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Specific binding and inhibition of 6-benzylaminopurine to catalase: Multiple spectroscopic methods combined with molecular docking study

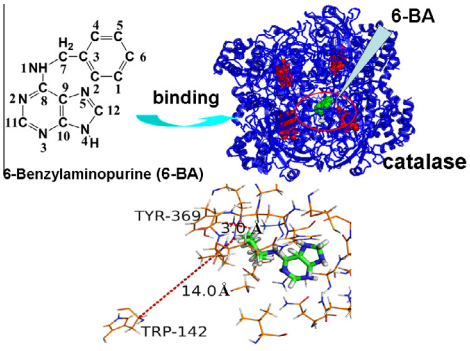
Qin Xu [a,*], Yanni Lu [a], Longyun Jing [a], Lijuan Cai [a], Xinfeng Zhu [b], Ju Xie [a], Xiaoya Hu [a,*]

[a] College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, China
[b] College of Information Technology, Yangzhou University, Yangzhou 225127, China

HIGHLIGHTS

- Fluorescence quenching mechanism between BLC and 6-BA was studied.
- 6-BA inhibited BLC activity via a noncompetitive manner.
- Molecular docking method was used to determine the location of 6-BA within BLC.

GRAPHICAL ABSTRACT



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ABSTRACT

6-Benzylaminopurine (6-BA) is a kind of cytokinin which could regulate the activities of the antioxidant defense system of plants. In this work, its interaction with and inhibition of beef liver catalase have been systematically investigated using spectroscopic, isothermal titration calorimetric and molecular docking methods under physiological conditions. The fluorescence quenching of beef liver catalase (BLC) by 6-BA is due to the formation of 6-BA–BLC complex. Hydrogen bonds and van der Waals interactions play major roles in stabilizing the complex. The Stern–Volmer quenching constant, binding constant, the corresponding thermodynamic parameters and binding numbers were measured. The results of UV–vis absorption, three-dimensional fluorescence, synchronous fluorescence and circular dichroism spectroscopic results demonstrate that the binding of 6-BA results in the micro-environment change around tyrosine (Tyr) and tryptophan (Trp) residues of BLC. The BLC-mediated conversion of H_2O_2 to H_2O and O_2, in the presence and absence of 6-BA, was also studied. Lineweaver–Burk plot indicates a noncompetitive type of inhibition. Molecular docking study was used to find the binding sites.

Introduction

The effects of cytokinin on cellular systems have received a great deal of attention in recent decades due to the increasing application of cytokinin in plant culture [1]. 6-Benzylaminopurine (6-BA) (Fig. 1), as one of the first-generation synthetic cytokinins, was used to stimulate cell division, lateral bud emergence (apples, oranges), basal shoot formation (roses, orchids), flowering (cyclamen, cacti) and fruit set (grapes, oranges, melons) [2,3]. It is also regarded as a good candidate for postharvest applications and used as a biopesticide in the USA and Canada [4]. Recent research shows that 6-BA would delay the production of reactive oxygen species, and regulate the oxidative status of tissues. It has a close relationship with the antioxidant defense system of plants [5–7].

Catalase is one of the most important proteins of the antioxidant defense system whose function is to protect cells from the toxic effects by catalyzing the decomposition of hydrogen peroxide

* Corresponding authors. Tel.: +86 514 87975244.
E-mail addresses: yz_xiner@163.com (Q. Xu), xyhu@yzu.edu.cn (X. Hu).

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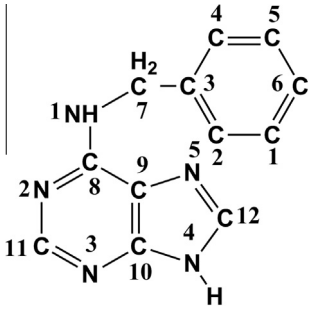

Fig. 1. Structure of 6-benzylaminopurine.

into molecular oxygen and water. Recently, there is increasing evidence that catalase is a major factor in a variety of pathological states such as diabetes, aging, oxidative stress, and cancer [8]. Intake of any extraneous chemical is likely to enhance or inhibit the catalytic activity of catalase in vivo. Understanding the interaction mechanism between catalase and various extraneous chemicals and how these interactions influence their biological functions would help us guide the proper use of these chemicals. In recent years, some researches on the interactions between catalase and chemicals have been developed. Sarmiento et al. have studied the interaction between nafcillin and catalase by equilibrium dialysis and ζ-potential measurements [9]. But this procedure could not provide the conformation changes information. Fluorescence spectroscopy can provide particularly useful information on binding modes by a simple procedure [10]. Liu et al. have reported the interaction mechanisms between catalase and different chemicals such as 4-aminoantipyrine [11], oxytetracycline [12] and fluoroquinolones [13] and characterized their harmful effects by spectroscopic and computational methods. 6-BA has been reported to adjust catalase activities. Díaz–Vivancos found that catalase activity in crocus sativus explants was reduced in the presence 6-BA [14]. Zavaleta–Mancera et al. reported that 6-BA caused the increase of the activities of catalase in the delayed-senescence tissue in dark [15]. However, all of the reported works studied the regulation of catalase activity by 6-BA in plants or plant tissues. Up to now there was no evidence whatsoever supporting the idea that 6-BA would interacts with catalase. Studying the interaction mechanisms between 6-BA and catalase and the structure changes of catalase in the presence of 6-BA could help us to better understand the influence of 6-BA in vivo at the molecular level and guide the proper use of 6-BA.

In this work, the interaction between 6-BA and a kind of catalase, beef liver catalase (BLC), has been investigated by UV–vis spectroscopy, fluorescence spectroscopy, circular dichroism (CD) spectroscopy, isothermal titration calorimetry (ITC) and molecular docking methods. The thermodynamic functions, energy transfer efficiency between the donor and acceptor, binding distances, and effect of 6-BA on the conformation of BLC were investigated. The inhibitory effects and inhibition type of 6-BA on BLC were evaluated. We also tried to locate the best binding sites and to construct the binding modes according to the molecular docking results.

Experimental

Reagents and solutions

Crystalline BLC (molecular weight 240 kD, Product No. C-9322, activity ⩾2000 U/mg, from Bovine Liver, Sigma) was directly dissolved in water to prepare stock solutions (2.0×10^{-5} M) and stored at 4 °C. 6-BA was purchased from Sangon Biotech in Shanghai (China). The stock solution of 6-BA was prepared by dissolving it in ethanol to a suitable concentration (6.0×10^{-3} M). 50 mM Tris–HCl buffer containing 0.1 M NaCl (pH 7.4) was used for all experiments. All chemicals were used without further purifications.

UV–vis absorption and fluorescence experiments

All UV–vis absorption spectra were measured on a Shimadzu UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) by using 1 cm quartz cells.

All fluorescence spectra were recorded on an F-4500 fluorescence spectrophotometer (Hitachi Japan) equipped with a 1 cm cell quartz cuvette, a thermostat bath (Model ZC-10) and a Xenon lamp. The excitation and emission slit widths were both set at 5 nm. The scanning speed was 1200 nm/min.

The fluorescence intensities used in this work were corrected to account for the inner filter effect by using Eq. (1) [16]:

$$F_{corr} = F_{obs} \times 10^{\frac{A_{ex}d_{ex}+A_{em}d_{em}}{2}} \quad (1)$$

where F_{corr} and F_{obs} are the corrected and observed fluorescence intensities respectively. A_{ex} and A_{em} are the absorbance values at excitation and emission wavelengths respectively, and d_{ex} and d_{em} are the cuvette path lengths in the excitation and emission direction (in cm) respectively.

In a typical fluorescence measurement, 1.0 mL of 1.0×10^{-5} M BLC solution, and different concentrations of 6-BA were added into a 10 mL colorimetric tube, successively. The samples were diluted to scaled volume with 0.1 M pH 7.40 Tris–HCl, mixed thoroughly by shaking, and kept static for 15 min at different temperatures (293, 303, and 313 K). The excitation wavelength for BLC was 280 nm, with the excitation and emission slit widths set at 5 nm. The solution was subsequently scanned on the fluorophotometer and the fluorescent intensity at 350 nm was determined. The synchronous fluorescence spectra were recorded when the $\Delta\lambda$ value between the excitation and emission wavelengths was stabilized at 15 and 60 nm, respectively.

To confirm the quenching mechanism, the fluorescence quenching data were analyzed using the Stern–Volmer equation (Eq. (2)) [16].

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \quad (2)$$

where, F_0 and F are emission intensities of BLC in the absence and presence of 6-BA respectively, K_{SV} is the Stern–Volmer constant related to the bimolecular quenching rate constant (k_q) by $K_{SV} = k_q \cdot \tau_0$, and τ_0 is the excited state lifetime of BLC which is 10^{-8} s [17], and [Q] is the concentration of 6-BA.

For the binding-related quenching process, the binding constant K_a and Hill's coefficient n can be determined on the basis of the following Eq. (3) [18]:

$$\lg[(F_0 - F)/F] = \lg K_a + n\lg[Q] \quad (3)$$

This form of Hill's equation is used to estimate the cooperativity in multi-subunit BLC.

The thermodynamic parameters, enthalpy changes (ΔH^θ), and entropy changes (ΔS^θ), can be calculated from the thermodynamic Van't Hoff equation:

$$\ln K_a = \frac{-\Delta H^\theta}{RT} + \frac{\Delta S^\theta}{R} \quad (4)$$

where K_a is the associate constant at the corresponding temperature and R is gas constant. The temperatures used were 298, 308, and 318 K. There is a linear relationship between $\ln K^\theta$ and $1/T$. The enthalpy change (ΔH^θ) can be obtained by the slope, and ΔS^θ