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STRUCTURE, ANTIGENIC ACTIVITY, AND BIOLOGICAL PROPERTIES OF WATER-SOLUBLE COTTON MARKER PROTEINS

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The water-soluble fraction of cotton-seed auxiliary proteins contains species-specific proteins designated as H-0.13 and B-0.18 [1, 2]. The existence of two forms of H-0.13 that contain carbohydrate component was also reported. Practical recommendations for their use in cotton selection and seed production have been developed.

The present communication discusses results from certain structural and biological studies of these proteins by column and ion-exchange chromatographies, ultracentrifugation, and antigenic and hemagglutinating activities.

Water-soluble protein fractions from *G. hirsutum* and *G. barbadense* were investigated.

Removal of husks, defatting, and extraction of the total water-soluble fraction were carried out as before [3]; electrophoresis in 7.5 and 17% PAAG, according to Davis [4]; gel filtration and ion-exchange chromatography, over Sephadex G-150 and DEAE cellulose columns.

The elution profiles of the total water-soluble protein fractions from *G. hirsutum* and *G. barbadense* led to the conclusion that the fractions are similar. The tryptic hydrolysates of the native marker proteins H-0.13 and B-0.18 from *G. hirsutum* and *G. barbadense* were separated and compared in order to determine if the marker properties of these cotton proteins are a consequence of differences in the primary or tertiary structure. The proteins were hydrolyzed for 6 h at 37° C by adding aqueous trypsin twice with a 3-hour interval at a dose of 0.5 μg per 100 mg of protein. The modification was carried out in Tris-HCl (0.5 M) at pH 8.5 containing EDTA (0.25 M), guanidine chloride (6 M), and β-mercaptoethanol (8 mM).

The tryptic hydrolysates of marker proteins were analyzed by two-dimensional electrophoresis and ascending chromatography on thin-layer silica-gel plates (20×20 cm) and electrophoresis of peptides in CH_3COOH:HCOOH:water (100:3:897) at pH 6.5 and 1000 V in the cold for 80-90 min. The peptides fluoresced at 360 nm after spraying the dried plates with triethylamine solution (10%), fluorescamine in acetone (0.1%), and again triethylamine.

Despite differences found in the molecular weights by sedimentation analysis, 120 kDa for H-0.13 and 112 kDa for B-0.18, thin-layer chromatography of the tryptic hydrolysates of *G. hirsutum* and *G. barbadense* native marker proteins on silica-gel plates revealed no differences between them.

We also found the common antigenic parts of these proteins. Antibodies were obtained by intramuscular immunization of rabbits with these proteins prepared by preparative electrophoresis three times at a dose of 5-8 mg/kg over 21 days without using Freund's adjuvant. Results from electrophoresis of marker and other water-soluble proteins in the presence of SDS and β-mercaptoethanol and double immunoelectrophoresis indicated that they have similar polypeptide organization and antigenic properties.

Erythrocytes for investigation of the hemagglutinating activity were prepared analogously to the literature method [5]. The reaction of erythrocytes with proteins was observed under a microscope with preliminary equilibration of [NaCl] to 0.9% by dialysis. Solutions of bovine serum albumin and physiological saline were used as controls.

Results for the hemagglutinating activity of marker protein H-0.13 for erythrocytes of two human blood groups indicated that they react selectively.

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Thus, the polypeptide organization of marker proteins H-0.13 and B-0.18 and the principal components of the water-soluble fraction of *G. hirsutum* and *G. barbadense* cotton seeds have similar structures. Differences in the molecular weights and electrophoretic mobilities are due to varying amounts of subunits in the native molecules.

A result of the identical polypeptide organization is the expression of similar antigenic properties for the principal protein components of the water-soluble fraction from *G. hirsutum* and *G. barbadense* cotton seeds.

Marker protein H-0.13 has selective hemagglutinating activity for human erythrocytes.

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