CARBOHYDRATE-PROTEIN COMPOUNDS OF Stigmata maydis

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It has been shown previously [1] that maize stigmata, Stigmata maydis, contain a phytohemagglutinin (PHA). The present paper gives an account of its chemical characteristics.

We used two methods of extraction to obtain the PHA after preliminary treatment of the maize stigmata with methanol: 1) extraction with dilute hydrochloric acid followed by precipitation with ammonium sulfate (sample A), and 2) extraction with ammonium oxalate followed by precipitation with ethanol (sample B).

A comparative study of the two samples was performed. A preliminary investigation showed that they contained carbohydrates and protein components. Hydrolyzates of both samples were found to contain the following monosaccharides: galactose, mannose, glucose, arabinose, xylose, traces of rhamnose, a uronic acid, glucosamine, and galactosamine. In addition, amino acids were identified: asparagine, glutamic acid, glycine, alanine, lysine, proline, serine, threonine, cysteine, valine, methionine, leucine, isoleucine, tyrosine, phenylalanine, histidine, arginine, and cystine.

Analytical results for the carbohydrate-protein compounds from maize stigmata are given below (%): Spectral analysis showed the presence of Ca and Mg ions in samples A and B.

Sample	Monosaccharides	Protein	*N*	*P*	*S*	*OAc*	OCH_3	Ash
A	26.5	50.0	8.0	—	1.0	...	2.5	2.0
B	28.5	46.8	7.5	0.2	0.25	0.6	2.6	2.2

Great interest was presented by a study of the homogeneity of the samples obtained. The results of gel filtration of Bio-Gel P-150 are shown in Fig. 1. As can be seen from the figure, sample A is distinguished by a lower homogeneity, although the formation of two main peaks is observed in both cases. Analysis of the compounds corresponding to the two peaks showed that they contained carbohydrate and protein components. Hydrolyzates of these compounds were found to contain the same monosaccharides and amino acids as the initial samples A and B.

The results of chromatography on Sephadex G-200, which are given in Fig. 2, also show the absence of the homogeneity of samples A and B, sample A being characterized by considerably greater nonhomogeneity. The compounds corresponding to peaks I and II (see Fig. 2) were isolated. Their hydrolyzates were found to contain the same monosaccharides and amino sugars as the initial samples A and B.

Chromatography on DEAE-cellulose (Fig. 3) led to the formation of three fractions, the first being eluted with phosphate buffer, the second with a 0.01 N solution of hydrochloric acid, and the third with a 0.1 N solution of caustic soda. As can be seen from Fig. 3, the first fraction gives absorption at 280 nm and by the phenol–sulfuric acid method (at 490 nm), which shows the presence of carbohydrate and protein components in it. The hydrolysis of this fraction gave the same monosaccharides and amino acids as the initial samples A and B. The second fraction gave practically no absorption by the phenol–sulfuric acid method. A hydrolyzate of this fraction was found to contain amino acids with only traces of monosaccharides. Finally the third fraction was strongly colored and its hydrolyzate was practically free from amino acids and monosaccharides. It probably represents some decomposition products and components accompanying the main compounds. Similar results were obtained when chromatography was performed on DEAE-Sephadex A-25.

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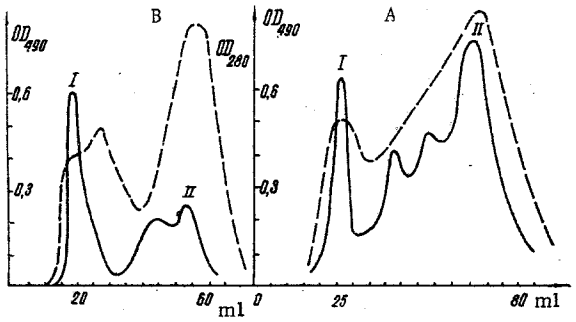

Fig. 1. Gel filtration of the carbohydrate–protein compounds of maize stigmata on Bio-Gel P-150: A) sample A: B) sample B: OD = optical density at 490 nm (phenol–sulfuric acid method). The absorption at 280 nm is shown by the broken line in each case.

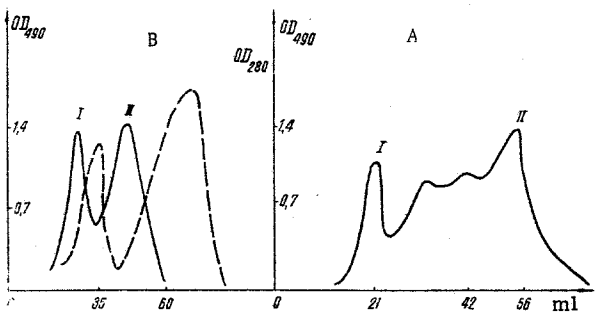

Fig. 2. Gel filtration on Sephadex G-200. Symbol the same as for Fig. 1.

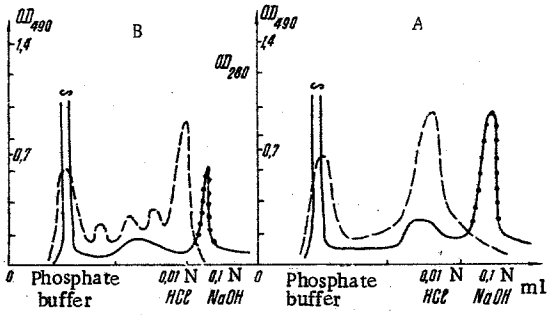

Fig. 3. Chromatography on DEAE-cellulose (the section of the curve with dots relates to visible light, colored fractions). Symbol the same as for Fig. 1.

Ion-exchange chromatography on IRC-50 (H^+) did not enable us to separate the protein and carbohydrate components. The substances were eluted almost completely by phosphate buffer, giving only a single peak (Fig. 4).

The caebohydrate–protein compounds of maize stigmata possess a well-defined agglutinating activity. The agglutination titer of the total fraction (a sample of 10 mg/1 ml of physiological solution) was 1:512, and those of fractions A and B after long storage 1/32 and 1/16, respectively. In addition, the PHA possesses mitotic activity. Depending on the concentration of material, we counted the following numbers of metaphase plates: at a concentration of 0.50-0.60 mg/ml–30-40; at 1.5-2.5 mg/ml–40-65; and at 2.0-2.4 mg/ml–80-85. Under the same conditions, the activity of a PHA preparation of the film "Difco" (USA) was about 70 metaphase plates at a concentration of PHA of 0.02 mg/ml of standard solution.

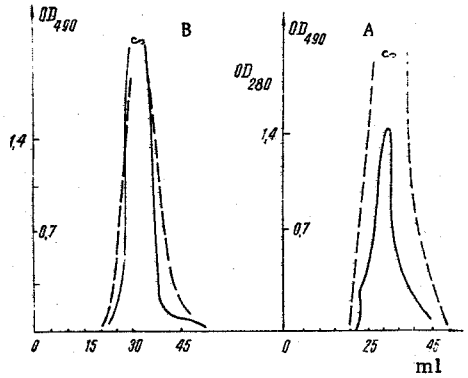

Fig. 4. Ion-exchange chromatography on IRC-50 cation-exchange resin (H^+) with elution by phosphate buffer (pH 6). Symbols the same as for Fig. 1.

EXPERIMENTAL

In order to determine their monosaccharide compositions, the phytohemagglutinins were hydrolyzed with 2 N sulfuric acid at 100°C for 6 h. The hydrolyzates were neutralized with barium carbonate and deionized with KU-2 cation-exchange resin (H^+). The solutions were concentrated in vacuum at 35°C.

Partition paper chromatography (PC) was performed on FN-12 and FN-3 papers with the following solvent systems: 1) butan-1-ol–pyridine–water (6 : 4 : 3); and 2) the Partridge system [butan-1-ol–acetic acid–water (4 : 1 : 5), upper layer]. Aniline phthalate was used to reveal the monosaccharides.

Gas–liquid chromatography (GLC) was performed on a Vyrukhrom chromatograph with a flame-ionization detector using spiral stainless-steel columns (100 × 0.3 cm); rate of flow of N_2 33 ml/min, of H_2 33 ml/min, and of air 300 ml/min. The stationary phase was HIEEF-8-Bp on Gas-Chrom Q (100-200 mesh). The monosaccharides in the form of the aldononitrile acetates were chromatographed (GLC) with programming of the temperature from 160 to 225°C (program rate 4 deg/min).

The total amount of monosaccharides was determined by the phenol–sulfuric acid method [2], the amount of proteins from the nitrogen content, and the ash by igniting samples at 600°C to constant weight. The amino sugars were determined by performing hydrolysis with 6 N hydrochloric acid at 110°C for 4 h, and the amino acids by hydrolyzing under similar conditions for 24 h. The hydrochloric acid was eliminated by repeated evaporation in vacuum and the residue was used for analysis on a "Jeol" (Japan) type JIC-6AH amino-acid analyzer.

For the gel chromatography of aqueous solutions (1 ml) of samples of phytohemagglutinin (10-20 mg) we used columns with Bio-Gel P-150 (36 × 1.5 cm) and with Sephadex G-200 (38.5 × 1.5 cm). Elution was carried out with water, 3-ml fractions being collected. The results of gel filtration are shown in Figs. 1 and 2.

Isolation of Sample A. Isolation of sample was accomplished in the following way: extraction with water acidified to pH 4-5 at room temperature; fractionation with ethanol of increasing concentration; further purification–salting out with ammonium sulfate at 0.4 saturation (saturated aqueous solution of ammonium sulfate previously diluted 2.5-fold).

Isolation of Sample B. Comminuted air-dry maize stigmata (18 g) that had previously been treated with methanol and with hydrochloric acid (pH 5) at 60°C for 4 h were extracted with a 1% solution of ammonium oxalate on the boiling-water bath for 10 h. The extract obtained was evaporated to small volume and poured into ethanol. The resulting precipitate was separated off and washed with ethanol. The precipitate after gentle drying in air was dissolved in water, dialyzed against distilled water, and freeze-dried. This gave 1.2 g (6.5%) of sample B.

Ion-Exchange Chromatography of Samples A and B on DEAE-Cellulose. The total carbohydrate-protein fraction (60 mg) was deposited on a column (37 × 1.8 cm) of DEAE-cellulose in the phosphate form. Elution was carried out by the following scheme: phosphate buffer, pH 4.5; phosphate buffer, pH 4.5, in 0.1 M NaCl solution; phosphate buffer, pH 4.5, in 1 M NaCl; 0.2 M phosphate buffer + phosphoric acid to pH 2.9; 0.01 N hydrochloric acid solution; 0.1 N NaOH solution.

The rate of elution was such as to give a 15-ml fraction every hour, and the fractions were analyzed by the phenol–sulfuric acid method and by their absorption at 280 nm. The results are given in Fig. 3.

Chromatography on Cation-Exchange Resin. The total carbohydrate-protein fraction (160 mg) was deposited on a column (40 × 1.5 cm). Elution was performed with phosphate buffer, pH 6, and then with phosphate buffer, pH 6, in 1 M NaCl solution. The fractions were analyzed as described above. The results are given in Fig. 4.

The determination of the hemagglutinating activity of the initial carbohydrate-protein compounds of maize stigmata and of the fractions isolated by chromatography on various adsorbents were determined in a dilution series by the method of Dubois et al. [3].

SUMMARY

It has been shown that the phytohemagglutinins of maize stigmata consist of carbohydrate-protein compounds and that the carbohydrate and protein components have a fairly strong bond. The nature of this bond remains unknown.

The phytohemagglutins isolated possess a well-defined agglutinating and mitotic activity.

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A NEW PREPARATIVE METHOD FOR ISOLATING CEREBROSIDES AND SPHINOGOSINE BASES FROM CATTLE BRAIN

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The majority of preparative methods for isolating the cerebrosides of the brain described hitherto are based on the poor solubility of these compounds in ether and cold ethanol and their good solubility in hot ethanol [1, 2]. For the final purification of the cerebrosides, crystallization from glacial acetic acid has been recommended [3]. The isolation of cerebroside fractions has also been performed by the chromatography of the total lipids on silica gel or on alumina [4-6]. Another method for the preparative isolation of the cerebrosides of the brain [7, 8] is based on the elimination of the bulk of the phospholipids by precipitation with acetone and filtration through a column packed with Florisil. The acidic and basic phospholipids remaining in the extract were removed by ion-exchange chromatography on a column containing a mixture of cation- and anion-exchange resins. The final stage of purification of the cerebrosides was crystallization from a mixture of chloroform and methanol [7, 8]. Finally, the chromatography of the total lipids of the brain on DEAE-cellulose and on magnesium silicate has been recommended for the isolation of the cerebrosides [9, 10]. The best of the preparative methods described in the literature is that of Wells and Dittmer [11], which successfully combines the preliminary purification of the total sphingolipids by extraction with cold ether, the cleavage of the phospholipids by mild alkaline hydrolysis [12], and column chromatography on silica gel, leading to the isolation of the three main types of brain sphinogolipids: cerebrosides, sulfatides, and sphingomyelin.

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