KINETIC CHARACTERIZATION OF β-GLUCOSIDASE FROM ALMOND

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ABSTRACT

β-glucosidases (3.2.1.21) constitute a group of enzymes that catalyze the hydrolysis of nonreducing terminal glucosyl residues from saccharides and glycosides. They are found in all domains of living organisms performing a wide range of biologycal functions. They have many aplications in industrial and scientific fields. This report covers the kinetic characterization of β-glucosidase from almond (*Prunus dulcis*). Optimal assay conditions were detemined, the same as kinetic parameters of the reaction using p-nitrophenyl β-D-glucoside (pNPG) as substrate. Temperature assays were performed, obtaining an optimal temperature for catalysis of 60 ºC and the reaction activation energy (12 kJ/mol). Inhibitions studies lead to the conclusion that glucose and δ-gluconolactone act as competitive inhibitors of the enzyme. It is proposed that the enzyme operates by a non-secuential or ping –pong mechanism where pNPG binds first. After that pNP is released and a molecule of water binded to allow the releasing of the second product, glucose.

INTRODUCTION

β-glucosidases (β-D-glucoside glucohydrolases, EC 3.2.1.21) constitute a widely group of enzymes from the family of glycosidases (EC 3.2.1.). Glycosidases catalyze the hydrolysis between a carbohydrate and other molecule, that may be another carbohydrate or a molecule form other nature, named aglycone, breaking O- or S- glycosidic bonds. β-glucosidases catalyze the hydrolysis of nonreducing terminal glucosyl residues from saccharides and glycosides. They are also able of transglycosilation reactions [1]. They take part in the metabolism of many different organisms. In microorganisms such as bacteria or fungi they participate in biomass conversion, being part of celulases, celulose breaking down complexes. [2,3,4]. In mammals they perform an importante role in xenobiotics metabolism and the lack of this enzyme cause Gaucher's disease [5]. In plants they have various roles in the realeasing of secondary metabolites [6,7].

Taking in account the great industrial and scientific interest of this family of enzymes, this report presents a kinetic characterization of β-glucosidase from sweet almond (Prunus Dulcis), using p-nitrophenyl- β-D-glucoside as substrate. The reaction consists in the hydrolysis of pNPG in pNP and glucose. The aim of the report is the optimization of an enzymatic assay for the previous enzyme in order to determine their kinetic parameters. It is also studied the temperature effect in catalysis and the effect of two inhibitors of the enzyme, glucose and δ-gluconolactone. Finally, a kinetic mechanism of the reaction is proposed.

METHODS

1. Enzymatic activity valoration method

0.9 mL of pNPG+ buffer 100mM, pH 5.0

↓ Incubate at 40ºC, 5 minutes

+0.1 ml of enzyme

↓ 10 minutes, +1.5 ml of NaOH

Absorbance measurements at 410 nm

2. pNP standard curve elaboration

A standard curve of product (pNP) is needed in order to quantify the product obtained in following assays.

3. Standarization of enzymatic assay conditions

Assay conditions are optimized to measure β-glucosidase activity. Were considered:

- Optimal enzyme concentration
- Aproximated Km
- Time constant progress

4. Enzymatic assay to determine kinetic parameters

Assay conditions:

[E] = 2.6 nM
[S] = 0.5 mM – 14 mM
T = 40 ºC
pH 5.0
t = 10 min

Equations:

Michaelis - Menten
Lineweaver - Burk
Eadie - Hofstee
Hanes - Woolf

5. Temperature studies

To study the effect of temperatura in β-glucosidase activity various assays at different temperatures, among 20 – 70 ºC, were performed. It was also determined the activation energy of the reaction.

6. Inhibition studies

For this studies two inhibitiors were used, glucose and δ-gluconolactone. Glucose is one of the products and δ-gluconolactone is a transition state analog. Various assays using different inhibitor concentrations.

RESULTS

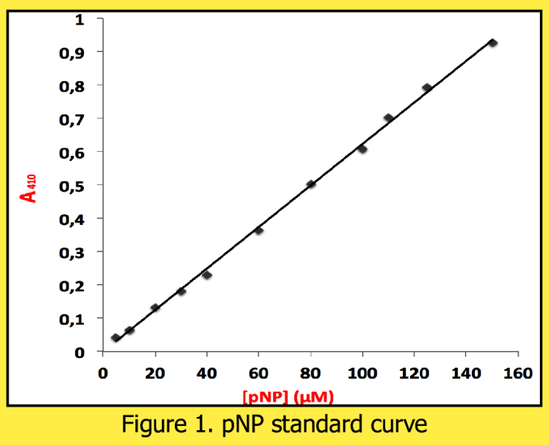


Figure 1. pNP standard curve

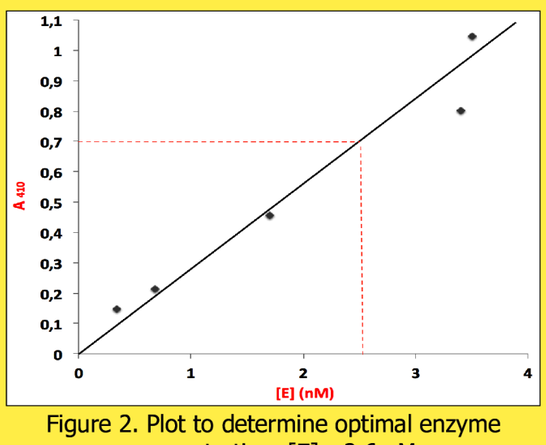


Figure 2. Plot to determine optimal enzyme concentration. [E]= 2.6 nM

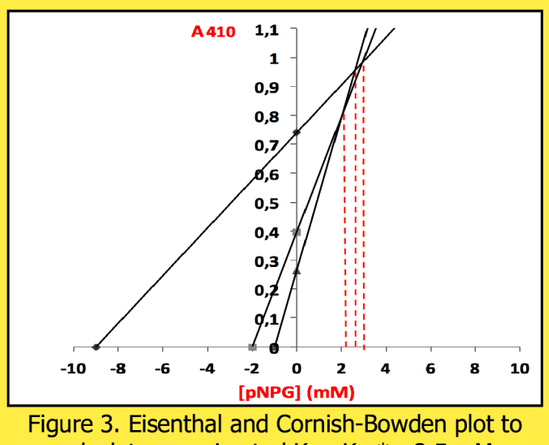


Figure 3. Eisenthal and Cornish-Bowden plot to calculate aproximated Km. Km*= 2.5 mM

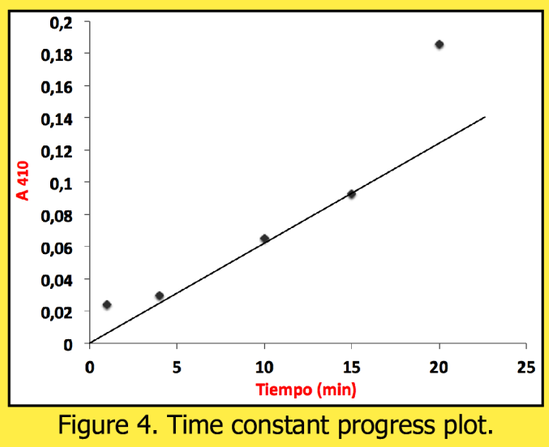


Figure 4. Time constant progress plot.

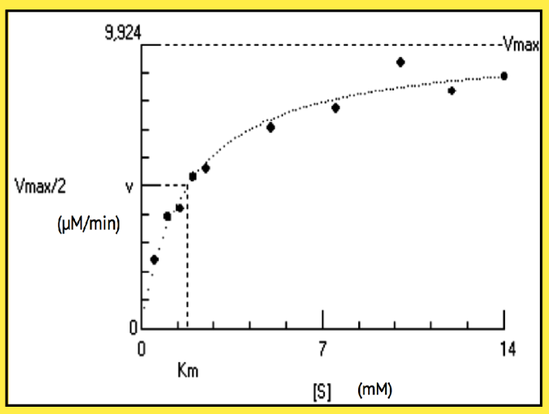


Figure 5. Michaelis-Menten plot to calculate kinetic parameters: Km=1.8 mM and Vmáx=9.9 µM/min

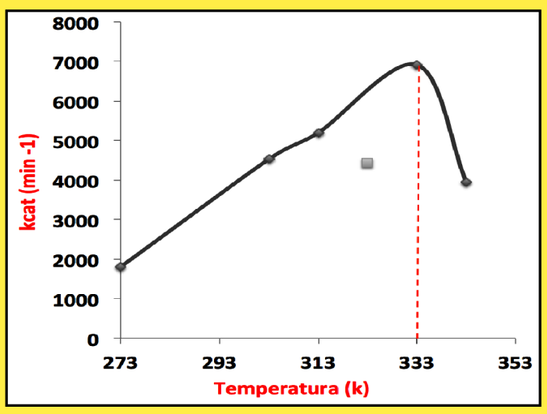


Figure 6. Plot to determine optimal temperature of the enzyme. T=60ºC

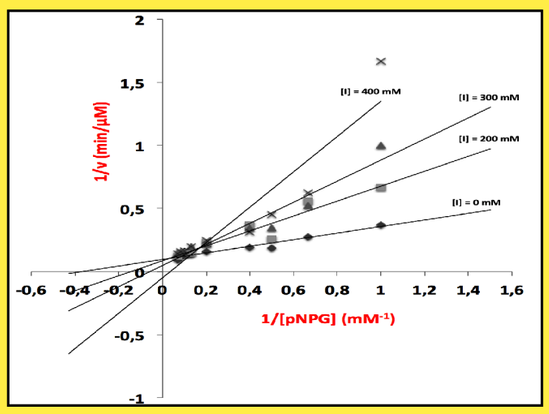


Figure 7. Lineawer-Burk plot to B-glucosidase of almond using glucose as inhibitor. Kis= 100 mM

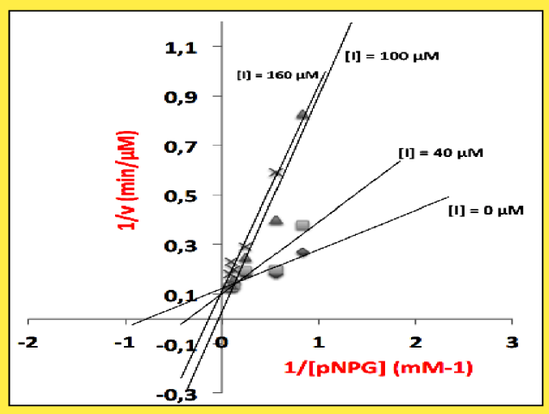


Figure 8. Lineawer-Burk plot to B-glucosidase of almond using δ-gluconolactone as inhibtor. Kis=0.064 mM

CONCLUSION

-Kinetic parameters of the reaction:
Km = 1.8 mM
Vmáx= 9.9 µM/min
Kcat = 3817 min-1
Catalytic efficiency= 2118 mM^{-1}

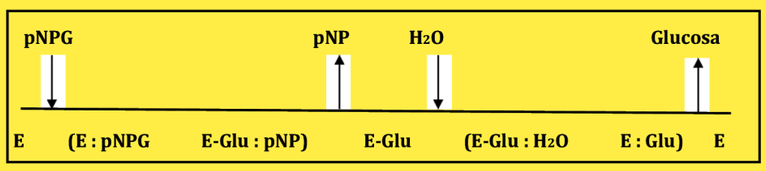
-60 ºC is the temperature at which the enzyme reachs its highest activity. Nevertheless, this temperature is near to the denaturalization temperature, so optimal assay temperature was defined as 50 ºC.

-The activation energy of the reaction is 12 kJ/mol.

-Glucoseis a competitive inhibitor of the enzyme, showing low levels of inhibition (Kis = 100 mM). Thisisdueto the factthatglucoseis a product of the reaction, that practise a negative feedback effect over β-glucosidases. It is a more regulator than an inhibitor of the enzyme.

-δ-gluconolactone acts as a competitive inhibitor of the enzyme, with high levels of inhibition (Kis = 0.064 mM). This is because the inhibitor is a transiton state analog, which is similar to the activated substrate. This is the form for which the enzyme shows higher affinity, being the inhibition practically irreversible.

-The kinetic mechanism of the reaction is proposed as a non-secuentialor ping–pong mechanism where pNPG binds first. After that pNP is released and a molecule of water binded to allow the releasing of the second product, glucose.



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