β-glucosidase characterisation

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Abstract

β-glucosidase (EC: 3.2.1.21) from almond will be studied and characterized so it can be correctly treated on future experiments focused on different applications, such as the food industry. It catalyses the hydrolysis of terminal non-reducing residues in β-glucosidases and transpeptidation as well. B-glucosidases have multiple functions, such as defence on plants and forming cellulases on bacteria and fungus. For this, the most accurate assay conditions for the aforementioned enzyme, using ρ-nitropenyl-β-D-glucopyranoside as substrate, will be found out, so its kinetic parameters will be calculated. Inhibitions assays will take place as well, using glucose and δ-glucogalactone as inhibitors. With the results given, ideal conditions will be proposed for β-glucosidase from almond, and resemblances between this enzyme and β-glucosidase from other sources, studied previously, will be stated.

Materials and methods

Standardisation. It has been realised a Standard curve with pNP measured at 410 nm according to its concentration. Also, it has been calculated the optimum enzyme. Finally, it has been calculated approximate Km and linearity with time.
Determination of the kinetic parameters. The kinetic parameters has been calculated using the Lineweaver Burk, Eadie Hofstee, Hanes-Woolf and direct plot.
Temperature assays. It has been done using temperatures between 20 and 70ºC, and they have been calculated for each of them.
Inhibition. The inhibitors used to the experiment are Glucose and δ-gluconolactone. Measuring absorptions and calculating velocities of reaction, a double reciprocal plot will be represented to clear up what kind of inhibition takes place in each case. After that, secondary plots will be used to determinate constants of inhibition.

Conclusion

β-glucosidase characterisation has shown that this enzyme catalyses a reactor with a lot of efficacy. It is shown that it is a thermostable enzyme. Given the results exposed, an ordered Uni Bi ping –pong mechanism can be proposed: finally, glucose and δ-gluconolactone act as competitive inhibitors.

Results

Standardisation.

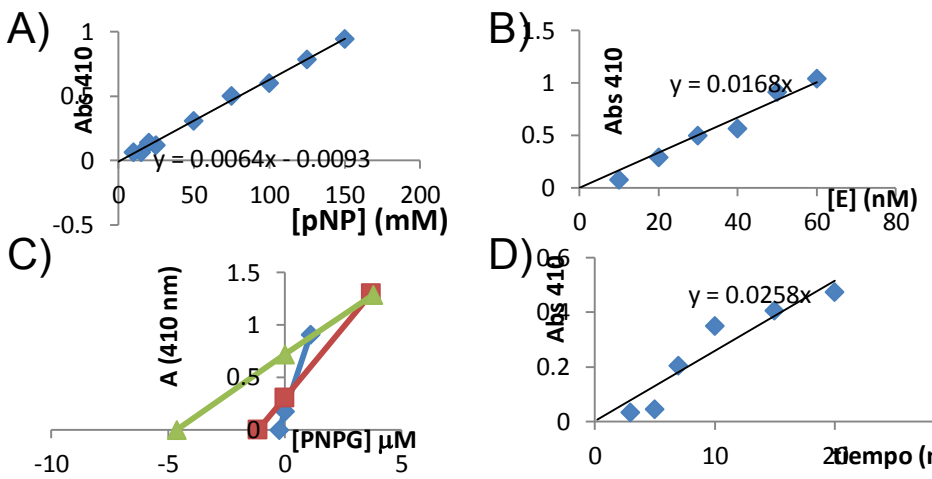

Figure 1. A) standard curve of pNP; B) optimum β-glucosidase (3,4 nM; C) approximate Km (1 mM); D) linearity with time.

Determination of the kinetic parameters.

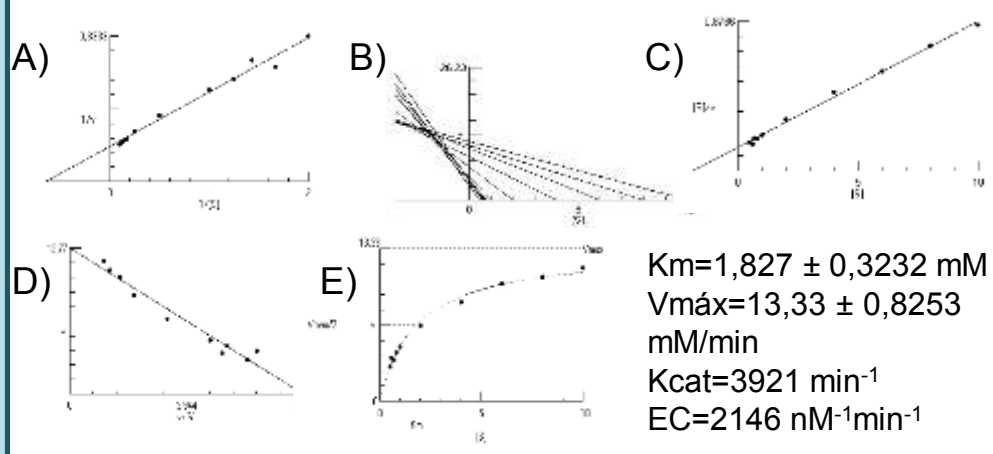

Figure 2. A) Lineweaver-Burk; B) Eadie-Hofstee; C) Eisenthal y Cornish-Bowden ; D) Hanes-Wolf; E) hyperbola

Temperature assays.

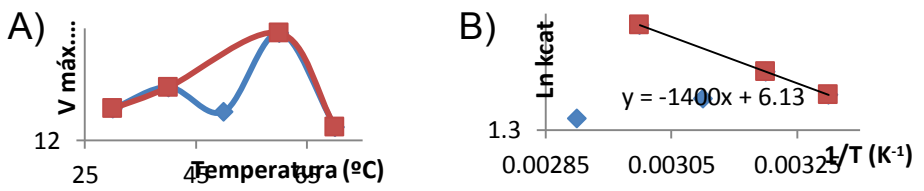

Figure 3. A) variation of Vmax with time: B) linearization for Arrhenius equation (Ea=1164J/mol; Q10(30/40)=1,5; Q10(40/50)=0,85.

Inhibition.

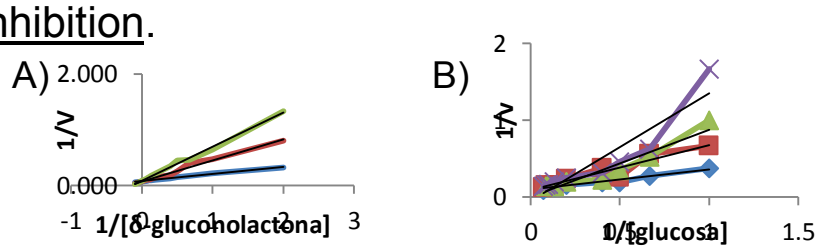

Figure 4. A) inhibition with δ-gluconolactona (competitive, Ki=13,64 mM); B) inhibition with glucose (competitive, Ki=62,96 mM).

Cleland representation.