AFFINITY CHROMATOGRAPHY OF BIOSYNTHETIC THREONINE DEAMINASE OF *ESCHERICHIA COLI*

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1. Introduction

Biosynthetic threonine deaminase (L-threonine hydrolyase deaminating, EC 4.2.1.16) is the first enzyme in the pathway of isoleucine biosynthesis and is subject to feedback inhibition by this amino acid. The effect of isoleucine is reversed by valine. Threonine deaminase has been purified from *Salmonella typhimurium* and some other microorganisms [1–3]. Attempts to purify the enzyme from *E. coli* were hampered by the instability of the enzyme [4].

We made an attempt to purify threonine deaminase of *E. coli* by means of affinity chromatography. The allosteric effectors isoleucine and valine and the substrate threonine, respectively, were coupled to Sepharose [5] and tested for their ability to remove threonine deaminase from protein solutions. Leucine as a structural analogue of isoleucine and glycine which does not interact specifically with threonine deaminase were included in this study.

Of the amino acids tested, isoleucine bound to Sepharose showed the strongest affinity for threonine deaminase in the chromatographic experiments. The strength of interactions between threonine deaminase and the Sepharose-linked amino acids increases with the molarity of phosphate buffer used.

2. Materials and methods

2.1. *Preparation of enzyme extracts*

The leucine-deficient strain L 3 was isolated after treatment of *E. coli* B with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [6]. The strain was grown in mineral-salt medium [7] supplemented with 5 mg of L-leucine per liter (threonine deaminase is derepressed by growth on limiting concentrations of leucine). Cells were harvested by centrifugation and disrupted by sonication in a solution containing 1.0 M potassium phosphate buffer pH 8.0, 5×10^{-3} M L-isoleucine, 10^{-3} M EDTA, and 10^{-3} M 2-mercaptoethanol. The enzyme was partially purified according to the procedure of Changeux [4]. Threonine deaminase was determined as described by Umbarger and Brown [8]. Protein concentrations were measured by the Biuret method or by the procedure of Lowry et al. [9]. Specific activity is expressed as μM of product formed per min per mg protein.

2.2. *Affinity chromatography*

L-amino acids (Merck AG, Darmstadt) were coupled to Sepharose 4 B (Pharmacia) as described by Cuatrecasas [5]. 5 ml of the enzyme solution were applied to 1.2 × 16 cm columns. The columns were equilibrated and developed with a solution containing 1.0 or 0.2 M potassium phosphate buffer pH 8.0, 10^{-3} M EDTA, and 10^{-3} M 2-mercaptoethanol. Fractions of 4 ml were collected.

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3. Results

The mutant strain *E. coli* L 3, leu⁻ was grown with limiting concentrations of leucine (5 mg per liter) resulting in derepression of threonine deaminase (specific activity 1.0). Enzyme extract was prepared and submitted to heat treatment and fractionation by ammonium sulfate [4]; the resulting enzyme solution containing 1 mg of protein per ml (specific activity 4.0) was used for the chromatographic experiments.

3.1. *Affinity chromatography in 0.2 M phosphate buffer*

The enzyme solution was applied to an isoleucine-Sepharose column equilibrated with 0.2 M phosphate buffer pH 8.0 (fig. 1A). The column was developed with the same buffer. A protein peak was eluted, which contained little enzyme activity. The enzyme emerged behind the bulk of protein. 50–60% of threonine deaminase were recovered. The enzyme activity is completely recovered if 10^{-3} M isoleucine is added to the elution buffer. This is consistent with the protective activity of isoleucine described by Changeux [4]. A 6-fold purification was achieved by this procedure. Similar experiments were performed with Sepharoses substituted with leucine, valine, threonine and glycine, respectively. Threonine deaminase is retarded by leucine-Sepharose but not by the other Sepharose-preparations. The retarding effect is less pronounced with leucine-Sepharose as compared to isoleucine-Sepharose.

3.2. *Affinity chromatography in 1.0 M buffer*

The same type of experiment as described above was performed with isoleucine-Sepharose equilibrated with 1.0 M buffer. 60% of the protein applied to the column was eluted with 1.0 M buffer. This protein fraction contained no threonine deaminase activity. Similarly, threonine deaminase was not eluted by buffer containing 10^{-3} M isoleucine. However, subsequent development of the column with 0.2 M buffer resulted in the elution of threonine deaminase. The yield was 50%. The enzyme was completely recovered if elution was performed with a solution of 0.2 M buffer and 10^{-3} M isoleucine (fig. 1B). Under the experimental conditions 1 ml of isoleucine-Sepharose binds 200 units. Optimal results are obtained, if the quantity of enzyme applied corresponds to the total capacity of the column.

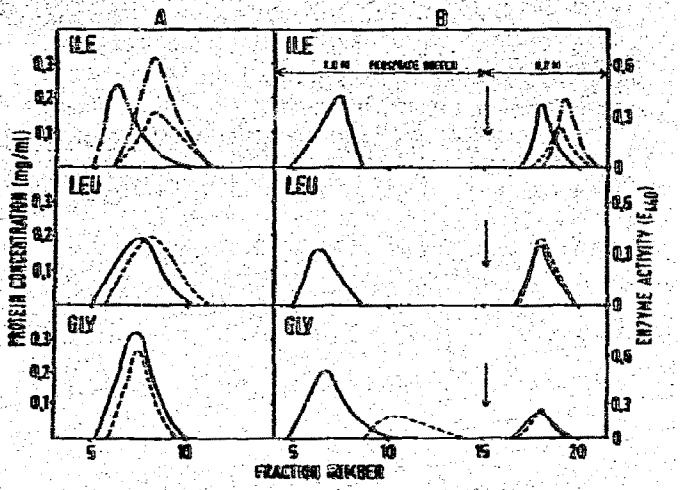

Fig. 1. Affinity chromatography of threonine deaminase. 5 ml of the enzyme solution were applied to columns (1.2 × 16 cm) of Sepharose 4 B conjugated with isoleucine (ILE), leucine (LEU), or glycine (GLY). The columns were equilibrated and developed with 0.2 M (A) or with 1.0 M phosphate buffer pH 8.0 (B); when no further protein was eluted with 1.0 M buffer, 0.2 M buffer was applied (B). (——) Protein concentration; (– – –) enzyme activity; (–.–.–) enzyme activity, when buffer contained isoleucine (10^{-3} M).

Sepharoses substituted with leucine, valine, or threonine behaved similar as isoleucine-Sepharose in analogous experiments, i.e. threonine deaminase was tightly bound in 1.0 M buffer and was eluted with 0.2 M phosphate buffer. Fig. 1B shows the results of the experiments with leucine-Sepharose. With valine-Sepharose a 4-fold purification with complete recovery of the enzyme was achieved by this procedure.

Glycine-Sepharose did not bind threonine deaminase tightly in 1.0 M buffer, but the enzyme was retarded as compared to the bulk of proteins (fig. 1B).

The threonine deaminase eluted from the Sepharose columns was tested with respect to its kinetic properties. Inhibition by isoleucine and substrate saturation kinetics were not altered as compared to the native enzyme.

4. Discussion

Purification of threonine deaminase from *E. coli* has proved difficult by conventional procedures. The present results indicate that affinity chromatography may be a useful procedure for purification of

threonine deaminase in a good yield and without alteration of the kinetic properties of the enzyme.

In buffer of low ionic strength threonine deaminase is retarded on isoleucine- and leucine-Sepharose, but not on the other Sepharose preparations tested. The retardation is caused by specific interaction of threonine deaminase with the Sepharose-bound amino acids. This is concluded from the finding that both isoleucine and leucine inhibit threonine deaminase (K_i 2.2×10^{-4} M and 2.1×10^{-2} M, respectively, at a threonine concentration of 2.5×10^{-2} M).

The inactivation of threonine deaminase during chromatography on isoleucine-Sepharose was reduced by the presence of isoleucine in the elution buffer. However the pattern of elution of threonine deaminase was not influenced by isoleucine suggesting that there is no competition between free and bound isoleucine for threonine deaminase.

In buffer of high ionic strength threonine deaminase is tightly bound to isoleucine-, leucine-, valine-, and threonine-Sepharose, respectively. This is in part due to two unspecific effects; i) not only threonine deaminase but an appreciable quantity of other proteins is bound. ii) Glycine-Sepharose retards threonine deaminase although glycine presumably does not interact specifically with the enzyme.

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