ENZYMATIC CHARACTERIZATION OF β-GLUCOSIDASE FROM ALMOND

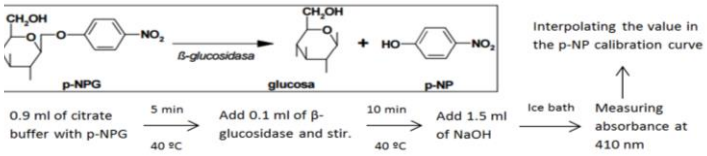
Quesada C., Pérez M. and Romero A.
Laboratory of Biochemistry and Molecular Biology I. Faculty of Chemistry. Complutense University of Madrid, Spain.

INTRODUCTION

The β-glucosidases form a big group of enzymes within the glycosidases. These enzymes catalyze the hydrolysis of non-reducing terminal glucose residues with the release of β-D-glucose. The β-glucosidase has a great interest to the biotechnology industries [1]. In this study, a kinetic characterization of almond β-glucosidase was carried out in order to propose a model for the catalytic mechanism with pNPG as a substrate. In this experiment, assay conditions were standardized, macroscopic kinetic parameters were determined, the effect of temperature in the catalysis was studied and reversible inhibition experiments with glucose and β-gluconolactone as inhibitors were conducted.

MATERIALS AND METHODS

The procedure is the following:



Chemicals: p-nitrophenil-β-D-glucosidase (pNPG), p-nitrophenol (p-NP), glucose and δ-gluconolactone, pH 5.0 sodium citrate buffer.
Biological material: commercial solution of β-glucosidase isolated from almond. [2]

RESULTS

1. Standardization of assays conditions

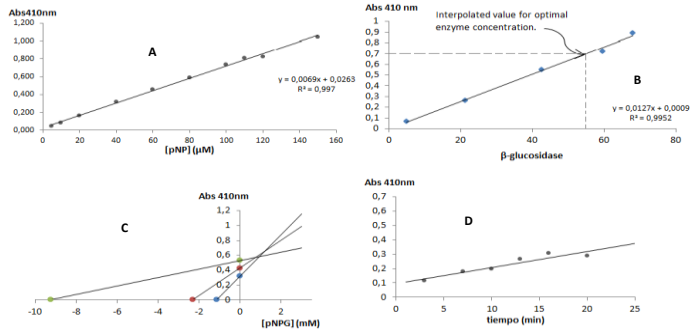
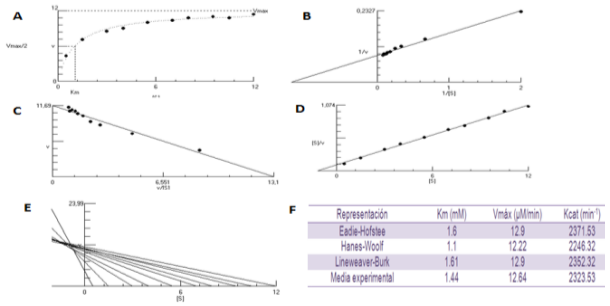


Figure 1. A. Standard curve for pNP. **B**. Optimal concentration of β-glucosidase. **C**. Eisenthal and Cornish-Bowden plot. **D**. Time linearity.

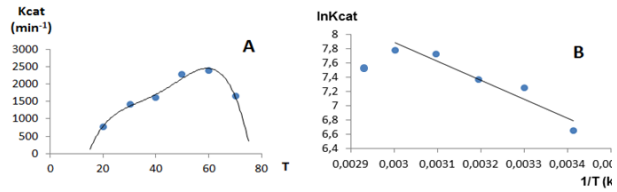
2. Determination of kinetic parameters



Representación	Km (mM)	Vmáx (µM/min)	Kcat (min⁻¹)
Eadie-Hofstee	1.8	12.9	2371.53
Hanes-Woolf	1.1	12.22	2246.32
Lineweaver-Burk	1.81	12.9	2352.32
Media experimental	1.44	12.64	2323.53

Figure 2. A. Michaelis-Menten plot. **B**. Lineweaver-Burk plot. **C**. Eadie-Hofstee plot. **D**. Hanes-Woolf plot. **E**. Hyperbola adjustment. **F**. Calculated kinetic parameters.

3. Temperature assays



C

Ea (kJ/mol)	Q10
21.6	1.13

Figure 3. A. Variation of Kcat with temperature. **B.** Linearization for Arrhenius equation. **C.** Calculated activation energy and Q_{10} factor.

4. Inhibition studies

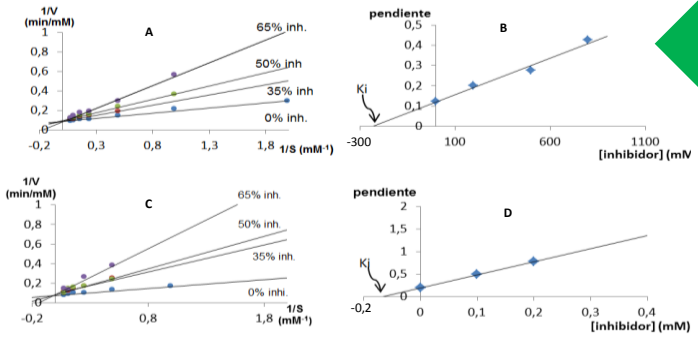
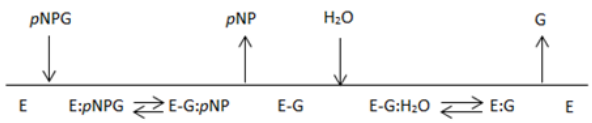


Figure 4. A. Lineweaver-Burk plot for glucose inhibition. **B**. Dixon plot for glucose inhibition. **C.** Lineweaver-Burk plot for δ-gluconolactone inhibition. **D**. Dixon plot for δ-gluconolactone inhibition.

Cleland mechanism:



CONCLUSION

- Temperature studies confirm that Kcat depend on temperature, while Km does not.
- β-glucosidase was classified as an aril-β-glucosidase due to its high affinity for pNPG.
- The enzyme showed activity up to 60ºC under the assay conditions.
- It was found that δ-gluconolactone is a much more powerful inhibitor (Ki=0.1mM) than glucose (Ki=210mM). Both of them behave as competitive inhibitors.
- Finally, a crypto ping-pong kinetic mechanism is suggested. [3]

References:
[1] Singhania, R. R.; Patel, A. K.; Sukumaran, R. K.; Larroche, C.; Pandey, A., Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresource Technology 2013, 127, 500-507.
[2] Rye, C. S.; Withers, S. G., Glycosidase mechanisms. Current Opinion in Chemical Biology 2000, 4, 573-580.
[3] Rye, C. S.; Withers, S. G., Glycosidase mechanisms. Current Opinion in Chemical Biology 2000, 4, 573-580.