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CRYSTALLINE ORNITHINE TRANSCARBAMYLASE

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SUMMARY

Ornithine transcarbamylase (carbamoyl phosphate:L-ornithine carbamoyl transferase, EC 2.1.2.2) from arginine grown Streptococcus D_{10} cells was purified to homogeneity by acid, solvent, heat, and salt fractionation. The enzyme was then crystallized from an ammonium sulfate solution. Some molecular properties of the enzyme are described.

INTRODUCTION

Ornithine transcarbamylase (EC 2.1.3.3) has been prepared from a number of sources; although highly purified and/or homogeneous, it has never been crystallized. We have found it possible to crystallize ornithine transcarbamylase as a by-product of our previously described procedure for the preparation of crystalline carbamate kinase[1]. The method together with some of the molecular properties of the crystalline enzyme, are presented here.

PROCEDURE

All operations were performed at 0° and all precipitates were collected by centrifugation at 15 000 × *g* for 15 min unless otherwise stated. Potassium acetate buffer, 0.25 M, pH 3.8 was used. $(NH_4)_2SO_4$, enzyme grade, was from Mann Research Laboratories; all $(NH_4)_2SO_4$ solutions were saturated at pH 5.5. Acetone was spectral grade and measured, then added at −20°.

Streptococcus D_{10} was grown and sonicated as previously described[1]. The following description applies to purification from one 32-l batch of culture. After harvesting and sonication, the sonicate was centrifuged. The clear supernatant (*ca.* 800 ml) was diluted to 1200 ml (Crude fraction) and mixed with 400 ml of acetate buffer. After standing for 20 min the precipitate was collected and homogenized by hand with 500 ml of 0.4 M $(NH_4)_2SO_4$ (in about 50 ml portions) and centrifuged. This

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extraction was repeated and the two extracts were combined. 360 g of $(NH_4)_2SO_4$ were stirred into the supernatant fluid, the precipitate collected, and then dissolved in 200 ml of 0.05 M $NaHCO_3$ (about 20 mg protein/ml, Fraction 1). 60 ml of acetone were added (dropwise with stirring) as the preparation was cooled at −10° and an additional 100 ml of acetone added. The precipitate was collected by centrifugation (10 000 × *g*, 20 min at −10°), resuspended in 120 ml of cold water, and any insoluble material removed by centrifugation. This preparation (Fraction 2) contains the bulk of the ornithine transcarbamylase (carbamate kinase can be obtained by further fractionation of the acetone supernatant[1]). Fraction 2 was heated to 65° and held for 7 min then cooled in an ice bath to 4° and centrifuged (Fraction 3). An equal volume of the saturated $(NH_4)_2SO_4$ solution was stirred into Fraction 3 and the precipitate removed by centrifugation. Acetate buffer was added to the supernatant fluid until faintly turbid (about 4 ml to pH 4.5) and then stirred for 4 h; the turbidity increased with a faint silky appearance. The precipitate was collected, dissolved in 6 ml of water at room temperature (Fraction 4) and one ml saturated $(NH_4)_2SO_4$ solution was stirred in slowly. Some turbidity occurred and was removed immediately by centrifugation. Acetate buffer (0.2 ml) was added dropwise until turbid and then stirred slowly at 4°. A drop of acetate buffer was added every hour (for 6 h) and the stirring continued overnight. After centrifugation the precipitate was dissolved in 6 ml of water and dialyzed overnight against 1 l of 0.05 M sodium succinate, pH 5.5 (Fraction 5). This fraction is essentially homogeneous when analyzed in the analytical ultracentrifuge or with disc electrophoresis. A resume of the purification is presented in Table I.

TABLE I

SUMMARY OF PURIFICATION PROCEDURE

Enzyme dilutions were made in 0.01 M Tris–maleate, pH 7.5, 0.01 M ornithine. One unit is the amount of enzyme which will form one μmole of citrulline/min in 0.05 M Tris–maleate pH 8.55 from 0.01 M dilithium carbamyl phosphate and 0.01 M ornithine at 38°. Citrulline was measured as described previously[2].

Fraction	*Units (10^5)*	*ml*	*Protein (mg/ml)*	*Specific activity (units per mg of protein[3])*
Crude	9.9	1200	14.6	56
1	7.8	210	19.3	195
2	5.8	125	16	290
3	5.0	118	7.7	550
4	4.0	9	59	750
5	3.2	9.5	34	1000

Crystallization

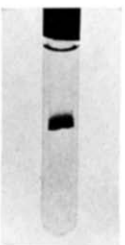
Although schlieren was often observed in the last two steps of the purification procedure, the preparations were usually amorphous. An equal volume of a saturated $(NH_4)_2SO_4$ solution was added to a 15 mg/ml solution of the enzyme (Fraction 5). $MnCl_2$ (0.1 M) was added to a final concentration of 0.002 M, then 0.1 M sodium EDTA, pH 5, was added dropwise to a concentration of 0.002 M. The enzyme precipitated immediately. If EDTA was added first, then the enzyme precipitated on the addition

of $MnCl_2$. After centrifugation, the enzyme (slightly soluble in water) was dissolved in 0.1 M Tris–HCl, pH 8.5, at a protein concentration of 30 mg/ml. Five volumes of saturated $(NH_4)_2SO_4$ solution were added and the pH adjusted to 5 with the acetate buffer. The precipitate was collected and dissolved in water to a concentration of 15 mg/ml. 1.2 volumes of the saturated $(NH_4)_2SO_4$ solution were added slowly with gentle swirling, then water dropwise until the solution was at incipient turbidity at room temperature. The preparation was stirred in the cold room at 4°. Crystals (Fig. 1) began to form in 1 h. An equal volume of the saturated $(NH_4)_2SO_4$ solution was added slowly and the preparation stirred overnight. The specific activity was unchanged with crystallization and the yield of enzyme varied from 70–80%. Precipitation of the enzyme with MnEDTA, seemed essential for crystallization. When $MnCl_2$–EDTA precipitation of the enzyme was attempted on Fractions 3 and 4, crystallization was possible but the preparations were not homogeneous on disc electrophoresis, contained a fairly large nucleic acid contaminant and recovery was usually below 50%.

Fig. 1. Photomicrograph of crystalline ornithine transcarbamylase (× 275).

RESULTS AND DISCUSSION

Chromatography of the enzyme on DEAE-cellulose at pH 5.5 in a stepwise elution gradient of sodium succinate[4] from 0.05 to 0.35 M indicated a single protein eluting at 0.14 M. Disc electrophoresis at pH 8.5 (Fig. 2) and the sedimentation pattern in the analytical ultracentrifuge (Fig. 3) suggest a single protein. The s_{20} in 0.05 M sodium succinate, pH 5.5, at infinite protein dilution was 8.95. The s_{20} increased only 7% with a four-fold increase in concentration to 10 mg/ml and was unchanged with the addition of *p*-hydroxymercuribenzoate at $7 \cdot 10^{-4}$ M. When chromatographed on



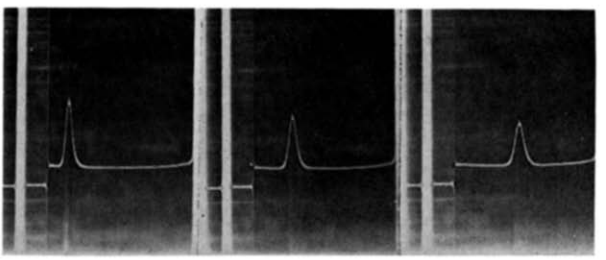


Fig. 2. Protein pattern obtained after subjecting a 35-μg sample of crystalline ornithine transcarbamylase to disc electrophoresis[5] at pH 8.5. Staining was with aniline black.

Fig. 3. Sedimentation pattern of crystalline ornithine transcarbamylase at 8.4 mg/ml in 0.05 M sodium succinate, pH 5.5. Speed was 59 780 rev./min using rotor AN-D in the Model E Spinco ultracentrifuge at 20°. Exposures from left to right are at 8, 20 and 36 min after speed equilibrium at a bar angle of 70°.

Bio-Gel P-300, the enzyme was included with an R_F of 0.8. The absorbance ratio, $A_{280\ m\mu}:A_{260\ m\mu}$ was 1.93 at pH 5.5. The preparation catalyzed the synthesis of δ-acetyl ornithine at 0.3–0.5% the rate of citrulline synthesis at pH 8.5 when acetyl phosphate replaced carbamyl phosphate; this is as was found in crude preparations[6].

The specific activity of the crystalline enzyme is equal to that described for the enzyme purified from *Staphylococcus lactis*[7] and *Escherichia coli*[8] and about one-third that of the purified enzyme from *Staphylococcus faecalis* R (ATCC 8043, ref. 9). The s_{20} is similar to that reported for the derepressed enzyme from *E. coli*[8], and the molecular weight appears similar to that of the rat liver[10] ornithine transcarbamylase. On the other hand, the enzyme from Mycoplasma[11] has about six times higher specific activity; while these variations could reflect assay conditions, it is of interest that the s_{20} of the Mycoplasma enzyme is twice that reported here for the *Staphylococcus faecium* enzyme.

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