KINETIC CHARACTERIZATION OF ALMOND β-GLUCOSIDASE

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INTRODUCTION

β-glucosidases (also known as β-D-glucoside glucohydrolases or EC 3.2.1.21) are a large group of enzymes within the glycosidases. These enzymes catalyse the hydrolysis of the O-β-glycosidic bond located at the non-reducing terminus of short chain oligosaccharides, disaccharides and aryl- or alkyl-β-D-glucosides, releasing β-D-glucose. The β-glucosidases are found in practically all areas of life and are widely distributed in nature. In bacteria and fungi, β-glucosidases are part of the multienzymatic systems called cellulases. In plants, these enzymes are involved in chemical defence mechanisms, activation of phytohormones' precursors and aglycone's release. In mammals, cytosolic β-glucosidases take part in the xenobiotic metabolism. Moreover, in humans, the lysosomal acid β-glucosidase deficiency origins the Gaucher disease. β-glucosidase is very important in textile, food and biotechnological industries.

The final objective is the proposal of a model for the catalytic mechanism of β-glucosidase. In order to do this, it is necessary to complete some specific objectives like the standardization of the assay conditions of β-glucosidase, macroscopic kinetic parameters determination, effect of the temperature and inhibition studies.

MATERIALS AND METHODS

The biological material is a commercial solution of β-glucosidase 170 nM isolated from almond emulsin (*Prunus dulcis*) supplied by FLUKA. The rest of solution are given in 100 mM, pH 5.0 citrate buffer: *p*NP (*p*-nitrophenol) 25 mM, *p*NPG (*p*-nitrophenil-β-D-glucoside) 50 mM, glucose 2.0 M and δ-gluconolactone 20 mM. They were supplied by FLUKA too. Other reagents like NaOH or HCl were supplied by PANREAC.
With regard to the method used, the following scheme is used:

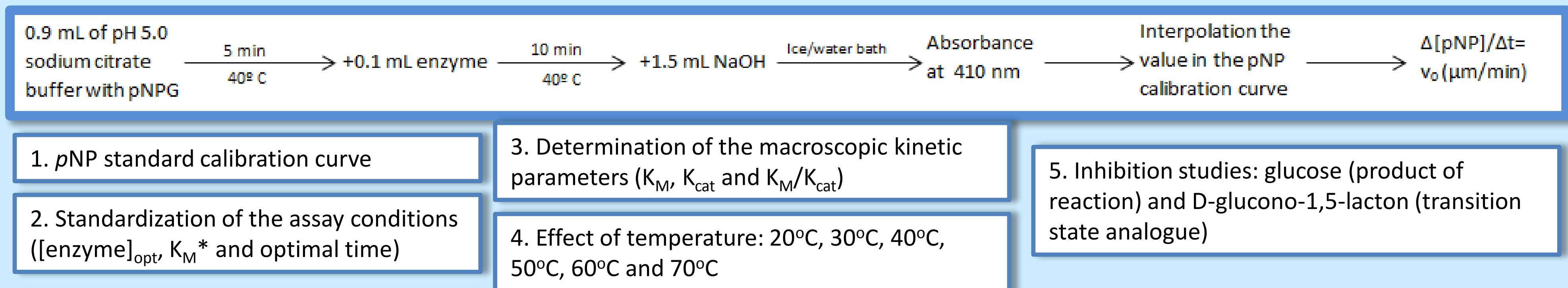

Figure 1. General scheme followed in each enzymatic assay.

RESULTS. According to the method used, the following data are obtained:

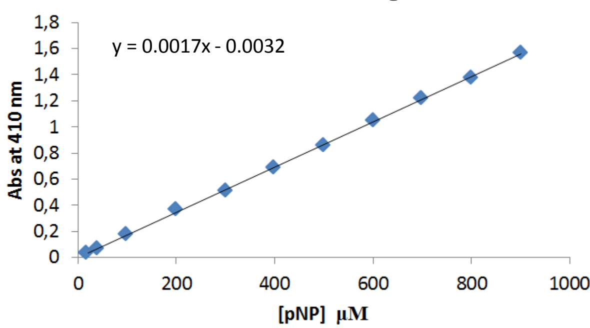

Figure 2. Standard curve for *p*NP

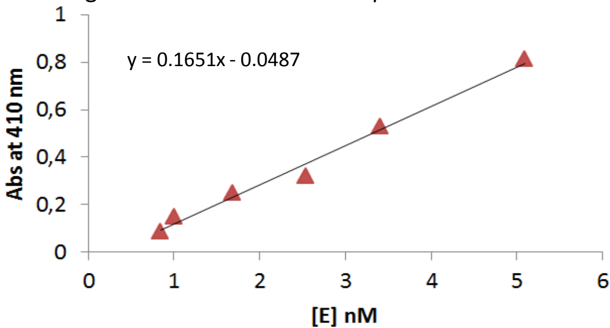

Figure 3. Linearity with the enzyme concentration

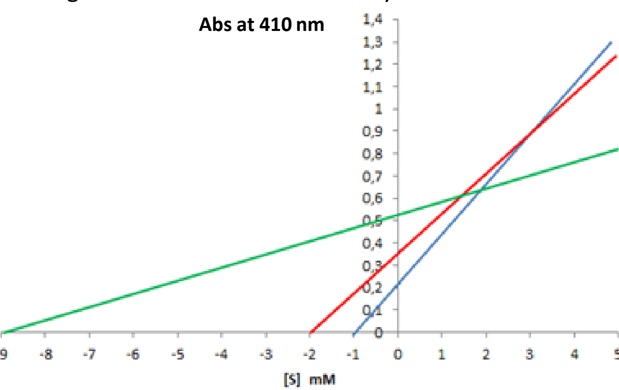

Figure 4. Eisenthal and Cornish-Bowden plot

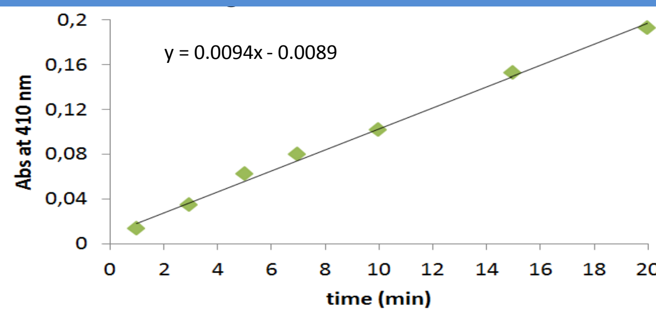

Figure 5. Linearity with the time

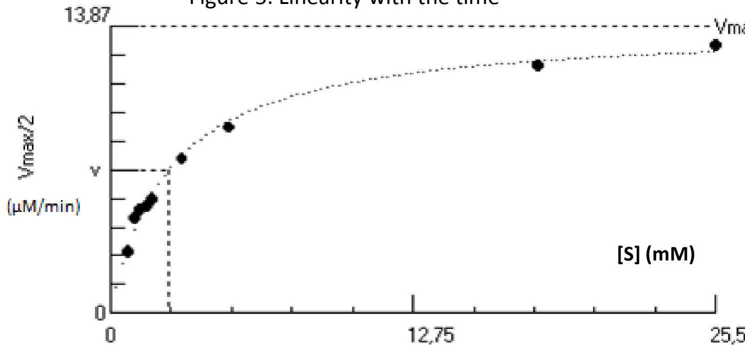

Figure 6. Dependence of the initial velocity of catalysis with substrate concentration. The macroscopic kinetic parameters obtained are: K_M= 2.468 mM, K_{cat}=2,951 min^{-1} and K_M/K_{cat}=1,195.7 $mM^{-1}min^{-1}$

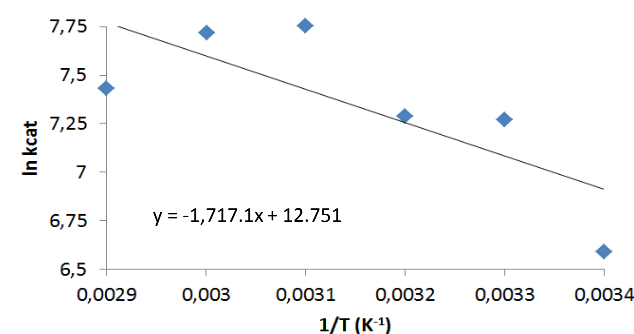

Figure 7. Variation of the kinetic parameters with the temperature. Values obtained: Q10=0.82 and Ea= 19.92 KJ/mol

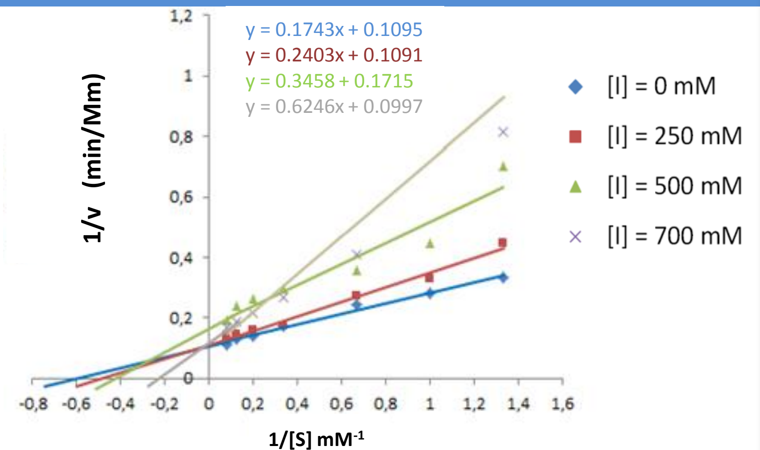

Figure 8. Lineweaver-Burk plot for glucose inhibition: K_{ic}=275 mM

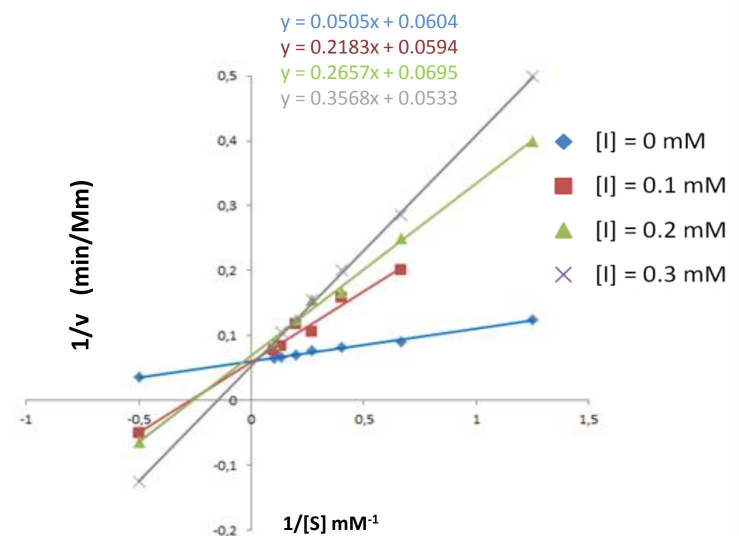

Figure 9. Lineweaver-Burk plot for δ-gluconolactone inhibition: K_{ic}=0.079 mM

CONCLUSION

- As stated in inhibition studies, glucose and δ-gluconolactone are competitive inhibitors.
- δ-gluconolactone presents a lower inhibition constant which means it is needed a lower concentration to inhibit the enzyme.
- The results support the idea that the catalytic mechanism of β-glucosidase is Uni-Bi crypto ping-pong.
- This proposed mechanism has two stages. The first product to be released is *p*NP and a covalent intermediate is formed by the enzyme and the glucose. In the second stage, water hydrolyses the bond between the enzyme and glucose. Then, glucose is released.
- To check, if the catalytic mechanism is correct, it would be necessary to do an additional inhibition study with *p*NP, the other product.

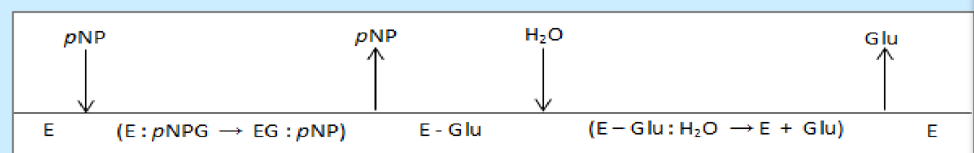

Figure 10. Cleland scheme for β-glucosidase