KINETIC CARACTERIZATION OF THE ALMONF β-GLUCOSIDASE

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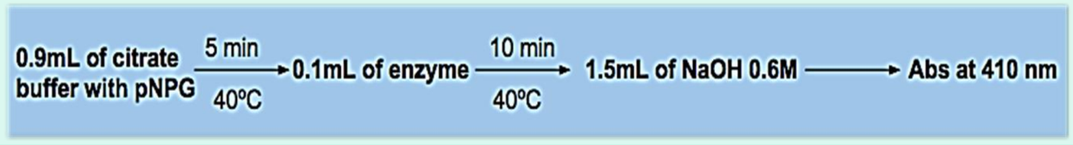
INTRODUCTION

β-glucosidases (C 3.2.1.21) are enzymes that catalyze the hydrolisis of a O-β-glycosidic bond at the non-reductive terminal end extreme of oligosaccharides, disaccharides and aril- or aquil-β-D-glucosides, releasing β-D-glucose as a product. **(1)** The chosen glucosidase comes from sweet almond (*Prunus dulcis)* belonging to the GH-1 family (glycoside hydrolases) **(2)** On plants, these enzymes perform different functions as the remodeling of the cellular wall, the mechanism of chemical defense against pathogens or the participation in the metabolism through an activation of different metabolic intermediates **(3)** The goal of this projects is to propose a kinetic mechanism for the beta glucosidase.

MATHERIALS AND METHODS

The materials used for the experiment were: commercial solution of β-glucosidase from sweet almonds (biological material), p-nitrophenol, p-nitrophenyl-β-D-glucoside, glucose and δ-gluconolactone from the chemical producer FLUKA. Also, others chemical reagents were employed, such as the NaOH, HCl, citric acid and phosphate salts, supplied by PANREAC. The same general diagram was used to carry out each enzyme assay:



RESULTS

1. STANDARIZATION OF ASSAYS CONDITIONS

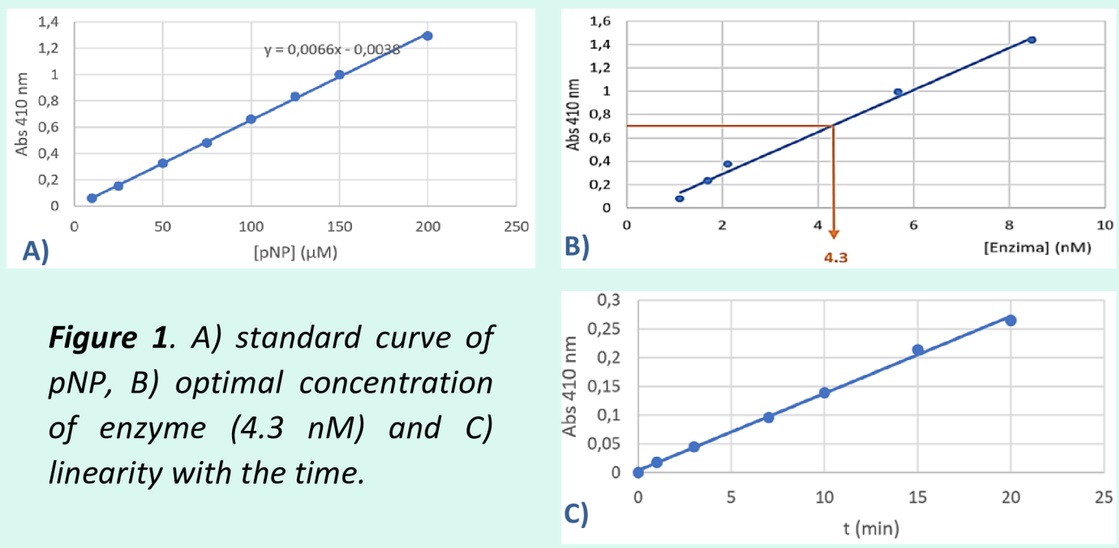


Figure 1*. A) standard curve of pNP, B) optimal concentration of enzyme (4.3 nM) and C) linearity with the time.*

3. TEMPERATURE EFFECT

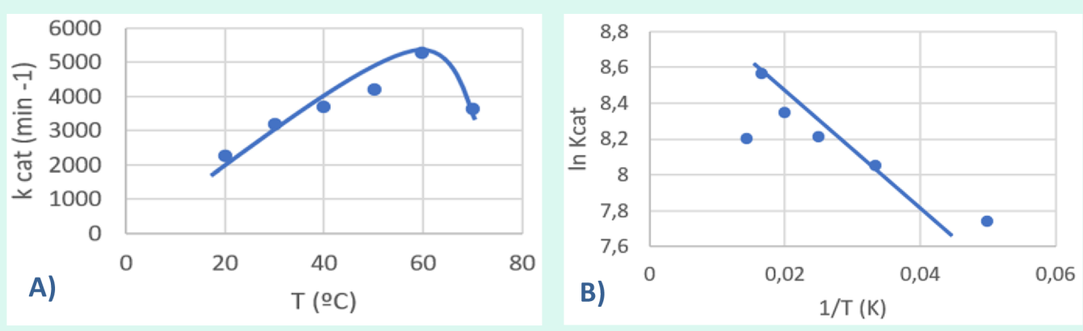
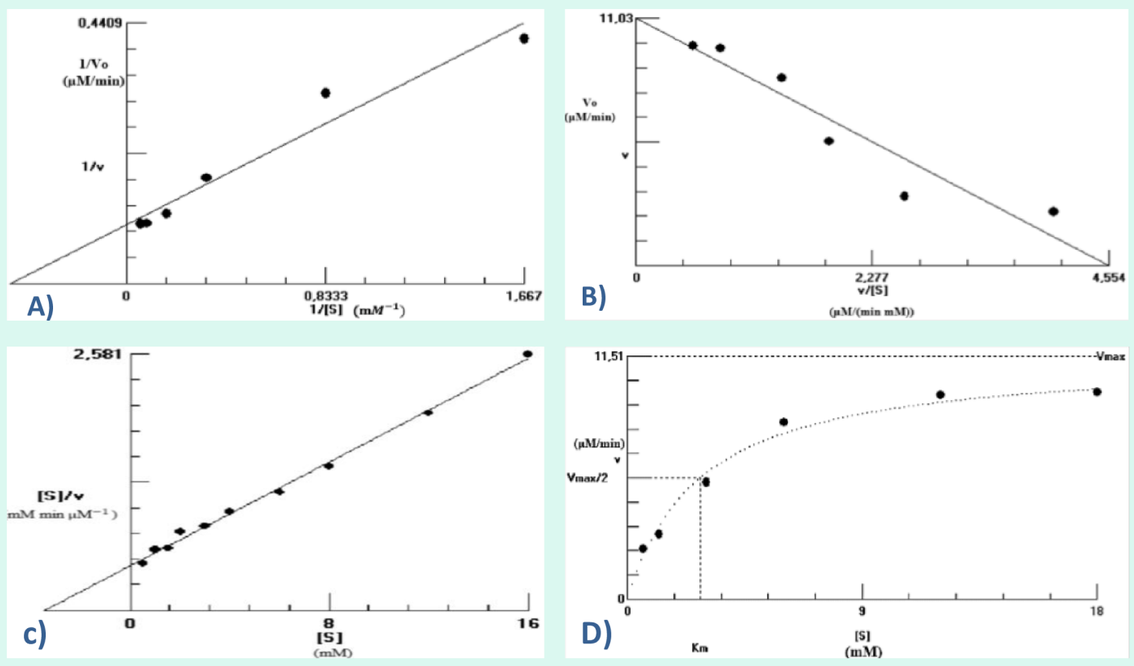


Figure 2*. A) and B) show the relationship of kcat versus temperature*

K cat increases with linearity versus temperatura, but from 60 degrees, this parametrre decays and it loses its activity. Q_{10} value selecting 20º and 30º is 1.37.

2. DETERMINATION OF KINETIC



	V máx (mM/min)	Km (mM)
HANES-WOOLF	11,52	2,715
LINEWEAVER-BURK	10,03	2,054
EADIE-HOFSTEE	11,03	2,422
DIRECT REPRESEN-TATION	11,6	2,289
MICHAELIS-MENTEN	11,51	2,784

Table 1*. Kinetic parameters.*

Figure 3*. A) Lineweaver-Burk, B) Eadie-Hofstee, C) Hannes-Woolf, D) Direct representation and E) Michaelis-Menten.*

4. INHIBITION STUDIES

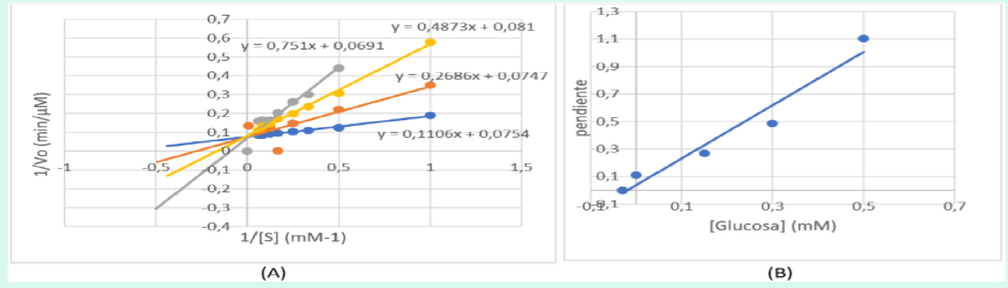


Figure 4*. A) Lineweaver-Burk for some inhibitor concentration (blue 0 M , orange 0.15 mM , yellow 0.3 mM and grey 0.5 mM) and B) Dixon plot.*

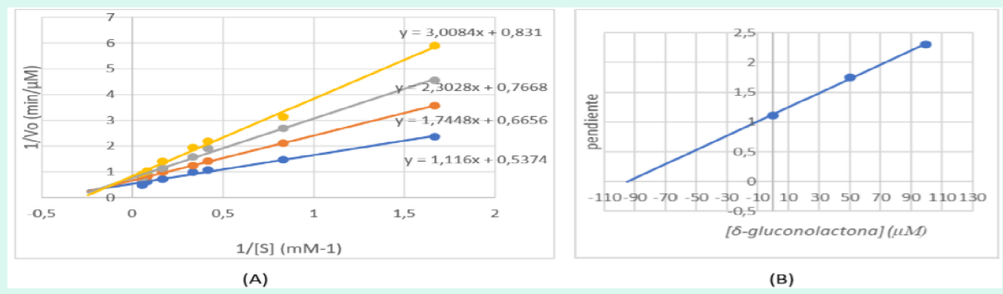


Figure 5*. A) Lineweaver-Burk for some inhibitor concentration (blue 0 µM, orange 50 µM, yellow 100 µM and grey200 µM) and B) Dixon plot.*

CONCLUSION

Knowing that the enzyme shows a mechanism with two steps and that β-D-glucose interacts with the enzyme in its free state, we know that this product will be the last one to be released, after pNP. So we will have one first step where pNPG will react with the enzyme and after the reaction, the pNP will be released leaving behind an intermediate form of the enzyme. Then, the second and final step begins when H_2O enters the catalytic site of the enzyme releasing β-D-glucose and regenerating the original state of the enzyme. All of these deductions indicate that our enzyme follows a Ping-Pong mechanism.

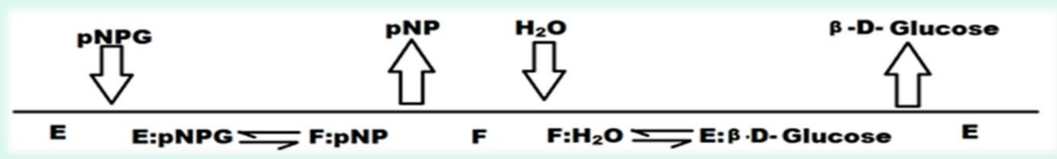


Figure 6*. Mechanism Ping-Pong of β-glucosidase.*

REFERENCES

1*. Opassiri, R.; Hua, Y.L.; Wara-Aswapati, O.; Akiyama, T.; Svasti, J.; Esen, A.; Cairns, J.R.K. (2004). "β-Glucosidase, exo-β-glucanase and pyridoxine transglucosylase activities of rice BGlu1" Biochem. J. 379: 125–131.*

2*. Carbohydrate-Active enzymes. CAZy. (http://www.cazy.org/)*

3*. Morant AV.; Jørgensen K.; Jørgensen C.; Paquette SM.; Sánchez-Pérez R.; Møller BL.; Bak S. (2008). "beta-Glucosidases as detonators of plant chemical defense". Phytochem 69: 1795-1813*