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COMPARATIVE CHARACTERISTICS OF PROTEINS OF COTTONPLANT LINES DIFFERING IN THE STRENGTH OF THE FIBER

A. A. Akhunov, F. A. Ibragimov, Z. Golubenko, N. A. Abdurashidova, É. Ch. Mustakimova, and Yu. V. Beresneva

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The enzymes and proteins of the fibers of two lines of cotton plant differing in the strength of the fiber have been investigated. It has been shown that the activities of glucan synthetase and peroxidase rise as the fiber matures, while the activities of β-(1-3)-glucanase and cellulase fall. The specific enzymatic activities of peroxidase and glucan synthetase in the L-175 line, distinguished by a stronger fiber, are higher than for the L-466 line with a weaker fiber. The activity of glucanase changes according to the strength of the fiber. In a study of the protein composition of cotton fibers, polypeptides with molecular masses of 28 and 39 kDa were found among the proteins responsible for the strength of the fiber.

It is known that the proteins of various species and varieties of plants differ by the presence or absence of individual components. The revelation in hybrid forms of a plant of proteins that were absent from the parental forms may be a consequence of the activation of genes previously recessive in the parental forms [1]. It has been shown that the genetic material is the same in all the cells of an organism but the mechanism of differentiation shown in the activity of individual sections of the genome in different organs is still unknown [2, 3].

One of the main enzymes taking part in the synthesis of cellulose is glucan synthetase, located in the thickness of the plasmalemma of the cell wall. The glucan synthetase activity differs in various lines and experimental plants and is closely connected with the characteristics of the elements of the fruit: the better the technological quality of the fiber, the higher the enzymatic activity [4].

Among the multitude of protein systems the greatest interest is aroused by peroxidase, which participates in the transformation reactions of phenolic acids associated with the polysaccharides of the cell walls [5]. Being distinguished by a clearly expressed polyfunctionality, a high molecular homogeneity, and an effectiveness of electrophoretic separation, peroxidase completely corresponds to the requirements set for protein markers [6, 7].

A special position among the enzymes of cotton fiber is occupied by β-glucanases bound to the cell membrane [8]. The main detectable activity is that of an exo-β-(1-3)-D-glucanase that also has the characteristics of a β-glucosidase [9].

Our task was to reveal proteins in lines of the cotton plant differing in the strength of the fiber and also to study the peroxidase, glucanase, cellulase and glucan synthetase activities in the same lines.

By disk electrophoresis (anodic) in 10% PAAG we found in the fiber of line L-175 three isoforms of peroxidase with relative electrophoretic mobilities (REMs) of 95, 66, and 58, while characteristic for the L-466 line was the presence of a fourth broad minor zone with the REM 115, that was absent from L-175.

A comparison of the spectra of peroxidases in the cottonplant lines L-175 and L-466 taking into account the dynamics of the development of the fiber showed that they differed not only in electrophoretic mobility but also in the intensity of staining (Fig. 1). However, in the investigation of the peroxidase activities in lines L-175 and L-466, differing in fiber strength, we established that the specific activity in L-175 was higher than in L-466 (Table 1). Consequently, it is not the amounts of protein that determine the activities of peroxidase isoenzymes but, probably, the catalytic state of the enzymes.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 162 70 71. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 530-536, July-August, 1998. Original article submitted October 21, 1997.

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TABLE 1. Total Activities and Component Compositions of the Peroxidases of Developing Fibers of Cottonplant Lines L-175 and L-466

Cottonplant line	Total activity of the fiber peroxidase, nanokat			Number of components
	10-day	20-day	30-day	
L-175	379.6	480.5	290.5	3
L-466	175.0	289.4	150.3	4

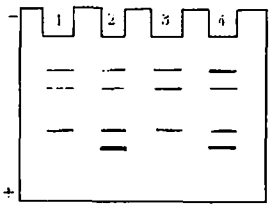

Fig. 1. Isozyme composition of the fiber peroxidase of cotton plants of the L-175 and L-466 lines: 1) 10-day L-175 fiber; 2) 10-day L-466 fiber; 3) 20-day L-175 fiber; 4) 20-day L-466 fiber.

The cellulase activities of cotton fibers of 10 and 20 days' growth were determined from a colored substrate. In the lines investigated, the cellulase activity of a 10-day fiber was higher than that of a 20-day fiber. In a comparison it was found that the L-175 line had a lower cellulase activity than the L-466 line, at 470 and 510 sp. units/mg of protein, respectively.

There were no absolute β-glucanase activities, determined with a buffer solution having a low ionic strength, in the supernatants from either 10- or 20-day developing fibers. When the enzyme was extracted with buffer systems having a high ionic strength, fractions were obtained with slight β-glucanase activity, amounting to 0.0035-0.004 IU/mg of protein.

In an investigation of absolute endo-β-(1-3)-glucanase activity using soluble CM-cellulose as substrate, no significant differences were observed in 10- and 20-day cotton fibers of the lines L-175 and L-466 because of the low activity of the enzyme. A slight activity of the enzyme was confirmed in a determination by the glucose oxidase-peroxidase method of the glucose formed as a result of the reaction. The β-glucanase activity in the 10-day fiber considerably exceeded that in the 20-day fiber. The maximum activity was observed in the 10-day fiber, after which the activity gradually diminished.

It is possible that the β-(1-3)-glucanase activity plays a role in the lengthening of the fiber, since it exhibits its activity mainly in the construction of the primary cell wall. By using different substrates (soluble CM-cellulose, laminarin, cellobiose) it was established that up to the 15th day the β-glucanase activity in the fiber functions mainly as a hydrolytic enzyme while in a more mature fiber it acts as a transglucosidase [9]. The use of laminarin as a substrate to determine exo-β-(1-3)-glucanase showed that in the L-175 and L-466 lines it was about 29 and 38 IU/mg of protein, respectively.

It is known that during the development of the fiber the component composition of the cell wall changes. A not unimportant role in the development of the fiber is played by proteins taking part in the regulation of the strength of the cell walls [5]. Investigations in the field of marker proteins of the cotton plant are sparse and have been conducted mainly on the seeds of various cottonplant species [1, 2]. Marker proteins responsible for a definite characteristic are extremely convenient in the zoning and creation of new varieties of cotton plant with the highest yield and best quality of the fiber.

To investigate marker proteins, we isolated the water- and salt-soluble proteins, the main ones being glycoproteins, from fibers of the cottonplant lines L-175 and L-466. From the results of electrophoresis (Fig. 2) it is possible to note the highest content of protein zones in the water-soluble fraction, and this was confirmed by the quantitative protein content. During the development of the fiber a change in the protein spectrum takes place. As can be seen from the phoregram, the composition of the protein isolated from a 20-day fiber differed from that of a 10-day fiber by a larger number of bands. In all probability, this is connected with the formation of the secondary cell wall and enhanced protein synthesis [5].

It has been found that the spectra of the water- and salt-soluble fractions differ by the number and intensities of appearance of the protein zones. The component compositions of the proteins of 10-day fibers of the lines studied were identical. Differences were observed in the fractions of proteins from a 20-day fiber. Thus, polypeptides with molecular masses

TABLE 2. Influence of Pix on the Total Activity and the Component Composition of the Peroxidase of Cotton Fibers of the Lines L-175 and L-466

Line of cotton plant	Total peroxidase activity of the fiber, nanokat			Number of components
	10-day	20-day	30-day	
L-175 control	379.6	480.5	290.5	3
L-175 experiment	380.2	510.3	310.0	4
L-466 control	175.0	289.4	150.3	4
L-466 experiment	176.1	345.1	170.4	5

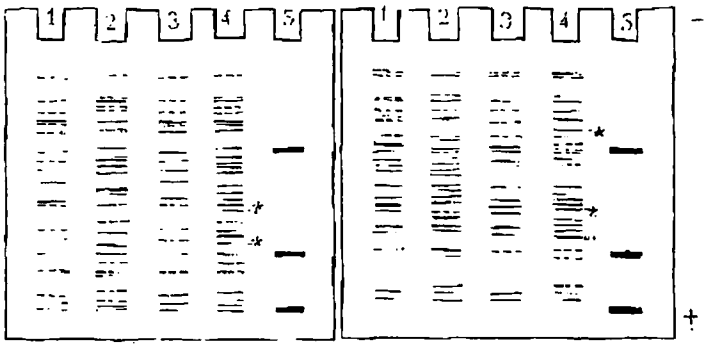

Fig. 2. Electrophoregram of the water- and salt-soluble proteins of fibers from different lines of cotton plant: A) water-soluble fraction; B) salt-soluble (10% NaCl) fraction; 1) 10-day fiber; 2) 20-day fiber of L-466; 3) 10-day fiber; 4) 20-day fiber of L-175; 5) markers (bovine serum albumin (BSA), chymotrypsinogen, cytochrome C); asterisks denote polypeptides characterizing the strength of the fiber.

of 28 and 39 kDa are characteristic for the protein spectrum of the line with the greater fiber strength, L-175, and molecular masses of 28, 39, and 90 kDa for the salt fraction. In the water-soluble fraction of line L-466 with the weaker fiber, the 28- and 39-kDa polypeptides were absent while the presence of peptides with molecular masses of 52 and 58 kDa was characteristic for the salt fraction. In the water-soluble fractions of both lines a greater level of clearly expressed high-molecular-mass proteins was observed, while in the salt fraction medium-molecular-weight polypeptides were clearly expressed. This fact can be explained by the assumption that a buffer with a higher ionic strength is required for the isolation of the medium-molecular-mass polypeptides.

Thus, differences between the proteins of fibers of different cottonplant lines and also different intensities of the manifestation of polypeptides with the same electrophoretic mobility have been revealed for the first time with the use of electrophoretic analysis on polyacrylamide gel plates in the presence of sodium dodecyl sulfate. It may be assumed that polypeptides characteristic for lines controlled by specific genes are responsible for the strength of the fiber.

It is known that a requirement of marker proteins is their stability under the action of various factors of the external medium and chemical and other reagents. The stabilities of the isoforms of peroxidase and marker proteins characteristic for the lines investigated were studied by means of the action on the cotton plants of the synthetic growth regulator Pix, which is used in the cotton-planting enterprises of Uzbekistan.

We studied the action of the retardant on the activity and composition of the isoforms of peroxidase in 20-day cotton fibers of lines L-175 and L-466 that had been treated with Pix in various concentrations. It was found that when a cotton plant was treated with a 0.10% solution of Pix the peroxidase activity rose and its iso composition changed, i.e., one additional isoform of peroxidase appeared in each line (Table 2). With an increase in the concentration of Pix (0.016, 0.020%) the activity fell, while the number of isoforms remained unchanged.

To study the influence of a stress factor on the stability of the proteins found that were characteristic for the L-175 line (28 and 39 kDa), cotton plants in the mass flowering phase were treated with Pix in the concentration used in cotton growing (0.013%). The results of the investigation showed that the above-mentioned polypeptides that are characteristic for the L-175 line and possibly responsible for the quality of the fiber were preserved under the action of a chemical factor and

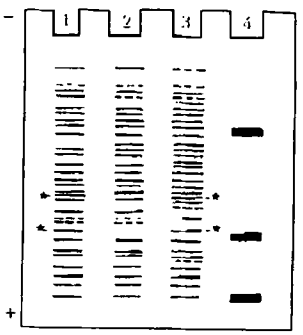

Fig. 3. Electrophoregram of the water-soluble proteins of fibers of lines L-175 and L-466 and a hybrid (L-175 × L-466); 1) line L-175; 2) line L-466; 3) the hybrid (L-175 × L-466); 4) markers (BSA, chymotrypsinogen, cytochrome C); asterisks denote polypeptides characterizing fiber strength.

can be assigned to the marker proteins. No appreciable influence on the protein spectrum and the component composition of the proteins was observed.

Markers are widely used in modern genetics both for prokaryotes and for eukaryotes with the aim of their genetic mapping and analysis. They are convenient in those cases where the gene under study codes a qualitative characteristic. By selecting in the progeny genotypes with the marker it is possible with a definite probability to expect that they will inherit the characteristic with respect to which selection is being performed. Here, the closer the gene under investigation is to the marker in the chromosome the more likely is their combined inheritance.

In the light of what has been said above, it appeared to us to be of interest to investigate the inheritance of marker proteins in the line L-175 that was distinguished by the strength of the fiber. For this purpose we obtained a hybrid by crossing lines L-175 and L-466. On an electrophoregram (Fig. 3) the protein compositions of fibers from the hybrid and parental forms showed the passage of the 39- and 28-kDa bands of the proposed marker proteins responsible for strength from the L-175 line into the progeny.

In a comparative study of the activity of the enzyme glucan synthetase in the genetic lines investigated and their hybrid the following results were obtained. In the L-175 line, the incorporation of the label into the newly synthesized cellulose took place more intensively, while in L-466 the formation of a labeled product proceeded at a lower rate (3910 and 1465 pulses/min, respectively). In the hybrid, the biosynthesis of cellulose took place at a greater rate than in the parental forms (4235 pulses/min).

On comparing the results obtained it may be concluded that the fiber with the greater strength had an increased glucan synthetase activity and that this property was inherited by the progeny. Analogous results were obtained in a determination of peroxidase activities in the parental and the hybrid forms. The glucanase activity in the parental forms was an order of magnitude higher; it corresponded to 2.7 IU/mg of protein in the hybrid form.

A comparative characterization of the activities of the fiber enzymes studied from the parental (L-175 and L-466) and hybrid forms is given in the diagram (Fig. 4). The technological characteristics obtained for the fibers of the L-175 and L-466 lines and their hybrid confirmed the results of the electrophoretic analysis of protein compositions and those of the determination of enzymatic activities (Table 3). Thus, the main properties of the hybrid fibers were retained, and the relative breaking load and the weight of the seeds had improved in comparison with the parental forms.

A comparison of the results obtained on the stability of the proteins, hybridization, electrophoretic analysis, and the biochemical and technological characteristics of the lines studied permits the conclusion that polypeptides with molecular masses of 39 and 28 kDa are among the marker proteins responsible for the strength of a fiber.

TABLE 3. Technological Characteristics of Cotton Fibers of Lines L-175 and L-466 and Their Hybrid

Line	Fiber yield, %	Fiber weight, mg	Fiber length, mm	Breaking load, gf	Linear density	Metric number, m/tex	Rel. breaking load
L-175	38.0	11.7	34.9	4.4	188	5318	23.4
L-466	33.3	12.0	35.5	3.6	148	6750	24.3
Hybrid	35.0	12.2	33.6	3.9	149	6692	26.1

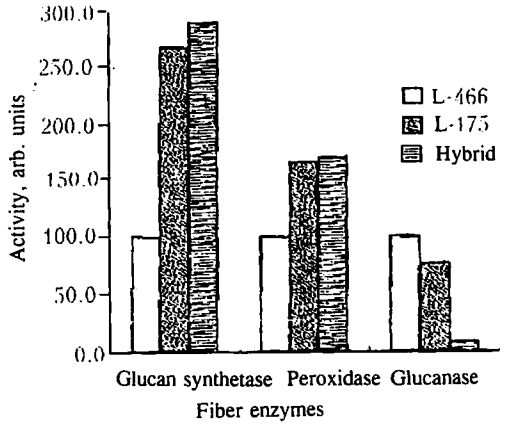

Fig. 4. Comparative characterization of the activities of the finer enzymes of parental and hybrid forms of cotton plant.

EXPERIMENTAL

We investigated fibers obtained 10 and 20 days after flowering from two lines of the cotton plant *Gossypium hirsutum* L. differing in fiber strength (L-175 — high strength, and L-466 — low strength). The plants were grown under phytotron conditions, in a field at the Institute for Cottonplant Selection and Seed Production, and also in an experimental field of the biological faculty of Tashkent State University and experimental plots of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan. The cell walls of the fibers were disrupted with liquid nitrogen, and the water- and salt-soluble fractions were extracted [10] and were subsequently, after lyophilization, used for the electrophoretic investigation of the protein spectrum of the cotton fiber in the presence of sodium dodecyl sulfate [11]. Protein contents were determined by Lowry's method [12].

To determine absolute glucanase activities we used an Na acetate buffer, pH 4.5-5.5, with various ionic strengths and the addition of NaCl. Absolute glucanase activities were measured by the differentiated viscosimetric method using soluble CM-cellulose as substrate.

Cellulase activities were determined in relation to a colored insoluble substrate. At 40°C on a shaking machine (100 rpm), 2 ml of a solution of the enzyme (activity 0.1-1 IU/ml in terms of endoglucanase) was added to 150 mg of substrate in 5 ml, the mixture was incubated for 20 min and it was then filtered and the absorption at 490 nm was determined. To prepare a control, a solution of the enzyme was added to a suspension of the substrate and the mixture was filtered immediately.

The extraction of the peroxidase was conducted with the aid of an alkaline tris-glycine buffer, pH 8.3. The fiber was disrupted in a mortar with the addition of liquid nitrogen and was homogenized in cold buffer solution at a ratio of 0.5 g to 2 ml of buffer. Then the homogenate was centrifuged at 7000 rpm and the peroxidase activity in the supernatant was determined by Boyarkin's method [13]. The electrophoretic separation of the peroxidases was conducted in an alkaline 10% PAAG gel according to [14]; spots revealed with benzidine.

Glucan synthetase activities were determined from the incorporation of a labeled precursor, uridine phosphate glucose, in the growing cellulose chain [15].

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