Reaction of the Sulfhydryl Group with an Oxidation Product of β-3,4-Dihydroxyphenyl-L-alanine

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The sulfhydryl group plays a role in the biological formation of melanin. Mechanisms suggested for the inverse relationship between the sulfhydryl group and the formation of melanin in mammalian skin include inhibition of tyrosinase and reaction with precursors of melanin (1, 2). However, knowledge in this field is fragmentary, and definitive proof of mechanism is lacking (3). The present work is a study in vitro of the reaction between the sulfhydryl group and an oxidation product of β-3,4-dihydroxyphenyl-L-alanine and the sulfhydryl group of cysteine or glutathione in the presence of tyrosinase at pH 7.4.

MATERIALS AND METHODS

Separate solutions containing 0.76 × 10⁻⁴ mole of dihydroxyphenylalanine, 0.83 × 10⁻⁴ mole of cysteine, 0.98 × 10⁻⁶ mole of glutathione, or 100 units of tyrosinase per ml of distilled water were prepared immediately before each experiment. The reaction proceeded at 25°C in a total volume of 4 ml of solution buffered at pH 7.4 by 1 ml of 0.1 M sodium phosphate. The absorbancy was measured in the Beckman model DU spectrophotometer, with use of the tungsten or mercury arc lamp. The absorbancy of blanks formed by omission of dihydroxyphenylalanine yielded the net absorbancies of the experimental solutions. The absorbancies of cysteine, cystine, reduced and oxidized glutathione, and tyrosinase were relatively small over the whole range of wave lengths studied. Measurement of the -SH group followed the method of Ellman (5).

RESULTS

At 480 μm, the absorbancy of dihydroxyphenylalanine was essentially zero, whereas the absorbancy of dopachrome (2-carboxy-2,3-dihydroindole-5,6-quione) was at a maximum level. During formation of the pink dopachrome, the absorbancy at 480 μm increased for approximately 7 minutes after the addition of tyrosinase (Fig. 1). Then it remained essentially constant for 12 to 14 minutes, and finally increased by 10% during the subsequent 10 minutes, with increasing formation of melanin. Inhibition of tyrosinase and addition of potassium ferricyanide, either initially or after 7 minutes of reaction, did not change the plateau level of absorbancy by dopachrome.

Addition of cysteine as well as tyrosinase to dihydroxyphenylalanine caused a decrease in the plateau level of absorbancy at 480 μm (Figs. 1 and 2). The time pattern of the variation in absorbancy, however, remained unchanged. Increase in tyrosinase either initially or after attainment of the plateau did not change the final amount of dopachrome. The SH group of cysteine disappeared after 3 minutes of reaction (Fig. 1). The plateau level of absorbancy, which had an inverse linear relationship to cysteine concentration, became essentially zero when the molarity of the cysteine in the solution was equal, within 2%, to the molarity of the dihydroxyphenylalanine (Fig. 2). Cysteine had no effect upon the absorptive pattern of dihydroxyphenylalanine in the absence of tyrosinase. Thus, also, no effect upon preformed dopachrome in the presence of tyrosinase.

The ultraviolet absorbancies of separate solutions containing dihydroxyphenylalanine alone, dihydroxyphenylalanine and tyrosinase, and dihydroxyphenylalanine and cysteine in equal molarity together with tyrosinase are presented in Fig. 3. Dihydroxyphenylalanine and the cysteine-derived compound had slight absorbancy above 350 μm. The absorbancy of the same dopachrome solution as in Curve 2 of Fig. 3 decreased gradually to 0.040 at 370 μm, then increased to a maximum of 0.105 at 480 μm, and, finally, decreased to 0.084 at 525 μm and 0.023 at 700 μm.

At 250 μm, the absorbancies of dihydroxyphenylalanine and dopachrome were near their minima. In addition, at this wave length, the value of the ratio formed by division of the absorbancy of the cysteine-derived compound by the absorbancy of dopachrome was nearly maximal (Fig. 3), but the sum of the rates of change of the absorbancies with variation in wave length was minimal. As the amounts of cysteine and dihydroxyphenylalanine varied, various amounts of dopachrome and the cysteine-derived compound were formed. The contribution of dopachrome to the total plateau absorption at 250 μm was found by multiplication of the plateau reading of dopachrome itself at 250 μm by the relative percentage of dopachrome in the final solution. This percentage was calculated from the absorbancy at 480 μm, where absorption by dopachrome mainly occurred. The absorbancy remaining at 250 μm after subtraction of the dopachrome contribution was due to the cysteine-derived compound and had a linear relationship to the cysteine present (Fig. 2).

The results obtained with glutathione were very similar to those obtained with cysteine. The effect of glutathione at 480 μm was essentially the same as that of cysteine, 1 mole of glutathione eliminating the absorption of 1 mole of dihydroxyphenylalanine. Up to 265 μm, the absorbancy of the glutathione-derived compound was very close to that of dopachrome. However, above 265 μm, the glutathione curve approached that of the cysteine compound. A linear relationship existed between the net absorbancy of the glutathione-derived compound at 250 μm and the amount of glutathione.

DISCUSSION

The present study demonstrates the formation of reaction products between sulfhydryl compounds and derivatives of di-
hydroxyphenylalanine in essentially two ways—the first, indirect, and the second, direct. In the first place, decrease in dopachrome indirectly shows diversion of dihydroxyphenylalanine in the direction of the sulfhydryl compound. Secondly, the distinctive absorption properties of the cysteine-derived compound permit its direct determination between 230 and 260 mp in the presence of dopachrome and other substances. Each approach demonstrates the reaction of equimolar amounts of the sulfhydryl group and an oxidation product of dihydroxyphenylalanine. On the one hand, increase in sulfhydryl concentration leads to an equimolar decrease in dopachrome formation. Dopachrome is not formed when the molar concentrations of the sulfhydryl group and dihydroxyphenylalanine are equal. On the other hand, the final concentration of the sulfhydryl reaction product and the initial sulfhydryl concentration are the same. The rapid disappearance of the sulfhydryl group points to its participation in the chemical bonding. The other reactant may be an o-quinone intermediate between dihydroxyphenylalanine and dopachrome.

The inverse biological relationship between the sulfhydryl group and pigmentation may be due to the formation of sulfhydryl-containing compounds. Melanin would, then, appear only when the -SH group has largely reacted. As in the case of epinephrine and norepinephrine (4), this group tends to prevent the oxidation of dihydroxyphenylalanine to the dopachrome stage. Demonstration by Mason (8) of chemical reactions between oxidation products of catechol and certain polypeptides and proteins suggests directions of investigations on dihydroxyphenylalanine. The present investigation is being extended to a study of the physiological implications of the reaction of sulfhydryl compounds and oxidation products of dihydroxyphenylalanine.

**SUMMARY**

Formation of new chemical compounds resulted from the presence of $\beta$-3,4-dihydroxyphenyl-$\tau$-alanine, cysteine or glutathione, and tyrosinase in solution at pH 7.4. The experimental evidence demonstrated the existence of chemical bonding between the
sulfhydryl group and an oxidation product of dihydroxyphenylalanine. The distinctive patterns of absorbancy of the reaction products in the ultraviolet range permitted their measurement.

REFERENCES
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