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ISOLATION OF β-(1→4)-GLUCAN SYNTHETASE FROM COTTONPLANT SHOOTS

A. A. Akhunov, F. A. Ibragimov, and É. Ch. Mustakimova

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An enzyme complex including β-(1→4)-glucan synthetase has been isolated from cottonplant shoots by chromatography and electrophoresis. Accrding to electrophoretic analysis under denaturing conditions, the molecular masses of the polypeptides presumably present in in the glucan synthetase complex amounted to 74, 50, and 30 kDa.

One of the main enzymes participating in the synthesis of cellulose is β-(1→4)-glucan synthetase, which is localized in the active form in the plasmalemma of the plant cell. The glucan synthetases of pea shoots [1, 2], of onion stems [3, 4], and of the developing cotton fiber have been investigated. Of particular interest are papers by Benziman et al. [5] on the purification and partial characterization of a native activator of membrane β-glucan synthestase. In them it is shown that the active form of cellulose synthetase is an oligomeric protein consisting of two different subunits (67 and 57 kDa) obtained from a 90-kDa precursor.

The present work was devoted to isolation and characterization of a β-(1→4)-glucan synthetase from cottonplant shoots.

The presence of a glucan synthetase in the isolated enzyme preparaion was confirmed by incubation with the labeled cellulose precursor uridine diphosphate glucose (UDPG-^{14}C): the specific activity of the enzyme in the initial material was 7.4×10^{-7} act. units/mg of protein. The nature of the newly synthesized labeled polymer was determined by TLC. The highest radioactivity was found in he section of the chromatogram corresponding to cellobiose. An appreciable amount of radioactivity was detected at the point of deposition, which showed the presence of chromatographically immobile material.

To free the glucan synthetase from accompanying proteins and enzymes, the crude membrane preparation obtained after centrifugation at 4000 rpm was chromatographed on a column of Sepharose 4-B in a 0—1 M NaCl gradient. The enzyme preparation was eluted as three fractions (I—III, Fig. 1). The specific activities of fractions I and II were 1.2×10^{-6} and 9.1×10^{-6} act. units/mg of protein, respectively; fraction III possessed no glucan synthetase activity. The active fractions were deposited on a column of TSK gel, and, after fractionation with distilled water, three fractions possssing glucan synthetase activity were obtained: I-1 — 4.0×10^{-8}; I-2 — 3.6×10^{-6}; and II-1 — 2.0×10^{-5} act. units/mg of protein (Fig. 2). Fractions I-1 and I-2 were isolated from the first peak, and II-1 from the second.

An electrophoretic investigation of the fractions possessing the highest activities showed the presence in the protein spectrum of high- and low-molecular-mass components, the amounts of which depended on the degree of purification. For example, in fraction II-1 the presence was detected of three well-defined proteins with molecular masses of 74, 50, and 30 kDa (Fig. 3).

Thus, a protein fraction possessing a high glucan synthetase activity has been isolated by chromatography and gel filtration. Further purification and a determination of the physicochemical properties of this fraction have permitted its assignment to the β-(1→4)-glucan synthetase.

EXPERIMENTAL

Isolation of the Enzyme Preparation. Seeds of the cotton plant *Gossypium hirsutum*, variety Andizhan-9, were freed from fuzz with concentrated sulfuric acid, moistened for a day, and germinated in paper cases in the dark at 27°C for 4 days. The etiolated hypocotyls were comminuted with knives, ground in liquid nitrogen, and homogenizd in three volumes of 0.01

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax 627071. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 87—90, January-February, 1994. Original article submitted July 13, 1993.

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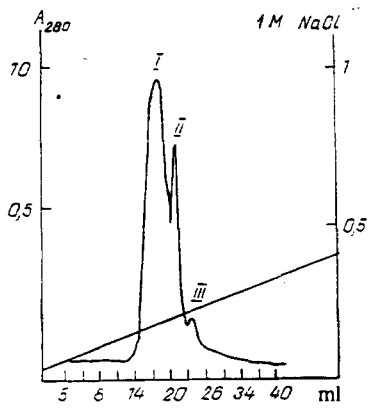

Fig. 1

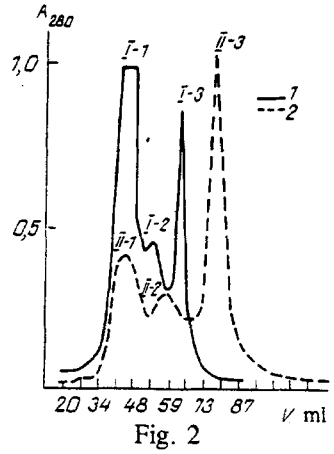

Fig. 2

Fig. 1. Chromatographic separation of the enzyme complex on a Sepharose 4-B column.

Fig. 2. Gel filtration of fractions possessing glucan synthestase activity on a TSK-HW-55 column: 1) fraction I; 2) fraction I.

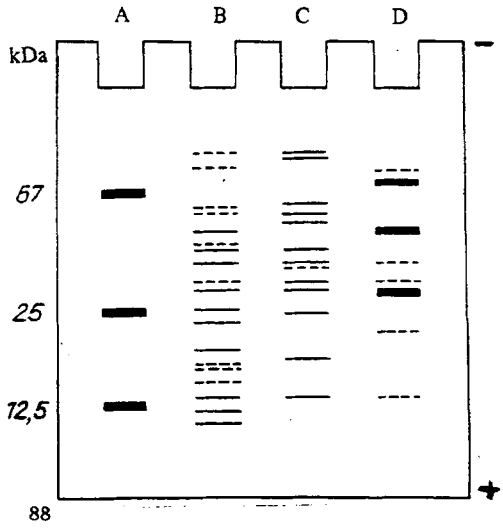

Fig. 3. Electrophoretic analysis of the activity of fractions: A) control; B) original material; C) fraction I-1; D) fraction II-1.

M Tris-HCl buffer, pH 7.8, containing 250 mM sucrose and 3 mM EDTA, with the addition of PVP and $NaHCO_3$ The homogenate was filtered through four layers of gauze and was centrifuged at 15,000 rpm for 45 min. The depoosit was used for chromatography

Chromatogrphy on Sepharose 4-B. The deposits obtained were dissolved in 0.01 M Tris-HCl buffer, pH 7.8, the solution was centrifuged at 5000 rpm for 10 min, and the supernatant was deposited on a column (8 × 47 cm) of Sepharose 4-B equilibrated with the above-mentioned buffer. Elution was conducted in a rising gradient of 1 M NaCl at a rate of 20 ml/h. The eluted proteins were recorded on a Uvicord instrument at 280 nm.

Purification of the β-(1→4)-glucan synthetase complex on a column of TSK-HW-55 gel. After desalting, the fractions obtained were freeze-dried, dissolved in distilled water, and chromatographed on a column (1.6 × 35 cm) at the rate of 20 ml/h.

Electrophoresis was conducted in a gradient PAA gel (from 10 to 15%) in the presence of sodium dodecyl sulfate [6]. BSA, chymotrypsinogen, and cytochrome C were used as molecular-mass markers.

Determination of Glucan Synthetase Activity. The intial crude membrane preparation and the fractions obtained after chromatography and gel flitration were incubated in a medium containing UDPG-^{14}C at 25—27°C for 2 h [7]. The reaction was stopped by the addition of hot 96% ethanol, and the unbound label was eliminated by four washing with 70% C_2H_5OH. An 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.

Identification of the Product of Synthesis. The nature of the polymer newly synthesized from the cellulose precursor UDPG-^{14}C 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 solution was a 0.5% solution of potassium permanganate in 1 N sodium hydroxide. After spraying, the plate was heated at 100°C. Glucose and cellobiose were used as markers. The bands with radioacive material were cut into transverse strips 0.5 cm wide, and the silica gel from them was transferrd into bottles and was counted in the β-analyzer. ZhS-T was used as scintillator.

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