Studies on Melanin Formation in Animals (II) : The Acceleration of Tyrosine Oxidation by Copper-Ammine Complex

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Studies on Melanin Formation in Animals
II. The Acceleration of Tyrosine Oxidation by Copper-Ammine Complex*

By

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Concerning the mammalian melanin formation, it has been stated by LERNER (1953) that the oxidation of tyrosine catalyzed by the copper enzyme, tyrosinase, is accelerated in the presence of 3, 4-dihydroxyphenylalanine, dopa, but that tyrosinase can not act on tyrosine in the absence of dopa. In his studies of non-enzymatic oxidation of tyrosine, FOSTER (1950) pointed out that tyrosine could be oxidized in the presence of both cupric ions and a trace of dopa. Henceforth, it has been accepted by many workers in this field that the presence of a "threshold concentration" of dopa is indispensable for the reaction from tyrosine to dopa.

In our previous paper, it was reported that the ethylenediamine, a chelating substance, acted according to its concentration inhibitively or acceleratively upon the non-enzymatic oxidation of monophenol when it was catalyzed by copper-ammine, and that the different action was due to both (1) higher stability constant between chelating substance and $Cu^{(II)}$ portion of the complex than the stability constant between substrate and the $Cu^{(II)}$, and (2) amount of the chelating substance against the copper complex. From the results obtained, we have proposed a plausible scheme of tyrosinase reaction. The mechanism in the accelerating autoxidation of tyrosine caused by the presence of a trace of dopa will be discussed in this paper.

Here we wish to thank Professor Dr. M. ICHIKAWA for his help for the preparation of the manuscript. Our thanks are also due to Dr. Y. FUJIMURA of the Research Institute for Food for his generous supply of oxidized form of ascorbic acid. We are also indebted to Professor Dr. S. TANAKA of the Biochemical Institute in the College of Science, Mr. S. KITAOKA of the Biochemical Laboratory in the College of Agriculture, for their valuable suggestions.

Experimental
Material and Methods

Accelerating effect of catechol, dopa and ascorbic acid upon the autoxidation

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of tyrosine catalyzed by copper-ammine: The main part of each manometrical vessel contained 1.5 ml of phosphate buffer (pH 10), 1 ml of 0.1 M copper-ammine and 1 ml of 0.04 M *l*-tyrosine. The centre well contained 0.3 ml of 10% KOH. The side arm contained 0.5 ml of such solution as 0.0025 M catechol, 0.0005 M *l*-dopa or 0.005 M ascorbic acid. These solutions were replaced by distilled water in their blank test. The gas space of the vessel was filled with air. The cock was closed after the vessel reached the thermal equilibrium, and then the solution of the materials was tipped from the side arm into the main part. The vessel was shaken for a given period at a rate of about 110 strokes per minute at 35°C, and the volume of oxygen-uptake in the buret was read at appropriate intervals.

Accelerating effect of oxidized forms of catechol, dopa and ascorbic acid upon the autoxidation of tyrosine catalyzed by copper-ammine: The main part of each manometrical vessel contained 1.5 ml of phosphate buffer (pH 10), 1 ml of 0.1 M copper-ammine and 0.5 ml of such solution as 0.0025 M catechol, 0.005 M dopa or 0.005 M oxidized form of ascorbic acid. The side arm contained 1 ml of 0.04 M *l*-tyrosine. Other conditions are the same as previous. After shaking at an aforesaid rate for 40 minutes in the case with catechol, and for 80 minutes in the case with dopa, when the rate of oxygen-uptake became constant (zero), the cock was opened, and then the tyrosine solution was tipped from the side arm into the main part. (The constant oxygen-uptake in these cases means the formation of the oxidized forms of the chelating substances.) The volume of oxygen-uptake was then estimated successively at appropriate intervals. In blank test the tyrosine solution was replaced by distilled water. In the case with the oxidized form of ascorbic acid (prepared by Dr. Y. FUJIMURA) the experimental procedures and conditions were the same as in the case of ascorbic acid.

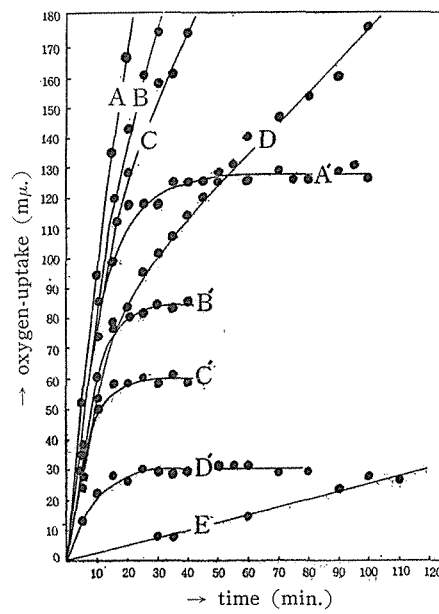

Fig. 1. Accelerating effect of catechol on autoxidation of tyrosine catalyzed by copper-ammine complex (pH 10, 35°C).

A', *B'*, *C'* and *D'*: oxygen-uptake in the system of catechol involving various concentrations of o-benzoquinone and copper-ammine.

A, *B*, *C* and *D*: oxygen-uptake in each system of the catechol, tyrosine and copper-ammine; *A* corresponds to *A'* and so on.

E: oxygen-uptake in the system of tyrosine and copper-ammine.

Results

Accelerating effect of catechol upon the autoxidation of tyrosine catalyzed by copper-ammine: As a preliminary experiment the autoxidation of the catechol catalyzed by copper-ammine was examined in the presence of the various concentrations of o-benzoquinone. The results are indicated by A', B', C' and D' in Fig. 1. The figure indicates that the rates of autoxidation of these solutions decrease in the order, $A'>B'>C'>D'$, and that the concentrations of o-benzoquinone before the autoxidation increase in the reversed order: $A'<B'<C'<D'$.

Next, the rates of oxygen consumption in the system of copper-ammine, tyrosine and catechol were measured, and the results obtained are shown by A, B, C and D in Fig. 1. The rates of tyrosine autoxidation in each system catalyzed by copper-catechol (1 : 1) complex increase in accordance with the decrease of o-benzoquinone concentration or with the increase of catechol concentration. These results reveal that the accelerated autoxidation of tyrosine may be dependent upon the concentration of catechol involved in the system, but not so much on the concentration of o-benzoquinone. The autoxidation of tyrosine catalyzed by copper-ammine complex is indicated by E in Figs. 1, 2 and 3.

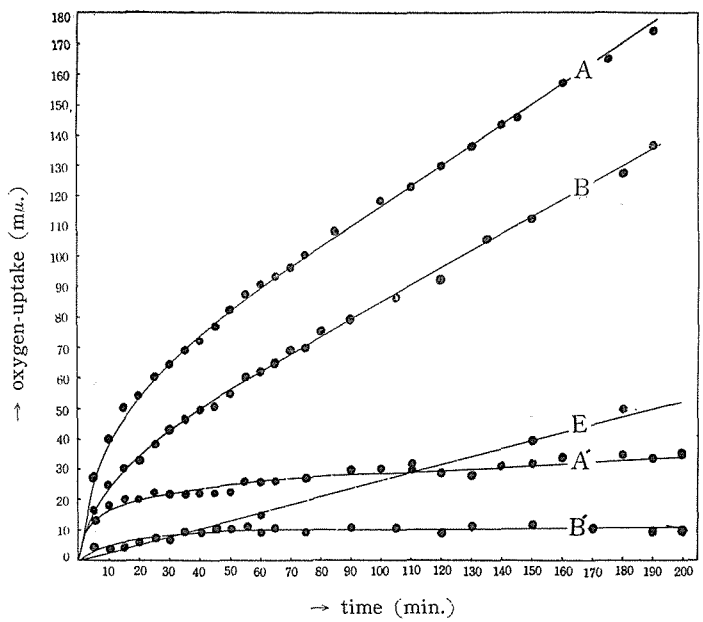

Fig. 2. Accelerating effect of dopa on autoxidation of tyrosine catalyzed by copper-ammine complex (pH 10, 35°C).

A and B: oxygen-uptake in the system of dopa (B; contained dopaquinone), tyrosine and copper-ammine.
A' and B': oxygen-uptake in the system of dopa (B'; contained dopaquinone) and copper-ammine.
E: oxygen-uptake in the system of tyrosine and copper-ammine.

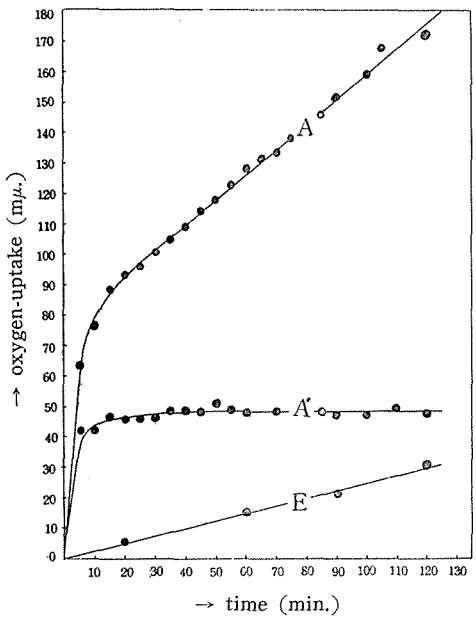

Fig. 3. Accelerating effect of ascorbic acid on oxidation of tyrosine catalyzed by copper-ammine complex (pH 10, 35°C).

A: oxygen-uptake in the system of ascorbic acid, tyrosine and copper-ammine.
A': oxygen-uptake in the system of ascorbic acid and copper-ammine.
E: oxygen-uptake in the system of tyrosine and copper-ammine.

Accelerating effect of dopa upon the autoxidation of tyrosine catalyzed by copper-ammine: *A'* and *B'* in Fig. 2 show the autoxidation (catalyzed by copper-ammine complex) of a freshly prepared colourless dopa and a brown coloured dopa which was exposed to air for three days at room temperature. The order of $A' > B'$ is parallel to the order in the case of catechol, i.e., it is general that oxygen-uptake of the reduced form is greater than that of the oxidized form.

A and *B* in Fig. 2 indicate the accelerating effect of the colourless fresh dopa and brown coloured dopa upon the autoxidation of tyrosine respectively. From the comparison of *A* and *B*, the former is obviously superior in effectiveness to the latter.

Accelerating effect of ascorbic acid upon the autoxidation of tyrosine catalyzed by copper-ammine: Autoxidation in the composite system of tyrosine, ascorbic acid and copper-ammine complex, and the autoxidation of ascorbic acid catalyzed by copper-

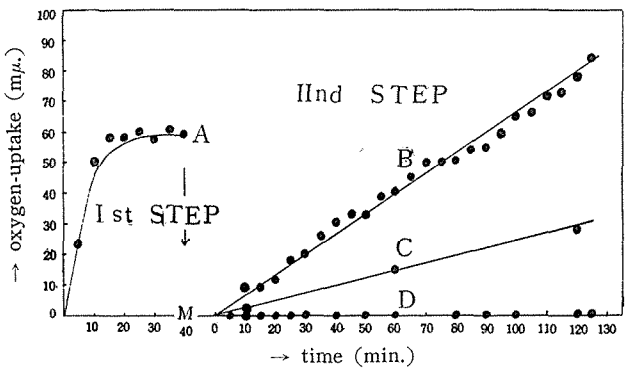

Fig. 4. Accelerating effect of oxidized form of catechol upon the autoxidation of tyrosine catalyzed by copper-ammine complex (pH 10, 35°C).

A: oxygen-uptake in the system of catechol and copper-ammine.
B: oxygen-uptake in the system of tyrosine, copper-ammine and oxidized form of catechol.
C: oxygen-uptake in the system of tyrosine and copper-ammine.
D: oxygen-uptake in the system of copper-ammine and oxidized form of catechol.
M: showing the time when the cock was opened and tyrosine was added.

ammine complex are given by A and A' in Fig. 3. E in the same figure represents the autoxidation of tyrosine catalyzed by copper-ammine complex. From the comparison of these three lines the accelerating effect is clear of the reducing agent on the autoxidation of tyrosine.

Accelerating effect of oxidized form of chelating substances upon the autoxidation of tyrosine catalyzed by copper-ammine: As is shown by A in Fig. 4, the rate

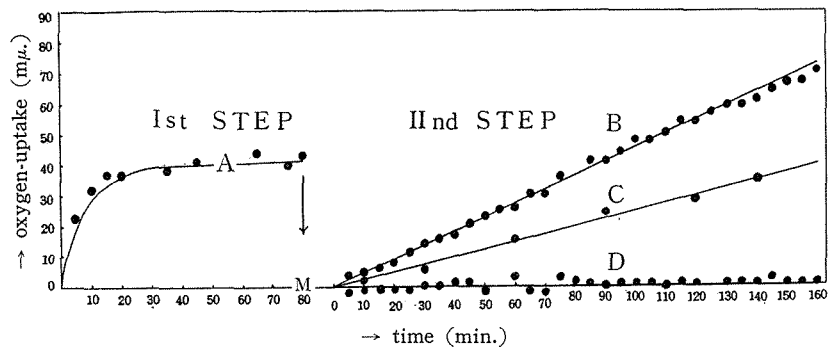

Fig. 5. Accelerating effect of oxidized form of dopa upon the autoxidation of tyrosine catalyzed by copper-ammine complex (pH 10, 35°C).

A: oxygen-uptake in the system of dopa and copper-ammine.
B: oxygen-uptake in the system of tyrosine, copper-ammine and oxidized form of dopa.
C: oxygen-uptake in the system of tyrosine and copper-ammine.
D: oxygen-uptake in the system of copper-ammine and oxidized form of dopa.
M: showing the time when the cock was opened and tyrosine was added.

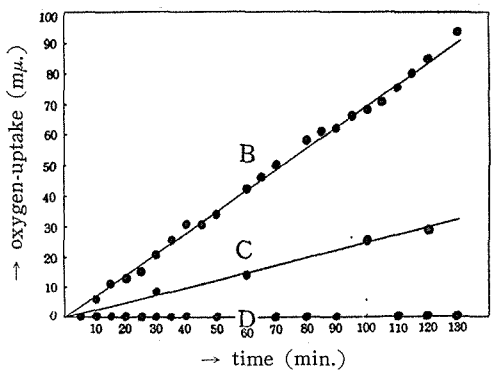

Fig. 6. Accelerating effect of oxidized form of ascorbic acid upon the autoxidation of tyrosine catalyzed by copper-ammine complex (pH 10, 35°C).

B: oxygen-uptake in the system of oxidized form of ascorbic acid, tyrosine and copper-ammine.
C: oxygen-uptake in the system of tyrosine and copper-ammine.
D: oxygen-uptake in the system of copper-ammine and oxidized form of ascorbic acid.

of oxygen-uptake due to the autoxidation of catechol reaches constant (zero) after about 40 minutes as in the case of *A'*, *B'*, *C'* and *D'* in Fig. 1. This means that the catechol becomes converted into the oxidized form. The rate of oxygen-uptake of the oxidized catechol thenceforth can be presented by *D* in Fig. 4. *C* in the same figure represents the rate of oxygen-uptake of tyrosine catalyzed by copper-ammine alone. When the two materials are mixed, the rate of oxygen-uptake is found remarkably enhanced synergistically, as is shown by *B* (tyrosine, oxidized form of catechol and copper-ammine).

The similar results have been obtained in the experiments using both oxidized form of dopa and ascorbic acid as shown in Figs. 5 and 6 respectively. From these results, it is clear that the autoxidation of tyrosine is accelerated by the oxidized form of chelating substances such as catechol, dopa and ascorbic acid. But, this accelerating effect is more marked when the reduced form of each substance is used.

Discussion and Summary

It has been pointed out by LERNER and FOSTER that dopa is converted into dopaquinone when it is participating in the accelerating oxidation of tyrosine (1, 2). Their view has been supported also in our previous paper (3, 4) in which we have concluded that this acceleration caused by a small amount of chelating substance would be due to the special property of a metal complex which increases the stability constant between tyrosine and $Cu^{(II)}$. But in the present experiments with catechol, dopa and ascorbic acid the autoxidation of tyrosine was more accelerated than in the experiment with the oxidized form of these substances, as is clearly understandable from the comparison of Figs. 1, 2 and 3 and Figs. 4, 5 and 6. This

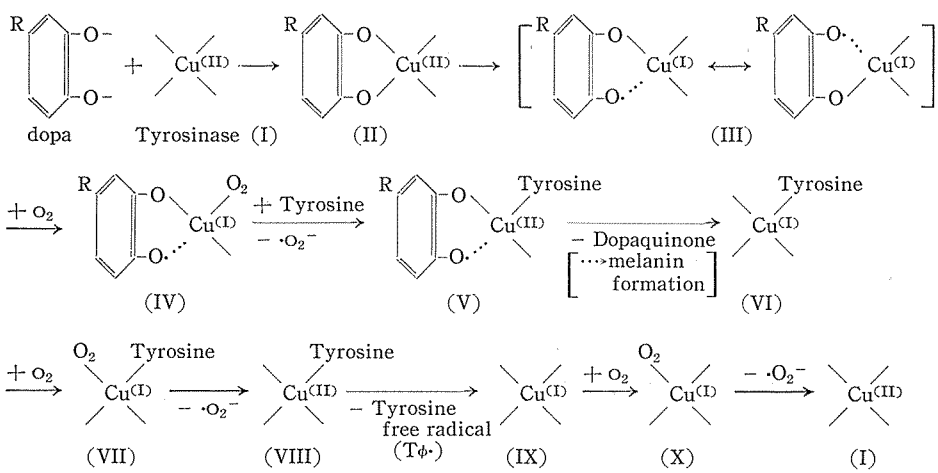

Fig. 7. Reaction mechanism of tyrosine oxidation accelerated by the presence of small amount of dopa. (*R*: $CH_2 \cdot CH \cdot NH_2 \cdot COOH$)

evidence indicates that the form of dopa, for instance, when participating in the accelerating reaction, would be dopasemiquinone as shown in Fig. 7.

The complex (II) in the Fig. 7 would be produced at first by the coordinative binding between dopa and $Cu^{(II)}$ portion of copper complex of tyrosinase (I). After one electron is transported from dopa to $Cu^{(II)}$ portion, the complex (II) will be converted into the unstable dopasemiquinone-$Cu^{(I)}$ complex (III). $Cu^{(I)}$ portion of the complex (III) will be attacked by oxygen molecule dissolved in the system to produce the complex (IV) and to set an $\cdot O_2^-$ ion free. The latter is formed from oxygen in the complex (IV) by addition of one electron from $Cu^{(I)}$ portion. The position occupied by this oxygen is coordinated by tyrosine as is shown in the complex (V). In the complex (V), one electron is transported from dopasemiquinone to $Cu^{(II)}$ portion, and the complex (V) separates into a dopaquinone and tyrosine-$Cu^{(I)}$ complex (VI). The dopaquinone will be consumed as mentioned by FOSTER for melanin formation as soon as it is produced. From the equation,

$$K = \frac{[\text{dopaquinone-Cu}^{(II)}]}{[\text{dopaquinone}][\text{Cu}^{(II)}]}$$

therefore, higher concentration of dopaquinone-$Cu^{(II)}$ complex will not be expected usually in these reaction systems.

The tyrosine-$Cu^{(II)}$ complex (VIII) will be formed from the O_2–tyrosine-$Cu^{(I)}$ complex (VII) which is produced through the interaction between a molecular oxygen and $Cu^{(I)}$ portion in complex (VI) (5). By transfering one electron from tyrosine to $Cu^{(II)}$ portion in the complex (VIII), a tyrosine free radical ($T\phi\cdot$), precursor of dopa, and $Cu^{(I)}$ complex (IX) will be produced, and the $Cu^{(I)}$ complex (X) will be returned to the initial form, the complex (I), through the interaction between a molecular oxygen and $Cu^{(I)}$ portion in complex (X).

Under these consideration, it is safe to say that an important meaning of the presence of a small amount of dopa for the autoxidation of tyrosine is in the formation of an unstable intermediate metal complex of dopasemiquinone.

In the oxidation from tyrosine to dopa catalyzed by tyrosinase, an accelerating mechanism by dopaquinone has been suggested by LERNER. He speculated also that the catalytic form of copper portion of tyrosinase is $Cu^{(I)}$ form in his scheme. But usually, the $Cu^{(II)}$ form of tyrosinase must be more suitable for transfering the electron from tyrosine to copper portion of tyrosinase. For the electron-transfer from tyrosine to $Cu^{(II)}$ portion of the complex, it will be required that the electron density of $Cu^{(II)}$ portion of the complex must be as small as possible. The electron distribution would be considered to be smaller in $Cu^{(II)}$ portion of dopaquinone complex. Namely, it would be that dopaquinone has higher oxidation potential than does dopasemiquinone.

Therefore, the participation of dopaquinone complex in the autoxidation of tyrosine should be easier than that of dopasemiquinone complex. Nevertheless, the results obtained in the present experiments were opposite against our expectation. Unfortunately we can not understand the reason for it.

A possible scheme of enzyme action due to the dopasemiquinone complex is represented in Fig. 8. And the reaction in the accelerating oxidation of tyrosine will be given in the cyclic form as is shown in Fig. 9.

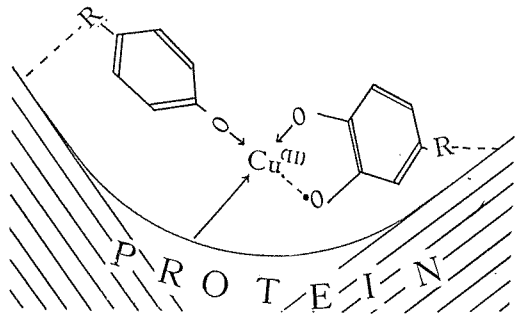

Fig. 8. Scheme of enzyme action.
R: $CH_2 \cdot CH \cdot NH_2 \cdot COOH$

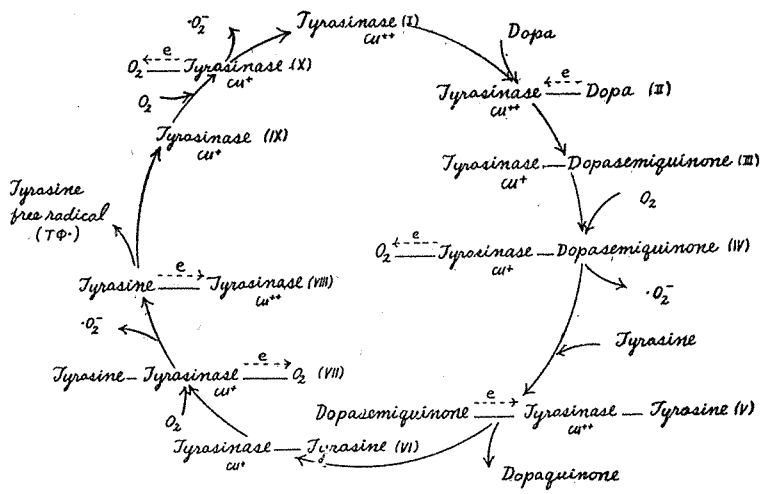

Fig. 9. Cycle of tyrosinase action.

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