

Supplementary information

Investigation the interactions between indole-3-acetic acid and catalase: A spectroscopic study in combination with second-order calibration and molecular docking methods

Xinfeng Zhu^{a,b}, Yanni Lu^c, Jiandong Wang^{a,*}, Qin Xu^{c,*}

^a *College of Computer Science and Technology, Nanjing University of Aeronautics and Astronautics, Nanjing 210016, China*

^b *College of Information Technology, Yangzhou University, Yangzhou 225127, China*

^c *College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, China*

Corresponding author's E-mail address: xuqin@yzu.edu.cn (Qin Xu)

1. Experiment Section

1.1 Isothermal Titration Calorimetry (ITC)

ITC was performed with a VP-ITC calorimeter (Micro-Cal Inc., Century City, CA) at 25 °C. Samples were buffered at pH 7.4 with 50 mM Tris-HCl solution. In a typical experiment, CAT solution (2.0×10^{-5} M) was placed in the 1.439 cm³ sample cell of the calorimeter and IAA solution (2.5×10^{-4} M) was loaded into the injection syringe. IAA was titrated into the sample cell as a sequence of 25 injections of 250 µl aliquots. The time delay between successive injections was 3 min. The contents of the sample cell were stirred throughout the experiment at 307 rpm to ensure thorough mixing. Raw data were obtained as a plot of heat (µJ) against injection number. Control experiments included the titration of 2.5×10^{-4} M IAA into buffer, buffer into CAT and buffer into buffer. The last two controls resulted in small and equal enthalpy changes for each successive injection of buffer, and therefore, were not further considered in the data analysis. Corrected data refer to experimental data after subtraction of the IAA into buffer control data. The first injection was ignored in the final data analysis.

Integration of the peaks corresponding to the injections and correction for the baseline were carried out using Origin[®] scientific plotting software provided by the manufacturer. All measurements were repeated three times.

1. 2. CAT activity assay and inhibition study

CAT activity was determined by detecting H₂O₂ consumption according to the procedure by Beers and Sizer¹. In a total volume of 3 mL sample, 1.0 × 10⁻⁷ M of CAT was incubated together with 0.018 M H₂O₂ and different concentrations of IAA in 50 mM Tris-HCl buffer (pH 7.4). The absorbance of the reaction mixture at 240 nm was recorded for 1 min every 10 sec. One unit of CAT activity is defined as the amount of enzyme that decomposes 1.0 mMol H₂O₂ per min. Controls incubated without IAA were also included and the results were expressed as the relative percentage of activity in respect to the control.

For inhibition studies, a matrix of substrate (H₂O₂) between 0.005 and 0.03 M and different concentrations of inhibitor (IAA) was generated. Values of the absorbance of H₂O₂ ($A^{H_2O_2}$) were determined after short reaction times in triplicate at each H₂O₂ concentration. The Lineweaver-Burk plot of 1/ $A^{H_2O_2}$ against 1/[H₂O₂] was used to identify the inhibition type².

2. Discussion

Isothermal titration calorimetry (ITC) is a technique used to measure the heat exchange associated with molecular interactions at constant temperature, most often as a direct method for finding thermodynamic parameters associated with complex formation³. It could provide thermodynamic profile, including binding affinity/association constant (K) and stoichiometry (n)⁴. A representative calorimetric titration profile of titration of CAT with IAA at pH 7.4 and 298 K is shown in Fig. S1. Each peak in the binding isotherm (see Fig. S1, panel A) represents a single injection of the IAA into the CAT solution. Panel b of the figure shows the plot of the amount of heat liberated per injection as a function of the molar ratio of the IAA to CAT. The

stoichiometry (n), equilibrium binding constant (K) can be analyzed by fitting the data to various interaction models in the Origin[®] scientific plotting software. Fig. S1 B shows that exothermic heats accompanied the binding of IAA to CAT. The data clearly demonstrate that one IAA binds to one CAT. The equilibrium binding constant K was $1.51 \times 10^5 \pm 1.1 \times 10^3 \text{ L} \cdot \text{mol}^{-1}$. The binding constant K and stoichiometry n were in agreement with those obtained by fluorescence methods combined with PARAFAC. The results indicate that the EEM combined with PARAFAC analysis method is suitable for studying the overlapped fluorescence system.

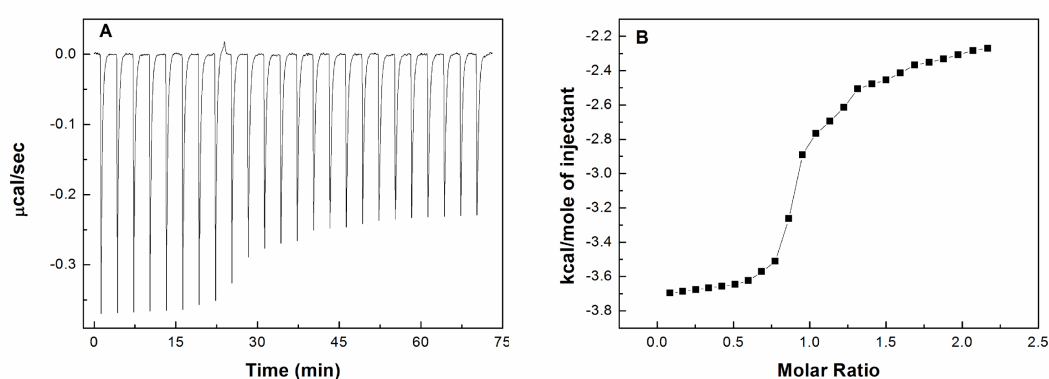


Fig. S1 ITC raw data for the titration of $2.5 \times 10^{-4} \text{ M}$ IAA into $2.0 \times 10^{-5} \text{ M}$ CAT in 50 mM Tris, pH 7.4. (A) the baseline-corrected raw data; (b) the peak-integrated, concentration normalized heats of reaction versus the molar ratio of IAA per CAT.

The effect of IAA on the activity of CAT was also studied in this work. **Fig. S2 A** shows the alterations in enzymatic activity of CAT as a function of IAA concentration. The activity of CAT decreases with increasing amount of IAA in vitro under physical conditions, which suggests the toxicity of IAA to CAT. The double-reciprocal (also known as the Lineweaver-Burk) plot was used to study the inhibition type. **Fig. S2 B** shows a series of lines converging on the same point on the X while the Y-intercept of the plots increases with the increase of IAA concentration. For Lineweaver-Burk plot, the slope of the resulting line is $K_M/V_{\max}$, the y-intercept is $1/V_{\max}$, and the x-intercept is $-1/K_M$. The inhibition of CAT by IAA causes a decrease in $V_{\max}$ value while K_M is unaffected. The hallmarks of noncompetitive

inhibition are an unchanging Michaelis constant (K_M) and a decrease of the maximum velocity (V_{max}) when the inhibitor is present⁵. So IAA acts as a noncompetitive inhibitor by binding to CAT. It binds to CAT at a location other than the active site. The K_m calculated from the x-intercept of **Fig. S2 B** is 74.85 mM, which is close to the reported value of 69.96 mM⁶.

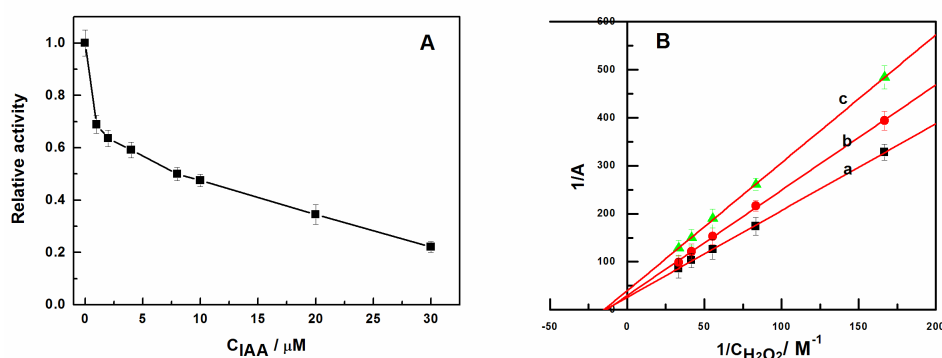


Fig. S2 (A) Changes of CAT activity with the increase of the concentration of IAA. Conditions : 3 ml assay solution containing 50 mM Tris-HCl buffer (pH 7.4), 18 mM of H_2O_2 , 0.1 μM of CAT and different concentrations of IAA at 298 K. (B) Double-reciprocal Lineweaver–Burk plot. Results are mean $\pm$ S.D. of three independent experiments. (a) 0.1 μM CAT; (b) 0.1 μM CAT+ 2.0 μM IAA; (c) 0.1 μM BLC+ 8.0 μM IAA. The data are collected at pH 7.4, 298 K.

Reference

1. R. F. Beers and I. W. Sizer., *J. Biol. Chem.*, 1951, **195** 133-140.
2. A. G. Marangoni, ed., *Enzyme Kinetics, A Modern Approach*, John Wiley & Sons, Hoboken, New Jersey, 2002.
3. R. Perozzo, G. Folkers and L. Scapozza, *J. Recept. Signal Transduction*, 2004, **24**, 1-52.
4. W. B. Peters, V. Frasca and R. K. Brown, *Comb. Chem. High Throughput Screening*, 2009, **12**, 772-790.

5. A. Fersht, ed., *Enzyme Structure and Mechanis*, W.H. Freeman, New York, 1985.
6. A. Fernandes and G.Gregoriadis, *Biochim. Biophys. Acta*, 1996, **1293**, 90-96.