Characterization and determination of kinetic parameters of β-glucosidase

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Introduction

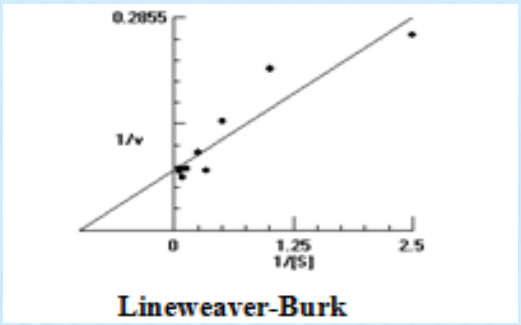
β-glucosidase (E.C. 3.2.1.21) is an enzyme whose function is the degradation of cellulose in order to obtain glucose. This enzyme appears in organisms that eat plants (in addition to degrade cellulose, it also regulates some metabolic pathways) and in plants (it helps the organism to defend itself from predators[1] and also control the liberation of phytohormones). Furthermore, it is known that β-glucosidase has some importance in industry (food processing industry, pharmacological industry[2], etc.) In this experiment, almond β-glucosidase and its kinetic parameters have been characterized.

Materials and methods

For the determination of the kinetic parameters and the determination of the effect of the temperature on catalysis, a few experiments have been made, changing the amount of substrate, enzyme , the temperature and the time of the reaction. The components used for this experiment have been 4-nitrophenyl- β-D-glucopyranoside, β-glucosidase, citrate buffer and NaOH 0.2 M.
For the inhibition assays, glucose and δ-gluconolactone have been used, making its reaction in similar assays conditions, changing only the concentration of inhibitor added to the assay. The materials used for this experiment have been the same used in the last experiment and, also, glucose and δ-gluconolactone.

Results

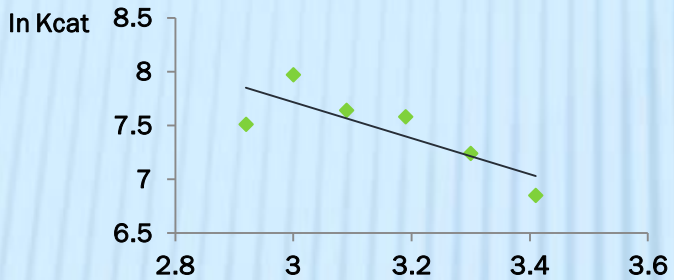
Kinetic parameters



Lineweaver-Burk

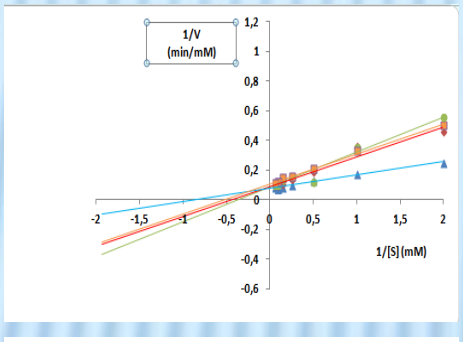
$V_{máx}$=14.09 mM/min
K_m=1.131 mM

Temperature assays

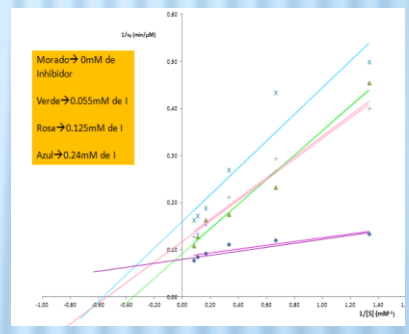


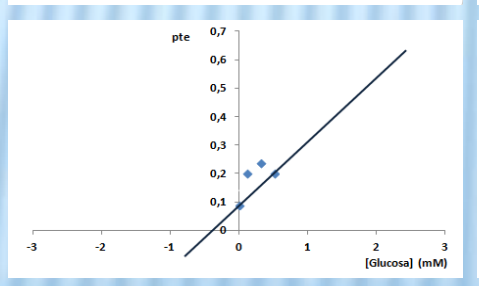
Inhibition assays (up) and Dixon diagram (down)

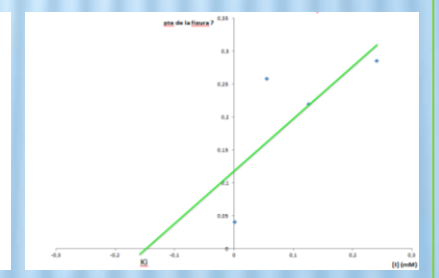
Glucose



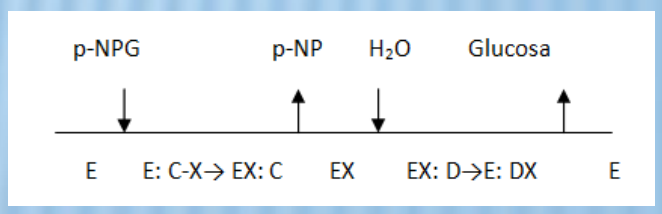
δ-gluconolactone







Cleland diagram



Conclusion

To optimize the assay, the optimal concentration of enzyme (4,5 nM), the optimal testing time (10 min), the approximate Km (2,18 mM) and the optimum temperature (40-60 ºC) were determined. The inhibiting behaviour of glucose and δ-gluconolactone was studied and it showed a competitive inhibition with Ki^E of 0,1 mM. δ-gluconolactone is confrimed to behave as a transition state analog while glucose turns out to be the last product to come out of the β-glucosidase active site. The patterns of inhibition are consistent with an ordered secuencial uni-bi kinetic mechanism. This is showed in the Cleland diagram. Studies from other authors suggest a doublé displacement mechanism with retention of configuration.[3]

References

[1] Morant, A. V.; Jorgensen, K.; Jorgensen, C.; Paquette, S. M.; Sanchez-Perez, R.; Moller, B.L.; Bak, S., beta-glucosidases as detonators of plant chemical defense. *Phytochemistry* **2008,** 69, 1795-1813..
[2] Bhatia, Y.; Mishra, S.; Bisaria, V. S., Microbial beta-glucosidases: Cloning, properties, and applications. *Critical Reviews in Biotechnology* **2002,** 22, 375-407.
[3] Rye, C. S.; Withers, S. G., Glycosidase mechanisms. *Current Opinion in Chemical Biology* 2000**,** 4, 573-580