Spectrophotometric Determination of the Kinetic Parameters of β-fructofuranosidase and the Mechanism of Inhibition by Copper (II) Sulfate

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1 Abstract

Excessive dosages of heavy metals can be hazardous to organisms and the environment. This is due to the ability of heavy metal ions to inhibit several kinds of biological enzymes. In this experiment, the kinetic parameters of β-fructofuranosidase, a fungal enzyme that catalyzes the breakdown of sucrose, were quantified through dinitrosalicylic acid assay and nonlinear regression. Furthermore, the activity of β-fructofuranosidase was measured in 6 different concentrations of copper (II) sulfate, an inhibitor, to determine the nature of inhibition. From the experiment, the results show that the nature of inhibition is noncompetitive at $[CuSO_4] < 0.0022M$ and competitive at $[CuSO_4] > 0.0044M$, V_{max} is $0.441 \pm 0.0354 \frac{mmol}{min}$, K_m is $17.6 \pm 2.6mM$, and K_i is 0.00863 ± 0.00101.

2 Introduction

2.1 Background

Sucrose is a disaccharide composed of α-glucose and β-fructose joined by an α-1,4-glycosidic linkage (Freeman *et al.*, 2011). This bond can be hydrolyzed by β-fructofuranosidase, an enzyme commonly known as invertase, to yield glucose and fructose in equal proportions (Greenwood Health Systems, 2009).

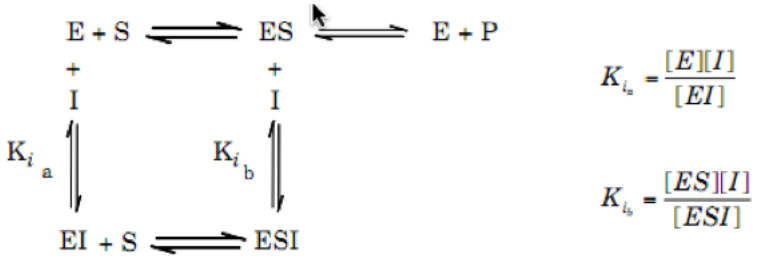
The presence of heavy metal ions such as Cu^{2+} may inhibit the activity of invertase. This is due to the high affinity of the sulfhydryl group on cysteine and methionine residues in a protein for the Cu^{2+} ion (Greenwood Health Systems, 2009). Soft bases, including sulfhydryl groups, have a high affinity for soft acids, such as the Cu^{2+} ion (Cotton *et al.*, 1976). When copper ions form complexes with sulfhydryl groups in amino acid residues, disulfide bridges are disrupted, modifying the enzyme's tertiary structure. Since the functions of an enzyme are highly correlated with its form, a change in the tertiary structure of invertase would likely inhibit its function (Cotton *et al.*, 1976).

As a result of the close proximity of methionine residues to the glycosylation sites of invertase, $CuSO_4$ is suspected to be a noncompetitive inhibitor–one that slows product formation, while not affecting substrate binding (http://www.civil.uwaterloo.ca/enve375). In order to test this hypothesis, the rates of sucrose hydrolysis by invertase at various concentrations of copper sulfate were experimentally determined, and a Lineweaver-Burk plot was used to determine the mechanism of inhibition.

2.2 Michaelis-Menten Kinetics

Michaelis-Menten kinetics were used throughout the experiment to model the reaction rate as a function of the inhibitor concentration, and to test whether the mechanism of inhibition was noncompetitive, competitive, or mixed. Noncompetitive inhibition refers to the process by which an inhibitor binds to a non-active site in an enzyme, and slows down the rate of product formation. However, noncompetitive inhibitors do not change the affinity, as measured by the Michaelis-Menten constant, K_m, of an enzyme for its substrate. On the other hand, competitive inhibition refers to the type of inhibition in which an inhibitor binds to an enzyme's active site and lowers the affinity of the enzyme for its substrate. Competitive inhibition does not change the maximal rate of reaction, measured as V_{max}, due to the fact that the presence of a large

amount of substrate can saturate the active sites, preventing the inhibitor from binding. Mixed inhibition is a combination of competitive and noncompetitive inhibition; both V_{max} and K_m are changed.
The general kinetic scheme for noncompetitive inhibition is as follows (http://www.civil.uwaterloo.ca/enve375):



The reaction velocity, v, is:

$$v = \frac{V_{max}[S]}{(K_m + [S])(1 + \frac{[I]}{K_i})}$$

where K_i is the kinetic constant of inhibition, which measures the ratio between the concentration of the enzyme-inhibitor complex and free inhibitor (http://www.civil.uwaterloo.ca/enve375). Rearranging,

$$\frac{1}{v} = (1 + \frac{[I]}{K_i})\frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{(1 + \frac{[I]}{K_i})}{V_{max}}$$

is a linear equation in terms of $[S]$ and v. This equation serves as the basis for the Lineweaver-Burk plot ($1/v$ vs. $1/[S]$), for noncompetitive inhibition. Similar equations for competitive and mixed inhibition can be found in literature (http://www.civil.uwaterloo.ca/enve375).

2.3 Applications

The study of enzyme kinetics is essential in the fields of medicine, biology, and chemistry. Baker's yeast (scientific name: *Saccharomyces cerevisiae*), a species of fungi that produces invertase, can be used to detect trace amounts of heavy metal ions, like cadmium and mercury, by analyzing the yeast's enzymatic activity over a fixed period of time, in solution (Narayana & Kumar, 2010). Detecting heavy metal concentrations is vital in testing water quality.
Enzyme inhibitors can also be used in antibiotics. Most antibiotics are administered with β-lactamase inhibitors, such as tazobactam and clavulanic acid, to prevent the bacterial enzyme β-lactamase from degrading antibiotics (Kuck *et al.*, 1989). By using an approach similar to the one carried out in this experiment, the enzyme kinetics of certain types of β-lactamases can be studied in order to elucidate the amount of tazobactam or clavulanic acid that should be administered to patients so as to minimize the probability of bacterial survival while maintaining reasonable production costs.

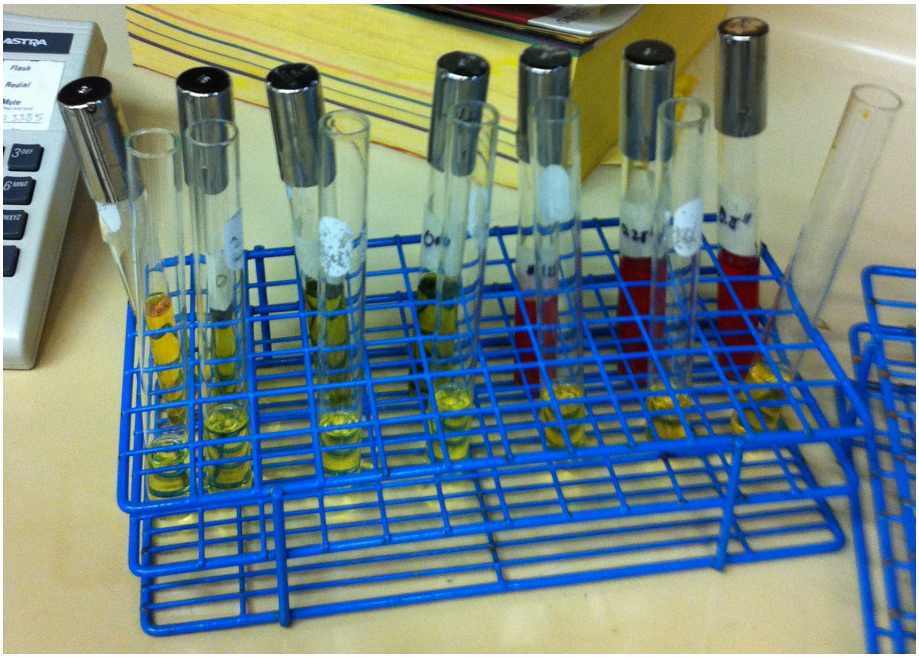


Figure 1: Diagram of the experimental setup for the treatment with no inhibitor. DNS reagent was added to each of the test tubes, and the test tubes were immersed in the 95°C water bath.

3 Methods

3.1 Beer's Law Calibration

Various amounts of deionized water and glucose-fructose solution were added to 7 labelled test tubes to generate the glucose-fructose concentrations specified in **Table 1**. 1.5mL of DNS reagent was then added to each test tube, and all of the test tubes were transferred to a $95-100$°C water bath. The test tubes were collected after 10 minutes and transferred to a cold water bath.

After the test tubes returned to room temperature, the contents in each of the test tubes were diluted 100 times by mixing 0.1 mL of test tube solution with 9.9mL of deionized water. An aliquot of the diluted content for each test tube was transferred into individual borosilicate test tubes in order to measure the absorbance of the diluted contents at 540nm with a spectrophotometer. The blank used was the same as that for **Measuring Enzyme Activity without Inhibitor** (see below). All empty borosilicate test tubes were chosen within a range of 0.073-0.077A absorbances at a wavelength of 540nm for consistency.

Table 1. Summary of the concentrations of glucose-fructose solution in each of the test tubes.

	Test tube 1	Test tube 2	Test tube 3	Test tube 4	Test tube 5	Test tube 6	Test tube 7
[Glucose-Fructose] (M)	3.5×10^{-5}	7.0×10^{-5}	1.75×10^{-4}	2.34×10^{-4}	3.51×10^{-4}	8.78×10^{-5}	2.0×10^{-3}

3.2 Measuring Enzyme Activity without Inhibitor

Various amounts of deionized water and 50mg/mL sucrose solution were added to 6 labelled test tubes, exact quantities are specified in **Table 2**. 1.5 mL of 0.04 mg/mL invertase solution (from Sigma-Aldrich I4504 Grade VII invertase) was then added to each test tube before the test tubes were transferred to a 55°C water bath. The sudden rise in temperature facilitated the reaction between invertase and sucrose. After 5 minutes, the test tubes were taken out of the 55°C water bath and 1.5 mL of the DNS Reagent was added to each test tube. Upon the addition of the DNS Reagent, the test tubes were immediately transferred to a $95 - 100$°C water bath; the second rise in temperature was intended to denature invertase and facilitate the bonding of DNS Reagent to glucose and fructose. The test tubes were collected after 10 minutes and transferred to a cold water bath.
When the test tubes returned to room temperature, the content of each test tube was diluted 100 times by mixing 0.1 mL of the content with 9.9 mL of deionized water. An aliquot of the diluted content of each test tube was transferred into individual borosilicate test tubes in order to measure the absorbance of the diluted contents at 540nm, using a spectrophotometer; Test tube 1 was used as the blank for calibration. Furthermore, all empty borosilicate test tubes were chosen within a range of 0.073A to 0.077A absorbances at 540nm for consistency.

Table 2. Summary of the amounts of reagents added to each of the test tubes (see **Measuring Enzyme Activity without Inhibitor**).

	Test tube 1	Test tube 2	Test tube 3	Test tube 4	Test tube 5	Test tube 6
Deionized H_2O (mL)	1.5	1.375	1.25	1.125	1.00	0.75
50mg/ml Sucrose (mL)	0	0.125	0.25	0.375	0.5	0.75

3.3 Measuring Enzyme Activity with Varying Concentrations of Inhibitor ($CuSO_4$)

Similar to the setup of the previous section, various amounts of deionized water and 50mg/mL sucrose solution were added to labelled test tubes with new additions of 0.1 M $CuSO_4$ at different treatments levels. During the experiment, this section was conducted in sets of 6 test tubes; all sets contained the amounts of sucrose specified in **Table 2** and the amount of $CuSO_4$ specified for each treatment level in **Table 3**. Although the amount of sucrose in each test tube remained constant from the last section, as illustrated by **Table 2**, the amount of deionized water required by each test tube varies between different treatments levels of $CuSO_4$. After sucrose and $CuSO_4$ were added, the volume of each test tube was adjusted up to 1.5 mL with deionized water.
For each set, an additional 1.5 mL of 0.04 mg/mL invertase solution was added before the test tubes were transferred to a 55°C water bath. The sudden rise in temperature facilitated the reaction between invertase and sucrose. After 5 minutes, the test tubes were taken out of the 55°C water bath and 1.5 mL of the DNS Reagent was added to each test tube. Upon the addition of the DNS Reagent, the test tubes were immediately transferred to a $95 - 100$°C water bath; the second rise in temperature was intended to denature invertase and facilitate the bonding of DNS Reagent to glucose and fructose. The test tubes were collected after 10 minutes and transferred into a cooling water bath. When the test tubes returned to room temperature, the

content of each test tube was diluted 100 times by mixing 0.1 mL of the content with 9.9 mL of deionized water. An aliquot of the diluted content of each test tube was transferred into individual borosilicate test tubes in order to measure the absorbance of the diluted contents at 540nm, using a spectrophotometer; Test tube 1 was used as the blank for calibration. Furthermore, all empty borosilicate test tubes were chosen within a range of 0.073A to 0.077A absorbances at 540nm for consistency.

Table 3. Summary of the volumes of 0.1M $CuSO_4$ in each of the treatment levels with inhibitor.

Treatment level (mLs of $CuSO_4$)	0.025	0.050	0.1	0.2	0.3

4 Results & Discussion

4.1 Measurements

Table 4. Beer's Law Analysis. The following data was taken with a spectrophotometer by pouring the invertase solutions into borosilicate test tubes, and recording the absorbances at 540nm. The solutions in the test tubes were diluted 100X from the values listed below. The borosilicate test tubes had absorbances that ranged from 0.073A to 0.077A. The uncertainties were determined by taking half of this range (0.002A).

[Glucose] (M)	Absorbance	Uncertainty	[Glucose] × Absorbance	[Glucose]^2(M)	(Model(Abs.)-Actual)^2(M^2)
3.5×10^{-5}	0.007	0.002	2.46×10^{-7}	1.24×10^{-9}	3.24×10^{-6}
7.0×10^{-5}	0.01	0.002	7.03×10^{-7}	4.94×10^{-9}	1.61×10^{-7}
1.8×10^{-4}	0.027	0.002	4.74×10^{-6}	3.09×10^{-8}	9.92×10^{-7}
2.3×10^{-4}	0.033	0.002	7.73×10^{-6}	5.49×10^{-8}	2.79×10^{-6}
3.5×10^{-4}	0.057	0.002	2.00×10^{-5}	1.23×10^{-7}	2.56×10^{-5}
8.8×10^{-4}	0.128	0.002	1.12×10^{-4}	7.71×10^{-7}	3.70×10^{-6}
2.0×10^{-3}	0.296	0.002	5.92×10^{-4}	4.01×10^{-6}	2.21×10^{-9}

The linear regression line has to go through the origin (the blank), so slope (m) and the uncertainty in slope (σ_m) were determined by minimizing χ^2 with:

$$m = \frac{\sum_{i=1}^{N} x_i y_i}{\sum_{i=1}^{N} (x_i)^2} = \frac{7.38 \times 10^{-4} M}{4.99 \times 10^{-6} M^2} = 148.0 M^{-1}; \sigma_m = \sqrt{\frac{\frac{1}{N} \sum_{i=1}^{N} (y_i - m x_i)^2}{\sum_{i=1}^{N} (x_i)^2}} = 1.2 M^{-1}$$

Table 5. Rates of sucrose hydrolysis by invertase under different concentrations of $CuSO_4$. The sucrose solutions were diluted 100X from the values listed. The rate of hydrolysis were determined using the absorbances and Beer's Law calibration techniques (see below). All rates listed below are in units of mM/min. Absorbances listed below are the means of three replicates for each treatment. The uncertainties (δ) were determined by taking the standard error for each of the sets of three replicates.

	No inhibitor			$25\mu l$ $CuSO_4$			$50\mu l$ $CuSO_4$			$100\mu l$ $CuSO_4$		
[Sucrose] (M)	Abs.	δ	Rate	Abs.	δ	Rate	Abs.	δ	Rate	Abs.	δ	Rate
0	0	0.001	0.000	-0.002	0.002	-0.002	-0.002	0.001	0.001	0.001	0.001	0.002
0.004	0.064	0.001	0.086	0.053	0.001	0.072	0.050	0.001	0.067	0.041	0.002	0.056
0.008	0.114	0.002	0.154	0.095	0.001	0.128	0.085	0.002	0.115	0.073	0.000	0.099
0.012	0.151	0.002	0.204	0.137	0.003	0.185	0.119	0.006	0.161	0.093	0.002	0.126
0.016	0.168	0.001	0.227	0.149	0.004	0.201	0.146	0.003	0.197	0.110	0.003	0.148
0.024	0.174	0.001	0.235	0.178	0.001	0.241	0.161	0.003	0.218	0.146	0.003	0.197

	$200\mu l$ $CuSO_4$			$300\mu l$ $CuSO_4$		
[Sucrose] (M)	Abs.	δ	Rate	Abs.	δ	Rate
0	0.002	0.000	0.000	0	0.001	0.000
0.004	0.036	0.001	0.048	0.031	0.001	0.042
0.008	0.069	0.002	0.093	0.058	0.002	0.079
0.012	0.083	0.003	0.112	0.076	0.001	0.103
0.016	0.104	0.002	0.140	0.093	0.001	0.126
0.024	0.133	0.003	0.179	0.124	0.002	0.168

The rates of hydrolysis were calculated from the absorbances with the slope from the Beer's Law plot (**Table 4**), as follows:

$$R = \frac{Abs}{(148.0M^{-1}) \times 5min} \times \frac{1000mM}{M}$$

4.2 Uncertainty Calculations

Table 6. Summary of the uncertainty calculations for the rate of sucrose hydrolysis under different concentrations of copper (II) sulfate. These uncertainties were obtained from propagating the uncertainties listed in **Table 5** with the results from the Beer's Law calibration in **Table 4**.

	Uncertainty in Rate of Hydrolysis (mmol/min)					
[Sucrose] (M)	No inhibitor	$25\mu l$ $CuSO_4$	$50\mu l$ $CuSO_4$	$100\mu l$ $CuSO_4$	$200\mu l$ $CuSO_4$	$300\mu l$ $CuSO_4$
0	0.00197	0.00316	0.00197	0.00119	0.000451	0.000781
0.004	0.00208	0.000977	0.00131	0.00278	0.00167	0.00200
0.008	0.00302	0.00171	0.00257	0.000808	0.00285	0.00259
0.012	0.00291	0.00440	0.00764	0.00293	0.00411	0.00214
0.016	0.00270	0.00556	0.00435	0.00388	0.00317	0.00187
0.024	0.00226	0.00251	0.00385	0.00425	0.00412	0.00303

The rate uncertainties (δR) were calculated in the same way as the rates of hydrolysis R were calculated, with a few changes. A sample calculation for the no inhibitor, $0.004M$ sucrose

treatment, using the method of partial derivatives, is shown below:

$$\begin{aligned}\delta R &= \sqrt{(\frac{\delta Abs}{m})^2 + (\frac{(Abs)(\delta m)}{m})^2} \times \frac{1000mM}{5min \times M} \\ &= \sqrt{(\frac{0.001}{148.0M^{-1}})^2 + (\frac{(0.064)(1.2M^{-1})}{148.0M^{-1}})^2} \times \frac{1000mM}{5min \times M} \\ &= 0.00208\frac{mmol}{min}\end{aligned}$$

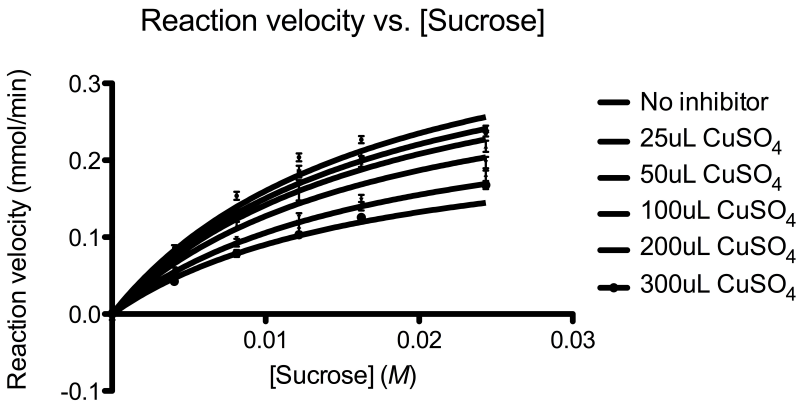


Figure 2: Graph of the changes in the hydrolysis rate of sucrose by invertase at 6 different concentrations of sucrose and 6 different concentrations of $CuSO_4$ ($n = 3$). This graph was plotted with the data in **Table 5**, and the uncertainties in **Table 6**.

4.3 Lineweaver-Burk Plot

A Lineweaver-Burk plot is a method for linearizing the Rate vs. [Substrate] plots in **Figure 2**. If the lines intersect at the x-axis ($\frac{1}{[S]}$), then copper (II) sulfate is a noncompetitive inhibitor. This is due to the fact that the x-intercept will be $-\frac{1}{K_m}$ (http://www.civil.uwaterloo.ca/enve375). Likewise, if the lines intersect at the y-axis ($\frac{1}{Rate}$), then copper (II) sulfate is a competitive inhibitor, as the y-intercept will be $\frac{1}{V_{max}}$ (http://www.civil.uwaterloo.ca/enve375). If the lines intersect somewhere in the $(-x, +y)$ quadrant, then the inhibition method is mixed.

Table 7. Summary of the calculations used to construct the Lineweaver-Burk plots (**Figure 2**). The uncertainties were calculated through propagation; smaller uncertainties indicate higher precision. All values for $\frac{1}{V}$ (1/rate) are in units of $\frac{min}{mmol}$.

$\frac{1}{S}$ (M^{-1})	No inhibitor		$25\mu l$ $CuSO_4$		$50\mu l$ $CuSO_4$		$100\mu l$ $CuSO_4$		$200\mu l$ $CuSO_4$		$300\mu l$ $CuSO_4$	
	$\frac{1}{V}$	95% C.I.	$\frac{1}{V}$	95% C.I.	$\frac{1}{V}$	95% C.I.	$\frac{1}{V}$	95% C.I.	$\frac{1}{V}$	95% C.I.	$\frac{1}{V}$	95% C.I.
246	11.6	0.282	14.0	0.190	14.9	0.291	17.9	0.890	20.7	0.719	23.6	1.11
123	6.51	0.128	7.78	0.104	8.66	0.193	10.1	0.0829	10.8	0.330	12.7	0.416
82.1	4.91	0.0701	5.40	0.128	6.21	0.295	7.92	0.184	8.94	0.329	9.69	0.201
61.6	4.41	0.0526	4.97	0.138	5.06	0.119	6.74	0.177	7.13	0.161	7.95	0.118
41.1	4.25	0.0410	4.15	0.0434	4.58	0.0892	5.08	0.109	5.57	0.128	5.96	0.108

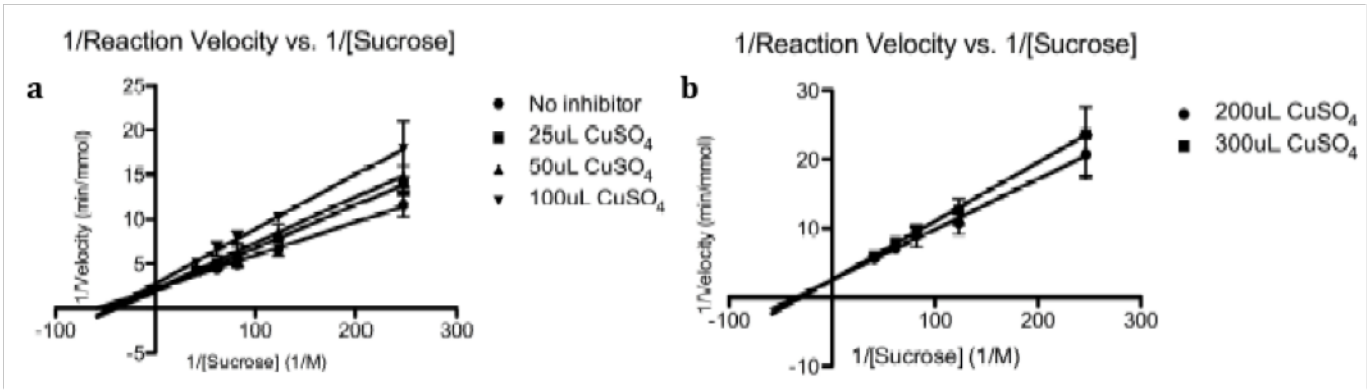


Figure 3: Linearized plots of 1/Hydrolysis Rate vs. $1/[Sucrose]$, commonly referred to as Lineweaver-Burk plots. Plots were constructed from the data in **Table 7**. **2a** Lineweaver-Burk plot for the first four concentrations of $CuSO_4$, with the point of intersection near the $\frac{1}{[Sucrose]}$-axis. **2b** Lineweaver-Burk plot for the two highest concentrations of $CuSO_4$; the point of intersection is at the $\frac{1}{V}$-axis.

4.4 Nonlinear Regression Results

Due to the fact that using the Lineweaver-Burk method of linearizing to determine K_m and V_{max} magnifies experimental errors, GraphPad's Prism 5® nonlinear regression software was used to determine the kinetic parameters of invertase.

Table 8. Summary of the values of the kinetic parameters for invertase (with sucrose as a substrate and copper (II) sulfate as the inhibitor) as determined by nonlinear regression analysis with GraphPad's Prism 5® software.

	V_{max} (mmol/min)	K_m (mM)	K_i
Best Fit Values	0.441	17.6	0.00863
Standard Error	0.0179	1.30	0.000507
95% Confidence Intervals	0.406-0.477	15.0-20.1	0.00763-0.00964
Overall R-squared		**0.978**	

The R-squared value of 0.978 is close to 1.00, which provides confidence in our regression lines.

4.5 Data Analysis

4.5.1 Kinetic Parameters

From nonlinear regression analysis, it was found that $K_m = 17.6 \pm 2.6mM$ (**Table 8**). This is comparable to the value of 18.3mM reported by Ribeiro and Vitolo (2006) for soluble invertase at pH 4.5. On the other hand, the value of V_{max} obtained in this study, $0.441 \pm 0.0354\frac{mmol}{min}$ (**Table 8**), was not in agreement with the results of the same study ($V_{max} = 0.0450\frac{Units}{mL}$) (Ribeiro & Vitolo 2006). This can be explained by the different experimental conditions in which the two studies were conducted. K_i, the kinetic constant of inhibition by copper (II) sulfate, was determined to be 0.00863 ± 0.00101 (**Table 8**).

4.5.2 Mechanism of Inhibition

When interpreting the data in **Figure 2**, it is interesting to note that the shape of the rate-substrate curve differs between concentrations of inhibitor. As inhibitor concentration is increased, the curve gradually becomes more sigmoidally (S) shaped, as opposed to the hyperbolic shape for the noninhibited reaction curve. This indicates that there may have been cooperative or noncooperative binding–when the substrate of the reaction serves to facilitate or inhibit the binding of other substrate molecules–or feedback inhibition (Combes & Monsan, 1983). In fact, both the substrate, sucrose, and the products of the reaction, glucose and fructose, inhibit invertase (Combes & Monsan, 1983). Despite this, inhibition due to glucose and fructose likely plays a minor role in the kinetics of the enzyme, as the affinity of sucrose for invertase ($K_m = 17.6 \pm 2.6mM$) is greater than that for fructose ($K_m = 128mM$) and glucose ($K_m = 270mM$) (Combes & Monsan, 1983).

Furthermore, from the Lineweaver-Burk plot in **Figure 3a**, it is apparent that the best fit lines intersect close to the x-axis, which is indicative of the noncompetitive nature of copper (II) sulfate inhibition, as K_m stays nearly constant while the inhibitor concentrations are varied; this is in agreement with the hypothesis. However, the Lineweaver-Burk plot in **Figure 3b** shows that the best fit lines for the $200\mu l$ copper (II) sulfate and $300\mu l$ copper (II) sulfate treatments actually intersect at the y-axis. This suggests that, at high concentrations, copper (II) sulfate competitively inhibits invertase. While this apparent discrepancy can be attributed to experimental error as well as the non-Michaelis-Menten (sigmoidal) nature of inhibition, it is also possible that Cu^{2+} ions interact with the active sites of invertase when the allosteric sites are saturated. Research shows that one methionine residue (residue number 40) is in close proximity to one of the active sites of invertase (residue number 42); furthermore, residue number 224, cysteine, is located next to another substrate binding site in invertase (residue number 223) (Taussig & Carlson, 1983). Therefore, it can be conjectured that Cu^{2+} ions preferentially bind to the allosteric sites in invertase (noncompetitive inhibition), but at high concentrations of Cu^{2+}, the ions may also bind to the active sites in invertase (competitive inhibition).

4.5.3 Biological and Chemical Applications

From the results of this study, $CuSO_4$ has been classified as a noncompetitive inhibitor of invertase, even when $[CuSO_4]$ is low. While there are inherent differences among invertase and other biological enzymes, other sources have reported that heavy metal ions are noncompetitive inhibitors for most enzymes (Narayana & Kumar, 2010). Therefore, it is likely that the ingestion of a substantial amount of Cu^{2+}, and possibly also other heavy metal ions may be life-threatening for organisms (heavy metal poisoning), as the essential enzymes that support life may be denatured in the presence of metal ions.

With the kinetic parameters determined in this experiment (see **Kinetic Parameters**), a precise value for the decrease in the activity of invertase in the presence of a certain amount of $CuSO_4$ can be elucidated. Consequently, $[Cu^{2+}]$ can be determined by adding known amounts of invertase and sucrose into a solution, assuming it does not contain any other inhibitors. A similar experiment and analysis to the ones used in this study can be carried out, for other heavy metal ions. Through this process, the qualities and metal ion contents of water samples can be ascertained.

Furthermore, due to the noncompetitive nature of invertase inhibition by $CuSO_4$, the Michaelis-Menten model of enzyme inhibition can be extended to an enzyme-inhibitor system involv-

ing certain types of β-lactamases and tazobactam, a noncompetitive inhibitor of particular β-lactamases (Kuck *et al.*, 1989). After determining the various kinetic constants–K_m, V_{max}, and K_i–for the β-lactamase-tazobactam system, the degree of inhibition at various concentrations of tazobactam can be related to the reaction rates between β-lactamases and their substrates.

5 Conclusion

Throughout the experiment, the kinetic parameters of invertase with respect to its substrate, sucrose, were determined as $K_m = 17.6 \pm 2.6mM$ and $V_{max} = 0.441 \pm 0.0354\frac{mmol}{min}$. Furthermore, it was found that $CuSO_4$ is a noncompetitive inhibitor of invertase at low $[CuSO_4]$ (below $0.0022M$), and $CuSO_4$ is a competitive inhibitor of invertase at higher $[CuSO_4]$ (above $0.0044M$). The inhibition constant, K_i, was found to be 0.00863 ± 0.00101. However, further studies should be conducted, with more precise equipment, to determine the kinetic constants to a greater degree of accuracy, and to confirm or refute our conjecture (see **Data Analysis**).

6 Acknowledgements

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