EXP. NUMBER	EXPERIMENT/SUBJECT	DATE
5	Kinetic Analysis of Tyrosinase	7/8+15/09

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
Jason Largen	Tim Van		Bio Chem

Title: Kinetic Analysis of Tyrosinase Enzyme

Reference: Expirement #5 in Modern Exp. Biochem 3rd
Slides on blackboard.

Synopsis: To examine enzyme kinetics using mushroom Tyrosinase enzyme's reaction with Dopa substrate. Ideal concentrations were first determined by measuring the reactions rate via 475nm absorbance (dopachrome, the product, ideal absorbane). Once determined, the ideal enzyme + substrate concentrations were reacted with an inhibitor (~~e~~ cinnamic acid). The results were used to compare the stereospecific preference of tyrosinase to either L or D Dopa, and to determine the degree and type of inhibition cinnamic acid is. -1/4 & to determine kinetic constants Km, Vmax, & KI

Procedure

Tyrosinase Concentration was predetermined by Bush using UV 280nm absorbance

Observations

$$\frac{1.0\%}{24.9} = \frac{x\%}{0.008} \cdot 3.213 \times 10^{-4}\% \ g/100mL$$

5 assays were set up w/ varying levels of Tyrosinase. a steady level of 3.0mL total was maintained using Phosphate buffer. Absorance @ 475nm was noted every 30 seconds for 5 min, ~~as well as a~~.

assay #	1	2	3	4.	*
buffer (mL)	1.45	1.3	1.1	1.0	
L-Dopa (mL)	1.5	1.5	1.5	1.5	
enzyme (mL)	.05	.2	.4	.5	

* - note, only 4 assays done to save enzyme

the buffer + substrate were added first to validate no reaction took place for a minute, then enzyme added.

~~Slope | .0385 | .1053 | .1~~ .0809

Slope	.0102	.0385	.0809	.1053

the slopes were analysed ~~B~~ looking for a $\frac{\Delta A}{min} > .10$

See data table for full results.

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Procedure

Once ideal enzyme amount determined, ideal substrate range was analyzed, using 0.5mL of enzyme.
Validating linearity by looking at R^2 of graph.

observations

assay	1	2	3*
buffer(mL)	2.4	1.5	1.0
L-Dopa(mL)	.1	1.0	1.5
tyrosinase(mL)	.5	0.5	0.5
slope	.054	.121	.105
R^2	~~.993~~	.998	.9973

* - used from enzyme conc determination.

Using the ideal enzyme concentration (0.5mL) + a good range of substrate (.1 to 1.5mL) measure uninhibited & inhibited rates.
Validate the inhibitor amount reduces rate by at least 20%

uninhibited
inhibited
See data tables.

Calculations - Note all performed in excel. sample calculations given & excel printout provided

Substrate Concentration

$$[S] = \frac{(\text{substrate mL in assay})(\text{stock conc mg/mL})}{3.00\text{mL}}\left(\frac{1\text{mol DOPA}}{197.2\text{g}}\right)$$

Sample calc. assay #1, uninhibited

$$[S] = \left(\frac{(0.1\text{mL})(1.515\ \text{mg/mL})}{3.00\text{mL}}\right)\left(\frac{1\text{mol}}{197.2\text{g}}\right) = 2.56\times10^{-4}\ \text{mol/L}$$

Rxn Rate, V_0, Calculation

$$V_0 = \left(\frac{(\Delta A/\text{min})}{(3600\ \text{L/mol}\cdot\text{cm})(1.0\text{cm})}\right)\underbrace{(0.003\text{L})}_{\text{total volume}}\left(\frac{10^6\ \mu\text{mol}}{1\text{mol}}\right) = \frac{\mu\text{mol}}{\text{min}}$$

Sample calc, assay #1, uninhibited

$$V_0 = \left(\frac{.087}{(3600)(1.0)}\right)(0.003)(10^6) = 0.725\ \frac{\mu\text{mol}}{\text{min}}$$

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	K[illegible]	7/[illegible]

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J[illegible] Larsen	[illegible]		Biochem

Calculations (cont)

Vmax + Km, based on uninhibited plot

constant units? -1/2

~~Km =~~

$$V_{max} = \frac{1}{y_{int}} = \frac{1}{0.7529} = 1.328$$

$$K_m = (\text{slope})(V_{max}) = \frac{1.328}{(.0002)(1.328)} = \underline{2.656 \times 10^{-4}}$$

Concentration of inhibitor

$$[I] = \frac{\left(\begin{matrix}\text{Inhibitor mL}\\ \text{in assay}\end{matrix}\right)(\text{Stock } [I])}{3.00 \text{ mL}} = \frac{(0.5 \text{ mL})(3.37 \times 10^{-3} \text{ M})}{3.00 \text{ mL}} = 5.62 \times 10^{-4} \text{ M}$$

Ki, noncompetitive

2 ways to calc.

$$\text{Ki slope}_{inh} = \text{slope}_{uninh}\left(1 + \frac{[I]}{K_i}\right) = .0005 = .0002\left(1 + \frac{5.62 \times 10^{-4}}{K_i}\right) = 3.747 \times 10^{-4} \text{ M}$$

$$\frac{1.2405}{\cancel{0.7529}} = \frac{0.7529}{\cancel{1.2405}}\left(1 + \frac{5.62 \times 10^{-4}}{K_i}\right) = 8.678 \times 10^{-4} \text{ M}$$

or

$$(y_{int})_{inh} = (y_{int})_{uninh}\left[1 + \frac{[I]}{K_i}\right]$$

$$\text{average } K_i = \frac{(3.747 \times 10^{-4}) + (8.678 \times 10^{-4})}{2} = 6.212 \times 10^{-4} \text{ M}$$

14/15

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