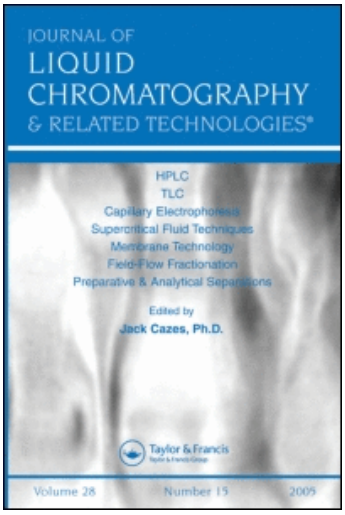

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Identification of Products and Intermediates During L-Dopa Oxidation to Dopachrome Using High Pressure Liquid Chromatography with Electrochemical Detection

Jianyong Li[a]; Bruce M. Christensen[a]
[a] Department of Animal Health and Biomedical Sciences, College of Agricultural and Life Sciences University of Wisconsin-Madison, Madison, Wisconsin

To cite this Article Li, Jianyong and Christensen, Bruce M.(1993) 'Identification of Products and Intermediates During L-Dopa Oxidation to Dopachrome Using High Pressure Liquid Chromatography with Electrochemical Detection', Journal of Liquid Chromatography & Related Technologies, 16: 5, 1117 — 1133
To link to this Article: DOI: 10.1080/10826079308019575
URL: http://dx.doi.org/10.1080/10826079308019575

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(5), 1117-1133 (1993)

IDENTIFICATION OF PRODUCTS AND INTERMEDIATES DURING L-DOPA OXIDATION TO DOPACHROME USING HIGH PRESSURE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

JIANYONG LI AND BRUCE M. CHRISTENSEN
Department of Animal Health and Biomedical Sciences
College of Agricultural and Life Sciences
University of Wisconsin-Madison
Madison, Wisconsin 53706

ABSTRACT

Products and intermediates produced during L-dopa oxidation by sodium periodate and mushroom tyrosinase were studied using high pressure liquid chromatography with electrochemical detection (HPLC-ED). The oxidation products and intermediates, including dopaquinone, leukodopachrome, 2,4,5-trihydroxyphenylalanine and its quinone species, were readily separated on a reverse-phase column with 15 mM citrate buffer, pH 3.0, containing 35 mM NaCl, 0.25 mM octyl sulfate and 0.7 mM Na_2EDTA and detected at either a reductive (-100 mV) or an oxidative potential (750 mV) of the working electrode. Using these techniques, the effect of base and acid on the chemical reaction of dopaquinone to dopachrome was demonstrated.

INTRODUCTION

Because of its physiological importance in living organisms, melanin and the process of melanogenesis have been extensively studied. Tyrosinase (EC

1.14.18.1) catalyzes several key steps of melanogenesis, the hydroxylation of tyrosine or other monophenols to L-dopa or related *o*-diphenols, and the oxidation of *o*-diphenols to their respective *o*-quinones. Because L-dopa is the most important precursor in the melanogenesis pathway, a significant amount of work has been conducted on tyrosinase using L-dopa as substrate. It generally has been accepted that the product of L-dopa oxidation by tyrosinase is *o*-dopaquinone. Because of its instability, *o*-dopaquinone, once formed, is rapidly converted to leukodopachrome, and then to dopachrome. Although, dopaquinone is the direct enzymatic product, this compound has not been detected and isolated during enzyme-mediated oxidation of this compound. Recently, a branched pathway (Fig. 1) of L-dopa oxidation to dopachrome was suggested to be significant when the oxidation of L-dopa, either by tyrosinase or sodium periodate, occurs in an acidic environment (1, 2). The branched pathway proposed suggests that at acidic conditions, the oxidation of L-dopa using either mushroom tyrosinase and sodium periodate results in the formation of dopaquinone-H, the hydroxylation of dopaquinone-H produces topa, and the oxidation of topa results in the formation of *p*-topaquinone (1, 2). These assumptions have been based on the matrix analysis of spectrophotometric data, in addition to cyclic voltammetric data (1, 2). However, no direct evidence has been provided to support the existence of a branched pathway in melanin biosynthesis. Because the reactions of L-dopa to dopachrome are either an oxidation or reduction process, we critically analyzed the possible intermediates during L-dopa oxidation by mushroom tyrosinase and sodium periodate using HPLC-ED. Because L-dopa, dopaquinone and other intermediates of the L-dopa oxidation pathway to dopachrome can be separated and detected by HPLC-ED, either at reductive or oxidative potentials, the HPLC-ED system makes it possible to identify oxidation products and other possible intermediates individually. Herein we report our data and suggest the potential usefulness of this described method for the identification of individual oxidizable and reducible species, and the dynamic changes of the individual species in the biochemical pathway of catecholamine metabolism.

MATERIALS AND METHODS

Chemicals

3,4-dihydroxyphenylalanine (L-dopa), 2,4,5-trihydroxyphenylalanine (topa), acetic acid, ascorbic acid, calcium chloride, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), 3-[N-morpholino]propanesulfonic acid (MOPS), calcium chloride, potassium chloride, sodium periodate ($NaIO_4$), sodium borohydride ($NaBH_4$), sodium chloride, sodium phosphate and mushroom tyrosinase were purchased from Sigma Chemical Co (St. Louis, MO).

HPLC-ED apparatus

The HPLC-ED system consisted of a Gilson 302 pump, a Rheodyne (Cotati, CA) Model 7125 injector fitted with a 20-µl sample loop, and a Bioanalytical System (West Lafayette, IN) Model LC-4B amperometric detector fitted with a glassy carbon electrode. The working electrode was maintained at either a oxidative potential of +750 mV or a reductive potential of -100 mV versus a Ag-AgCl reference electrode and a sensitivity of 200 - 500 nA full scale. Separation of L-dopa and dopaquinone and other intermediates of the L-dopa oxidation pathway was achieved at 30°C by reverse-phase chromatography on a BAS (West Lafayette, IN) 3-µm ODS column (3.2 mm i.d. x 10 cm). The mobile phase consisted of 15 mM citrate buffer (pH 3.0) containing 35 mM NaCl, 0.7 mM Na_2EDTA and 0.25 mM octyl sulfate. The flow rate was 0.5 ml/min.

HPLC-ED analysis of sodium periodate and mushroom tyrosinase mediated L-dopa oxidation

Because pH of the buffer used to prepare L-dopa solution has a significant effect on the chemical reactions of dopaquinone to dopachrome (1,2), L-dopa

solution (1 or 2 mM) was prepared using different buffers, including 20 mM citrate (pH 2.5 - 6.5), phosphate (pH 6.5), MOPS (pH 6.0), HEPES (7.2), and acetate (pH 4.0 - 4.5). The L-dopa reaction mixture also was prepared using ether distilled water or 20 -100 mM neutral salt solutions, including NaCl, KCl and $CaCl_2$ or 100 mM NaCl containing varying concentration of phosphate buffer (1.25 - 20 mM, pH 6.5) to determine the effects of ionic strength on the chemical reactions of dopaquinone to dopachrome.

Oxidation of L-dopa was initiated by incorporation of solid $NaIO_4$ (5.4 µg/10 ml, equivalent to a 2.5 mM final concentration of this chemical oxidant) into a 1 mM L-dopa solution. The L-dopa/$NaIO_4$ reaction mixture was shaken briefly and incubated at 30°C for 30 sec or 1 min and then was filtered through a 13 mm Acrodisc (0.2 µm pore). Filtrate samples (5 µl) were injected for HPLC-ED analysis using either an oxidative potential (750 mV) to assess substrate depletion and electrochemically oxidizable species formation during L-dopa oxidation, or a reductive potential (-100 mV) to determine oxidized products derived from L-dopa. Time elapsing from filtration to injection for HPLC-ED analysis was less than 15 seconds. Initial results showed that oxidation of L-dopa was virtually complete in less than 30 sec in 20 mM phosphate buffer (pH 6.0), and about one min under acidic conditions or in water. Compounds detected within 1 or 2 min of incubation in the L-dopa/$NaIO_4$ reaction mixture using HPLC-ED at a reductive potential (-100 mV) were considered to be the initial products of the L-dopa oxidation pathway. The filtrate also was incubated at 30°C and injected for HPLC-ED analysis at a reductive potential (-100 mV) at different incubation times to monitor dynamic changes in the individual electrochemically reducible species.

To verify the identities of the detected electrochemically reducible species, the filtered samples were treated with either ascorbate (10 mM) or $NaBH_4$ (1 mg/ml) at different periods of incubation, and then the ascorbate or $NaBH_4$ treated samples were analyzed using HPLC-ED at an oxidative potential (750 mV) of the working electrode. Because ascorbate can quickly reduce quinone species derived from L-dopa and other *o*-quinone species back to their respective *o*-diphenolic

compounds, with virtually no effect on the reduction of the aminochromes (demonstrated in this study), a concomitant disappearance of the specific reducible species and an increase in L-dopa concentration (determined using HPLC-ED analysis at the respective reductive and oxidative potential of the working electrode) made it possible to identify the detected species that corresponded to dopaquinone. In contrast, $NaBH_4$ is able to reduce both dopaquinone and dopachrome back to L-dopa and leukodopachrome (see Fig. 1). Therefore, differentiation of dopaquinone from dopachrome was achieved by this differential reduction using either ascorbate or $NaBH_4$.

Identification of topa and the quinone species derived from topa oxidation in the L-dopa/$NaIO_4$ reaction mixture was based on coelution of these detected

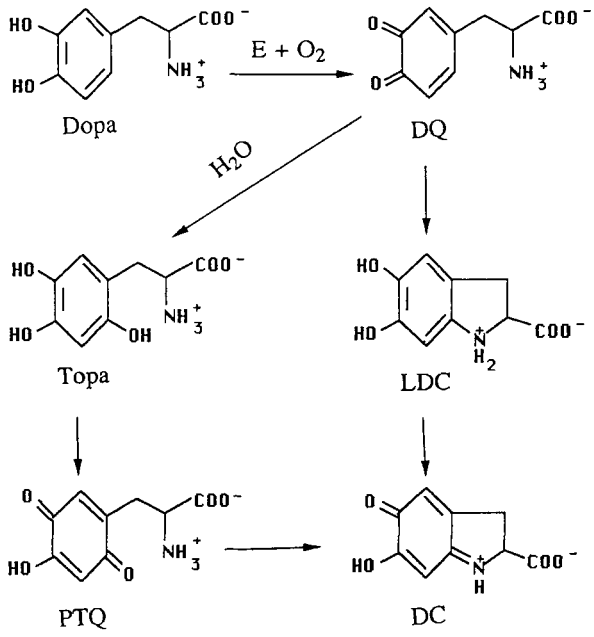

Figure 1. The biochemical pathways of L-dopa oxidation to dopachrome. Dopa=L-dopa; E=mushroom tyrosinase; DQ=*o*-dopaquinone; DC=dopachrome; LDC=leukodopachrome; PTQ=*p*-topaquinone.

intermediates under different conditions of chromatography using authentic topa standard and topaquinone obtained from the oxidation of topa (1 mM) using mushroom tyrosinase (40 µg/ml).

The product and intermediates formed during the enzymatic oxidation of L-dopa also were assessed using the same techniques employed in the analysis of the L-dopa/$NaIO_4$ reaction mixture. A reaction mixture of 800 µl, consisting of 0.8 µmol of L-dopa and 80 µg of mushroom tyrosinase in 20 mM citrate buffer (pH 4.0 - 6.0), was incubated at 30°C. The reaction mixture was filtered through a 13 mm Acrodisc (0.2 µm pore) after 2 and 6 min of incubation, and the filtrate samples (4 - 8 µl) were injected for HPLC-ED analysis at a reductive potential (-100 mV) or an oxidative potential (750 mV) following reduction using $NaBH_4$.

RESULTS

L-dopa (1 mM), prepared in distilled water and subjected to oxidation using solid $NaIO_4$, was virtually depleted in one minute (Fig. 2A-B). HPLC-ED analysis at a reductive potential (-100 mV) of the working electrode revealed the production of four distinct electrochemically reducible species in the reaction mixture with retention times of 1.8, 2.7, 3.6 and 5.8 min (Fig. 2C). The peak detected at 1.8 min was $NaIO_4$ or its ionized forms in the reaction mixture, because injection of $NaIO_4$ solution alone for HPLC-ED analysis at reductive potential also resulted in the detection of this early peak (not shown). The detected reducible species with retention times of 2.7, 3.6 and 5.8 min were considered to be derived from L-dopa oxidation (Fig. 2C). As the incubation proceeded, there was a concomitant increase and decrease of the detected peaks with retention times of 2.7 and 3.6 min (not shown). In about 20 min the 3.6 min peak became undetectable and the concentration of reducible species with a retention time of 2.7 min accumulated to a high concentration (Fig. 2D).

When the reaction mixture was reduced using ascorbate at 2 min following L-dopa oxidation by $NaIO_4$ in distilled water, the detected reducible species with

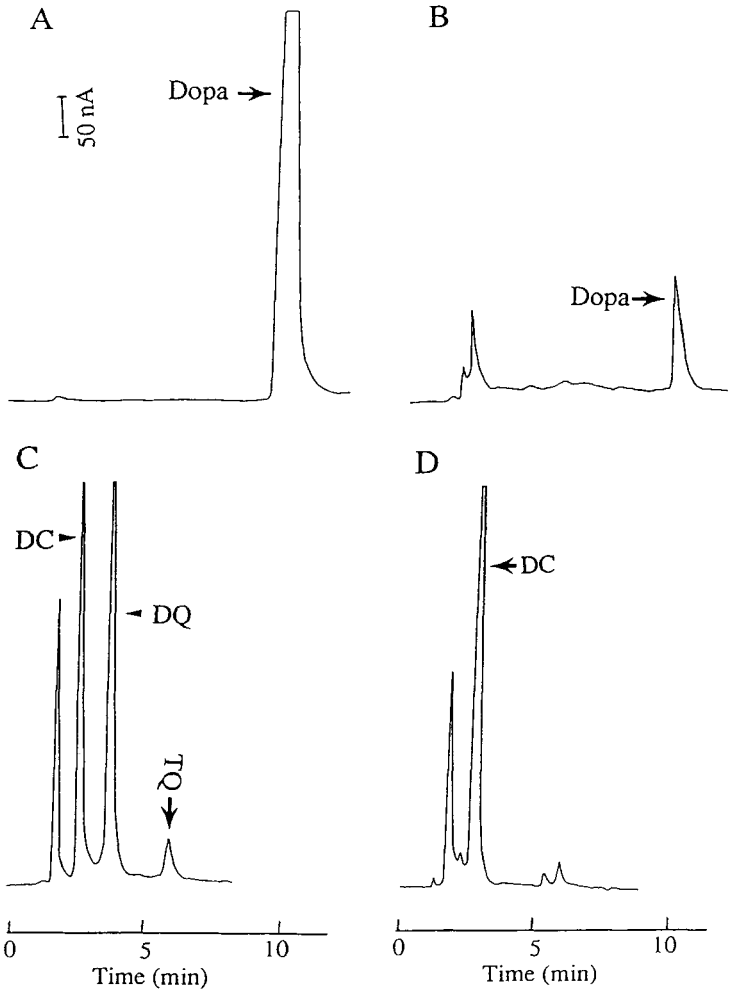

Figure 2. HPLC-ED Analysis of L-dopa depletion and intermediate/product formation during $NaIO_4$-mediated oxidation of L-dopa. Chromatograms showing the detection of L-dopa (1 mM) before chemical oxidation (A) and one min after chemical oxidation (B) determined using an oxidative potential (750 mV) of the working electrode. Chromatograms showing the formation of dopaquinone, dopachrome and topaquinone (C) and the total transformation of dopaquinone to dopachrome (D) after 20 min incubation determined using a reductive potential (-100 mV) of the working electrode. The mobile phase consisted of 15 mM citrate buffer (pH 3.0) containing 35 mM NaCl, 0.7 mM Na_2EDTA and 0.25 mM octyl sulfate at a flow rate of 0.5 ml/min. Dopa=L-dopa; DC=dopachrome; DQ=dopaquinone; TQ=*o*-topaquinone.

a retention time of 3.6 min disappeared (Fig. 3A); however, ascorbate had no noticeable effect on the 2.7 and 5.8 min peaks (Fig. 3A). HPLC-ED analysis of the ascorbate-treated reaction mixture at an oxidative potential of the working electrode (750 mV) showed the reappearance of a high concentration of L-dopa in the reaction mixture (Fig. 3B, see Fig. 1B to compare the amount of L-dopa left in the reaction mixture), which suggested that the detected reducible species with a retention time of 3.6 min (Fig. 3C) was dopaquinone. When the oxidized L-dopa solution was reduced using $NaBH_4$ at 2 min after initiation of chemical oxidation, all detected reducible species with retention times of 2.7, 3.6 and 5.8 min disappeared in the reaction mixture when assessed by HPLC-ED at a reductive potential (not shown), and analysis of the same $NaBH_4$ treated reaction mixture at an oxidative potential (750 mV) of the working electrode resulted in the detection of three reduced compounds with retention times of 2.9 and 6.2 and 9.6 min (Fig. 3C). The detected electrochemically oxidizable species with a retention time of 2.9 min was considered to be the reduced form of the compound with a retention time of 2.7 min (see Fig. 3A) detected using a reductive potential of the working electrode. Because L-dopa oxidation resulted in the formation of dopachrome as a stable product under neutral conditions, the detected species with a retention of 2.7 min was considered to be dopachrome, and the detected species with retention time of 2.9 min following $NaBH_4$ reduction using an oxidative potential of the working electrode, was its reduced form, leukodopachrome. The small amount of electrochemically reducible compound with a retention time of 5.8 min was verified to be topaquinone, derived from topa that was produced due to nucleophilic addition of a water molecule on the benzene ring of dopaquinone molecules (see Fig. 1).

The pH of the buffer solution used to prepare the reaction mixture had a significant effect on the fate of dopaquinone formed in the reaction mixture. Oxidation of L-dopa using $NaIO_4$ in highly acidic conditions (20 mM citrate buffer, pH 2.5) resulted in the detection of dopachrome, dopaquinone, and two additional electrochemically reducible species with retention times of 4.7 and 5.8

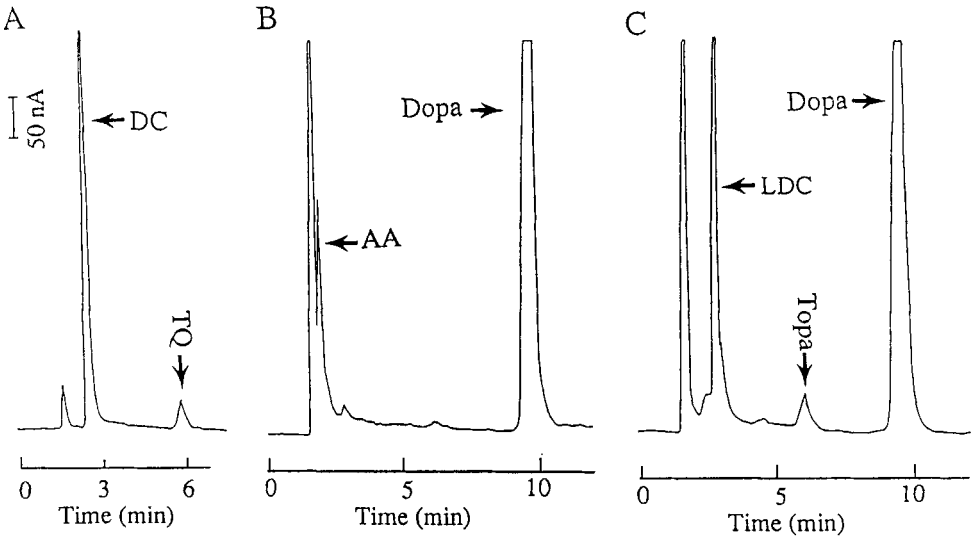

Figure 3. HPLC-ED analysis of ascorbate or $NaBH_4$ treated L-dopa/$NaIO_4$ reaction mixture. Chromatograms showing the concomitant disappearance of dopaquinone (DQ) determined using a reductive potential (-100 mV) of the working electrode (A) and reappearance of high concentration of L-dopa determined using an oxidative potential (750 mV) of the working electrode (B) following addition of ascorbate at one min after L-dopa oxidation using $NaIO_4$. Treatment of L-dopa/$NaIO_4$ reaction mixture using $NaBH_4$ resulted in the disappearance of dopachrome, dopaquinone and topaquinone (not shown) determined using a reductive potential of the working electrode and formation of leukodopachrome (LDC), topa and L-dopa detected using an oxidative potential (750 mV) of the working electrode (C). Other conditions of HPLC-ED analysis were identical as in Fig. 2.

min (Fig. 4A). The detected species with a retention time of 5.8 min was considered to be *o*-topaquinone derived from topa, because oxidation of topa by mushroom tyrosinase resulted in the formation of an electrochemically reducible species with the same retention time (Fig. 4B), and they coeluted at different conditions of chromatography (not shown). As the incubation proceeded, *o*-topaquinone accumulated to its highest concentration at about 10 min (Fig. 4C), and then its concentration gradually decreased in the reaction mixture (Fig. 4D).

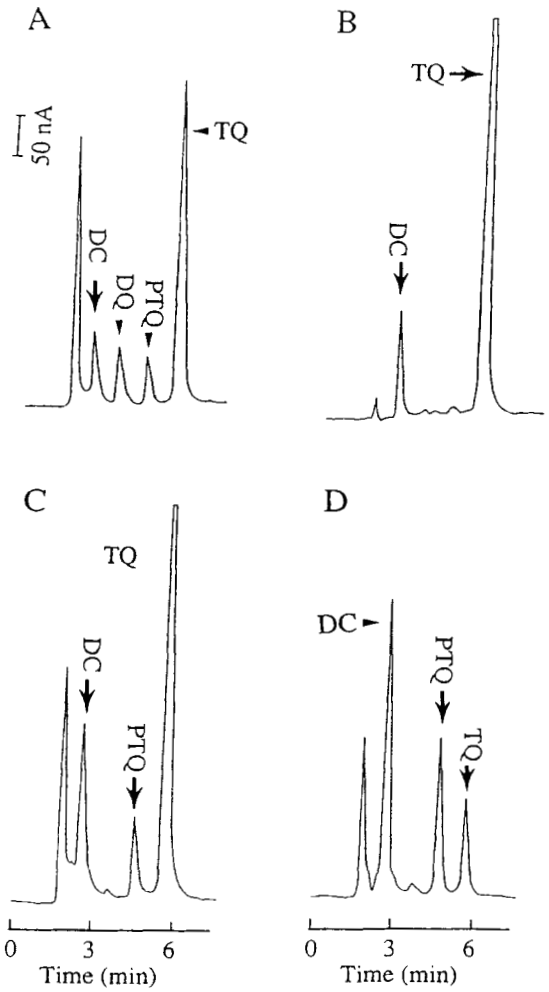

Figure 4. HPLC-ED analysis of product and intermediates during L-dopa oxidation by $NaIO_4$ under strong acidic conditions (20 mM citrate buffer, pH 2.5) using a reductive potential (-100 mV) of the working electrode. Chromatograms showing the detection of dopachrome (DC), dopaquinone (DQ), *o*-topaquinone (TQ) at one min after chemical oxidation (A), *o*-topaquinone formed during oxidation of authentic topa (1 mM) using mushroom tyrosinase (40 μg/ml) after 10 min incubation (B), and dynamic changes of individual components at 10 (C) and 40 min (D) after chemical oxidation of L-dopa using $NaIO_4$ under strong acidic conditions. The reducible species with a retention time of 4.7 min (C&D) was considered to be *p*-topaquinone (PTQ) derived from the *o*-topaquinone.

Accompanying the decrease of *o*-topaquinone, another electrochemically reducible species with a retention time of 4.7 min gradually increased in the reaction mixture (Fig. 4D). There also was an increase in the concentrations of dopachrome in the reaction mixture, which suggested that dopachrome could be produced from the quinone species derived from topa (Fig. 4B-C). The compound detected at 4.7 min was tentatively considered to be *p*-topaquinone derived from *o*-topaquinone, because *p*-topaquinone is structurally more stable than *o*-topaquinone and because $NaBH_4$ treatment of the reaction mixture containing both electrochemically reducible species with retention times of 4.7 and 5.8 min resulted in the detection of topa (see Fig. 5).

There was a small amount of L-dopa left in the L-dopa/$NaIO_4$ reaction mixture containing citrate buffer (pH 2.5) after 1 min of oxidation (Fig. 5A). HPLC-ED analysis (at an oxidative potential of the working electrode) of the $NaBH_4$ treated L-dopa/$NaIO_4$ reaction mixture prepared with citrate buffer (pH 2.5) resulted in the detection of leukodopachrome (2.9 min), L-dopa (9.6 min) and an oxidizable species with a retention time of 6.2 min (Fig. 5B-D). The compound with a retention time of 6.2 min was identified as topa reduced from its respective topaquinones (see Fig. 4A,C&D), because this peak coeluted with authentic topa at all chromatography conditions used (data not shown). It also is clear from the chromatograms that the concentrations of detected leukodopachrome, topa and L-dopa (Fig. 5B-D) correlated well with the concentrations of dopachrome, topaquinone and L-dopa detected at a reductive potential of the working electrode (Fig. 4A,C&D). Accumulation of topaquinone became significant only under strong acidic conditions ($pH \leq 3.5$).

Phosphate buffer (pH 6.5) had a significant effect on the chemical reaction of dopaquinone to dopachrome. Oxidation of L-dopa using $NaIO_4$ resulted in a significant accumulation of dopaquinone in the reaction mixture that did not contain phosphate buffer (Fig. 6A), and dopaquinone accumulation decreased substantially as phosphate buffer concentration increased (Fig. 6B-D). Oxidation of L-dopa using $NaIO_4$ in either 20 mM phosphate buffer or citrate buffer (pH 6.0)

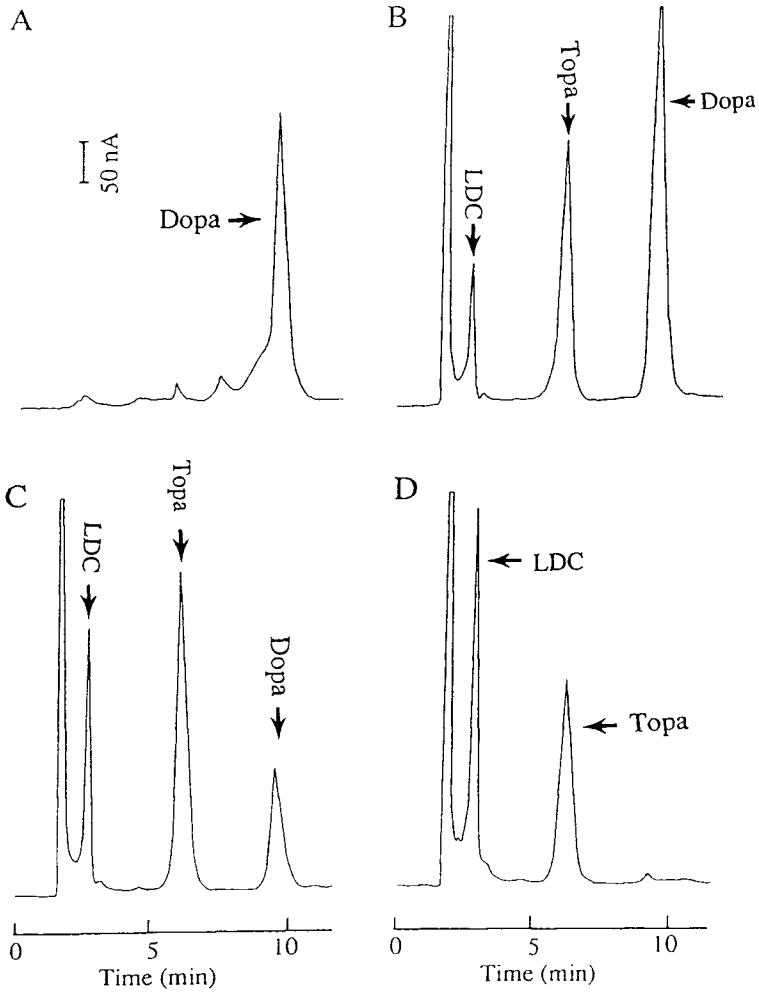

Figure 5. HPLC-ED analysis of $NaBH_4$ treated L-dopa/$NaIO_4$ reaction mixture (oxidized under acidic conditions, pH 2.5) using an oxidative potential (750 mV) of the working electrode. Chromatograms showing relative amount of L-dopa left after 1 min oxidation (A), and formation of leukodopachrome (LDC), topa and L-dopa from their respective oxidized forms due to $NaBH_4$ reduction at 1 (B), 10 (C) and 40 min (D) following chemical oxidation of L-dopa using $NaIO_4$. The conditions of L-dopa oxidation were identical as in Fig. 4.

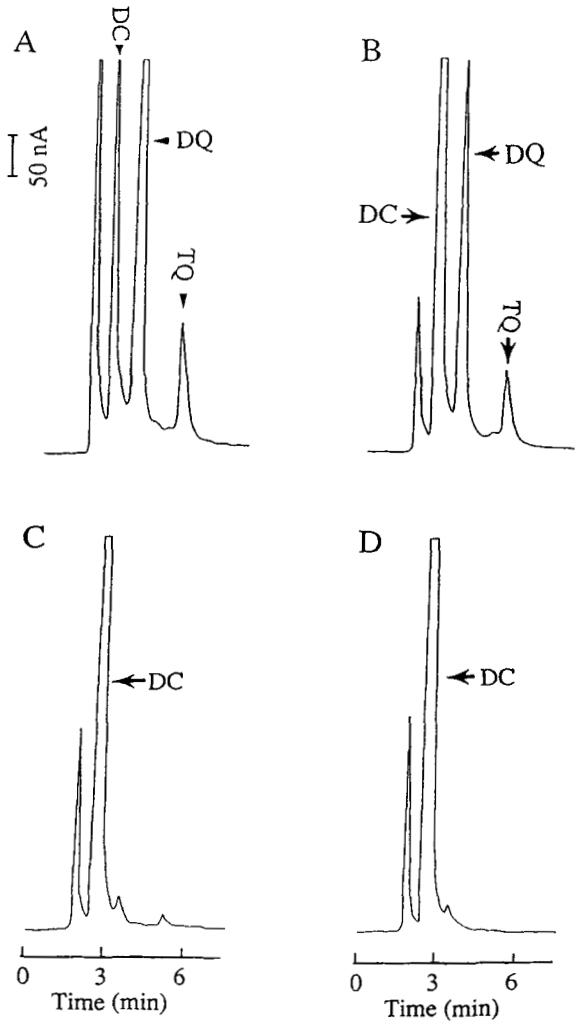

Figure 6. Determination of the effect of phosphate buffer on the chemical reaction of dopaquinone to dopachrome. L-dopa solution (2 mM) prepared using 100 mM NaCl containing varying concentration of phosphate buffer (0 - 5 mM), pH 6.5, was oxidized using $NaIO_4$ (10.7 mg/10 ml). The reaction solution was filtered at 30 sec after incorporation of $NaIO_4$ and 4 µl of the filtrate was injected for HPLC-ED analysis using a reductive potential (-100 mV) of the working electrode at 40 sec after initiation the chemical oxidation. High concentrations of dopaquinone (DQ) and small amounts of topaquinone (TQ) accumulated in the L-dopa/$NaIO_4$ reaction mixture prepared using NaCl alone (A), the incorporation of phosphate buffer (pH 6.5) at a concentration of 1.25 mM greatly decreased the concentration of dopaquinone in the reaction mixture (B), and an increase of phosphate buffer to 2.5 mM (C) and 5 mM (D) resulted in the detection of a small amount of dopaquinone in the reaction mixture.

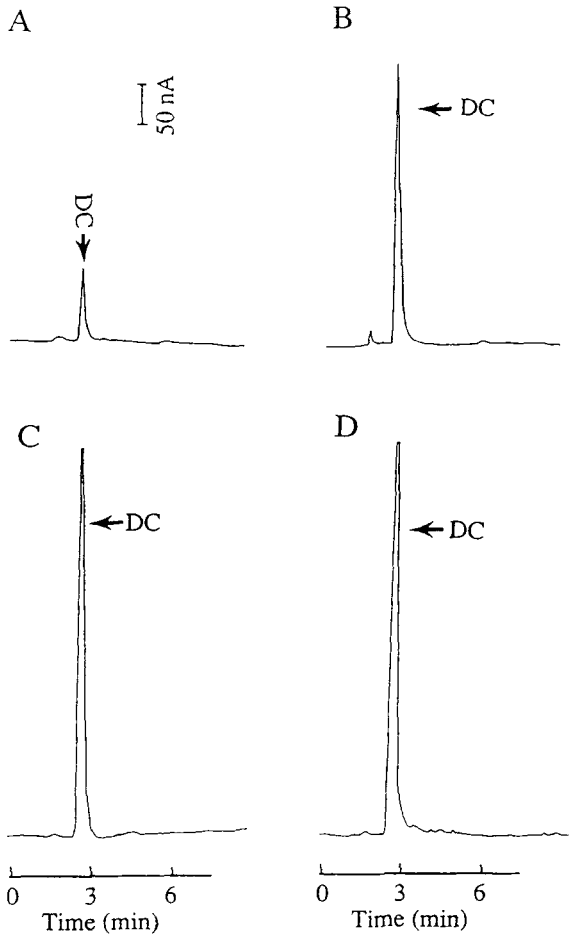

Figure 7. HPLC-ED analysis of L-dopa oxidation using mushroom tyrosinase at a reductive potential (-100 mV) of the working electrode. Reaction mixture of 800 µl, consisting of 0.8 µmol of L-dopa and 80 µg of mushroom tyrosinase in 20 mM citrate buffer (pH 4 - 6), was incubated at 30°C. The reaction solution was filtered after 6 min incubation and 5 µl of the filtrate was analyzed using HPLC-ED at identical conditions as in Fig. 2. Chromatograms showing formation of dopachrome (DC) in L-dopa/mushroom tyrosinase reaction mixture prepared in 20 mM citrate buffer, pH 4.0 (A), pH 4.5 (B), pH 5.0 (C) and pH 6.0 (D) after 6 min incubation.

alone resulted in no accumulation of dopaquinone at any time during the L-dopa oxidation. Likewise, the incorporation of 20 mM phosphate buffer (pH 6.5) into a L-dopa/$NaIO_4$ reaction mixture (prepared in either distilled water or 100 mM NaCl solution) at 1 min after oxidation resulted in the instant disappearance of dopaquinone and the accumulation of high concentrations of dopachrome (data not shown). Other buffers (HEPES and MOPS) under neutral conditions had the same effect on catalyzing the chemical reactions of dopaquinone to dopachrome, but neutral salts (NaCl, KCl and $CaCl_2$ at 20 - 100 mM) had no effect (see Fig. 6A as reference), thereby suggesting that the accelerated transformation of dopaquinone to dopachrome was not due to the ionic strength of the reaction solution. However, when mushroom tyrosinase was used to oxidize L-dopa in citrate buffer (pH 4.0 - 6.0), the only detectable product was dopachrome. No accumulation of dopaquinone was detected under the applied pH conditions (Fig. 7A-D). Reduction of the mushroom tyrosinase/L-dopa reaction mixture using $NaBH_4$ resulted in the formation of leukodopachrome as the detectable product (not shown).

DISCUSSION

Electrochemistry of catacholamines, such as L-dopa, dopamine, norepinephrine etc, has been extensively studied using cyclic voltammetry (3) to determine their oxidation and reduction potentials and the intermediates that occur during oxidation and reduction. The *o*-quinone species derived from catecholamine oxidation generally are unstable; therefore, several steps of chemical reactions often follow quinone formation. Cyclic voltammograms of catacholamines can often supply sufficient information to enable one to suggest the identities of intermediates formed during the electrochemical oxidation and reduction of catacholamines. However, if the components formed during electrochemical oxidation have similar oxidation or reduction potentials, it is difficult to distinguish these individual species using the cyclic voltammetric

method. Although our approach using HPLC-ED analysis for the identification of oxidized or reduced species is similar to cyclic voltammetry on the basis of the detection method, separation of individual components in the L-dopa/$NaIO_4$ or L-dopa/mushroom tyrosinase reaction mixture, and the selective detection using either an oxidative or reductive potential of the working electrode, make it possible to identify the individual species and monitor their dynamic changes during incubation.

The HPLC-ED method we used provided important information regarding the effect of pH on the biochemical pathway of L-dopa oxidation to dopachrome. Phosphate and other buffers, used around neutral pH, greatly accelerated the chemical reactions of dopaquinone to dopachrome (Fig. 6A-D). It is likely that the negatively charged phosphate groups function as hydrogen proton acceptors, thereby accelerating the deprotonation of the side chain amino group of dopaquinone. This is the first step in the intramolecular cyclization of dopaquinone to dopachrome (1,2,4). Therefore, the buffer enhanced dopachrome formation from dopaquinone is a base-catalyzed chemical reaction.

Our results also suggest a significant difference between mushroom tyrosinase and $NaIO_4$ catalyzed oxidation of L-dopa. $NaIO_4$ often has been used to oxidize L-dopa and its derivatives in attempts to delineate the possible biochemical pathways and the individual intermediates formed during melanin biosynthesis in living organisms (1,2). Because the formation of topa from dopaquinone is dependent on strong acidic conditions ($pH \leq 3.5$), and because tyrosinase is not biologically functional at $pH \leq 4.0$ (Fig. 7A), topa and topaquinone are likely not involved in the enzyme-mediated biochemical oxidation of L-dopa. Therefore, care should be taken when evaluating the biochemical pathway of melanogenesis in living organisms using results obtained from nonenzymatic, chemical-mediated L-dopa oxidation.

Our data strongly suggest that the accumulation of dopaquinone is a prerequisite for the formation of topa, which is readily oxidized to topaquinone, but the accumulation of dopaquinone itself does not result in the formation of high concentrations of topaquinone in the reaction mixture (Fig. 2C-D and Fig. 6A-B).

These results suggest that hydroxylation of dopaquinone to produce topa is an acid catalyzed reaction, because topaquinone (a direct indicator of topa production) became obvious only under conditions of higher acidity (pH$\leq$3.5). It also is evident that the stability of dopaquinone, derived from L-dopa oxidation, and its further chemical reactions depends largely on pH and buffer concentration. Therefore, half lives of the quinone species reported (3) and the kinetic studies of L-dopa oxidation (1, 2) are difficult to evaluate without consideration of the reaction conditions used in the oxidation of L-dopa.

In summary, using HPLC-ED analysis we were able to (1) identify individual species formed during L-dopa oxidation to dopachrome, (2) determine dynamic changes of individual species in the L-dopa/$NaIO_4$ reaction mixture, (3) demonstrate the effects of base on acceleration of dopaquinone to dopachrome, (4) determine the effect of acid on hydroxylation of dopaquinone to topa, and (5) distinguish the differences in enzymatic and chemical-mediated oxidation of L-dopa.

ACKNOWLEDGEMENTS

This work was supported by the NIH grant AI 19769 and the College of Agricultural and Life Sciences.

REFERENCES

1. J.N. Rodríguez-López, J. Tudela, R. Varón, F. García-Cánovas, Biochim. Biophys. Acta, 1076: 379-386 (1991)

2. F. García-Cánovas, F. García-Carmona, J. Vera-Sánchez, J.L. Iborra-Pastor, J.A. Lozano-Teruel, J. Biol. Chem., 257: 8738-8744 (1982)

3. G. Dryhurst, K. M. Kadish, F. Scheller, R. Renneberg, Biological Electrochemistry, Academic Press, Inc., New York, 1982.

4. Cabanes, J., F. García-Cánovas, J.A. Lozano, F. García-Carnoma, Biochim. Biophys. Acta, 923: 187-195 (1987)

Received: July 30, 1992
Accepted: August 11, 1992