KINETIC CARACTERIZATION OF THE ALMOND β-GLUCOSIDASE

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ABSTRACT

A series of enzyme assays were carried out in order to study the kinetic parameters, the environmental effect of temperature and the effect of two inhibitors on the enzyme β-glucosidase from almonds, catalyzing the hydrolysis of p-nitrophenol-β-D-glucose is the last one to leave the active site.

INTRODUCTION

β-glucosidases (3.2.1.21) are enzymes which catalyze the hydrolysis of non-reducing terminal glucosyl residues from saccharides and glycosides. In unfavourable conditions, this enzyme can catalyze inverse reaction, transglycosilation **[1]**. They get involved in many physiological roles **[2]**. They are divided into families, such as GH1 (plants and mammals) and GH3 **[3].**

MATERIALS AND METHODS

1. Enzymatic essay

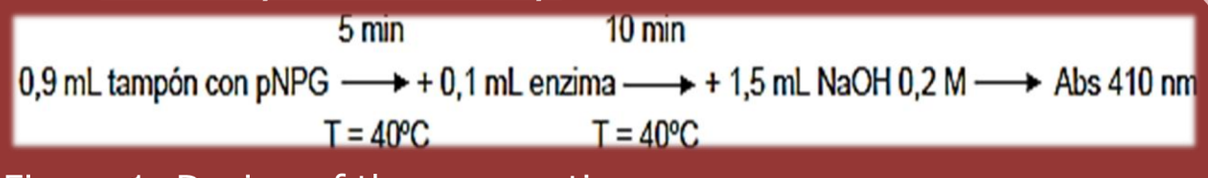


Figure 1. Design of the enzymatic essay

2. Standardization of essay conditions

Essay conditions were standardized using pNPG as substrate and considering optimal concentration of enzyme, an approximate Km and linearity with time.

3. Determination of kinetic parameters

They have been calculated using the representations of Lineweaver-Burk, Eadie-Hofstee, Hanes-Woolf and direct plot.

4. Temperature essay

To study this effect, various essays at different temperatures (20-70 ºC) were carried out. The activation energy of the reaction was also determined.

5. Inhibition essay

For this essay two inhibitors were used, D-Glucose and δ-gluconolactone, with different concentrations.

RESULTS

1. Standarization of assay conditions

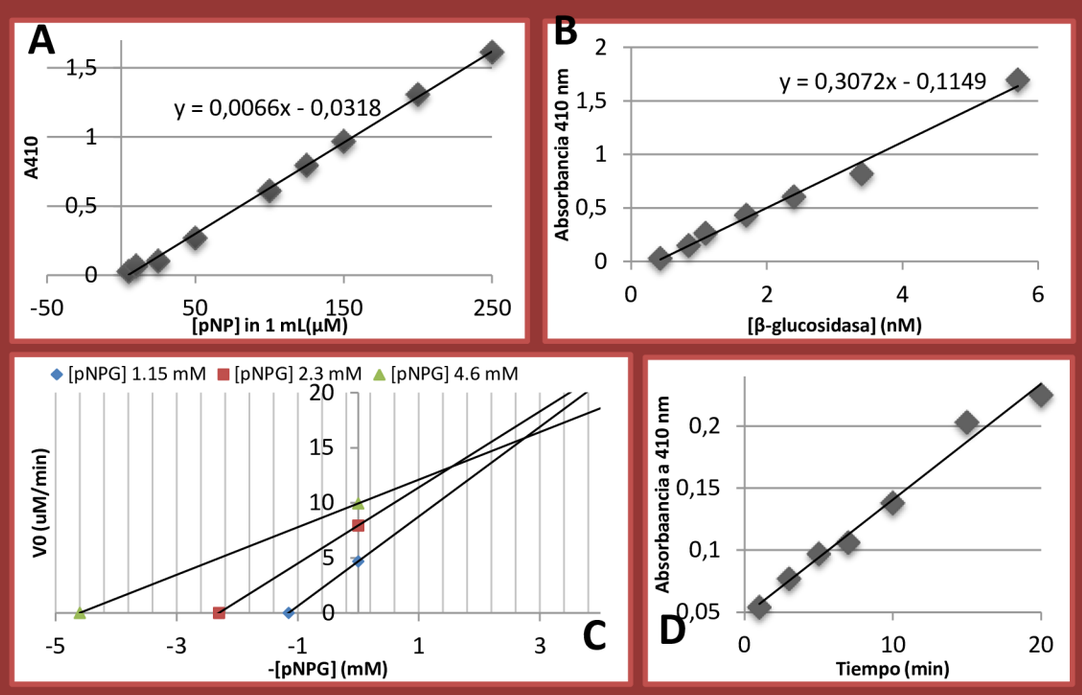


Figure 2. A. Standard curve of pNP. B. Optimum β-glucosidase (3.4 nM). C. Approximated Km (2.75 mM). D. Linearity with time

2. Kinetic parameters

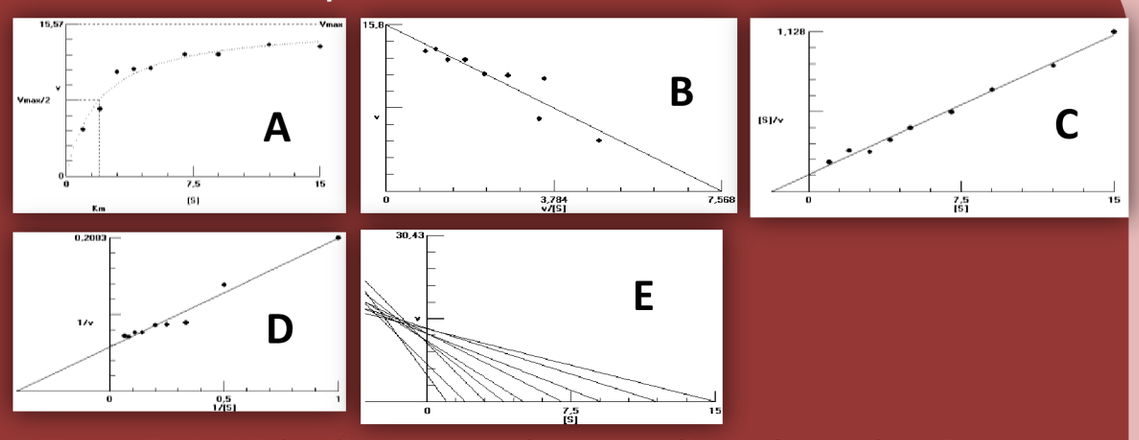
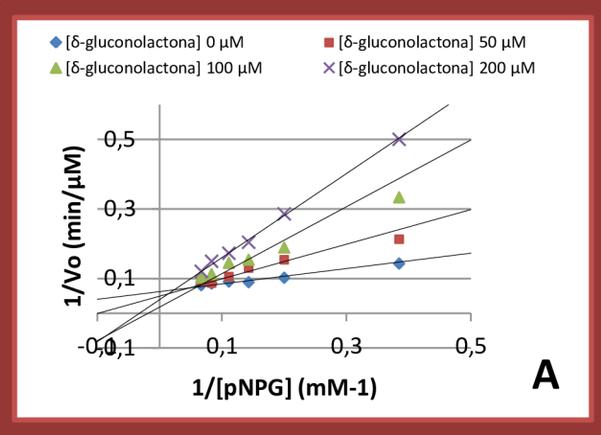
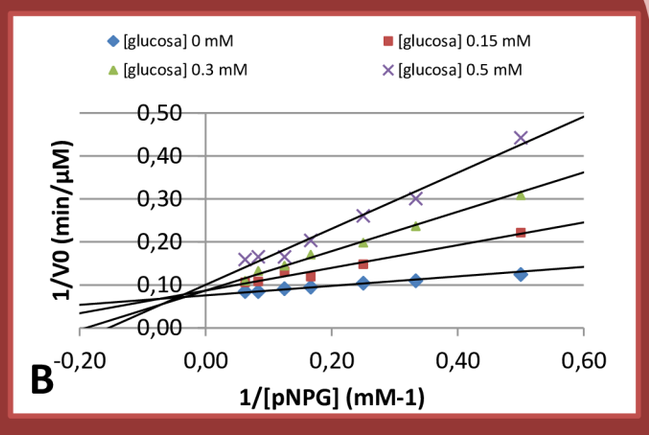


Figure 3. A. Michaelis-Menten plot. B. Eadie-Hofstee plot. C. Hanes-Woolf plot. D. Lineweaver-Burk plot E. Eisenthal and Cornish-Bowden plot

3. Temperature and inhibition assays





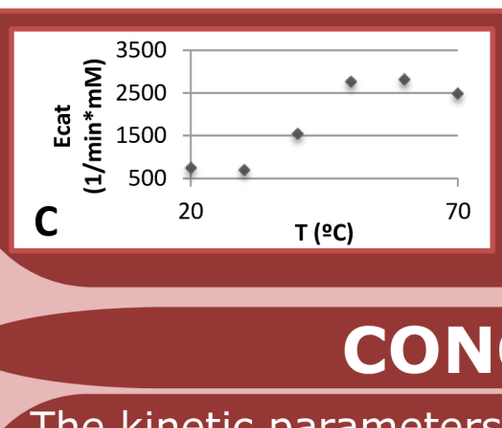


Figure 4. A. Gluconolactone inhibition. B. glucose inhibition. C. Variation of kcat/Km with temperature

CONCLUSIONS

The kinetic parameters obtained were K_M=1.94 mM, $v_{máx}$=15.6 µM/min and k_{cat}=4580 min^{-1} and the optimal temperature is thought to be around 40-50ºC. Due to retention of the anomeric carbon configuration, the reaction must occur in two steps. The reaction has two transition states and the gluconolactone is an analog for the first one, it is also a better inhibitor than glucose because it has a lower K_{IS}. The reaction has a ping-pong mechanism, with an ordered output of products where the glucose is the last one to leave the active site (Figure 5).

Figure 5. Cleland's scheme

REFERENCES

1. Mladenoska, I.; Grey, C. E.; Winkelhausen, E.; Kuzmanova, S.; Adlercreutz, P., Competition between transglycosylation and hydrolysis in almond beta-glucosidase-catalyzed conversion of p-nitrophenyl-beta-D-glucosidase in monophasic water/alcohol mixtures, *Biocatalysis and Biotransformation, 2007, 25, 382 - 385.*
2. Bhatia, Y.; Mishra, S.; Bisaria, V. S., Microbial beta-glucosidases: Cloning,properties, and applications, *Critical Reviews in Biotechnology, 2002, 22, 375 - 407.*
3. Henrissat, B., A classification of glycosyl hydrolases base don amino-acid-sequence similarties, *Biochemical Journal, 1991, 280, 309 - 316.*