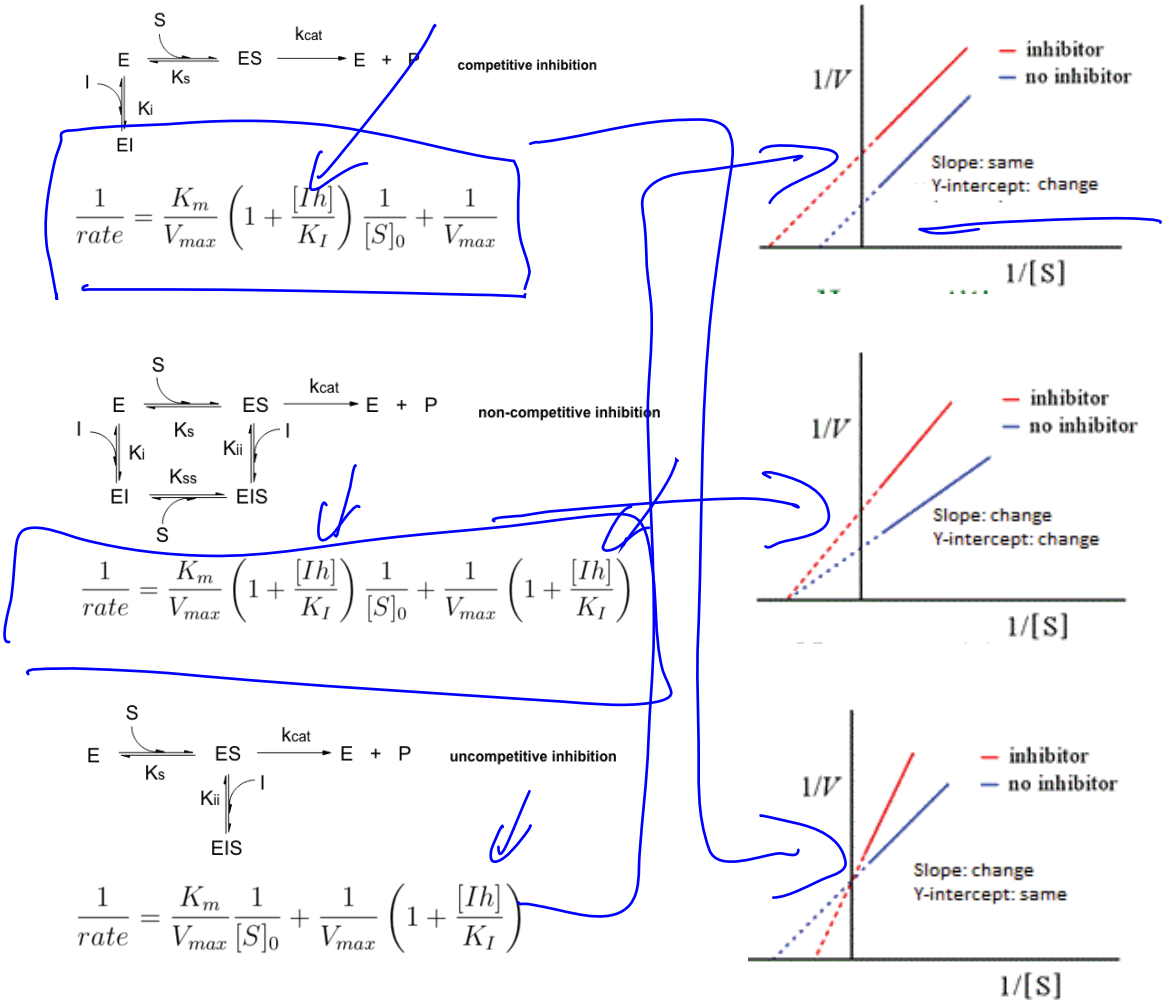
CHEM 361A - Lecture 19 Activity

Enzyme Kinetics

In Class

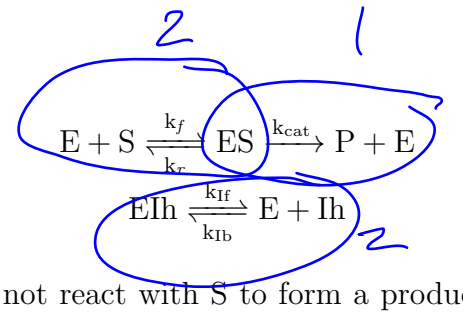
1. An inhibitor, (Ih), is a substance that decreases the rate of an enzyme-catalysed reaction. There are three types of reversible inhibition for Michaelis-Menten enzyme kinetics. Match each type with the correct Lineweaver-Burke plot.



Recall that the Lineweaver-Burke relationship when no inhibitor is present is

$$\frac{1}{rate} = \frac{K_m}{V_{max}}\frac{1}{[S]_0}+\frac{1}{V_{max}}$$

2. In this problem we will determine the Lineweaver-Burke relationship for reversible competitive inhibition. The reaction scheme for competitive inhibition is



The compound EIh does not react with S to form a product. For our purposes, let's assume that $k_{cat} \gg k_f, k_r$ and that the equilibrium between E, Ih and EIh is robust meaning that

$$\frac{[E][Ih]}{[EIh]} = K_I$$

(a) Write down a rate law expression for P, E, S and ES for this reaction mechanism. Apply any valid approximations.

(b) Write down conservation of moles expressions for everything that includes the Enzyme and relate it to $[E]_0$ (don't forget that you have an inhibitor complex). Do the same thing for the substrate and relate it to $[S]_0$. For both expressions, solve for [E] and [S] respectively. Express the [E] in terms of $[E]_0$, [ES] and [Ih].

(c) Determine the following expression for [ES]:

$$[ES] = \frac{[E]_0[S]_0}{K_m\left(1 + \frac{[Ih]}{K_I}\right) + [S]_0 + [E]_0}$$

During this process assume that [P] is very small and that [ES] is small (which means you neglect [P] and $[ES]^2$ terms).

(d) Substitute the expression you found in 2c into the rate law expression one you wrote in 2a for P. You should have

$$\frac{d[P]}{dt} = \frac{k_{cat}[E]_0[S]_0}{K_m\left(1 + \frac{[Ih]}{K_I}\right) + [S]_0 + [E]_0}$$

(e) Express the rate you just determined in 2d for the case that $[S]_0 \gg [E]_0$.

(f) Given that when no inhibitor is present, regular Michaelis-Menten kinetics apply

$$\frac{d[P]}{dt} = \frac{V_{max}[S]_0}{K_M + [S]_0}$$

Determine the ratio of the initial product formation rate for the case when no inhibitor is present to that with competitive inhibition and argue how one could overcome the effect of the inhibitor.

(g) Based on your rate law expression in 2e, determine the Lineweaver-Burk Equation for competitive inhibition. You should get

$$\frac{1}{rate} = \frac{K_m}{V_{max}}\left(1 + \frac{[Ih]}{K_I}\right)\frac{1}{[S]_0} + \frac{1}{V_{max}}$$

3. Applying the Lineweaver-Burk Equation for competitive inhibition

You were doing an experiment with and without an inhibitor present on an enzyme catalysed reaction. Unfortunately, you didn't label your reaction vessels so you don't know which one you put in the 8.0×10^{-3} M concentration of inhibitor. The data you collected from your experiment is found in Table 2 and a fit of this data according to the Lineweaver-Burk Equation for competitive inhibition can be found in Figure 1

Table 1: Data Collected from Reversible Inhibitor Experiment

$[S]_0$ (M)	rate$_A$ (M s^{-1})	rate$_B$ (M s^{-1})
5.0×10^{-4}	1.25×10^{-6}	5.8×10^{-7}
1.0×10^{-3}	2.0×10^{-6}	1.04×10^{-6}
2.5×10^{-3}	3.13×10^{-6}	2.00×10^{-6}
5.0×10^{-3}	3.85×10^{-6}	2.78×10^{-6}
1.0×10^{-2}	4.55×10^{-6}	3.57×10^{-6}

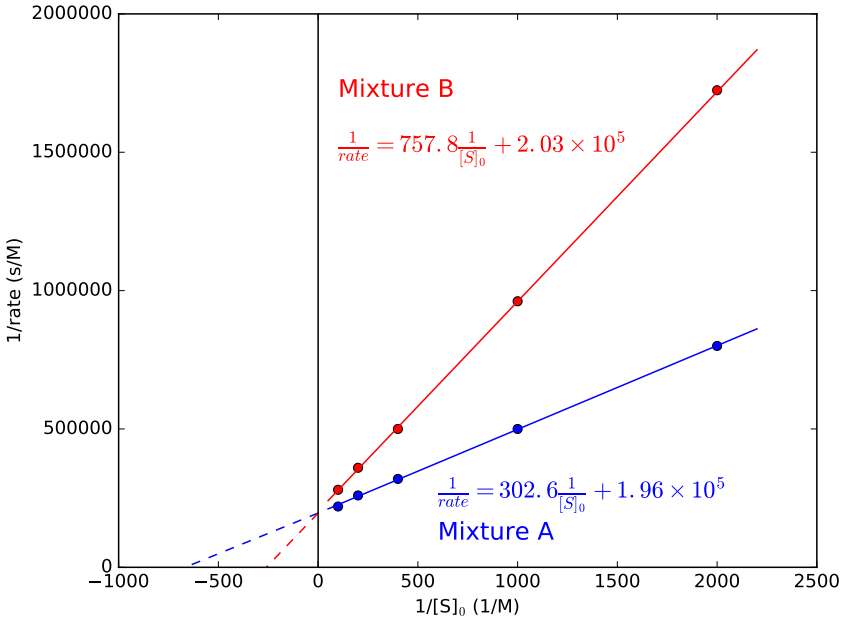


Figure 1: Lineweaver-Burk fit for reversible inhibition experiment using the data found in Table 2

(a) Which vial (A or B) is the one with the inhibitor in it? Do this by just looking at the fit of the data to the Lineweaver-Burk Equation.

(b) Determine the values K_m and V_{max} of the enzyme

(c) Determine K_I for the inhibitor

Homework

You decide to use the same enzyme tested in the previous question and test it with a second inhibitor. The $[Ih] = 8.0$ mM for all trials. The following data was measured:

Table 2: Data Collected from Second Inhibitor Experiment

$[S]_0$ (M)	rate_I (M s^{-1})
5.0×10^{-4}	3.8×10^{-7}
1.0×10^{-3}	6.3×10^{-7}
2.5×10^{-3}	1.00×10^{-6}
5.0×10^{-3}	1.25×10^{-6}
1.0×10^{-2}	1.43×10^{-6}

(a) Plot the data from this second inhibitor with the data from the uninhibited enzyme and determine the type of inhibitor present in this second experiment.

(b) Determine K_I for this second inhibitor. ($K_I = 3.3 \times 10^{-3}$)