SHORT REPORTS

Inactivation of Human Tyrosinase by Cysteine. Protection by Dopa and Tyrosine

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Jergil B, Lindbladh Ch, Rorsman H, Rosengren E. Inactivation of human tyrosinase by cysteine. Protection by dopa and tyrosine. Acta Derm Venereol (Stockh) 1984; 64: 155–157.

Human tyrosinase prepared from cultured melanoma cells is inactivated by 10 mM cysteine. The inactivation of the enzyme by cysteine is less pronounced in the presence of catalase and superoxide dismutase. Thus, oxygen radicals and/or hydrogen peroxide may contribute to the inactivation of human tyrosinase by cysteine. Dopa and/or tyrosine protects tyrosinase against inactivation by cysteine. The protection observed with tyrosine alone indicates that oxidation of substrate is not necessary for the protection. The effect of dopa and/or tyrosine is probably due to steric hindrance at the active site preventing the access of cysteine to the copper. *Key words: Oxygenase; Oxygen radicals; Melanin; Cysteinyldopa.* (Received December 13, 1983.)

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The biosynthesis of melanin in melanocytes is dependent on the action of the copper-containing enzyme tyrosinase. The isolated enzyme is inactivated by sulfhydryl-containing compounds that interact with copper (1). The inactivation of tyrosinase by such compounds is of physiological interest, because cysteinyldopas are found in melanocytes indicating that tyrosinase in vivo acts in the presence of cysteine or glutathione (2). We have recently studied the inactivation of mushroom tyrosinase by thiols, and found that the addition of substrate protected the enzyme against such inactivation (3). We also found that hydrogen peroxide interfered with this inactivation.

We now report on inactivation of human tyrosinase by cysteine and show that tyrosine in the presence or absence of dopa protects the enzyme against this inactivation. In addition, we show that the inactivation in part is caused by reaction products of oxygen.

MATERIAL AND METHODS

Tyrosinase was purified from the medium of cultured human melanoma cells (4). The effect of various substances on tyrosinase activity was examined by incubating the substances under investigation with a constant amount of tyrosinase at 25°C for 30 min in 0.05 M sodium phosphate buffer, pH 7.4. Immediately after incubation tyrosinase was separated from the low molecular weight reagents by gel filtration on Sephadex G-25 (the bed volume was 9 ml). After application of the sample the column was washed with one bed volume of the phosphate buffer before elution of tyrosinase in 5 ml of buffer. Tyrosinase activity was measured in the eluate (4) and compared with the activity obtained in an identical experiment but after incubation with buffer alone.

The effect of the following substances in concentrations and combinations described in the results section was examined, L-cysteine, L-tyrosine, L-dopa, catalase, and superoxide dismutase.

RESULTS

Preincubation of purified tyrosinase with 10 mM cysteine resulted in a 90% loss of the enzyme activity (Table I), whereas 1 mM cysteine had no significant effect. The enzyme

Table I. *Effect of various additions on tyrosinase activity. Incubations were as described in Methods. Number of experiments in brackets*

Incubation additions	Tyrosinase activity (% of control)
1 mM cysteine	98±8 (4)
10 mM cysteine	9±1 (9)
10 mM cysteine, catalase, superoxide dismutase	19±2 (9)
10 mM cysteine, L-dopa, L-tyrosine	66±5 (8)
10 mM cysteine, L-tyrosine	47±4 (8)

was partly protected from inactivation by 10 mM cysteine when 0.3 mM L-tyrosine was present in the incubation mixture, either alone or in combination with 0.1 mM L-dopa. The protecting effect of L-dopa and L-tyrosine together was more pronounced than the effect of L-tyrosine alone.

Catalase (Sigma) (25 μg/ml) and superoxide dismutase (Sigma) (50 μg/ml) together also protected purified tyrosinase against cysteine-dependent inactivation, although to a considerably lower degree than L-tyrosine (Table I).

DISCUSSION

The results show that high concentrations of cysteine are required to inactivate human tyrosinase. The human enzyme is much more resistant to cysteine treatment than mushroom tyrosinase (5).

The inactivation of the human enzyme by cysteine was to some extent prevented by superoxide dismutase and catalase. In the absence of dismutase and catalase the superoxide anion (O_2^-), hydrogen peroxide, and hydroxyl radical ($OH^\cdot$) should be formed (6, 7). The protection of tyrosinase by the dismutase–catalase system indicates that part of the inactivation of tyrosinase by cysteine is due to oxygen radicals or hydrogen peroxide. We have previously demonstrated that catalase augments the cysteine-dependent inactivation of mushroom tyrosinase at high cysteine concentrations, but counteracts the inactivation at low cysteine concentrations (0.1 mM). Oxygen radicals or hydrogen peroxide clearly influence the cysteine-dependent inactivation of both human and mushroom tyrosinase, although the inactivation mechanisms for the two enzymes seem to differ (3). Tyrosine and dopa, or tyrosine alone, protected human tyrosinase efficiently against inactivation by cysteine. This protection of tyrosinase by dopa and tyrosine may partly be attributed to adduct formation between cysteine and dopaquinone. However, tyrosine also protected the enzyme in the absence of dopa when no quinone formation occurred. Dopa and tyrosine therefore seem to protect tyrosinase by preventing the access of cysteine to the copper of the active site of the enzyme.

The present finding that human tyrosinase is protected by its substrates against inactivation by cysteine provides an explanation for the virtually non-inhibited action of tyrosinase in phaeomelanosomes in which cysteine concentrations should be high.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Cancer Society (project 626-B84-12XC), the Swedish Medical Research Council, the Walter, Ellen and Lennart Hesselman Foundation for

Scientific Research, the Edvard Welander Foundation for Scientific Research, the donation funds of the University Hospital at Lund, and the donation funds of the faculty of Medicine, University of Lund, and the Finsen Foundation for Scientific Research.

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