CRYSTALLIZATION OF L-ARGININE DEIMINASE FROM *PSEUDOMONAS PUTIDA*

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1. Introduction

L-Arginine deiminase (EC 3.5.3.6) catalyzes the degradation of L-arginine to L-citrulline and ammonia.

Many reports have appeared on the occurrence of this enzyme, but there are only two reports on the exhaustive purification of the enzyme [1, 2].

As reported in our previous report [3], we found that *Pseudomonas putida* produced markedly high activity of L-arginine deiminase and the enzyme is useful for the production of L-citrulline from L-arginine.

This report describes the crystallization of L-arginine deiminase of *P. putida.*

2. Materials and methods

P. putida ATCC 4359 was cultured under aerobic conditions at 30° for 24 hr in 8 l of a medium containing 2% glucose, 0.5% L-arginine hydrochloride, 0.5% yeast extract, 0.5% peptone, 0.5% $(NH_4)_2HPO_4$, 0.002% NaCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% $MnSO_4 \cdot 4H_2O$ and 0.0005% $FeSO_4 \cdot 7H_2O$ (pH 7.0). The harvested cells were washed with water and lyophilized. The lyophilized cells (about 30 g) were suspended in 1000 ml of 0.01 M potassium phosphate buffer (pH 7.0), sonicated for 15 min at 10 kc, and the supernatant solution was collected by centrifugation (step 1).

Ammonium sulfate was added to the solution, and the fraction precipitated between 40 and 60% saturation was collected. The precipitate was dissolved in 120 ml of 0.01 M potassium phosphate buffer (pH 7.0) and dialyzed overnight against the same buffer (step 2).

To the dialyzed solution was added protamine sulfate (from salmon, Sigma Chemical Company) in a proportion of 1 mg of protamine sulfate per 5 mg of protein. The resulting precipitate was removed by centrifugation (step 3).

The supernatant fluid was run on a DEAE-cellulose column (OH^- form, 3 × 90 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 7.0). The column was washed with the same buffer until no more material having absorption at 280 nm appeared, and then eluted by a linear gradient formed with 1 l of 0.01 M and 1 l of 1 M potassium phosphate buffer (pH 7.0). The enzyme activity was found in the fractions eluted by 0.1–0.2 M buffer (step 4).

The active fractions were passed through a Sepharose 6B column (3 × 90 cm) equilibrated with 0.01 M buffer and the fractions containing L-arginine deiminase were collected (step 5).

The fractions were then passed through a hydroxyl apatite column (1.6 × 32 cm) equilibrated with 0.1 M potassium phosphate buffer (pH 7.0). The column was washed with the same buffer until no more material having absorption at 280 nm appears, and then eluted by a linear gradient formed with 500 ml of 0.1 M and 500 ml of 1 M potassium phosphate buffer (pH 7.0). The enzyme was eluted by 0.2 M buffer as a well defined peak with a sharp leading edge (step 6).

Ammonium sulfate was added until the solution became slightly turbid, and the insoluble matter was discarded by centrifugation. The enzyme was crystallized from the supernatant solution by standing overnight at 4° (step 7).

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3. Results and discussion

The purification procedure is summarized in table 1. This procedure resulted in a 34-fold purification with an overall yield of 28%, corresponding to a yield of 4 mg crystalline enzyme per g of lyophilized cells. The purified enzyme had an activity of 57 units per mg of protein. Previous investigators reported activities of 41 and 53 units per mg of protein for purified enzymes from *Streptococcus faecalis* [1] and *Mycoplasma hominis* [2].

Fig. 1 is a photograph of the crystalline enzyme. The purified enzyme had an absorbance maximum at 278 nm and no absorption in the visible region. Besides column chromatography, the homogeneity of the enzyme was confirmed by criteria such as ultracentrifugation ($S^0_{20,w}$ = 10.2 S) and electrophoresis. The molecular weight of this enzyme was calculated to be about 120,000 from high speed sedimentation equilibrium measurements by the method of Chervenka [4].

It is interesting that the molecular weight of L-arginine deiminase obtained from *P. putida* is higher than that of *M. hominis* (78,300) [2].

Enzymatic properties of this enzyme will be presented subsequently.

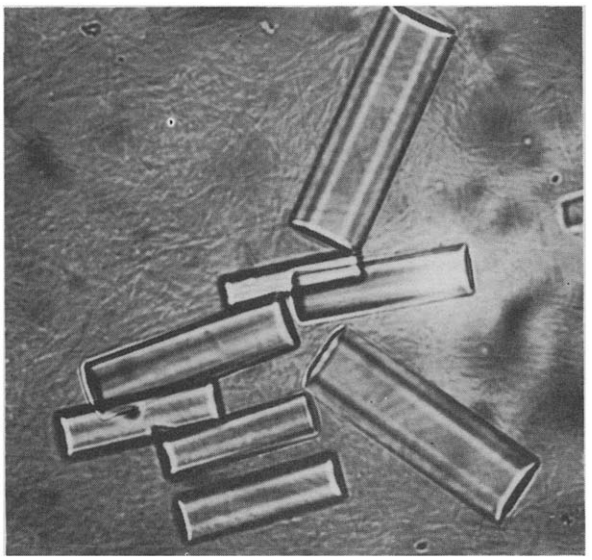

Fig. 1.

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Table 1
Purification of arginine deiminase from *Pseudomonas putida*.

Step no.	Step and fraction	Volume (ml)	Protein (mg)	Enzyme activity		
				total (μ)	specific (μ/mg)	yield (%)
1	Crude extract	1000	15,350	25,800	1.68	100.0
2	$(NH_4)_2SO_4$ 0.4–0.6 sat.	150	3470	17,535	5.05	68.0
3	Protamine sulfate	145	3178	17.531	5.52	53.0
5	DEAE-cellulose column	120	643	13,668	21.25	53.0
5	Sepharose 6B column	70	508	12,894	25.41	50.0
6	Hydroxyl apatite column	110	134	7480	55.74	29.0
7	Crystallization	20	127	7222	57.05	28.0

The assay of arginine deiminase activity was carried out by photometric measurements of citrulline liberated from L-arginine. The reaction mixture containing 4.5 ml of 30 mM L-arginine hydrochloride in 1 M sodium acetate buffer (pH 6.0) and 0.5 ml of enzyme solution was incubated for 15 min at 37°. L-Citrulline formed was assayed by the method of Archibald [5] using diacetylmonoxime. A unit of activity is defined as that amount of enzyme which catalyzes the liberation of 1 μmole of L-citrulline per min under the conditions of the assay.

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