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Tyrosinase-catalyzed hydroxylation of hydroquinone, a depigmenting agent, to hydroxyhydroquinone: A kinetic study

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ABSTRACT

Hydroquinone (HQ) is used as a depigmenting agent. In this work we demonstrate that tyrosinase hydroxylates HQ to 2-hydroxyhydroquinone (HHQ). Oxy-tyrosinase hydroxylates HQ to HHQ forming the complex met-tyrosinase-HHQ, which can evolve in two different ways, forming deoxy-tyrosinase and *p*-hydroxy-*o*-quinone, which rapidly isomerizes to 2-hydroxy-*p*-benzoquinone or on the other way generating met-tyrosinase and HHQ. In the latter case, HHQ is rapidly oxidized by oxygen to generate 2-hydroxy-*p*-benzoquinone, and therefore, it cannot close the enzyme catalytic cycle for the lack of reductant (HHQ). However, in the presence of hydrogen peroxide, met-tyrosinase (inactive on hydroquinone) is transformed into oxy-tyrosinase, which is active on HQ. Similarly, in the presence of ascorbic acid, HQ is transformed into 2-hydroxy-*p*-benzoquinone by the action of tyrosinase; however, in this case, ascorbic acid reduces met-tyrosinase to deoxy-tyrosinase, which after binding to oxygen, originates oxy-tyrosinase. This enzymatic form is now capable of reacting with HQ to generate *p*-hydroxy-*o*-quinone, which rapidly isomerizes to 2-hydroxy-*p*-benzoquinone. The formation of HHQ during the action of tyrosinase on HQ is demonstrated by means of high performance liquid chromatography mass spectrometry (HPLC–MS) by using hydrogen peroxide and high ascorbic acid concentrations. We propose a kinetic mechanism for the tyrosinase oxidation of HQ which allows us the kinetic characterization of the process. A possible explanation of the cytotoxic effect of HQ is discussed.

1. Introduction

Tyrosinase (E.C. 1.14.18.1) is a copper-containing enzyme widely distributed in nature. It catalyses two types of reactions: (a) the *ortho*-hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and (b) the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity). Both types of reaction require molecular oxygen as the second substrate of the enzyme.[1–3]

The first studies on the action of tyrosinase on hydroquinone (HQ) suggested that the enzyme does not act directly on this compound but that, in contrast, HQ was oxidized by the *o*-quinone generated by the enzyme acting on a *o*-diphenol substrate.[4–6] More recent studies, using tyrosinase from the Harding-Passey melanoma, showed that HQ inhibits the melanin biosynthetic pathway from L-tyrosine and L-Dopa[7–10] and, in subsequent studies, it was proposed that HQ inhibited the melanin biosynthesis pathway by acting as an alternative substrate to L-tyrosine and L-Dopa.[11] As a consequence, it was suggested that the adverse physiological effects of HQ may be due to its oxidation by tyrosinase.[12] These

Abbreviations: HQ, hydroquinone; HHQ, 2-hydroxyhydroquinone; PB, *p*-benzoquinone; HPB, 2-hydroxy-*p*-benzoquinone; D, *o*-diphenol; M, monophenol; AH_2, ascorbic acid; E_m, met-tyrosinase; E_{ox}, oxy-tyrosinase; E_d, deoxy-tyrosinase; V_0^{HPB}, initial rate of HPB formation; V_{max}^{app}, apparent maximum velocity of tyrosinase acting on HQ; K_M^{app}, apparent Michaelis constant of tyrosinase acting on HQ; *R* ratio between $[H_2O_2]_0$ and $[HQ]_0$, $V_0^{HPB,R}$, initial rate of HPB formation at a constant ratio $[H_2O_2]_0/[HQ]_0$; $V_{max}^{app,R}$, apparent maximum velocity of tyrosinase acting on HQ at a constant ratio $[H_2O_2]_0/[HQ]_0$; $K_M^{app,R}$, apparent Michaelis constant of tyrosinase acting on HQ at a constant ratio $[H_2O_2]_0/[HQ]_0$.

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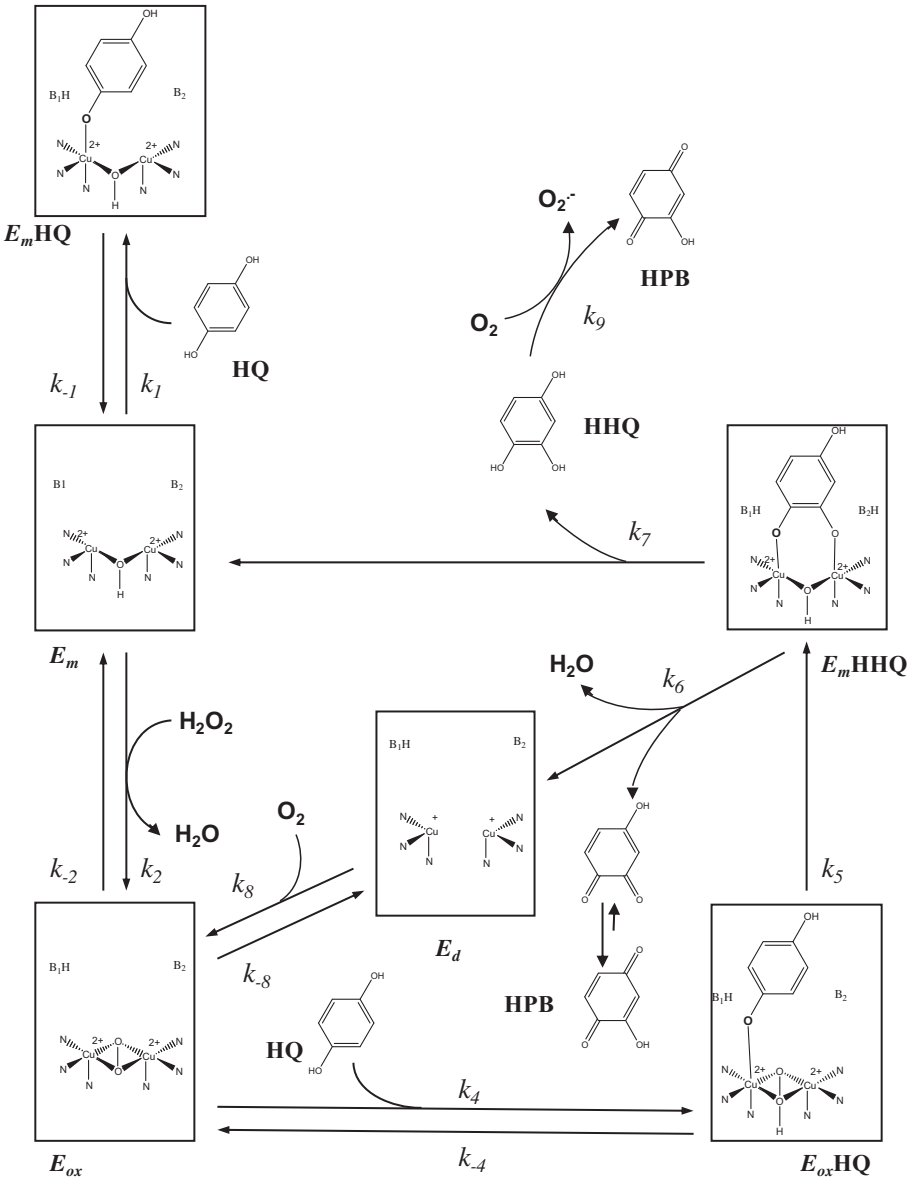
and subsequent studies suggested that HQ was hydroxylated to 2-hydroxyhydroquinone (HHQ) through the action of tyrosinase, although the direct enzymatic formation de HHQ has not been demonstrated experimentally.[13,14]

Serious problems attributed to HQ are pigmentation of the eye and, in a small number of cases, permanent corneal damage.[15] Due to this, dermatological depigmented treatments without HQ have been developed, which have been contrasted with others containing the compound.[16–18] Attempts to inhibit HQ cytotoxicity have used resveratrol as an additional component.[19] Actually, pharmaceutical products (creams and gels) contain up to 4% HQ are used, for example, despigmental topical gel (4%), melanase cream (2%), licoforte topical gel (4%), and pigmentase cream (4%).[20]

HQ, is an isomer of catechol, a known substrate of tyrosinase.[21] However, it has recently been suggested that HQ is neither a substrate nor inhibitor of tyrosinase.[22] The same authors suggest that these results agree with previous ones[4–6] and conclude that HQ is oxidized in the presence of tyrosinase substrates so that the oxidation is vicarious.[22] However, our studies about the action of tyrosinase on monophenols and *o*-diphenols[21,23–29] suggest that HQ is a substrate of tyrosinase but that the enzymatic activity is not evident because HQ is not capable of closing the enzymatic catalytic cycle by transforming the met-tyrosinase (E_m) form to oxy-tyrosinase (E_{ox}). This can be done by using an *o*-diphenol as a reductant.[21,23–26] E_m (inactive on monophenols) is transformed into E_d, which, by binding with oxygen, is transformed into E_{ox} (active on monophenols). Alternatively, E_m is converted into E_{ox} by the addition of H_2O_2,[28,30–32] as demonstrated by Mason (Schemes 1 and 2).[33,34] Moreover, the addition of ascorbic acid, which acts as substrate of the enzyme,[35] is capable of reducing E_m to E_d, facilitating the transformation into the E_{ox} which acts on HQ (Scheme 3).

The action of the enzyme on HQ in the presence of H_2O_2 is depicted in Scheme 1. Note that in the proposed mechanism HHQ is not accumulated in the medium and so the experiments show no lag period. Hence HQ is the only monophenol that does not show a lag in the presence of H_2O_2.[28] A kinetic analysis of



Scheme 1. Structural mechanism proposed to explain the catalytic pathway of tyrosinase in its action on HQ in the presence of H_2O_2/E_m met-tyrosinase, E_m HQ met-tyrosinase HQ complex axially bound to Cu atom with protonated base (B_1H), E_{ox} oxy-tyrosinase, E_{ox} HQ oxy-tyrosinase HQ complex axially bound to Cu atom with protonated base (B_1H), and the proton transferred to the peroxide, E_m HHQ met-tyrosinase/2-hydroxyhydroquinone complex, E_d deoxy-tyrosinase, HQ = hydroquinone, HHQ = 2-hydroxyhydroquinone, HPB = 2-hydroxy-*p*-benzoquinone.

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