino acids analysis. Each part was extracted with hot water. Precipitates produced by the addition of trichloroacetic acid solution to the extracts⁴⁾ were hydrolyzed with 6 N HCl for 20 hr at 120°C and the hydrolyzate was analyzed.

RESULTS AND DISCUSSION

Contents of free amino acids and γ -glutamyl peptides of green gram seeds and seedlings are listed in Table I. Free amino acids were increased during germination especially in hypocotyl and root. γ -Glutamylmethionine which was contained at high concentration in seeds was not detected in any part of seedlings. γ -Glutamylleucine also disappeared in seedlings.

Four kinds of γ -glutamyl peptides were isolated from kidney bean (*Phaseolus vulgaris*),⁵⁾ and the name and amount of them are given in Table II with those of green gram seeds. γ -Glutamyl-S-methylcysteine which is the most abundant peptide in kidney bean has not been detected in green gram, whereas γ -glutamyl-

TABLE I. CONTENTS OF FREE AMINO ACIDS AND γ -GLUTAMYL PEPTIDES IN GREEN GRAM SEED AND SEEDLING

	Seed	Seedling	
	μ mole/10 seeds	Cotyledon plus first leaves μ mole/10 samples	Hypocotyl plus root μ mole/10 samples
Aspartic acid	0.82	1.46	7.05
Threonine	^{a)}	0.55	9.56
Serine	0.19	1.01	12.37
Glutamic acid	1.03	0.88	1.69
Proline	0.40	0.31	2.82
Glycine	0.09	0.16	1.13
Alanine	0.33	1.04	8.74
Valine	0.22	1.56	21.71
Methionine	0.79	0.05	1.41
Isoleucine	0.04	0.78	12.41
Leucine	0.05	0.68	8.74
Tyrosine	0.04	0.21	3.38
Phenylalanine	0.10	1.20	12.69
β -Alanine	0.04	—	—
γ -Aminobutyric acid	0.05	0.57	1.13
Tryptophan	0.56	0.10	1.41
Lysine	0.04	0.47	8.18
Histidine	0.04	0.99	8.46
Arginine	0.26	0.83	11.28
Amide-ammonia	4.28	18.56	177.43
γ -Glutamylleucine	0.12	—	—
γ -Glutamylmethionine	2.61	—	—
γ -Glutamylphenylalanine	$\pm$	—	—
γ -Glutamyltyrosine	$\pm$	—	—
N-Carboxymethyl- β -alanine	^{a)}	^{a)}	^{a)}

^{a)} Insufficient separation.

TABLE II. CONTENTS OF γ -GLUTAMYL PEPTIDES OF GREEN GRAM, KIDNEY BEAN⁵⁾ AND SOYBEAN.²⁾ (mg/10 g seeds)

	Green gram (<i>Phaseolus radiatus</i> L. var. <i>typicus</i> Prain)	Kidney bean (<i>Phaseolus vulgaris</i>)	Soybean (Glycine max.)
γ -Glutamylmethionine	18.2	1.4	—
γ -Glutamylleucine	0.7	3.6	—
γ -Glutamyl-S-methylcysteine	—	30.3	—
γ -Glutamyl-S-methylcysteine sulfoxide	—	2.8	—
γ -Glutamylphenylalanine	$\pm$	—	2.9
γ -Glutamyltyrosine	$\pm$	—	1.8

4) M. Kandachi and T. Yasui, *Nippon Nogeikagaku Kaishi*, **25**, 27 (1949).

5) R. M. Zacharius, *Phytochem.*, **9**, 2047 (1970).

TABLE III. CONTENTS OF PROTEIN AMINO ACIDS IN GREEN GRAM SEED AND SEEDLING

	Seed	Seedling	
	μ mole/10 seeds	Cotyledon plus first leaves μ mole/10 samples	Hypocotyl plus root μ mole/10 samples
Aspartic acid	76.5	9.2	9.0
Threonine	19.0	3.4	4.4
Serine	22.0	3.0	4.2
Glutamic acid	88.0	9.4	8.4
Proline	27.3	4.0	5.2
Glycine	47.2	7.6	8.4
Alanine	45.1	6.8	8.0
Valine	47.2	7.2	7.4
Methionine	$\pm$	—	—
Isoleucine	35.6	4.4	5.4
Leucine	60.8	9.4	8.8
Tyrosine	6.3	1.6	2.2
Phenylalanine	30.4	3.6	3.4
Lysine	70.2	6.7	7.2
Histidine	27.2	1.9	1.8
Arginine	40.9	3.9	3.0

Tryptophan was decomposed during hydrolysis with HCl.

methionine which is the most abundant peptide in green gram is the least one in kidney bean.

The contents of protein amino acids are listed in Table III. Protein amino acids were decreased during germination. No significant relationship was found between the composition of free amino acids and that of protein amino acids in both seeds and seedlings as in the case of kidney bean⁵¹ and soybean.¹¹

N-Carboxymethyl- β -alanine, the isolation and identification of which were reported in Part I of this series, was not determined due to the overlapping of its peak with a large peak of aspartic acid on analyzer chart.