Biological Sciences
UNIVERSITY OF TORONTO
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BIOC23 Lab 6

Alkaline Phosphatase Report

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Introduction

Enzyme kinetics is the study of the rate of a chemical reaction catalyzed by an enzyme by measuring the reaction rate and studying the effect of changing the reaction conditions(Srinivasan 2022). Alkaline phosphatase (ALP) is an orthophosphate monoester phosphohydrolase. It is found in many tissues throughout the body and also involved in many biological processes (Sardiwal et al. 2013). In this experiment, we used ALP to study its enzyme kinetics in different substrate, enzyme and inhibitor concentrations.

Standard curve of Extinction Coefficient (ε)

Spectrophotometry is commonly used to observe changes in light absorption between products and reactants(Bzura, Fiedoruk-Pogrebniak, and Koncki 2018). In this experiment, we used p-nitrophenyl disodium orthophosphate (pNPP) as ALP substrate, which does not absorb 405 nm wavelength but ALP promotes hydrolysis to produce yellow p-nitrophenol anion (pNP), which has absorption at 405 nm (Bell 2023).

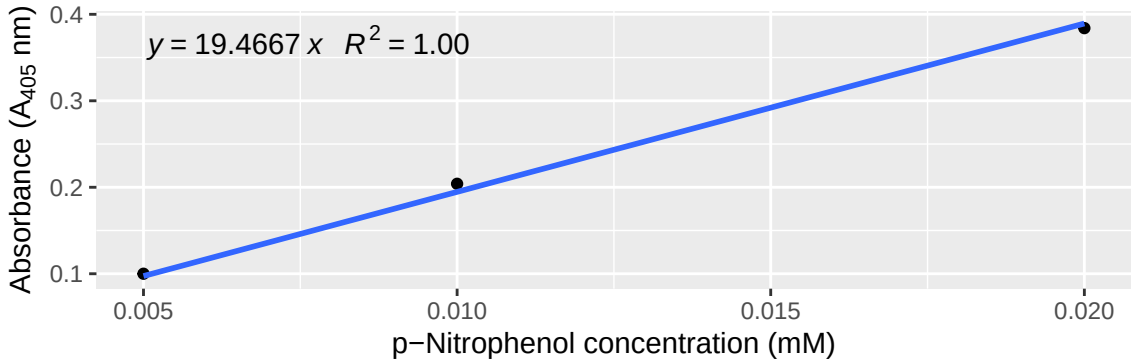

Figure 1: Standard curve for p-nitrophenol with extinction coefficient. The abscissa (x) of this figure represents the concentration of pNP (mM), and the ordinate (y) represents the absorption value of pNP at a wavelength of 405 nm. The regression equation through the origin and the coefficient of determination (R^2) for the data are shown in the upper left corner of the figure.

By Beer-Lambert law ($A = \varepsilon b \times C$) and $b = 1\ cm$, the slope is the desired extinction coefficient ε (*Fig.1*). The ε of PNP is 19.4667 $mM^{-1}cm^{-1}$, slightly larger than the value in the literature 15.3 $mM^{-1}cm^{-1}$. It is still acceptable because different glass tubes may have different refraction and absorption rate of light.

v_0 *vs.* enzyme concentration

From *Fig.2*, when $pH = 8.0$ and the substrate content is the same, v_0 increases with the increase of enzyme concentration, which is in line with our expectation.

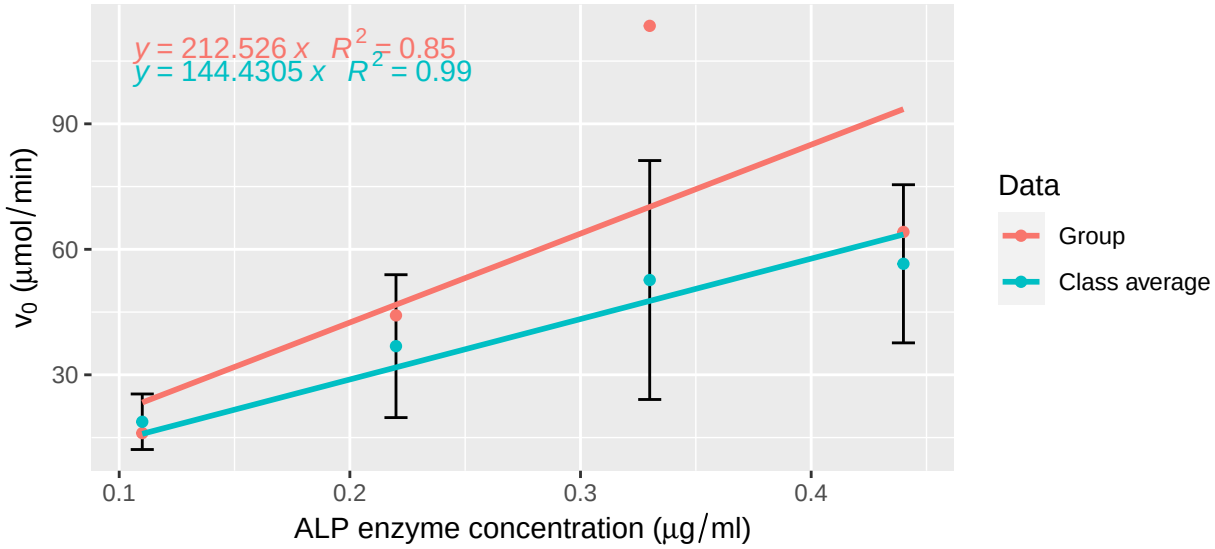

Figure 2: Group and class average initial velocity at different enzyme concentration. The x-axis of this graph represents ALP enzyme concentration ($\mu g/ml$), and the y-axis is v_0 ($\mu mol/min$). The upper left corner of the picture shows the coefficient of determination and the linear regression equation through the origin. The two sets of data in the figure are the results of our group and the average value of the class. The average value of the class has an error bar, and the data comes from the standard deviation.

v_0 *vs.* substrate concentration

In *Fig.3*, we use the logarithm to represent the relationship between the substrate and v_0 because the pairing of the enzyme and the substrate has an upper limit, and increasing the concentration of the substrate after that will not lead to an increase in the initial reaction rate, because the enzyme has already Saturated.

Linweaver-Burk plot

The regression equation in the Lineweaver–Burk plot below in *Fig.4* shows the Lineweaver–Burk equation: $\frac{1}{v_0} = (\frac{K_m}{V_{max}})\frac{1}{[S]} + \frac{1}{V_{max}}$. Based on the equation, we can calculate the K_m and V_{max}. The process and result is shown in *Table 1*.

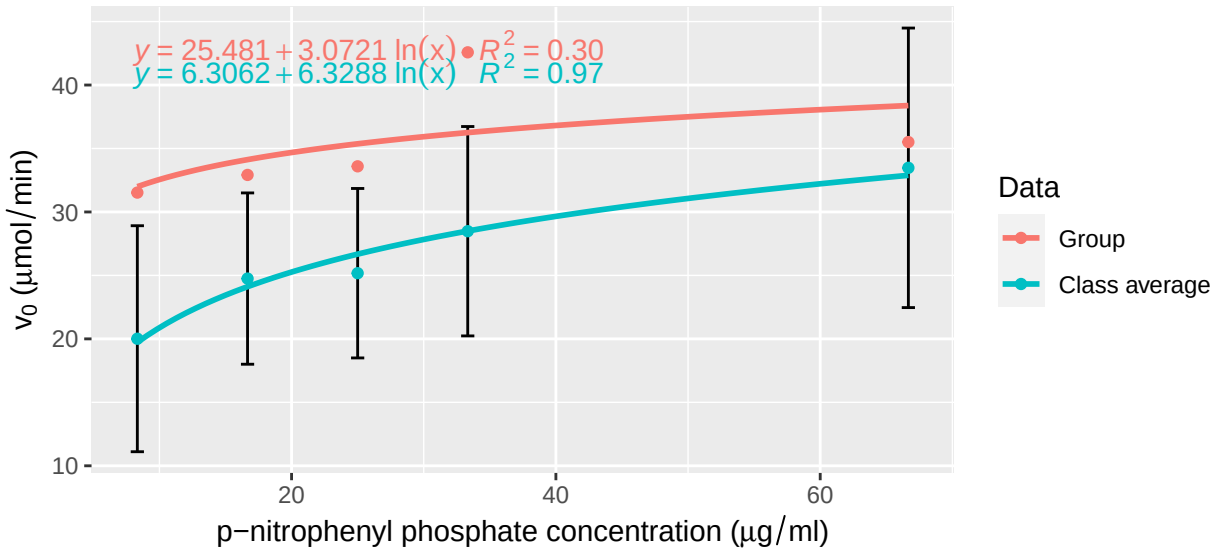

Figure 3: Group and class average initial velocity at different substrate concentration. The x-axis in this figure represents the pNPP concentration ($\mu g/ml$), and the y-axis is the initial velocity ($\mu mol/min$). The upper left corner of the picture shows the logarithmic regression equation through the origin and its R^2. The two sets of data in the figure are the results of our group and the average value of the class. The average value of the class has an error bar, and the data comes from the standard deviation.

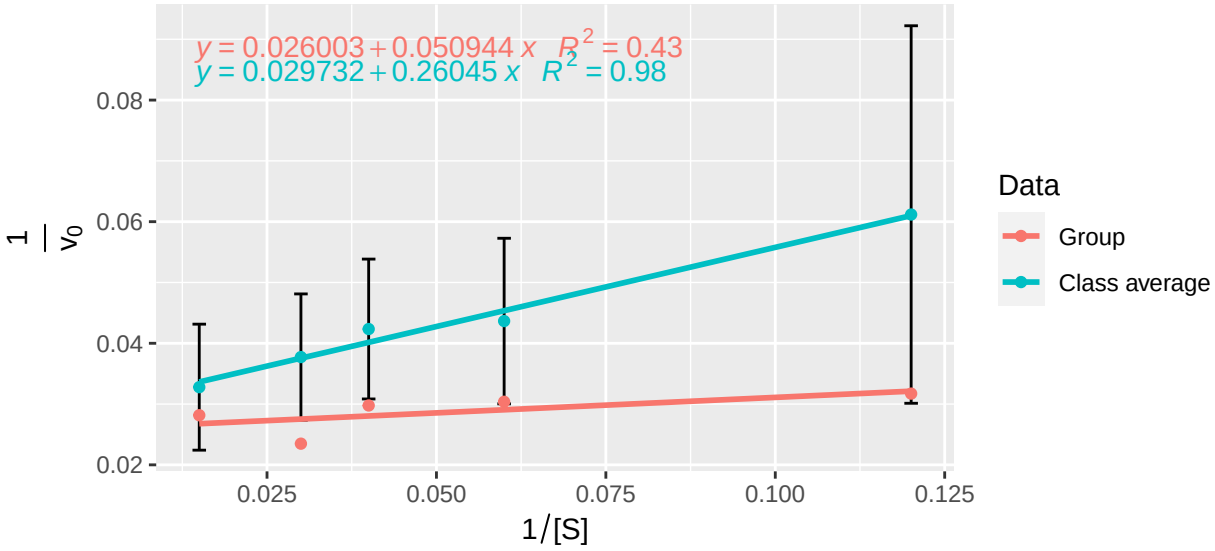

Figure 4: Linweaver-Burk plot from group and class average v_o of different substrate concentrations. In the LB plot, the x-axis represents the reciprocal of the substrate concentration, and the y-axis represents the reciprocal of the initial velocity. Both the regression equation and R^2 are shown in the upper left corner of the plot. Data sources for this figure are group and class means (with error bars) respectively.

Table 1: Calculation of K_m and V_{max}

Data \ Value	$\frac{K_m}{V_{max}}$	$\frac{1}{V_{max}}$	K_m $(\frac{K_m}{V_{max}}/\frac{1}{V_{max}})$	V_{max} $((\frac{1}{V_{max}})^{-1})$
Group data	0.05094	0.026002	$0.05094/0.026002 = 1.95908$	$1/0.026002 = 38.45858$
Class average	0.26045	0.029732	$0.26045/0.029732 = 8.759922$	$1/0.029732 = 33.6338$

V_{max} is the maximum reaction speed, K_m represents the Michaelis constant, which is an index to measure the enzymatic ability (Srinivasan 2022). A lower K_m means that less substrate is needed to reach half the maximum rate of the enzyme, the results of our group show that we have a higher V_{max} and a lower K_m than the class average, which explains why in previous figures our group are having a higher v_0.

Effect of EDTA on v_0

EDTA is a chelating agent that can combine with divalent metal ions to form a stable and inert complex (Ghosh et al. 2013). For ALP, divalent metal ions are an activator (Bell 2023), EDTA as an inhibitor snatches away the divalent metal ions that should be combined with ALP, so it can be seen from *Fig.5* that the initial speed of the reaction increases with EDTA decrease with increasing concentration.

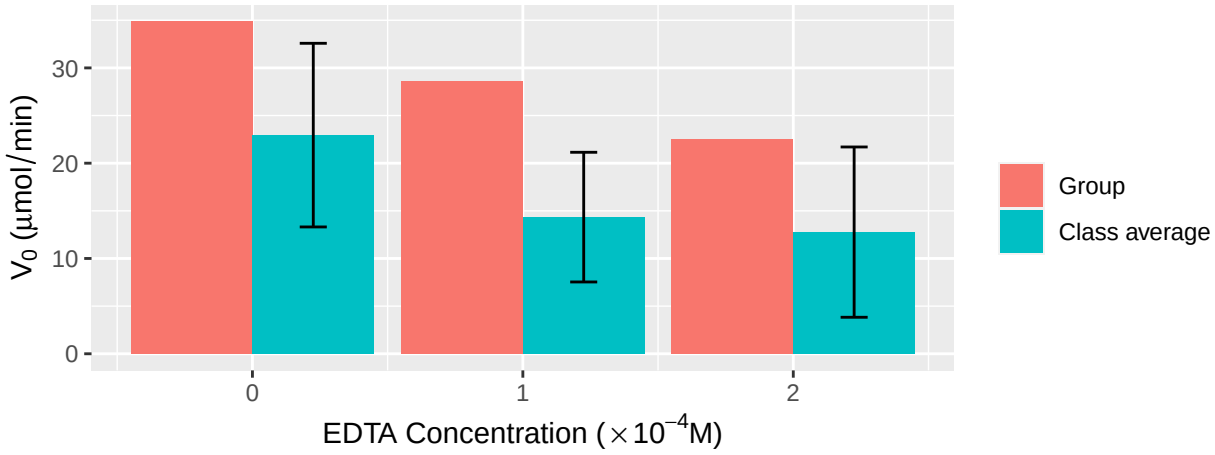

Figure 5: Effect of EDTA on v_0. The x-axis of this figure represents the number of parts of EDTA (multiplied by the concentration of one part of EDTA to get the total concentration), and the y-axis is the initial velocity. The two sets of data in the figure are the results of our group and the average value of the class respectively. The average value of the class has data from the standard deviation error bar.

Acknowledgement

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Reference

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