The Role of pH in the Melanin Biosynthesis Pathway*

(Received for publication, November 24, 1981, and in revised form, March 15, 1982)

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Having oxidized 3,4-dihydroxyphenylalanine (dopa) with sodium periodate or mushroom tyrosinase in a pH range from 3.5 to 6.0, it has been possible to detect spectrophotometrically 4-(2-carboxy-2-aminoethyl)-1,2-benzoquinone with the amino group protonated (o-dopaquinone-H+), a postulated intermediate in the melanogenesis pathway. When the pH value was greater than 4, the final product obtained was 2-carboxy-2,3-dihydroindole-5,6-quinone (dopachrome); however, for pH values lower than 4, two different products were identified by means of cyclic voltammetry: 5-(2-carboxy-2-aminoethyl)-2-hydroxy-1,4-benzoquinone and dopachrome. These products appeared when oxidation was achieved with the enzyme as well as with periodate. This suggests that two chemical pathways can arise from o-dopaquinone-H+, whose relative importance is determined by the pH. The steps of these pathways would be dopa → o-dopaquinone-H+ → o-dopachrome → leucodopachrome → dopachrome, for the first one, and dopa → o-dopaquinone-H+ → 2,4,5-trihydroxyphenylalanine → 5-(2-carboxy-2-aminoethyl)-2-hydroxy-1,4-benzoquinone very slowly → intermediate compound → dopachrome, for the second one. The stoichiometry for the conversion of o-dopaquinone-H+ into dopachrome for pH values greater than 4 followed the equation 2 o-dopaquinone-H+ → dopa + dopachrome. No participation of oxygen was detected in the conversion of leucodopachrome (2,3-dihydro-5,6-dihydroxyindole-2-carboxylate) into dopachrome.

Fig. 1 depicts the pathway of melanin synthesis from dopa following the scheme proposed by Lerner and Fitzpatrick (1) and based upon studies by Raper (2), Mason (3), and Lerner et al. (4). The two intermediates proposed before the formation of dopachrome, namely o-dopaquinone-H+ and leucodopachrome, have never been observed as direct products of dopa oxidation by tyrosinase (5, 6). The existence of dopaque- none-H+ as an intermediate was inferred from the action of tyrosinase on a variety of catechols. Evidence has also been presented for the formation of a dopaqueine analogue upon oxidation by tyrosinase of tyrosine-containing oligopeptides in which the α-amino group of tyrosine is substituted (7). The other intermediate in the pathway, named leucodopachrome, has only been observed as a product of dopachrome reduction (5).

The identification of topa as a product of the action of tyrosinase on tyrosine (8) has led to some authors' attempts to include this product in the melanin biosynthesis pathway. In this way, Lunt and Evans (9) proposed the following scheme: tyrosine → dopa → topa → 2,3-dihydro-5,6-dihydroxyindole-2-carboxylate → dopachrome → 5,6-dihydroxyindole-2-carboxylate → melanin. These authors did not include any quinonic compound as intermediates between dopa and dopachrome. An alternative reaction sequence for the transformation of topa into dopachrome was given by Swan (10), who showed that p-topaqueine is slowly converted into dopachrome.

Taking into account these results as well as the graphical analysis data, Greham and Jeffs (5) proposed another possible pathway that included topa as an intermediate: tyrosine → dopa → topa → 5-(2-carboxy-2-aminoethyl)-4-hydroxy-1,2-benzoquinone → p-topaqueine → intermediate compound → dopachrome. Neither of these authors consider the appearance of o-dopaquinone-H+, but they stated that tyrosine should be directly converted into topa as a result of tyrosinase action and that topa would be converted into its quinones.

On the other hand, a consideration of oxidation-reduction potentials for the pairs o-dopaquinone-H+/dopa and dopachrome/leucodopachrome led to the proposal by Lerner and Fitzpatrick (1) that leucodopachrome could be oxidized into dopachrome by o-dopaquinone-H+. Nevertheless, this suggestion has not been taken into account (5, 6, 11). The great instability of the intermediates in this pathway as well as the difficulty of their isolation also hindered their study.

In the present study, dopa was oxidized by sodium periodate and tyrosinase for several different pH values (3.5-6.0) in an attempt to determine whether o-dopaquinone-H+ and topa could be considered as intermediates in the pathway of melanin synthesis. It is proved that the pathway is dependent on pH with regard to its intermediate products. When reactions are achieved at neutral or slightly acid pH, the Raper-Mason pathway (Fig. 2A) is the main one. However, for strongly acid pH values lower than 4, two pathways should compete from o-dopaquinone-H+ (Fig. 2B). The first one is similar to that described in Fig. 2A and should include the deprotonation of o-dopaquinone-H+, yielding o-dopaquinone; the cyclization of the latter should give leucodopachrome, whose oxidation by o-dopaquinone-H+ should give dopachrome and dopa. The
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**Oxidation by Periodate**—Oxidation of dopa by sodium periodate was carried out at pH 4.8 at three different \([\text{dopa}]/[\text{NaIO}_4]\) ratios: 4.4, 1.0, and 0.1. When dopa concentration was greater than sodium periodate \((\text{dopa})/[\text{NaIO}_4] = 4.4\), a yellow compound appeared quickly with \(\lambda_{\text{max}}\) at 390 nm that was ascribed to o-dopaquinone-H⁺, in agreement with Graham and Jeffs (5); this peak shifted with time towards another one of \(\lambda_{\text{max}}\) at 475 nm, characteristic of dopachrome. Recordings are present in Fig. 3A showing an isosbestic point at \(\lambda = 416\) nm whose appearance suggested, as a first approximation, the occurrence of two species kinetically related.

The graphical analysis of the recordings in the visible spectrum by the matrix method of Coleman et al. (13) gave a good fit for two absorbing species in solution, as is shown in Fig. 4A. Nevertheless, as will be discussed later, in this case three kinetically related species should exist.

The appearance of the isosbestic point seems to suggest that an equivalent quantity of dopa to that of periodate has been converted into o-dopaquinone-H⁺, which in turn yields dopachrome. In this way, Fig. 5 shows a plot of maximal concentrations of o-dopaquinone-H⁺ at the beginning of the reaction against periodate concentrations; a straight line of slope 1 was obtained \((\text{dopa})/[\text{NaIO}_4] = 1250 \text{ M}^{-1} \text{ cm}^{-1}\) suggesting that periodate was fully consumed following a 1:1 stoichiometry. In this case, it seemed interesting to study whether oxygen does participate in the conversion of leukodopachrome into dopachrome, following the scheme of Raper (2) (Fig. 1). However, oxygen consumption through reaction time was measured in the same experiments, and no consumption was detected.

Bearing in mind the considerations of oxidation-reduction potentials for the pairs o-dopaquinone-H⁺/dopa and dopachrome/leukodopachrome indicated by Lerner and Fitzpatrick (1) and assuming that in the experimental conditions described above leukodopachrome was not oxidized by sodium periodate, the latter being fully depleted previously, nor by oxygen as it does not participate in this step, investigation was made to ascertain whether leukodopachrome oxidation was achieved by o-dopaquinone-H⁺. Thus, concentration of dopachrome was measured when it reached a constant value; it was found that this concentration was half of the periodate concentration and therefore half that of o-dopaquinone-H⁺. These results suggested that o-dopaquinone-H⁺ actually oxidizes leukodopachrome, being reduced to dopa. The slope of the straight line resulting from the plot of dopachrome concentration versus periodate concentration was also equal to 0.5 (Fig. 5).

When concentration of sodium periodate was greater than that of dopa \((\text{dopa})/[\text{NaIO}_4] = 0.1\), a set of recordings was obtained which showed the presence of two isosbestic points, at \(\lambda = 362\) nm and \(\lambda = 398\) nm, as is shown in Fig. 3B. The graphical analysis of the spectra gave a good fit for two absorbing species in solution, kinetically related (Fig. 4B).

Finally, when the oxidation was carried out in equimolar conditions, \([\text{dopa}]/[\text{NaIO}_4] = 1\), no isosbestic point appeared, as can be seen in the recordings of Fig. 3C. The graphical analysis of the spectra showed the presence of three absorbing species kinetically related (Fig. 4C).

In an attempt to define whether or not o-dopaquinone-H⁺ and dopa are intermediates in the melanin biosynthesis pathway, their spectrophotometric detection was carried out upon oxidation of dopa by sodium periodate and tyrosinase.

**EXPERIMENTAL PROCEDURES**

_Mushroom tyrosinase_ (monophenol monooxygenase, EC 1.14.18.1, 2230 units/mg) and tops were purchased from Sigma, and dopa was from Roche. Sodium metaperiodate was analytical grade. Spectra were recorded with an Aminco DW-2 spectrophotometer with scanning speeds up to 20 nm/s. Reference cuvettes contained in all cases all the components except substrate. Cyclic voltammograms were obtained using a Tektronik from Chemtrix, model 564 with memory, with an HMDE work electrode. Potential values were taken in reference to SCE saturated with KCl. Oxygen consumption was followed by a Rank oxygen electrode (Rank Brothers). Temperature was controlled by using a Gilson bath and a digital Cole-Parmer thermistor with a SR ± 0.1 °C. Protein concentration was determined by the method of Lowry et al. (12).

**RESULTS**

In an attempt to define whether or not o-dopaquinone-H⁺ and tops are intermediates in the melanin biosynthesis pathway, their spectrophotometric detection was carried out upon oxidation of dopa by sodium periodate and tyrosinase.
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Oxidation by Tyrosinase—When the oxidation of dopa was achieved by mushroom tyrosinase for slightly acid pH values, it was again possible to record a peak at 390 nm that was coincident with the maximum obtained previously upon oxidation by periodate. This maximum has been related to o-dopaquinone-H" and is shifted during reaction time to give another peak at 475 nm corresponding to dopachrome. In this case, no isosbestic point was defined, as can be observed in Fig. 6A; the analysis of the different recordings gave a good fit for three absorbing species kinetically related (Fig. 7A).

When the same assays were performed, but the absorbance increase with time was recorded at a fixed wavelength, the

![Figure 2: Pathways proposed for the oxidation of dopa to dopachrome catalyzed by tyrosinase for different pH values.](image)

**Fig. 2.** Pathways proposed for the oxidation of dopa to dopachrome catalyzed by tyrosinase for different pH values. E = tyrosinase. A, pH values greater than 4. The intermediates are 1) o-dopaquinone-H"; 2) o-dopaquinone; and 3) leukodopachrome. B, pH values lower than 4. The intermediates are 1) o-dopaquinone-H"; 2) o-dopaquinone; 3) topa; 4) leukodopachrome; 5) p-topaquinone.

![Figure 3: Spectrophotometric recordings for the oxidation of dopa by sodium periodate from 350 to 550 nm at 10 °C in 0.01 M sodium acetate buffer, pH 4.8.](image)

**Fig. 3.** Spectrophotometric recordings for the oxidation of dopa by sodium periodate from 350 to 550 nm at 10 °C in 0.01 M sodium acetate buffer, pH 4.8. Scan speed was up to 20 nm/s, and the first scan is started at 20 s from the beginning of the reaction. A, dopa at 1.52 mM was oxidized with 0.35 mM NaIO₄. B, dopa at 0.25 mM was oxidized with 2.5 mM NaIO₄. C, dopa at 0.35 mM was oxidized with 0.35 mM NaIO₄.
following results were obtained. When the recording was made at $\lambda = 390$ nm, corresponding to the maximum of o-dopaquinone-$H^+$, an apparent burst was detected (Fig. 8B). However, when the recording was made at $\lambda = 475$ nm, a wavelength coincident with the dopachrome maximum, a lag time appeared (Fig. 8A). This lag period is not dependent on the enzyme concentration, but it is due to the series of chemical reactions arising between o-dopaquinone-$H^+$ and dopachrome (Fig. 24); and thus, it is dependent on pH. This lag period has the value of $1/k$, where $k$ is the rate constant of the conversion of o-dopaquinone-$H^+$ to dopachrome.$^3$ Note that o-dopaquinone-$H^+$, the direct product of the enzymatic reaction, was accumulated following an apparent burst and the reaching of a steady state (Fig. 8C).

The kinetic analysis of a system of chemical reactions coupled to an enzymatic reaction$^4$ predicts that the rate of formation of the final product, when the system has reached the steady state, should be the same as the initial velocity of the enzymatic step provided that the chemical reactions are of first order or pseudo-first order; when an intermediate undergoes a second order reaction, this rate is half of the initial velocity of the enzyme. It is important to point out that, in the first case, the stoichiometry would be 1:1, while it would be 2:1 in the second case. From the results presented in Fig. 8, it is possible to calculate that the rate of dopachrome accumulation in the steady state is half of the initial velocity of o-dopaquinone-$H^+$ formation by the enzyme, suggesting that a 2:1 stoichiometry must exist in the pathway of coupled chemical reactions with respect to dopachrome formation.

Similar assays were performed with a 5-fold greater enzyme amount so that oxygen was depleted in the solution. In these conditions, it was possible to determine the same isosbestic point that appeared for a [dopa]/[NaIO$_4$] ratio of 4.4, and the matrix analysis applied to this anoxia situation showed the occurrence of two species kinetically related. However, as well

\[ \frac{1}{k} \]

as in the experiment shown in the Fig. 3A, three species are actually present, as will be discussed later. On the other hand, the lack of participation of oxygen in the oxidation of leukodopachrome into dopachrome was verified since the amount of oxygen in solution was determined to be null (starting from trace 6 of Fig. 6B).

\[ \text{Cyclic Voltammetry} \]

The two pathways of oxidation of dopa proposed by Graham and Jeffs (5) that can be catalyzed by tyrosinase lead to p-topaquinone and dopachrome. Since the $\lambda_{\max}$ for these compounds are very similar (485 nm and 475 nm), it is difficult to determine spectrophotometrically whether the two products could be formed at the same time for the same pH values. However, cyclic voltammetry allows

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$^3$ F. G. Cánovas, F. G. García-Carmona, J. V. Sánchez, J. L. Iborra Pastor, and J. A. Lozano Teruel, unpublished observations.

$^4$ F. García, F. G. Cánovas, and J. A. Lozano Teruel, in press.
a possible approach to this problem. When oxidation of dopa was achieved by means of tyrosinase or sodium periodate for pH values greater than 4, cyclic voltammograms (Figs. 9 and 10) showed, respectively, a cathodic peak and an anodic peak. The cathodic peak arose from the dopachrome reduction to leukodopachrome, while the anodic peak appeared for a potential value slightly more positive and should be explained by the oxidation of leukodopachrome into dopachrome (15). However, cyclic voltammograms obtained for pH values lower than 4, both for tyrosinase and sodium periodate, were constituted by two cathodic peaks and two anodic peaks, respectively (Fig. 11, A and B).

The cathodic peak obtained for the more negative potential of −0.10 V versus SCE arose from the reduction of dopachrome into leukodopachrome; its corresponding anodic peak appeared at 0.05 V versus SCE and was due to the oxidation of leukodopachrome into dopachrome. The cathodic peak located at a more positive potential, 0.05 V versus SCE, can be ascribed to the reduction of p-topaquinone into topa, which shows an anodic peak at the potential value of 0.12 V versus SCE, corresponding to the oxidation of topa into p-topaquinone, as was assessed by reference to the peaks obtained in the cyclic voltammograms of dopachrome and topa recorded separately for the same pH value. The separation between the potentials of the oxidation and reduction peaks is almost equal to the theoretical value for reversible processes.

**Leukodopachrome**—Leukodopachrome is another intermediate in the pathway leading to dopachrome. The graphical analysis of the spectra never shows the presence of leukodopachrome since its concentrations in the media are very low due to its rapid oxidation by o-dopaquinone-H⁺. Leukodopachrome can be obtained, in agreement with Graham and Jeffs (5), by means of reduction of dopachrome by NaBH₄.

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**Fig. 6.** Spectrophotometric recordings for the oxidation of dopa by tyrosinase from 350 to 550 nm at 10 °C in 0.01 M acetic-acetate buffer, pH 4.8. Scan speed was 20 nm/s. The first recording was started at 20 s from the beginning of the reaction. A, dopa at 1.52 mM was oxidized with tyrosinase (0.02 mg/ml). B, dopa at 1.52 mM was oxidized with tyrosinase (0.1 mg/ml).

**Fig. 7.** Graphical analysis of spectra recorded during tyrosinase oxidation of dopa. Spectra were recorded from 350 to 550 nm at 21 s intervals during the oxidation of 1.52 mM of dopa. A, tyrosinase concentration, 0.02 mg/ml. The same test as in Fig. 4C was applied. B, tyrosinase concentration, 0.1 mg/ml. The same test as in Fig. 4A was applied, starting in tracing number 6. The meanings of different magnitudes of graphical analysis are defined in Fig. 4 (see miniprint).

**Fig. 8.** Products formation with time for the dopa oxidation with tyrosinase. A, representation of absorbance of dopachrome accumulation against time followed at λ = 475 nm during reaction of 0.02 mg/ml of tyrosinase with 1.52 mM dopa at 25 °C, pH 4.8. B, representation of ΔA against time at λ = 390 nm for the same reaction as in A. C, o-dopaquinone-H⁺ (micromolar) accumulation against time followed at λ = 390 nm for the same reaction as in A. A correction for dopachrome absorbance at 390 nm was made using the relation: 

\[
A_{\text{dopa}} = \frac{\epsilon_{520}}{\epsilon_{475}} A_{\text{dopachrome}} - A_{\text{dopachrome}} = 970 \text{ M}^{-1} \text{ cm}^{-1}
\]
The identification of intermediates in the pathway of melanin biosynthesis has been hard to establish because of the great reactivity of these short half-life compounds. Two intermediates have been proposed for the steps included between dopa and dopachrome, namely o-dopaquinone-H' and leukodopachrome.

The occurrence of a maximum centered at 390 nm when tyrosinase acts on tyramine (linked to other amino acids by means of its a-amino group) led to the proposal that o-dopaquinone-H' was the product of the enzymatic action. Nevertheless, identification of this compound as the direct product of the enzyme action on dopa has never been achieved (5, 6).

It was possible to observe the appearance of o-dopaquinone-H' working with tyrosinase at acid pH values from 3.5 to 6.0 and with a rapid scan spectrophotometer (Fig. 6).

As is shown in the scheme of Fig. 2A, the formation of dopachrome from o-dopaquinone-H' is achieved by the cyclisation of the molecule following a Michael intramolecular 1,4 addition; this is only possible when the amino group is unprotected so that the reaction is dependent on pH. For pH values close to 7, the o-dopaquinone-H' intermediate could not be detected by means of graphical analysis due to the rapidity of cyclisation and the consequently very low concentration of the intermediate. For lower pH values (less than 6), the reaction became greater, making possible its detection by graphical analysis (Fig. 7A). As time elapsed, the system reached a steady state, and dopachrome was accumulated after a lag period at a constant rate (Fig. 8A).

From the experiments of dopa oxidation by sodium periodate with dopa in excess (Fig. 5), where no oxygen consumption was detected and where it was determined that the amount of dopachrome formed was half that of o-dopaquinone-H', and from the experiments realized with tyrosinase (Fig. 8), where it can be seen that the rate of dopachrome accumulation is half that of o-dopaquinone-H', it can be inferred that the following reaction should occur: 2 o-dopaquinone-H' 7.0, at 25 °C (A) and with 0.25 mm NaI04, (B). The enzyme was incubated with the substrate and then the solution was located into the cell with the proper electrodes. Nitrogen was bubbled through the solution before starting the record. The magnitude *I* is the current intensity expressed in microamperes and *E* is the voltage expressed in volts (V). Working electrode: hanging mercury electrode; scan rate, 0.5 V/s⁻¹.

In view of the preceding consideration, we believe that the potential differences between the pairs o-dopaquinone-H'/dopa and dopachrome/leukodopachrome pointed out by Ler- A. & Fitzpatrick (1) should be borne in mind and that this step should be added as an obligatory one to the pathway of melanization.

When the oxidation of dopa is achieved in defect of sodium periodate, the appearance of an isosbestic point at λ = 416 nm can be seen, being εo-dopachrome = 1.806 m⁻¹ cm⁻¹ (Fig. 3A). When the analysis of the spectra were performed, two species were detected (Fig. 4A). However, from the considerations outlined above, it can be inferred that three species kinetically related are actually present: dopa, o-dopaquinone-H', and dopachrome. In fact, from 2 mol of o-dopaquinone-H', 1 mol of dopa, and another of dopachrome were released in a constant ratio so that the analysis detected these compounds as a single species. A similar situation can be obtained with the enzyme tyrosinase when the reaction is achieved with very high concentrations of tyrosinase. In this way, the oxygen was depleted in the medium, and the graphical analysis at short times gave a good fit for three species: dopa, o-dopaquinone-H', and dopachrome. Nevertheless, from the number 6 tracing in Fig. 6B, the test for two species was accomplished: o-dopaquinone-H' and dopachrome (Fig. 7B).

The experiments performed with sodium periodate in excess showed the appearance of an isosbestic point different from the preceding one, at λ = 398 nm, εo-dopachrome = 1.100 m⁻¹ cm⁻¹ (Fig. 3B), that can be explained by the transformation of o-dopaquinone-H' into dopachrome following 1:1 stoichiometry. The excess of periodate prevents the accumulation of dopa in the medium, and this excess can also oxidize the leukodopachrome formed, preventing the action of o-dopaquinone-H' as an oxidant. In this case, the graphical analysis (Fig. 4B) gave a good fit for two species: o-dopaquinone-H' and dopachrome.

When the oxidation is performed in stoichiometric conditions for dopa and periodate, the cyclization and oxidation reactions take place at the same time, periodate can also oxidize leukodopachrome so that no well defined isosbestic point appears (Fig. 3C). The graphical analysis was in accord-
ance with three species (Fig. 4C) which, in agreement with Graham and Jeffs (5), are supposed to be dopa, o-dopaquinone-H⁺, and dopachrome. The same result is obtained with low amounts of tyrosinase provided that the oxygen in the solution is not depleted (Fig. 6 and 7, A).

The third intermediate of the pathway leading to dopachrome is leukodopachrome. This compound can be a substrate for the enzyme. However, the possibility that the enzyme may act again in this step is negligible since high concentrations would be required in order to compete with dopa. In these conditions, leukodopachrome would be detected by graphical analysis, and this was never the case in the present study.

Assays performed for pH values lower than 4 allow investigation as to whether topa participates in the melanin biosynthesis pathway, following a different approach than that proposed by Graham and Jeffs (5). These authors proposed a minority melanogenesis pathway that includes the participation of topa, suggesting that this compound should arise from the direct action of the enzyme on dopa and should be transformed later into p-topaquinone. So, the participation of o-dopaquinone-H⁺ is not considered in this scheme.

We have always detected the formation of o-dopaquinone-H⁺ as the first oxidation product, irrespective of pH value. Nevertheless, the formation of p-topaquinone from o-dopaquinone-H⁺ can be achieved by means of solely chemical reaction. For strongly acid pH values, the cyclization reaction of o-dopaquinone-H⁺ is very slow due to the distance of the pK range for the amino group. In these conditions, the addition of water to the ring can take place with the formation of topa, which in turn can be oxidized by o-dopaquinone-H⁺, leading to p-topaquinone that slowly yields dopachrome (5) (Fig. 2B). This pathway would compete with the cyclization one, and the relative importance of each one would be dependent on pH.

As we pointed out previously, the maxima for p-topaquinone and dopachrome are very near so that their spectrophotometric identification becomes very difficult. Nevertheless, in the results obtained in cyclic voltammetry experiments, the peak potentials are separate enough as to allow the identification of these compounds. Cyclic voltammograms (Fig. 11A) showed the formation of two products when we worked with tyrosinase with strongly acid pH values (3.5); the corresponding peaks were ascribed to p-topaquinone and dopachrome by means of comparison with the standards of these compounds. The evidence that these products are not formed directly by the enzyme arises from the fact that both p-topaquinone and dopachrome were obtained when the oxidation of dopa was performed with sodium periodate (Fig. 11B).

Cyclic voltammograms also showed that for pH values greater than 4.0 only dopachrome was obtained as final product when the oxidation was achieved by tyrosinase as well as by sodium periodate (Figs. 9 and 10, A and B). It must be noted that topa does not accumulate in the medium because this product is in a similar situation to that of leukodopachrome, and thus, it is rapidly oxidized by o-dopaquinone-H⁺. Topa has also been obtained by reduction of p-topaquinone with NaBH₄ (5).

Our experiments also would explain the initial results obtained by Lissitzky and Rolland (8) with respect to the topa synthesis by tyrosinase action on tyrosine. These authors worked in unbuffered aqueous solution; hence, the addition of ascorbic acid should decrease the pH of the reaction medium, enhancing the addition of water into the ring. On the other hand, cyclization should be prevented by the o-dopaquinone-H⁺ reduction by ascorbic acid, and in this way, only the topa formation would progress.

From all the preceding considerations, it can be inferred as a conclusion that the formation of intermediates in the melanin biosynthesis pathway is dependent on pH. The o-dopaquinone-H⁺ is always the direct product of the enzymatic action of dopa, and from this compound, two competitive pathways arise, the one yielding dopachrome and the other p-topaquinone. The pathway of p-topaquinone (Fig. 2B) is the major one for very acid pH values, while for a physiologic pH, the pathway of dopachrome formation (Fig. 2A) is the main one.

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