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# AFFINITY CHROMATOGRAPHY OF THE CHORISMATE MUTASE FROM *CLAVICEPS PASPALI* BASED ON SPECIFIC INTERACTION WITH THE ACTIVATOR

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## 1. Introduction

Certain enzymes can be efficiently enriched by affinity chromatography [1]. For this purpose, compounds with a high affinity to the enzyme are covalently attached to a suitable carrier. We studied the purification of chorismate mutase from *Claviceps paspali* strain Pb 156 by this procedure.

## 2. Materials and methods

Preparation of the cell-free extracts and the assay of the chorismate mutase are already described [2]. The crude enzyme extract was partially purified by chromatography on DEAE-cellulose. The purified fractions were concentrated by precipitation with ammonium sulfate (0.9 sat. $(NH_4)_2SO_4$) and finally dialysed against phosphate buffer (0.01 M, pH 6.9). The crude extract previously dialysed was also occasionally used. Sepharose 4 B purchased from Pharmacia was activated with cyanogen bromide according to the procedure of Cuatrecasas et al. [1]. The pretreated Sepharose was stirred at pH 9 for 20 hr at 4°C with L-Trp and L-Phe, respectively, using 1 mmole of the amino acids per ml of Sepharose. The Sepharose was washed with a large excess of $NaHCO_3$ solution (0.01 M) and water. The substituted Sepharose was resuspended in phosphate buffer and packed into a column (1.5 X 9 cm). The enzyme extract was applied and washed with buffer. The chorismate mutase was eluted from the column with phosphate buffer (0.01 M pH 6.9) supplemented with $10^{-3}$ M L-Trp.

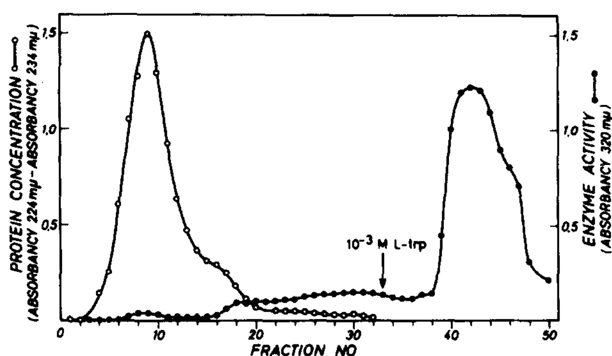


Fig. 1. Chromatography of a DEAE-cellulose purified *claviceps* extract at 4°C on L-Trp-substituted Sepharose. The column (1.5 X 9 cm) was equilibrated with 0.01 M potassium phosphate buffer pH 6.9. The sample consisted of 3 ml *claviceps* extract (9 mg protein) also in the same buffer. 3 ml fractions were collected. After 100 ml buffer had passed through the column, $10^{-3}$ M L-Trp-solution was added to elute chorismate mutase.

## 3. Results and discussion

Chorismate mutase from *Claviceps paspali* is an allosteric enzyme with binding sites for the substrate, chorismic acid, for the inhibitors, L-Phe and L-Tyr, and for the activator, L-Trp. Chorismic acid is a very labile compound and therefore not suitable for affinity chromatography. Therefore the two effectors L-Trp and L-Phe were coupled with Sepharose. L-Trp was especially convenient due to its strong affinity for the enzyme. Activation by L-Trp has already observed at concentrations above $10^{-7}$M. A specific interaction between the effectors attached to Sepharose and the

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chorismate mutase from *Claviceps* is not unlikely, since the enzyme with a molecular weight of $6 \times 10^4$ is not too large. As already demonstrated [3] analogs and homologs of the effectors are capable of interacting with the inhibitor and activator site. It was especially convenient that amino-*N*-substituted tryptophan compounds also act as activators, since the effectors are covalently linked by the amino groups to Sepharose. Fig. 1 shows that chorismate mutase is indeed retarded by Sepharose substituted with L-Trp. An approximately quantitative adsorption of the enzyme is only possible at low protein concentration. At higher amounts of protein (10–15 mg) chorismate mutase activity in the most prominent protein peak was not observed but the succeeding fractions contained part of the chorismate mutase activity. The recovery of the chorismate mutase eluted from the column with phosphate buffer supplemented with $10^{-3}$ M L-Trp was greater than 80%. Corresponding results were obtained when dialysed crude enzyme extracts were applied to the column. Dialysis was essential because the crude extract containes L-Trp.

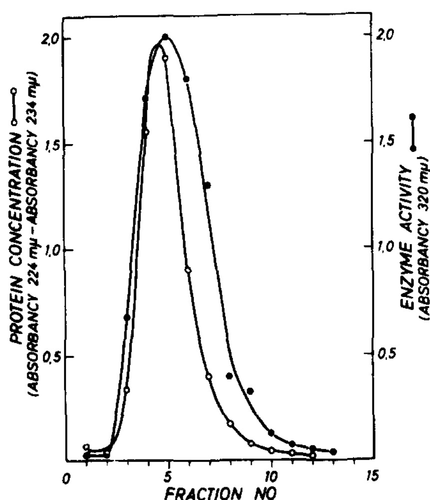


Fig. 2. Chromatography on L-Phe-substituted Sepharose.

Similar experiments with Sepharose substituted with L-Phe were unsuccessful. The activity of the chorismate mutase is mainly observed in the prominent protein peak. There is possibly a little retardation, but this is insufficient for a separation (fig. 2).

The protein concentration of the chorismate mutase

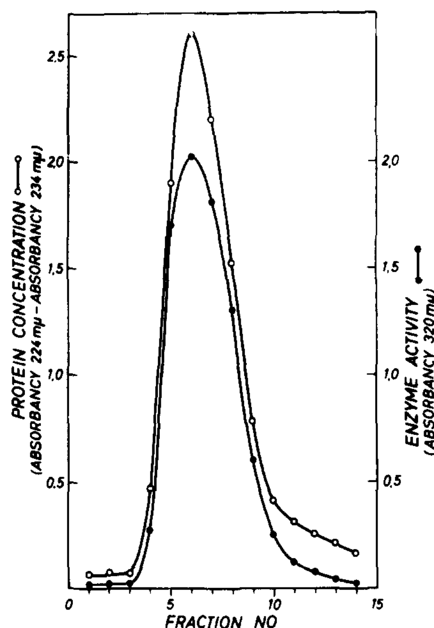


Fig. 3. Chromatography on unsubstituted Sepharose.

peak could not be determined since L-Trp in such a high concentration ($10^{-3}$ M) could not be completely removed by dialysis and the remaining Trp interferes with the protein determination [4, 5]. For this reason the specific activity of the chorismate mutase cannot be determined. We assume that the enrichment of the enzyme is high since all proteins which do not show affinity to L-Trp are not retarded as demonstrated in an experiment with unsubstituted Sepharose (fig. 3) The Sepharose substituted with L-Trp was used 9 times for the enrichment of chorismate mutase.

DAHP-synthetase from *Claviceps paspali* Pb 156 was neither separated from other proteins on Sepharose substituted with L-Trp nor on Sepharose substituted with L-Phe (experiments by J.Eberspächer [6]).

We hope that a higher column capacity for the separation of the chorismate mutase and an enrichment for the DAHP-synthetase by covalently binding the effectors to the Sepharose by means of a $C_6$-chain can be achieved.

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