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EFFECT OF PENICILLAMINE ON THE CONVERSION OF DOPA TO DOPACHROME IN THE PRESENCE OF TYROSINASE OR CERULOPLASMIN

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Abstract—The effect of penicillamine on the tyrosinase- and ceruloplasmin-catalyzed oxidation of dopa to dopachrome was studied. Penicillamine, a one-electron donor, prevents the production of dopachrome by reducing an oxidation product of dopa, probably a free radical. During the reaction the –SH group of penicillamine is oxidized. It is suggested that this mechanism of penicillamine action can play a role in the decrease of the skin pigmentation observed in schizophrenic patients on penicillamine therapy.

It has been reported that schizophrenic patients have an increased melanogenesis [1, 2], which is further increased by the phenothiazine treatment [1–5]. Melanin is produced by the tyrosinase-catalyzed oxidation of dopa to dopaquinone and dopachrome, followed by polymerization of the indol derivatives formed. This reaction is also catalyzed by ceruloplasmin *in vitro* [6].

In schizophrenic patients treated with low copper diet and penicillamine (β,β-dimethylcysteine), a copper chelating agent, the abnormal skin pigmentation is decreased [2, 4]. It is assumed that penicillamine inhibits the melanin synthesis by binding to tyrosinase [4], a copper containing enzyme. In the present communication the effect of penicillamine on the oxidation of dopa to dopachrome and hence melanin in the presence of tyrosinase and ceruloplasmin has been investigated.

MATERIALS AND METHODS

Human ceruloplasmin was purchased from AB Kabi and crystallized according to the method of Deutsch [7]. The purified enzyme had an absorbance ratio, A_{610}/A_{280}, of 0·041. Enzyme concentrations were calculated from the 610 nm absorption (ϵ = 10,900 M^{-1} cm^{-1}) [7]. Mushroom tyrosinase (EC 1.10.3.1), D-penicillamine, L-dopa (3,4-dihydroxyphenylalanine), NADH, *p*-hydroxymercuribenzoate and adrenaline were obtained from Sigma Chem. Co. and promazine from AB Ferrosan.

The enzyme-catalyzed production of dopachrome from dopa was followed spectrophotometrically at 475 nm (ϵ = 3,875 M^{-1} cm^{-1}) [8], using a Beckman DK 1 recording spectrophotometer equipped with a thermo cell. The rate of oxygen uptake during the tyrosinase- or ceruloplasmin-catalyzed oxidation of dopa was measured with a Clark oxygen electrode (Yellow Springs Instr. Co., Inc.) connected to a W + W 3012 recorder. The temperature was kept at 30° in all experiments. Aqueous solutions were prepared using deionized, glass-distilled water.

RESULTS AND DISCUSSION

In the presence of tyrosinase or ceruloplasmin dopa is rapidly converted to dopachrome (Fig. 1), which is an essential intermediate in the melanin synthesis. During the process molecular oxygen is reduced to water [9]. The dopachrome formation ceases after a while, due to lack of oxygen, but continues when oxygen is introduced into the reaction mixture. When penicillamine is added to the system some time elapses before the dopachrome formation starts (Fig. 1), the length of the lag period increasing with increasing penicillamine concentration. Less dopachrome is formed in the presence of penicillamine, suggesting that an oxygen consuming reaction takes place during the lag period. This was verified with oxygen electrode experiments (Fig. 1). At high penicillamine concentrations no dopachrome was formed at all. Similar results were obtained when the effect of cysteine and glutathione on the aminochrome formation was investigated [10]. Fig. 2 shows that the concentration of penicillamine-SH steadily decreases during the lag

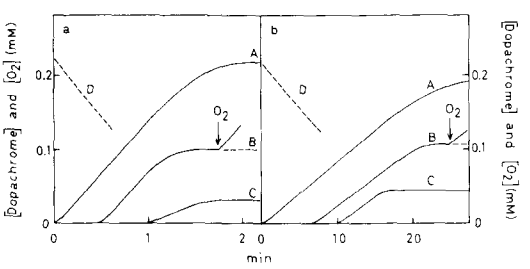


Fig. 1. (a) Effect of penicillamine on the dopachrome formation in the presence of tyrosinase. The reaction mixture contained 60 units/ml tyrosinase, 2·5 mM dopa and penicillamine (0·5 mM (B), 0·75 mM (C)) in 0·25 M sodium-acetate buffer, pH 6·2. Curve A shows the dopachrome formation in the absence of penicillamine and curve D the oxygen uptake when 0·5 mM penicillamine was present. (b) Effect of penicillamine on the dopachrome formation in the presence of ceruloplasmin (8·3 μM). The experimental conditions were as described above.

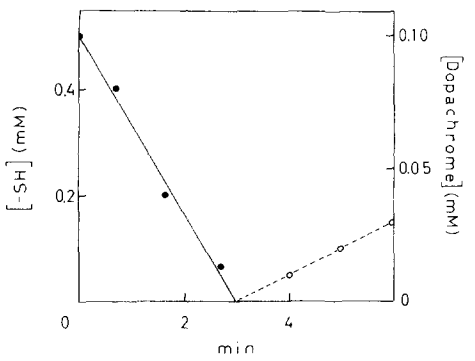


Fig. 2. Time course of the decrease in penicillamine—SH groups during the tyrosinase-catalyzed oxidation of dopa (●). The reaction mixture contained 12 units/ml tyrosinase, 2·5 mM dopa and 0·5 mM penicillamine in 0·25 M sodium-acetate buffer, pH 6·2. The method of Boyer was used for determining the —SH concentration [11]. The time course of the dopachrome formation is shown in the figure (○).

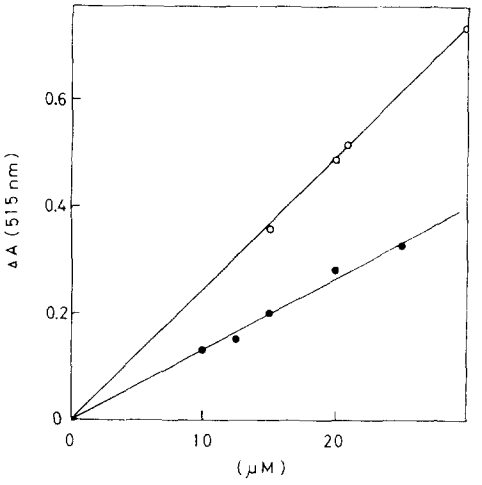


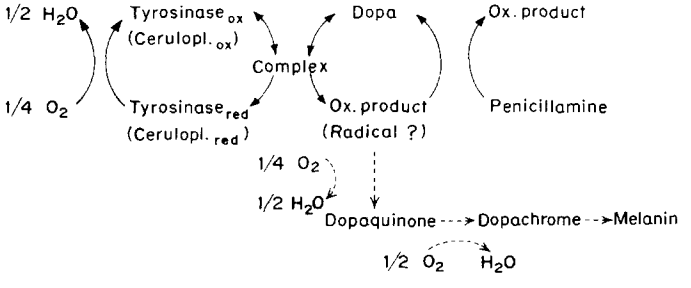
Fig. 3. The decrease in the 515 nm absorption of promazine radicals plotted against the concentration of penicillamine (●) and NADH (○) added. The method for producing free promazine radicals is described elsewhere [13].

period, and that the dopachrome production does not start until all the –SH groups are oxidized. Since penicillamine alone is not catalytically oxidized by tyrosinase or ceruloplasmin, the results suggest that penicillamine acts by reducing an oxidation product of dopa.

The amount of oxygen used for penicillamine oxidation is calculated by subtracting the amount of oxygen used for dopachrome formation (Fig. 1) from the initial amount of oxygen present (0·22 mM). One oxygen molecule is consumed per aminochrome molecule formed [12]. The ratio, [Penicillamine]/[O_2], is 4·2, suggesting that penicillamine is a one-electron donor in the process, since it takes four electrons to reduce one molecule of oxygen to water.

Penicillamine spontaneously reduces the red coloured promazine radicals (R. A. Lovstad, unpublished data), which have an absorption maximum at 515 nm (ϵ = 11,500 M^{-1} cm^{-1}) [13]. From Fig. 3 it is calculated that one molecule of penicillamine reduces one radical molecule, confirming the one-electron donor property of the chelator. In comparison NADH, a two-electron donor, reduces twice as many radicals as penicillamine.

Heacock and Scott [14] found that penicillamine had a reducing effect on adrenochrome, and the possibility existed that penicillamine had a similar effect on dopachrome. When 0·25 mM penicillamine was added to 0·1 mM dopachrome in 0·25 M sodium acetate buffer, pH 6·2, a reduction did take place. The rate of reduction was 0·7 μM/min, while the rate of dopachrome formation in the presence of tyrosinase (Fig. 1a) was 140 μM/min and ceruloplasmin 10 μM/min (Fig. 1b), suggesting that the effect of penicillamine on dopachrome is too slow to account for the lack of dopachrome formation in the presence of the chelating agent (Fig. 1). Ceruloplasmin oxidizes *p*-phenylenediamines [15] and phenothiazines [13] to free radicals by a one-electron transfer. The interaction of catecholamines with ceruloplasmin results in the formation of an activated product [15], probably a free radical, which rapidly oxidizes NADH and NADPH. Transitory free radicals were reported to be formed from catechols when they were acted upon by a polyphenoloxidase [16]. It seems therefore likely that penicillamine, being a one-electron donor, prevents the dopachrome synthesis by reducing an active oxidation product of dopa, which is probably a free radical. A satisfactory explanation for the experimental facts can be made with the following mechanism:



When all the penicillamine is oxidized the reaction leading to melanin starts. It is suggested that this mechanism of penicillamine action can play a role in the reduction of melanin synthesis *in vivo*. The tyrosinase activity was almost the same in the absence and presence of penicillamine, indicating that in the present experiments no significant inhibition of tyrosinase by the chelator occurs.

Penicillamine treatment markedly improves the mental state of the schizophrenic patients [2]. Hoffer and Osmond [17, 18] proposed that the oxidation of

adrenaline to adrenochrome may play a role in the etiology of schizophrenia. In this connection it is of interest that penicillamine also prevents the formation of adrenochrome from adrenaline in the manner described in this paper.

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