KINETIC CHARACTERIZATION OF ALMOND β-GLUCOSIDASE

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INTRODUCTION

β-glucosidases are enzymes that hydrolyze O-glycosidic bonds of glycosidic residues' non-reducing ends from oligosaccharides and aryl- or alkyl-β-D-glucosides, releasing β-D-glucose as a product. If the saccharide possess an aglycone, this will be released too. These enzymes have been isolated from many sources and have a large potential in industrial processess: biomass conversion, beverage processing, paper treatment, biofuels, clothing industry, etc. The aim of these experiments is to propose a catalytic mechanism model for β-glucosidase from sweet almond (*Prunus dulcis*) by kinetic characterization. To study so, we carried out various experiments: standarization of assay conditions, estimation of macroscopic kinetic parameters, denaturation study respect to temperature, and inhibition assays with glucose and D-glucono-1,5-lactone.

MATERIALS AND METHODS

β-glucosidase was isolated from almond emulsin (supplied by *FLUKA*). As substrate, *p*-nitrophenyl-β-D-glucopyranoside has been used; *p*-nitrophenol, as product for the standard curve; and glucose and δ-gluconolactone, both as inhibitor compounds. The rest of general reactants, such as citric acid, NaOH or HCl, have been supplied by *PANREAC*. Kinetic and inhibition assays have been carried on at a temperature of 40 ºC, while denaturation studies were performed at six different values of temperature: 20, 30, 40, 50 and 60 ºC. The assays lasted 10 minutes, carried on after 5 minutes of incubation of the reaction medium. The reaction detention was triggered upon pouring in 1 mililiter of 0.2 M NaOH, at the same time the assay tubes were put into a basin filled with ice. Concentration of *p*-NP was quantified using a *Spectro 22* spectrophotometer (LABMED).

RESULTS

1. Standardization of assay conditions

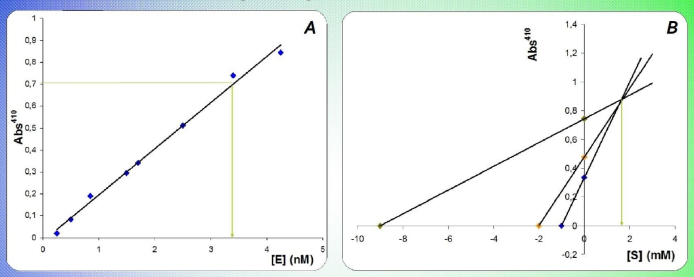


Figure 1: A) Absorbance of tested solutions at each different enzyme concentration. Interpolating at absorbance 0.7, we get an optimum enzyme concentration of 3.4 nM. B) Absorbance of tested solution at substrate concentrations of 1, 2 and 9 mM. Intersection of lines shows us the estimated value for K_M of 1.7 mM. Absorbances have been corrected with substrate controls.

2. Determination of kinetic parameters

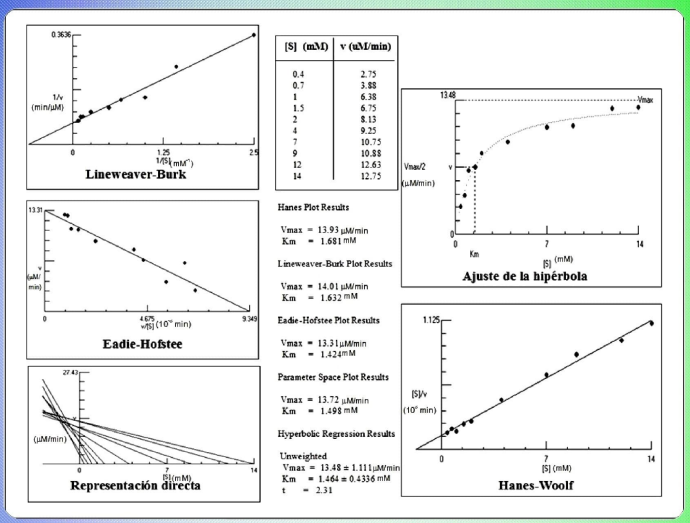


Figure 2: Computational plots (*Hyper 32*) of kinetic equations from activity assays at 40 ºC, enzyme concentration of 3.4 nM and different substrate concentrations. Such substrate concentrations and their respective velocities are found in the table at the top. By means of statistical studies, the estimated values for kinetic parameters are shown for each one of the plots in the middle of the figure. For further assays, estimated values from hyperbolic regression were chosen.

3. Thermal denaturation and inhibition assays

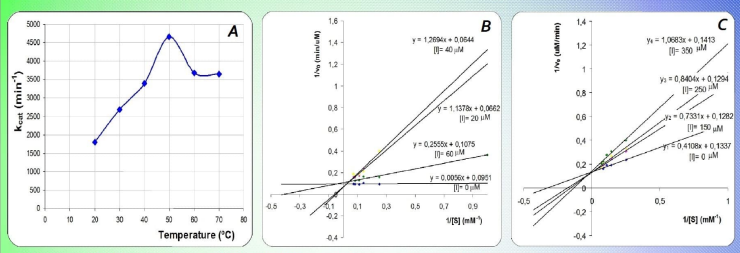
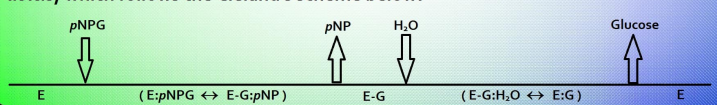


Figure 3: A) Enzymatic activity at different temperatures. It shows an optimum temperature for catalysis of approximately 50 ºC. The right downward curve shows the beginning of the denaturation process. B) Lineweaver-Burk plot for enzymatic assay with δ-gluconolactone as inhibitor molecule. The interception of lines shows a competitive mechanism. C) Lineweaver-Burk plot for enzymatic assay with glucose as inhibitor. The interception shows a competitive behavior again. Thus, both glucose and δ-gluconolactone bind the free enzyme competing with the substrate. Their K_{is} values are 234.56 and 121.57 mM, respectively.

CONCLUSIONS

· We estimated values for v_{max} and K_M of 13.48 µM/min and 1.464 mM, respectively.
· The optimum temperature value is approximately 50 ºC. However, it is better to perform assays at 40 ºC because the enzyme is completely stable.
· Inhibition assays show a competitive behavior for both compounds. Moreover, δ-gluconolactone exhibited a stronger inhibition, due to its analogous form to the transition state.
· We propose a Uni-Bi crypto Ping-Pong mechanism for β-glucosidase from *Prunus dulcis*, which follows the Cleland's scheme below:



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