MAMMALIAN TYROSINASE AND DOPA OXIDASE

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Most of the experimental work concerning the mechanism of melanin production in mammals indicates that the pigment is formed from the amino acid, dihydroxyphenylalanine (dopa), by the action of a specific oxidative enzyme. The evidence for this reaction has in large part been presented by Bloch (1), whose experiments were confirmed by Laidlaw and others (2). Bloch treated frozen sections of skin with a 1 per cent aqueous solution of dihydroxyphenylalanine buffered at pH 7.4, and over a period of 24 hours noted the appearance of a dark pigment in the cytoplasm of the melanoblasts. The reaction was inhibited by cyanide ion and by heat and failed to occur with a number of other substrates, including tyrosine. Bloch concluded that a specific enzyme, which he named dopa oxidase, is responsible for the formation of melanin.

Although several tyrosine-oxidizing enzymes have been extracted from certain plants and insects, there is no conclusive evidence for the existence of a mammalian tyrosinase. A number of attempts to demonstrate this enzyme in extracts of mammalian skin by means of a color reaction have been made, but the results are contradictory and inconclusive (3-9). Color reactions with dihydroxyphenylalanine have usually been obtained with such extracts, but the amount of dopa oxidase extractable is apparently very small. It seems evident, therefore, that normal pigmented tissues do not provide a rich enough source for any detailed study of this enzyme system.

The melanoma, a tumor composed chiefly of melanin-producing cells, might be expected to provide a richer source of the enzyme. Several experiments (10, 11) have indicated that extracts of melanomata possess activity against catechol derivatives, but the presence of tyrosinase has not been proved. The melanoma which we have utilized arose spontaneously in the skin of the ear of a chocolate-brown mouse and was reported by Harding and Passey in 1930 (12). It was found to be easily transplantable to mice of all colors, including albinos, and it has been carried for a number of years in the Rockefeller Institute strain of albino mice. Although slow growing, the neoplasm attains a relatively enormous size, often as much as one-third that of its host, in about 3 months. Grossly, it is a soft, encapsulated, jet-black nodule. Microscopically, it is made up of two cellular elements, one a cuboidal cell containing a relatively
small amount of pigment and comprising the bulk of the tumor, and the
other a large cell which is loaded with pigment, seems to lie in the inter-
stices, and is probably a macrophage.

EXPERIMENTAL

As a prospective source of melanin-producing enzymes, the transplant-
able melanoma is ideal, both because of its uniformity and because of the
fact that it can be grown in theoretically unlimited quantity. It therefore
seemed feasible to test its enzymatic activity. Accordingly, a tumor was
removed, ground thoroughly with sand, and made into a thin brei with
saline. The substrates L-phenylalanine, L-tyrosine, and L-dihydroxyphenyl-
alanine were added to the brei in the Warburg apparatus and the oxygen up-

![Fig. 1. The oxidation of tyrosine and dihydroxyphenylalanine by tumor brei. Each flask contained 2.5 cc. of tumor brei or the equivalent of 0.5 gm. of tumor. Curve 1, tyrosine 1.0 mg., pH 7.4; Curve 2, dihydroxyphenylalanine 1.0 mg., pH 6.5; Curve 3, phenylalanine 1.0 mg., control, pH 7.4; Curve 4, control, pH 6.5.](http://www.jbc.org/)

take measured. The temperature was maintained at 38°. When dihy-
droxyphenylalanine was the substrate, a phosphate buffer at pH 6.5 was
used, because this amino acid undergoes appreciable autoxidation at
physiological pH. With the remaining substrates, a buffer at pH 7.4 was
employed.

As can be seen in Fig. 1, no oxidation of phenylalanine occurred. This
has been the case in several other experiments lasting as long as 8 hours.
Both tyrosine and dihydroxyphenylalanine underwent vigorous oxidation
in the presence of the tumor brei. When the oxygen absorption ceased,
the samples were removed and centrifuged. The supernatant fluids from the
phenylalanine and control samples were light tan in color; those from the
tyrosine and dihydroxyphenylalanine samples were quite black, indicating
that melanin was produced.
Since the tissue suspension failed to catalyze the oxidation of phenylalanine, it seemed probable that we were not dealing with an amino acid oxidase or amine oxidase, but rather with a phenolic oxidase. The melanoma brei possessed a considerable oxygen absorption in the absence of added substrate; consequently, fractionation and partial purification were desirable before further study of the properties of the enzyme was undertaken. The degree of enzyme activity during the fractionation procedure was followed by the establishment of a convenient activity unit, which was defined as that amount of enzyme required to catalyze the absorption of 1 c.mm. of oxygen per minute by 1 mg. of appropriate substrate.

**Fractionation**—Trial experiments indicated that, upon centrifugation of the melanoma brei at 20,000 R.P.M. for 10 minutes, most of the enzyme activity remained in solution, the insoluble tissue debris being relatively inactive. Neither dialysis nor precipitation by the addition of ammonium sulfate caused destruction of the enzyme.

On the basis of the trial experiments, the fractionation procedure outlined in the accompanying diagram was adopted. The fraction insoluble

**Fractionation of Melanoma Oxidases**

- 70 gm. melanoma ground with sand and Ringer's solution, 65 units tyrosinase,
- 225 units dopa oxidase (centrifuge)

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1 4
Insoluble material Solution
20 units tyrosinase 60 units tyrosinase
20 units dopa oxidase 195 units dopa oxidase

Ppt.*
55 units tyrosinase 38 units dopa oxidase

Solution
(5 saturated (NH₄)₂SO₄)

Ppt.
No tyrosinase
65 units dopa oxidase

Supernatant
Inactive

(5 saturated (NH₄)₂SO₄)

Solution†
No tyrosinase
90 units dopa oxidase

(repeat (NH₄)₂SO₄ fractionation)
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* Tyrosinase fraction.
† Dopa oxidase fraction.

In one-third saturated ammonium sulfate contained all of the tyrosinase but relatively little of the dopa oxidase activity. This precipitate could not be redissolved and retained a residuum of autoxidation even after dialysis. It will be referred to as the tyrosinase fraction.
The fraction soluble in one-third saturated ammonium sulfate contained 40 per cent of the dopa oxidase activity of the original tissue, but no tyrosinase. It was completely soluble in water and rather deeply pigmented. A 10-fold purification was attained, since this fraction showed 160 units of activity per gm. of dry weight, whereas the original tumor brei contained only 16 units per gm. of dry weight. This enzyme preparation, the dopa oxidase fraction, maintained its activity for more than 2 weeks.

Properties of the Dopa Oxidase Fraction—In Fig. 2 the rate of oxidation of 1 mg. of dihydroxyphenylalanine, catalyzed by varying amounts of the dopa oxidase fraction, is indicated. The maximum reaction velocity is proportional to the amount of enzyme added. When relatively large amounts of the enzyme are present, the rate of oxidation falls off sharply, indicating exhaustion of substrate, since the rapid uptake of oxygen was resumed when more dopa was added. The total oxygen uptake after completion of the reaction approximates 4 atoms of oxygen per molecule of dopa added as substrate. The end-product of the oxidation of dopa by the melanoma oxidase is the insoluble black pigment, melanin.

The catalytic activity of the dopa oxidase fraction was destroyed by heating for 10 minutes at 100° and was completely inhibited by 0.001 M cyanide ion. The activity was lost at pH 4, much reduced at pH 6, and appeared to be maximum between pH 7 and 8. It should be noted that autoxidation of dopa becomes an important factor in alkaline solution.

The dopa oxidase fraction is apparently very highly specific for dihydroxyphenylalanine. At the concentration of enzyme available, it was completely unable to catalyze the oxidation of tyrosine, hydroquinone,
p-benzylhydroquinone, and p-cresol. Catechol was oxidized at one-seventh and adrenalin at one-eighth the rate of dopa.

Properties of the Tyrosinase Fraction—Investigation of the tyrosinase fraction has been less satisfactory, since there is relatively little tyrosinase present in the tumor and since the fractionation procedure apparently rendered the tyrosinase insoluble. This fraction contained the bulk of the originally water-soluble proteins and still absorbed a small amount of oxygen in the absence of substrate. It was possible to demonstrate, however, that the melanoma tyrosinase catalyzed the aerobic oxidation of tyrosine to melanin with the absorption of 5 atoms of oxygen per molecule of substrate.

The catalytic activity of the tyrosinase fraction was destroyed by boiling and completely inhibited by 0.01 M but not inhibited by 0.001 M KCN. This tyrosinase at the concentration available failed to oxidize phenol, hydroquinone, and p-cresol. The further investigation of its properties must wait until a relatively large quantity of melanoma is available.

Effect of p-Benzylhydroquinone on Melanoma Tyrosinase and Dopa Oxidase—An occupational leucoderma investigated by Schwartz, Oliver, and Warren (13) was found by these authors to be due to the action of monobenzylhydroquinone on the melanoblasts of the skin. Peck and Sobotka (14) found that the action of potato oxidase on tyrosine and dopa is interrupted by this substance at a stage prior to the formation of melanin. When melanoma dopa oxidase is the catalyst, the addition of monobenzylhydroquinone to the dopa solution greatly slows the rate of oxidation, the final product formed being a soluble red pigment rather than the insoluble black melanin otherwise produced. Monobenzylhydroquinone completely inhibits the oxidation of tyrosine by the tyrosinase fraction, no oxygen being absorbed. Mushroom tyrosinase, on the contrary, oxidizes monobenzylhydroquinone itself to a red pigment. Our results in general agree with those of Peck and Sobotka (14) and confirm their explanation of the relationship between monobenzylhydroquinone and leucoderma.

DISCUSSION

In many respects, the melanoma tyrosinase and dopa oxidase show considerable similarity to the tyrosinas isolated from plants and insects. The absorption of 5 and 4 atoms of oxygen per molecule of tyrosine and dopa respectively has been shown by Duliere and Raper (15) to occur when these substances are oxidized to melanin in the presence of meal worm tyrosinase. This finding has been repeatedly confirmed with the use of tyrosinas from such sources as mushrooms and potatoes. The loss of activity with heat and marked reduction of activity in slightly acid solution are properties common to all known tyrosinas. The sensitivity of the
melanoma enzymes to cyanide ion indicates that they are iron- or copper-containing catalysts. All the phenolic oxidases are inactivated by cyanide, and those which have been sufficiently purified have been proved to be copper proteins.

The apparent high specificity of the two fractions is of interest, however, in that it contrasts sharply with the broad specificity of tyrosinases from other sources. The melanoma dopa oxidase, for example, failed to catalyze the oxidation of tyrosine, p-benzyldihydroquinone, and p-cresol and promoted the oxidation of catechol and adrenalin at very slow rates. Mushroom tyrosinase, on the other hand, not only oxidizes all of these compounds, but oxidizes catechol twice as rapidly as it does dopa. The high specificity of the dopa oxidase fraction confirms the findings of Bloch.

It does not necessarily follow from our experiments that these enzymes extracted from a tumor occur in a normal melanoblast. The fact remains, however, that no enzyme system peculiar to tumor has thus far been demonstrated, and until such a discovery is made, we are probably safe in assuming that the production of melanin by this tumor cell is a normal mechanism. The advantages of the transplantable melanoma which is made up essentially of a single cell type, the tumor melanoblast, as a source of enzyme are obvious. It seems conceivable that other neoplasms possessing active and specialized enzymatic function could be utilized in similar studies of other enzyme systems.

SUMMARY

The above experiments demonstrate conclusively that a mouse tumor, which in all probability arose from the skin melanoblast, contains extractable enzymes which catalyze the oxidation of both tyrosine and dihydroxyphenylalanine to melanin. The presence of one of these enzymes, dopa oxidase, has been demonstrated by Bloch to occur in normal mammalian skin, but not in sufficient quantity to allow a detailed study of its properties. The other enzyme, a tyrosinase, has not previously been clearly shown to occur in mammalian tissue.

BIBLIOGRAPHY

5. Yamasaki, Y., Biochem. Z., 147, 203 (1924).
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