KINETIC CARACTERIZATION OF SWEET ALMOND β-GLUCOSIDASE

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1. Introduction

β-Glucosidases (3.2.1.21) are enzymes that cleaves β-O and β-S glycosidic linkages in glucose-substituted molecules. This enzymes can also catalyze transglycosilation reactions under certain conditions [1]. β-Glucosidases are widely distributed in different species, and they are involved in many biological roles [2,3]. Due to its properties, this enzyme is widely used in many industrial processes, such as biomass conversion, textile and food industry, or medicine production.

The objective of this study is to determinate the kinetic parameters of *Prunus dulcis* β-Glucosidase and to figure out it's catalytic mechanism, using *p*-Nitrophenyl-β-D-glucosyde as a substrate model.

2. Materials and methods

All enzymatic assays were performed according to the following diagram:

0.9 mL Substrate + Citrate buffer (0.1 M, pH = 5.0) —5 min, 40 ºC→ + 0.1 mL enzyme —10 min, 40 ºC→ + 1,5 mL NaOH 0.2 M → Absorbance meassurement at 410 nm

At first, experiments to establish the optimal assay conditions were carried out (Optimal enzyme and substrate concentration and testing if reaction rate presents a linear dependence over time). Also, a standard curve of p-Nitrophenol absorbance at 410 nm in presence of citrate buffer and NaOH was made, in order to determinate the amount of product obtained in the enzyme assays.

Once optimal assay conditions were determinated, steady state kinetic studies were performed with the aim of determinating kinetic parameters of β-Glucosidase with pNPG as substrate. In order to establish the effect of temperature on enzymatic catalysis, kinetic studies at different temperatures were carried out. Also, reversible inhibition studies in presence of glucose and Glucono δ-lactone were made with the objetive to determinate the type of inhibition induced by these substances, and also obtain information about the kinetic mechanism of the enzyme.

3. Results

a) Optimal assay conditions

Molar attenuation coefficient of pNP in pressence of citrate buffer and NaOH was determined to be 16650 $M^{-1}cm^{-1}$.

It was established that optimal enzyme concentration is 2.83 nM. Km aproximated value obtained in pre-kinetic assays was 3.17 mM.

Also it was proved that reaction rate keeps constant in time up to 20 minutes.

b) Determination of kinetic parameters

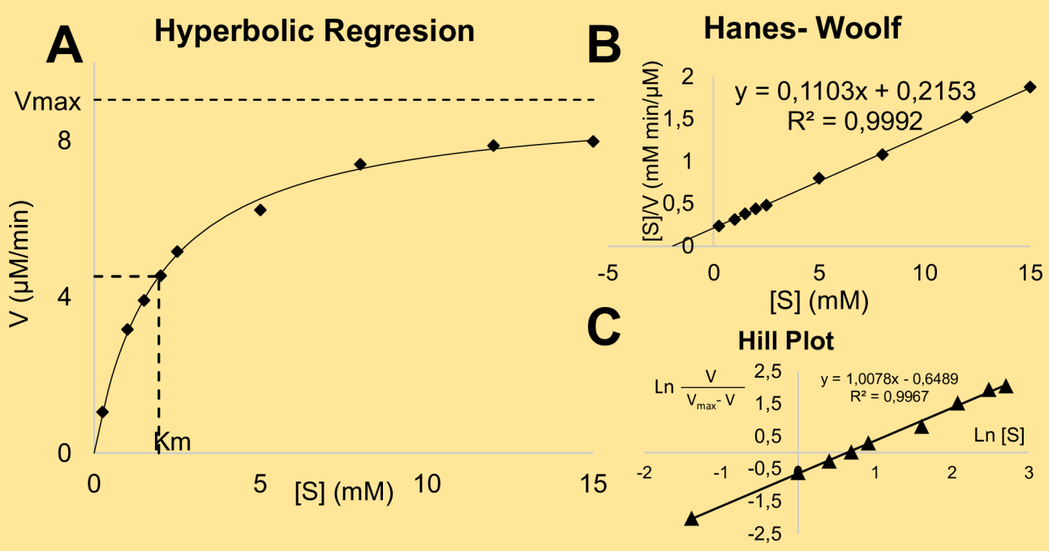

Figure 1. **A)** Hyperbolic regression acording to Michaelis-Menten equation. **B)** Hanes-Woolf linear plot. **C)** Hill plot for determination of Hill coeficient.

c) Temperature effect

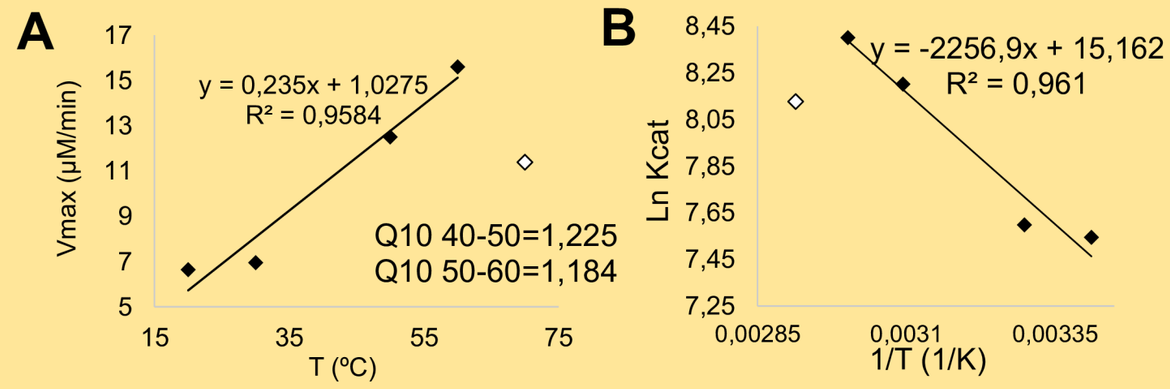

Figure 2. **A)** Variation of Vmax value at different temperatures. Q10 calculated values for two different temperature ranges appear in the graphic. **B)** Linearization of Arrhenius equation.

d) Reversibe inhibition studies

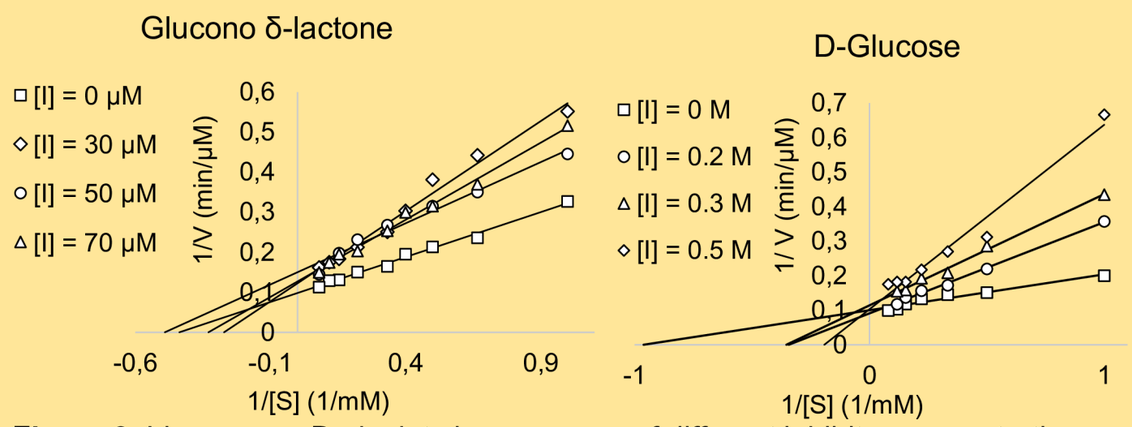

Figure 3. Lineweaver-Burk plots in pressence of different inhibitor concentrations, using Glucono δ-lactone (left) and D-Glucose (Right)

4. Conclusions

Table 1. Kinetic parameters of *Prunus dulcis* β-Glucosidase using pNPG as substrate. This values were calculated by means of Hyper program.

Km (mM)	Vmax (µM/min)	K_{cat} (min^{-1})	k_{cat}/Km ($mM^{-1}min^{-1}$)
1.915	9.004	3177.79	1659.42

-The value of Hill coeficient is 1, which means that the enzyme is not regulated by cooperative effects.

-Energy of activation value, calculated from Arrhenius equation (Fig 2.B) was 18.76 kJ/mol. It can be observed a decrease of Vmax value at 70 ºC (point represented as ◇) due to enzyme's thermal denaturalization. Therefore, it is established that the optimal temperature for this enzyme is 60 ºC.

-It was determined that both Glucono δ-lactone and D-Glucose are competitive inhibitors for β-Glucosidase, being its Kis values 99.27 µM and 203.21 mM, respectively. As Glucose, one of the products of the reaction, acts as a competitive inhibitor, it can be deduced that it binds to the same enzyme form as substrate does: free enzyme. Therefore, D-glucose shall be the last product released from the catalytic center.

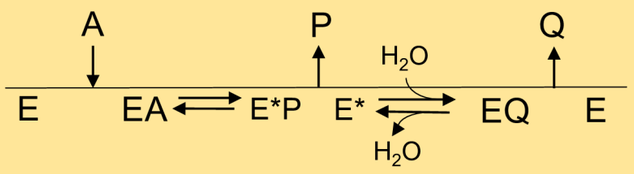

Figure 4. Cleland scheme of the kinetic mechanism of β-Glucosidase. A represents the substrate (β-D-Glucoside), P represents the aglycone and Q represents D-Glucose [4-6]

5. References

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