CHARACTERIZATION OF ALMOND ß -GLUCOSIDASE

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ABSTRACT

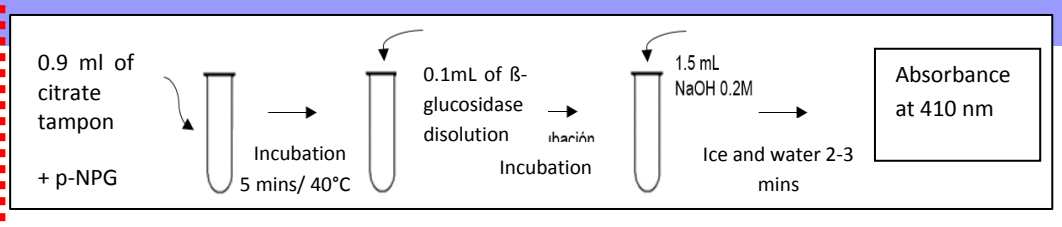
ß -glucosidases are found in all domains of living organisms. This report describes the kinetic characterization of almond β-glucosidase. It includes a first step of assay conditions standardization to define the enzyme activity, using p-NPG as substrate, determination of the kinetic parameters, a study about the temperature effect on the catalysis and 2 inhibition experiments by glucose and δ-gluconolactone. We acquired the following kinetic parameters for the reaction catalyzed by β-glucosidase on p-NPG: Km = 2.689 mM and Vmax = 7.8228 µM/min. Moreover, the enzyme had its optimum temperature at 50°C and inhibition results assays supported an Uni Bi mechanism.

INTRODUCTION

ß-glucosidases (***EC*** 3.2.1.21) are enzymes which catalyze the hydrolysis of O-ß-glycoside bond at the non-reducing terminal end of short chain oligosaccharides, disaccharides, aryl- or alkyl-β-D-glucosides, in order to release β-D-glucose. ß-glucosidases perform different physiological functions **[1]** in different living beings: in bacterium and fungus are part of the multienzymatic complex of cellulose **[2]**, which degrades cellulose in ß-D-glucose units. In plants, these hydrolytic enzymes participate in mechanisms of chemical defense against pathogens through the release of toxic glycosides **[3].** In mammals, a cytosolic ß-glucosidase of liver and intestine is involved in the metabolism of dietary xenobiotics, such as vitamin B6. They also have a biotechnological interest in the textile or energy industry. In this study, sweet almond ß-glucosidase is kinetically characterized by different experiments.

MATERIALS AND METHODS

The materials used, both biological and chemical, are almond ß-glucosidase, p-nitrophenol, p-nitrophenil-ß-D-glucoside, glucose and δ-gluconolactone, they are all from FLUKA trading house. In addition, analytical grade reagents such as NaOH, HCl, citric acid and phosphate salts come from PANREAC. To determinate kinetic parameters and temperature effect on the catalysis, the same experiment scheme (Fig.1) was followed but changing the enzyme, substrate and temperature. For the inhibition assay, two inhibitors were studied: glucose and δ-gluconolactone, the scheme of the experiment was the same as that of the previous case, but this time changing inhibitor concentrations.



RESULTS:

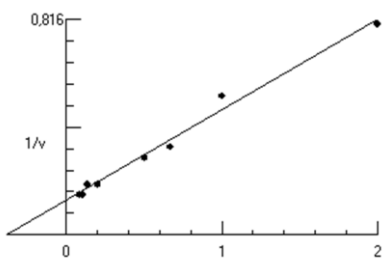


***Figure 1**: **Lineweaver-Burk plot**, representing 1/v vs. 1/[S], to calculate Vmax and Km*

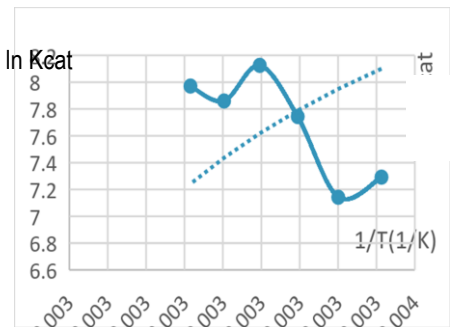
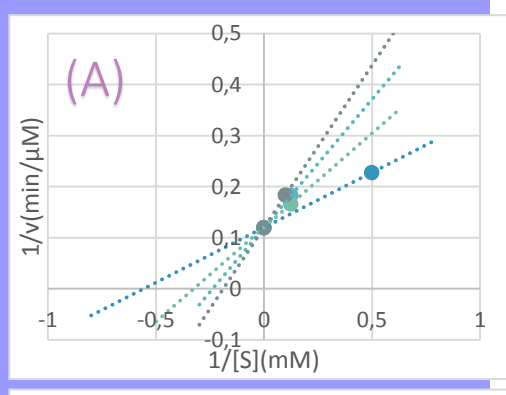
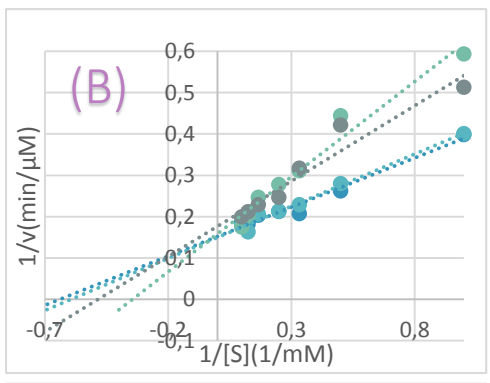


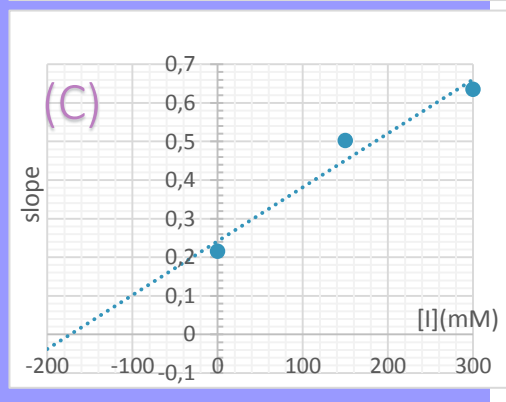
***Figure 2. Arrhenius plot** to calculate activation energy and the enzyme's activity for each temperature*

***Table 1**. Kinetic parameters obtained*

Vmax(µM/min)	Km (mM)	Kcat(1/min)	Kcat/Km (1/min.mM)	Ea(KJ/mol)
7.844	2.705	2307.06	852.89	26.4







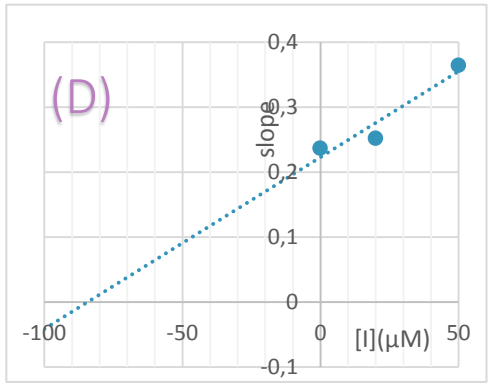


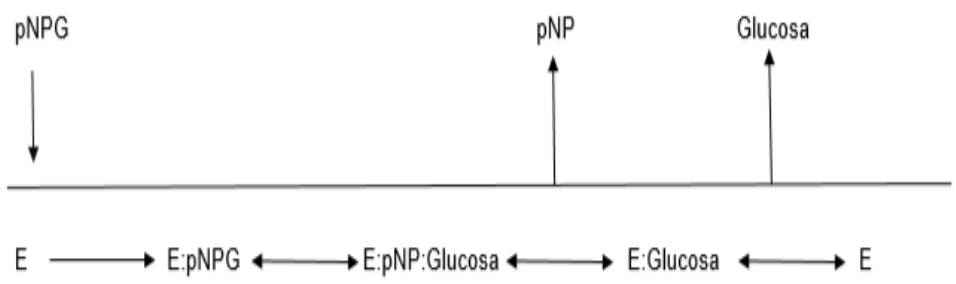
***Figure 3. Inhibition Results. (A)** Lineweaver-Burk plot for glucose. **(B)** Lineweaver-Burk plot for δ-Glucolactone. **(C)** Dixon plot to calculate k_{is}, (k_{is}=170mM). **(D)** Dixon plot for δ-Glucolactone. (k_{is}= 85 µM)*

CONCLUSIONS

The optimal conditions for the assay were determined: the concentration of the enzyme used was 2,43nM, the optimal reaction time was 10 min and the temperature in which the ß-glucosidase had its maximum of activity was 50 ºC, a higher temperature than the one used in the whole experiment (40 ºC).

Doing the inhibition assay, it was possible to determine the mechanism of reaction: on the one hand, the glucose was used as one of our inhibitors, as it is a product of the reaction catalysed by the enzyme studied, and due to the competitive inhibition it causes, the glucose must be only combined with the free enzyme. Therefore, the glucose is the last product to be released. On the other hand, δ-Glucolactone was neccesary as it is strongly linked to the p-NPG, being an anologue of the latter. It also produces competitive inhibition, so the pNPG joins the free enzyme before the water does.

On the whole, and trying to summarize the reaction mechanism, it can be classified as **uni bi ordered**, with the next Cleland diagram:



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