CRYSTALLIZATION OF ORNITHINE TRANSAMINASE AND ITS PROPERTIES

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Received June 1, 1968

It is well known that ornithine-ketoacid transaminase(OKT) catalyzes the interconversion of ornithine and gluaamic-δ-semialdehyde with concomitant interconversion of α-ketoglutarate and glutamate in animal tissues. In 1963 Peraino and Pitot reported some properties of OKT using partially purified enzyme. In 1964 we reported the purification of the enzyme to a single component by ultracentrifugal and electrophoretic analysis. Strecher also reported some important kinetical properties using partially purified enzyme.

Recently, we purified and crystallized the enzyme in good yiels from rat liver. This is reported in this paper with some physicochemical properties of OKT.

Experimental

Enzyme assay-------Enzyme activity was assayed as reported previously. The reaction mixture usually contained 20 μmoles of ornithine, 20 μmoles of α-ketoglutarate, enzyme and 1.0 ml of 0.1 M phosphate buffer(pH 8.0) in a total volume of 2 ml. Incubation was carried out at 37°C for 30 minutes and terminated by addition of 1 ml of 1% o-aminobenzaldehyde in N HCl. Samples were immersed in boiling water for 5 minutes to allow development of color and then centrifuged. The absorbancy of the yellow color

of quinazolium in the supernatant was measured at 440 mμ. Protein was measured by the method of Lowry et al(Lowry et al., 1951).

Enzyme--------As reported previously (Katunuma et al., 1964), liver ornithine transaminase is strongly induced by a high protein diet. Rats were fed on a 70 % casein diet for a week. Then their livers were removed and homogenized and the mitochondrial fraction was obtained by the method of Hogeboom (Hogeboom, 1955) because this enzyme is localized entirely in the mitochondria.

Preparation of anti-OKT Serum--------OKT was injected subcutaneously into rabbits by the method of Leskowity and Waksman(1960), with 10 mg of protein in complete Freund's adjuvant. At intervals of 1 to 4 weeks, the animals were bled. Control antiserum was obtained from rats which had received Freund's adjuvant only. The serum was purified to a single protein as judyed by electrophoresis by the Kekwick method(1960). The antisera were stored at -15°C until used.

Estimation of Enzyme Activity by the Precipitate Reaction with Anti Serum--------Volumes of 1 ml of antigen and antibody were mixed at room tempereture, incubated for 30 minutes at 37°C, and stored at 5°C for over 48 hours. Then the samples were centrifuged at about 5000 xg for 15 minutes. The precipitates thus obtained, were washed twice with chilled saline and then 1 ml of N-NaOH was added and tubes were stored for an hour at room temperature. The absorbancy was measured at 280 mμ in a Beckman spectrophotometer.

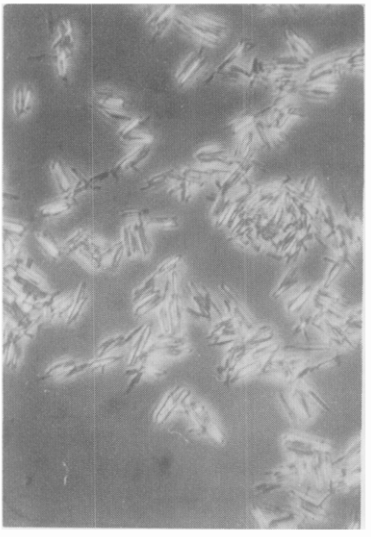
Purification--------The procedure for purification of the enzyme is given in Table 1. All purification procedures were performed at about 5°C. The sample was homogenized in 4-5 volumes of water

with a Waring Blendor run at maximum speed for 20 minutes. The enzyme, which is localized in the mitochondria was solubilized quite easily. This homogenate was ajusted to pH 5.5 with 1 % acetic acid. It was then centrifuged for 10 minutes at 10000 xg and the supernatant was adjusted to pH 6.8 with N-NaOH. The fraction precipitating with 50 % saturation of ammonium sulfate was dissolved in water and dialysed against tap water over night. The precipitate which formed was removed. To the solution 5 μmoles of α-ketoglutarate, 5 μmoles of EDTA and 50 μg of pyridoxal phosphate(PALP) per ml. were added to stabilize the enzyme, the solution was heated at 60°C for 1 minute, the precipitate was removed and the supernatant was fractionated by addition of 30% saturation of ammonium sulfate. The resulting precipitate was washed with a small amount of water, and the solution which had little activity was removed, because the enzyme was very difficult to dissolve in water. The precipitate was dissolved in 0.1 M phosphate buffer, pH 7.5, containing 5 μg PAL-P per ml and the solution was treated by DEAE-cellulose column chromatography. The enzyme was obtained from the column by elution with 0.1 M buffer. The eluate had a specific activity of 700 to 950 and was about 2000 fold purer than the initial homogenate.

Table 1. Purification of Ornithine Transaminase from Rat Liver

Steps	Specific Act. GSA formed μmole/ hour/mg protein	Total Activity
Homogenate Supernatant	0.4 - 0.6	—
1 st. Amm. Sulf.	2.9	100 %
Heat Treat.	10.5	55
2nd Amm. Sulf.	120	35
DEAE cellulose column	700	26
Acetone Fraction	950	17
Crystalline	1050	10
Recrystallization	1000	

Fig. 1



Crystallization--------The fraction was concentrated by acetone fractionation (75 %). And the precipitate was dissolved in the small amount of water. Then to the solution was gradually added solid ammonium sulfate until just before a slight turbidity appeared under constant stirring on a magnetic stirrer in the cold. Long rod shaped (10μ) yellow crystals appeared after 40 hours in the cold. Recrystallization was carried out in the same way as the first crystallization and the recrystallized enzyme had the same specific activity as the first crystals. A photograph of the crystalline enzyme is shown in Fig. 1.

Molecular Weight and other Characters--------The recrystallized enzyme shows a single symmetrical boundary on ultracentrifugation. The sedimentation constants (S 20w) were 10.20 S and 10.25 S for the PAL-P form enzyme and PAM-P form enzyme, respectively. The molecular weight was calculated to be 160,000 -180,000 by the Archibald method. The electrophoretic patterns on cellulose acetate at pH 6.5 and pH 8.6 also indicate that it is a single protein.

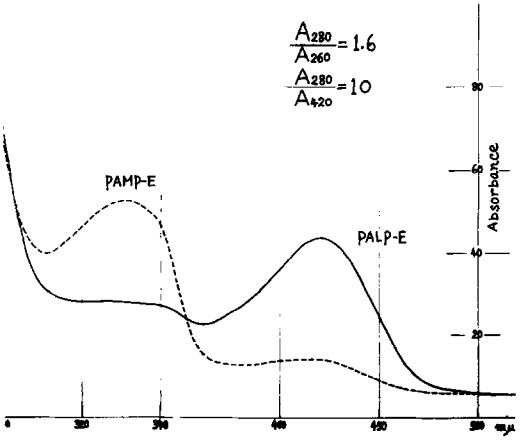
PAL-P Content--------To estimate the PAL-P content the following procedure was used. A solution of the crystalline enzyme was deproteinized and PAL-P was resolved from the apoenzyme by addition of hydrochloric acid. The PAL-P content of the solution was determined by the cyanohydrine method and by microbioassay.

The value by the cyanohydrine method gave 1.92-2.18 moles and by microbioassay after hydrolysis by potato acid phosphatase gave 2.38 moles per mole. These data show that the PAL-P content two moles per molecule of the enzyme.

Absorption Spectra-------

A concentrated solution of the crystalline enzyme was deep yellow. It had an absorption maximum at 420-430 mμ due to the bound PAL-P. No remarkable change of the spectrum was observed at different pH values. On addition of 0.1 M L-ornithine to the PAL-P form of enzyme a new peak appeared at 330 mμ with a corresponding decrease of in the absorption at 420 mμ indicating a conversion of the PAL-P form to the pyridoxamine phosphate form.

Fig. 2 Absorption Spectrum



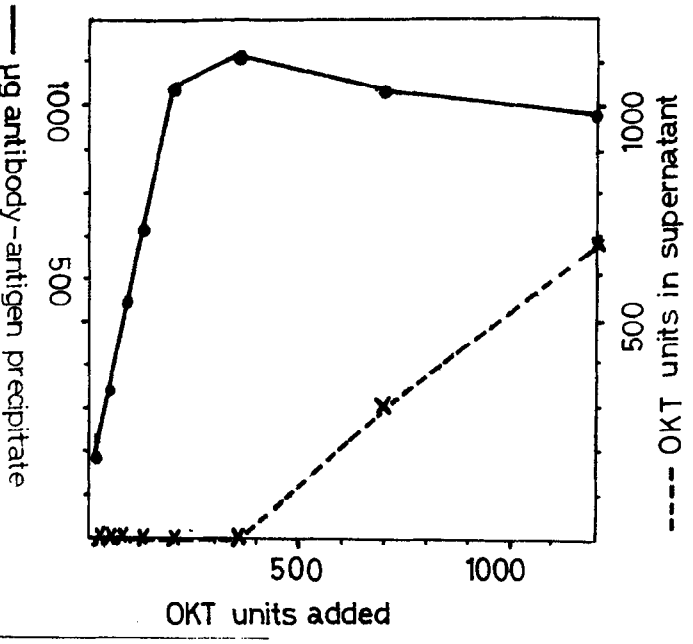
The 280/420 ratio of the PAL-P form was about 10 at pH 8.0 in 0.1 M phosphate buffer (Fig. 2).

Immunochemical Studies-------The results of quantitative precipitation tests with antiserum are shown in Fig. 3. Ornithine transaminase activity was completely inhibited by addition of the antiserum, but not of control serum. In this it is exceptional for it is rarely found that an enzyme-antibody complex loses almost all enzyme activity.

Varying amounts of OKT were added to 0.1 ml of anti-OKT serum and to control serum, and mixtures were incubated for 30 minutes at 37°C. No precipitation occurred in any of the control tubes. The tubes were stored at 5°C for over 45 hours, and the precipi-

tates were collected and washed twice with chilled saline. The supernatant fluids were assayed for OKT activity, and the protein contents of the washed precipitates were measured.

Fig. 3 Quantitative Precipitin Reactions of Purified Ornithine Transaminase



During prepare this manuscript, we have had self communication from Dr. T. Jenkins regarding crystallization of this enzyme from pig kidney.

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