Chemistry of Natural Compounds, Vol. 36, No. 5, 2000

ROLE OF PHYTOHORMONES IN COTTON-FIBER FORMATION

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UDC 577.124.5:633.511

The effect of exogenic phytohormones (indolylacetic and naphthylacetic acids) on key enzymes for cotton-fiber biosynthesis, peroxidase, glucansynthetase, and cellulase, was studied. The phytohormones affect differently fiber formation and the protein composition.

Key words: phytohormones, cotton, peroxidase, glucansynthetase, cellulase.

Hormonal regulation of cellulose synthesis in cotton has been studied mainly by analyzing the levels of endogenous hormones in cells of cottonseed pilae [1-3]. The effect of exogenous hormones is unknown. Considering that the reaction of plant cells to treatment with growth regulators depends on the conditions under which the experimental plants are kept and their ontogenetic state, we studied the effect of hormones on cotton cellulose synthesis at various ontogenetic states.

The physiological effect of phytohormones on various plant organs is known to depend on the concentration [2]. Therefore, it seemed interesting to study the modulation of glucansynthetase, peroxidase, and cellulase activities by various concentrations of auxin-like compounds in cotton sprouts and fiber during formation of the secondary cell wall.

An enzyme system that oxidizes indolylacetic acid (IAA) has been found in tissues of many plants, for example, etiolated pea plants. According to some reports [3, 4], this system contains a flavoproteide that is involved in H_2O_2 production and peroxidase, which oxidizes IAA to physiologically inactive 3-methyleneoxyindole. It has been proposed [5] that peroxidase is involved in auxin formation.

The peroxidase activity of cotton sprouts doubled (*o*-dianisidine substrate) under the influence of IAA at a concentration of 10^{-7} M (control 77.79 nkat, experimental 166.69 nkat). The overall peroxidase activity decreases to 111.13 nkat (Fig. 1A) if the IAA concentration increases to 10^{-6} and 10^{-5} M. The increase of peroxidase activity indicates that the enzyme and phytohormone interact. Our results are consistent with an inverse relationship of phytohormone with peroxidase involved in a cascade of redox reactions.

According to the results, IAA at concentrations of 10^{-6} and 10^{-5} M increased the glucansynthetase activity (control 3.2×10^{-6} act. units/mg protein; 10^{-6} M, 6.7×10^{-6}; 10^{-5} M, 3.1×10^{-6} act. units/mg protein). At 10^{-7} M, the activity is close to that of the control (Fig. 1A). The cellulase activity dropped almost in half with a IAA concentration of 10^{-7} and 10^{-5} M (138 arb. units for the control; 56, for 10^{-7} M and 78, for 10^{-5} M). At a concentration of 10^{-6} M, the enzyme activity increased insignificantly (146 arb. units).

The peroxidase activity of cotton sprouts under the influence of naphthylacetic acid (NAA) increased by 1.3 times (control, 122.25 nkat; 10^{-7} M, 166.69 nkat). At 10^{-6} M, the activity decreased (10^{-6} M, up to 85.57 nkat; 10^{-5} M, up to 61.125 ncat relative to the control) (Fig. 1B). We investigated the range of isozymes by electrophoresis because IAA and NAA regulate differently the activity of cotton-sprout peroxidase. Figure 2 shows the spectra of peroxidase isozymes affected by IAA at 10^{-7} M. Polypeptides with a relative electrophoretic mobility (REM) of 0.29, 0.37, and 0.49 appear whereas NAA (10^{-7} M) produces polypeptides with REM 0.28, 0.30, and 0.32.

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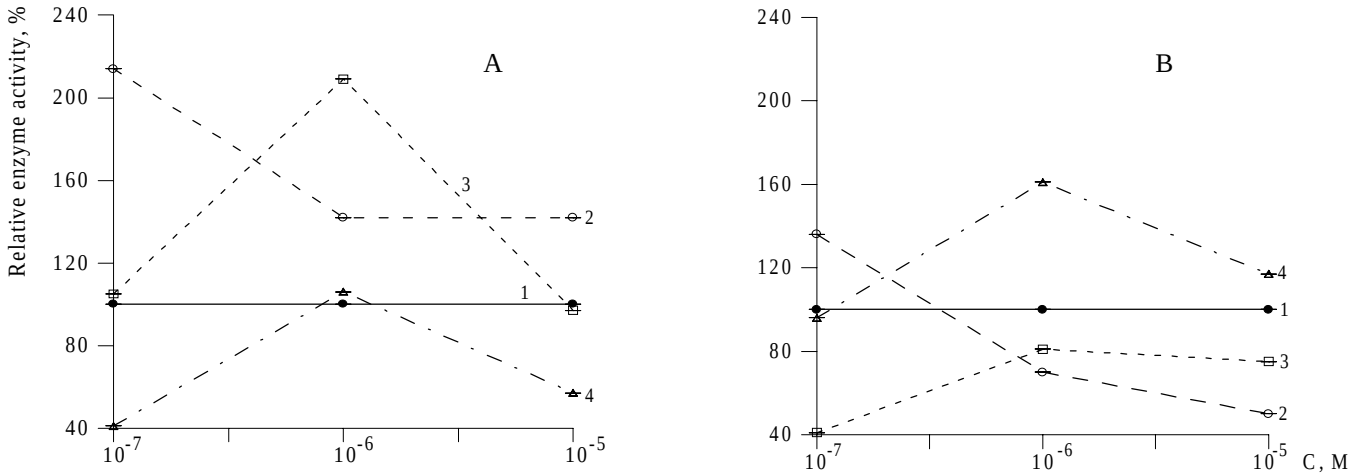

Fig. 1. Activity change of enzymes isolated from cotton sprouts at various concentrations of phytohormones IAA (A) and NAA (B): control (1), peroxidase (2), glucansynthetase (3), cellulase (4).

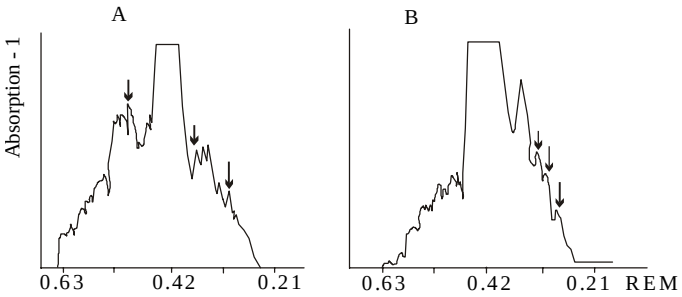

Fig. 2

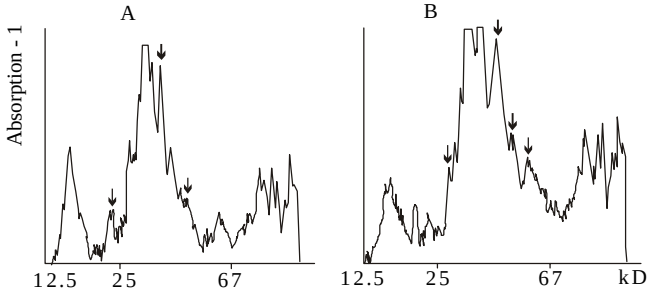

Fig. 3

Fig. 2. Electrophoregrams in 7.5% PAAG of peroxidase isozymes from cotton sprouts grown in the presence of IAA (A) and NAA (B).

Fig. 3. Electrophoregrams of proteins isolated from cotton sprouts grown in the presence of IAA (A) and NAA (B).

NAA, which is a synthetic auxin, inhibited glucansynthetase activity (control, 6.9×10^{-6}; 10^{-7} M, 2.8×10^{-6}; 10^{-6} M, 5.6×10^{-6}; 10^{-5} M, 5.2×10^{-6} act. units/mg protein) (Fig. 1B). At low NAA concentration (10^{-7} M), cellulase activity remained almost constant (control 240 act. units/mg protein; 10^{-7} M, 231 act. units/mg protein). Increasing the concentration to 10^{-6} M increased the activity to 386 act. units/mg protein. Increasing the NAA concentration further (to 10^{-5} M) decreased the positive effect of NAA on cellulase activity to 281 act. units/mg protein (Fig. 1B).

Electrophoresis studies of sprout proteins showed the appearance of "de novo" proteins under the influence of IAA and NAA. At 10^{-7} M IAA, polypeptides with molecular weight 24, 37, and 47 kD appear (Fig. 3A); at 10^{-7} NAA, 27, 42, 47, and 54 kD (Fig. 3B).

A comparative analysis of the activity of the studied hormones showed that the use of the natural auxin IAA gives the greatest positive effect. The synthetic analog NAA acts more as an inhibitor of cotton-fiber-forming enzymes.

The effect of IAA and NAA on enzymes of developing cotton fiber was studied. The peroxidase activity of the fiber increased by 2 and 1.3 times for IAA and NAA, respectively. The phytohormones had the greatest effect at a concentration of 10^{-7} M. Electrophoresis of the isoperoxidases revealed that new isozymes with REM 0.58 and 0.62 appear under the influence of IAA and NAA (Fig. 4).

The glucansynthetase activity of 10-day cotton fiber (formation of primary cell wall) increased by 54% under the influence of 10^{-7} M IAA (control, 6.9×10^{-7}; experimental, 1.04×10^{-6} act. units/mg protein). Increasing the concentration to 10^{-6} M inhibited the activity, decreasing it to 1.3×10^{-7} act. units/mg protein. The effect of IAA on 20-day fiber (formation of secondary cell wall) was analogous. The lower concentration (10^{-7} M) increased the enzyme activity (control, 1.1×10^{-6}; experimental, 1.3×10^{-6} act. units/mg protein). The activity decreased at 10^{-6} M (9.3×10^{-7} act. units/mg protein). The activity

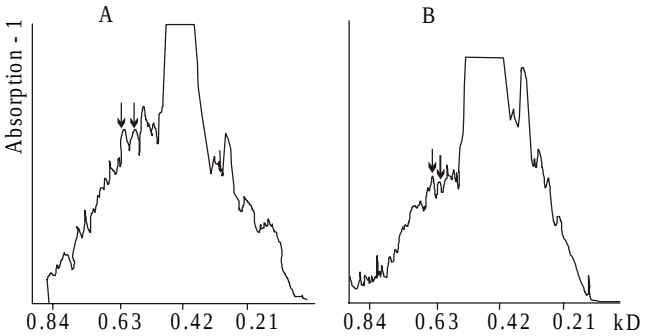

Fig. 4

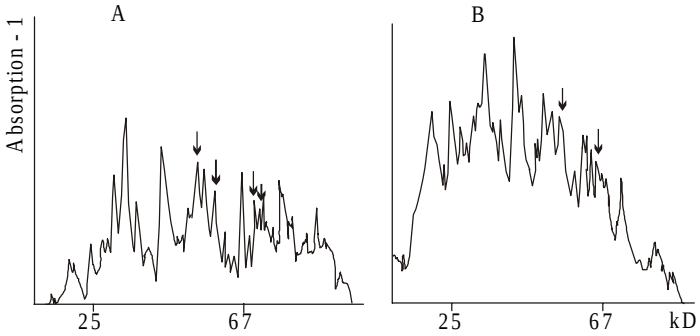

Fig. 5

Fig. 4. Electrophoregrams of peroxidase from 20-day fiber of cotton treated with phytohormones IAA (A) and NAA (B).
Fig. 5. Electrophoregrams of proteins from 20-day fiber of cotton treated with IAA (A) and NAA (B).

decrease of glucansynthetase in developing cotton fiber under the influence of high doses of IAA is possibly related to the demands of cellulose biosynthesis for additional phosphates and carbohydrates. The enzyme activity of fiber glucansynthetase, like for cotton sprouts, was inhibited by NAA.

The activity of enzymes that hydrolyze structural polysaccharides has been reported to increase under the influence of auxins on the cells. Apparently this results from acidification of the cell walls. Auxin had an activating effect on one of the two forms of cellulase [1]. Ethylene activated the other form of the enzyme [3]. Induced degradation of xyloglucans (the "backbone" of the structure is β-1,4-glucan; the "ribs," xyloglucan side chains) by hormones can be expected because cellulases hydrolyze β-1,4-bonds of not only cellulose but also xyloglucans.

Thus, the studied phytohormones at concentrations of 10^{-7} and 10^{-6} M had no effect on the cellulase activity of 20-day cotton fiber. At a concentration of 10^{-6} M they increased the activity of 10-day fiber, IAA by 42%; NAA by 62%. The cellulase activity increased by 24 and 4%, respectively, at a concentration of 10^{-7} M.

Electrophoresis of the protein content of 20-day fiber showed (Fig. 5) that polypeptides with molecular weights 47, 54, 70, and 71 kD appear under the influence of 10^{-7} M IAA; 70 and 54 kD, of NAA.

Thus, it can be concluded that auxins act as regulators of the activity of the principal enzymes involved in fiber formation, peroxidase, glucansynthetase, and cellulase, and change the protein composition. Stimulation by the phytohormones is probably related to increased protein synthesis that is regulated by the genetic apparatus of the plant cell. The optimal concentrations of physiologically active substances that do not suppress biosynthesis of proteinaceous and nucleic compounds in cotton sprouts and fiber can be chosen based on the results.

EXPERIMENTAL

Indolylacetic and naphthylacetic acids were used at concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M. Cotton (*Gossypium hirsutum* L., variety C-6524) sprouts (6- and 7-day) and fiber (10- and 20-day) were used. Plants were grown under phytotron conditions and on a field of the Institute of Cotton Selection and Seed Production.

Cottonseeds were cleaned with concentrated H_2SO_4, washed with a stream of cold water, and left for 1 h in tapwater. The swelled seeds were placed in paper cartridges and sprouted for 5-6 days at 27°C in a moist dark chamber. Sprouts were kept for 1 d in solutions of phytohormones at given concentrations. Sprouts and fiber were ground in liquid N_2 with added buffers to isolate each enzyme separately.

Glucansynthetase Activity Determination. The crude membrane preparation was homogenized with *Tris*-HCl buffer at pH 7.8. The homogenate was centrifuged at 2000 rpm for 5 min. The supernatant was centrifuged at 15,000 rpm for 45 min. The enzyme preparation isolated from the sprouts was incubated in a medium containing ^{14}C-glucose or uridinediphosphate-^{14}C-glucose (UDP-^{14}C-G) for 2 h at 27°C. The reaction was stopped by adding hot (96°C) ethanol. Nonbound tracer was removed by washing with C_2H_5OH (70%). The control was an enzyme preparation inactivated by heating for 5 min on a boiling water bath before addition of tracer. The radioactivity was counted in a β-analyzer.

Identification of Synthesis Product. The nature of the polymer newly synthesized from cellulose precursor UDP-^{14}C-

G and the cotton-enzyme complex was determined by TLC on Silufol plates (15×7.5 cm) using 1-propanol—ethylacetate—water (7:1:2). Glucose and cellobiose were used as standards. Bands with radioactive material were cut in 0.5-cm transverse strips. Each strip silica gel was placed in a separate vial and counted in the β-analyzer.

Isolation of Peroxidase Enzyme Preparation. Fiber specimens for isolation were collected at the moment that primary and secondary cell walls formed. Cell walls were disrupted by liquid N_2. The resulting powder was treated with 5-7 volumes of *Tris*-glycine buffer at pH 8.3 containing NaCl (1 M). The extract was centrifuged at 10,000 rpm for 30 min. The supernatant was dialyzed against distilled water and dried by lyophilization.

The peroxidase activity was determined by the Boyarkin spectrophotometric method [6].

Electrophoretic separation of peroxidase was performed in 7.5% PAAG [7, 9] according to the Davis method [7] with development by benzidine.

The protein composition was found by electrophoresis in gradient PAAG (from 10 to 15%) in the presence of sodium dodecylsulfate according to the Laemmly method [8]. Tracers for determining the molecular weight were bovine serum albumin, chymotrypsin, and cytochrome C. The protein content was measured by the Lowry method [9].

The cellulase activity was determined relative to colored insoluble substrate by adding enzyme solution (2 ml, 0.1-1 ME/ml of endoglucanase) to substrate (150 mg) in acetate buffer (5 ml, 0.1 M, pH 4.5) in conical 50-ml tubes at 40°C on a shaker. The mixture was incubated for 20 min and filtered. Absorbance at 490 nm was determined. The control was prepared by adding enzyme solution to substrate suspension and quickly filtering. One arbitrary activity unit (A_{AC}) is equal to 0.1 optical unit at 490 nm per minute.

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