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Exploration of the active site specificity of MalA, a glucosidase from the predatory bacterium Bdellovibrio bacteriovorus

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Background

Bdellovibrio Bacteriovorus is a motile, predatory gram-negative bacterium that preys on other gram-negative bacteria including *E. coli*, salmonella, and other medically relevant organisms.[1, 2, 3] This bacterium has the potential to be used as a living antibiotic agent[3], but in order for safe application, a thorough understanding of the predator's molecular biochemistry is required.

The 2005 sequencing of *B. bacteriovorus'* HD100 genome revealed genes whose metabolic roles are difficult to rationalize, including a gene for a the putative maltase, MalA.[1, 2, 3] Given the bactirum's observed disuse of prey carbohydrates as an energy source[4], this enzyme is of particular interest for studies of activity and specificity in the context of the bacterium's predatory lifestyle.

MalA has been placed in the glycoside hydrolase (GH) family 13 of α-retaining enzymes based on sequence homology.[5] While placement in a family of enzymes may help reflect structural features and reveal evolutionary relationships, it is not particularly useful in determining substrate specificity. Therefore, further investigation is necessary to determine the kinetic parameters and native substrate of this enzyme in the context of the *B. bacteriovorus* genome.

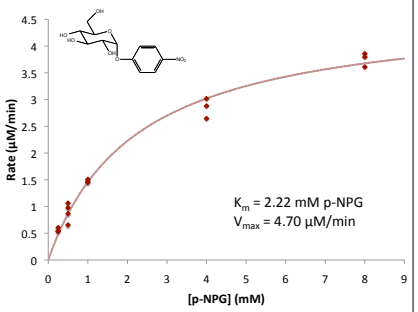
Previous Research

Using TLC and H-NMR spectroscopy, Greg Kirkpatrick and Jordan Carelli showed that MalA catalyzes the hydrolysis of maltose to glucose. They also showed that MalA also has glycosyltransferase activity -- maltotriose and higher oligosaccharides are transiently produced when maltose is treated with MalA.[6, 7]

Enzyme Prep

Fellow student Jared Trecker is working toward purification of MalA in the Grinstead Lab. Top10 *E. coli* containing the pMalA gene were cultured and the cells were collected and lysed. Supernatants were collected and purified on a GE HiTrap DEAE FF ion exchange column. This partially purified enzyme was used for the studies described here.

p-NPG as Substrate of MalA



All assays performed in pH 6.9 100 mM phosphate buffer

pH Profile of MalA

•The relative rate of hydrolysis of p-NPG

pH	Relative Rate
4	0
4.8	6
6	100
6.9	90
8	0

•Extinction coefficients: pH 4.8 = 142, pH 6 = 2207, pH 6.9 = 8090, pH 8 = 12560 AU/M
•Assay A (pH 6, 6.9, 8): continuous assay at A_{405} of reaction consisting of pH 6.9 phosphate buffer (100 mM) and p-NPG (1 mM) to calculate rate.
•Assay B (pH 4, 5, 6): 3 mL reaction started with buffer (100 mM), p-NPG (1 mM), and enzyme. At 0, 10, 20 and 30 minutes, 500 μL reaction mixture was transferred to a cuvette containing 500 μL 0.2 M NaOH and the A_{405} was measured. A linear fit was used to determine rate (AU/min).

Stability of MalA

•Crude preps of MalA were stable at 4ºC for several days
•Tests with calcium chloride (0-100 μM) did not indicate that calcium increases rate of hydrolysis of p-NPG.
•At 25ºC, enzyme activity is stable for greater than 3 hours.
•At 37ºC, enzyme activity is stable for greater than 30 minutes.

Inhibition of p-NPG Hydrolysis

Assuming competitive inhibition, an average dissociation constant, K_i, was calculated for each sugar. Lower K_i means better inhibition. All inhibition studies performed at 1mM p-NPG in 100 mM pH 6.9 phosphate buffer.

Inhibitor	Average K_i
Maltose	1.8
Glucose	2.4
Isomaltose	4.6
Sucrose	11.6
Trehalose	17.0
Cellobiose	>1000

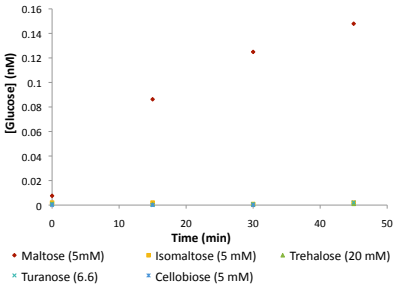
•MalA is inhibited by a variety of α-linked disaccharides, but not by a β-linked disaccharide.

Time Dependent Inhibition of p-NPG hydrolysis

Inhibitor	K_i (mM)	
Turanose	6.3 (initial) 1.6 (final)	Rate of p-NPG hydrolysis decreased from 60% to 27% in ten minutes
Fructose	>100 (initial) 4.4 (final)	Rate of p-NPG hydrolysis decreased from 100% to 58% in five minutes

Activity Studies

Various disaccharides were incubated with MalA and the formation of glucose was monitored using a glucose oxidase test kit.



•Maltose is the only disaccharide hydrolyzed by MalA.
•Despite binding a variety of α-linked disaccharides as inhibitors, MalA only shows hydrolytic activity with maltose as a substrate.

LC/MS

•Preliminary tests using the LC/MS carbohydrate detection method developed by Jordan Carelli[7] showed formation of pseudo-molecular ions [M+Cs^+] for glucose and maltose in solution.
•Pseudo-molecular ion detected for glucose from a sample containing 6 mM Glucose 0.1 M pH 6.9 phosphate buffer, 50 μL enzyme stock, and 40 μM cesium acetate.

Future Research

•Develop an LC/MS method for quantitating the carbohydrate products of the enzymatic reaction.
•H-NMR assay for determining the stereochemistry of hydrolysis by MalA.
•Use of maltose sugars with modified glucose substituents to identify enzyme-substrate interactions.
•Long term storage of enzyme.

Literature Cited

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