BBA 61186

A kinetic study of particulate bovine adrenal tyrosine hydroxylase

The enzyme tyrosine hydroxylase which catalyzes the tetrahydropteridine(2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine)-dependent hydroxylation of tyrosine to DOPA was first partially purified from the supernatant solution of adrenal homogenates[1]. More recently the enzyme was solubilized by incubation with trypsin and partially purified from the adrenal particles[2]. Kinetic studies were previously carried out with enzyme preparations obtained from the supernatant fractions of the adrenal homogenates.

In the present study we have investigated the kinetic properties of the enzymatic tyrosine hydroxylation with a more purified enzymatic preparation obtained from the adrenal particles. The results to be reported below suggest that the kinetic mechanism of action of particulate tyrosine hydroxylase differs from the mechanism reported for the soluble enzyme[3].

The purification and the solubilization of the particulate enzyme was carried out as previously described[2]. An additional 4–5-fold purification was achieved by adsorption of the enzyme on alumina C_γ gel. The $(NH_4)_2SO_4$ fraction was diluted with 4 vol. of ice-cold water, and 3.0 ml of alumina C_γ gel were added to 100 ml of the diluted enzyme preparation (100 mg of protein per 3 ml of alumina C_γ gel). The mixture was stirred for 5 min and centrifuged at 0°. The supernatant fluid was discarded and the gel eluted successively with 10 ml of 0.02–0.1 M phosphate buffer (pH 6.5). The highest activity was obtained in the fraction eluted with 0.05 M phosphate buffer. The enzyme at this stage was stable only for a few days at −20°. The enzyme activity was measured as described previously by determining the release of ^3H from [3,5-$^3H_2$]tyrosine into water[4].

Reciprocal velocities were plotted graphically against the reciprocals of substrate concentration. When these plots were linear, the data were fitted using the least-square method of WILKINSON[5]. These calculations were performed on an Olivetti Underwood Programma 101.

The effect of different O_2 concentrations on the enzymatic tyrosine hydroxylation at different concentrations of tetrahydropteridine. Fig. 1 shows double-reciprocal plots of velocity against O_2 concentration for different concentrations of tetrahydropteridine at a constant concentration of tyrosine. The lines intersect at a point left of the vertical axis. It is evident that O_2 affects the affinity of tetrahydropteridine for the enzyme and *vice versa*. The intersection point is above the horizontal axis, which indicates that the presence of one substrate enhances the binding of the other. The apparent K_m value for O_2 at tetrahydropteridine concentration of 0.05 mM is 26.4%, while at a concentration of 0.1 mM it is 8.8%. At high tetrahydropteridine concentration O_2 has only a marginal effect on the enzymatic tyrosine hydroxylation, while at a low concentration increased O_2 concentration results in a considerable increase in the enzyme activity.

The effects of different tyrosine concentrations on the enzymatic tyrosine hydroxylation at different tetrahydropteridine concentrations. Fig. 2 shows double-reciprocal plots of velocity against tyrosine concentration for different concentrations of tetrahydropteridine at a constant concentration of O_2. It can be seen that the results consist

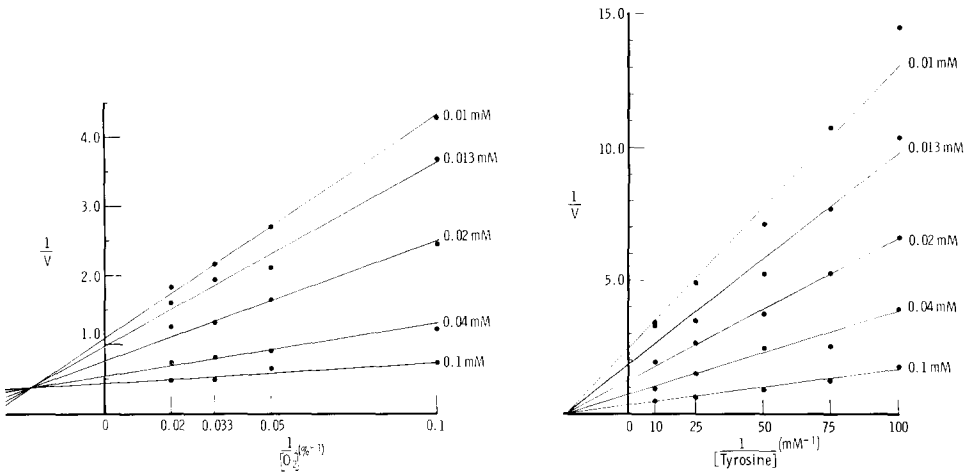

Fig. 1. Double-reciprocal plots of velocity against O_2 concentration in the presence of different concentrations of tetrahydropteridine. The incubations were carried out in a mixture that contained the following components: sodium acetate (pH 6), 200 mM; potassium phosphate (pH 6), 10 mM; 2-mercaptoethanol, 50 mM; $FeSO_4$, 0.5 mM; [3,5-$^3H_2$]tyrosine, 0.1 mM; tetrahydropteridine, as indicated in the figure. The final volume was 1 ml, and the incubations were carried out at 30°. After the addition of enzyme and acetate buffer, different concentrations of O_2 gas were delivered for 5 min prior to the addition of the other components, and the incubations were carried out for 4 min. The reaction was terminated by the addition of 0.05 ml of glacial acetic acid

Fig. 2. Double-reciprocal plots of velocity against tyrosine concentration in the presence of different concentrations of tetrahydropteridine. The experiments were performed in the incubation mixtures with the same compositions as indicated in Fig. 1 except that all the incubations were carried out in the presence of 20% O_2, and the tyrosine and tetrahydropteridine concentrations were varied as indicated above.

of a series of straight lines which intersect near the horizontal axis. The apparent K_m value for tyrosine was found to be almost constant for various tetrahydropteridine concentrations and lies within the range of $3.0 \cdot 10^{-5}$–$6.0 \cdot 10^{-5}$ M. If the concentration of tyrosine or tetrahydropteridine is raised, the lines become more parallel and the apparent K_m for tyrosine changes with the tetrahydropteridine concentration. However, at higher substrate concentrations the reciprocal plots become curved, most likely as a result of substrate inhibition.

The intersecting initial velocity pattern shown in Fig. 1 implies a sequential mechanism in which tetrahydropteridine and O_2 add to the enzyme before a product is released. The intersecting initial velocity patterns shown in Fig. 2 suggest a sequential mechanism in which both substrates, tetrahydropteridine and tyrosine, add to the enzyme before a product is released. When tyrosine was the variable substrate at different concentrations of O_2 and at a constant concentration of tetrahydropteridine an intersecting initial velocity pattern was also obtained. Since all three initial velocity patterns intersect, our present results suggest the possibility of a mechanism in which a quaternary complex is formed, with the rate-limiting step being the conversion of the complex into products and enzyme[6]. The present results differ in certain aspects from the previously reported kinetic data which were performed with enzyme preparations obtained from the supernatant fractions of the adrenal glands[3]. In these studies the double-reciprocal plots of velocity against tyrosine concentrations for

different concentrations of tetrahydropteridine resulted in a series of parallel lines, and the formation of a reduced enzyme intermediate *via* the "ping-pong" mechanism was postulated[3]. Thus, the kinetic mechanism of action of tyrosine hydroxylase from the solubilized and from the particulate preparation does not appear to be the same. However, it is possible that the difference in the initial rate patterns is due to a difference in the experimental conditions such as differences in incubation periods or in the range of substrate concentrations. A subsequent detailed paper will further explore the mechanism of the enzymatic tyrosine hydroxylation based on numerical values of the constants obtained from studies with enzyme substrates and inhibitors.

One of us (M.G.) is a Research Scientist Awardee of the U.S. Public Health Service (grant No. 5 K05 MH-14918-02). This work was supported by the National Institutes of Health (grant No. MH-02717-10) and the National Science Foundation (grant No. GB 5941).

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Received October 14th, 1968

Biochim. Biophys. Acta, 171 (1969) 378–380