Kinetic and thermodynamic characterizations of thermal inactivation of the inulinase produced by *Kluyveromyces latics*

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Abstract. The present study evaluated the kinetics and thermodynamic parameters for the thermal inactivation of inulinase. The thermoinactivation behaviours of inulinase under 40, 50, 60 and 70°C were observed. Residue activity of inulinase after incubated for a certain time under the tested temperatures, respectively. The inactivation rate constants (k_{in}) of inulinase under different temperatures were calculated and used to estimate the activation energy for irreversible inactivation $E_{a(in)}$ of inulinase, which was 236.9 kJ mol⁻¹.

1. Introduction

Bioethanol, considered as the recommended alternative fuel for gasoline, has been into human sights for decades of years. The energy crops that are used as the raw materials of bioethanol should be non-food crop, which can be planted in nonfarm land [1]. Jerusalem artichoke (Helianthus tuberosus L), tuberous-rooted perennial of the family Asteraceae, is a kind of non-food bioenergy crop which can flourish on most soils even on the tidal flat [2] or desert. Inulin, the main component of the Jerusalem artichoke tuber, is employed to produce fermentable sugar which is the raw material of bioethanol fermentation. Inulin consists of linear chains of β -2 \rightarrow 1-D-fructosfuranose with a glucose molecule at the end. Inulinase hydrolysis of inulin was recommended due to its environmentally sound and no formation of by-products.

To the best of our knowladge, the enzyme employed as the catalyst for hydrolysis of inulin is too expesive to be applied in industrial production. Moreover, inulinase is hard to recover, therefore, the high utilization efficiency of inulinase become a key issue for the fermentable sugar production. A higher reaction temperature is required for the desired fast hydrolysis rate[3], however, the thermo inactivation of inulinase during the hydrolysis reaction will increase the cost of inulinase [4, 5]. In the present work, the thermo inactivation behaviour of inulinase was observed, and provide a basis for the optimization of inulin hydrolysis by inulinase accounting for both the hydrolysis time and the thermo inactivation of inulinase.

2. Theoretical

The thermodynamics of the irreversible thermoinactivation of inulinase was determined by rearranging the Eyring's absolute rate equation, derived from the transition state theory[6],

where, *h* represents the Planck's constant (6.63×10⁻³⁴ J s), $k_{\rm B}$ is the Boltzman's constant (1.38×10⁻²³ J K⁻¹), *R* is the gas constant (8.314 J.K⁻¹ mol⁻¹), ΔH^* , ΔG^* , and ΔS^* represent enthalpy, free energy, and entropy of inactivation respectively and are introduced in Eqs. (2) – (4).

$$\Delta H^* = E_{a(\text{in})} - RT \qquad (2)$$

| $\Delta G^* = -\mathrm{R}T \ln(k_{\mathrm{in}}h) / (k_{\mathrm{B}}T)$ | | (3) |
|---|--|-----|
|---|--|-----|

$$\Delta S^* = \left(\Delta H^* - \Delta G^*\right) / T \tag{4}$$

The energy barriers of thermoinactivation $Ea_{(in)}$, was estimated by applying the Arrhenius equation (5) and plot.

 $\ln k_{\rm in} = -E_{\rm a(in)} / R T$ (5)

3. Materials and methods

3.1 Materials.

Inulin (derived from Jerusalem artichoke purchased from Gansu Likang nutrition and Food Co, Ltd.). Inulinase used in this study was produced by the *Kluyveromyces lactis MW270-7B* strain [7], Chemicals were purchased from Oxoid Ltd., Basingstoke, Hampshire, England.

3.2 Microorganism and enzyme production.

The liquid media for inulinase production contains 1% yeast extract, 2% glucose and 2% peptone. A 500 ml Erlenmeyer flask with 200 ml medium was inoculated with 4 ml seed culture medium and incubated in a rotator shaker at 30 °C with the stirring speed of 170 rpm for 120 h.

3.2 Analysises and tests.

Inulinase solution were incubating in a thermostated bath at three temperature values (40, 50, 60 and 70 °C) to observe the thermal inactivation behaviour, respectively. A certain amount of inulinase was sampled to test the inulinase avtivity at every certain time. Inulinase activity was assayed in a reaction mixture containing 0.5 ml appropriately diluted enzyme solution and 0.5 ml 50 mM sodium acetate buffer, pH 4.6, containing 2 wt% inulin. After the mixture was incubated at 55 °C for 15 min, it was heated for 5 min in boiling water to terminate the reaction. Then, the reducing sugar concentration liberated in the reaction mixture was confirmed by DNS method[8]. One unit (U) inulinase activity was defined as the amount of enzyme required for hydrolyzing inulin to produce 1 μ M reducing sugar per minute in the reaction mixture [9].

4. Results and discussion

4.1 Inulinase thermal inactivation behaviour

Fig.1 shows the inulinase inactivation behaviour at different temperatures. Inulinase almost inactivated completely within 20 minutes under 70° C, but only lost about 10% activity when it prolonged to 100 min under 50°C. Further more, after being heated under 40°C 100min, there was no inactivation of the inulinase observed.



Fig. 1 Inulinase activity changes with time during the inactivation process

4.2 Thermodynamics analysis

 $E_{a(in)}$ is an important indicator of the thermostability of an enzyme. Fig. 5 shows the parameter $E_{a(in)}$ obtained by applying an Arrhenius plot to the k_{in} values (-0.001, -0.016, -0.1701 for 50, 60, 70°C, respectively) obtained from Fig. 2-4. The energy barrier of the thermal inactivation process was 236.9kJ mol⁻¹, and was used to calculate the thermodynamic parameters inulinase inactivation using Eqs. 2–4.



Fig 2. The natural logarithm of residual activities of inulinase as a function of the incubation time under (a) 50° C, (b) 60° C and (c) 70° C; and (d) the determination of the inactivation energy based on Atthenius plot.

Table 1 shows the calculated value of ΔH^* , ΔG^* , and ΔS^* at different temperature (50, 60, 70°C), respectively. It is clear that, ΔG^* increased with the temperature but ΔH^* and ΔS^* decreased with the temperature.

| | ΔH^* (kJ mol ⁻¹) | ΔG^* (kJ mol ⁻¹) | ΔS^* (kJ mol ⁻¹ K ⁻¹) |
|------|--------------------------------------|--------------------------------------|--|
| 50℃ | 234.21 | 249.6 | -0.047 |
| 60°C | 234.13 | 269.9 | -0.107 |
| 70℃ | 234.05 | 284.7 | -0.148 |

Table 1 Thermodynamic parameters for irreversible inactivation of inulinase

For the inulinase used in this study, the most recommended application temperature was between 50-60 °C, which was decided by considering both the hydrolysis rate and the enzyme cost. If the temperature is lower than 50 °C, although the cost of enzyme is negligible, the hydrolysis reaction of

inulin will take a long time, leading to the high time-cost. When the hydrolysis temperature is carried out at the temperature higher than 60 $^{\circ}$ C, the initial hydrolysis rate will be very fast. However, along with the reaction time, the inactivation of inulinase occurs fastly, the residual inulinase activity is lower and lower, that may caused slow hydrolysis rate even the incomplete conversion of inulin.

5. Summary

The thermal inactivation of inulinase under 50, 60 and 70 °C was observed, respectively. The $E_{a(in)}$ of inulinase produced by *Kluyveromyces lactis MW270-7B* was calculated. The results shows that the inulinase sustain a stable activity under 50 °C, but inactvated fast under 60 and 70 °C. That is to say, 50 °C may be the recommend temperature for hydrolysis reaction of inulin when considering the cost of inulinase.

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