Rate sensitivity of Tyrosinase-catalyzed L-DOPA and D-DOPA reactions under concentration changes in enzymes and under Benzoic acid, Cinnamic acid and Thiourea

Beatriz Gascón Lourenço

Lab partner: Francesco Pasqualotto

TA: Robert Mothersole

BIOC 393, L07

The University of British Columbia

Rate sensitivity of Tyrosinase-catalyzed L-DOPA and D-DOPA reactions under concentration changes in enzymes and different inhibitors

Abstract

This paper reports on the characterization of the kinetics of the Tyrosinase-catalyzed reaction of L-DOPA and D-DOPA under changes in enzyme and substrate volume, as well as after change in substrate isomer and under inhibition of Benzoic acid, Cinnamic acid and Thiourea. A direct correlation between enzyme concentration and reaction rate was determined in the first section of the paper. Analysis of the Michaellis-Menten and the Lineweaver-Burk plots also allowed for estimation of K_m and V_{max} values for both of the isomers, and consequently, for the determination that Tyrosinase had a higher specificity towards D-DOPA than towards L-DOPA, despite not having obtained particularly accurate measurements for the L-DOPA isomer. The analysis of the Lineweaver-Burk plots for all three inhibitors led to the conclusion that both Benzoic acid and cinnamic acid acted as competitive inhibitors, while Thiourea acted as a non-competitive inhibitor; the conclusions obtained from the data were also later confirmed by recent literature review on all three inhibitors.

Keywords: Tyrosinase, enzyme kinetics, L-DOPA, D-DOPA, inhibition, Michaellis-Menten, Lineweaver-Burk.

1. Introduction

Enzymes are defined as proteins that have the function of accelerating the rate of unfavourable reactions, usually by forming a complex with its substrate and decreasing the amount of free energy required for a reaction to proceed. According to the lab protocol written by the coordinators Dr. D'Souza and Dr. Julien Gibon (2020) these types of proteins were extensively studied due to their important role and power in biological systems.

From the years of intense study on enzymes, equations such as the Michaellis-Menten and the Lineweaver-Burk were developed as a way to better understand enzyme kinetics and reaction rates.

The Michaellis-Menten plot is described by the equation (I) below:

$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

Where V_o and V_{max} are defined as the initial and final velocity, respectively, [S] represents substrate concentration, and K_m represents the Michaellis constant, defined as the result obtained from the formula below:

$$\frac{k_2 + k_{-1}}{k_1}$$

In the Michaellis-Menten plot, the variable in the y-axis is velocity (V), whereas the variable plotted in the x-axis is substrate concentration (S) as shown by Figure 1 below. This plot is used as a hyperbolic representation of an enzyme's saturation function.

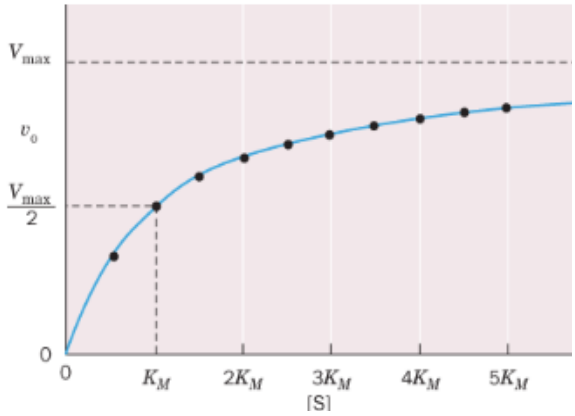


Fig.1. Standard Michaellis-Menten plot. Image retrieved from (Voet et al, 2006).

The Lineweaver-Burk or double reciprocal equation consists of a reciprocal version of the Michaellis-Menten equation as its own name suggests. The formula is shown by equation (II) below:

$$\frac{1}{V_0} = \frac{K_m}{V_{max}\,[s]} + \frac{1}{V_{max}}$$

in the Lineweaver-Burk plot, the variable in the y-axis is 1/V, while the variable on the x-axis is 1/S and the slope is $\frac{K_m}{V_{max}}$. The plot formed by this equation is linear and directed at making the determination of maximum velocity (Vmax) and the Michaelis constant (Km) easier, as shown by Figure 2.

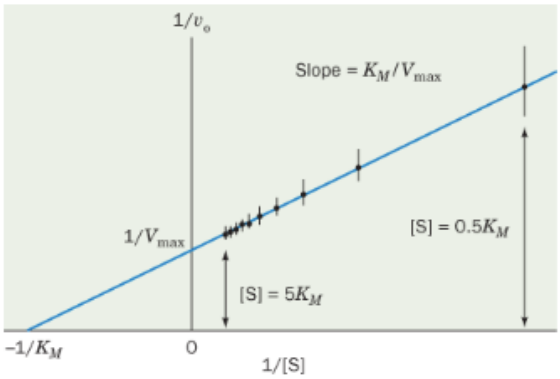


Fig.2. Standard Lineweaver-Burk plot. Image retrieved from (Voet et al, 2006).

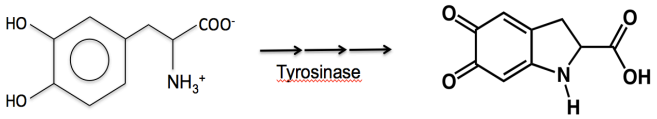
The presence of inhibitors can heavily decrease reaction rates, once they block the enzyme from perfoming its catalytic role, this phenomenon can happen either through competitive, non-competitive or mixed inhibition action.

According to the textbook Fundamentals of Biochemistry, published in 2006 by Voet et al; competitive inhibitors act by directly binding to the enzyme's active site, thereby blocking the actual substrate from binding and carrying on with the reaction, which ultimately causes an increase in K_m.

In contrast, uncompetitive inhibitors act by binding to the enzyme-substrate complex and causing for enough distortion to the point of rendering the enzyme inactive, which ultimately causes for a decrease both in K_m and V_{max}. Whereas a mixed inhibitors act by performing a mixture of both types of inhibitions, ultimately causing for a decrease in V_{max} and either a decrease or an increase in K_m.

Tyrosinase enzymes are mostly known as copper-enzymes, commonly found in nature, not particularly keen on substrate specificity, and main catalyzers of quinone oxidations, as well as monophenol and diphenol reactions (Ortiz-Ruiz et al, 2015). This enzyme has also been determined as a fundamental step for the melanogenesis pathway (Biswas et al, 2017), performing the role of oxidizing the amino acid precursor of dopamine, Levodopa or (S)-2-amino-3-(3,4-dihydroxyphenyl)-propanoic acid (L-DOPA), as well as L-tyrosine (Biswas et al, 2017).

In this experiment, the kinetics of tyrosinase were characterized with respect to its catalysis role in the L-DOPA reaction, shown below by Figure 3.



L-DOPA
(dihydroxyphenylalanine)
(no colour)

Dopachrome
(orange colour)
λmax = 475 nm

Fig.3. Reaction mechanism for L-DOPA reaction. Image retrieved and modified from lab manual (D'Souza & Gibon, 2020).

This was done by repeatedly monitoring rate sensitivity of the reaction with different enzyme and substrate concentrations, after addition of an inhibitor (Benzoic acid), and after substitution of L-DOPA by its isomer, (R)-2-amino-3-(3,4-dihydroxyphenyl)-propanoic acid (D-DOPA).

Given the changes enzyme concentration, it was expected that the reaction rate would increase proportionally to the amount of enzyme in solution. Regarding the changes in substrate concentration for both isomers, it was expected that the reaction rate would increase proportionally to substrate until it eventually reached a plateau once the enzyme became saturated. It was also expected that this reaction would be more efficient for one of the isomers, indicating enzyme specificity towards either L-DOPA or D-DOPA.

Given the molecular structures of the inhibitors used, as shown by Figure 4, it was expected that the type of inhibition taking place would be non-competitive for Thiourea and competitive for the other two inhibitors, since the Thiourea didn't seem similar enough to the L-DOPA molecular structure in contrast to the other two inhibitors, whose substituted benzene seemed similar enough to the structure of L-DOPA.

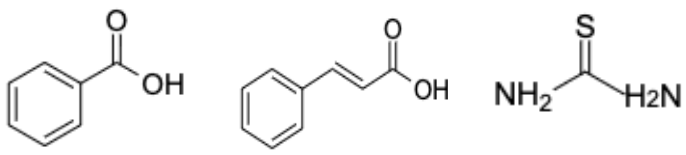


Fig.4. Molecular structures of the inhibitors used in the experiment, Benzoic acid, Cinnamic acid and Thiourea respectively.

2. Materials and Methods

The Experiment was carried on in accordance with the laboratory manual (D'Souza & Gibon, 2020).

When testing the rate sensitivity under different substrate concentrations, the solutions were made with L-DOPA and D-DOPA concentrations of 0.05, 0.1, 0.4, 0.6, 0.8, and 1.5 mL instead of the recommendation of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.5 mL made by the laboratory manual, still while maintaining the total volume of the solution fixed at 3mL . This was done since the initial concentration recommended by the manual was deemed to be too high to set the base value for the assay and as a way to avoid reaching the saturation point too quickly.

Having performed the experiment, the data was analysed by first determining the enzyme activity using A_{280} as stated by the laboratory manual (D'Souza & Gibon, 2020. This was done by using the equation shown below:

$$\frac{c_1}{A_1} = \frac{c_2}{A_2}$$

where c_1 and c_2 represent initial and final concentration of the tyrosinase and the unknown enzyme solution in % (w/v), respectively; while A_1 and A_2 represent the initial and final absorbance readings for the standard tyrosinase and the unknown solutions, repectively.

Following this, the change in absorbance values previously calculated in $\Delta A/minute$ for each of the assays was converted into the amount of product formed/time through the following equation:

$$\Delta c = \frac{\Delta A/\min}{E \times l}$$

Where E is a constant, representative of the molar absorption coefficient for dopachrome (3600 M^{-1} cm^{-1}) and l is also a constant vaue, representative of the length of the light path through the cuvette (1 cm). The resultant value from this equation was then converted from M/minute into µM/minute.

The two calculations made above were used to determine the reaction velocities for L-DOPA and D-DOPA followed by plotting the Michaellis-Menten curve for the two isomers.

Following this plot, the reciprocal was taken from the obtained V_0 and [S] for plotting the Lineweaver-Burk graphs. Both isomers were plotted in the same image for easy comparison.

The last section of data analysis consisted of calculating the reaction velocities and the axis values for Lineweaver-Burk plots just as done above for all the three inhibitors and determining the nature of the inhibitors (competitive, non-competitive or mixed). In order to fulfill this step accurately, the data for Benzoic Acid inhibition, Cinnamic Acid, and Thiourea were obtained from our lab partners Nakya and Simon, Helena and Taylor, and Alexandra and Adeel respectively.

3. Results

The analysis of rate sensitivity while changing volumes of Tyrosinase were used to plot for the effect of enzyme concentration on reaction rates, as shown by Figure 5.

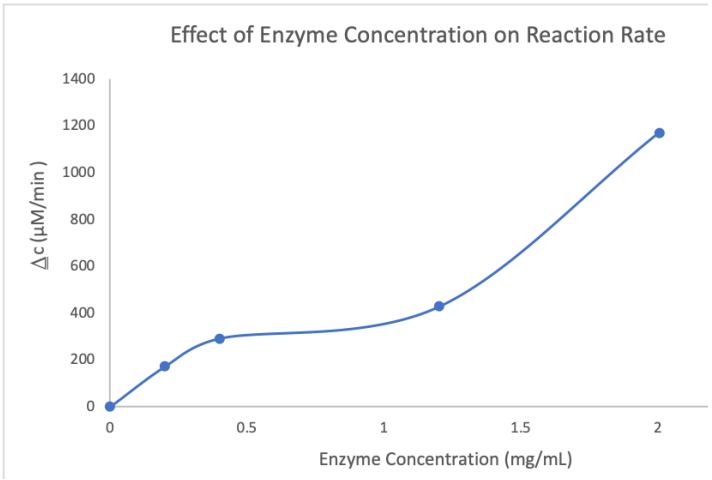


Fig.5. Plot for effect of enzyme concentration on reaction rate. The x-axis represents enzyme concentration and the y-axis represents reaction rate.

The graph was plotted with the assay data tabulated in Table 1. Similarly, the analysis of reaction velocities under changes of L-DOPA concentration and D-DOPA concentrations were used in order to plot a Lineweaver-Burk plot and a Michaellis-Menten curve, as shown by Figure 6 and 7 respectively. For the Menten plot specifically, the estimations for $\frac{V_{max}}{2}$, V_{max}, and K_m could only be made for the D-DOPA reagent, once the curves for L-DOPA were deemed much too inaccurate for any estimations to be made.

Table 1. *Effect of Tyrosinase concentration on reaction rate*

Assay number	Tyrosinase Vol. (mL)	Rate (ΔA/min)	Δc (μM/min)	Enzyme Conc. (mg/mL)
1	0	0	0	0
2	0.05	0.618	171.666	0.2005
3	0.1	1.05	291.666	0.401
4	0.3	1.542	428.333	1.203
5	0.5	4.212	1170	2.005

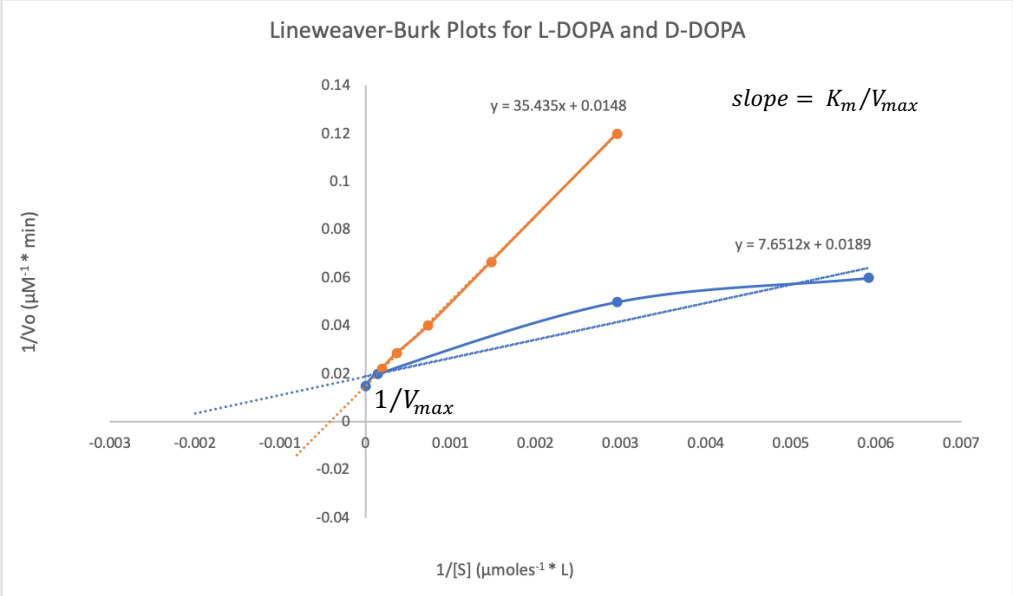


Fig.6. Lineweaver-Burk plot for L-DOPA (Blue line) and D-DOPA (Orange line). The lines were accomplished by backwards extrapolation of the trendlines (dashed lines) for each data series given the errors in measurements made for the L-DOPA reagent

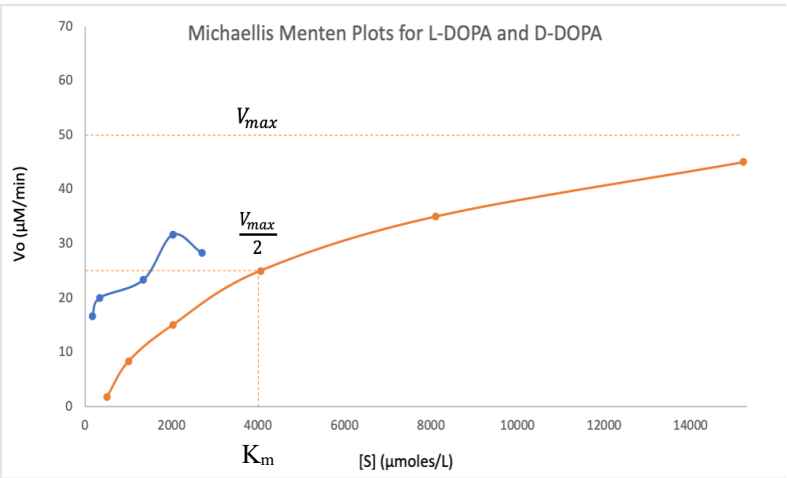


Fig.7. Michaellis-Menten plot for L-DOPA (Blue line) and D-DOPA (Orange line). The dashed lines are representative of the estimations for $\frac{1}{V_{max}}$, V_{max}, and K_m made for the D-DOPA reaction only.

In the Lineweaver-Burk plot, the values for V_{max} and K_m were estimated through labelling the Y-intercept as $1/V_{max}$, and finding the K_m by calculating the slope of the line; as indicated by the textbook (Voet et al, 2006).

Whereas in the Michaellis-Menten plot, V_{max} was estimated through drawing a line (dashed line) at the point in the graph where the curve was supposed to reach a plateau. Having done that, the value for K_m was the x-axis value at which the velocity was half-maximal ($V_{max}/2$). This estimation was also done as indicated by the textbook (Voet et al, 2006).

As done for the plot in Figure 1, the calculated data for the Michaellis-Menten and Lineweaver-Burk plots of L-DOPA and D-DOPA were tabulated in Tables 2 and 3, respectively.

Table 2. Tabulated data for reaction velocities of variable L-DOPA volumes and fixed tyrosinase volume.

Assay	ΔA/minute	ΔC (μM/min)	L-DOPA Vol. (mL)	L-DOPA Conc. (μmoles/L)	1/[L-DOPA]	1/Vo
1	0.06	16.66	0.05	169.04	0.0059	0.06
2	0.072	20	0.1	338.08	0.0029	0.05
3	0.084	23.33	0.4	1352.34	0.00073	0.04
4	0.114	31.66	0.6	2028.52	0.00049	0.03
5	0.102	28.33	0.8	2704.69	0.00036	0.03

Table 3. Tabulated data for reaction velocities of variable D-DOPA volumes and fixed tyrosinase volume.

Assay	ΔA/minute	ΔC (μM/min)	D-DOPA Vol. (mL)	D-DOPA Conc. (μmoles/L)	1/[L-DOPA]	1/Vo
1	0.006	1.66	0.05	169.043	0.0059	0.60
2	0.03	8.33	0.1	338.087	0.0029	0.12
3	0.054	15	0.2	676.174	0.0014	0.066
4	0.09	25	0.4	1352.34	0.00073	0.04
5	0.126	35	0.8	2704.69	0.00036	0.028
6	0.162	45	1.5	5071.30	0.00019	0.022

Lastly, the plots for the solutions containing variable amounts of the inhibitors Benzoic acid, Cinnamic acid, and Thiourea are plotted in Figures 8, 9 and 10, respectively; their data is tabulated by Tables 4, 5 and 6 respectively. All plots have been extrapolated in order to adjust for measurement errors made during the experiment.

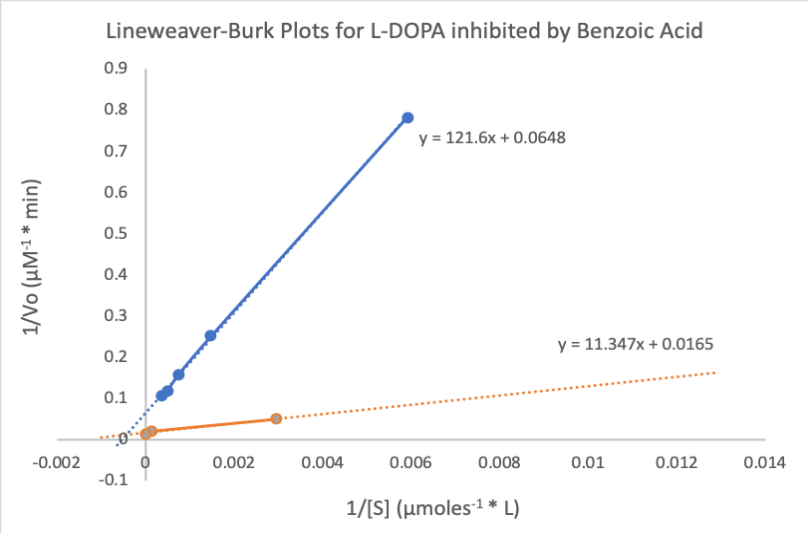


Fig. 8. Lineweaver-Burk plot for the tyrosinase-catalyzed reaction of L-DOPA inhibited by Benzoic acid (Blue line). The plot for uninhibited L-DOPA is also plotted (orange line) for comparison purposes. The dashed lines both represent the trendlines for the plots.

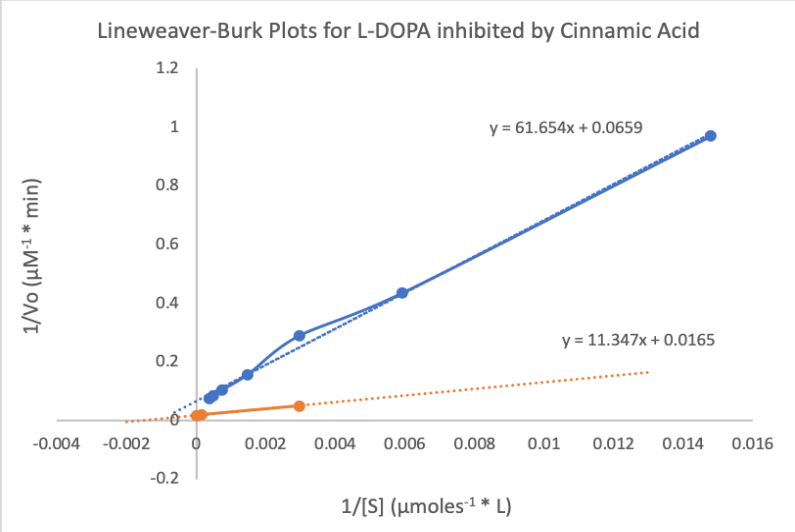


Fig. 9. Lineweaver-Burk plot for the tyrosinase-catalyzed reaction of L-DOPA inhibited by Cinnamic acid (Blue line). The plot for uninhibited L-DOPA is also plotted (orange line) for comparison purposes. The dashed lines both represent the trendlines for the plots.

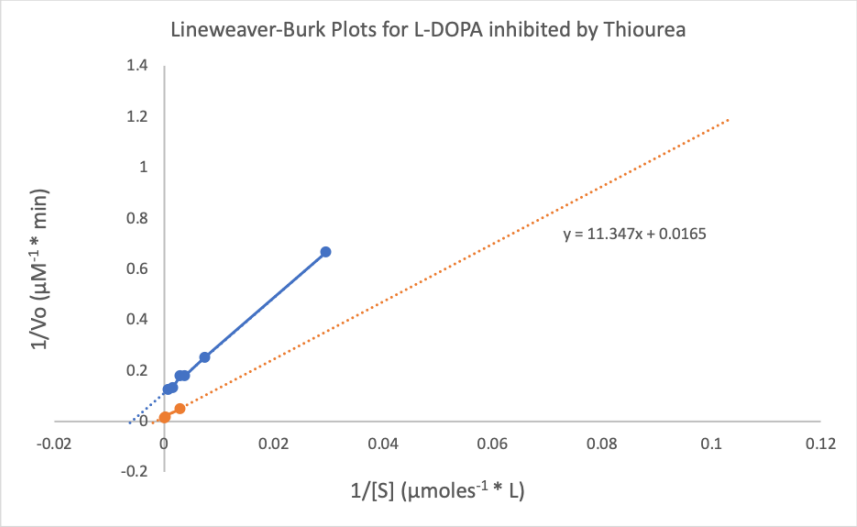


Fig. 10. Lineweaver-Burk plot for the tyrosinase-catalyzed reaction of L-DOPA inhibited by Thiourea (Blue line). The plot for uninhibited L-DOPA is also plotted (orange line) for comparison purposes. The dashed lines both represent the trendlines for the plots.

Table 4. Tabulated data for reaction velocities of Tyrosinase- catalyzed L-DOPA inhibited by Benzoic acid. Data obtained from lab partners Nakya and Simon

Assay	ΔA/minute	ΔC (μM/min)	L-DOPA Vol. (mL)	L-DOPA Conc. (μmoles/L)	1/[L-DOPA]	1/Vo
1	0.004	1.27	0.05	169.043	0.0059	0.782
2	0.014	3.97	0.2	676.174	0.0014	0.251
3	0.022	6.30	0.4	1352.34	0.0007	0.158
4	0.030	8.38	0.6	2028.52	0.0004	0.119
5	0.034	9.44	0.8	2704.69	0.0003	0.105
6	0.046	12.88	1.0	3380.87	0.0002	0.077

Table 5. Tabulated data for reaction velocities of Tyrosinase- catalyzed L-DOPA inhibited by Cinnamic acid. Data obtained from lab partners Helena and Taylor.

Assay	ΔA/minute	ΔC (μM/min)	L-DOPA Vol. (mL)	L-DOPA Conc. (μmoles/L)	1/[L-DOPA]	1/Vo
1	0.003	1.03	0.02	67.617	0.0147	0.969
2	0.008	2.30	0.05	169.043	0.0059	0.434
3	0.012	3.46	0.1	338.087	0.0029	0.288
4	0.022	6.38	0.2	676.17	0.0014	0.156
5	0.034	9.61	0.4	1352.34	0.0007	0.103
6	0.043	11.98	0.6	2028.52	0.0004	0.083

Table 6. Tabulated data for reaction velocities of Tyrosinase- catalyzed L-DOPA inhibited by Thiourea. Data obtained from lab partners Alexandra and Adeel.

Assay	ΔA/minute	ΔC (μM/min)	L-DOPA Vol. (mL)	L-DOPA Conc. (μmoles/L)	1/[L-DOPA]	1/Vo
1	0.0054	1.5	0.01	33.808	0.02957	0.666
2	0.0142	3.94	0.04	135.234	0.00739	0.253
3	0.0201	5.58	0.08	270.469	0.00369	0.179
4	0.0201	5.58	0.1	338.087	0.00295	0.179
5	0.0267	7.41	0.2	676.174	0.00147	0.134
6	0.0287	7.97	0.4	1352.348	0.00073	0.125

4. Discussion

The analysis of the results section leads to the conclusion that the reaction rate is directly proportional to the enzyme concentration, which would make logical sense, since more enzymes would mean more binds to substrate per minute until saturation, thereby causing for a faster reaching of the maximal velocity faster.

Nevertheless, it is safe to assume that factors, such as enzyme specificity, also influence the velocity of the enzyme-substrate formation, once more specificity of tyrosinase towards one of the isomers would cause for better and faster binding of the enzyme, and consequently a faster reaction.

Given the measurement errors made when collecting the L-DOPA data for the Michaellis-Menten and Lineweaver-Burk plots, the conclusions made on this report are not guaranteed to be accurate comparisons the efficiencies of catalysis between the L-DOPA and D-DOPA isomers. In an ideal situation, the Km values for both of the isomers should be taken into consideration as direct indications of binding affinity, and ultimately the more efficient binding can be accurately determined by the highest Km value.

According to the data obtained in the linear-Burk plots, the isomer with more efficient binding to tyrosinase is D-DOPA, with a K_m value of 1417.4, whereas L-DOPA Lineweaver-Burk line indicates a K_m value of 382.56. This result would indicate that Tyrosinase holds a reasonably higher specificity towards D-DOPA than L-DOPA. Whether this result is an accurate representation of the truth remains unclear, once there seems to be very few to no articles making a distinction between the two isomers and the measurements errors do not allow for a certainty that the data obtained on the L-DOPA absorbance values is accurate enough be taken as a relevant result.

As expected, the V_{max} values for the isomers confirm higher tyrosinase specificity towards D-DOPA, which shows a V_{max} value of 50 in contrast to the V_{max} value of 40 observed in the L-DOPA Lineweaver-Burk plot.

Regarding the irregularity of the L-DOPA Menten curve and Lineweaver plots, it could be hypothesized that either the wrong absorbances values might have been recorded, that the samples were contaminated with the tyrosinase enzyme before undergoing analysis in the spectrophotometer, or that the spectrophotometer was not calibrated properly before starting the analysis; all of which would ultimately cause for the big discrepancy observed between the data points and the standard Michaellis-Menten and Lineweaver plots.

Among the plethora of possibilities, it is more plausible to assume that it the wrong absorbance were recorded, once contaminating the substrate long before analyzing for its absorbance would definitely cause for a linear-like Menten, indicative of the enzyme reached the point of saturation before the start of absorbance analysis. In addition to this, it is also unlikely that the spectrophotometer was wrongly calibrated, once the absorbance values observed from second assay run, the D-DOPA solutions, formed a very standard Menten curve, which would not have happen had the spectrophotometer been poorly calibrated since the beginning of the L-DOPA analysis; in this case, both of the curves would show irregular plots.

According to the images provided by the textbook "Fundamentals of Biochemistry: Life at the Molecular level" (Voet et al, 2006). The Lineweaver-Burk plot for inhibition of Benzoic acid and Cinnamic are both indicatives of competitive inhibition, whereas the plot for Thiourea is indicative of uncompetitive inhibition. This information would correspond to the hypothesis made in the introduction section, meaning that both Benzoic acid and Cinnamic acid have molecular structures that are similar enough to L-DOPA to form a complex with Tyrosinase's active site, while Thiourea performs inhibition by binding directly to the L-DOPA-Tyrosinase complex, since it is not similar enough to L-DOPA.

On competitive inhibition article written by Kochana et al in 2009 on Benzoic acid inhibition of tyrosinase confirms the competitive inhibition of Benzoic acid on Tyrosinase when explaining why it was decided to monitor the influence of catechol concentration in the biosensor response toward Benzoic acid. In addition, Cinnamic acid was also determined to be a reversible competitive inhibitor of various diphenolase and monophenolase activities catalyzed by tyrosinase (Garcia-Jimenez et al, 2018)

On non-competitive inhibition, other articles such as the "repositioning of Thiourea-Containing Drugs as Tyrosinase Inhibitors" (Choi & Jee, 2015) also determine Thiourea to be a non-competitive inhibitor of Tyrosinase just as determined by the obtained Lineweaver-Burk plots of this experiment as hypothesized in the introduction section.

5. Conclusion

In summary, despite the irregular Menten curve from L-DOPA, the experiment was successful in its initial goal of characterizing the kinetics of tyrosinase with respect to its catalysis role in the L-DOPA reaction, and resulted in reasonably accurate Lineweaver-Burk plots, as well as accurate patterns of inhibition, later confirmed by literature.

6. References

Biswas, R., Chanda, J., Kar, A., & Mukherjee, P. K. (2017). Tyrosinase inhibitory mechanism of betulinic acid from dillenia indica. Food Chemistry, 232, 689-696. doi: 10.1016/j.foodchem.2017.04.008

Choi, J., & Jee, J. (2015). Repositioning of thiourea-containing drugs as tyrosinase inhibitors. International Journal of Molecular Sciences, 16(12), 28534-28548. doi:10.3390/ijms161226114

D'Souza, B., Gibon, J. (2020, January). Lab 3: Enzyme Kinetics. Retrieved from https://canvas.ubc.ca/courses/31767/files/6655010?module_item_id=1601958.

Garcia-Jimenez, A., García-Molina, F., Teruel-Puche, J. A., Saura-Sanmartin, A., Garcia-Ruiz, P. A., Ortiz-Lopez, A., . . . Munoz-Munoz, J. (2018). Catalysis and inhibition of tyrosinase in the presence of cinnamic acid and some of its derivatives. International Journal of Biological Macromolecules, 119, 548-554. doi:10.1016/j.ijbiomac.2018.07.173

Kochana, J., Kozak, J., Skrobisz, A., & Woźniakiewicz, M. (2012). Tyrosinase biosensor for benzoic acid inhibition-based determination with the use of a flow-batch monosegmented sequential injection system. Talanta, 96, 147-152. doi:10.1016/j.talanta.2011.12.009

Ortiz-Ruiz, C. V., Maria-Solano, M. A., Garcia-Molina, M. D. M., Varon, R., Tudela, J., Tomas, V., & Garcia-Canovas, F. (2015). Kinetic characterization of substrate-analogous inhibitors of tyrosinase. IUBMB Life, 67(10), 757-767. doi:10.1002/iub.1432

Pratt, C. M., Voet, D., & Voet, J. G. (2006). 12.2 Enzyme Inhibition. In Fundamentals of Biochemistry: Life at the Molecular Level (5th ed.). New Jersey, NY: Wiley

7. Appendix

A. Absorbance Plots

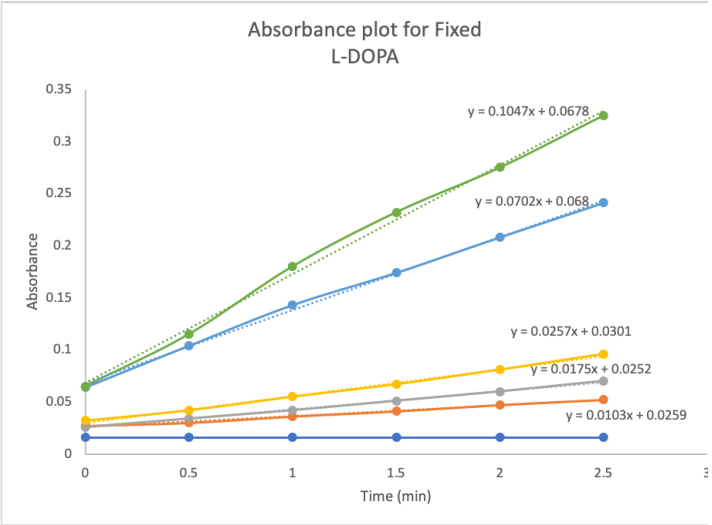


Fig.A1. Absorbance plot for assay run keeping L-DOPA volume fixed and variable tyrosinase volume. Different colours represent different assay runs.

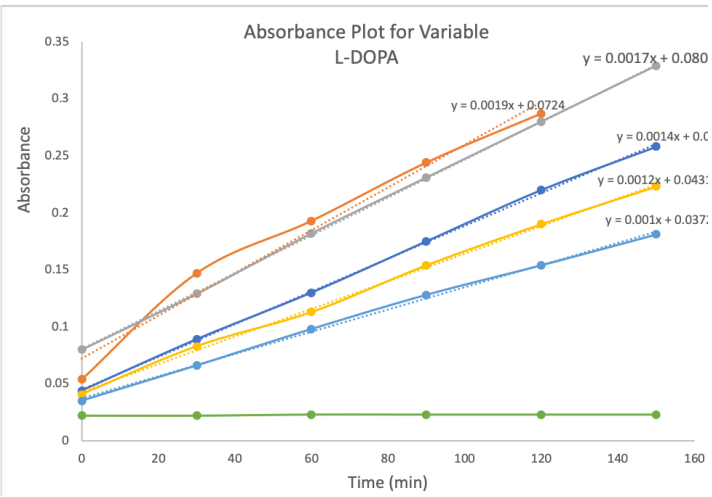


Fig.A2. Absorbance plot for assay run keeping tyrosinase volume fixed and variable L-DOPA volume. Different colours represent different assay runs.

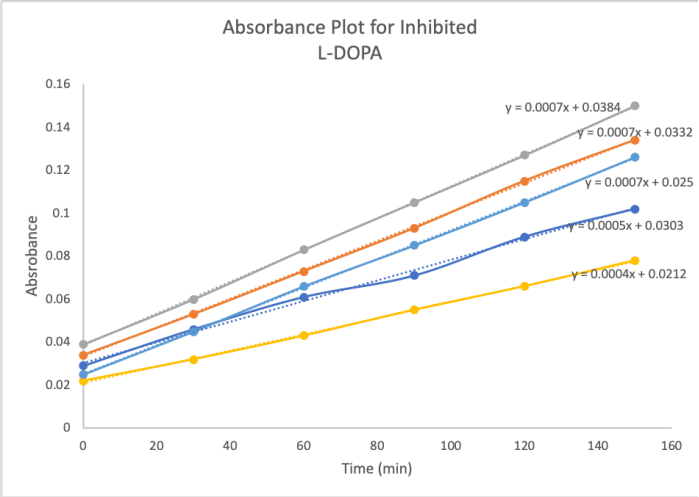


Fig.A3. Absorbance plot for assay run with inhibited tyrosinase and L-DOPA catalysis. Different colours represent different assay runs.

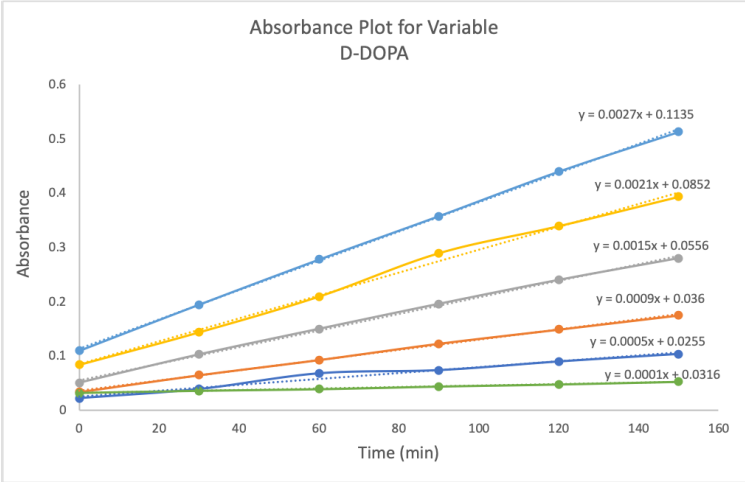


Fig.A4. Absorbance plot for assay run keeping tyrosinase volume fixed and variable D-DOPA volume. Different colours represent different assay runs.

B. **Tabulated Raw Data**

Table B1. Raw Data for assay runs of Fixed L-DOPA and variable Tyrosinase volume

Time (s)	Control Assay	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
0	0.016	0.027	0.026	0.032	0.064	0.065
0.5	0.016	0.03	0.034	0.042	0.104	0.115
1	0.016	0.036	0.042	0.055	0.143	0.18
1.5	0.016	0.041	0.051	0.067	0.174	0.232
2	0.016	0.047	0.06	0.081	0.208	0.275
2.5	0.016	0.052	0.07	0.096	0.241	0.325

Table B2. Raw Data for assay runs of variable L-DOPA and fixed Tyrosinase volume

Time (s)	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Control
0	0.044	0.054	0.08	0.041	0.035	0.022
30	0.089	0.147	0.129	0.083	0.066	0.022
60	0.13	0.193	0.182	0.113	0.098	0.023
90	0.175	0.244	0.231	0.154	0.128	0.023
120	0.22	0.287	0.28	0.19	0.154	0.023
150	0.258	------	0.329	0.223	0.181	0.023

Table B3. Raw Data for assay runs of Tyrosinase reaction with L-DOPA inhibited by Benzoic acid

Time (s)	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
0	0.029	0.034	0.039	0.022	0.025
30	0.046	0.053	0.06	0.032	0.045
60	0.061	0.073	0.083	0.043	0.066
90	0.071	0.093	0.105	0.055	0.085
120	0.089	0.115	0.127	0.066	0.105
150	0.102	0.134	0.15	0.078	0.126

Table B4. Raw Data for assay runs of variable L-DOPA and fixed Tyrosinase volume

Time (s)	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Control
0	0.022	0.034	0.051	0.084	0.11	0.032	0.018
30	0.039	0.065	0.103	0.144	0.195	0.036	0.018
60	0.068	0.093	0.15	0.21	0.278	0.039	0.018
90	0.074	0.123	0.196	0.29	0.358	0.044	0.018
120	0.09	0.149	0.24	0.34	0.44	0.048	0.018
150	0.104	0.175	0.28	0.394	0.513	0.053	0.019