

On the Seed Globulin of Cucurbitaceae*

Report 2. Amino Acid Composition of the Globulin**

Yoshihiro OKUDA

From the Laboratory for Protein Chemistry

(Head: Prof. R. Hirohata)

Yamaguchi Medical School, Ube, Japan

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The globulin of the cucurbitaceous seeds are easily extracted with 10% solution of NaCl from the defatted meal of the seed. They are obtained in crystalline form either by dialysis or dilution with water^{1,2)}. Hirohata³⁾ has reported that eight genera of the family *Cucurbitaceae* resemble one another in the chemical and physicochemical properties of their seed globulin, with an exception of *Momordica*, but that the precipitin reaction points to the existence of a slight difference in those properties between the seed globulins from the genera *Luffa* and *Trychosanthes* and those from the genera *Benincasa*, *Citrullus*, *Cucumis* and *Cucurbita*. The amino acid composition of the seed globulin of these kinds has so far been determined fragmentally by microbiological method mainly by Smith, Greene and coworkers^{4,5,6,7,8)}. They stated that, in point of the amino acid composition of their seeds, the pumpkin (*Cucurbita*) resembles the squash (*Cucurbita*), and that the cucumber (*Cucumis*), the water melon (*Citrullus*) and *Cucurbita* differ from one another. According to Anderson *et al.*^{9,10)}, who examined the sedimentation and electrophoretic properties of the globulin of different seeds, closely related species are similar to each other, and remotely related species measurably different from one another, in these properties of their seed globulin.

Of particular interest is to study the amino acid composition of many different seed globulins in *Cucurbitaceae* and to pursue comparatively the generic difference of them. The present author examined for comparison of the amino acid composition and the terminal amino acid, of the seed globulin from four different genera of the cucurbitaceous family.

MATERIALS AND METHODS

Preparation of the cucurbitaceous seed globulin.

The seeds obtained for this purpose from a seeds-raising firm (the Takii Shubyo Co., Ltd.) were those of these eight different varieties:—

Cucumis melo, L. (common melon)

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- Cucumis melo*, *L. var.* (muskmelon)
Cucumis melo var. conomon f. albus Makino (white cucumber)
Cucumis sativus, *L.* (cucumber)
Lagenaria vulgaris var. Gourda Ser. (bottle gourd)
Luffa cylindrica (sponge gourd)
Citrullus vulgaris (water melon)
Cucurbita pepo, *L. var. Toonas Makino* (pumpkin).

The kernels of these seeds were ground in a mortar. The meal obtained was defatted with petroleum ether and the globulin of the defatted meal was extracted thrice over with a 10% solution of NaCl in a water bath kept at 50°. The extract was filtered through a mixed layer of filter paper pulp with Hyflo super-Cel and the clarified filtrate was submitted to 24 hours' dialysis against running water. The precipitate was thrice repeatedly redissolved in a 10% solution of NaCl and then dialyzed against distilled water for further purification. The purified precipitate, now occurring mainly as regular octahedral crystals, and partly as twin crystals of the globulin, was washed with water and dried with ethanol and ethyl ether.

The nitrogen content of the seed globulin obtained was quantitatively determined by the micro-Kjeldahl method.

Quantitative analysis of amino acids.

The amino acid content of each sample was estimated by elution analysis on column of Dowex 50W x 8¹¹⁾. Samples of 10 mg. of the seed globulin were each hydrolyzed with 1 ml. of redistilled hydrochloric acid in a sealed tube kept at 100° for 24 and 72 hours, respectively. After removal of hydrochloric acid *in vacuo*, 5 mg. of the hydrolysate was applied on the 100 cm. column which was used for determination of the neutral and acidic amino acids. The basic amino acids were determined on the 15 cm. column applied 5 mg. of the hydrolysate. The effluent was collected in fraction of 1 ml. The quantity of amino acid in each fraction was estimated by the photometric ninhydrin method¹²⁾, and the proline content of its fraction was estimated by Chinard's method¹³⁾.

As cystine, methionine and tryptophan are destroyed partly under the above conditions of hydrolysis, they were determined individually. Tryptophan was estimated by Spies' method¹⁴⁾, cystine by the hydrazinolysis method of Kuratomi *et al.*¹⁵⁾ The seed globulin was reducibly decomposed with hydrazine hydrate at 120° for 8 hours. H₂S yielded from cystine in the protein was reacted with dimethyl-*p*-phenylenediamine, and methylene blue formed was determined colorimetrically and the quantity of cystine was estimated. Total sulfur in the seed globulin was titrimetrically determined¹⁶⁾ after it was decomposed by micro-Carius method. Methionine was calculated from the total sulfur and cystine sulfur in the protein.

Determination of the terminal amino acid.

N-terminal amino acid of the seed globulin was analyzed by the use of the FDNB technique¹⁷⁾; DNP-globulin was hydrolyzed for 24 hours at 100° in a sealed tube. The qualitative analysis of DNP-amino acid was carried out on two dimensional paper chromatography, using toluene-chloroethanol-pyridine-0.8N ammonia (5: 3: 1.5: 3) and 1.5M phosphate buffer¹⁸⁾ as solvents.

C-terminal amino acid of the seed globulin was analyzed by the hydrazine method¹⁹⁾ for its qualitative determination. A sample of 30 mg. of the seed globulin was sealed up with 0.5 ml. of anhydrous hydrazine in a tube; the tube was heated at 100° for 8 hours and its contents evaporated *in vacuo* over sulfuric acid. The residue was dissolved in water to be passed over Amberlite IR-410 (OH-form). The terminal amino acid fraction of the final residue was eluted with 2N ammonia and the terminal amino acid was identified on two dimensional paper chromatography, using acetic acid-butanol and an 80% solution of phenol as solvents.

RESULTS AND DISCUSSION

The nitrogen content of the seed globulin. The proportion in which nitrogen was contained in each anhydrous ash-free sample of the seed globulin, ranged from 17.94% to 18.61% as follows:—

Common melon	18.47%	Bottle gourd.....	18.40%
Muskmelon	18.61	Sponge gourd	18.16
White cucumber	18.51	Water melon	17.94
Cucumber	18.07	Pumpkin	18.39

In the relative nitrogen contents of the seed globulin obtained from *Cucumis melo* varieties were thus found to be much the same with one another and a variety *Cucumis sativus* was differed from the other varieties of the same genera. The seed globulins of four other genera, *Lagenaria*, *Luffa*, *Citrullus* and *Cucurbita* were varied slightly in the nitrogen contents.

Amino acid composition. In Table 1 and 2 are shown the amino acid compositions of different cucurbitaceous seed globulins determined in the protein submitted to 24- and 72-hours hydrolysis -- the values shown in Column (1) are expressed as gram of amino acid per 100 g. of protein and those in Column (2) the nitrogen contents as percent of total nitrogen in the seed globulin. The nitrogen recoveries from the hydrolyzed samples were as follows:—

Common melon	100.10%	Bottle gourd.....	95.34%
Muskmelon	99.10	Sponge gourd	97.12
White cucumber	103.37	Water melon	98.83
Cucumber	102.49	Pumpkin	101.13

The figures will show that nitrogen is recoverable in sufficient quantities from the seed globulin. It was generally seen that the cucurbitaceous seed globulin is rela-

Table 1. Amino Acid Composition of the Seed Globulins of *Cucumis*.

	<i>C. melo</i> (Common melon)		<i>C. melo</i> var. (Muskmelon)		<i>C. melo</i> var. <i>albus</i> (White cucumber)		<i>C. sativus</i> (Cucumber)	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Aspartic acid	11.49	6.54	10.95	6.16	11.66	6.63	10.57	6.17
Threonine	3.58	2.28	4.08	2.57	3.65	2.32	3.67	2.40
Serine	7.00	5.05	7.31	5.21	6.30	4.54	7.35	5.44
Glutamic acid	20.40	10.51	20.44	10.40	19.21	9.88	20.24	10.69
Proline	5.62	3.70	4.96	3.23	5.21	3.43	4.98	3.36
Glycine	5.14	5.19	4.89	4.90	4.67	4.71	4.80	4.97
Alanine	5.24	4.46	4.93	4.14	5.24	4.45	4.89	4.27
Valine	5.91	3.83	5.11	3.27	6.13	3.96	6.18	4.10
Isoleucine	4.83	2.79	4.40	2.51	4.89	2.82	4.77	2.83
Leucine	9.34	5.40	8.65	4.94	8.57	4.94	8.39	4.97
Tyrosine	3.22	1.35	3.24	1.34	2.75	1.15	3.27	1.40
Phenylalanine	7.64	3.51	6.85	3.11	7.91	3.62	7.35	3.46
Histidine	2.57	3.77	2.83	4.10	3.05	4.46	2.68	4.03
Lysine	3.00	3.11	3.00	3.07	3.31	3.43	3.43	3.65
Arginine	16.15	28.12	16.97	29.17	17.76	30.85	15.89	28.36
Ammonia (NH ₃)	1.32	5.90	1.46	6.47	1.75	7.80	1.70	7.76
Methionine	3.19	1.62	3.32	1.67	2.89	1.47	3.05	1.59
Cystine	2.09	1.32	2.12	1.32	1.73	1.09	1.65	1.08
Tryptophan	2.22	1.65	2.07	1.52	2.46	1.82	2.58	1.96
Totals	119.95	100.10	117.58	99.10	119.14	103.37	117.44	102.49

(1) gram of amino acid per 100 g. of protein

(2) nitrogen as per cent of total nitrogen

tively rich in arginine, glutamic acid and aspartic acid.

Four varieties of *Cucumis melo* and *Cucumis sativus* were found to resemble one another in the amino acid composition of their seed globulin, though *Cucumis melo albus* was slightly higher than the varieties of *Cucumis melo* and *Cucumis sativus* in the arginine content. The pumpkin (*Cucurbita*) was similar in the amino acid composition to 4 species of *Cucumis*, showing apparently that the more closely related species of the genus *Cucumis* are alike in the amino acid composition of their seed globulin.

The water melon (*Citrullus vulgaris*) appeared to differ in the amino acid composition of its seed globulin from any other species in *Cucurbitaceae*, and the bottle gourd (*Lagenaria vulgaris*) and the sponge gourd (*Luffa cylindrica*) from the other genera of the family *Cucurbitaceae*. Thus the author's work is fully compatible with Smith and Greene's⁶⁾ in having disclosed that the pumpkin, cucumber and water melon are measurably different from one another in the kind of the globulin

Table 2. Amino Acid Composition of the Seed Globulins of *Lagenaria*, *Luffa*, *Citrullus* and *Cucurbita*.

	<i>Lagenaria vulgaris</i> (Bottle gourd)		<i>Luffa cylind.</i> (Sponge gourd)		<i>Citrullus vulgaris</i> (Water melon)		<i>Cucurbita pepo</i> (Pumpkin)	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Aspartic acid	10.81	6.18	9.84	5.70	9.28	5.45	10.63	6.08
Threonine	3.12	1.99	2.67	1.73	3.17	2.08	3.53	2.26
Serine	5.02	3.64	5.21	3.83	4.86	3.62	7.48	5.42
Glutamic acid	18.41	9.52	19.83	10.40	17.00	9.04	19.49	10.09
Proline	5.35	3.54	5.01	3.36	5.05	3.43	4.66	3.08
Glycine	4.93	5.00	5.53	5.68	4.94	5.15	4.86	4.93
Alanine	4.62	3.94	5.48	4.74	5.32	4.67	4.90	4.19
Valine	5.45	3.54	5.43	3.58	5.23	3.49	6.43	4.18
Isoleucine	4.62	2.93	5.02	2.95	4.99	2.98	4.82	2.80
Leucine	7.85	4.56	8.52	5.01	8.08	4.81	8.75	5.08
Tyrosine	3.07	1.29	3.89	1.66	3.62	1.56	3.46	1.46
Phenylalanine	7.07	3.26	5.77	2.70	6.95	3.20	8.05	3.71
Histidine	2.78	4.09	2.64	3.94	2.77	4.19	2.45	3.61
Lysine	2.71	2.82	2.88	3.04	2.53	2.71	3.10	3.23
Arginine	16.36	28.60	15.94	28.23	18.35	32.95	17.44	30.50
Ammonia (NH ₃)	1.36	6.10	1.40	6.36	1.07	4.92	1.27	5.70
Methionine	2.77	1.41	2.32	1.20	2.98	1.57	3.92	2.01
Cystine	1.71	1.08	1.89	1.21	1.87	1.22	1.65	1.05
Tryptophan	2.43	1.85	2.38	1.80	2.34	1.79	2.34	1.75
Totals	110.49	95.34	111.65	97.12	110.40	98.83	119.23	101.13

(1) gram of amino acid per 100 g. of protein

(2) nitrogen as per cent of total nitrogen

their seeds contain.

The terminal amino acid. All seed globulin from plants of different species of the family *Cucurbitaceae* contained in equal rations glycine, alanine and glutamine as N-terminal group and the same three amino acids as the C-terminal group.

The polycomponent nature of the seed globulin, on the electrophoretic studies, indicated that the crystalline form is no proof of its homogeneity²⁰. Other investigators^{9,10,21} have also stated that the seed globulin are not a homogeneous protein. Therefore, the terminal amino acid must be examined in the homogeneous form in future.

According to Bodo²², the amino acids of the seed globulin varies in its pattern with fertilization of the original plant. If so, these pattern of the cucurbitaceous seed globulin may better be determined by obtaining seeds from many different species and genera of the family cultured all alike in one and the same field.

SUMMARY

1. A comparative study by ion exchange chromatography was made of the amino acid composition of the seed globulin from different species in the family *Cucurbitaceae*, comprising *Cucumis melo*, *L.*, *Cucumis melo*, *L. v.*, *Cucumis melo v. conomon f. albus*, *Cucumis sativus*, *Lagenaria vulgaris var.*, *Luffa cylindrica*, *Citrullus vulgaris* and *Cucurbita pepo*.

2. Most of the seed globulins so examined were relatively rich in arginine, aspartic acid and glutamic acid.

3. The amino acid composition of the seed globulin was nearly the same for four different varieties of the genus *Cucumis melo* and *Cucumis sativus*. *Cucumis*, *Citrullus*, *Lagenaria* and *Luffa* seemed to differ one from the other.

4. The total nitrogen contents of the seed globulin were ranged from 17.94 to 18.61 per cent. In the nitrogen content of their seed globulins, the varieties of *Cucumis melo* were the same with one another and *Cucumis sativus* variety differed from *Cucumis melo*.

5. In all of them glycine, alanine and glutamine were found as N-terminal amino acid and glycine, alanine and glutamine as C-terminal amino acid, thus no considerable difference in the terminal amino acid was recognizable.

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