CRYSTALLIZATION AND PROPERTIES OF CYSTEINE DESULFHYDRASE FROM *AEROBACTER AEROGENES*

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1. Introduction

Cysteine desulfhydrase is an enzyme which catalyzes degradation of L-cysteine into pyruvate, ammonia and hydrogen sulfide. The enzyme was first demonstrated using cell extracts from *Proteus vulgaris* by Tarr [1] and subsequently from *Escherichia coli* by Fromageot and Desnuelle [2]. The latter authors reported the stoichiometry of the overall reaction as follows.

$$HSCH_2CH(NH_2)COOH + H_2O \longrightarrow CH_3COCOOH + H_2S + NH_3$$

The enzyme was found in numbers of bacteria [1–4], in yeasts [5] and in higher animals [6,7].

Recently, Collins and Monty reported about the effect of the cryolysis on the enzyme from *Salmonella typhimurium* [8], about the kinetics and catalytic properties of the enzyme [9]. Kredich et al. have purified the enzyme from the same bacterium to a state of near homogeneity and reported its physical and chemical properties [10,11].

The occurrence of the enzyme activity in bacteria has recently been investigated in our laboratory with various bacterial strains and the markedly high activity was found in a strain isolated from soil. This strain was identified as *Aerobacter aerogenes* from the results of taxonomical studies, and the cultural conditions for the formation of the enzyme were studied with the strain [12].

The present communication reports the preparation and crystallization of cysteine desulfhydrase from *A. aerogenes* and some properties of the enzyme.

2. Materials and methods

All chemicals used were the best available commercial products.

Cysteine desulfhydrase activity was assayed by measuring the amount of sulfide formed from L-cysteine, according to Kredich et al. [11] with a slight modification. The reaction mixture contained 5 μmol of L-cysteine, 0.2 μmol of pyridoxal phosphate, 100 μmol of Tris–HCl buffer, pH 9.0, 100 μmol of KCl and enzyme in a total vol of 2.0 ml. The reaction was carried out at 30°C in capped test tubes (12 × 100 mm) for 5 min and terminated by the addition of 0.5 ml of 0.02 M *N,N'*-dimethyl-*p*-phenylene-diamine sulfate in 7.2 N HCl followed immediately by 0.5 ml of 0.03 M $FeCl_3$ in 1.2 N HCl. The absorbance at 650 nm was determined spectrophotometrically after storage in the dark for 20 min. A unit of enzyme activity was defined as the amount of enzyme which produced 1 μmol of sulfide per min.

Protein concentration was determined spectrophotometrically by measuring its absorbance at 280 nm. An E value of 13.1 for 10 mg/ml and for 1-cm light path, which was used throughout, was obtained by absorbance and protein determination by the method of Lowry et al. [13] using bovine serum albumin as the standard. Specific activity was expressed as units per mg of protein.

3. Results and discussion

3.1. *Purification of the enzyme*

Aerobacter aerogenes was cultivated in a 50-liter

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fermentor containing 40 liters of bouillon–peptone medium supplemented with 0.2% L-cysteine·HCl·H_2O and other materials as previously reported [12]. The cultivation was carried out at 30°C for 8 hr with aeration (10 liters per min.). All subsequent procedures were performed at 0–5°C.

Step 1: The cells were harvested and suspended in the dilution buffer which consists of 0.05 M potassium phosphate, pH 7.0, 10^{-5} M pyridoxal phosphate, 10^{-4} M EDTA, 10^{-2} M β-mercaptoethanol and 20% glycerol (v/v). The suspension was disrupted continuously by a DYNO-MILL KDL at 3000 rev/min (W. A. Bachofen, Switzerland) with 0.25–0.5 nm glass beads. Cells and debris were removed by centrifugation.

Step 2: The supernatant solution was fractionated with ammonium sulfate (30–50% saturation), followed by dialysis against the dilution buffer.

Step 3: The dialyzed solution was applied to a DEAE-Sephadex column (15 × 40 cm) equilibrated with the dilution buffer. After the column was washed with 20 liters of the dilution buffer, the enzyme was eluted with 0.1 M potassium phosphate buffer, pH 7.0, containing 0.15 M KCl, 10^{-5} M pyridoxal phosphate, 10^{-4} M EDTA, 10^{-2} M β-mercaptoethanol and 20% glycerol (v/v). The active fractions were combined and concentrated by the addition of ammonium sulfate. The precipitate was collected and dialyzed against the dilution buffer.

Step 4: The dialyzate was fractionated with ammonium sulfate again (30–40% saturation).

Step 5: The precipitated protein was dissolved with a small amount of dilution buffer and passed through a Sephadex G-200 column (2 × 100 cm) equilibrated with the dilution buffer. Active fractions were combined and concentrated by the additions of ammonium sulfate.

Step 6: Dialyzed enzyme solution was fractionated with ammonium sulfate (30–40% saturation).

Step 7: Concentrated active fractions were subjected to gel filtration through Sephadex G-200.

Step 8: Dialyzed active fraction was applied to a DEAE-Sephadex column (2 × 40 cm) equilibrated with the dilution buffer and eluted with a gradient of potassium chloride in a concentration from 0 to 0.35 M in the dilution buffer. Active fractions were combined and concentrated by the addition of ammonium sulfate. The precipitated protein was collected and dissolved with a minimal amount of 0.05 M potassium phosphate buffer containing

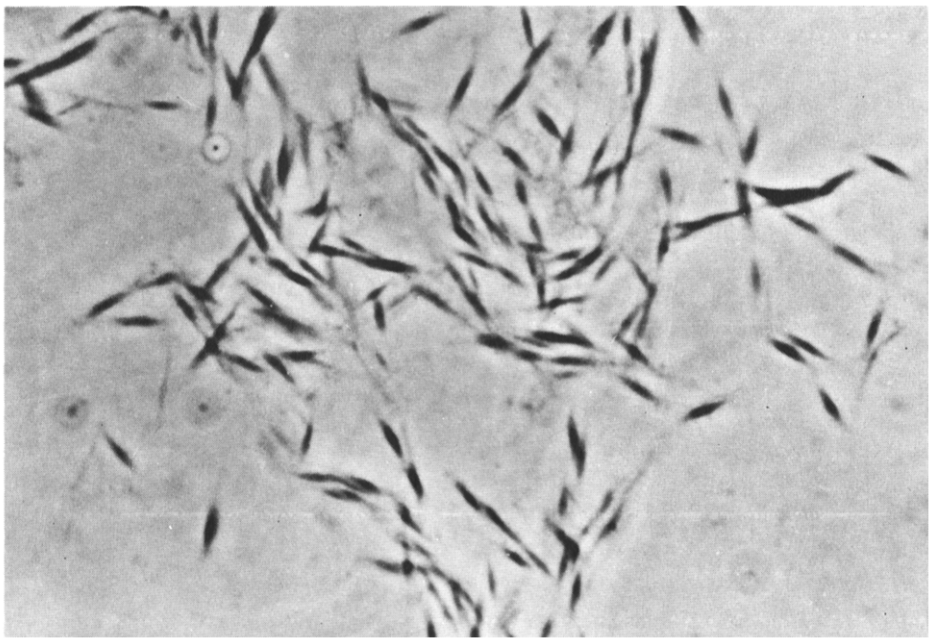

Fig.1. Photomicrograph of crystalline cysteine desulfhydrase. Magnified 600-fold.

Table 1
Purification of cysteine desulfhydrase from *Aerobacter aerogenes*

Step	Total protein mg	Total units	Specific activity units/mg
1. Cell extract	97 800	35 900	0.37
2. 1st Ammonium sulfate	86 600	37 000	0.43
3. 1st DEAE-Sephadex chromatography	2270	27 700	12.2
4. 2nd Ammonium sulfate	403	21 600	53.6
5. 1st Sephadex G-200 filtration	159	18 400	116
6. 3rd Ammonium sulfate	57.0	13 000	228
7. 2nd Sephadex G-200 filtration	30.7	11 500	375
8. 2nd DEAE-Sephadex chromatography	20.0	9860	493
9. Crystallization	19.2	9540	497

10^{-4} M pyridoxal phosphate, 10^{-4} M EDTA and 10^{-4} M dithiothreitol.

Step 9: Finely powdered ammonium sulfate was cautiously added to the enzyme solution until it became slightly turbid, and the mixture was placed in an ice bath. Crystallization began after about 2 days and was virtually completed within two weeks. A photomicrograph of the crystalline enzyme is shown in fig.1. A summary of typical purification procedures is presented in table 1.

3.2. *Properties of enzyme*

The crystalline cysteine desulfhydrase gave a single band on disc gel electrophoresis carried out at pH 8.3 [14]. The enzyme preparation sedimented as a single symmetric peak under ultracentrifugation performed at pH 7.0 in the dilution buffer without glycerol. Extrapolation of the data obtained from three ultracentrifuge runs to zero protein concentration gave an $S^0_{20,w}$ of 10.5 S. A diffusion constant, $D_{20,w}$, of 3.68×10^{-7} cm^2/sec was determined for a 2.11 mg/ml solution of protein. A value of 253 000 was calculated [15] for the mol. wt of the enzyme assuming a partial specific vol of 0.74.

The absorption spectrum of the enzyme has maxima at 330 and 415 nm (fig.2), indicating that the formyl group of the bound pyridoxal phosphate forms an azomethine link to an amino group of the protein, as in other pyridoxal enzymes. The pyridoxal phosphate content of the enzyme was determined after dialysis of the enzyme, against the dilution buffer. After 24 hr, the concentration of pyridoxal phosphate inside and outside the dialysis bag was determined fluorometrically according to Adams [16]. An excess concentration of pyridoxal phosphate was found within the dialysis bag, corresponding to the binding of 1 mol of pyridoxal phosphate by 39 000 *g* of the apoenzyme. This value is indicating that the enzyme contains 6 mol of pyridoxal phosphate per mole of enzyme.

In the stoichiometrical examination on the degradation of L-cysteine by the purified enzyme, a value of approx. 5 was found for the ratio of sulfide to

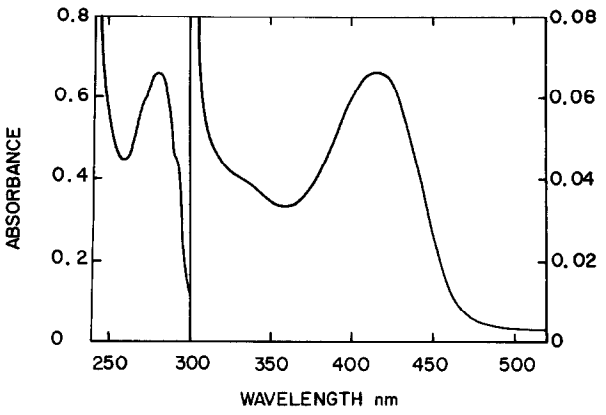

Fig.2. Absorption spectra of crystalline cysteine desulfhydrase. Crystalline enzyme, 0.51 mg, was dissolved in 1 ml of 0.05 M potassium phosphate buffer, buffer, pH 7.0, containing 10^{-5} M pyridoxal phosphate, 10^{-4} M EDTA, 10^{-2} M β-mercaptoethanol and 20% of glycerol. The absorption spectra was taken against a reference of the same buffer with a Shimazu MPS-5000 recording spectrophotometer.

pyruvate production and the same value for the ratio of ammonium to pyruvate production. Unusual stoichiometry in cysteine desulfhydrase was reported by many workers [5,8,10,11,17], and was explanated by Kredich [11], based on the isolation and identification of a product of the enzyme reaction. Pyruvate formation by the enzyme of *A. aerobacter* was also observed from L-serine, L-tryptophan and S-methyl-L-cysteine at the relative rate of 2.5, 1.5, 1.4% of sulfide production from L-cysteine. The purified enzyme catalyzed stoichiometric degradation of β-chloro-L-alanine into pyruvate, ammonia and chloride at the relative rate of 167% of sulfide production from L-cysteine.

The purified enzyme preparation was found to catalyze the synthesis of L-cysteine from pyruvate, ammonia and sodium sulfide in the presence of high concentration of these substrates. The enzymatically synthesized L-cysteine was identified by an amino acids analyzer. The enzymatic synthesis of *S*-methyl- or *S*-ethyl-L-cysteine was carried out in the same manner except that sodium sulfide was replaced by methyl- or ethyl-mercaptan, respectively [18].

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