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KINETIC CHARACTERIZATION OF THE SYNTHESIS OF COTTON CELLULOSE

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The influence of the temperature, pH, and inhibitors on the synthesis of cellulose from cottonplant shoots has been investigated. The maximum activity of glucan synthetase was exhibited at 27°C, pH 8.0. The greatest inhibition of the formation of cellulose was shown by EDTA.

Biological systems, especially living cells, are extremely sensitive to changes of temperature and pH and a number of other factors, and this is basically a reflection of the properties of the enzymes upon which the functioning of biological systems depends. A knowledge of the kinetics of the action of enzymes will be of assistance in the analysis of many biological phenomena. In view of this, it appears of interest to study the influence of inhibitors, the pH, and the temperature on the glucan synthetase participating in the synthesis of cotton cellulose.

Three groups of inhibitors of cellulose synthesis are known, and these have different mechanisms of their action on the laying down of this polymer in cell walls [1], affecting: the regular change in the orientation of the microfibrils (colchicine); the synthesis of the polyglucan chains (EDTA); and preventing the crystallization of the cellulose chains (Congo Red). In the present paper we give the results of a study of the action of the temperature factor, the pH, and the above-mentioned inhibitors on the synthesis of cellulose.

An investigation of the inhibiting action of colchicine, EDTA, and Congo Red on the *in vitro* synthesis of cellulose by an enzyme preparation from shoots of a cotton plant of the Andizhan-9 variety using [^{14}C]glucose as substrate showed the absence of a pronounced suppression of glucan synthetase activity. These results are comparable with those obtained previously, according to which the rate of the cellulose-synthesizing reaction is an order of magnitude lower when glucose is used as the source of glycosyl radicals. In the case of uridine diphosphate [^{14}C]glucose, EDTA, colchicine, and Congo Red suppressed the synthesis of the labeled polymer, the greatest inhibiting effect being shown by EDTA. The enzymatic activity of the glucan synthetases measured from the inclusion of the label in the newly synthesized alcohol-insoluble polymer, identified as cellulose, was 70% suppressed at an EDTA concentration of 3.0 mM, 55% in the case of colchicine, and 20% in the case of Congo Red (Fig. 1). In view of the fact that EDTA affects the synthesis of the glucan chains, the increased inhibition of the *in vitro* synthesis of cellulose with the participation of glucan synthetase from cottonplant shoots and a labeled cellulose precursor — UDPG — is explicable.

The various enzymes differed strongly from one another with respect to the pH dependence of their action. In the study of cellulose synthesis in shoots, the highest activity of the enzyme preparation was observed at pH 8.0 — 15.0×10^{-6} activity units/mg of protein. At more acidic and more alkaline values, the activity was extremely low — 0.5×10^{-6} and 3.0×10^{-6}, respectively (Fig. 2, *a*).

In a study of the influence of the temperature factor — one of the most important parameters — on cellulose synthesis in the cotton fiber, it was found that the optimum temperature for this process is 30°C [2]. In Tarchevskii's studies [3], this index is given as 40°C. On the incubation of an enzyme preparation from cottonplant shoots under various temperature regimes, we observed the formation of newly synthesized labeled polymer at 27°C. The synthesis of cellulose slowed down at lower and higher temperatures (Fig. 2, *b*).

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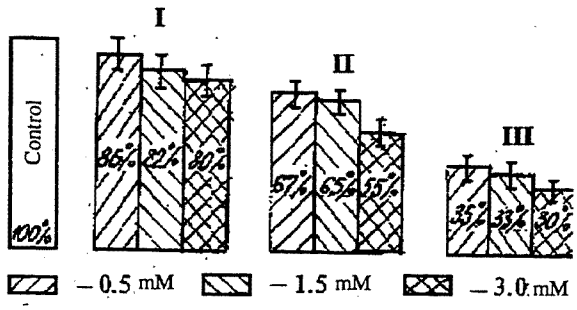

Fig. 1. Influence of the inhibitors Congo Red (I), colchicine (II), and EDTA (III) in various concentrations on the inclusion of labeled glucose in newly synthesized cellulose.

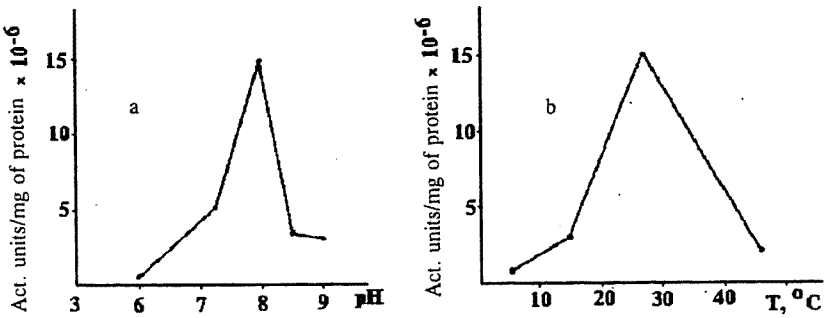

Fig. 2. Dependence of the change in the glucan synthetase activity of cottonplant shoots on the pH (*a*) and on the temperature (*b*).

Thus, the optimum values of the temperature and of the pH at which the maximum activity of glucan synthetase participating in the synthesis of cellulose is manifested have been established (27°C and 8.0, respectively), and the influence of inhibitors on the activity of this enzyme has been determined, which will make it possible to regulate the kinetics of the catalytic reaction.

EXPERIMENTAL

Isolation of an Enzyme Preparation from Shoots. Seeds of a cotton plant, *Gossypium hirsutum*, of the Andizhan-9 variety were stripped with concentrated sulfuric acid, washed rapidly under a jet of cold water, and left for a day in mains water. The swollen seeds were placed in paper cases and grown for four days in a thermostat at 27°C in a moist humid chamber. The etiolated hypocotyls were ground in a porcelain mortar with the addition of liquid nitrogen to disrupt the cell walls and also of 1.0 g of $NaHCO_3$ and 5 g of polyvinylpyrrolidone (PVP) per 100 g of plant material. During the grinding process, 0.01 M Tris-HCl buffer, pH 7.8, was added to give a ratio of 1:4. The resulting mass was pressed through four layers of gauze and was centrifuged at 2000 rpm for 5 min. The supernatant was then centrifuged at 15,000 rpm for 45 min. The deposit was used in the subsequent investigations.

Determination of Glucan Synthetase Activity. The enzyme preparation isolated from shoots was incubated in a medium containing [^{14}C]glucose or UDP[^{14}C]G at 27°C for 2 h, unless stated otherwise [4]. The reaction was stopped by the addition of hot 96° ethanol, and the unbound label was eliminated by four washings with 70% C_2H_5OH. The enzyme preparation inactivated by heating in the boiling water bath for 5 min before the addition of the label was used as control. Radioactivity was measured in a β-analyzer. Protein was determined by the Lowry method [5].

Identification of the Product of Synthesis. The nature of the polymer newly synthesized from the cellulose precursor UDP[^{14}C]G and the enzyme complex from cottonplant shoots was established by TLC on Silufol plates (15 × 7.5 cm) in the solvent system *n*-propanol–ethyl acetate–water (7:1:2). The revealing agent was a 0.5% solution of potassium permanganate

in 1 N sodium hydroxide. After being sprayed, the plate was heated to 100°C. Glucose and cellobiose were used as markers. The bands with radioactive material were cut into transverse strips 0.5 cm wide and the silica gel from them was transferred into bottles and counted in the β-analyzer. ZhS-T was used as the scintillator.

A Study of the Action of Various Factors on Glucan Synthetase Activity. The inhibition of the activity of glucan synthetase participating in cellulose synthesis was investigated *in vitro* by the addition to the incubation mixture of EDTA, colchicine, and Congo Red in concentrations of 0.5, 1.5, and 3 mM. The influence of the temperature was studied at 4, 15, 27, and 45°C, and that of the pH at pH values of 6.0, 7.0, 8.0. 8.5, and 9.0. In Figs. 1 and 2 the mean values of four replicates are given.

REFERENCES

1. D. C. Robinson and H. Quader, Eur. J. Cell. Biol., **84**, No. 1, 229 (1980).
2. S. L. Naithani, N. Rama Rao, and J. D. Singh, Physiol. Plant., **54**, No. 2, 225 (1982).
3. I. A. Tarchevskii and G. N. Marchenko, The Biosynthesis and Structure of Cellulose [in Russian], Moscow (1985), p. 108.
4. B. N. Stepanenko and A. V. Morozova, Dokl. Akad. Nauk SSSR, **187**, No. 6, 1425 (1969).
5. O. H. Lowry, N. T. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, No. 1, 265 (1951).