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Microcalorimetric Study on Tyrosine Oxidation Catalyzed by Tyrosinase

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Abstract: Through the method of initial heat release rate, the kinetic property of tyrosine oxidation catalyzed by tyrosinase from *Pseudomonas maltophilia* was investigated using a LKB-2107 batch microcalorimeter. Tyrosine was catalyzed and oxidized into L-dopa, then into melanin catalyzed by tyrosinase. We found that the tyrosinase reaction obeyed the Michaelis-Menten kinetics, and at 298.15K and pH 7.0, the initial exothermic rate (Ω_0) are in the range of 0.1567~0.5704 mJ • s^{-1}, the maximum exothermic rate (Ω_{max}) are in 0.4152 ~ 0.8143mol • L^{-1}, and mean value of the Michaelis constant (K_m) is $2.199 \pm 0.105 \times 10^4$ mol • L^{-1}.

Keywords: Tyrosine, tyrosinase-catalyzed oxidation reaction, microcalorimetry, thermokinetics.

At present, several methods are available for kinetic studies of enzyme-catalyzed reactions, but study on the reaction of *Pseudomonas maltophilia* tyrosiase by microcalorimetry has not been reported yet.

Tyrosinase, ecoded by mel gene, is copper-containing enzyme, which can catalyze and oxidize tyrosine L-dopa to melanin. It exists in the organism of fungi, actinomycetes, bacteria, gymnosperms, angiosperms, insects, chordata, mammals, and human *etc*.. Tyrosinase from different source has different molecular character. Tyrosina can be oxidized to melanin, and provide protection for organism, but its biological function on bacteria has not been fully understood. Due to its unique structure and property, it can be used as UV absorbent, amorphous semiconductor and new-style natural drug carrier, it can resist γ–ray, X-ray and some toxic materials, tyrosina hold antioxidation and resistance to HIV. It also can protect of cosmetic and living insecticide from photolysis. In the past few years, interests in melanin have been grown because melanin has the ability of anti-radiation of UV and is scavenger of the free radicals[1].

The thermokinetic method is one of the most powerful tools for kinetic studies of chemical processes because it provides on-line, quasi-continuous, non-invasive and accurat measurement of the thermodynamic and kinetic data of the reactions which may help to understand the reaction mechanisms[2]. No solvent, spectral, analysis,

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electrochemical, or other properties of the reaction system are required. Owing to these advantages, the thermokinetic method has recently attracted increasing attention in many fields.

In this paper, using the method of initial heat release rate, the kinetic property of tyrosine oxidation catalyzed by tyrosinase from *Pseudomonas maltophilia* was investigated by means of microcalorimetry. By analyzing the calorimetric curves of these reactions, the thermokinetic models can be used to calculate the initial exothermic rate (Ω_0), the maximum exothermic rate (Ω_{max}) and the kinetic parameters (K_m). The oxidation of tyrosine catalyzed by tyrosinase as a well-studied single-substrate enzymatic reaction, was employed to test the validity of these thermokinetic models. The thermokinetic parameters of this reaction were determined.

Results and Discussion

For a single-substrate enzyme-catalyzed reaction, the exothermic rate of the reaction Ω and the reaction rate υ exhibit the following relation[3],

$$\Omega = \Delta_r H_m . V_c . \upsilon \quad (1)$$

The maximal exothermic rate of the reaction Ω_{max} can be calculated as follows[4];

$$\Omega_{max} = -\Delta_r H_m \cdot V_c \cdot \upsilon_{max} \quad (2)$$

here, V_c represents the volume of solution in the reaction system.

Determination of the Michaelis constant (K_m) in accordence with the Michaelis-Menten mechanism, is in response to the following formula[4]

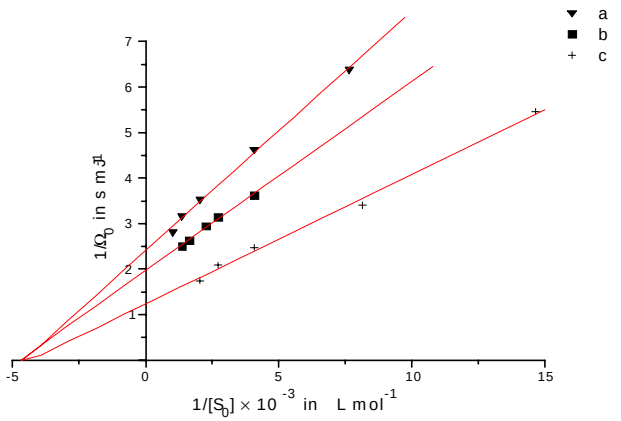
$$1/\upsilon_0 = 1/\upsilon_{max} + (K_m/\upsilon_{max}) \cdot (1/[S_0]) \quad (3)$$

from (2) and (3), we have

$$1/\Omega_0 = 1/\Omega_{max} + (K_m/\Omega_{max}) \cdot (1/[S_0]) \quad (4)$$

This is a linear equation called the Lineweaver-Burk equation in which Ω_0 is the initial exothermic rate of the reaction (represented by mJ s^{-1} in the enthalpic determination) and $[S_0]$ is intitial concentration of the substrate. The maximum exothermic rate, Ω_{max}, and the michaelis constant K_m can be calculated from the intercept of $1/\Omega_{max}$ and the slope K_m/Ω_{max}, respectively. **Figure 1** shows a plot of $1/\Omega_0$ *versus* K_m/Ω_{max} at different enzyme concentrations according to the data in **Table 1**. It can be seen from **Figure 1** that the curves are linear and intersect the abscissa in a point. The point stands for $-1/K_m$. The slope is proportional to the reciprocal of the enzyme concentration. The results are shown in **Table 1**. From **Table 1**, we can see that the repeatability of K_m is in agreement with each other; correlation coefficient R is larger than 0.9960; With double concentration of enzyme, the valuse of υ_{max} increased by 2 times approximately. Therefore, the results demonstrated the feasibility of the method of enzyme-catalyzed reactions. It also showed that tyrosine oxidation catalyzed by tyrosinase obey Michaelis-Menten kinetics.

Figure1 Lineweaver-Burk plot of $1/\Omega_0$ *vs.* $1/[S_0]$ at different tyrosinase concentrations



(The initial tyroniase volume and correlation coefficient is: a. 0.2 mL, R=0.9983, b. 0.25 mL, R=0.9967, c. 0.40 mL, R=0.9978)

Table 1 Determination of K_m, Ω_{max} of tyrosine oxidation reaction catalyzed by tyrosinase using a Lineweaver-Burk plot

L_0 mL	$[S]_0\times10^4$ mol • L^{-1}	Ω_0 mJ • s^{-1}	$1/[S]_0\times10^{-3}$ L • mol^{-1}	$1/\Omega_0$ s • mJ^{-1}	$K_m\times10^4$ mol • L^{-1}	Ω_{max} mJ • s^{-1}
	1.307	0.1567	7.651	6.382		
	2.455	0.2163	4.073	4.623		
0.2	4.911	0.2823	2.026	3.531	2.185	0.4152
	7.374	0.3165	1.358	3.160		
	9.823	0.3554	1.018	2.814		
	7.364	0.3998	1.358	2.501		
	6.138	0.3784	1.629	2.629		
0.25	4.419	0.3400	2.263	2.941	2.091	0.5061
	3.683	0.3183	2.715	3.142		
	2.455	0.2756	4.073	3.628		
	0.6822	0.1830	14.66	5.463		
	1.228	0.2941	8.314	3.400		
0.40	2.455	0.4087	4.073	2.447	2.320	0.8143
	3.683	0.4788	2.715	2.088		
	4.911	0.5704	2.036	1.753		

mean value $\overline{K_m}$ =2.199±0.105×10^{-4}mol • L^{-1}. *[L_0] is the initial tyrosinase volume.

Under the catalyzation of tyrosinase, tyrosine was oxidized into L-dopa by oxygen,

$$\text{Tyrosine} + O_2 \xrightarrow{tyrosinase} \text{L-dopa} \longrightarrow \text{Melanin} \qquad (6)$$

then into melanin[5]. The result from spectrographic determination is not accurcy because this enzyme catalyzed reaction was very complicated, and the enzyme solution was a suspended liquid, and the mycelium must be treated by ultrasonic. We used the method

of initial heat release rate to determine the kinetic property of this reaction by microcalorimetry. The results indicated that the reaction of tyrosine oxidation catalyzed by tyrosinase is in accordence with Michaelis-Menten kinetic model.

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