Evidence of the Indirect Formation of the Catecholic Intermediate Substrate Responsible for the Autoactivation Kinetics of Tyrosinase*

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Tyrosinase (EC 1.14.18.1) exhibits unusual kinetic properties in the oxidation of monohydric phenol substrates consisting of a lag period that increases with increasing substrate concentration. The cause of this is an autocatalytic process dependent on the generation of a dihydric phenol substrate, which acts as an activator of the enzyme. Experiments with N-substituted dihydric phenol substrates (N-methyldopamine, N-acetyldopamine) demonstrate that oxygen consumption is retarded in the N-acetyl substituted material due to a diminished rate of cyclization. The oxygen uptake exhibited a similar pattern when N-acetyltyramine was oxidized, and this was reflected by a prolongation of the lag period. N,N-Dipropyltyramine was oxidized with normal kinetics but with an oxygen stoichiometry of 0.5 mol of oxygen/mol of substrate. We show that this is the result of the formation of a stable indoliumolate product with the formation of dopaminochrome, thus blocking further stages in the tyrosinase-catalyzed oxidation.

Evidence that the indoliumolate product is formed by cyclization of the ortho-quinone is presented by pulse radiolysis studies, which demonstrate the formation of the ortho-quinone (by disproportionation of the corresponding semiquinones), which cyclizes to give the indoliumolate. The rate constant for cyclization was shown to be 48 s⁻¹ (at pH 6.0).

Tyrosinase-catalyzed oxidation of the monohydric phenol analogue, N,N-dimethyltyramine, was shown to require the addition of a dihydric phenol. Oxygen utilization then exhibited a stoichiometry of 1.0, indicating that the reactions proceed only as far as the cyclization. The analogous stable cyclic indoliumolate product was shown to be formed, with UV absorption and NMR spectra closely similar to the indoliumolate derived from N,N-dipropyltyramine. This material was methylated by catechol O-methyltransferase but was unreactive to reduct reagents. The formation of the cyclic product accounts for the indefinite lag when N,N-dimethyltyra-

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Tyrosinase (EC 1.14.18.1) is an enzyme widely distributed in nature that catalyzes the oxidation of monohydric phenols (such as tyrosine). It exhibits unusual kinetic properties. Its natural substrate is considered to be tyrosine, yet it exhibits an induction period or a lag phase in the oxidation of this substrate (1). The lag phase is explained by an autocatalytic mechanism that depends on the elaboration of dihydroxyphenylalana-

† The abbreviations used are: DOPA, 3,4-dihydroxyphenylalanine; DQ, dopaquinone; DPDA, N,N-dipropyl dopamine; NMDA, N-acetyl dopamine; DMTA, N,N-dimethyltyramine; COMT, catechol O-methyltransferase; HPLC, high pressure liquid chromatography; Gy, gray.
to prevent the autocatalytic activation of tyrosinase by inhibiting or modifying the intramolecular ring formation of dopaquinone. There are several possible approaches to this including the following.

(a) Acidification of the microenvironment of the reactants will protonate the side chain amino group and hinder intramolecular reductive addition. We have previously demonstrated that the autocatalytic activation of tyrosinase is progressively abolished by acidification (4). The importance of acidification of the milieu interieur of the melanosome in the control of melanogenesis has been recognized but misinterpreted by most commentators (6). However, acidification also inhibits the rate of oxidation by tyrosinase and is thus unsatisfactory.

(b) Inhibition of cyclization by exclusion of reactive nucleophilic groups from the reaction system should be readily achieved by the choice of analogue substrates that lack appropriately placed side chain functionalities, but the presence of nucleophilic groups on the enzyme means that reductive addition to the quinone by external nucleophiles can still occur.

(c) Inhibition of cyclization by ring substitution has been found unsatisfactory because ring substitution alters the ease of oxidation, as in the case of substitution of the ring hydrogens by fluorine, or it prevents access to the active site of the enzyme, as in the case of ring methylation (7).

(d) Consideration of the process of intramolecular cyclization of DOPA suggests that substitution of the hydrogen atoms on the amino group will interfere with the hydrogen redistribution following the lone pair electronic attack on the quinone ring. This paper reports the results of experiments with N-substituted tyrosinase substrates.

**Experimental Procedures**

**Chemicals and Reagents**

N-2-(4-Hydroxyphenyl)ethyl-N,N,N-trimethylammonium Bromide (N,N,N-Trimethyltyramine)—Tyramine hydrochloride (Sigma, 1.0 g, 5.76 mmol) was suspended in methanol (10 cm$^3$), and aqueous ammonia (5.4 cm$^3$) was added dropwise until the solid dissolved. Bromomethane (4 cm$^3$) was added, and the solution was allowed to evaporate over 1 week. Large pale brown crystals (778 mg, 52% yield) were obtained (melting point of 257.6 °C, $^1$H NMR (Me$_2$SO-d$_6$, 400 MHz) 9.36 s (1H), 7.09 d (8.4 Hz, 2H), 6.73 d (8.4 Hz, 2H), 3.46 cm (2H), 3.11 s (9H), 2.91 m (2H)).

N-2-(4-Hydroxyphenyl)propyl-N,N,N-trimethylammonium Bromide—This compound was prepared from 4-(3-bromopropyl)phenol (8) in the following way. 4-(3-Bromopropyl)phenol (310 mg) in chloroform (2 cm$^3$) was added to trimethylamine (2 cm$^3$). After 24 h, the mixture was diluted with chloroform (5 cm$^3$), and the product was filtered to give a white solid (386 mg) in quantitative yield that was characterized by $^1$H NMR (D$_2$O, 400 MHz), 7.66dd (8.4 Hz, 2H), 7.35dd (8.4 Hz, 2H), 3.58dd (2H), 3.54 s (9H), 3.12t (7.2 Hz, 2H), 2.54s (2H).

N,N-Dipropydopamine Hydrobromide (DPDA)—DPDA was obtained from Research Biochemicals International (Natick, MA). All other substrates were from Sigma, and other reagents were of analytical grade.

Tyrosinase from *Agoricus bisporus* (3400 units/mg) was obtained from Sigma.

**Combined Oximetry and Spectrophotometry**

Tyrosinase was dissolved in 0.1 M phosphate buffer (pH 7.4) and frozen in aliquots of 5 ml. Aliquots were warmed to room temperature before use. Substrates were made up freshly in phosphate buffer; normally 2 mM stock solutions were used, from which small volumes (10–200 μl) were added to the reaction chamber.

Spectrophotometry was carried out using a Hewlett-Packard diode array spectrometer (model 8452A). A cuvette holder was constructed to position a modified silica glass cuvette in the light path so that the beam passed through the solution just above the tip of a Clark-type oxygen electrode inserted through a 8-mm orifice cut in the side of the cuvette 1 cm from the bottom of the chamber. The electrode was connected to an oximeter (Yellow Springs Instruments Co., model 5300) with an analogue output to a chart recorder. The solution was stirred with a small magnetic stirrer bar inserted into a slightly ribbed 8-mm diameter polypropylene "flying saucer" (Lendenz Apparatus). The oximeter was calibrated with air (100%) and 1% sodium dithionite (0%), and the voltage-independence of the electrode current was checked before each set of assays. All assays were conducted at room temperature (approximately 24 °C).

Oxygen levels were calculated with a temperature correction from the tabulated oxygen partial pressure in 0.1 M buffer (9). The spectral data were recorded using the Hewlett-Packard single cell kinetics program from which kinetic data were calculated for absorption at specified wavelengths. The tabulated values together with the oxygen utilization data derived from the oximeter recording were used to derive relative rates of change and stoichiometric ratios. The stoichiometry of oxygen utilization was expressed as the ratio of mol of oxygen consumed (100% O$_2$ = 820 mmol) to mol of substrate calculated from the concentration and volume added.

**Pulse Radiolysis**

The pulse radiolysis experiments were performed with a 9–12-meV Vickers linear accelerator, using 50–200-ns pulses with doses up to 30 Gy and quartz capillary cells of optical path 2.5 cm (10, 11). Absorbed doses were determined from the transient (SCN$^-$_ formation in air-saturated KSCN solutions (10 μm) using a G of 0.30 μs/Gy and e (500 nm) = 7100 M$^{-1}$ cm$^{-1}$ (12). Generation of the one-electron oxidizing species Br$_2^*$ or N$_3^*$ was achieved by irradiating nitrous oxide-saturated aqueous solutions of 100 mM KBr or 3 mM NaN$_3$. Under such conditions, Br$_2^*$ or N$_3^*$ radicals are formed within ~0.1 μs after the radiation pulse via Reactions 3–7.

**Tyrosinase Assay**

Substrates (1 mM) were dissolved in PBS (pH 7.4) and incubated with 14.9 units of mushroom tyrosinase in a water bath at 37 °C. Generation
of a quinone product was measured using Besthorn’s hydrazine (3-methyl-2-benzothiazolinone hydrazine), which forms a colored adduct with quinones. The extinction of the adduct was measured at 505 nm (15).

**Catechol O-Methyltransferase (COMT) Assay**

The assay of catechol O-methyltransferase activity was essentially as described by Smit et al. (14). HPLC separation used a Bondapak TM C18 column with a mobile phase of 50 mM sodium acetate/acetic acid (pH 4.7), 10 mM Na2S2O5, 1 mM EDTA, and 25% methanol, infused by following six successive steps.

![Diagram of DOPA oxidation kinetics](image)

**Fig. 1. DOPA oxidation kinetics.** The plot shows the oxygen uptake as a function of time during the oxidation of 360 nmol of DOPA by tyrosinase. Three kinetic phases are indicated: phase Ia, phase Ib, and phase II.

**RESULTS**

**Dihydric Phenol Oxidation by Tyrosinase**—The set of reactions involved in tyrosinase-catalyzed oxidation of natural dihydric phenolic substrates can be conveniently summarized in the following six successive steps.

- D + 0.5 O2 → Q + H2O (tyrosinase-catalyzed oxidation) (step 1)
- Q → CD (spontaneous reductive cyclization) (step 2)
- CD + Q → CQ + D (redox exchange) (step 3)
- D + 0.5 O2 → Q + H2O (tyrosinase-catalyzed oxidation) (step 4)
- CQ → DHI (spontaneous tautomerization) (step 5)
- DHI + 0.5 O2 → IQ + H2O (tyrosinase-catalyzed oxidation) (step 6)
- D + 1.5 O2 → IQ + 3H2O (overall)

where D represents dihydric phenol (e.g., DOPA); Q is the corresponding ortho-quinone (e.g., dopaquinone); CD is cyclic dihydric product (e.g., cyclodopa); CQ is cyclic quinone (e.g., dopachrome); DHI is dihydroxyindole derivative (e.g., 5,6-dihydroxyindole-2-carboxylic acid); and IQ is corresponding indole-quinone (e.g., indole-2-carboxylic acid-5,6-quinone).

We have examined the overall oxygen stoichiometry of DOPA and dopamine oxidation (Table I) and obtained values of 1.42 and 1.46, respectively, consistent with the theoretical ratio of 1.5. The rate-limiting reactions are likely to be the spontaneous steps (steps 2, 3, and 5). In the case of dopaquinone it is known that the rate of reaction (step 3, the redox generation of dopachrome) is exceptionally rapid, with a rate constant of >10^9 M^-1 s^-1 (5). Under neutral conditions the reductive cyclization of dopaquinone is also fast (k ~ 3–4 s^-1) (16) so that the initial four steps in DOPA oxidation generally proceed rapidly. Tautomerization of dopachrome in the absence of the enzyme dopachrome tautomerase is a slow reaction and constitutes the rate-determining step leading to indolequinone (and the subsequent reactions of melanogenesis). Examination of the oxygen utilization kinetics of DOPA oxidation (Fig. 1) shows that there is a rapid initial phase (phase Ia) of oxygen utilization with a stoichiometry of approximately 0.5 mol of oxygen/mol of substrate followed by a slower phase (phase Ib) accounting for a further 0.5 mol of oxygen/mol of substrate corresponding to steps 1 and 4. Since 5,6-dihydroxyindole oxidation in the presence of tyrosinase is rapid, the slow oxygen utilization rate (~5 nmol of O2/min in phase II) is a measure of the rate of spontaneous tautomerization (step 5) under the experimental conditions. The oxidation kinetics of dopamine show the same pattern (data not shown).

To investigate the conditions affecting the cyclization reaction, we examined phase I tyrosinase-catalyzed oxidation of three substituted dopamine analogues: N-methyl dopamine (NMDA), N-acetyldopamine (NADA) and N,N-dipropyl dopamine (DPDA). The comparative behavior of these substrates when oxidized by tyrosinase is illustrated in Fig. 2. These patterns were independent of the substrate concentration over the limited range possible with the apparatus used (maximum oxygen content was 820 nmol). The patterns of oxygen consumption are consistent with differences in the rates of the cyclization step (step 2). NMDA exhibited rapid phase I oxidation, whereas NADA exhibited a
fast oxygen utilization to a stoichiometric ratio of approximately 0.5 followed by a nearly linear subsequent oxidation to reach a stoichiometry of nearly 1.0 (Table I) after a 7.5-min incubation. Since step 4 is identical to step 1 (the tyrosinase-catalyzed oxidation of the dihydric phenol substrate), the slow rate of oxygen uptake is indicative of a slower rate of secondary substrate generation. DPDA was rapidly oxidized with a final oxygen stoichiometry of 0.495 ± 0.08 (Table I). This result indicates that steps 3 and 4 do not take place when DPDA is used as substrate. Simultaneous spectrophotometry showed the formation of a product with a maximum absorbance at 290 nm (Fig. 3) The molar extinction coefficient of the product, assuming complete conversion of the substrate to the product, was estimated as 4100 at 290 nm. The rate of product formation was double the rate of oxygen consumption (Fig. 3).

The product from DPDA was found to be stable over a period of 48 h at room temperature. The UV absorption spectrum was sensitive to pH. Titration with molar HCl diminished the absorbance in the shoulder regions (246 and 306 nm) (Fig. 4).

The enzyme-generated product was isolated by evaporation at 0.01 mm Hg to give a crystalline solid, which was extracted with methanol. Filtration and evaporation of the filtrate at 0.01 mm gave a pale brown solid. Column chromatography on Silica Gel 60 eluting with 5:1 chloroform/methanol and then with 20:4:1 chloroform/methanol/acetic acid gave two fractions that, when analyzed by TLC (aluminum foil-backed Silica Gel 60, visualized with iodine vapor), showed separation of a single product (Rf 0.19) from the starting material (R factor 0.35). Evaporation of the appropriate fractions gave colorless oils that solidified. The 1H NMR spectra were examined in CD3OD. Electron impact mass spectrometry at 70 eV of the fractions recovered from the NMR solvent showed small peaks at m/e 237, 238, and 239 for the starting material (C10H11NO2 = 239) and m/e 236 and 237 for the product (C10H11NO2 = 237).

The NMR spectrum of the product showed that it is a cyclic indolene. The aromatic ring protons were singlets at 6.94 and 6.80 ppm; the indole ring protons were coupled triplets at 4.13 and 3.18 ppm; and the propyl groups showed two coupled doublets (4.9 Hz) of quartets (12.5 Hz) at 3.67 and 3.51 ppm, 2H multiplets at 1.73 and 1.42 ppm, and a methyl group triplet (7.4 Hz) at 0.95 ppm. A COSY spectrum showed the expected proton coupling pattern.

These data are identical to those obtained from synthetically prepared material2 and are consistent with the indolium-5-olate structure shown in Fig. 5a. AM1 molecular orbital calculations indicate that this structure (Fig. 5a) is more stable than the isomeric indolium-6-olate (Fig. 5b). This result is in agreement with expectations, since for the indolium-5-olate a favorable resonance structure can be written that places the negative charge on a carbon atom adjacent to the positively charged nitrogen atom (Fig. 5a). At pH >7 we therefore expect the betaine to be present in solution as the indolium-5-olate isomer.

**Fig. 2. The kinetics of oxidation of NMDA, NADA, and DPDA by tyrosinase.** The plot shows the oxygen consumption as a function of time during the oxidation of 800 nmol of the N-substituted dopamine analogues.
radiolytically with that obtained enzymatically at physiological pH, the above experiment was repeated at pH 7.2. Based on a yield of N$_3^*$ radicals of 0.62 µm/Gy (20), the absolute spectrum of the DPDA-derived oxidation product, corrected for parent DPDA depletion, presented in Fig. 4, can be seen to be identical to that of the product found by enzymatic oxidation.

**Monohydric Phenol Oxidation by Tyrosinase**—The general set of reactions involved are the same as those set out for dihydric phenol substrates except that the first step (1a) involves the generation of the corresponding ortho-quinone from the monophenol and involves two atoms of oxygen: M + O$_2$ → Q + H$_2$O (tyrosine-catalyzed oxidation), where M represents monohydric phenol substrate (e.g. tyrosine).

This results in an overall oxygen stoichiometry for the six reactions leading to indolequinone of 2.0 mol of oxygen/mol of substrate. We examined the tyrosinase-catalyzed oxidation of tyrosine and tyramine and confirmed this stoichiometric ratio (Table I). In the case of monohydric phenol substrates, the kinetics of oxygen utilization showed a pronounced lag period but were in other respects similar to DOPA and dopamine, as were the corresponding spectral changes (data not shown).

We examined the oxidation of N-substituted monohydric phenols, including N-acetytyrosine, N,N-dimethyltyramine (DMTA), and N,N,N-trimethyltyramine. The phase I oxygen uptake rates are shown in Fig. 7. N,N,N-Trimethyltyramine showed the shortest lag and the most rapid phase I oxidation. This was expected, since the charged quaternary nitrogen terminus of the side chain prevents cyclization and, therefore, the substrate behaves like other noncyclizing compounds such as 4-hydroxyanisole, where attack on the ring by nucleophilic
groups on the enzyme accounts for the activation (4). N-Acetyltyrosine oxidation exhibited a pronounced lag period followed by rapid oxidation to a relative oxygen stoichiometry of approximately 1.0. Spectrophotometry showed coincident rates of formation of a (quinone) product with an absorption peak at 420 nm. This was succeeded by a slow oxidation, accounting for a further 0.5 mol of oxygen/mol of substrate. This observation is consistent with the results of tyrosinase-catalyzed oxidation of N-acetyltyrosine (NAT) and N,N,N-trimethyltyramine (TMTA) is shown. DMTA is not oxidized under these conditions.

FIG. 5. Resonance forms of indolium-5-olate (a) and indolium-6-olate (b). The calculated dipole moments of the N,N-dimethylindoliumolates (R represents methyl) were 13.2 Debyes in each case. The calculated ionization potentials were 6.87 and 6.71 eV, and the calculated heats of formation (ΔHf) were −7.54 and −4.03 kcal/mol for the a and b forms, respectively.

FIG. 6. Absorption changes at various times after pulse radiolysis of N2O-saturated aqueous solutions of 1.0 mM DPDA containing 100 mM KBr and 10 mM phosphate buffer, pH 6.0 (dose, 30 Gy). Inset, time profiles of transmission changes (vertically upwards displacements show increasing transmittance). From the profile at 295 nm, it can be seen that at this pH the semiquinone decay is fast compared with the ortho-quinone decay.

FIG. 7. Oxygen utilization kinetics of tyrosinase-catalyzed oxidation of 400 nmol of N,N-disubstituted monohydric phenol substrates with differing cyclization products. The oxygen uptake by tyrosinase-catalyzed oxidation of N-acetyltyrosine (NAT) and N,N,N-trimethyltyramine (TMTA) is shown. DMTA is not oxidized under these conditions.
oxidation rate this DOPA-stimulated oxidation of DMTA was independent of whether the DOPA was added before or after DMTA was introduced into the reaction vessel (Fig. 8). The oxygen stoichiometry of DMTA oxidation in the presence of trace amounts of DOPA was independent of the substrate concentration. The DMTA oxygen uptake was half that of tyramine (Fig. 9). Other dihydric phenol substrates, including DPDA, were also effective in activating tyrosinase oxidation of DMTA. Preincubation of tyrosinase with DPDA was able to initiate the subsequent oxidation of DMTA (Fig. 10).

The oxidation product of DMTA using dihydric phenol (DOPA)-activated tyrosinase gave no color with 3-methyl-2-benzothiazolinone hydrazone, which is consistent with the short lifetime of the quinone. The absorption spectrum of the DMTA oxidation product was indistinguishable from that of the indoliumolate product of DPDA oxidation and exhibited a closely similar extinction coefficient at 290 nm.

The indoliumolate, separated by HPLC, was insensitive to oxidation by silver nitrate in alkaline solution (using a 1:5 mixture of 0.1 M silver nitrate and 5 M ammonium hydroxide). The DMTA oxidation product was also unreactive to an excess of sodium thiosulfate or dithiothreitol. We conclude that the indoliumolate generated by tyrosinase oxidation of DMTA exhibits oxidation-reduction properties that differ significantly from those of catechols or ortho-quinones. Nevertheless, the indoliumolate was capable of methylation by COMT. Prolonged incubation led to the formation of a single major product with a retention time of 7.1 min with concurrent loss of the substrate (retention time of 4.8 min), suggesting that only one methylation product is formed (Fig. 11).
DISCUSSION

Our data demonstrate that dialkyl substitution of the side-chain amino group of \( N,N \)-dipropyl dopamine results, on oxidation by tyrosinase, in the formation of a stable product. Pulse radiolysis experiments show that a product with the same spectral absorption is rapidly formed from the corresponding ortho-quinone. The compound has a peak absorption at 290 nm with an estimated molar extinction coefficient of 4500 \( \pm 400 \, M^{-1} \, cm^{-1} \). The proton NMR spectrum is consistent with a cyclic product with a structure represented in Fig. 5. The cyclic product is not readily susceptible to oxidation or reduction, and there is no evidence that it reacts with the corresponding dopaminequinone. The failure to undergo redox exchange with \( N,N \)-dipropylindoliumolate, where \( \text{DPDQ} = \text{N,N-dipropyl dopaminequinone} \), is consistent with the observed oxygen stoichiometry of 0.5, which accounts for the following reaction:

\[
\text{DPDA} + 0.5 \, \text{O}_2 \rightarrow \text{DPDQ} \rightarrow N,N\text{-dipropylindoliumolate, where}
\]

\[
\text{DPDQ} = N,N\text{-dipropyl dopaminequinone}.
\]

This contrasts with the oxidation of DOPA or dopamine in which the kinetics are complicated by further oxidation reactions, which account for the observed oxygen stoichiometry. In these cases the rate-limiting steps are the cyclization of dopaquinone and the conversion of dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (or the corresponding decarboxylated derivatives), which account for the kinetics of oxygen utilization (Fig. 1). The spectral data are consistent with the initial rapid tyrosinase-catalyzed oxidation yielding dopaquinone and subsequently dopachrome as the major chromogens followed by a slower conversion to indolequinone.

Oxidation of tyrosine and tyramine were found to proceed with characteristic lag kinetics. The oxygen stoichiometry was 2.0, consistent with the reaction scheme outlined (Scheme 1). When \( N,N \)-dimethyl tyramine was used as substrate, no significant oxidation took place. However, if the enzyme was premixed with a trace amount of DOPA \((1/1000 \, \text{of the DMTA concentration})\) the substrate was oxidized with an oxygen stoichiometry of 1.0. The oxygen consumed by oxidation of the added DOPA was negligible. DOPA initiated the oxidation of DMTA when added either prior or subsequent to the monohydric phenol substrate. Dopamine and DPDA also acted as activators of DMTA oxidation by tyrosinase.

The stoichiometry data show that, once the enzyme is activated by a dihydric phenol substrate, DMTA is oxidized to the corresponding ortho-quinone, which, by analogy with DPDA, forms a stable cyclic product, DMTA + \( \text{O}_2 \rightarrow \text{Q} \rightarrow N,N\text{-dimethylindoliumolate, where} \text{Q represents} \, N,N\text{-dimethyl dopaminequinone.} \)

This product has an absorption spectrum closely similar to the DPDA cyclic product. It is separable by HPLC from a reaction system consisting of DMTA and activated tyrosinase as a single peak, and this material was shown to be monomethylated by COMT. These results indicate that the reactions involved terminate with the formation of the stable cyclic product (Scheme 2).

The failure of autoactivation of tyrosinase when DMTA is used as substrate is only explicable in terms of the theory that the dihydric phenol activator is formed indirectly by the disproportionation of the ortho-quinone, since this reaction does not occur in the case of the \( N,N \)-dialkyl-substituted substrates. If the dihydric phenol substrate were to be formed directly as the hydroxylation product of the monohydric substrate, the oxidation of DMTA would exhibit kinetic characteristics similar to tyrosine or tyramine oxidation. Thus, the failure of autocatalytic oxidation of DMTA constitutes proof that the activating dihydric phenol is not directly generated.

We conclude that tyrosinase does not possess two separable
activities, i.e. tyrosine hydroxylase and DOPA oxidase; the enzyme catalyzes the production of ortho-quinones from both monohydric and dihydric phenols. This is entirely consistent with the studies of Solomon and Lowery (22), which show that the oxidation product is released as the quinone from a catecholate coordination complex with the copper atoms at the active site of the enzyme. The unusual kinetic behavior of tyrosinase is due to its activation by dihydric phenol substrates. Our data show that these are indirectly formed in a manner essentially as proposed by Evans and Raper (23). The mode of action of dihydric phenol substrates could be by an allosteric mechanism (e.g. as proposed by Hearing et al. (24), but there are good mechanistic reasons for supposing that it is due to recruitment of met-enzyme by reduction of the copper atoms at the active site as shown by Lerch (25). We have previously published evidence to support this second mechanism of tyrosinase autocatalysis (4).

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