KINETIC CHARACTERIZATION OF β-GLUCOSIDASE FROM *Prunus dulcis*, SWEET ALMOND

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Abstract

β-Glucosidases (EC 3.2.1.21) are found in all domains of living organisms, playing key roles in the removal of nonreducing terminal glycosyl residues from saccharides and glycosides. A series of assays were run on the β-glucosidase from *Prunus dulcis* (sweet almond), using pNPG (p-nitrophenyl-β-D-glucopyranoside) as a substrate, which undergoes hydrolysis in the presence of β-glucosidase, resulting in pNP (p-nitrophenyl) and β-D-glucose. This Uni-Bi reaction was used to determine the kinetic parameters of the enzyme and establish a kinetic mechanism of action.

Standard Assay Protocol

A standard assay protocol was established for all experiments. The assays were run at 40ºC for 10 minutes at pH 5.0 with varying concentrations of pNPG and β-glucosidase depending on the specific aim of each experiment. In every case the reaction was started with the addition of enzyme and stopped with NaOH 0.2 M. The reaction of pNP with NaOH resulted in a colorimetric product which could be quantified at A_{410}. The standard assay conditions were determined as [β-glucosidase]=3.0 nM and an incubation time of 10 minutes.

Results

Kinetic parameters:

Hyperbolic regression curve (Figure 3). K_M =2.55 mM, V_{MAX}=10.90 µM/min, k_{cat}=3632 min^{-1}.

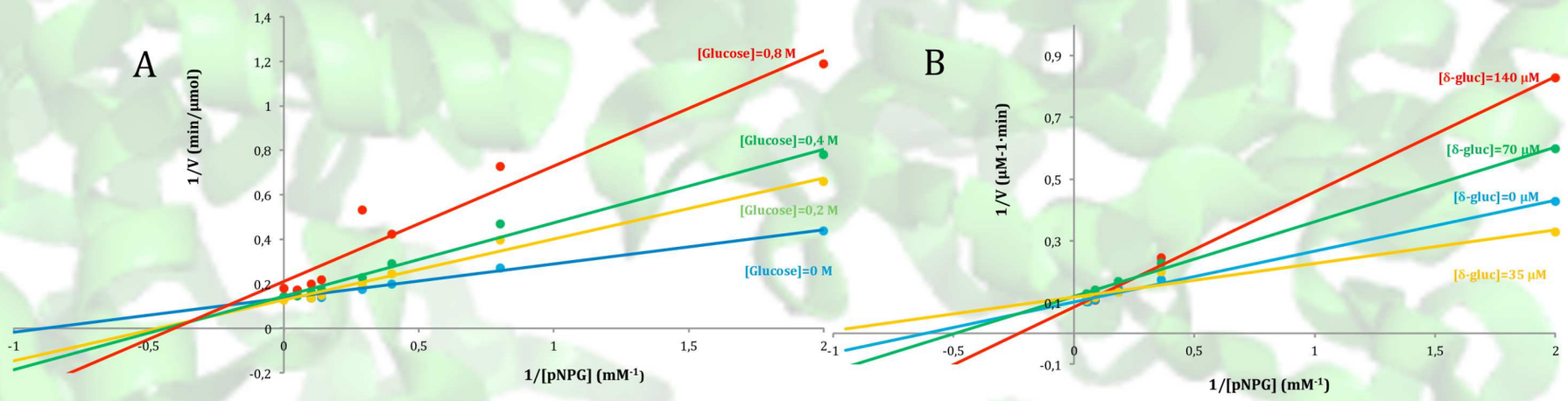


Figure 1: Lineweaver-Burk plots for both (A) product inhibition with glucose and (B) inhibition with δ-gluconolactone. Both reveal competitive inhibition.

Temperature effect:

Maximum activity at 50ºC and thermic denaturalization from here on.

Ea=23.69 kJ/mol.

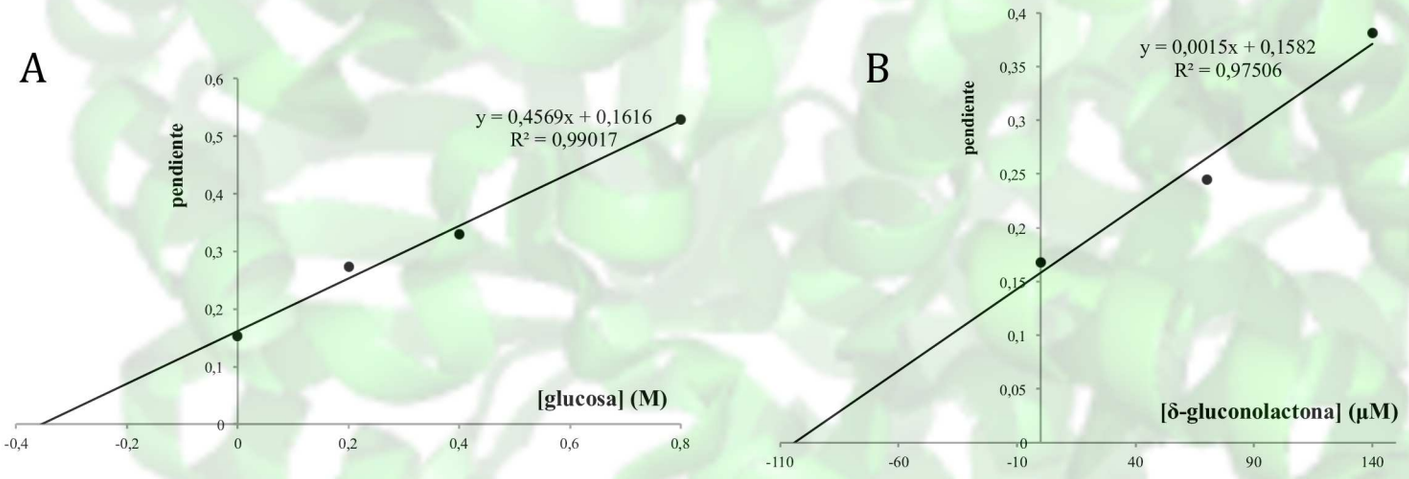


Figure 2: Secondary representation for the calculation of Kis. (A) product inhibition with glucose, Kis=457 mM and (B) inhibition with δ-gluconolactone, Kis=0.105mM.

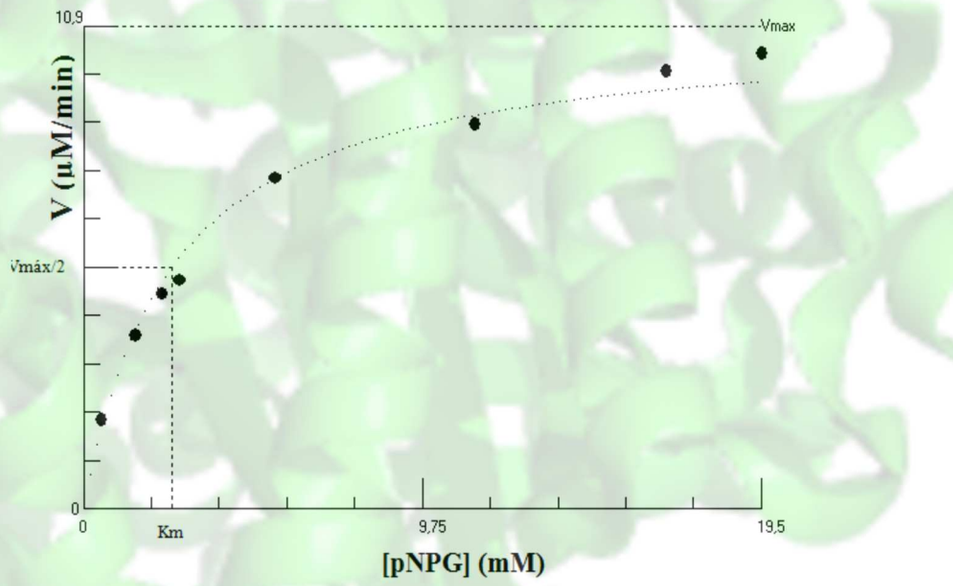
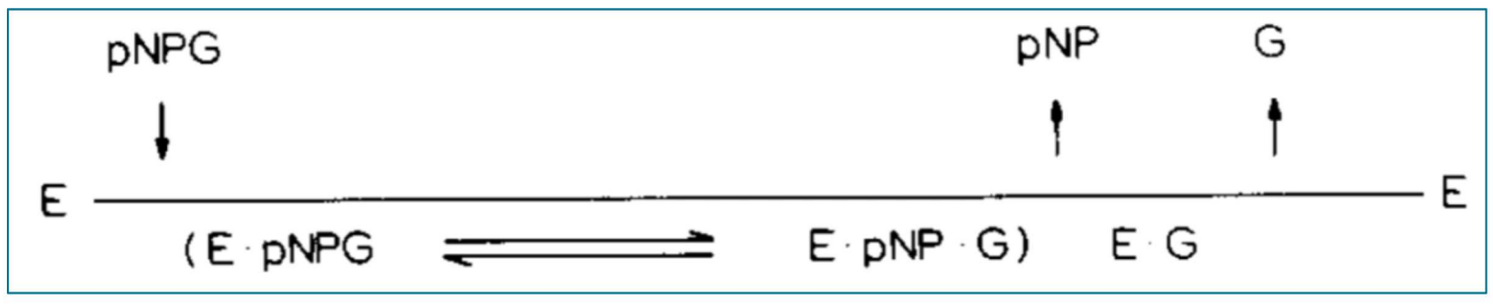


Figure 3: Hyperbolic regression curve.

Conclusions

The competitive inhibition observed for glucose (Figure 1 A) suggests that β-glucosidase hydrolyses pNPG by an ordered and sequential mechanism, as shown in the Cleland diagram.

The *Kis* for δ-gluconolactone is lower (0.105 mM) than the *Kis* for glucose (457 mM). This indicates that δ-gluconolactone binds to the enzyme with much higher affinity, which is consistent with the fact that it is an analogue of the transition state.



Cleland diagram for the reaction catalysed by β-glucosidase.