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Short Communication

Thermal Investigation of three n-alkyl Xanthates binding with Mushroom Tyrosinase

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Abstract

The interaction between three iso-alkyldithiocarbonates (xanthates), as sodium salts, $C_3H_7OCS_2Na$ (I), $C_4H_9OCS_2Na$ (II), $C_5H_{11}OCS_2Na$ (III) and mushroom tyrosinase enzyme, MT, have been investigated by isothermal titration calorimetry to clarify thermodynamics of these bindings as well as structural changes of the enzyme due to its interaction with xanthates at 27 °C in phosphate buffer (10 mmol.L^{-1}; pH=6.8). The extended solvation model was used to elucidate the effect of these xanthates on the stability of the enzyme. The values of δ^{θ}_A and δ^{θ}_B were attributed to the type of inhibition for I, II and III. The obtained results indicate that there are two identical and non-cooperative binding sites for three xanthates.

Keywords: Mushroom tyrosinase, iso-propyl xanthate, iso-butyl xanthate, iso-pentyl xanthate.

Introduction

Tyrosinase is ubiquitously distributed among animals, plants and fungi and plays a pivotal role in catalysis the hydroxylation of monophenols and the oxidation of o-diphenols to o-diquinones[1]. Tyrosinase is a bifunctional, copper-containing enzyme involved in pigment biosynthesis of various organisms such as melanin[2,3]. Besides, tyrosinases are important subjects of many ongoing researches mainly due to their key role in the enzymatic browning phenomenon which affects the quality of fruits, vegetables and crop products[4]. The inhibitors of this enzyme have been utilized in cosmetics, especially as depigmenting agents in the case of hyperpigmentation[5, 6]. The inhibitory effect of xanthates on mushroom tyrosinase was elucidated, which is related to the chelating of the copper ions at the active site by a negative head group (S^-) of the anion xanthate. The inhibitory effects of three synthetic n-alkyl xanthates, sodium salts, with different aliphatic tails of C_3, C_4 and C_5 were described. Lineweaver-Burk plots showed different patterns of mixed for iso-propyl and competitive inhibition for iso-butyl and iso-pentyl xanthate [3, 4]. To see whether three new n-alkyl xanthates (iso-propyl, iso-butyl and iso-pentyl xanthate) induced structural changes of tyrosinase and how thermodynamical changes by ligand binding are occurred, the analysis of isothermal titration calorimetric data by the extended solvation model is conducted.

Material and Methods

Mushroom tyrosinase was purchased from Sigma and iso-propyl xanthate, iso-butyl xanthate, iso-pentyl xanthate sodium salts were synthesized[4]. All other materials and reagents were of analytical grade, and solutions were made in 10 mmol.L^{-1} 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. Injection of iso-propyl, iso-butyl or iso-pentyl xanthate solution was repeated 20 times by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL tyrosinase (8.3 µmol.L^{-1}) and phosphate buffer solution (10 mmol.L^{-1}; Ph 6.8). Each injection included 20 µL xanthate solution. The measurements were performed at constant temperature of 27°C. The heat of each injection was calculated by the "Thermometric Digitam 3" software.

Results and Discussion

The interaction between a protein and ligand in the aqueous solvent system can be analyzed by the extended solvation equation[7-20]:

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

x'_B is a fraction of bound ligand with the protein molecule and x'_A is the fraction of unbound ligand. 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 concentration of ligand after every injection divided by the maximum concentration of 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 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:

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

Recovered values of δ^{θ}_A and δ^{θ}_B from the coefficients of the second and third terms of equation 1, are indexes of MT structural changes due to the reaction with xanthates in the low and high concentrations, respectively. It is possible to attribute the approximately identical values of δ^{θ}_A and δ^{θ}_B for iso-propyl xanthate (table-1) to the mixed inhibition, whereas the different δ^{θ}_A and δ^{θ}_B values for iso-butyl and iso-pentyl xanthate (table-1) can be related to the cooperative manner of inhibition. These interpretations are in agreement with previous reports[3,4]. The negative values of δ^{θ}_A and δ^{θ}_B exhibit that iso-propyl, iso-butyl and iso-pentyl xanthates destabilize MT structure.

A simple graphical method was applied for ITC data analysis in the protein-ligand interaction for a set of identical and independent binding sites to provide the number of binding sites (g), the dissociation binding constant (K_d) and the molar enthalpy of binding site (ΔH°). using Eq. 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 $(\frac{-K_d}{g})$ [11-13]:

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

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} indicates 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 ($\Delta G°$) is determined by using the association binding constant (K_a), obtained from the inverse of K_d value, in the Eq. 6, where R is the gas constant and T is the absolute temperature[14]:

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

The change in standard entropy ($\Delta S°$) of this binding can be calculated as Eq. 7[15]:

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

All calculated thermodynamic parameters are reported in table-1.

Conclusion

The extended solvation theory was used to predict enzyme destabilization and binding non-cooperativity in two identical binding sites. The close agreement is found between the calculated and experimental results (figure-1) and gives considerable support to the use of theory. The binding processes for three xanthates are spontaneous in the forward direction ($\Delta G° < 0$). Three xanthate binding processes are both enthalpy and entropy driven, with negative δ^{θ}_A and δ^{θ}_B values, indicating that three xanthates destabilize mushroom tyrosinase structure. It is possible to attribute the values of δ^{θ}_A and δ^{θ}_B to the type 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 but the electrostatic interactions are more important than hydrophobic forces

parameters	I	II	III
p	1±0.01	1±0.01	1±0.01
G	2±0.02	2±0.02	2±0.02
K_a / M^{-1}	9.07×10^4±24	1.26×10^5±12	1.68×10^5±12
$\Delta H°$/ kJ.mol^{-1}	-18.70±0.06	-19.30±0.07	-1.16±0.03
$\Delta G°$/ kJ.mol^{-1}	-28.47±0.12	-29.28±0.14	-30.02±0.13
$\Delta S°$/ kJ.mol^{-1}.K^{-1}	0.03±0.01	0.03±0.01	0.10±0.02
δ_A^θ	-4.99±0.02	-4.47±0.06	-4.23±0.06
δ_B^θ	-4.23±0.02	-6.58±0.08	-8.66±0.08

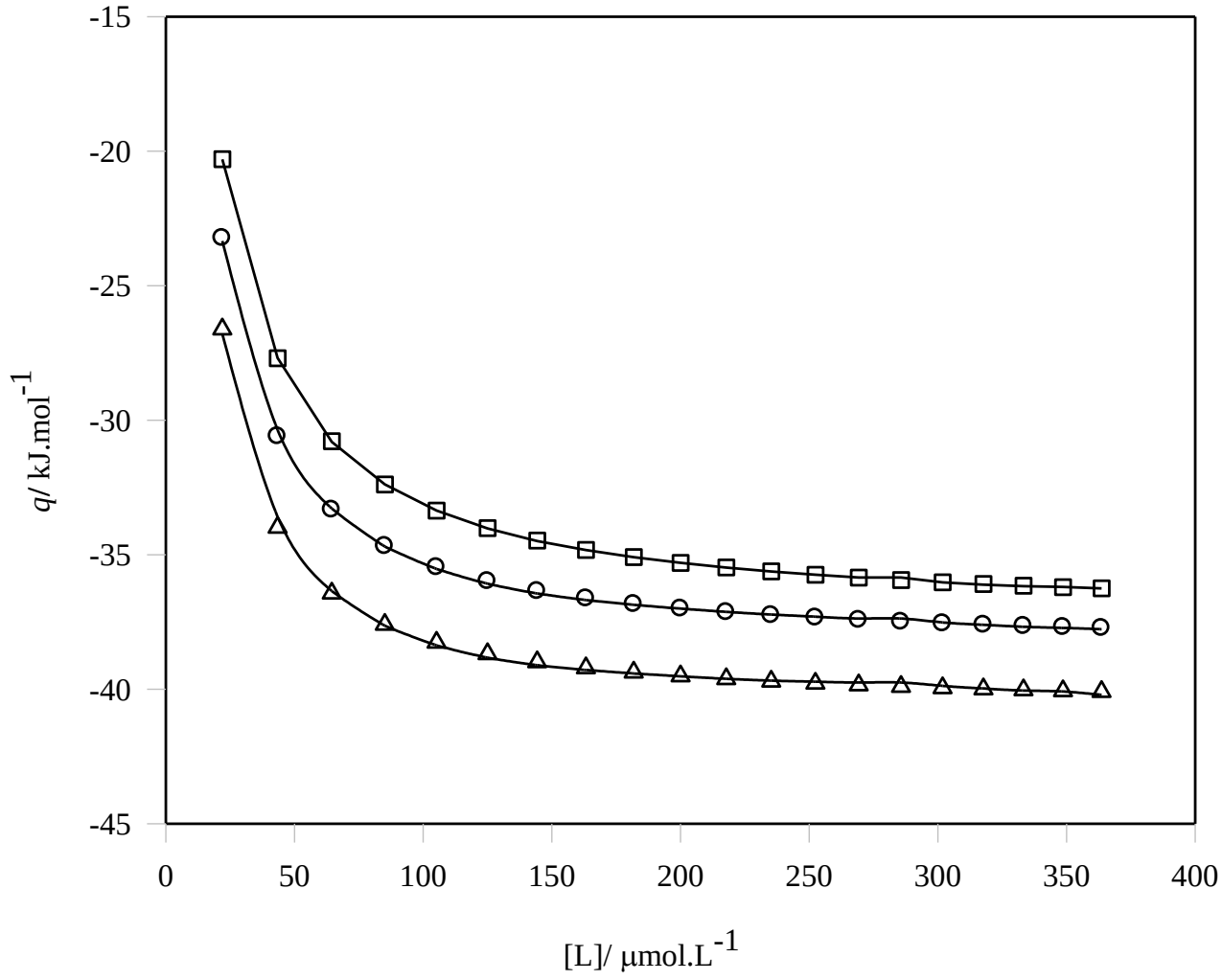

Figure-1
Comparison between the experimental heats, q, for the interaction between mushroom tyrosinase and iso-propyl xanthate (ϒ), iso-butyl xanthate (⊗) and iso-pentyl xanthate (⊄) at 27 °C and calculated data (lines) via equation 1