ISOLATION AND STUDY OF THE PEPTIDES FROM AN ALCOHOLIC EXTRACT OF THE SEEDS OF GLYCINE HISPIDA MAX.

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The proteins of Glycine hispida Max. (soybean) are rich in lysine [1, 2]; they have been studied by numerous investigators [3-6]. Only free amino acids have been found in alcoholic extracts of seeds of the leguminosae [4]. We have succeeded in showing that, in addition to amino acids, these extracts also contain a number of peptides of different degrees of complexity.

From soybean flour, about 10% of nitrogen-containing compounds is extracted with 70% alcohol and precipitated with acetone. Some authors have regarded these substances as free amino acids [4] and, on paper chromatography, have assigned them to "an unidentified spot" [7].

The aim of the present work is to consider the chromatographic and electrophoretic behavior of the total peptide fraction and its components and to establish their amino acid composition. Kubanskaya soybean of variety 276 (1962 harvest, Central Asian Station of the All-Union Institute of Plant Breeding) was used in the experiments.

Cases are known in which peptides have been discovered, or their presence has been assumed [9], in seeds of leguminosae, for example, lupin [8]. However, the free amino acids [4, 5] and other nitrogen-containing components [10] have usually been investigated.

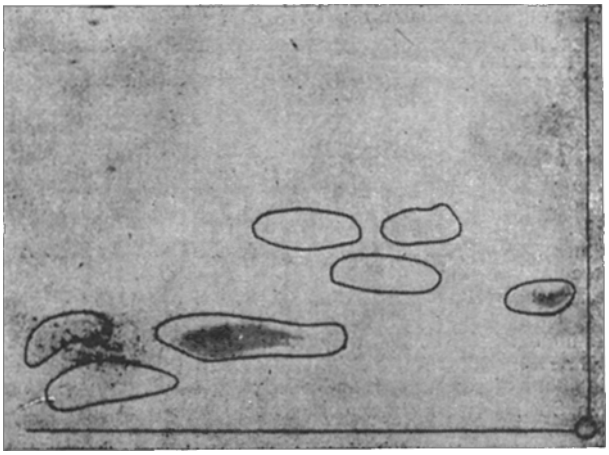

Fig. 1. Two-dimensional chromatogram of the alcoholic fraction; right to left - phenol-water; bottom to top - butan-1-ol-acetic acid-water (4:1:5).

We have found no report of a method of obtaining peptide fractions from an alcoholic extract of leguminosae seeds in the literature available to us. We have determined the peptides extracted by alcohol and precipitated by acetone in four species of the family leguminosae (soybean, mung bean, cow pea, chick pea) and one species of the malvaceae (cotton family). Various peptides may apparently be present in the seeds of other plants, as well.

The mixture of peptides was freed from the uncombined amino acids by means of acetone. On the chromatogram, this mixture showed the presence of seven individual peptides (Fig. 1). After hydrolysis of the peptide mixture with hydrochloric acid, twelve amino acids were found by chromatography (table).

The mixture of peptides was separated into seven fractions by preparative chromatography (Fig. 2). Each fraction was subjected to hydrolysis. The amino acids formed were chromatographed on paper with reference samples. The amounts of the amino acids were determined both visually [4, 6, 11, 12] and spectrophotometrically by Bohley's method [13].

Thus, the soybean peptides contain 12 amino acids in the following sequence with respect to the size of the spots: glutamic acid, glycine and cystine, alanine, lysine, and so on. In determining the food value of the seeds, the presence of a considerable amount of lysine in them must be taken into account.

Amino Acid Composition of the Peptide Fractions of Soybean

Amino acid	Presence of free amino acids [2]	Amount of amino acid residues found in the individual fractions								
		fraction no.								
		I	II	III	IV	V		VI	VII	
		visually				visually	by the SF-4	visually	visually	by the SF-4
Glycine	—	—	2	—	—	1	1.02	—	4	2.78
Alanine	+	—	—	—	2	—	—	2	1	1.00
Serine	+	—	—	1	—	—	—	—	2	1.51
Threonine	—	—	—	—	—	3	1.78	—	—	—
Cystine	—	1	—	3	—	2	1.77	1	—	—
Valine	+	—	—	—	—	—	—	—	—	—
Leucine, isoleucine	+	—	—	—	—	—	—	—	—	—
Aspartic acid	+	—	1	1	—	—	—	—	—	—
Glutamaric acid	+	3	—	2	—	—	—	3	—	—
Arginine	+	—	—	1	—	—	—	—	—	—
Lysine	—	—	—	—	3	1	1.00	—	—	—
Histidine	—	—	—	1	—	—	—	—	—	—
Tyrosine	+	—	—	—	—	1	1.62	—	—	—
Proline	—	—	—	—	—	3	3.50	—	—	—
Number of amino acid residues in the peptide subfraction	—	4	3	9	5	11	10.7	6	7	5.3

Experimental

The seed lobes freed from the coat and germ part, were ground to a flour and exhaustively defatted with ether and then with acetone. The flour was extracted with 70% alcohol (1:5 and 1:3) until the extract gave a negative reaction with the Folin-Lowry reagent [14]. The extracts were combined, filtered, and evaporated in vacuum at 40-50°C to a volume of 65-70 ml. Part of them were dried (P_2O_5, 99°C, 30-50 mm). The yield of extracted substances calculated on the air-dry flour was 18.2%.

A double volume of acetone was added to the concentrate and after the mixture had stood for 15 hr in the cold it was centrifuged for 50 min at 3600-3800 rpm. A viscous mass separated out. The upper layer (alcohol-acetone) was decanted off and the residue was treated with a five-fold amount of acetone. After vigorous shaking, the mixture was left to stand for 2-2.5 hr and was then centrifuged again. The sticky hygroscopic mass was triturated with dry acetone to form a free-flowing powder. The faintly yellowish mixture of peptides without free amino acids obtained was readily soluble in water and 70% alcohol. A considerable part of the substances from the solution passed through a colloid membrane. The solutions gave a positive reaction with ninhydrin and with the Folin-Lowry reagent, and also the reaction for carbohydrates with malonic ester [15].

The aqueous extracts were subjected to paper electrophoresis in an EFA-1 apparatus with two buffer solutions: medialveronal buffer – pH 8.6; 0.1 μ, 150 V; 0.625 ma; borate buffer – pH 12.37; 0.1μ 200 V, 2.2 ma. The electrophoregrams had dimensions of 3 × 26 cm. The substances were found to be clearly separated after phoresis for 4 hr with both buffers. The mixture of peptides consisted of seven components of which five migrated to the anode and two to the cathode on the electrophoregrams. The

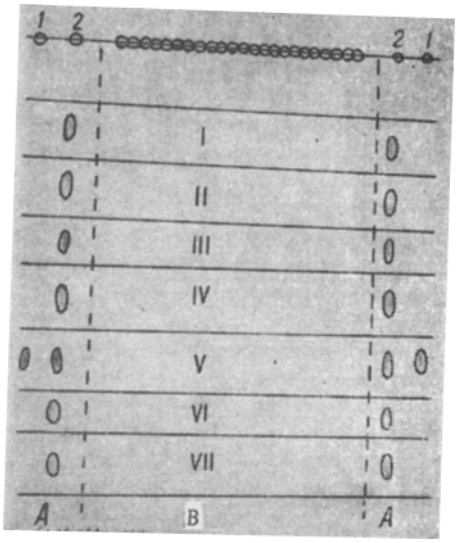

Fig. 2. One-dimensional descending chromatogram of the total peptide fraction: 1) Standard, glycine; 2) guide trace of substance under investigation; the broken line shows where the paper was cut. A) Detected (with ninhydrin); B) undetected; I-VII) cutting of strips with the seven peptides.

electrophoregrams were obtained with a 0.2% solution of ninhydrin in butan-1-ol saturated with water and containing 4% of acetic acid; three spots were brighter and two showed up very weakly.

One-dimensional chromatography. A solution of 20 mg of the mixture of peptides in 20 µl of water was deposited on five strips of chromatographic paper (Leningrad type "B") with dimensions of 8 × 60 cm together with reference samples of glycine and tyrosine. The descending method of chromatography in the butan-1-ol-acetic acid-water (4:1:5) system was used, and runs were continued for 24, 48, 72, 96, and 120 hr. The chromatograms were revealed by a ninhydrin solution. The optimum running time was 96 hr, under which conditions glycine had moved 34 cm and tyrosine had been eluted from the paper. By this means, the mixture was separated into seven fractions (I-VII) with the following R_f values: I-0.23, II-0.28, III-0.32, IV-0.38, V-0.51, VI-0.63 and VII-0.65. In this way the necessary amount of the individual fractions for further investigation was obtained.

Elution of the substances from the chromatograms was carried out by the Dent-Ioffe method [11]. The fact that each fraction consisted of a single component was shown by electrophoresis.

All the peptides were individually hydrolyzed for 24 hr in the boiling water bath in a sealed capillary with a 5.7 N solution of hydrochloric acid. After hydrolysis, the hydrochloric acid was eliminated by repeated distillation with water, and the residue was analyzed by paper chromatography.

Summary

Seven peptides have been isolated from soybean seeds. Twelve amino acids have been detected in the hydrolysis products of the peptides.

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