BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 2620—2622 (1970)

The Reaction of 3,4-Dihydroxyphenylalanine with *p*-Benzoquinone under Physiological Conditions

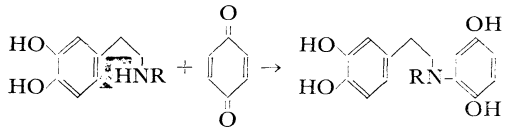
Aizo HIKOSAKA and Ju KUMANOTANI

Engineering Research Institute, Faculty of Engineering, University of Tokyo, Bunkyo-ku, Tokyo

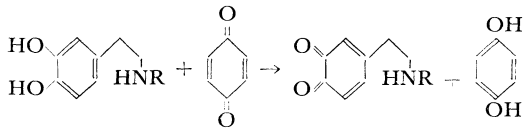
(Received December 23, 1969)

When 3,4-dihydroxyphenylalanine (dopa) is oxidized in the presence of tyrosinase, the oxidation is considerably enhanced by *p*-benzoquinone.[1] However, the stimulation by the quinone has not yet been elucidated.

This study was undertaken in order to examine the interaction of dopa and *p*-benzoquinone *in vitro* under physiological conditions. Two types of reactions were expected to occur; one of these was the ring amination of the quinone with dopa (Eq. (1)), and the other was the oxidation of dopa with the quinone (Eq. (2)).



(1)



(2)

In this study, the spectrum for the enzymic oxidation of dopa[2] was compared with that of the reaction of dopa with *p*-benzoquinone. It was found that these spectra were essentially identical.

The scheme for the enzymic oxidation of dopa by tyrosinase[3] has been confirmed polarographically.[4] The oxidation products of dopa give polarographic waves. In this study, by a comparison of the observed polarogram for the enzymic oxidation of dopa with that for the reaction of dopa with *p*-benzoquinone *in vitro* under physiological conditions (*i.e.*, in a buffer solution of pH 7.0 at ordinary temperatures), it was found that the polarograms (Fig. 2) were essentially identical, except that the anodic-cathodic wave for the hydroquinone and the quinone appeared in the dopa-*p*-benzoquinone reaction.

In view of the above facts, it appears that the reaction of dopa with *p*-benzoquinone *in vitro* under physiological conditions involves mainly the redox reaction.

The other expected course of the reaction was the amination of the quinone ring by the amino group of dopa. Polarographic investigation showed, however, that the rate of the reaction was much smaller than that of the redox reaction of dopa with *p*-benzoquinone. It is likely that the contribution of amination to the overall reaction was very small.

Experimental

Materials. Dopa (mp 271—272°C decomp. Found: C, 54.73; H, 5.59; N, 6.89%. Calcd for $C_9H_{11}O_4N$: C, 54.82; H, 5.62; N, 7.10%) was obtained commercially. No polarographic reduction wave was found in a solution of dopa. *p*-Benzoquinone (mp 116°C) was purified by the sublimation of commercially available chemicals. The buffers used were prepared according to the method of Sörensen in our laboratory. A tyrosinase preparation from a mushroom was kindly supplied by Prof. Y. Ogura.

Apparatus. The visible spectra were recorded on a Hitachi Recording Spectrophotometer, Model EPS-2U. The polarographic determinations were made with a Yanagimoto Pen Recording Polarograph, PA-102.

The Determination of 2,3-Dihydroindol-5,6-quinone (Red Quinone). 1) *Visible Spectroscopy. Enzymic Oxidation of Dopa*: To 3.0 m*l* of a solution buffered at pH 6.8 and containing 0.214 mg (1.09×10^{-6} mol) of dopa per m*l*, we added about 0.1 m*l*, of a tyrosinase solution. The absorption spectra were then immediately measured. The results are depicted in Fig. 1. The absorption spectra of intermediates between dopa and the pigment did not become evident. The experiment was conducted at room temperatures.

Reaction of Dopa with p-Benzoquinone: To 2.0 m*l* of a solution buffered at pH 6.8 and containing 0.106 mg (9.8×10^{-7} mol) of dopa per m*l*, we added 1.0 m*l* of a solution buffered at pH 6.8 and containing 0.229 mg (2.1×10^{-6} mol) of *p*-benzoquinone per m*l*. The experiment was conducted at room temperatures. The absorption spectrum of the pigment obtained in this manner was the same as that found in the enzymic oxidation.

2) *Polarography*. The experimental conditions were the same as those of the spectrophotometric studies.

1) Private communication.
2) H. S. Mason, *J. Biol. Chem.*, **172**, 83 (1948).
3) H. S. Mason, "Advances in Enzymology," Vol. 16, Interscience, New York, London (1955), p. 105.
4) H. S. Raper, *Biochem. J.*, **21**, 89 (1943).
5) K. Wiesner, *Biochem. Z.*, **314**, 2214 (1943).

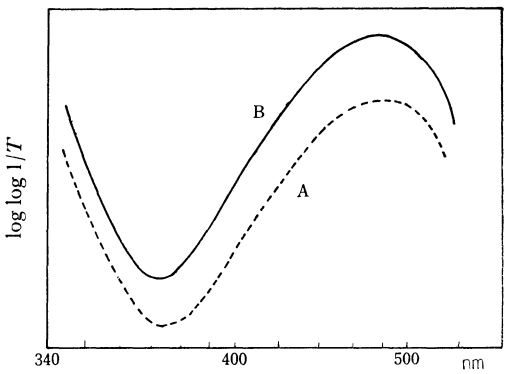


Fig. 1. Absorption spectra of oxidized dopa.
A: Enzymically catalyzed oxidation of dopa. Three m*l* of a solution containing 0.624 mg of dopa buffered at pH 6.8 and 0.1 m*l* of enzyme solution.
B: Oxidation of dopa with *p*-benzoquinone. Two m*l* of a solution containing 0.212 mg of dopa buffered at pH 6.8 and 1.0 m*l* of a solution containing 0.229 mg of *p*-benzoquinone.

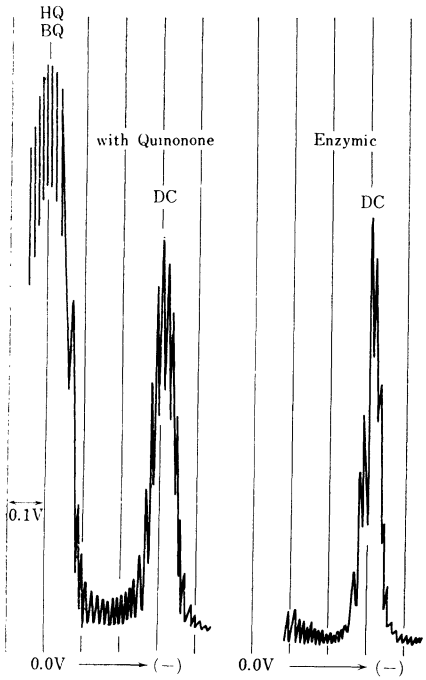


Fig. 2. Polarograms of dopachrome from tyrosinase catalyzed oxidation of dopa and from dopa-*p*-benzoquinone reaction.
DC; Dopachrome, BQ; *p*-Benzoquinone, HQ; Hydroquinone

Enzymic Oxidation of Dopa: The reaction mixtures showed a polarographic wave at −0.20 V.

Reaction of Dopa with p-Benzoquinone: The reaction mixtures showed two polarographic waves, at −0.20 and at 0.0 V. The polarograms are all essentially identical with the exception of the anodic-cathodic wave (at 0.0 V) for the hydroquinone and the quinone.

Reaction of Dopa with *p*-Benzoquinone. The buffered solution of dopa and that of the quinone were deoxygenated by passing nitrogen through, and then they were mixed in a polarographic cell in order to initiate the reaction. Nitrogen was bubbled through the reaction mixture in order to remove any oxygen and agitate the solution. The bubbling was stopped during the recording of the polarograms. Polarographic curves were registered at regular intervals, and the amounts of the unchanged quinone and its hydroquinone were determined by means of the polarograms.

To 3.0 m*l* of an oxygen-free solution buffered at pH 7.0 (phosphate buffer) and containing 0.091 mg (4.6×10^{-7} mol) of dopa per m*l*, we added 3.0 m*l* of an oxygen-free solution buffered at pH 7.0 (phosphate buffer) containing 0.15 mg (1.4×10^{-6} mol) of *p*-benzoquinone per m*l*. The rate of the reduction of the quinone was obtained at 28°C by polarography. The results are given in Fig. 3.

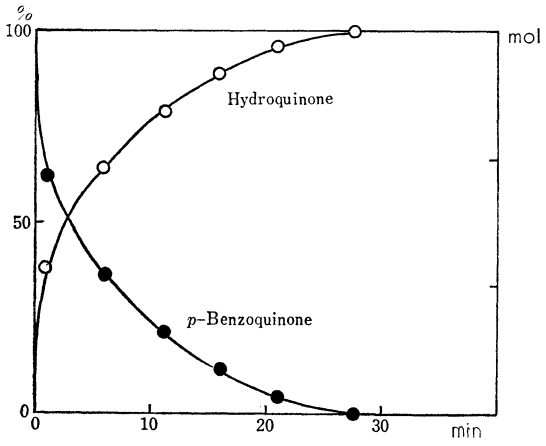


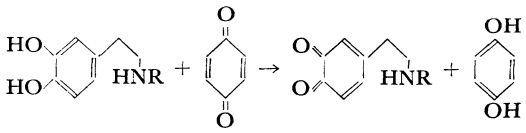
Fig. 3. Change of amount of *p*-benzoquinone and that of hydroquinone in dopa-*p*-benzoquinone reaction. The final reaction mixtures contained 4.6×10^{-7} mol of dopa and 1.4×10^{-6} mol of the quinone in a total volume 6.0 m*l* in 0.03 M phosphate buffer at pH 7.0. The experimental was run in a polarographic cell at 25°C.

The experimental results show that, at any instant, the amount of hydroquinone formed was equal to the amount of quinone that had disappeared. Three moles of the quinone were quantitatively reduced to hydroquinone by one mole of dopa. Therefore, one might conclude that the redox reaction took place. When more than three moles of the quinone were used, hydroquinone was not quantitatively formed, although the amount of excess quinone decreased.

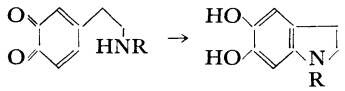
Results

The experimental results showed that dopa was oxidized to indol-5,6-quinone by *p*-benzoquinone. The oxidation was similar to the oxidation by phenoloxidase or that by inorganic oxidants.

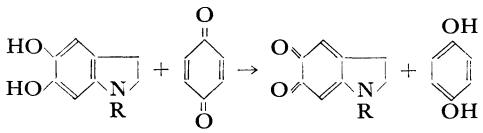
One molecule of dopa is capable of reducing 3 molecules of the quinone. The following scheme can be put forward to explain the reaction:



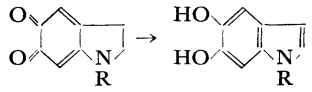
Next, the quinon undergoes an intramolecular change, with the production of 5,6-dihydroxy-2,3-dihydroindol derivatives:



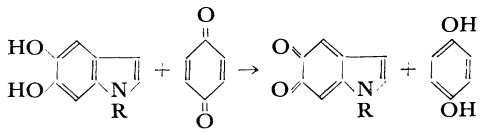
This is in turn oxidized to its quinone, which is the red pigment which appears in the first of the reaction stages:



This pigment undergoes further change on standing and yields 5,6-dihydroxyindole derivatives:



These are oxidizable in the air or by the quinone and yield indol-5,6-quinone derivatives:



The problem of the fate of indol-5,6-quinone derivatives is not discussed in the present paper.