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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Type of maize	Component	D_{260}/D_{280}	φ	r
A 204+/+	I	1.02	0.080 ± 0.005	0.061 ± 0.003
	II	0.92	0.084 ± 0.005	0.062 ± 0.003
A 204 o2/o2	I	1.11	0.060 ± 0.005	0.061 ± 0.003
	II	1.03	0.077 ± 0.005	0.056 ± 0.003

The fluorescence characteristics of the components are determined by the tryptophan fluorophore: At an excitation wavelength of 280 nm the fluorescence maximum of each of the proteins was observed at 346 nm (half-width of the maximum 62 nm). The identical position of the fluorescence maxima showed that the low-molecular-mass components (I) were not products of the organic proteolysis of the high-molecular-mass components (II), since the positions of the fluorescence maxima of proteins change substantially as the result of proteolysis [7]. The values of the quantum yields φ of the molecular forms of the trypsin inhibitors of the normal and mutant maizes differed, which may be connected with the nature of the microenvironment of the tryptophan residues in the protein macromolecule. The values found for the fluorescence anisotropy r were low, which, with a low viscosity of the microenvironment in dilute solution, may indicate a flexibility of the sections of the polypeptide chain at the tryptophan-binding sites and their possible localization on the surface of the macromolecule.

Thus, the spectral-fluorescent investigation has confirmed the appearance of structural differences of the individual molecular forms of the trypsin inhibitor from maize grains with a change in genotype.

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