ASPARTATE AMINOTRANSFERASE FROM COTTON SEEDS

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UDC 577.15.0.72

It is known [1] that L-aspartate, 2-oxoglutarate aminotransferase (EC 2.6.1.1), is one of the most active enzyme systems of dormant cotton seeds.

The present paper gives information on the isolation of this enzyme from cotton seeds of variety 108-F. We extracted the defatted flour obtained from the seeds [2] with a buffer solution, pH 6, containing 10^{-3} M glutathione, sodium pyruvate, EDTA, and Tween-80 (detergent). The extract was brought to pH 6 and fractionally precipitated with ammonium sulfate. The 40% to 70%-saturation fraction possessed the highest aspartate aminotransferase activity. Ion-exchange chromatography on CM-cellulose was used for the further purification of the protein fraction. The initial 0.01 M acetate buffer, pH 5.4, eluted a protein fraction from the column (peak I) which possessed no activity. Then the concentration of the buffer was increased from 0.01 M to 0.04, 0.06, and 0.08 M. The 0.08 M buffer eluted two more protein fractions (peaks II and III), which possessed aspartate aminotransferase activity (Fig. 1).

The active fractions from the CM-cellulose were concentrated and dialyzed against 0.01 M phosphate buffer, pH 7.6, and each separately was transferred to a column of DEAE-cellulose equilibrated with the same buffer.

The enzyme was eluted from the column with 0.02 M phosphate buffer, pH 7.6; the colored impurities remained at the top of the column.

The high degree of purity of the enzyme was confirmed by the results of rechromatography on a column of DEAE-cellulose. Information on the isolation and purification of the enzyme are given in Table 1.

The protein fractions obtained in all the stages of purification were studied by electrophoresis in acrylamide gel [3]. The fraction precipitated at 70% saturation exhibited two bands, and on separation on CM-cellulose the fraction eluted in peak I gave seven bands, that in peak II three bands, and that in peak III one band. After chromatography on a column of DEAE-cellulose, the electrophoretic composition of the fractions had not changed. On electrophoresis, all the components migrated towards the anode (Fig. 2).

On determining aspartate aminotransferase activities during the work, it was found that with a decrease in the concentration of enzymes in the solution its activity rose. For the animal enzyme it has been reported [4] that in relatively concentrated solutions aspartate aminotransferase is predominantly in a catalytically poorly active associated form which, on dilution, dissociates into subunits with a relatively higher activity. This situation requires further study for the plant enzyme.

EXPERIMENTAL

The activities were determined by the colorimetric method with 2,4-dinitrophenylhydrazine [5]. At all stages of purification the concentration of protein was determined by the Warburg-Christian method [6].

Defatting of the Seeds. One hundred grams of the seeds was colled with nitrogen and crushed in a mortar, after which they were sieved from hulls and were ground. Then they were extracted with four volumes of acetone cooled to −30 to −40°C for 1.5-2 h; after filtration, the residue was washed with a

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Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 735-738, November-December, 1970. Original article submitted June 11, 1970.

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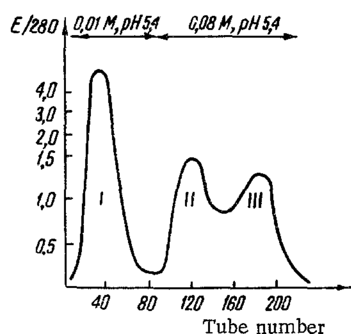

Fig. 1

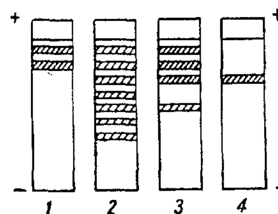

Fig. 2

Fig. 1. Separation of the protein fraction corresponding to 40-70% saturation with $(NH_4)_2SO_4$ on a column of CM-cellulose.

Fig. 2. Electrophoregrams of the active fractions obtained during purification: 1) fraction precipitated with 40- to 70%-saturated $(NH_4)_2SO_4$; 2) fraction eluted in peak I from the CM-cellulose; 3) peak II; 4) peak III.

TABLE 1

Purification stage	Vol., ml	Activity: total no. of units	Activity: specific activity, units/mg	Yield, %	Deg. of purification
Initial extract	150	4332	0,19	100	1
Precipitate with 40-70% $(NH_4)_2SO_4$	50	2999,5	4,87	69	25
Chromatography on CM-cellulose, peak II	36	1805,4	109	41,6	574
Chromatography on CM-cellulose, peak III	25	790,25	104	18,2	548
Chromatography on DEAE-cellulose, peak III	10	390	300	9,03	1579

cooled mixture of acetone and ether (1 : 2) until the filtrate no longer had a yellow color. The solvent residues were eliminated under vacuum. This gave 60 g of defatted flour.

Extraction. Forty g of defatted flour was stirred with five volumes of a solution containing 10^{-3} M glutathione, sodium pyruvate, EDTA, and Tween-80 at 4°C for 12-18 h. The suspension was centrifuged for 30 min at 6000 rpm and then for 30 min at 18,000 rpm. The supernatant liquid obtained was fractionally precipitated with ammonium sulfate. The precipitate from 0-40% saturation was rejected, and that from 40-70% saturation was centrifuged at 18,000 rpm for 15 min. The residue was dissolved in the minimum amount of 0.01 M acetate buffer, pH 5.4, and dialyzed against the same buffer until the ammonium sulfate had been completely eliminated.

Separation on a Column of CM-Cellulose. The dialysate was centrifuged at 18,000 rpm from the precipitate that had deposited during dialysis for 15 min, and was transferred to a column of CM-cellulose equilibrated with 0.01 M acetate buffer, pH 5.4 (column dimensions 16 × 4.5 cm, rate of elution 16 ml/h). Four-milliliter fractions were obtained in a collector. The extinction of the protein fractions at 280 nm was determined on an SF-4 spectrophotometer. A graph was plotted from the spectrophotometer readings (see Fig. 1). The fractions belonging to one peak according to the graph were combined, and their concentration of protein and activity were determined. Then they were concentrated by means of Sephadex G-25.

Separation on DEAE-Cellulose. The protein fractions after concentration with Sephadex G-25 were dialyzed against 0.01 M phosphate buffer, pH 7.6, and were transferred to a column of DEAE-cellulose equilibrated with the same buffer (column dimensions 17 × 1.5 cm, rate of elution 12 ml/h). Three-milliliter fractions were obtained in a collector.

Electrophoresis. The micromethod in a thin layer of polyacrylamide gel was used. The dimensions of a plate were 8.5 × 2 × 0.15 cm, the voltage 300-350 V, the current 7 mA per plate and the time of electrophoresis 30 min. Tris-citrate buffer, pH 9.3, was used.

SUMMARY

Using fractionation with ammonium sulfate and ion-exchange chromatography on CM- and DEAE-celluloses, two fractions with aspartate aminotransferase activity have been obtained from cotton seeds.

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