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Short Communication

Thermal investigation of the interaction between Phenyl Dithiocarbamate and mushroom tyrosinase

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Abstract

A comprehensive, simple and rapid thermodynamic study on the interaction of Mushroom Tyrosinase (MT) with Phenyl Dithiocarbamate by using isothermal titration calorimetry at 27 and 37°C in phosphate buffer (10 mM) at pH 6.8, was carried out to see whether Phenyl Dithiocarbamate induced conformational change of Mushroom Tyrosinase and how changes by ligand binding were occurred. The extended solvation theory can be used to elucidate the stability of enzyme by Phenyl Dithiocarbamate. The negative change of the Gibbs free energy at two temperatures of 27 and 37°C shows that the binding process in both temperatures are spontaneous. The obtained results indicate that there are two identical and non-cooperative binding sites for Phenyl dithiocarbamate.

Keywords: ushroom tyrosinase; phenyl dithiocarbamate; isothermal titration calorimetry.

Introduction

Tyrosinase is a bifunctional, copper-containing monooxygenase catalysing the *o*-hydroxylation of monophenols to the corresponding catechols and the oxidation of catechols to the corresponding *o*-quinones[1]. *o*-Quinones follow some reactions, which result in formation of biopolymers like melanin[2]. In mushrooms as well as in fruits and vegetables, this enzyme is responsible for browning, a commercially undesirable phenomenon[3]. Therefore, Tyrosinase inhibitors have attracted interest recently due to undesired browning in vegetables and fruits in post-harvest handling. Among inhibitors, a distinction can be made between chelators of two Cu^{2+} in the active site. The well-known heavy metal chelators, derivatives of dithiocarbamate have been found to possess a wide range of biological activities of Mushroom Tyrosinase. In view of the increasing importance of controlling Tyrosinase activities, we applied isothermal titration calorimetry (ITC) to obtain thermodynamic parameters for the interaction between Mushroom Tyrosinase and Phenyl Dithiocarbamate at two temperatures. We attempted to apply the extended solvation model to allow one to interpret the enzyme stability after binding to a ligand.

Material and Methods

Experimental: Mushroom tyrosinase was obtained from sigma and phenyl dithiocarbamate was synthesized. All other materials and reagents were of analytical grade, and solutions were made in 10 mM phosphate buffer using double-distilled water.

The isothermal titration calorimetric experiments were performed with the four channel commercial calorimetric system, thermal activity monitor 2277, thermometric, sweden. The microcalorimeter is composed of two identical cells, reference cell and a sample cell of 1.8 mL in volume which made of a highly efficient thermal conducting material surrounded by an idiabetic jacket. The sample cell was loaded with mushroom tyrosinase solution (8.3 μM) and phosphate buffer sollution (10 mM) and the reference cell contained buffer sollution. The solution in the cell was stirred at 307 rpm by the syringe filled with phenyl dithiocarbamate solution (25 mM) to ensure rapid mixing. Injection of phenyl dithiocarbamate solution in to the perfusion vessel was repeated 20 times, with 20 μL per injection. To correct the thermal effects due to ligand dilution, control experiments were done in which identical aliquots were injected into the buffer solution with the exception of enzyme. The measured enthalpy changes associated with processes occurring at two constant temperatures of 27^0C and 37°C in kJ mol^{-1} are shown in figure-1.

Results and Discussion

Our efforts aimed at elucidation of whether Phenyl Dithiocarbamate induced conformational change of Mushroom Tyrosinase and how thermodinamical changes by ligand binding were occured. Obtained heats from the extended solvation model (Eq. 1) for interactions between a protein and ligand in the aqueous solvent systems, as we have shown before, are in principle compatible with ITC enthalpies[4-12]:

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

Where x'_B , x'_A can be defined as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \qquad x'_A = 1 - x'_B \quad (2)$$

x_B is equal to the ligand concentrations divided by the maximum concentration of the ligand upon saturation of all enzyme as follows:

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

It is worth noting that, the smallest relative standard coefficient error and the highest value of r^2 support p=1, this means that ligand binds at each site independently and the binding is non-cooperative. L_A and L_B are the contributions of unbound and bound ligand to the heats of dilution with the exclusion of enzyme and can be calculated from the heats of dilution of phenyl dithiocarbamate in buffer as follows:

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

δ^{θ}_A and δ^{θ}_B parameters have been optimized to fit the data and recovered from the coefficients of the second and third terms of Eq. 1, while they are indexes of mushroom tyrosinase structural changes due to the reaction with Phenyl Dithiocarbamate in the low and high concentrations, respectively. The superscript θ in all cases refer to the quantities in the infinite dilution of the solute. It is interesting to note that the positive values of δ^{θ}_A and δ^{θ}_B at 27^{0}C and 37°C, exhibit that phenyl dithiocarbamate is able to stabilize the enzyme. These results of $\delta^{\theta}_A > \delta^{\theta}_B$ at 27°C and $\delta^{\theta}_A < \delta^{\theta}_B$ at 37°C indicate that low phenyl dithiocarbamate concentration at 27°C and high phenyl dithiocarbamate concentration at 37°C cause more stabilization of MT. According to figure-1 the calculated heats show a good agreement with the experimental data.

For a set of identical and independent binding sites, we show three different methods of ITC data analysis for providing the dissociation binding constant (K_d). In the first method, using eq. 2, a plot of $\frac{\Delta q}{q_{max}} M_0$ *vs* . $(\frac{\Delta q}{q}) L_0$ should be a linear plot by a slope of $\frac{1}{g}$ and the vertical-intercept of $(\frac{-K_d}{g})$, which the number of binding sites (g) and K_d can be obtained:

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

Our results suggest a set of two binding sites with non cooperativity. M_0 and L_0 are total concentrations of enzyme and ligand, respectively. While q represents the heat value at a certain L_0 and q_{max} represents the heat value upon saturation of all enzyme, $\Delta q = q_{max} - q$.

The linearity of the plot has been examined by different estimated values for q_{max} to find the best value for the correlation coefficient. If q_{max} is calculated per mole of enzyme then the standard molar enthalpy of binding for each binding site will be $\Delta H^{\circ} = \frac{q_{max}}{g}$. The calculated K_d, g and $\Delta H°$ are reported in table 1.

The change of the standard Gibbs free energy of binding ($\Delta G°$), which is shown in table 1, is determined using K_a, the association binding constant (the inverse of the K_d), In the equation $\Delta G = -RT\mathrm{Ln}K_a$. Where R is the gas constant and T is the absolute temperature. To compare all thermodynamic parameters, the value of ΔG° can use in eq. 5 for calculating the change in standard entropy (ΔS°) of this binding.

$$\Delta G° = \Delta H° - T\Delta S° \quad (5)$$

The binding process in both two temperatures is spontaneous ($\Delta G° < 0$), which are only entropy driven that means the predominant interaction in the active site of the enzyme is hydrophobic.

Conclusion

p=1 indicates that the binding is non-cooperative in two binding sites. The positive values of δ^{θ}_A and δ^{θ}_B show that phenyl dithiocarbamate stabilizes the MT structure. The binding process for MT inhibition is only entropy driven, indicating that hydrophobic interaction is more important in the inhibition sites of MT.

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Table 1
Thermodynamic parameters for the interaction between Phenyl Dithiocarbamate and Mushroom Tyrosinase in Phenyl Dithiocarbamate solution with water

Parameters	T=300 K	T=310 K
δ_A^θ	3.91±0.01	4.73±0.02
δ_B^θ	3.00±0.02	5. 31±0.02
K_a / M^{-1}	2929.11±54	3471.02±62
g	2	2
P	1±0.04	1±0.04
ΔH / kJmol^{-1}	12.12±0.06	12.52±0.02
ΔG / kJmol^{-1}	-19.90±0.04	-21.00±0.05
ΔS / kJmol^{-1}K^{-1}	0.11±0.01	0.10±0.01

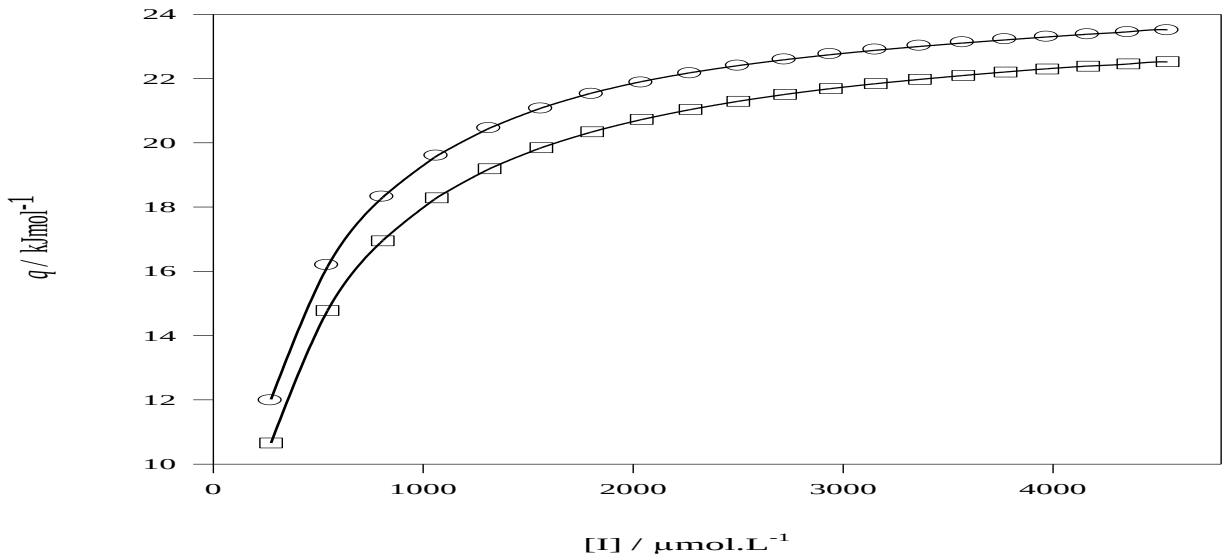

Figure-1
Comparison between the experimental heats, q, for the interaction between Phenyl Dithiocarbamate and Mushroom Tyrosinase at 27 °C (ϒ), 37 °C (O) and calculated datas (lines) at both temperatures via Eq. 1.