Kinetic characterization of almond β-glucosidase

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Introduction

β-Glucosidases (EC 3.2.1.21) have been the subject of intensive research due to the key role these enzymes play in biological processes (in plants, mammals and microorganisms) and potential biotechnological applications (Bhatt, 1997).
β-Glucosidase acts on the β(1-4) bond linking two glucose residues or glucose-substituted molecules. The action of the enzyme on such glucosides results in the release of sugar units (see Figure 1). The water molecule is used as a base for the nucleophilic attack on the positively charged anomeric carbon.

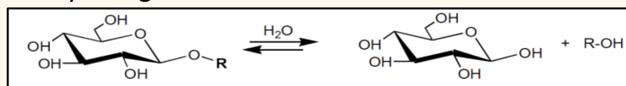

Figure 1: The general equation of the chemical reaction that-glucosidase catalyses (Vasella et al, 2002).

One striking feature of the enzyme is its broad specificity, which can catalyse a wide range of substrates. This broad specificity allows to explore both the active site topology and the mechanism of action of the enzyme (Dale et al, 1985).
The objective of this experiment is to propose a catalytic mechanism model for ß-glucosidase by kinetic characterisation. To perform this proposed objective, several studies such as determining the activity of the enzyme beta-glucosidase and the kinetic parameters at steady state, studying the effect on catalysis of environmental factors such as temperature, and analysis inhibiting performance of the reaction product and transition state analogues of the enzyme have been carried out.

Materials and Methods

Materials:

β-glucosidase from emulsion of almonds, which is commercially prepared from Sigma-Aldrich, will be used as a biological source of material **(Sigma-Aldrich, 2015)**, with p-Nitrophenol (pNP) 25 mM and p-Nitrophenol- β-D-glycoside (pNPG) 50 mM as product and substrate, respectively. Glucose 2 M, and Glucono-δ-lactone 20 mM are used as inhibitors in this study.

Method:

Using the schematic diagram below (Figure 2), a series of experiments have been carried with changing one condition and keeping the other conditions constant (for example: temperature, time, [S] and [E]…)

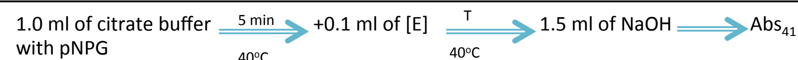

Figure 2: The assay that would be used throughout the experiment to work out key conditions ([S], [E], time), temperature and inhibition studies.

Results

Standardisation

The enzyme works best at a concentration of 6.5 nM, a Km substrate concentration of 2.1 mM, and a calibration curve was built to work out initial velocities for the remaining part of the investigation.

Kinetic Parameters

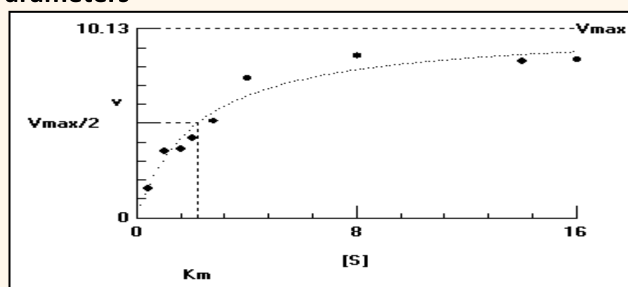

Figure 3: Graphical representations to determine kinetic parameters. A hyperbolic graph representation (or Michealis-Menten graph), can be created using the Hyperbolic Regression software by Dr Easterby. The kinetic parameters are: Vmax=10.13 mM/min, Km= 2.12 mM, kcat= 1558 min^{-1} and kcat/Km= 158 mM^{-1} min^{-1} .

Temperature

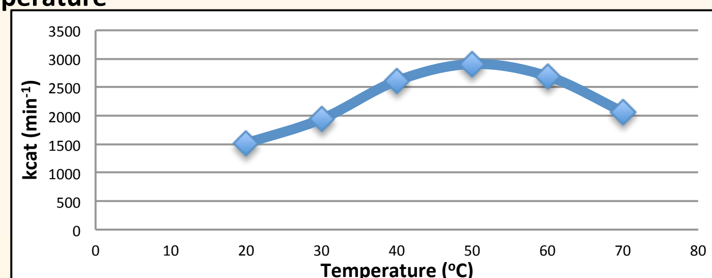

Figure 4: A graph representing kcat against temperature. The kcat value increases from 20 until 50ºC, and then starts to decreases at 60 and 70ºC at high temperature. This shows that the enzyme works best at a temperature of 50 ºC. The Q_{10} value can be calculated using this graph, and selecting two points (for example 30 and 20ºC in which the Vmax30ºC/Vmax20ºC= 1.28.

Inhibition Studies

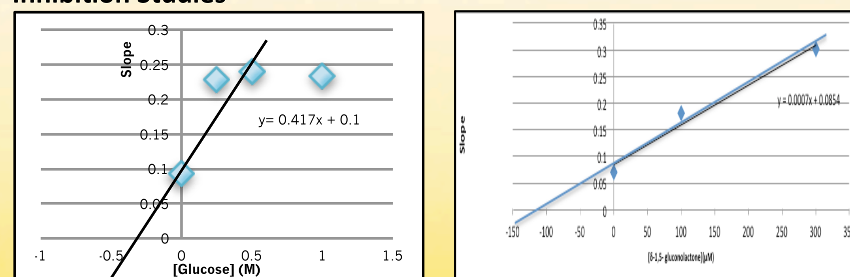

Figure 5: A secondary representation graph of each inhibitor, glucose and δ-1,5-gluconolactone. From a Lineweaver-Burk plot (not shown), both inhibitors have been proven to be competitive inhibitors. A secondary representation (also known as a Dixon plot) allows to work out the kis value for each inhibitor. Glucose has a kis of 240 mM whereas δ-1,5-gluconolactone has a kis of 0.120 mM.

Conclusion

Through a series of experiments, the best conditions of assays were defined through studying the kinetic parameters of β-glucosidase in the stationary phase: the incubation time of 10 minutes, the optimum [E] of 6.5 nM, a temperature of 50ºC and the approximate Km of 2.1 mM. The kinetic parameters were also defined, and the values from the hyperbolic graph were: a Km of 2.21 mM, a Vmax of 10.13 mM min^{-1} and a kcat of 1558 min^{-1}. The inhibition studies have shown that glucose and δ-1,5-gluconolactone have a competitive effect on the enzyme β-glucosidase. Glucose has a kis of 240 mM whereas δ-1,5-gluconolactone has a kis of 0.120 mM, meaning that glucose as a product will be released last, after the release of pNP.

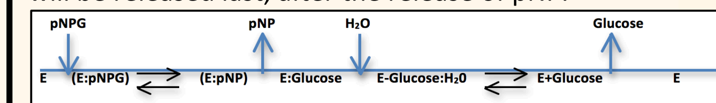

Figure 6: Proposed mechanism for β-glucosidase, using the experimental finding that glucose is a competitive inhibitor.

The proposed mechanism of the β-glucosidase is a sequential-ordered crypto ping-pong mechanism, due to taking water molecules into account and due to the overall retention of the configuration of the reactants (pNPG and glucose), which means that the β-form of the structure is kept from the substrate and when producing the product, while the intermediate produces the α configuration. . Further studies need to be done in order to verify the proposal.

Reference

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