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ISOLATION OF A CELLULASE FROM THE SEPARATING ZONES OF COTTONPLANT LEAVES

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As is known, the leaf-shedding process is induced by the phytohormone ethylene, which stimulates the secretion in the cell wall of a number of hydrolytic enzymes — in the first place, cellulase (β-1,4-glucan 4-glucanohydrolase) and pectinase [1, 2]. However, the hydrolytic enzymes from the separating zone of the cotton plant have not previously been isolated and characterized, although there are reports of the activation of a cellulase-pectinase complex by defoliants [3].

Our task was to develop an effective scheme for isolating the enzyme. Each individual stage — the extraction of the protein, its precipitation, desalting, and fractionation — was conducted by different methods with the aim of the optimum isolation. The results obtained are presented in Tables 1 and 2.

The best extractant is a buffer containing 1 M NaCl, 5 mM β-mercaptoethanol (to inactivate serine proteinases), and 5 mM EDTA (to bind heavy-metal ions), pH 5.0. The highest yield of enzyme is achieved at a high degree of comminution of the separating zone.

The most effective precipitant is ammonium sulfate. Enzymatic activity was determined by the viscosimetric method, using medium-viscosity CMC in 0.1 M acetate buffer containing 0.1 M NaCl, pH 4.5—5.0, at 40°C. The substrate concentration was 0.28—0.35%.

Activity was determined from a published formula [4, 5] in μmole/min per 1 g (or per 1 ml) of enzyme preparation:

$$A_{sp} = K(r_o - r_t)/r_b(r_o/r_b)^{7/8}[E](t + (r_t/120)),$$

where r_o, r_t, and r_b are the outflow times of the CMC solution, of a mixture of CMC and the enzyme preparation, and of the acetate buffer, respectively, s; t is the time that has passed from the moment of beginning hydrolysis to the beginning of the determination, min; and [E] is the concentration of the enzyme, g/liter or ml/liter.

The value of K for many types of CMC ranges between 10 and 15; in all cases, 0.1 ml of enzyme preparation containing 3 μg of protein was added to 10 ml of CMC solution.

Fractionation of the total proteins of the separating zones of cottonplant leaves was performed on the hydrophobic sorbent Polikhrom-1 in a straight linear concentration gradient of ethyl alcohol (0—96%, Fig. 1).

Enzyme activity appeared in fractions 4 (4.2 int. activity units) and 5 (about 2 int. activity units) Electrophoresis in a concentration gradient of PAAG with SDS showed that the fractions mainly contained a protein with MM 25—28 kDa but with minor impurities. For final purification with the aim of isolating an individual protein subsequent rechromatography was necessary.

Thus by chromatography on Sephadex G-25 and Polikhrom-1 we have isolated and purified a cellulase (β-1,4-4-glucanohydrolase) from the separating zones of cottonplant leaves. The influence of the conditions of extraction, precipitation and fractionation on the activity of the enzyme has been studied.

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TABLE 1. Influence of the Extraction Conditions on the Yield of Enzyme

Composition of the buffer	pH	Liquor ratio $m_{biol.\ mat}/m_{buffer}$	Yield of protein, mg	Specific activity, μmole/min per 1 g of enzyme prep.
0.05 M Tris-HCl	8	1:1	2.2	0.55
0.05 M NaAc (sodium acetate buffer)	5	1:1	2.9	0.33
0.05 M NaAc+1M NaCl	5	1:1	5.0	0.74

TABLE 2. Influence of the Conditions of Protein Precipitation on the Specific Activity of the Enzyme

Precipitant	Saturation	A_{sp}, μmole/min per 1 g of enz. prep.
$(NH_4)_2SO_4$	<=80% (m/v)	2.07
C_2H_5OH	<=40% (v/v)	1.28
Polyethyleneglycol, MM 6000	<=20% (m/v)	1.75

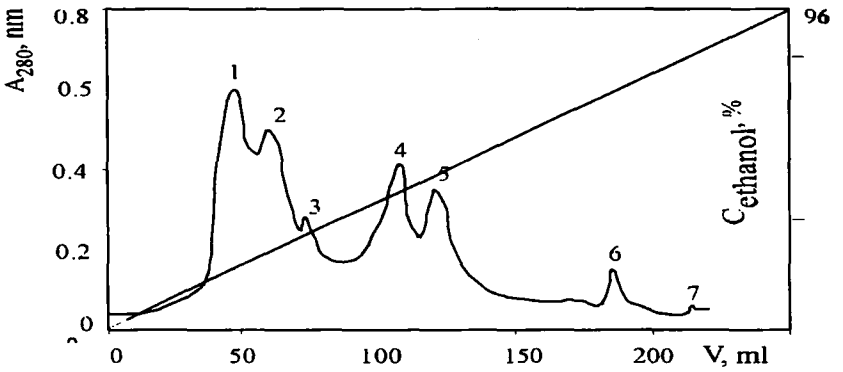

Fig. 1. Hydrophobic chromatography, on a column of Polikhrom-1 (2.5 × 50 cm) in a 0—96% concentration gradient of ethanol, of the proteins eluted from a column of Sephadex G-25 in the free volume; rate of flow of eluent 30 ml/h.

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