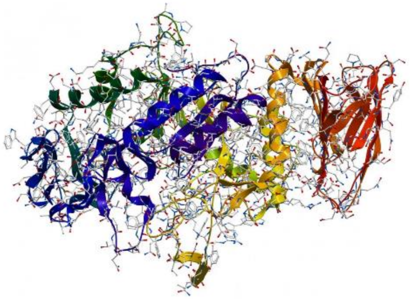


CEN-CHE 422
ENZYME ENGINEERING

Determination of K_m and r_{max}

$$r = \frac{r_{\max} C_S}{Km + C_S}$$

The graph of MM equation (r vs C_s) is not entirely satisfactory for the determination of r_{max} and K_m.

Michaelis-Menten equation is linearized by being inverted to *y*=*n*+*mx* form

$$\frac{1}{\underline{r}} = \frac{Km}{r_{\max}} \frac{1}{\underline{C_S}} + \frac{1}{r_{\max}}$$

✓Lineweaver-Burk Equation*
✓Eadie-Hoffstee Equation
✓Hanes-Woolf Equation
(Langmuir Equation)

$$\frac{r}{\underline{C_S}} = \frac{r_{\max}}{K_m} - \frac{1}{K_m}\underline{r}$$

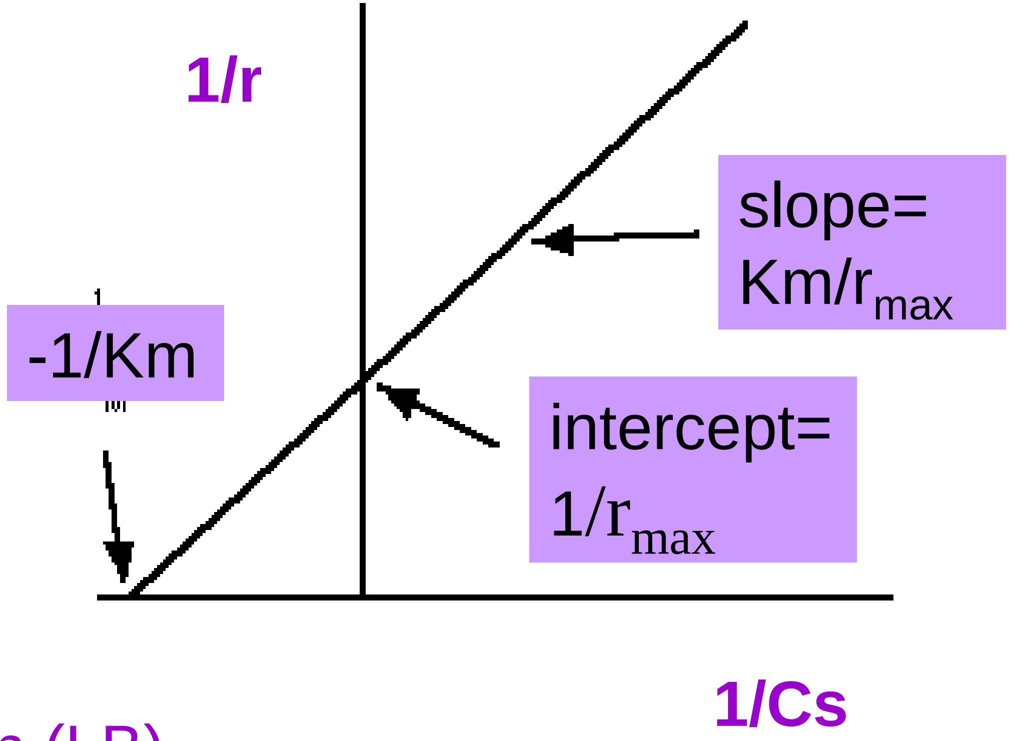
$$\left(\frac{C_S}{\underline{r}} = \frac{Km}{r_{\max}} + \frac{\underline{C_S}}{r_{\max}}\right)$$

Lineweaver-Burk Graph

$$r = \frac{r_{\max} C_S}{Km + C_S}$$

If MM equation is inverted: :

$$\frac{1}{\underline{r}} = \frac{Km}{r_{\max}} \frac{1}{\underline{C_S}} + \frac{1}{r_{\max}}$$



Lineweaver-Burk Equation (LB)

$$slope = \frac{K_m}{r_{max}} \qquad intercept = \frac{1}{rmax}$$

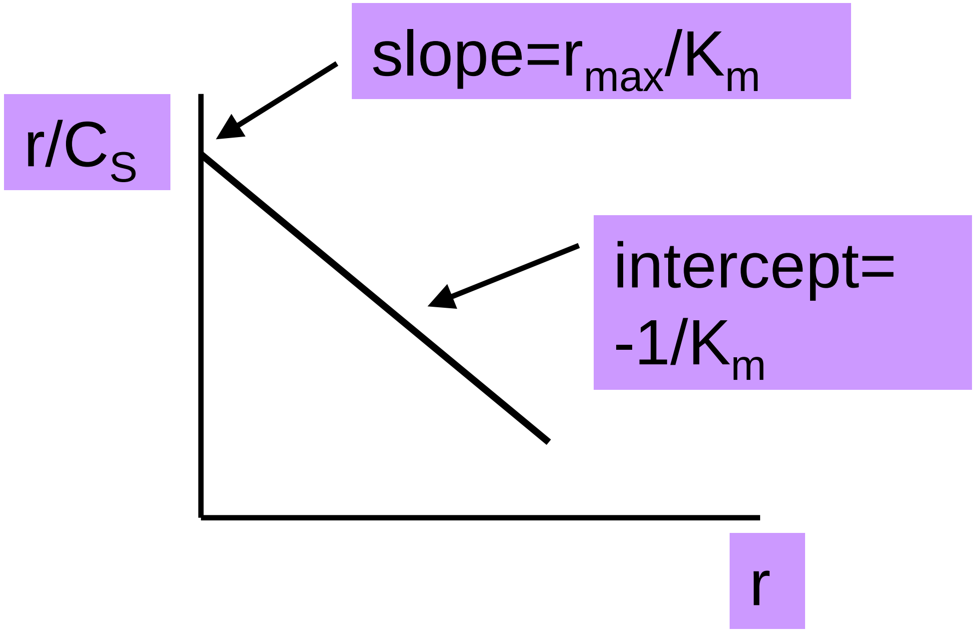
Eadie-Hoffstee Graph

$$r = \frac{r_{\max} C_S}{Km + C_S}$$

$$r(K_m + C_S) = r_{\max} C_S$$

$$rK_m = (r_{\max} - r) C_S$$

$$\frac{r}{\underline{C_S}} = \frac{r_{\max}}{K_m} - \frac{1}{K_m}\underline{r}$$



slope=r_{max}/K_m

r/C_S

intercept= -$1/K_m$

r

$$intercept = \frac{r_{max}}{K_m}$$

Eadie-Hoffstee Equation

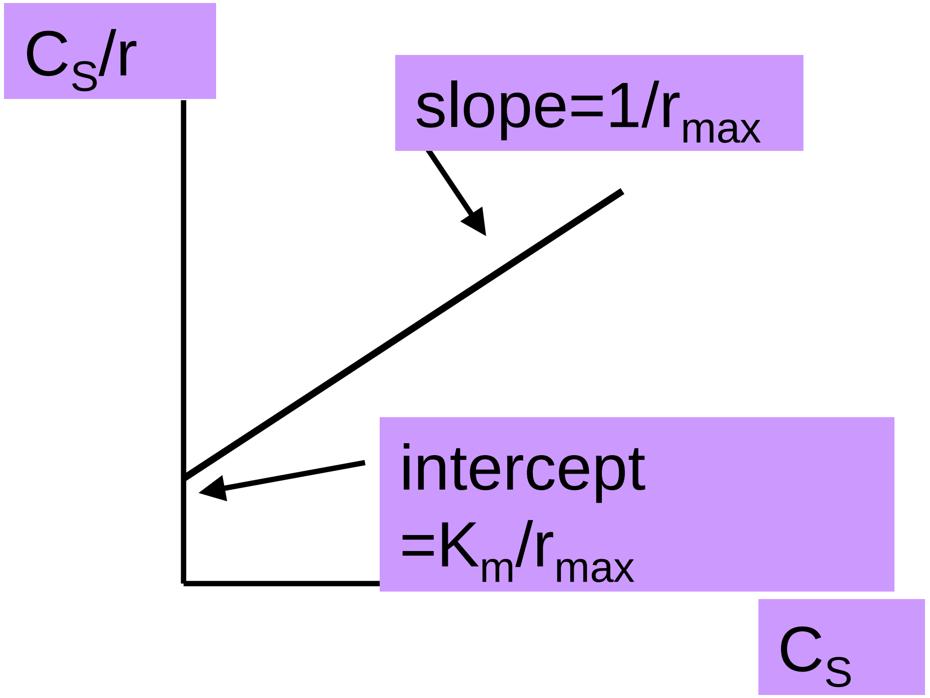
$$slope = \frac{1}{K_m}$$

Hanes-Woolf Graph

$$C_S x(\frac{1}{r} = \frac{Km}{r_{\max}}\frac{1}{C_S} + \frac{1}{r_{\max}})xC_S$$

$$(\frac{C_S}{\underline{r}} = \frac{Km}{r_{\max}} + \frac{\underline{C_S}}{r_{\max}})$$

Hanes-Woolf Equation



C_S/r

slope=1/r_{max}

intercept =K_m/r_{max}

C_S

$$slope = \frac{1}{r_{max}} \qquad intercept = \frac{K_m}{rmax}$$

ENZYME INHIBITION

Inhibitor(I): Inhibitors are substances which tend to decrease the rate of enzyme-catalysed reaction (by formig a complex, EI, ES, ESI, etc..)

1. Irreversible Inhibition
2. Reversible Inhibisyon

- Competitive Inhibition
- Uncompetitive Inhibition
- Noncompetitive Inhibition
- Mixed Inhibition
- Substrate Inhibition
- Partial Inhibition
- Allosteric Inhibition

Irreversible Inhibition

- There is no structural similarity between the substrate and the enzyme

- The active group of the enzyme undergoes chemical change. Therefore, the enzyme-inhibitor complex cannot re-dissociate to form an active enzyme.

- These inhibitors are known as enzyme poisons.

- Iodoacetate and some heavy metal ions metal cations

- *This type of inhibition is permanent*

Competitive Inhibition

Competitive inhibitors closely resemble in some respects the substrate whose reactions they inhibit.

Because of the similarity they may be compete for the same binding-site on the enzyme.

$$E + S \Leftrightarrow ES$$

$$E + I \Leftrightarrow EI \qquad Ki = \frac{k_{-i}}{k_i}$$

$$\mathrm{ES} \Rightarrow E + P$$

quasi-steady-state assumption

Inhibitor binds to active site on enzyme $$\frac{dC_{ES}}{dt} = 0$$

$$\frac{dC_{EI}}{dt} = 0$$

reaction rate: $$r = k_2 \underline{C_{ES}}\ ?$$

$$\frac{dC_{ES}}{dt} = k_1 C_E C_S - k_{-1} C_{ES} - k_2 C_{ES} = 0$$

$$C_{ES} = \frac{k_1}{k_{-1} + k_2} C_E C_S \quad \Rightarrow \quad C_{ES} = \frac{C_E C_S}{Km}$$ ✓

$$\frac{dC_{EI}}{dt} = k_i C_E C_I - k_{-i} C_{EI} = 0$$

$$C_{EI} = \frac{k_i}{k_{-i}} C_E C_I \quad \Rightarrow \quad C_{EI} = \frac{C_E C_I}{Ki}$$ ✓

Enzyme balance: $C_{Eo} = C_E + C_{ES} + C_{EI}$

$$C_{Eo} = C_E + \frac{C_E C_I}{Ki} + \frac{C_E C_S}{Km}$$

$$C_E = \frac{C_{Eo}}{1 + \frac{C_S}{Km} + \frac{C_I}{Ki}}$$ ✓

$$C_{ES} = \frac{C_E C_S}{Km}$$

If we substitute C_E into C_{ES}

$$C_{ES} = \frac{C_S}{Km} x \frac{C_{Eo}}{1 + \frac{C_S}{Km} + \frac{C_I}{Ki}} \Rightarrow C_{ES} = \frac{C_S C_{Eo}}{Km + C_S + \frac{Km}{Ki} C_I}$$

reaction rate:

✓

$$r = k_2 C_{ES} = \frac{k_2 C_{Eo} C_S}{C_S + Km(1 + \frac{C_I}{Ki})} \Rightarrow r = \frac{r_{\max} C_S}{C_S + \underbrace{Km(1 + \frac{C_I}{Ki})}_{K_m^*}}$$

If MM equation is linearized (LB):

$$\frac{1}{\underline{r}} = \frac{Km}{r_{\max}}\left(1+\frac{C_I}{Ki}\right)\frac{1}{\underline{C_S}} + \frac{1}{r_{\max}}$$

$$slope = \frac{K_m}{r_{max}}\left(1 + \frac{C_I}{K_i}\right)$$

$$İntercept = \frac{1}{r_{max}}$$

The lines intersect at the same point on 1/r axis (1/r_{max})

1/r_{max} is unchanged by the addition of inhibitör

If the lines drawn at different inhibitor concentrations intersect at the same point on the ordinate, the type of inhibition is competitive inhibition

Noncompetitive Inhibition

$$E + S \Leftrightarrow ES \qquad Ks = \frac{C_E C_S}{C_{ES}}$$

$$E + I \Leftrightarrow EI \qquad Ki = \frac{C_E C_I}{C_{EI}}$$

$$ES + I \Leftrightarrow ESI \qquad Ki = \frac{C_{ES} C_I}{C_{ESI}}$$

$$EI + S \Leftrightarrow ESI \qquad Ks = \frac{C_{EI} C_S}{C_{ESI}}$$

$$\mathrm{ES} \Rightarrow\!\!\gg E + P$$

reaction rate: $r = k_2 \underset{?}{C_{ES}}$

Michaelis-Menten assumption:

Enzyme balance:

$$C_{Eo} = C_E + C_{ES} + C_{EI} + C_{ESI}$$

Using dissociation constants:

$$C_{ES} = \frac{C_E C_S}{Ks} \checkmark \qquad C_{EI} = \frac{C_E C_I}{Ki} \checkmark \qquad C_{ESI} = \frac{C_{ES} C_I}{Ki} = \frac{C_E C_S C_E}{KiKs} \checkmark$$

Inserting in C_{Eo}

$$C_{Eo} = C_E + \frac{C_E C_S}{Ks} + \frac{C_E C_I}{Ki} + \frac{C_E C_S C_I}{KiKs}$$

$$C_{Eo} = C_E (1 + \frac{C_S}{Ks} + \frac{C_I}{Ki} + \frac{C_S C_I}{KiKs})$$

$$C_E = \frac{C_{Eo}}{(1 + \frac{C_S}{Ks} + \frac{C_I}{Ki} + \frac{C_I C_S}{Ki Ks}}$$

Inserting in C_{ES}

$$C_{ES} = \frac{C_E C_S}{Ks}$$

$$C_{ES} = \frac{C_S}{K_S} x \frac{C_{Eo}}{(1 + \frac{C_S}{Ks} + \frac{C_I}{Ki} + \frac{C_I C_S}{Ki Ks})}$$

$$C_{ES} = \frac{C_{Eo} C_S}{(K_S + C_S + \frac{C_I K_S}{Ki} + \frac{C_I C_S}{Ki})}$$ ✓

reaction rate: $$r = k_2 C_{ES} = \frac{k_2 C_{Eo} C_S}{K_S\left(1 + \frac{C_I}{K_i}\right) + C_S\left(1 + \frac{C_I}{K_i}\right)}$$

✓

$$r = \frac{C_S r_{\max}}{(K_S + C_S)\left(1 + \frac{C_I}{K_i}\right)}$$

r_{max}^*

If MM equation is linearized:

$$\frac{1}{r} = \frac{K_m}{r_{\max}}\left(1 + \frac{C_I}{K_i}\right)\frac{1}{C_S} + \frac{1}{r_{\max}}\left(1 + \frac{C_I}{K_i}\right)$$

$$slope = \frac{K_m}{r_{max}}\left(1 + \frac{C_I}{K_i}\right)$$

$$intercept = \frac{1}{r_{max}}\left(1 + \frac{C_i}{K_i}\right)$$

$$K_S = \frac{k_{-1}}{k_1}$$

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

$k_2 << k_{-1}$

↓

$K_m = K_S$

The lines intersect
at the same point
on $1/C_S$ axis ($1/K_m$)

If the lines drawn at different inhibitor concentrations intersect at the same point on the x axis, the type of inhibition is noncompetitive inhibition

In noncompetitive inhibition :

- ✓ ***K_m does not change***

Uncompetitive Inhibition

$$E + S \Leftrightarrow ES$$

$$ES + I \Leftrightarrow ESI$$

$$\mathrm{ES} \Rightarrow\!\!\gg E + P$$

quasi-steady-state assumption

$$\frac{dC_{ES}}{dt} = 0$$

$$\frac{dC_{ESI}}{dt} = 0$$

reaction rate: $r = k_2 C_{ES}$

?

$$\frac{dC_{ES}}{dt} = k_1 C_E C_S - k_{-1} C_{ES} - k_i C_{ES} C_I + k_{-i} C_{ESI} - k_2 C_{ES} = 0$$

$$C_{ES} = \frac{k_1 C_E C_S + k_{-i} C_{ESI}}{\left(k_{-1} + k_i C_I + k_2\right)}$$ ✓

$$\frac{dC_{ESI}}{dt} = k_i C_{ES} C_I - k_{-i} C_{ESI} = 0$$

$$C_{ESI} = \frac{C_{ES} C_I}{\left(k_{-i} / k_i\right)}$$ ✓

Inserting in C_{ES}

$$C_{ES} = \frac{k_1 C_E C_S + k_{-i} \dfrac{C_{ES} C_I}{\left(k_{-i} / k_i\right)}}{\left(k_{-1} + k_i C_I + k_2\right)}$$

$$\left[\left(k_{-1}+k_2+k_iC_I\right)-k_iC_I\right]C_{ES}=k_1C_EC_S \Longrightarrow C_{ES}=\frac{k_1C_EC_S}{\left(k_{-1}+k_2\right)}$$

Inserting in C_{ESI} $$C_{ESI}=\frac{C_I}{K_i}x\frac{k_1C_EC_S}{\left(k_{-1}+k_2\right)}$$ ✓

Enzyme mass balance: $C_{Eo}=C_E+C_{ES}+C_{ESI}$

$$C_{Eo}=C_E+\frac{k_1C_S}{\left(k_{-1}+k_2\right)}C_E+\frac{k_1C_IC_S}{K_i(k_{-1}+k_2)}C_E$$

$$C_E=\frac{C_{Eo}}{1+\frac{k_1C_S}{\left(k_{-1}+k_2\right)}+\frac{k_1C_IC_S}{K_i\left(k_{-1}+k_2\right)}}$$

Inserting in C_{ES} $$C_{ES}=\frac{k_1}{\left(k_{-1}+k_2\right)}x\frac{C_{Eo}}{1+\frac{k_1C_S}{\left(k_{-1}+k_2\right)}+\frac{k_1C_IC_S}{K_i\left(k_{-1}+k_2\right)}}$$

$$C_{ES} = \frac{C_{Eo} C_S}{\dfrac{k_{-1} + k_2}{k_1} + C_S + \dfrac{C_I C_S}{K_i}}$$

$$C_{ES} = \frac{C_{Eo} C_S}{K_m + C_S + \dfrac{C_I C_S}{K_i}}$$ ✓

reaction rate: $r = k_2 C_{ES}$

$$r = \frac{k_2 C_{Eo} C_S}{K_m + C_S + \dfrac{C_I C_S}{K_i}}$$

✓

$$r = \frac{r_{\max} C_S}{K_m + C_S \left(1 + \dfrac{C_I}{K_i}\right)}$$

$r_{\max}^*$

$$r = \frac{r_{\max} / \left[C_S \left(+ \dfrac{C_I}{K_i} \right) \right] (C_S)}{K_m / \left[C_S \left(+ \dfrac{C_I}{K_i} \right) \right] + C_S}$$

K_m^*

If MM equation is linearized:

$$\frac{1}{r} = \frac{K_m}{r_{\max}}\frac{1}{C_S} + \frac{1}{r_{\max}}\left(1 + \frac{C_I}{K_i}\right)$$

$$slope = \frac{K_m}{r_{max}}$$

$$Intercept = \frac{1}{r_{max}}$$

The lines are parallel to each other

If the lines drawn at different inhibitor concentrations are parallel to each other, the type of inhibition is uncompetitive inhibition