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Short Communication

Research on Thermodynamic aspect of the Binding of *p*-Phenylene-bis dithiocarbamate to Mushroom Tyrosinase

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Abstract

The binding properties and structural changes of mushroom tyrosinase enzyme, MT, due to its interaction with p-phenylene-bis dithiocarbamate (I) was investigated at 27 and 37°C in phosphate buffer (10 mmol.L^{-1}) at pH 6.8 by isothermal titration calorimetric (ITC). The extended solvation model was used to calculate the solvation parameters, which were attributed to the stability of enzyme. Thermodynamic analysis indicated that the binding of I to MT essentially depends on electrostatic interactions. It was concluded that MT has two distinct sites for p-phenylene-bis and phenyl dithiocarbamate.

Keywords: Mushroom tyrosinase; *p*-phenylene-bis dithiocarbamate; isothermal titration calorimetry.

Introduction

Tyrosinase is a copper monooxygenase, which catalyze the oxidation of mono- and o-diphenols to o-diquinones[1]. Tyrosinase plays an essential role in melanin production and responsible for the formation of pigment in the skin, hair and eye[2, 3]. The accumulation of an abnormal melanin amount in different specific parts of the skin, resulting in more pigmented patches, is an esthetic problem[4]. Tyrosinase inhibitors have attracted concern recently due to undesired browning in vegetables and fruits in post-harvest handling[3]. The di-copper centre of this enzyme has been the target of many inhibitors. Dithiocarbamate compounds act as inhibitors of mushroom tyrosinase due to their ability to chelate copper ion[5]. Certain dithiocarbamate derivatives have been found to possess a wide range of biological activities, i.e, anti-bacterial, tuberculostatic, anti-diuretic and anti-hypertensive. n-Butyl dithiocarbamate, n-hexyl dithiocarbamate and n-octyl dithiocarbamate, as sodium salts, show inhibitory effect on mushroom tyrosinase[6]. Among various dithiocarbamates, we tried to elucidate the effect of *p*-phenylene-bis dithiocarbamate on mushroom tyrosinase stability at 27 and 37 °C applying the extended solvation model for the data analysis.

Material and Methods

Experimental: Mushroom tyrosinase was obtained from Sigma, *p*-phenylene-bis dithiocarbamate was synthesized. All other materials and reagents were of analytical grade, and solutions were made in 10 mmol.L^{-1} phosphate buffer using double-distilled water. The isothermal titration calorimetric experiments were performed with the four channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The microcalorimeter is composed of two identical cell made of a highly efficient thermal conducting material surrounded by an adiabetic jacket. The sample cell contained 1.8 mL MT (8.3 μmol.L^{-1}) and phosphate buffer solution (10 mmol.L^{-1}; pH 6.8) and the reference cell filled with phosphate buffer. The titration of MT with *p*-phenylene-bis dithiocarbamate involved 20 consecutive injections of the ligand and each injection included 20 μL *p*-phenylene-bis dithiocarbamate (2.5 mmol.L^{-1}). The heat of injection was calculated by the 'Thermometric Digitam 3' software program. The measurements were performed at constant temperatures of 27 and 37°C and the temperature was controlled using a poly-science water bath. The microcalorimeter was frequently calibrated electrically during the course of the study.

Results and Discussion

The extended solvation model, the applicable, in principle, to a variety of protein + ligand systems, was used to analyze the heats of interaction of MT+II and MT+I mixtures as follows[7-10]

$$q = q_{max} x'_B - \delta^{\theta}_A (x'_A L_A + x'_B L_B) - (\delta^{\theta}_B - \delta^{\theta}_A)(x'_A L_A + x'_B L_B) x'_B \quad (1)$$

x'_B can be expressed as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \quad (2)$$

x'_B is a fraction of bound ligand with the protein molecule and $x'_A = 1 - x'_B$ is the fraction of unbound ligand. Where x_B can be defined as follows:

$$x_B = \frac{[L]}{[L]_{max}} \quad (3)$$

$[L]$ is the concentration of *p*-phenylene-bis dithiocarbamate after every injection and $[L]_{max}$ is the maximum concentration of *p*-phenylene-bis dithiocarbamate upon saturation of all MT. In the fitting procedure, *p* was changed until the best agreement between the experimental and calculated data was approached

(figure-1). If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity (p>1). Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, the enzyme exhibits negative cooperativity (p<1). If the ligand binds at each site independently, the binding is non-cooperative (p=1). L_A and L_B are the relative unbound and bound I contributions to the heats of dilution in the absence of MT.

$$L_A = q_{dilut} + x_B \left(\frac{\partial q_{dilut}}{\partial x_B}\right) \qquad L_B = q_{dilut} - x_A \left(\frac{\partial q_{dilut}}{\partial x_B}\right) \quad (4)$$

Stability of the MT is discussed on the basis of the obtained results for δ_A^θ and δ_B^θ from the coefficients of the second and third terms of eq. 1. δ_A^θ and δ_B^θ values reflect to the MT structural changes due to its interaction with p-phenylene-bis dithiocarbamate in the low and high concentrations, respectively. The negative values of δ_A^θ and δ_B^θ indicate that MT is destabilized by p-phenylene-bis dithiocarbamate. Eq. 5 was used for isothermal titration calorimetric data analysis to obtain the number of binding sites (g) and the dissociation binding constant (K_d) from the slope $(\frac{1}{g})$ and the vertical-intercept of $(\frac{K_d}{g})$ of the linear plot of

$$\left(\frac{\Delta q}{q_{max}}\right) M_0 \ vs. \ \left(\frac{\Delta q}{q}\right) L_0 .$$

$$\frac{\Delta q}{q_{max}} M_0 = \left(\frac{\Delta q}{q}\right) L_0 \frac{1}{g} - \frac{K_d}{g} \quad (5)$$

Where $\Delta q = q_{max} - q$ and q represents the heat value at a certain ligand and biomolecule concentration. M_0 and L_0 are total concentrations of enzyme and ligand, respectively. q_{max} represents the heat value upon saturation of all MT and the molar enthalpy of binding for each binding site ($\Delta H°$) will be $\Delta H° = \frac{q_{max}}{g}$. The linearity of the plot has been examined by different estimated values for q_{max} to find the best value for the correlation coefficient (near to one). The standard Gibbs free energy, $\Delta G°$, can be calculated from association constant $(K_a = \frac{1}{K_d})$ as follows: $\Delta G = -R\,T \ln K_a$ (6)

The negative values of $\Delta G°$ suggest that the binding process of MT to I proceeds spontaneously in the forward direction. $\Delta S°$ is directly calculated from $\Delta G°$ and $\Delta H°$ according to eq.7:

$$\Delta S° = \frac{(\Delta H° - \Delta G°)}{T} \quad (7)$$

All thermodynamic parameters of I binding to MT are summarized in table-1.

The decreasing K_a values (Table-1) with increasing temperature (table-1) indicates that the binding of I is an enthalpically driven process and consequently the electrostatic forces are dominant.

Conclusion

p=1 indicates that the binding is non-cooperative in two binding sites. The negative values of δ_A^θ and δ_B^θ show that p-phenylene-bis dithiocarbamate destabilizes the MT structure. The binding process for MT inhibition is only enthalpy driven, indicating that electrostatic interaction is more important in the inhibition sites of MT.

Acknowledgement

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Table-1
Binding parameters for *p*-phenylene-bis dithiocarbamate+MT interaction recovered from Eqs. 1, 5, 6 and 7. *p*=1 indicates that the binding is non-cooperative in two binding sites. The negative values of δ_A^θ and δ_B^θ show that *p*-phenylene-bis dithiocarbamate destabilizes the MT structure at 27 and 37 °C. The binding process for MT inhibition is only enthalpy driven, indicating that electrostatic interaction is more important in the inhibition site.

parameters	T=27 °C	T=37 °C
p	1	1
g	2±0.01	2±0.01
K_a / L.mol^{-1}	3.3×10^5 ±56	2.0×10^5 ±34
ΔH/ kJ mol^{-1}	-39.2	-39.8
ΔG/ kJ mol^{-1}	-31.7	-31.5
ΔS / kJ mol^{-1}K^{-1}	-0.025	-0.027
δ_A^θ	-7.4	-10.6
δ_B^θ	-16.7	-17.6

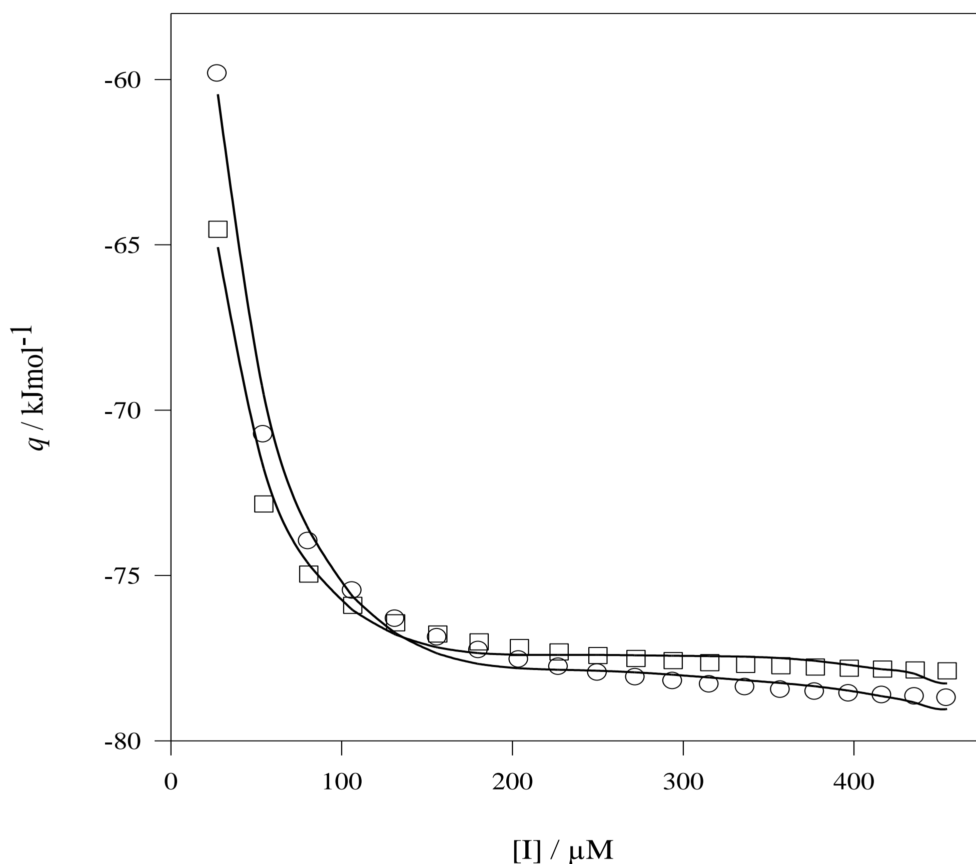

Figure-1
Comparison between the experimental heats, q, for the interaction between *p*-phenylene-bis dithiocarbamate and Mushroom Tyrosinase at 27 °C (□), 37 °C (O) and calculated datas (lines) at both temperatures via eq. 1