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ISOLATION OF HIGHLY SPECIFIC PROTEINS FROM COTTON SEEDS OF VARIETY C-6030

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In a comparative investigation of the seeds of cotton plants of the varieties Tashkent-1 (G. hirsutum) and C-6030 (G. barbadense) by electrophoresis in PAG [1], individual proteins specific for this species were detected with R_f 0.37 and 0.48 for G. barbadense (B. 0.37 and B. 0.48) and with R_f 0.43 and 0.51 for G. hirsutum (H. 0.43 and H. 0.51) [2]. In order to elucidate the differences in their physicochemical characteristics that are responsible for their high specificity it was necessary to isolate and purify them.

The individual proteins from cotton seeds of the variety C-6030 were isolated from the acetone-defatted flour. After three extractions of the flour with distilled water, which removed the bulk of the water-soluble proteins, the flour was stirred for an hour with 0.05 M Tris-HCl buffer, pH 8.9, in a ratio of 1:10 (weight/volume). The resulting suspension was centrifuged at 3000 rpm (K-70) for 30 min, and the supernatant was treated with dry ammonium sulfate to 80% saturation and was then kept in the refrigerator until a precipitate had formed. The precipitate contained the accompanying high-molecular-mass proteins. It was separated off by centrifugation, and the supernatant was again treated with dry ammonium sulfate, to 100% saturation, and was left in the refrigerator. The protein precipitate that deposited, enriched with fractions of the highly specific proteins B. 0.37 and B. 0.48, was separated off by centrifugation and was dissolved in 0.05 M Tris-HCl buffer, pH 8.9, and the solution was dialyzed against water and was lyophilized. Electrophoresis in PAG showed that the fraction obtained at 100% saturation contained other proteins besides the highly specific proteins.

The further purification of proteins B. 0.37 and B. 0.48 was performed by gel filtration on a column of Sephadex G-100. On chromatography in 0.05 M NH_4HCO_3 the proteins were separated into three peaks. After lyophilization and electrophoresis of the fractions obtained it was found that the highly specific proteins of G. barbadense had issued from the column in the second peak, without being separated from one another.

The highly specific proteins B. 0.37 and B. 0.48 were separated by preparative isoelectric focusing. The isoelectric points of these proteins were first determined by analytical isofocusing, which was conducted on a Multiphor instrument (LKB) in the prepared PAG gels associated with it. Pieces of filter paper were impregnated with 10 μl of protein solution (concentration 2 mg/ml) and were deposited on a gel with a pH gradient of from 3.5 to 9.5. A standard set of proteins with known isoelectric points (pI – Marker Proteins; Protein Test Mixture 9, Serva) was deposited in parallel. After cooling to 8-10°C, isofocusing was carried out at a voltage of 200-500 V for 2 h. The gels were fixed and stained and were then washed free from the excess of dye. The results of isofocusing showed that protein B. 0.37 had an isopoint of 6.2, and protein B. 0.48 one of 5.8.

Preparative isoelectric focusing was conducted in a LKB vertical column in a 5-60% gradient of sucrose solution containing ampholines with pH 5-7 in a concentration of 1%. The

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5-60% concentration gradient of sucrose was created in a column with a volume of 110 ml, and it was cooled to 6°C. Part of the gradient (3 ml) was taken off with a tube, the B. 0.37 and B. 0.48 proteins were dissolved in it, and the density of the solution was adjusted to the required value. The protein solution so obtained was returned to the sucrose gradient, and separation was carried out at 1500 V for 24 h at 6°C. During isoelectric focusing the proteins collected in the column in the form of narrow disks which were carefully sucked off separately with the aid of a micropump and were then dialyzed and lyophilized. Electrophoresis in PAG of the fractions obtained showed that the highly specific proteins B. 0.37 and B. 0.48 of G. barbadense were homogeneous.

To determine the molecular masses of the highly specific proteins from G. barbadense we used a column of Sephadex G-100 that had previously been calibrated with proteins having known molecular masses, such as blue dextran – $2 \cdot 10^6$ Da, immunoglobulin – 160,000 Da, bovine serum albumin – 67,000 Da, ovalbumin – 45,000 Da, and lactalbumin – 18,000 Da. According to the results of column gel chromatography, the molecular masses of proteins B. 0.37 and B. 0.48 were each 18,000. This was confirmed by the results of ultracentrifugation and of electrophoresis under denaturing conditions in the presence of 0.1% of sodium dodecyl sulfate and 1% of β-mercaptoethanol.

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SPECTROSCOPY OF MAIZE PROTEINS

I. MOLECULAR FORMS OF THE TRYPSIN INHIBITOR

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The polymorphism of plant proteinase inhibitors is determined by their genotypes [1-3]. We have previously [4] demonstrated differences in the component composition of trypsin inhibitors isolated from maize grains with different genotypes and have also determined the spectral characteristics of total preparations of inhibitors [5]. In view of information [6] on the possibility of using spectroscopy for establishing structural differences between isoenzymes, we have studied the spectral-fluorescent properties of the molecular forms of the trypsin inhibitors from normal (A 204+/+) and mutant (A 204 o2/o2) lines of maize. The low-molecular-mass and high-molecular-mass components [(I) and (II), respectively] were isolated from total preparations of the trypsin inhibitors by preparative isotachophoresis in a column of 4.5% polyacrylamide gel with 6 M urea (volume 5 ml) [4]. The conditions for recording the spectra have been described previously [5].

The nature of the appearance of the absorption maxima in the UV spectra of the substances under investigation differed: a structured band of a tryptophan chromophore (277 nm) was characteristic only for component (II) of the initial maize, while for the other components the maximum was appreciably broadened. The values of the optical density ratios D_{260}/D_{280} confirmed the differences in the individual molecular forms of the inhibitors:

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