The Tyrosine Hydroxylase Activity of Mammalian Tyrosinase*

(Received for publication, June 16, 1965)

SEYMOUR H. POMERANTZ
From the Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201

SUMMARY

The tyrosine hydroxylase activity of tyrosinase from hamster melanoma has been studied by measurement of the release of tritium from tyrosine-3,5-3H. Experiments with mixtures of tyrosine-3,5-3H and tyrosine-4C show that there is little or no tritium rate effect in this reaction. Dopa is the most effective hydrogen donor and will eliminate the lag period when used in catalytic amounts. 2-Amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine eliminates the lag when it is at a concentration of $2.4 \times 10^{-4}$ M and shortens the lag at lower concentrations. Substrate quantities of ascorbate, reduced tri- and diphosphopyridine nucleotide, 3,4-dihydroxyphenylethylamine, epinephrine, and norepinephrine do not abolish the lag. 3,4-Dihydroxyphenylalanine at high concentration is an inhibitor of tyrosine hydroxylation, while tyrosine acts as a substrate inhibitor at concentrations above $8 \times 10^{-4}$ M. The substrate inhibition can be partially reversed by increasing the concentration of 3,4-dihydroxyphenylalanine from the catalytic level to $8 \times 10^{-4}$ M. Ascorbate magnifies the inhibition by 3,4-dihydroxyphenylalanine. Inhibition by cyanide, diethylidithiocarbamate, 5-hydroxyindole, and 5-hydroxy-DL-tryptophan is also reported.

The hydroxylation of tyrosine to dopa is the initial reaction in the biosynthesis of melanin catalyzed by tyrosinases, as shown in Equation 1. $AH_{2}$ represents an electron donor.

$$\text{L-tyrosine} \rightarrow \frac{O_2}{AH_2} \rightarrow \text{L-dopa} \rightarrow \text{dopachrome} \rightarrow \rightarrow \text{melanin}$$  (1)

The hydroxylation step has been difficult to study because of the absence of an adequate assay. The studies are further complicated by the occurrence of an induction period when this reaction is measured manometrically or colorimetrically (by dopachrome formation). Dopa formation has also been used (1) to assay hydroxylation activity, but this method requires a high concentration of ascorbate or other reducing agent to convert dopachrome rapidly to dopa nonenzymatically. Large quantities of ascorbate have been reported to be effective in eliminating the lag entirely with some tyrosinases (1-3), but not with mammalian enzyme (4). Some success in assaying hydroxylation has been reported by measuring tyrosine disappearance (2, 3) but this method is obviously unreliable at very early stages of the reaction. Since the enzyme is a mixed function oxidase (5), it is likely that the lag may sometimes be caused by the absence of a suitable hydrogen donor. Even the presence of a catalytic amount of dopa, the best reducing agent, only shortens the lag when the hydroxylation is measured manometrically (6) because these methods measure tyrosine hydroxylation indirectly. When large quantities of dopa are used, the results are confusing because dopa is oxidized by the enzyme. The systematic study of other compounds that may shorten the lag has also been hampered.

In a preliminary report (4) it was shown that L-tyrosine-3,5-3H may be used to study the initial step by measuring the rate of formation of tritiated water. This permits, for the first time, a sensitive assay of the reaction shown in Scheme 1, and a means of studying the cofactor requirements. Nagatsu, Levitt, and Udenfriend (7) independently used this method to study the tyrosine hydroxylase of beef adrenal medulla, an enzyme which does not oxidize dopa.

This paper reports the use of the new method to study several aspects of tyrosine hydroxylation catalyzed by mammalian tyrosinase, in particular the action of dopa and other hydrogen donors in eliminating the lag; the action of dopa, tyrosine, and several other compounds as inhibitors; and the extent of a possible tritium isotope effect.

EXPERIMENTAL PROCEDURE

Materials—The enzyme used was purified about 250-fold from hamster melanoma and was the fraction designated Enzyme I in Reference 6. L-Tyrosine-3,5-3H and L-tyrosine-4C (uniformly labeled) were purchased from New England Nuclear. The tritiated tyrosine was purified by high voltage paper electrophoresis at pH 1.9 (8) and stored at $-20^\circ$. The tritium in this compound exchanges slowly with water, and so samples were evaporated to dryness shortly before use. Norit A was purchased from Pfanzelth, chromatographic grade aluminum oxide from Merck, and 2-amino-4-hydroxy-6,7-dimethylpteridine from Aldrich Chemical. This compound was reduced to the 5,6,7,8-tetrahydropteridine with $H_2$ and PtO$_2$ (9); the tetrahydropteridine was purchased from Calbiochem. In each case the preparation was dissolved in 0.001 N HCl and assayed for

\* This investigation was supported in part by Research Grant CA-07093 from the National Cancer Institute, National Institutes of Health.

\* The abbreviations used are dopa, 3,4-dihydroxyphenylalanine; dopamine, 3,4-dihydroxyphenylethylamine; dopachrome, 2,3-dihydroindole-3,6-quinone-2-carboxylic acid.
L-ascorbate were obtained from Nutritional Biochemicals. L-Dopa, DPNH, TPNH, dopamine, dl-epinephrine, and dl-norepinephrine were purchased from Sigma; L-tyrosine and L-ascorbate were obtained from Nutritional Biochemicals.

**Standard Reaction Mixture**—The standard reaction mixture contained the following for each 1.25-ml volume: L-tyrosine-3, 5-3H (5 to 20 × 10^6 dpm) or L-tyrosine-14C (414,000 dpm), 1.0 μ mole; L-dopa, 0.05 to 0.15 μ mole; sodium phosphate buffer, pH 6.8, 35 μ moles; and enzyme, 1 to 7 units (6). The incubation temperature was 37°, and the reactions were stopped with metaphosphoric acid (final concentration, 1.5%). In experiments designed to measure dopa-3H or dopa-14C, neutralized ascorbate (14.8 μ moles) was included.

To measure 3HOH, the acidified reaction mixture was passed onto a column 1.0 cm in diameter packed with a mixture of 100 mg of Norit A and 500 mg of Celite 545. The column was washed with water to make an effluent volume of 25 ml. An aliquot was dissolved in 20 ml of dioxane scintillator fluid (11) and counted. The counts obtained in boiled enzyme controls, washed with water to make an effluent volume of 25 ml, and n-butyl alcohol-acetic acid-water (80 : 20 : 20). The purified compound was identified as dopa by coincidence of counts with dopa chromatograms derived from experiments with mushroom tyrosinase.

The known recovery was then used to calculate the amount of radioactive dopa synthesized. It was assumed that dopa-3H had one-half the specific activity of tyrosine-3, 5-3H.

When it was necessary to count recovered tyrosine, the amount of charcoal which passed from the column did not reduce the counting efficiency.

When dopa-14C or dopa-3H was measured, 2 μ moles of dopa were added to the acidified reaction mixture, which was mixed with 2 ml of 0.5 M ammonium acetate, pH 6.1, and then passed through a column 1 cm in diameter containing 2 g of aluminum oxide suspended in 0.5 M ammonium acetate, pH 6.1. Tyrosine was washed through with the same buffer, and dopa was eluted with 0.5 N acetic acid. The dopa solution was acidified with dilute HCl, evaporated under reduced pressure at a bath temperature of 37°, and purified free of traces of tyrosine by chromatography on aluminum oxide.2 The radioactive compound was identified as dopa by coincidence of counts with dopa on high voltage paper electrophoresis at pH 1.9 (8) and by paper chromatography in tert-butyl alcohol-formic acid-water (70: 15: 15), methylethyl ketone-propionic acid-water (60: 20: 24), and n-butyl alcohol-acetic acid-water (80: 20: 20). The purified dopa solution was assayed colorimetrically (13) and counted.

When dopa solutions are repeatedly evaporated in this manner, considerable decomposition occurs unless the solutions contain HCl. Chromatography and electrophoresis of the materials obtained as a result of one or more evaporations in the absence of HCl show the presence of at least one major contaminant in addition to a smear of radioactivity. This might account for the additional spot observed by Lititsky and Rolland (12) on paper chromatograms derived from experiments with mushroom tyrosinase, tyrosine, and excess ascorbic acid.

When it was necessary to count recovered tyrosine, the amount of charcoal which passed from the column did not reduce the counting efficiency.

Counting Methods—All counting was done in polyethylene vials in a Packard model 314A spectrometer. With the exception of the double labeling experiments, standard 3HOH was added to duplicate vials of about 20% of the 3HOH samples from each experiment. The individual efficiencies were always in good agreement, and an average efficiency was calculated for all 3HOH samples of each experiment. To determine 14C counting efficiencies, L-tyrosine-14C of known activity was added to duplicate vials of each sample; and in a similar way standard 3HOH was used to determine the efficiencies of counting dopa-3H and tyrosine-3, 5-3H. Tritium was counted at efficiencies of 8 to 14% for samples with 1 ml of water; the efficiency of counting 14C was about 40%,.

In the double labeling experiments all samples (except 3HOH) were made in triplicate; 14C standard was added to one and standard 3HOH to another. All samples were counted in turn at the optimum voltages for tritium and for 14C, and appropriate corrections were made for efficiency, background, and contribution of each isotope to the count rate of the other. The counting efficiency for each 3HOH sample was determined.

### RESULTS

**Stoichiometry** Two products of the hydroxylation, dopa and water, were measured. In one experiment, shown in Fig. 1, the reaction was performed either with or without added ascorbate, and the 3HOH produced was assayed. The graph shows that large quantities of ascorbate do not influence the reaction rate in the presence of a catalytic quantity of dopa. No reaction occurs in this time period in the absence of both dopa and ascorbate. These experiments were carried out without preincubation of ascorbate and enzyme and were stopped within 10 min. Later experiments showed that the reaction rate in the presence of ascorbate dropped off on longer incubations. Further experiments with tyrosine and ascorbate are reported in a later section.

The graph shows that dopa-5-3H and 3HOH are synthesized at, nearly equal rates when excess ascorbate is used to prevent further utilization of dopa. The position of the dashed line indicates that dopa is apparently made at a slightly greater rate than water. This is to be expected if some tritium in the tyrosine is at positions other than 3 and 5, thus leading to a dopa with specific activity greater than one-half that of tyrosine.

**pH Optimum**—The pH optimum for the reaction, determined with phosphate buffers, was found as a low plateau over the pH range of 7.1 to 7.7. This is somewhat higher than the pH range of 6.2 to 7.4 found for dopa oxidation.

**Tritium Isotope Effect**—Since a carbon-hydrogen bond is broken in this reaction, the investigation of a tritium isotope effect is relevant to the mechanism of hydroxylation. If cleavage of the carbon-hydrogen bond is rate-limiting, one would expect a reduction in the rate of reaction of tyrosine-3, 5-3H as compared with that of the normal amino acid. This would lead to a decrease in the tritium content of dopa and an enrichment of monium acetate fractions were evaporated to dryness under a heat lamp or at 60° under reduced pressure. The samples were adjusted to a known volume, and aliquots were counted.
tritium in tyrosine. The results of experiments utilizing mixtures of tyrosine-3,5-3H and tyrosine-14C as substrate are reported in Table I. If there were no tritium isotope effect and all the tritium in the tyrosine were in the 3 and 5 positions, (1) the 3H:14C ratio in the dopa product would be one-half of that in the tyrosine substrate; (2) the amount of dopa synthesized as calculated from tritium content would equal the amount as estimated from 14C content; (3) the ratio of tritium in the dopa to that in the water would be 1.0; and (4) the 3H:14C ratio in the tyrosine would remain unchanged. The results shown in Table I support the conclusion that there is little or no tritium rate effect and that only a small fraction of tritium in the tyrosine could be in positions other than 3 and 5. The 3H:14C ratio for dopa in Experiment 2 is much higher than the calculated value. This is probably caused by an error in the 14C count because the calculation of the amount of dopa synthesized with the use of 14C data (0.29 μmole) diverges considerably from the value calculated from tritium content (0.38 μmole) and the amount of water synthesized (0.35 μmole). It is pertinent that Wood and Ingraham (14) reported a small isotope rate effect in the mushroom tyrosinase-catalyzed hydroxylation of 3,4-dimethylphenol-2,6-3H.

Dopa as Hydrogen Donor—The effectiveness of dopa as a hydrogen donor is demonstrated by the curves in Fig. 3. Dopa at 1.2 × 10⁻⁴ M completely eliminates the lag period, while extended induction periods occur in the absence of any hydrogen donor. With another enzyme preparation only 4 × 10⁻⁴ M dopa was required to abolish the lag.

Tetrahydropteridine, Ascorbate, TPNH, DPNH, Epinephrine, Norepinephrine, and Dopamine as Hydrogen Donors—The curves in Fig. 4 illustrate the ability of 2-amino-4-hydroxy-6,7-

### Table I

**Estimation of extent of tritium isotope effect with double labeling technique**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tyr 14C</th>
<th>Tyr 3H</th>
<th>Dopa synthesized</th>
<th>3H:W ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tyr 14C</td>
<td>Tyr 3H</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyr 14C</td>
<td>Tyr 3H</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyr 14C</td>
<td>Tyr 3H</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table I provides a summary of the experiments conducted to estimate the extent of tritium isotope effect with double labeling technique. The table includes the ratio of 3H to 14C in the dopa synthesized, the ratio of 3H to W in the water, and the ratio of 3H to dopa. The asterisk (*) indicates ratios calculated from average 3H:14C ratios of tyrosine samples. The dagger (†) indicates calculated from tritium data assuming loss of 1 tritium atom per mole.

* Ratios in parentheses calculated from average 3H:14C ratios of tyrosine samples.
† Calculated from tritium data assuming loss of 1 tritium atom per mole.
‡ Calculated from the micromoles of 3H-dopa and 3H-W.
dimethyl-5,6,7,8-tetrahydropteridine to remove the lag when used at the relatively high concentration of $2.4 \times 10^{-3}$ M and to shorten it at lower concentrations.

Fig. 5 shows that while low concentrations of ascorbate shorten the lag, the high concentration of $1.2 \times 10^{-2}$ M does not eliminate it entirely. The activities of TPNH and DPNH are given by the curves of Fig. 6. Although substrate levels of either of these compounds do not eliminate the lag, TPNH is more effective then DPNH in shortening it.

The ability of dopamine, nL-epinephrine, and nL-norepinephrine to shorten, but not eliminate, the induction period is shown by the curves of Fig. 7. Dopamine is the most effective com-
FIG. 7. The ability of epinephrine, norepinephrine, and dopamine to shorten the lag in tyrosine hydroxylation as compared with dopa. Standard reaction mixtures employing 0.71 unit of enzyme were used. Curve A, dopa at $1.2 \times 10^{-4}$ M; Curve B, dopamine at $8.43 \times 10^{-4}$ M; Curve C, dl-epinephrine at $8.75 \times 10^{-4}$ M; Curve D, dl-norepinephrine at $7.8 \times 10^{-4}$ M; TOH, SHOH.

FIG. 8. Reciprocal plots showing inhibition of tyrosine hydroxylation by dopa at the presence and absence of high concentrations of ascorbate. Standard reaction mixtures were used, with the tyrosine concentrations varied as noted on the abscissa, and the following modifications: Curve A, the uninhibited reaction with dopa at $1.2 \times 10^{-4}$ M (catalytic amount) in the presence or absence of ascorbate at $1.2 \times 10^{-3}$ M; Curve B, inhibition by dopa at $2.4 \times 10^{-3}$ M; Curve C, the same as Curve B except for the addition of ascorbate at $1.2 \times 10^{-3}$ M. Each point represents a reaction rate as determined by averaging the rates for three time intervals.

FIG. 9. Partial reversal of substrate inhibition by tyrosine by increasing the concentration of dopa. Standard assay conditions were employed except that the tyrosine was at $2.6 \times 10^{-3}$ M and the concentration of dopa was varied as follows: Curve A, $1.2 \times 10^{-4}$ M (catalytic amount); Curve B, $4 \times 10^{-3}$ M; Curve C, $8 \times 10^{-3}$ M; TOH, SHOH.

Fig. 8 shows that dopa at $2.4 \times 10^{-3}$ M is a competitive inhibitor of tyrosine hydroxylation with a $K_i$ of $4 \times 10^{-3}$ M. The figure also indicates that ascorbate at high levels ($1.2 \times 10^{-2}$ M), while not inhibitory itself under these conditions, increases the inhibition by dopa by reducing its $K_i$ to $2 \times 10^{-4}$ M. The increased inhibition could not be explained by the fact that dopa accumulates in the presence of ascorbate because the results are based on initial rates of hydroxylation.

Figs. 8, 10, and 11 reveal that tyrosine is a substrate inhibitor at concentrations of $8 \times 10^{-4}$ M and higher and has an apparent $K_i$ of $3 \times 10^{-4}$ M. The data of Fig. 9 show that it is possible to reverse partially the substrate inhibition by tyrosine (at $2.6 \times 10^{-3}$ M) by increasing the concentration of dopa from the catalytic level to about $8 \times 10^{-4}$ M. The alternate possibility, that a contaminant in L-tyrosine was the cause of the inhibition, was practically eliminated by the observation that dl-tyrosine at twice the concentration of L-tyrosine exhibited the same level of inhibition. It was also found that preincubation of enzyme with ascorbate at high concentration ($5.9 \times 10^{-3}$ M) led to an inhibition of hydroxylation ($K_i = 8.1 \times 10^{-3}$ M) and also to an elimination of substrate inhibition.

Inhibition by Cyanide, Diethyldithiocarbamate, 5-Hydroxyindole, and 5-Hydroxy-DL-tryptophan—Levels of inhibition by these compounds are reported in Table II. Generally they are not as effective against tyrosine hydroxylation as against dopa oxidation (6). It is interesting that diethyldithiocarbamate, which inhibits dopa oxidation very well with no preincubation with

\(^3\) The report (16) by the National Academy of Sciences shows that a likely impurity of L-tyrosine (prepared from casein or hair) is L-cystine, while dl-tyrosine is a synthetic substance not likely to contain cystine as an impurity. Sulfur compounds are generally quite inhibitory to tyrosinase.
Inhibition by cyanide, diethyldithiocarbamate, 5-hydroxy-DL-tryptophan, and 5-hydroxyindole

Standard reaction mixtures were employed with a dopa concentration of 1.2 × 10⁻⁴ M. In each case initial rates were determined from three time intervals. Where indicated, preincubation experiments were performed by incubating enzyme with diethyldithiocarbamate for 30 min before addition of tyrosine and dopa.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration</th>
<th>Preincubation</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanide</td>
<td>5.3 × 10⁻⁴ M</td>
<td>-</td>
<td>13%</td>
</tr>
<tr>
<td>Diethyldithiocarbamate</td>
<td>1.2 × 10⁻⁴ M</td>
<td>-</td>
<td>34%</td>
</tr>
<tr>
<td>5-Hydroxy-DL-tryptophan</td>
<td>1.2 × 10⁻⁴ M</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>5-Hydroxyindole</td>
<td>1.5 × 10⁻⁴ M</td>
<td>+</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>2 × 10⁻⁴ M</td>
<td>-</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>3 × 10⁻⁴ M</td>
<td>-</td>
<td>48%</td>
</tr>
</tbody>
</table>

The kinetics of inhibition by diethyldithiocarbamate and cyanide is given in Fig. 10. They both act as noncompetitive inhibitors of tyrosine hydroxylation with $K_i$ values of 2.0 × 10⁻⁴ M and 1.8 × 10⁻⁴ M, respectively. Diethyldithiocarbamate is also a noncompetitive inhibitor of dopa oxidation with a smaller $K_i$ of 8 × 10⁻⁵ M (6). Fig. 11 shows that 5-hydroxyindole, a noncompetitive inhibitor of dopa oxidation with a $K_i$ of 2 × 10⁻⁵ M (6), is a competitive inhibitor of tyrosine hydroxylation with the similar $K_i$ of 3 × 10⁻⁴ M. The kinetics of inhibition by 5-hydroxy-DL-tryptophan was also studied, but the inhibition is complex, and a straight line was not obtained in a double reciprocal plot.

**DISCUSSION**

Although the errors inherent in the method preclude an unequivocal statement at this time, there appears to be little or no tritium rate effect during the hydroxylation of tyrosine catalyzed by mammalian tyrosinase. The conclusion of Wood and Ingraham (14) was that the mushroom tyrosinase-catalyzed hydroxylation of 3,4-dimethylphenol-2,6⁻³H proceeds with a small, but real, tritium rate effect. Their calculation was based on a knowledge of the extent of the reaction and the increase in specific activity of the recovered substrate. They concluded further that the rate-determining step was one of proton removal and that the isotope rate effect was small because removal of the proton was facilitated by a base group on the enzyme.

The ratio of tritium in dopa to that in water was always greater than 1.0 (Table I, last column). These data and the data in Fig. 2 indicate that about 3 to 9% of the tritium in the tyrosine is in a position other than 3 and 5. The tyrosine-3,5⁻³H used in these experiments was made by an exchange reaction with tritiated water. Nagatsu et al. (7), with the use of L-tyrosine-3,5⁻³H prepared by catalytic reduction of 3,5-diido-L-tyrosine with tritium gas, found that between 10 and 14% of the tritium in their preparation was bound in positions other than the de-
sired ones. From their work it appears that there is also little or no tritium rate effect in the tyrosine hydroxylation catalyzed by tyrosine hydroxylase.

These results clearly establish dopa as a competitive inhibitor of tyrosine hydroxylation. Kim and Tchen (17) reached this conclusion from work with mushroom tyrosinase and the observation of a lag in the disappearance of tyrosine-\(^{14}C\) in the presence of high concentrations of dopa. Kendal (1) had earlier come to the opposite conclusion with the mushroom enzyme. However, his statement was based on experiments in which initial rates of tyrosine hydroxylation in the presence of dopa were not obtained; dopa was merely allowed to accumulate during the reaction and thereby affect the rate. In addition the true reaction rates were obscured because of enzymatic inactivation during the reaction.

The relationship between tyrosine-binding and dopa-binding by the enzyme appears complex at present. The facts which bear on this may be summarized as follows. Tyrosine is a competitive inhibitor of the hydroxylation but is a noncompetitive inhibitor of dopa oxidation and also exhibits substrate inhibition. The substrate inhibition is removed by preincubation of the enzyme with high concentrations of ascorbate. Under these conditions ascorbate is a weak inhibitor of hydroxylation. The substrate inhibition may also be partially reversed by increasing the level of dopa. Dopa at even higher concentrations is a competitive inhibitor of the hydroxylation reaction. This inhibition is magnified by ascorbate at high concentration, leading to a lower \(K_i\) for dopa. 5-Hydroxyindole is a competitive inhibitor of the hydroxylation but is a noncompetitive inhibitor of dopa oxidation. Diethyldithiocarbamate has a smaller \(K_i\) for dopa oxidation than for tyrosine hydroxylation, and, furthermore, a preincubation of diethyldithiocarbamate and enzyme is required for immediate inhibition of tyrosine hydroxylation. Some of the findings can be accommodated by the hypothesis that tyrosinase contains separate, but closely related, combining sites for dopa and tyrosine. Excess tyrosine could combine at the dopa site, as was suggested earlier (18), thus preventing dopa from acting as a hydrogen donor. This would account for substrate inhibition by tyrosine and for the partial reversal of this effect by higher concentrations of dopa. Excess dopa could combine with the tyrosine site and lead to inhibition of the hydroxylation reaction.

The data on inhibition by 5-hydroxyindole and diethyldithiocarbamate and the information on the effects of ascorbate are too complicated to be explained by our present knowledge and with a relatively crude enzyme. Since 1 molecule of dopa must be oxidized concomitantly with the hydroxylation of 1 molecule of tyrosine, it is difficult to understand the ability of these compounds to influence tyrosine hydroxylation and dopa oxidation in different ways.

It is possible that diethyldithiocarbamate might be employed to inhibit dopa oxidation differentially at levels which are too low to inhibit tyrosine hydroxylation initially. This might be an additional method of separating the hydroxylation reaction from further reactions on dopa.

Although the \(V_{max}\) for tyrosine can be estimated from the kinetic curves in Figs. 8, 10, and 11 as about 0.01 \(\mu\)mole per min per unit of enzyme, it is not possible to achieve these rates because of substrate inhibition above \(8 \times 10^{-4}\) M. Previous work (6) established the \(V_{max}\) for dopa oxidation (at 35°) as 0.13 \(\mu\)mole per min per unit of enzyme. Tyrosine hydroxylation is the rate-limiting reaction in the formation of pigment, but it is not certain what physiological role, if any, is played by tyrosine inhibition.

Several other hydroxylating enzymes, phenylalanine hydroxylase from animal (10, 19) and bacterial sources (20), adrenal tyrosine hydroxylase (21, 22) and tryptophan hydroxylase from rabbit brain (23), have all been shown to utilize tetrahydropteridines as cofactors, whereas a tetrahydropteridine is relatively ineffective with this enzyme.

Recently two tyrosinases from mouse melanoma have been purified to homogeneity and one has been crystallized (24), but no information is available yet about their properties.

A few comparisons can usefully be made between mammalian tyrosinase and the adrenal tyrosine hydroxylase (21, 22). The \(K_m\) value for tyrosine with mammalian tyrosinase is about \(3 \times 10^{-4}\) M by the tritium method (compared to \(6 \times 10^{-4}\) M found by dopachrome measurement (6)), while the \(K_m\) for tyrosine hydroxylase is about \(10^{-5}\) M (21). L-Dopa is an inhibitor of both enzymes (21), but it acts as a cofactor only in the tyrosinase reaction. Tetrahydropteridines are cofactors for tyrosine hydroxylase (21, 22) whereas one such compound is completely active only at high concentration with tyrosinase. It is quite possible that, with any cofactor, dopa is always the effective agent in the tyrosinase reaction once the reaction has started and some critical amount of dopa has been synthesized. Diethyldithiocarbamate is an effective copper-binding agent, and an inhibitor of tyrosinase, but it does not inhibit tyrosine hydroxylase (21). On the other hand, \(\alpha,\alpha\)-dipyridyl, an iron chelating agent, inhibits tyrosine hydroxylase (21) but does not inhibit the dopa oxidase activity of tyrosinase. These and other data on metal requirements of mammalian tyrosinase (6, 25) lead to the tentative conclusion that the enzymes also differ in metal component.

Acknowledgment—I wish to thank Mrs. Margaret Warner for expert technical assistance.

REFERENCES


\(^{4}\) Table X of Reference 21 summarizes information about mammalian tyrosinase derived from Reference 6 and states that dopa is not an inhibitor of mammalian tyrosinase. This is, however, incorrect. No direct information on dopa as an inhibitor of tyrosine hydroxylation by tyrosinase was shown in Reference 6, and information in the present report and in Reference 4 shows dopa to be an inhibitor of tyrosine hydroxylation.

\(^{5}\) S. H. Pomerantz, unpublished experiments.
The Tyrosine Hydroxylase Activity of Mammalian Tyrosinase
Seymour H. Pomerantz


Access the most updated version of this article at http://www.jbc.org/content/241/1/161

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/241/1/161.full.html#ref-list-1