Chemistry of Natural Compounds, Vol. 35, No. 6, 1999

EFFECT OF BENTONITE CLAY ON BIOSYNTHESIS OF COTTON CELLULOSE

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UDC 631.86+633.511

Bentonite clay affects biochemical processes occuring during synthesis of cotton-fiber cellulose by increasing the activity of glucansynthetase, peroxidase, and cellulase at 20% clay concentration. The content of soluble proteins increases and their composition changes.

Bentonite clay is a multicomponent system that includes various metal ions depending on the origin. Clay from the Khaudag deposit contains large quantities of Ca^{2+}, Mg^{2+}, and Fe^{3+}, which affect the metabolism of plant cells [1].

We studied the effect of bentonite clay on cotton-fiber cellulose synthesis, in particular, on the principal enzymes affecting the development of cell walls, glucansynthetase, cellulase, and peroxidase. In addition, the protein composition in the seedlings and fiber was studied.

Glucansynthetase, peroxidase, and cellulase, which are found in cell walls, actively participate in fiber formation. Glucansynthetase, which is involved in cellulose formation, transfers glucose units from the precursor uridinediphosphatoglucose (UDPG) to developing cellulose chains [2]. Peroxidase plays an important role in the thickening of the fiber sheath and in the conversion of phenolic acids associating into polysaccharides [3]. Cellulase in the plant cell acts as a protective barrier and also participates in the expansion of cell walls [4].

Glucansynthetase activity of cotton seedlings grown in suspensions of bentonite clay varied as a function of bentonite concentration. Formation of freshly synthesized cellulose was activated by 10 and 20% suspensions. However, increasing the concentration to 30% inhibited this process (Fig. 1).

The decreased cellulose formation can be explained as follows. Bentonite clay contains divalent cations (Ca, Mg) that stimulate cellulose biosynthesis [5]. However, increasing their content leads to supersaturation by the ions, which limits the rate of cellulose synthesis.

The cellulase activity of seeds soaked in a 10% suspension of bentonite clay reached 94 activity units per milligram of protein (AU/mg). This corresponded to 124% (the control cellulase activity was 76 AU/mg protein). Increasing the bentonite clay concentration to 20-30% has practically no effect on the cellulase activity. The values were 76 and 70 AU/mg protein, which correspond to the control.

Peroxidase activity for benzidine substrate in 10% bentonite clay suspension increased compared with the control by 1.2 times; in 20%, by 3 times; in 30%, by 2.5 times.

Comparison of the spectra of control and experimental isoperoxidases shows changes that reflect on the mobility of these enzymes. Isozymes with mobilities 0.31 and 0.67 appear. Also, isozymes in experimental samples are clearly evident upon coloration of the PAAG plates (Fig. 2). Spectrophotometric determination of the total peroxidase activity confirms these data.

Academician A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 789-794, November-December, 1999. Original article submitted May 10, 1999.

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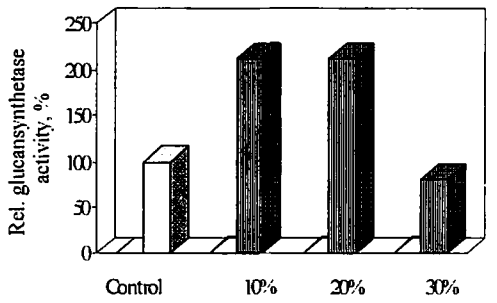

Fig. 1. Change of glucansynthetase activity of cotton seedlings grown in bentonite clay solutions of varying concentration.

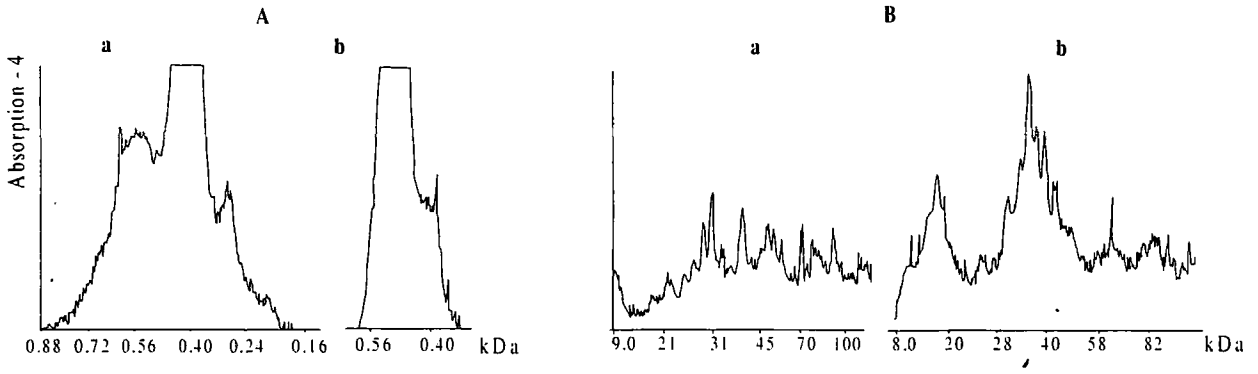

Fig. 2. Electrophoresis densitograms in 7.5% (A) and 10-15% (B) PAAG of peroxidase isozymes of 4-day seedlings (a) and 20-day cotton fiber (b) grown on bentonite clay.

Studies of the activity and electrophoretic behavior of peroxidase indicate that bentonite clay favorably affects metabolic processes occurring in the cell. A 20% suspension of bentonite clay was optimal in this experiment. Results from studies of the effect of various concentrations of bentonite clay on the content and composition of proteins in cotton seedlings are presented below:

Bentonite clay concentration, %	Protein content	
	µg/mg dry weight	%
Control	205	100
10	205	100
20	212	103.4
30	133	64.8

The protein content in seedlings did not change with 10% clay concentration. The highest content was observed for a 20% clay suspension.

Electrophoresis of experimental samples revealed a change in the protein composition and the appearance of polypeptides with molecular masses of 28, 38, 51, and 62 kDa (Fig. 2B).

The protein content and composition suggest that bentonite clay helps to increase the protein content and induces the synthesis of "de novo" proteins.

The enzyme activity of cotton fiber grown on soil with added bentonite clay was studied from the moment that primary (10-day fiber) and secondary (20-day) cell walls were formed.

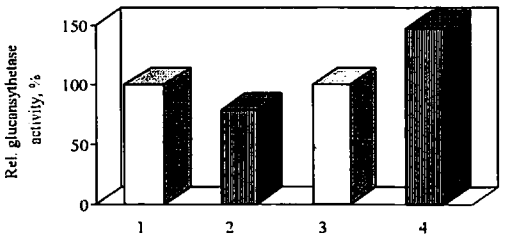

Fig. 3. Glucansynthetase activity of cotton fiber growing on bentonite clay: control, 10-day fiber (1); 10-day fiber + bentonite (2); control, 20-day fiber (3); 20-day fiber + bentonite (4).

It was found that the rate of cellulose synthesis in young fiber in the experimental sample decreased to 79% of the control value (Fig. 3). Glucansynthetase activity increases as the fiber develops (20-day) and exceeds the control value by 48%. These results confirm the dependence of glucansynthetase activity on divalent cations. The use of bentonite clay as a modulator of cotton productivity can be recommended.

We observed the following during studies of cellulase activity of developing cotton fiber in plants grown with added bentonite clay. Control plants had cellulase activity of 12,000 AU/mg protein during formation of the primary cell wall. However, the activity in experimental samples increased sharply to 38,000 AU/mg protein. This corresponds to 317% of the control. Experimental samples of 20-day cotton fiber had cellulase activity 140% of the control value. This corresponded to 1820 in the control and 2600 AU/mg protein in the experimental sample.

Peroxidase activity for benzidine substrate is 2.2 times higher then that of the control during formation of the primary cell wall and lengthening of the fiber. The coloration of isozymes in the electrophoregram of the experimental samples (Fig. 5) is more intense than that of the control. The peroxidase activity for benzidine substrate was 1.5 times higher then that of the control during formation of the secondary cell wall (20-day bolls). The electrophoretic study demonstrated that experimental isozymes are more intensely colored. A third minor band with mobility 0.32 was detected (Fig. 2A).

Mechanisms for controlling metabolism with basic and acidic peroxidases are known from the literature. External influences (stresses) activate different peroxidase isozymes [6]. In our experiment, basic peroxidase isozymes had the higher activity. The data suggest that bentonite clay stimulated peroxidase activity. In general, this has a very positive effect on cellulose biosynthesis in cotton fiber.

The studies of the effect of bentonite clay on the content and composition of soluble proteins in cotton fiber demonstrated that protein synthesis is induced during fiber development that is affected by bentonite clay. The quantitative protein content exceeded the control value by 20%:

Fiber growth from flowering	Protein content	
	µg/mg dry wt.	%
10-day, control	375	100
10-day, exptl.	300	80
20-day, control	415	100
20-day, exptl.	525	126.5

The protein composition of the experimental sample (20-day growth) also changed. Polypeptides of molecular mass 25, 30, and 36 kDa appeared. Thus, bentonite clay acts as a regulator that increases protein biosynthesis and as an expression agent for specific polypeptides that appear under the influence of external factors (Fig. 2B).

An analysis of the effect of various bentonite clay concentrations on cotton seedling enzymes (Fig. 4) showed that the optimal clay concentration for eliciting peroxidase and glucansynthetase activity is 20%; cellulase, 10%. A positive effect of bentonite clay on peroxidase and cellulase in developing cotton fiber was noted in the 10-day fiber. The activity of glucansynthetase was increased in the 20-day fiber (Fig. 5).

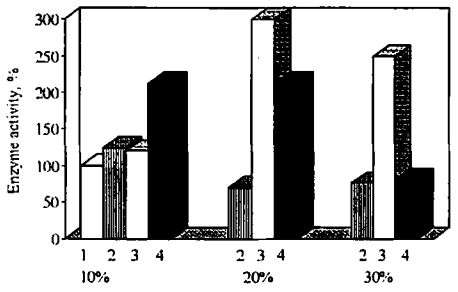

Fig. 4

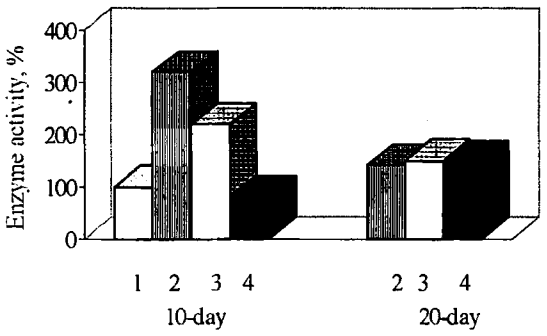

Fig. 5

Fig. 4. Effect of varying bentonite-clay concentration on enzyme activity of cotton seedlings: control (1), cellulase (2), peroxidase (3), glucansynthetase (4).

Fig. 5. Enzyme activity of cotton fiber growing on bentonite clay: control (1), cellulase (2), peroxidase (3), glucansynthetase (4).

It can be concluded that bentonite clay can be used as a regulator of cotton cellulose biosynthesis and is an economically lucrative mineral fertilizer that increases cotton productivity.

EXPERIMENTAL

Cotton seeds were soaked in 10, 20, and 30% clay suspensions for 12-15 h. The control was tapwater. Seeds in paper containers were placed in these same solutions and grown at 27°C for 7 days in a dark room.

The effect of bentonite clay on fiber formation was studied for Namangan-77 cotton grown at the Uzbekistan Scientific-Research Institute of Cotton. Control plots were treated with mineral fertilizer at 150 kg/hectare N, 60 kg/h P, and 50 kg/h K. Experimental plots were treated with the mineral fertilizer and bentonite clay at 3 t/h.

Homogenate from Cotton Seedlings and Fiber. Samples were ground in liquid nitrogen with added 0.01 M Tris-HCl buffer at pH 7.8 and homogenized. The homogenate was filtered through four layers of canvas and centrifuged at 1500 rpm for 5 min. The supernatant was centrifuged at 15,000 rpm for 45 min. The precipitate was then used for chromatography and determination of glucansynthetase activity. The supernatant after lyophilization was used for the electrophoretic study of the cotton-fiber protein spectrum.

Electrophoresis was performed in a PAAG gel gradient (from 10 to 15%) in the presence of sodium dodecylsulfate [8]. Bovine serum albumin (BSA) and chymotrypsin were used as markers for determining the molecular mass. PAAG plates were scanned on a Ultraskan 2202 (LKB) densitometer.

Glucansynthetase activity in cotton seedlings and fiber was determined by the Stepanenko method [9] using C-labeled UDPG. The nature of freshly synthesized polymer was established on Silufol plates [10].

Cellulase activity was determined spectrophotometrically relative to colored substrate. Substrate (150 mg) in acetate buffer (5 ml, 0.1 M) at pH 4.5 and 40°C was treated with constant stirring with enzyme solution (2 ml, 0.1-1 AU/ml endoglucanase activity). The mixture was incubated for 20 min and filtered. The absorption at 490 nm was determined. The control was prepared by rapidly adding a solution of enzyme to the substrate suspension and immediately filtering [11].

Peroxidase extraction was carried out using basic Tris-glycine buffer at pH 8.3. Fiber was ground with cold buffer (after preliminary destruction of cells by liquid nitrogen) at a ratio 0.5 g per 2 ml buffer. The homogenate was centrifuged at 7000 rpm for 20 min. Peroxidase activity in the supernatant was determined by the Boyarkin method [12].

Electrophoresis of peroxidase was performed in basic 10% PAAG buffer according to Davis [13] and developed with benzidine.

Protein content was determined according to Lowry [14].

ACKNOWLEDGEMENT

The work was supported by the Science and Technology Center of Ukraine (STCU).

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