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Short Communication

New methods for Data Analysis of Isothermal Titration Calorimetry for studying binding of two n-alkyl Xanthates to Mushroom Tyrosinase

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Abstract

A simple rapid direct isothermal titration calorimetry (ITC) method was applied to study the binding properties and structural changes of mushroom tyrosinase enzyme (MT) due to the interaction with two iso-alkyl dithiocarbonates (xanthates), $C_3H_7OCS_2Na$ (I) and $C_4H_9OCS_2Na$ (II) at 27 °C in phosphate buffer (10 mM) at pH 6.8. The extended solvation model provides more insights into this interaction for further understanding of the effect of iso-propyl and iso-butyl xanthate on the stability and the structural changes of MT. The solvation parameters derived from the solvation model can be related to the changes in the stability of enzyme and type of inhibition. ITC implies that there is a set of two binding sites for two new synthesized xanthates on MT with non cooperativity in the binding process.

Keywords: Mushroom tyrosinase, iso-propyl xanthate, iso-butyl xanthate, the extended solvation theory.

Introduction

Tyrosinase is a copper containing monooxygenase catalyzing the *o*-hydroxylation of monophenols to the corresponding catechols and the oxidation of catechols to the corresponding *o*-quinones[1]. mushroom tyrosinase is popular among researchers as it is commercially available and inexpensive[2]. In mushrooms as well as in fruits and vegetables, this enzyme is responsible for browning, a commercially undesirable phenomenon. Therefore, tyrosinase inhibitors have attracted interest recently due to this undesired browning in vegetables and fruits in post-harvest handling. The di-copper centre of this enzyme has been the target of many inhibitors[3]. Xanthate compounds act as inhibitors of mushroom tyrosinase due to their ability to chelate copper ion. Lineweaver-Burk plots showed different patterns of mixed and competitive inhibition for iso-propyl and iso-butyl xanthate, respectively[4,5]. We attempted to apply the extended solvation model to allow one to interpret the enzyme stability due to its binding with two new synthesized xanthates and to predict the inhibition type.

Material and Methods

MT was obtained from sigma, iso-propyl and iso-butyl xanthate was synthesized[5]. 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 microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The sample cell contained 1.8 mL MT (8.3 μM) and phosphate buffer solution (10 mM; pH 6.8) and the reference cell filled with phosphate buffer. The titration of MT with iso-propyl and iso-butyl xanthate involved 20 consecutive injections of the ligand and each injection included 20 μL iso-propyl or iso-butyl xanthate. The calorimetric signal was gauged by a digital voltmeter that was part of a computerized recording system. The heat of injection was calculated by the 'Thermometric Digitam 3' software program. The microcalorimeter was frequently calibrated electrically during the course of the study.

Results and Discussion

As we have shown before the heats of interactions between a protein and a ligand in the aqueous solvent systems, can be analyzed by the following equation[6-15].

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

The obtained heats from extended solvation model (equation 1) are in principle compatible with ITC enthalpies over the entire range of iso-propyl and iso-butyl xanthate, as shown in figure-1. Where x'_B and x'_A can be defined as follows:

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

x_B is equal to the total ligand concentrations divided by the maximum concentration of the ligand upon saturation of all enzyme. The obtained result of p=1 shows that ligand bind at each site independently. L_A and L_B are the relative of unbound and bound xanthate contributions to the enthalpies of dilution in the absence of tyrosinase.

$$L_A = q_{dilut} + x_B(\frac{\partial q_{dilut}}{\partial x_B}) \qquad L_B = q_{dilut} - x_A(\frac{\partial q_{dilut}}{\partial x_B}) \quad (3)$$

δ^{θ}_A and δ^{θ}_B values reflect the tyrosinase structural changes due to the interaction with xanthates in the low and high concentrations in infinite dilution of tyrosinase, respectively. The negative values of δ^{θ}_A and δ^{θ}_B show that tyrosinase is substantially destabilized by iso-propyl and iso-butyl xanthate at 27 °C. It is worth noting that, the approximately identical values of δ^{θ}_A and δ^{θ}_B for iso-propyl xanthate (table- 1) can be attributed to the mixed inhibition and the different δ^{θ}_A and δ^{θ}_B values for iso-butyl (table-1) can be related to the competitive inhibition, which are in good agreement with previous results[3,4].

For a set of identical and independent binding sites, the number of binding sites and dissociation equilibrium constant can be calculated by:

$$\frac{\Delta q}{q_{max}} M_0 = (\frac{\Delta q}{q}) L_0 \frac{1}{g} - \frac{K_d}{g} \quad (4)$$

q_{max} represents the heat value upon saturation of all biomacromolecule. $\Delta q = q_{max} - q$. 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. The molar enthalpy of binding for each binding site ($\Delta H°$) will be $\Delta H° = \frac{q_{max}}{g}$. The standard Gibbs free energy, $\Delta G°$, can be calculated from association constant $(K_a = \frac{1}{K_d})$ as follows:

$$\Delta G = -R\,T \ln K_a \quad (5)$$

The negative values of $\Delta G°$ suggest that the binding processes of MT to I and II proceed spontaneously, which are both enthalpy and entropy driven. $\Delta S°$ is directly calculated from $\Delta G°$ and $\Delta H°$ according to equation 6:

$$\Delta S° = \frac{(\Delta H° - \Delta G°)}{T} \quad (6)$$

All calculated thermodynamic parameters are summarised in table-1.

Conclusion

Considering the massive number of different formulations in the healthcare and cosmetic market which contain tyrosinase controlling substances, our focalization on the thermodynamic parameters of the interaction between MT and iso-propyl and iso-butyl xanthate as inhibitors, shows that there is a set of two binding sites with non cooperativity for both xanthates on MT and the binding processes of both ligands are spontaneous, which are both enthalpy and entropy driven. The agreement between the experimental heats and the calculated results via Eq. 1, support the extended solvation model. The discovered binding parameters from solvation model indicate that the stability of tyrosinase is decreased as a result of its interaction with iso-propyl and iso-butyl xanthate. The recovered δ^{θ}_A and δ^{θ}_B values can be used to predict the mode of inhibition.

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Table-1

Binding parameters for xanthates+MT interactions recovered from Eqs. 1, 4, 5 and 6. *p*=1 indicates that the binding is non-cooperative in two binding sites. The negative values of δ^{θ}_A and δ^{θ}_B show that xanthates destabilize the MT structure. The binding process for MT inhibition is both enthalpy and entropy-driven

parameters	I	II
p	1±0.01	1±0.01
g	2±0.02	2±0.02
K_a / M^{-1}	9.07×10^4±24	1.26×10^5±12
$\Delta H°$/ kJ.mol^{-1}	-18.70±0.06	-19.30±0.07
$\Delta G°$/ kJ.mol^{-1}	-28.47±0.12	-29.28±0.14
$\Delta S°$/ kJ.mol^{-1}.K^{-1}	0.03±0.01	0.03±0.01
δ^{θ}_A	-4.99±0.02	-4.47±0.06
δ^{θ}_B	-4.23±0.02	-6.58±0.08

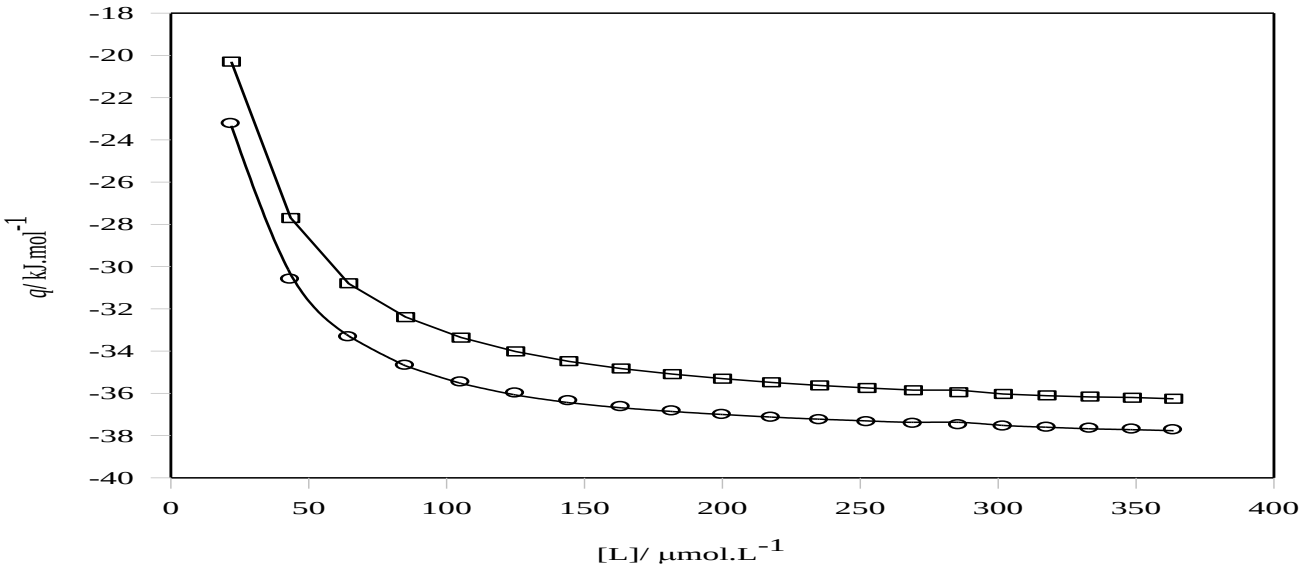

Figure-1
Comparison between the experimental heats, q, for the interaction between mushroom tyrosinase and iso-propyl xanthate (ϒ), iso-butyl xanthate (ⵔ) at 27 °C and calculated data (lines) via equation 1.

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