KINETIC CHARACTERIZATION OF ALMOND BETA-GLUCOSIDASE

José María Fernández Palacios. Diego Heras Márquez. Universidad Complutense de Madrid.

Abstract

The main aim of this project is to determine the kinetic parameters of almond β-glucosidase for the hydrolysis reaction of pNPG and study its variation with temperature. Inhibition assays that were also carried out supported an ordered Uni-Bi kinetic mechanism for the reaction.

Introduction

β-glucosidase is an ubiquitous protein and a key enzyme of the celulase complex that can hydrolyze O- and S- glycosidic bonds, with the release of D-glucose. Among its many industrial applications, biomass production and synthesis of flavoured molecules stand out.

Methods

All of the assays were carried out at 40 ºC, pH 5.0 and 1 mL of reaction volume for 10 minutes using β-glucosidase from sweet almond emulsin and p-nitrophenyl-β-D-glucoside as substrate. The reaction was stopped by adding 1.5 mL of NaOH 0.2 M. The rates were obtained by measuring the absorbance of the sample at 410 nm. Linearity of the product (p-nitrophenol) release along with changes in the enzyme concentration and assay time were checked and the optimal values were determined. Kinetic parameters of the enzyme were determined using the standard assay conditions. They were also determined at different temperatures were performed to stablish the best one for the reaction. Lastly, inhibition assays using glucose and δ -gluconolactone were carried out.

Results

Firstly, the optimal enzyme concentration and reaction time were stablished: 3.5 nM and 10 min. The kinetic parameters obtained are as follows: Km=2.28 mM, Vmax=9.47 μM/min, kcat==2.71 min^{-1}, specificity constant =1184.25 min^{-1} mM^{-1} and Hill coefficient, θ=1. The activation energy was also calculated: 34458 J/mol and so was the Q10 factor:1.664. Finally, the inhibition constants for both glucose and δ-gluconolactone were obtained: 384 mM and 0.266 mM, respectively.

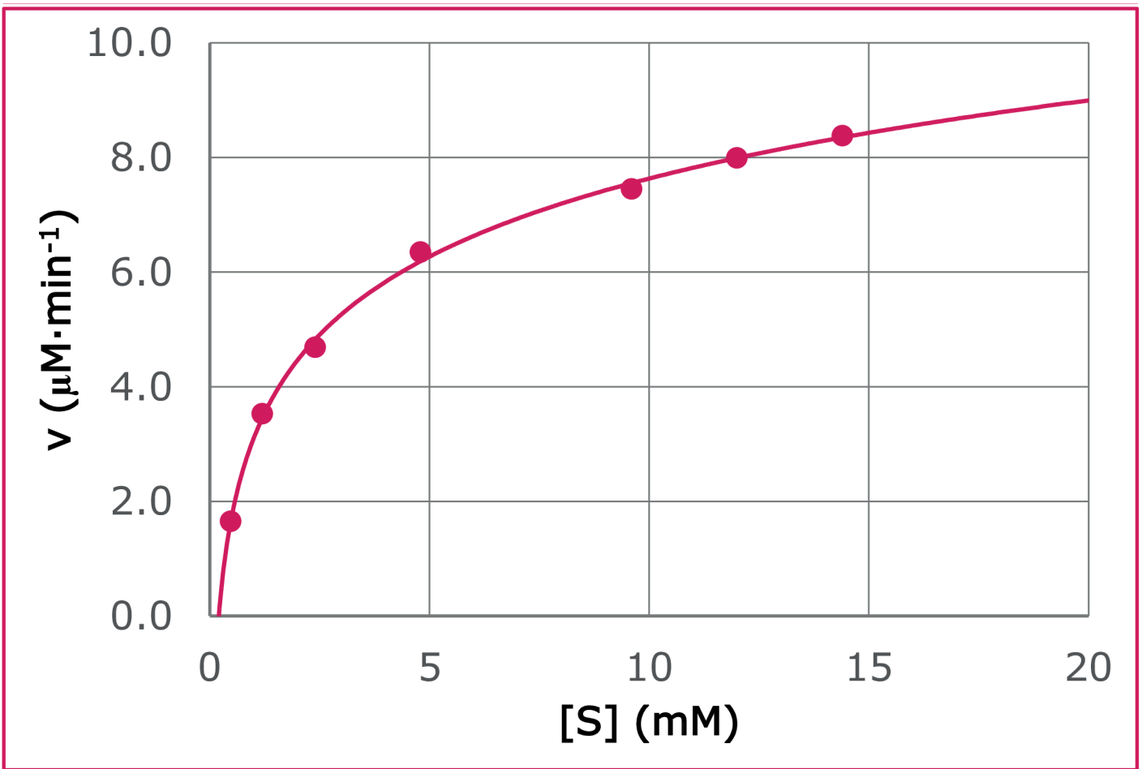

Figure 1. Michaelis-Menten plot of the enzymatic assay. The data were obtained using the standard assay conditions.

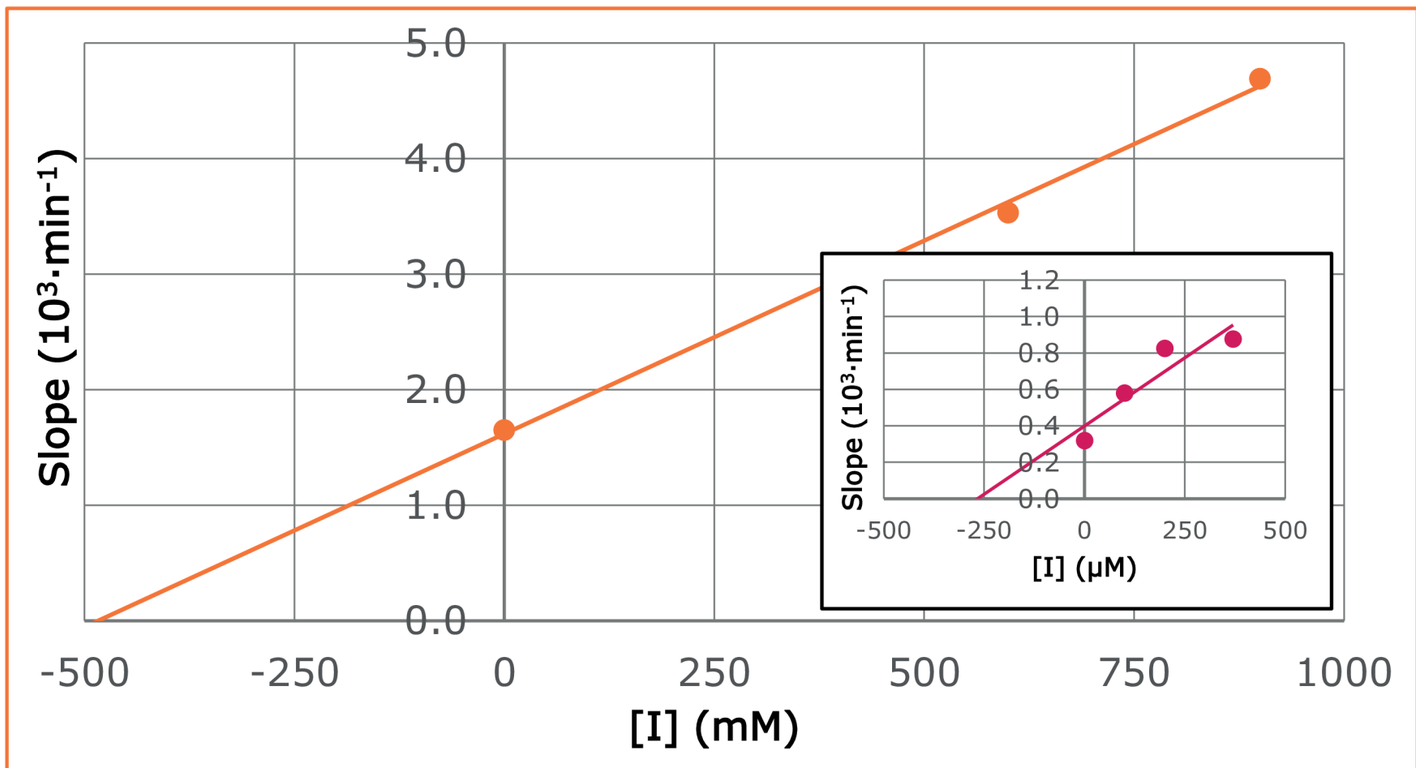

Figure 3. Secondary plot for the inhibition assay. The orange line stands for glucose and the red one stands for δ-gluconolactone.

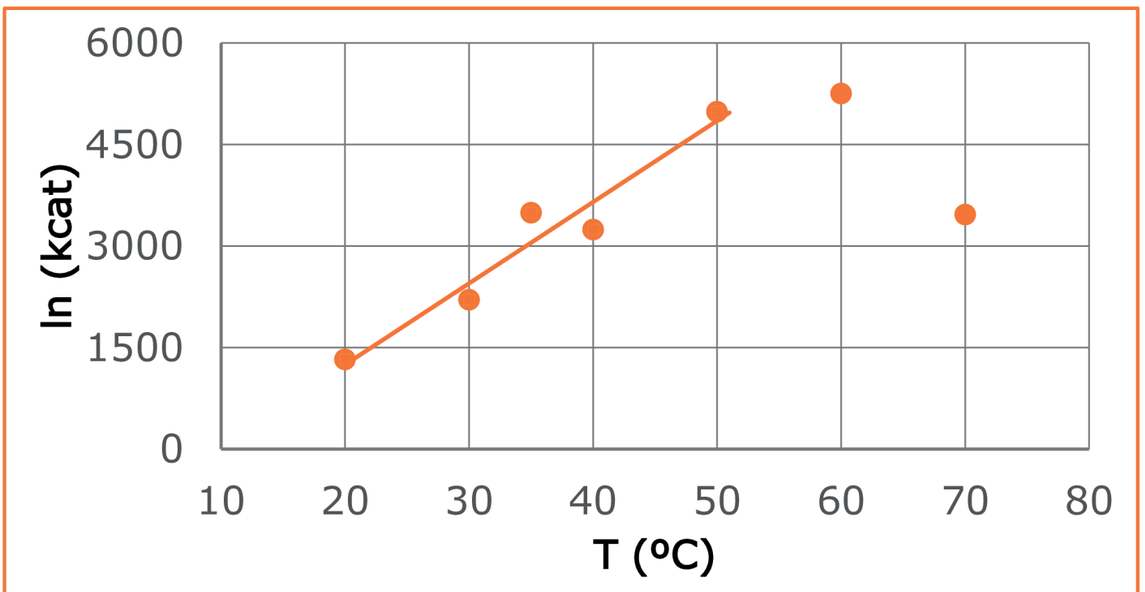

Figure 2. Variation of the kcat value with assay temperature. It increases linearly until about 50 ºC.

Conclusion

Although further studies are required to confirm the approaches exposed in this piece of work, we propose the optimal temperature for the catalysis as the one that corresponds to the highest reaction rate at which no deactivation occurs: 50 ºC. We also found that δ-gluconolactone is a much more effective inhibitor than glucose for the reaction, both behaving as competitive inhibitors. Supported by the data gathered with that assay, we propose an ordered Uni-Bi kinetic mechanism for the reaction, represented in the following Cleland diagram (Fig. 4).

Figure 4. Cleland diagram for the proposed mechanism. p-NPG stands for p-nitrophenylglucosyde, p-NP stands for p-nitrophenol, E for the enzyme and G for glucose.

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