KINETIC CHARACTERIZATION OF ALMOND β-GLUCOSIDASE

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1.INTRODUCTION

The β-glucosidase (EC 3.2.1.21) catalyzes the hydrolisis of the glycosidic bonds of terminal non-reducing residues in β-D-glucosides and oligosaccharides with release of β-D-glucose and the corresponding alcohol. It also catalyzes the inverse reaction characterised by the synthesis of a glycosidic bond between different molecules in order to increase the solubility, the stability and the activity of small molecules [1].

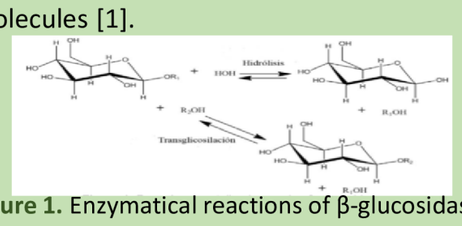


Figure 1. Enzymatical reactions of β-glucosidase

They are implicated in numerous physiological functions [2] in bacterias, fungus, plants, mammals and humans. Regarding their clasification, it follows rules of sequence and folding similarities [3,4] dividing glycoside hydrolases in more than a 100 families where the majority of beta glucosidases belong to GH1, GH2, GH3, GH5, GH30 and GH116. [5] Their 3D structure depends on their clasification. These enzymes have generate possible biotechnology uses.

The aim of this study is to propose a model for the kinetic mechanism of the reaction.

2. MATERIAL AND METHODS

2.1 Assay and material

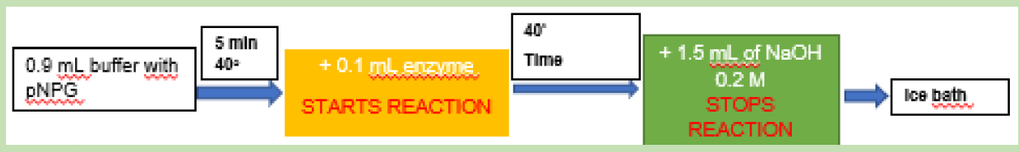


Figure 2. Assay protocol

As biological material, a commercial preparation of beta glucosidase isolated by the sweet almond(*Prunus dulcis)* emulsine provided by FLUKA. As chemical reactives: pNP(p-nitrophenol), pNPG(p-nitrophenol-β-D-glycoside), glucose and δ-gluconolactone provided by FLUKA; NaOH, HCl, citric acid and phospate salts provided by PANREAC.

2.2. Standarization

Make a model straight line by putting face to face [pNP] against speed (µM/min). Fix the substrate concentration (approximately Km from theorical Km) Check the linear appereance of product with the [E]. Obtain approximate Km by modifying [pNPG]. Check the linearity of appereance of product with the time by altering the assay time (1-20mins) Check in this last assay at ten minutes, the percentage of substrate consumed ($\%S_{transformed}$= ([P]/$[S]_o$) x 100) and the molar relation [S]/[E]

2.3 Kinetic parameters

Km, kcat and kcat/Km have been established by perfoming an assay with the conditions previously calculated and different [pNPG]. It is observed the dependence of initial velocity with [pNPG]. The values of initial velocity allows for getting the macroscopic kinetic parameters.

2.4 Inhibition studies

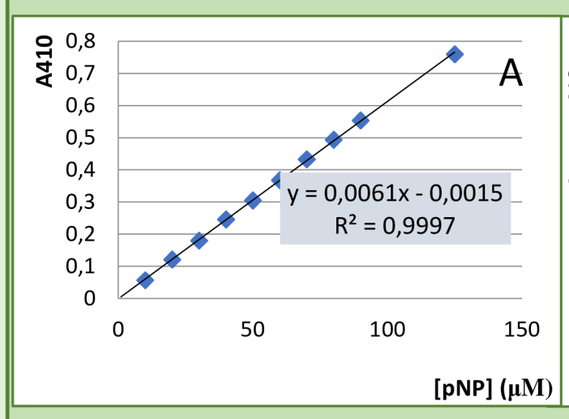
To study the effect of inhibitors, it has been used Glucose acting as a product inhibitior and δ-gluconolactone as a transition estate analogue. Their behaviour it is determined by studying the kinetic parameters of the enzyme with different inhibitor concentrations of each one.

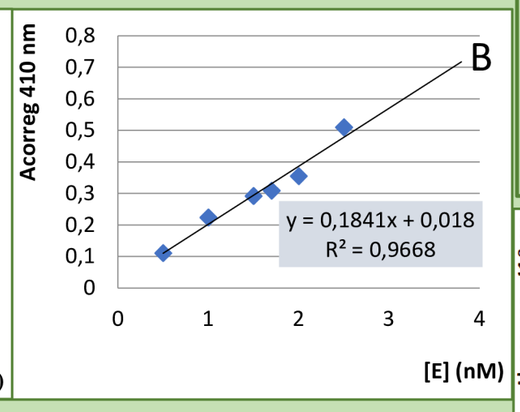
2.5 Temperature effect

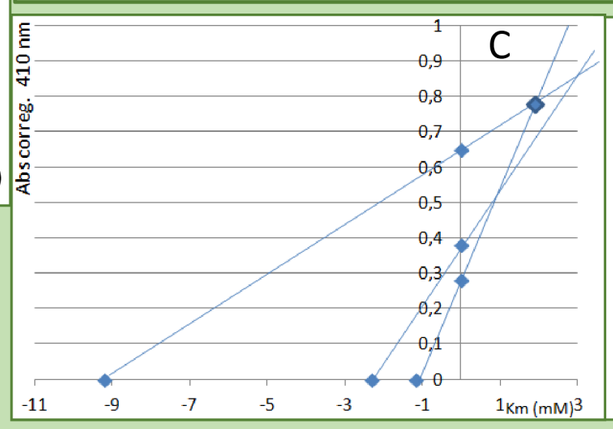
The kinetic parameters of the enzyme are analyzed at different temperatures. Q10 is calculated (times that increase velocity when the temperature grows 10 degrees)= Vmáx(T)/Vmáx(T+10º). Ln kcat against 1/T gives the activation energy value. Denaturation temperature could be observed.

3. RESULTS

3.1 Assay standardization







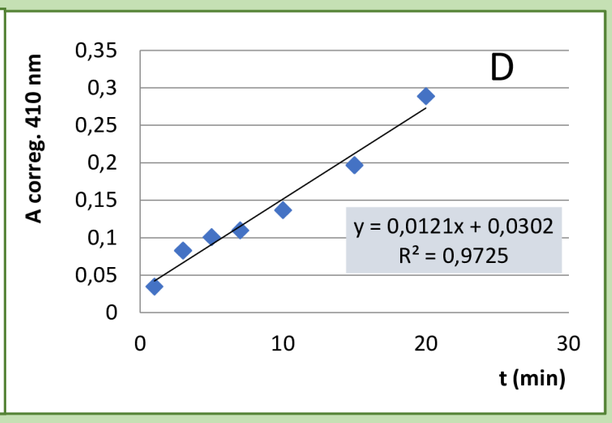


Figure 3. A) Calibration line **B)** Optimal concentration of enzyme **C)** Approximate Km **D)** Linearity with time

3.2. Kinetic parameters

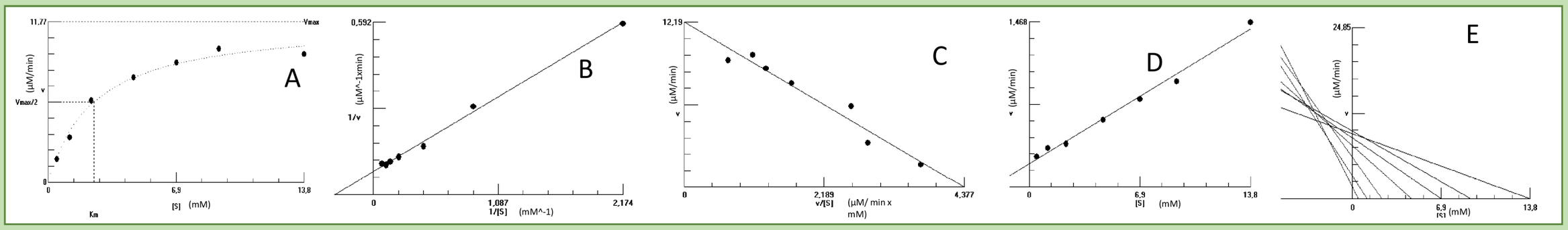


Figure 4. Representation of: **A)** Michaelis-Menten **B)** Lineweaver-Burk **C)** Eadie-Hofstee **D)** Hanes-Woolf **E)** Parameter

Table 1. Kinetic parameters for each representation

	Lineweaver-Burk	Eadie-Hofstee	Hanes-Woolf	Parameter Space	Regresión hiperbólica
Vmáx (µM/min)	12,42	12,19	11,49	12,42	11,77 ± 1,5
Km (mM)	2,923	2,785	2,379	2,787	2,457 ± 1,003
kcat (min^-1)	3548,571	3482,857	3282,857	3548,571	3362,857
kcat/Km (mM^-1 min^-1)	1214,017	1250,577	1379,932	1273,258	1368,684

3.3 Temperature effect

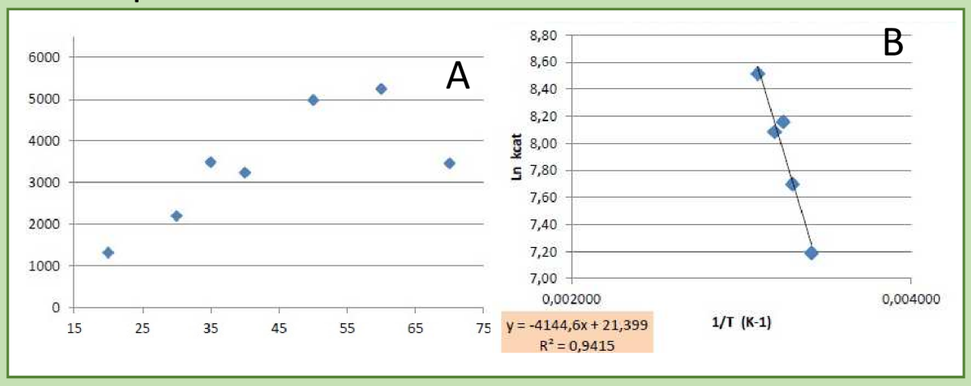


Figure 5. Temperature effect on activity. **A)** Kcat vs. Km **B)** Arrhenius representation between 20 – 50 °C

3.4. Inhibition studies

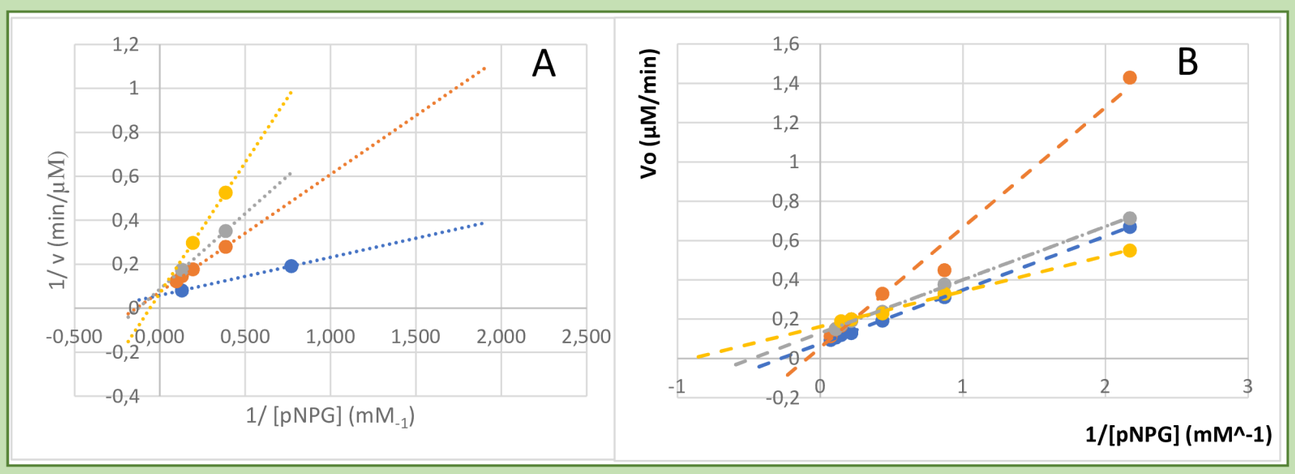


Figure 6. Inhibition studies using as inhibitor **A)** glucose, which Kis = 112 mM and **B)**δ-gluconolactone which Kis= 0,139 mM

4. CONCLUSION

The optimal conditions for this essay, obtained from the results of the standardization, were: pH 5,0; T=40°C; t=10 min; [E]=3,5 nM in the essay; [S]=0,2-4·Km. The results of the entire study shows the following kinetic parameters: Vmax=11,77 µM/min; Km=2,457 mM; kcat=3365,86 min^-1; kcat/Km=1368,68 min^-1 mM^-1. In second place, reversible inhibition studies have been carried out with glucose, a product of the enzymatic reaction and δ-gluconolactone, a transition state analog. These studies conclude that both inhibitors presents a competitive inhibition towards the substrate (pNPG), so it's concluded that the kinetic mechanism of the almond β-glucosidase is an ordered secuential mechanism, where the last product is the glucose.

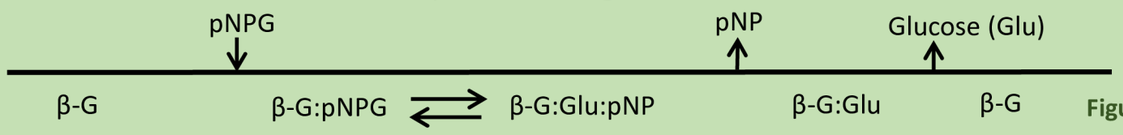


Figure 7. Cleland diagram

5. REFERENCES

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