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Short Communication

Calorimetric Studies on the Interaction between two n-alkyl Xanthates and Mushroom Tyrosinase

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Abstract

Thermodynamics of the interaction between two iso-alkyl dithiocarbonates (xanthates), $C_3H_7OCS_2Na$ (I), $C_5H_{11}OCS_2Na$ (II) with mushroom tyrosinase was investigated at 27°C, pH 6.8 and in phosphate buffer (10 mM) by isothermal titration calorimetry to clarify thermodynamics of this binding as well as structural changes of the enzyme due to its interaction with xanthates. These compounds are potent inhibitors of MT with K_a values of 9.07 x 10^4, 1.68 x 10^5 M^{-1} for I and II, respectively. The MT inhibition is related to the chelating of the copper ions at the active site by a negative head group (S-) of the anion xanthate. Different K_a values for MT inhibition are related to different interactions of the aliphatic chains of I and II with hydrophobic pockets in the active site of the enzyme. The obtained results indicate that there are two identical and non-cooperative binding sites for both xanthates. The extended solvation theory was used to elucidate the effect of these xanthates on the stability of enzyme. These compounds are potent inhibitors of MT with association equilibrium constant (K_a) values of 9.07 × 10^4 and 1.68×10^5 L.mol^{-1} for I and II, respectively. Different K_a values for MT inhibition are related to different interactions of the aliphatic chains of I and II with hydrophobic pockets in the active site of the enzyme. It is possible to ascribe the values of δ^{θ}_A and δ^{θ}_B for I and II to the type of inhibition. The obtained results indicate that there are two identical and non-cooperative binding sites for both xanthates.

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

Introduction

Tyrosinase is the common name for enzyme that is formally termed monophenol mono oxygenase and is listed as enzyme (1.14.18.1) in the standard enzyme nomenclature[1]. Tyrosinase is bifunctional metalloenzyme and deals with catalyzing ortho hydroxylation of monophenols to diphenols and the oxidation of o-diphenols to o-quinones, also it is a necessary enzyme for synthesizing melanin. Melanin is a pigment, which is responsible for hyper pigmentation in skin and the undesirable browning of fruits and vegetables after harvest-handling[2]. Tyrosinase has two Cu^{2+}, that are each coordinated by histidine residues in the active sites and these two Cu^{2+} are essential for the enzyme to participate in catalysis, so chelating the Cu^{2+} in this enzyme by different inhibitors has been embattled to reduce tyrosinase activity[3]. Incidentally, many investigators have been paying special attention to tyrosinase inhibition because of its potential use in medicinal and cosmetic applications as well as its usefulness for agricultural purposes[4]. Previous studies on the inhibitory effect of xanthates reported that iso-propyl xanthate and iso-pentyl xanthate show mixed and competitive inhibition, respectively[3,5]. Among many inhibitors of mushroom tyrosinase, we applied isothermal titration calorimetry (ITC) to obtain thermodynamic parameters of the interaction between two new n-alkyl xanthates (iso-propyl xanthate and iso-pentyl xanthate) and mushroom tyrosinase. To understand the relationship between the structure and stability of biological macromolecules, thermodynamic investigating of bindings is very useful.

Material and Methods

Mushroom tyrosinase was obtained from Sigma, iso-propyl xanthate and iso-pentyl xanthate sodium salts were synthesized[5]. All other materials and reagents were of analytical grade, and solutions were made in 10 mM buffer phosphate using double-distilled water.

The isothermal titration calorimetric experiments were performed with the four channel commercial calorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. A solution of ligand (20 µL) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL tyrosinase (8.3 µmol.L^{-1}). The heat of each injection was calculated by the "Thermometric Digitam 3" software.

Results and Discussion

We have shown previously that the heats of interactions between a protein and ligand in the aqueous solvent systems can be analyzed by the following equation[6-10]:

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

Where x'_B and 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 the extended solvation model (figure-1). p=1, this means that ligand binds at each site independently and the binding is non-cooperative. L_A and L_B are the relative contributions of unbound and bound ligand in the heats of dilution with the exclusion of enzyme and can be calculated from the heats of dilution of ligands in buffer as follows:

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

Parameters have been optimized to fit the data and recovered from the coefficients of the second and third terms of equation 1 (table-1), while they are indexes of MT structural changes due to the reaction with xanthates in the low and high concentrations, respectively. The negative values of δ^{θ}_A and δ^{θ}_B exhibit that iso-propyl and iso-pentyl xanthate destabilizes MT structure. From another point of view, the approximately identical values of δ^{θ}_A and δ^{θ}_B for iso-propyl xanthate (δ^{θ}_A=-4.99, δ^{θ}_B=-4.23), can be related to the mixed inhibition, whereas the large difference between the obtained δ^{θ}_A and δ^{θ}_B values for iso-pentyl xanthate (δ^{θ}_A=-4.23, δ^{θ}_B=-8.66), can be related to the competitive mode of inhibition. In the competitive inhibition type, similarities between substrate and inhibitor exclude simultaneous binding of inhibitor and substrate. Double reciprocal Lineweaver-Burk plots confirm mixed and competitive inhibitions for iso-propyl and iso-pentyl xanthate, respectively[3,5].

For a set of identical and independent binding sites, using equation 5, 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 $(\frac{-K_d}{g})$, so we can obtain the number of binding sites (g) and K_d[10]:

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

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. 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

$$\Delta H^{o} = \frac{q_{max}}{g}.$$

The change of the standard Gibbs free energy of binding (ΔG°), which is shown in table-1, is determined by using K_a, the association binding constant (the inverse of the K_d), in the equation 6:

$$\Delta G° = -R\,T \,\mathrm{Ln}\, K_a \quad (6)$$

The change in standard entropy ($\Delta S°$) of this binding can be calculated as equation 7:

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

All calculated thermodynamic parameters are reported in table-1.

Conclusion

The extended solvation theory was used to obtain the effect of iso-propyl and iso-pentyl xanthate on the stability of MT. The agreement between the experimental heats and the calculated results via equation 1 is strong and support the extended solvation model. The results of this study also show non-cooperative binding between two identical binding sites of MT. The binding processes of both ligands are spontaneous (ΔG°<0) and both enthalpy and entropy driven. Negative δ^{θ}_A and δ^{θ}_B values for the interaction, essential for many non-specific ligand-protein interactions (in the nonspecific interaction, ligand binds weakly to many different groups at the protein/water interface), indicating that both xanthates destabilize mushroom tyrosinase structure. The approximately identical values of δ^{θ}_A and δ^{θ}_B for iso-propyl xanthate can be related to the mixed inhibition, whereas the large difference between the obtained δ^{θ}_A and δ^{θ}_B values for iso-pentyl xanthate can be related to the competitive mode of inhibition.

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Table-1

Binding parameters for xanthates+MT interactions 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 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	1.68×10^5±12
$\Delta H°$/ kJ.mol^{-1}	-18.70±0.06	-1.16±0.03
$\Delta G°$/ kJ.mol^{-1}	-28.47±0.12	-30.02±0.13
$\Delta S°$/ kJ.mol^{-1}.K^{-1}	0.03±0.01	0.10±0.02
δ_A^θ	-4.99±0.02	-4.23±0.06
δ_B^θ	-4.23±0.02	-8.66±0.08

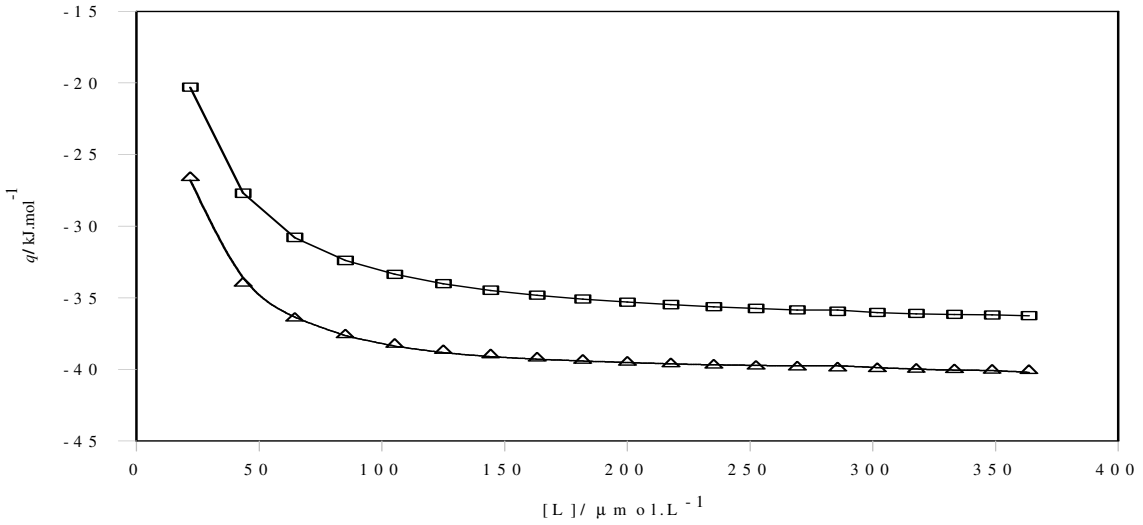

Figure-1

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