THE ISOLATION OF PIG LIVER MONOAMINE OXIDASE

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SUMMARY. Monoamine oxidase from pig liver has been isolated and purified approximately three hundred-fold. This enzyme has a molecular weight of 1,200,000, is highly polymeric, and contains subunits of molecular weight 146,000, as determined by Sephadex chromatography. The apparent K_m at 25°C is 1.28 x 10^{-6} M at pH 9.0 (0.05 M glycine) and 1.74 x 10^{-5} M at pH 7.2 (0.2 M phosphate) using benzylamine as a substrate. This enzyme contains approximately 8 copper(II) ions per 1,200,000 molecular weight.

INTRODUCTION. Liver mitochondrial amine oxidase has been isolated from a number of sources including rat (1-4), human (5), beef (6-8), guinea pig (9) and pig (10,11) liver. The enzyme catalyzes the oxidative deamination of amines in the following general reaction:

$$RCH_2NHR' + O_2 + H_2O \rightarrow RCHO + R'NH_2 + H_2O_2 \qquad (1)$$

In a previous studies (10,11) using pig liver as a source, a soluble amine oxidase of molecular weights 400,000, 290,000-275,000 and 115,000 were reported. An extraction procedure using 2-butanone was developed and criticism was offered concerning the use of detergents in amine oxidase preparations despite previous successes in this area. In this communication we report an alternate method using Triton X-100 which leads to the preparation of a highly purified monoamine oxidase of molecular weight 1,200,000.

ASSAY METHOD. The enzyme assay with benzylamine as a substrate was carried out at 25°C as described by Tabor, et al. (12) at 250 nm. The buffer system was 0.05 M glycine (pH 9.0) containing 1 mM EDTA and 10^{-4} M dithioerythritol. One unit of activity is defined as the amount of enzyme catalyzing

a change of 0.001 absorbance per minute per mg of protein. Protein concentrations were determined by the biuret method using bovine albumin from Sigma as a standard. A value of 10.9 for $E_{280}^{1\%}$ was established.

ISOLATION PROCEDURE. Liver obtained from a freshly slaughtered pig was cut into 1 cm cubes and frozen overnight. The liver was thawed at room temperature and 50 g amounts were homogenized in a Waring Blendor with 150 ml of water for 120 sec. The resulting solution was filtered through cheese cloth to remove large particles, and centrifuged at 6,000 x g for 1 hour at 4°C. The precipitate was discarded and ammonium sulfate (21 g/100 ml) was slowly added with gentle stirring. The solution was then allowed to stand for at least 4 hrs. at 4°C. The final suspension was centrifuged at 10,000 x g for 30 min. at 4°C, and the supernatant discarded. The precipitate was treated with Triton X-100 (4 drops Triton X-100/3 ml ppt) in a tissue homogenizer and ground by hand for approximately 2 min.

The resulting suspension was increased in volume by 50% using 0.2 M phosphate buffer (pH 7.0) and was stored overnight at 4°C. The solution was then centrifuged at 20,000 x g for 1 hr. at 4°C. Any suspended material and the ppt. were discard. The suspended matter was removed from the supernatant by filtering through nylon mesh or other porous material.

At this point, 20 mg of deoxyribonuclease (6,000 Kunitz units) was added and the solution stored at 4°C for 1 week. As in the isolation of sugar dehydrogenases (13) and catalase (14) from pig liver, the addition of deoxyribonuclease results in an overall increase in enzyme units. This is due to the breakdown of large nucleic acid particles which slowly precipitate from the solution. These large particles were apparently complexed with the enzyme of interest, thus reducing the effective number of catalytic sites per mg of total protein. At the end of 5 or more days, the solution was centrifuged at 20,000 x g for 30 min. and the supernatant added in 5 ml quantities to a Sephadex G-200 column (flow rate 0.25 ml/min).

The first G-200 treatment yielded two main components, one of molecular

weight 1,200,000 and the other 146,000 as determined on a standardized Sephadex G-200 column (13,15). The most active fractions of the high molecular weight component with respect to benzylamine were condensed using collodion membranes and chromatographed on a second Sephadex G-200 column. The column eluent was triply distilled water at pH 7.0 (0.001 M phosphate buffer) containing 1 mM EDTA, and 0.004% sodium azide.

Attempts to increase the specific activity beyond that obtained from the second column elution by ammonium sulfate recrystallization proved fruitless. This technique yielded samples showing little or no improvement over the chromatographed samples. In summary, the specific activity increased from 10.8 after centrifugation at 6,000 x g to 3067 after elution from the second Sephadex G-200 column.

RESULTS. The specific activity of the high molecular form of this enzyme was more than twice that of the 146,000 molecular weight form. The subunit form was concentrated by the use of collodion membranes and after centrifugation at 20,000 x g for twenty minutes, the supernatant (95% of the original sample) was rechromatographed on a Sephadex G-200 column. Approximately 70% of the sample was eluted in the 1,200,000 molecular weight region.

The pH of maximum activity was 9.0 at 25°C in either 0.05 M glycine or phosphate buffers. Less than one-half of this activity was obtained using 0.2 M phosphate buffer at pH 7.2 and 25°C. The activity of this enzyme increased 37% between 25 and 40°C. A similar result was obtained for the beef liver enzyme (6) of molecular weight 1,280,000.

The apparent K_m at 25°C was 1.28×10^{-6} M at pH 9.0 (0.05 M glycine) and 1.74×10^{-5} M at pH 7.2 (0.02 M phosphate) using benzylamine as a substrate. The recently isolated enzyme of molecular weight 275,000-290,000 (11) was reported to give a K_m of 4.5×10^{-4} M using a butanone extraction and a K_m of approximately 1.25×10^{-3} M if Triton X-100 was used. No physical properties were reported for the latter enzyme, and no indication of substrate inhibition was reported (11). We observed

substrate inhibition as is the case for this enzyme from human plasma (16), rabbit serum (17) and other sources. This latter fact fits in well with a subunit model containing allosteric sites and is consistent with our general observations.

As Cu(II) is found in most monoamine oxidases, it was determined by the use of dithionite and biquinoline (18,19). The amount of Cu(II) was 0.0434% or 8.09±0.29 copper atoms per enzyme unit of 1,200,000 molecular weight. The amount of Cu(I) was less than 0.50 atoms per enzyme molecule and apparently represents a trapped impurity.

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