DEUTERIUM SOLVENT ISOTOPE EFFECTS IN OXIDATION OF L-TYROSINE CATALYZED BY TYROSINASE

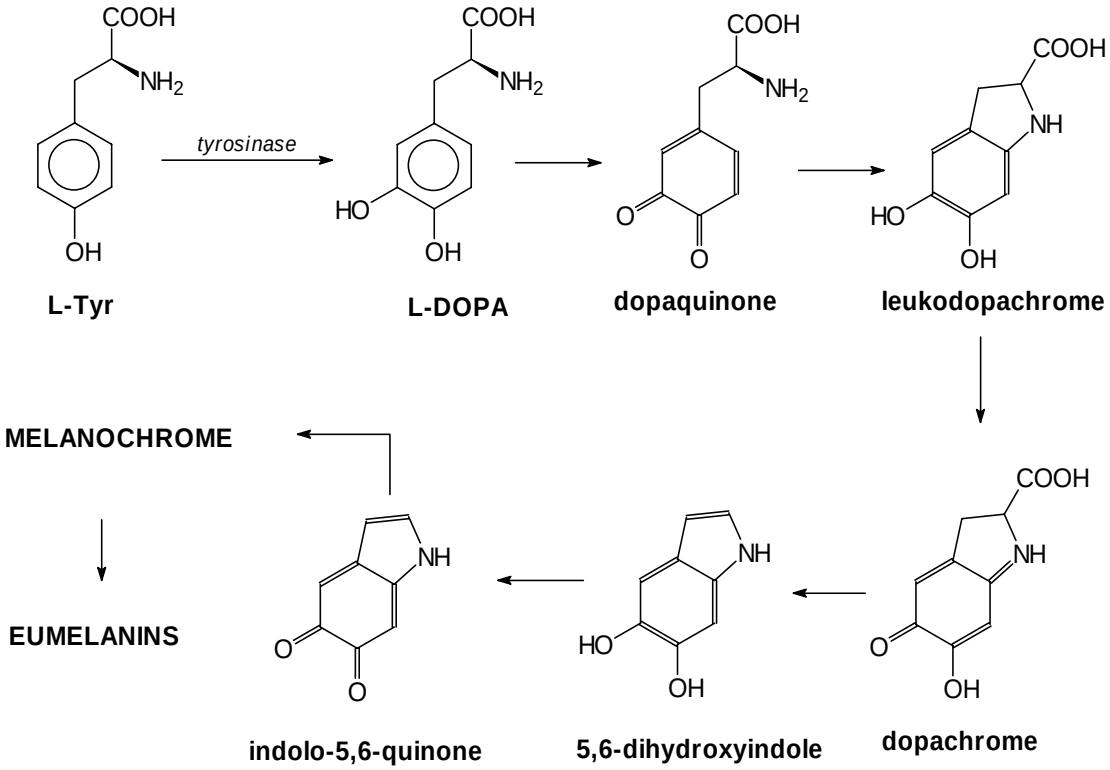
Małgorzata Kozłowska, Marianna Kańska
University of Warsaw, Department of Chemistry, Pasteur 1 Str., 02-093 Warsaw

Abstract

The solvent isotope effects on Michaelis, K_m, and maximum reaction rate, V_{max}, in the reaction of hydroxylation of L-tyrosine to L-DOPA catalyzed by enzyme tyrosinase (EC 1.14.18.1) were determined. Solvent isotope effects were obtained by noncompetitive method for L-tyrosine and its two deuteriated isotopomers: [3',5'-$^2H_2$]-, and [2',6'-$^2H_2$]-L-tyrosine.

Introduction

The enzyme tyrosinase (Tyrosinase EC 1.14.18.1) classified to oxyreductases catalyzed two followed (subsequent) oxidation reactions of L-tyrosine to L-DOPA (3',4'-dihydroxyphenylalanine), and next L-DOPA to dopaquinone [1,2]. This *o*-phenol nonenzymatically proceeds to several another unstable intermediates, which polymerize to yield melanins [3], scheme 1.



Scheme 1. Conversion of L-tyrosine into melanins in living cells.

Tyrosinase is widely distributed throughout the nature [4,5]. It plays very important role in browning of vegetable and fruits, it causes also pigmentation in vertebrates, its deficit in mammalian leads to albinism. Exact mechanism of the action of

enzyme tyrosinase is not clear up to now. It would be interesting to study this problem using isotope effect method, particularly investigating the deuterium solvent isotope effects. Kinetics of enzymatic reaction are usually characterized by Michaelis equation:

$$K_m = S\left(\frac{V_{max}}{v} - 1\right)$$

where ***v*** is the reaction rate at substrate concentration S, V_{max} is a maximum reaction rate, and K_m is a Michaelis constant. K_m and V_{max} are interrelated, and approximately speaking K_m is a measure of strength of enzyme-substrate binding, and V_{max} is a measure of the rate of reaction under conditions given. Interpretation of solvent isotope effects may bring new light on the mechanism of reaction studied. In the literature there are many papers concerning using of isotope methods for investigation of mechanism of action of enzyme tyrosinase [6-10]. Some of them report the data of SIE obtained for hydroxylation of L-tyrosine using natural and genetically modified enzymes [11]. The very similar enzyme tyrosine hydroxylase (EC 1.14.16.2), known also as tyrosine 3-monoxygenase or L-tyrosine tetrahydropteridine, was the subject of intensive investigations, and many data concerning isotope effects were obtained using isotope methods [12-14]. This enzyme oxidizes also L-tyrosine to dopachrome *via* L-DOPA, but its structure [15] differs considerably from tyrosinase (EC 1.14.18.1) studied currently by us.

In this work we study the influence of solvent on K_m and V_{max} in the reaction of hydroxylation of L-tyrosine to L-DOPA catalyzed by enzyme tyrosinase. Solvent isotope effects - SIE, were determined for L-tyrosine and its two deuteriated isotopomers, i.e., [3',5'-$^2H_2$]-L-tyrosine, which synthesis was described elsewhere [16] and commercial [2',6'-$^2H_2$]-L-tyrosine. The kinetic of conversion of L-tyrosine into L-DOPA were measured using noncompetitive method, and numerical values of SIE [17] were calculated by dividing kinetics parameters obtained in phosphate buffer by the ones obtained in fully deuteriated buffer. The progress of reaction was monitored indirectly by spectrophotometrical measurements of absorbance of dopachrome (Scheme 1) at λ = 475 nm (molar extinction coefficient for dopachrome $\varepsilon = 3600\ M^{-1}cm^{-1}$).

Experimental

Standard mixture contains 0.1M phosphate buffer (pH 7.0), or 0.1M fully deuteriated phosphate buffer (pD 7.0) prepared from 83% D_3PO_4/D_2O, 30% KOD and 99,9% heavy water, L-tyrosine and enzyme Tyrosinase EC 1.14.18.1 (0.02 U/ml). The concentration range of L-tyrosine, [3',5' -$^2H_2$]- L-tyrosine, [2',6'-$^2H_2$]- L-tyrosine was between 2.1 mM and 0.6 mM. The isotopomer [3',5'-$^2H_2$]- L-tyrosine has been synthesised in our laboratory [16] , and [2',6'-$^2H_2$]- L-tyrosine was purchased from Sigma. The total volume of reaction mixture was 2 ml. The progress of reaction was monitored spectrophotometrically by measuring the increasing of absorbance of dopachrome at λ=475 nm. These kinetic assays were carried out at the room temperature. The maximal velocity (V_{max}) and ratio of Michaelis constant per maximal velocity (K_m/V_{max}) were calculated using computer programme Enzfitter 1.05. The results of solvent kinetic isotope effects concerning of three mentioned above isotopomers of L-tyrosine are shown in Table 1.

Isotopomer of L-tyrosine	SIE on V_{max}	SIE on V_{max}/K_m
L-tyrosine	5.71 ± 0.131	9.33 ± 0.37
[3',5'-$^2H_2$]- L-tyrosine	1.89 ± 0.053	3.66 ± 0.142
[2',6'-$^2H_2$]- L-tyrosine	4.16 ± 0.036	9.95 ± 2.45

Table 1. Solvent Isotope Effects

Discussion

The oxidation of L-tyrosine in the presence of tyrosinase in phosphate buffer proceeds about 6 times faster than in fully deuteriated medium. Similarly the rates of oxidation for compounds of L-tyrosine labeled with deuterium in aromatic ring are also lower in deuteriated medium. Probably the H/D exchange of labile protons of enzyme causes the conformational changes in active site of enzyme, and this reflects on activity of this biological catalyst slowing considerably its activity.

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