



Role of cyclic alkyl group in conformational instability of tannase



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ARTICLE INFO

Article history:

Received 31 August 2015

Received in revised form 25 March 2016

Accepted 26 March 2016

Available online 26 March 2016

Keywords:

Biocatalysis

FT-IR

Conformational stability

Tannase

Cyclic structure

ABSTRACT

The conformational stability of enzyme has a crucial effect on its catalytic performance. The effects of six organic solvents with different structures on the conformational stability of tannase were studied using Fourier transform infrared spectroscopy in this work. This results indicated that the cyclic structure of organic solvent plays a negative role in the conformational stability of tannase. The alkyl group of organic solvent has an interaction with the groups of oxygen and nitrogen of tannase, and the interaction goes against the conformational stability. The findings potentially provide a deep insight into the relationship between the biocatalytic activity and conformational stability of enzymes and push the study on the interaction of enzyme with organic solvent.

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1. Introduction

Application of enzymes in synthetic organic chemistry gained importance in the latter half of the 20th century [1], while there must be two major limitations to be broken through. First, many enzymes with tolerance for organic solvent were not accessible for practical applications in large scale. Second, a few enzymes exhibit a low substrate affinity, and/or insufficient stability in organic phase, although they have been commonly employed in the enantioselective synthesis [2]. These are impeding industrial application of enzymes in organic synthesis. Accordingly, it will be remarkably necessary to study the effect of organic solvent on enzyme and to understand the relationship between conformational flexibility, stability, and activity of enzyme.

Tannase, a tannin acyl hydrolase (EC, 3.1.1.20), can hydrolyze the ester and depside linkages in hydrolysable tannins [3,4] and synthesize gallic acid esters (e.g., PG) [5] by catalyzing esterification/transesterification reaction in organic media [6]. However, tannase exhibits an insufficient stability as resulting in a low catalytic performance in organic synthesis. Such efforts as bioimprinting [7], immobilization [8,9], and optimization of reaction medium [10–12] were taken to overcome this. Still, its catalytic capability in organic phase is insufficiently stable. It is well known

from our previous report that the conformational structure of tannase in organic solvent can be changed, and the conformation variation closely correlates with the balance between hydrophilic solvents and hydrophobic solvents in reaction system [13]. It is concluded that the insufficient stability maybe results from organic solvent.

The effect of organic solvent on the conformational stability of tannase was investigated using Fourier transform infrared spectroscopy (FT-RT) in this present work. Expectedly, this work will be helpful to understand the relationship between organic solvents and enzymatic conformation well.

2. Materials and methods

2.1. Materials

Tannase was purchased from Jinan Huazuan trading Co., Ltd., China. Tannic acid (CB_{76B}HB_{52B}OB_{46B}), celite, and citric acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC), China. All of other solvents and reagents were obtained commercially and were of analytical grade.

2.2. Enzyme immobilization

50 mg tannases (16 IU) were uniformly dissolved in 5 mL of 90 mM citric acid buffer at pH 5.0 including 0.5 g celite, and maintained without agitation for 30 min at ambient temperature.

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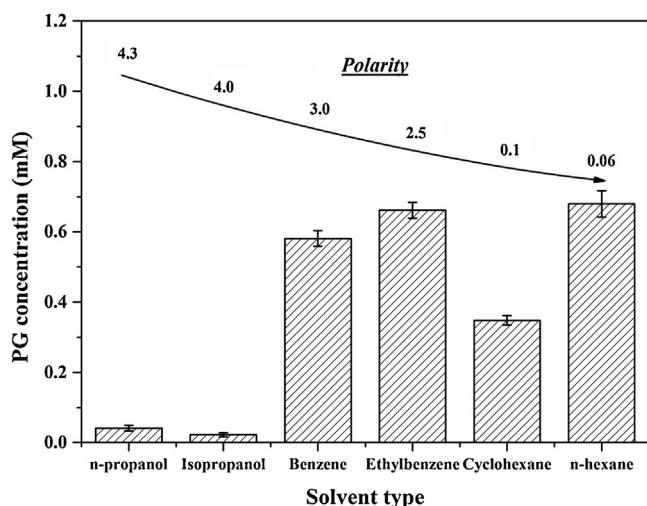


Fig. 1. Effect of organic solvents on the catalytic performance of tannase. The data above the line refer to the polarity values of the corresponding organic solvents on the horizontal axis (the data referred from the polarity chart of organic solvent).

Subsequently, the enzyme solution was frozen at -20°C overnight and lyophilized in a freeze dryer (LGJ-10, Beijing Songyuan Huaxing Sci. & Tech. Development Co., Ltd., China) for 24 h. The powder is an immobilized tannase and stored at 4°C until use.

2.3. Enzymatic reaction and assay of the biocatalytic performance

The immobilized tannase and 50 mg tannic acid were added into a 25 mL flask with approximate 20 mL reactive mixture composed of 2 mL *n*-propanol, 18 mL bulky medium (i.e., *n*-propanol, isopropanol, *n*-hexane, cyclohexane, benzene, and ethylbenzene), and 100 μL distilled water. The reaction was carried out at 40°C and 200 rpm for 24 h. The content of product, propyl gallate (PG), was determined by HPLC (Shimadzu LC-20A, Shimadzu, JPN) to estimate the catalytic performance of tannase. All experiments were performed in duplicates.

2.4. Enzyme treatment and conformation analysis by FT-IR spectrometry

Duplicate six copies of 50 mg tannase were added into organic solvents such as *n*-propanol, isopropanol, *n*-hexane, cyclohexane,

benzene, and ethylbenzene, respectively, and placed at ambient temperature for 1 h and 16 h. The treated enzymes were achieved by centrifugation at 5000 rpm for 5 min. The residual organic solvents were removed by air drying.

The secondary structure of the treated tannase was measured by FT-IR spectrometry detailed by Quiroga et al. [14]. The spectra was measured at 25°C by a FTIR-650 spectrometer (Tianjin Gangdong Sci. & Tech. Development Co., Ltd., China). The spectra in the region of $4000\text{--}400\text{ cm}^{-1}$ had a 1.5 cm^{-1} of spectral resolution. The secondary structure elements based on the information of amide III region and the band assignment were manipulated using Omnic 8.2.0.387 (Thermo Fisher Scientific Inc) coupled with PeakFit v4 (AISN software Inc.)

3. Results and discussion

The effects of *n*-propanol and isopropanol on tannase in the catalytic performance were investigated. The result showed that the catalytic activity of tannase in two alcohols is not high, and wherein tannase in isopropanol has a lower activity than the one in *n*-propanol (shown in Fig. 1). One likely cause is that the essential water for high conformational flexibility is deprived from the peripheral micro water layer around enzyme molecule by the polar alcohols. As compared to *n*-propanol, isopropanol with a higher polarity has a stronger capability for water absorbance, and thus it can reduce tannase activity. Therefore, it is interesting to modify the conformational flexibility of enzyme for the activity increment [15].

Hydrophobic solvents such as *n*-hexane and cyclohexane were chosen to testify the positive effect of low polar organic solvent on tannase in the catalytic activity. The result indicated *n*-hexane dramatically improved the catalytic performance by a factor of 16 relative to *n*-propanol (shown in Fig. 1). The first reason may be that *n*-hexane as a water-immiscible solvent cannot remove the essential water from the micro-water layer around enzyme molecule easily like polar alcohol. Secondly, polar solvents can penetrate deep into the protein's interior, and induce the secondary and tertiary structure to be changed more easily than nonpolar solvents [16,17]. Thirdly, the solvent can change the observed catalytic activity by impacting on the ground state of the reactants and products, and the ground state correlates nicely with the polarity of organic solvent [18]. Unexpectedly, tannase in cyclohexane with a similar polarity to *n*-hexane just has a low catalytic activity, approximately

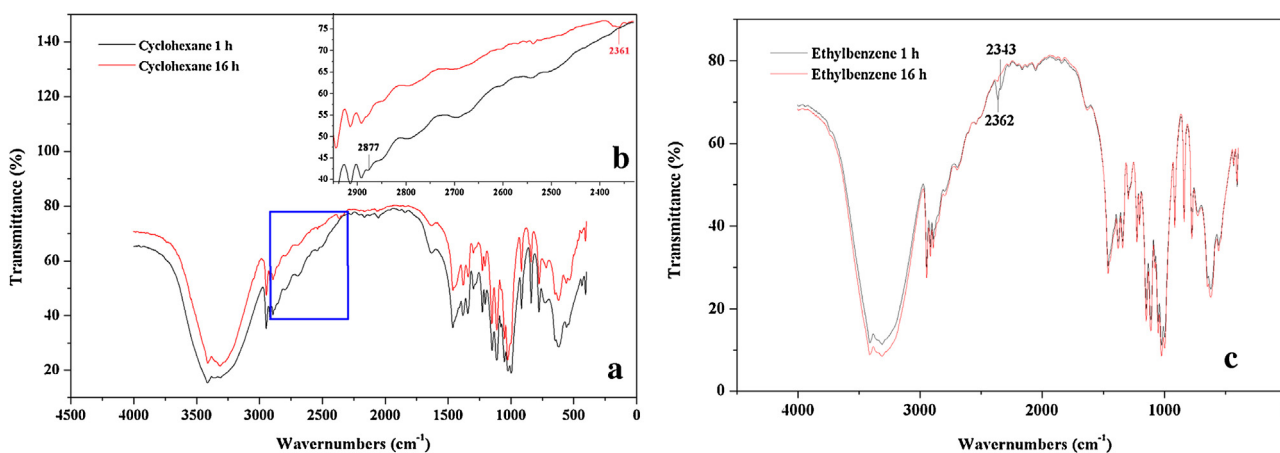


Fig. 2. Infrared spectrogram of tannase samples treated by cyclohexane (a and b) and ethylbenzene (c). The part of the box in (a) was magnified and seen in the embedded graph (b). The red and black lines in (a and b) denote the infrared spectrograms of the tannases treated by cyclohexane for 1 h and 16 h, respectively. The red and black lines in (c) denote the infrared spectrograms of the tannases treated by ethylbenzene for 1 h and 16 h, respectively.

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