Enzyme Inhibition

Imagination is more important than knowledge.

– *Albert Einstein*

Introduction

- A modulator (or effector) is a substance which can combine with enzymes to alter their catalytic activities.
- An inhibitor is a modulator which decreases enzyme activity. An inhibitor binds with the enzyme and decreases the catalytic activity of the enzyme.
- An inhibitor may be: *Organic* or *Inorganic* in nature.
- Enzyme inhibitors such as heavy metals (Pb, Cd, Hg) form stable complex with the enzyme and reduce the activity.
- Such enzyme inhibition may be reversed only by using the chelating agent such as EDTA (ethylene diamine tetraacetic acid) and citrate.

Introduction (2)

- The inhibitor can decrease the rate of reaction either competitively, noncompetitively, or uncompetitively.
- Basically, there are two types of inhibition observed, namely *reversible* and *irreversible*.
- In reversible inhibition, it is possible to eliminate the inhibitory action by decreasing its concentration (e.g. dilution).
- In irreversible inhibition, there is no reversal of the inhibitory action due to the formation of covalent bonds.

Several types of reversible inhibitions observed are discussed as follows:

Competitive Inhibition

Two types of inhibition are possible:

- *Active site binding*: In this case the inhibitor is a compound with a close structural and chemical similarity to the substrate of the enzyme. As a result, the inhibitor binds to the active site in place of the substrate.
  The inhibitor simply blocks the active site, and it is impossible for both of them to bind to the active site at the same time.

- *Conformational change*: In this type, the inhibitor binds to another site which causes a conformational change to the active site of the enzyme such that the substrate can no longer bind to the enzyme.

The mechanism of the reaction is:

$$E + I \rightleftharpoons EI$$

Competitive Inhibition (2)

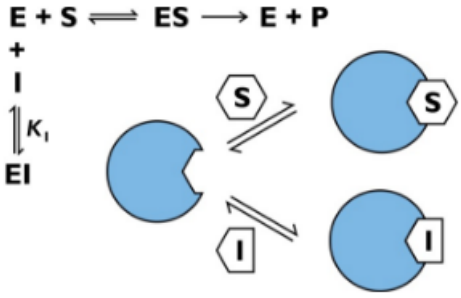

Figure 1: Competitive inhibition

Competitive Inhibition (3)

The inhibition constant

$$K_I = \frac{[E][I]}{[EI]} \tag{1}$$

The inhibitor binds only to the free enzyme, producing an inactive dead end complex.
The equation for the free enzyme becomes:

$$[E] = [E]_o - [ES] - [EI] \tag{2}$$

Substituting Eq. (1) in Eq. (2), we have

$$[E] = [E]_o - [ES] - \frac{[E][I]}{K_I} \tag{3}$$

Competitive Inhibition (4)

But the Michaelis-Menten constant for enzyme-substrate complex is

$$K_M = \frac{[E][S]}{[ES]} \tag{4}$$

Hence, the rate

$$v_o = k_3[ES] \tag{5}$$

Rewriting Eq. (3) in terms of $[E]$, we get

$$[E] = \frac{[E]_o - [ES]}{\left(1 + \frac{[I]}{K_I}\right)} \tag{6}$$

Competitive Inhibition (5)

substituting Eq. (6) in Eq. (4), we have

$$K_M = \frac{([E]_o - [ES])[S]}{\left(1 + \frac{[I]}{K_I}\right)[ES]} \tag{7}$$

$$K_M\left(1 + \frac{[I]}{K_I}\right) = \frac{[E]_o[S]}{[ES]} - \frac{[ES][S]}{[ES]} \tag{8}$$

Rearranging Eq. (8), we get

$$[ES] = \frac{[E]_o[S]}{[S] + K_M\left(1 + \frac{[I]}{K_I}\right)} \tag{9}$$

Competitive Inhibition (6)

The initial rate becomes

$$v_o = \frac{k_3[E]_o[S]}{[S] + K_M\left(1 + \frac{[I]}{K_I}\right)} \tag{10}$$

$$v_o = \frac{v_{max}[S]}{[S] + K_M\left(1 + \frac{[I]}{K_I}\right)} \tag{11}$$

Taking the inverse of Eq. (11), we have the Lineweaver-Burke plot (Fig. 2)

Deductions:

- The net effect of competitive inhibition is an increase in the value of K_M and therefore reduction in reaction rate.
- It can be overcome by high $[S]$.

Competitive Inhibition (7)

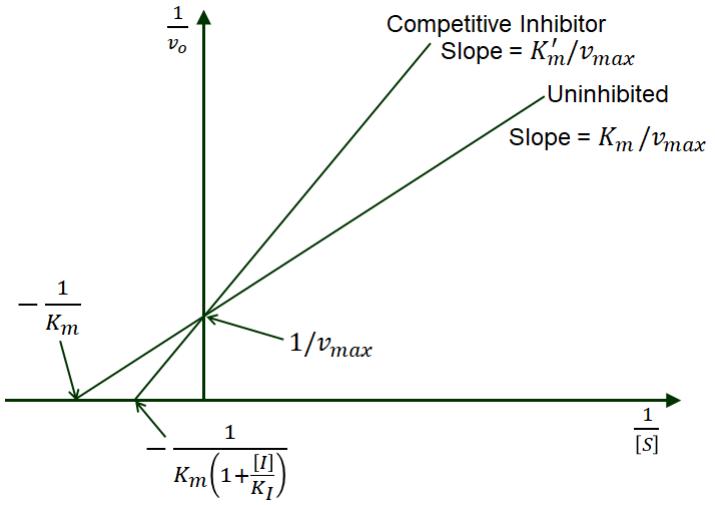

Figure 2: Lineweaver-Burke plot for reversible competitive inhibition

Uncompetitive Inhibition

- In this type of inhibition, the inhibitor binds only to the enzyme substrate complex producing a dead end complex, thereby preventing product formation.

The mechanism of the reaction is:

$$ES + I \rightleftharpoons ESI$$

The inhibition constant is

$$K_I = \frac{[ES][I]}{[ESI]} \tag{12}$$

The Michaelis-Menten Constant is

$$K_M = \frac{[E][S]}{[ES]} \tag{13}$$

The equation for the total enzyme becomes:

$$[E]_o = [E] + [ES] + [ESI] \tag{14}$$

Uncompetitive Inhibition (2)

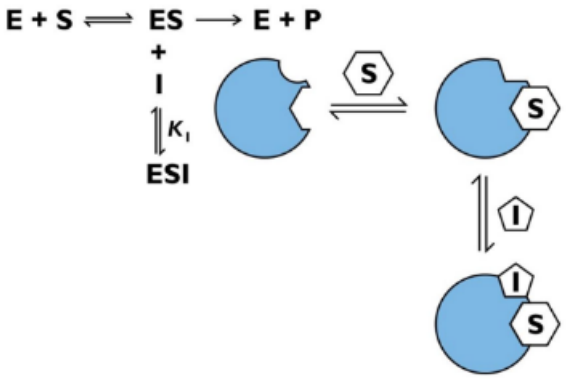

Figure 3: Uncompetitive inhibition

Uncompetitive Inhibition (3)

$$[ESI] = \frac{[ES][I]}{K_I} \tag{15}$$

Substituting Eq. (15) into Eq. (14), we have

$$[E]_o = [E] + [ES] + \frac{[ES][I]}{K_I} \tag{16}$$

Rearranging Eq. (16) and simplifying it, we get

$$[E] = [E]_o - [ES]\left(1 + \frac{[I]}{K_I}\right) \tag{17}$$

But

$$K_M = \frac{[E][S]}{[ES]} \tag{18}$$

Uncompetitive Inhibition (4)

$$K_M = \frac{\left[[E]_o - [ES]\left(1 + \frac{[I]}{K_I}\right)\right][S]}{[ES]} \tag{19}$$

$$K_M = \frac{[E]_o[S]}{[ES]} - \frac{[ES][S]\left(1 + \frac{[I]}{K_I}\right)}{[ES]} \tag{20}$$

$$\frac{[E]_o[S]}{[ES]} = K_M + [S]\left(1 + \frac{[I]}{K_I}\right) \tag{21}$$

$$[ES] = \frac{[E]_o[S]}{K_M + [S]\left(1 + \frac{[I]}{K_I}\right)} \tag{22}$$

But the initial rate, $v_o = k_3[ES]$

Uncompetitive Inhibition (5)

Hence,

$$v_o = \frac{k_3[E]_o[S]}{K_M + [S]\left(1 + \frac{[I]}{K_I}\right)} \tag{23}$$

$$v_o = \frac{v_{max}[S]}{K_M + [S]\left(1 + \frac{[I]}{K_I}\right)} \tag{24}$$

Taking the inverse of Eq. (24), we have

$$\frac{1}{v_o} = \frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right) + \frac{K_M}{v_{max}}\frac{1}{[S]} \tag{25}$$

Fig. 4 shows the Lineweaver-Burke plot with

$$\frac{1}{v'_{max}} = \frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

Uncompetitive Inhibition (6)

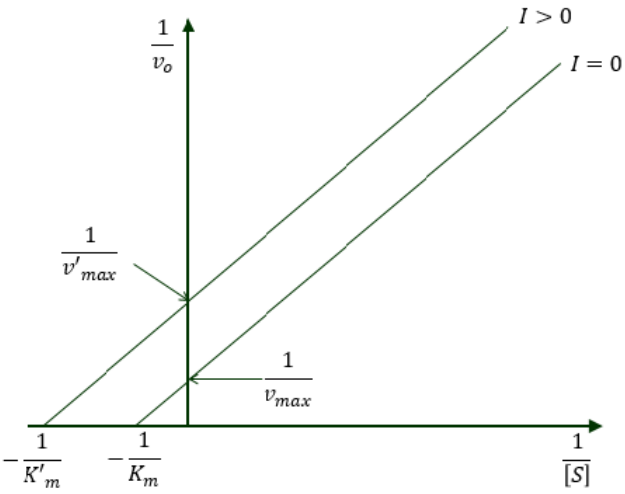

Figure 4: Lineweaver-Burke plot for reversible Uncompetitive inhibition

Noncompetitive Inhibition

- In this type of inhibition, the inhibitor is assumed to bind with equal affinity to both the free enzyme as well as substrate complex.
- Enzyme like *chymotrypsin*, which has an active site that can accept a proton can be inhibited by increasing hydrogen ion concentration.
- The mechanism of the reaction is:

$$E + I \rightleftharpoons EI$$
$$ES + I \rightleftharpoons ESI$$

- The inhibition constant is assumed the same for both reaction

$$K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]} \tag{26}$$

$$K_M = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]} \tag{27}$$

Noncompetitive Inhibition (2)

$$[EI] = \frac{[E][I]}{K_I} \tag{28}$$

$$[ESI] = \frac{[ES][I]}{K_I} \tag{29}$$

The equation for the total enzyme becomes:

$$[E]_o = [E] + [ES] + [ESI] + [EI] \tag{30}$$

$$[E]_o = [E] + [ES] + \frac{[ES][I]}{K_I} + \frac{[E][I]}{K_I} \tag{31}$$

$$[E]_o = [E]\left(1 + \frac{[I]}{K_I}\right) + [ES]\left(1 + \frac{[I]}{K_I}\right) \tag{32}$$

Noncompetitive Inhibition (3)

$$[E] = \frac{[E]_o - [ES]\left(1 + \frac{[I]}{K_I}\right)}{\left(1 + \frac{[I]}{K_I}\right)} \tag{33}$$

Substitute Eq. (33) into Eq. (27), we get

$$K_M = \frac{[E][S]}{[ES]}$$

$$K_M = \frac{\left([E]_o - [ES]\left[1 + \frac{[I]}{K_I}\right]\right)[S]}{\left(\left[1 + \frac{[I]}{K_I}\right]\right)[ES]} \tag{34}$$

$$K_M\left[1 + \frac{[I]}{K_I}\right] = \frac{[E]_o[S]}{[ES]} - \frac{[ES]\left[1 + \frac{[I]}{K_I}\right][S]}{[ES]} \tag{35}$$

Noncompetitive Inhibition (4)

$$\frac{[E]_o[S]}{[ES]} = K_M\left(1+\frac{[I]}{K_I}\right)+\left(1+\frac{[I]}{K_I}\right)[S] \tag{36}$$

$$\frac{[E]_o[S]}{[ES]} =\left(1+\frac{[I]}{K_I}\right)(K_M+[S]) \tag{37}$$

Hence

$$[ES] = \frac{[E]_o[S]}{\left(1+\frac{[I]}{K_I}\right)(K_M+[S])} \tag{38}$$

Therefore, the initial rate, $v_o = k_3[ES]$ becomes

$$v_o = \frac{k_3[E]_o[S]}{\left(1+\frac{[I]}{K_I}\right)(K_M+[S])} \tag{39}$$

Noncompetitive Inhibition (5)

$$v_o = \frac{v_{max}[S]}{\left(1 + \frac{[I]}{K_I}\right)(K_M + [S])} \tag{40}$$

Taking the inverse of Eq. (40), we have:

$$\frac{1}{v_o} = \frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right) + \frac{K_M}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{[S]} \tag{41}$$

Fig. 5 shows the Lineweaver-Burke plot with

$$\frac{1}{v'_{max}} = \frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

And the slope of the inhibited reaction becomes:

$$slope = \frac{K_M}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

Noncompetitive Inhibition (6)

Deductions:

- The net effect of noncompetitive inhibition is a decrease in v_{max}.
- High substrate concentration cannot overcome this type of inhibition.

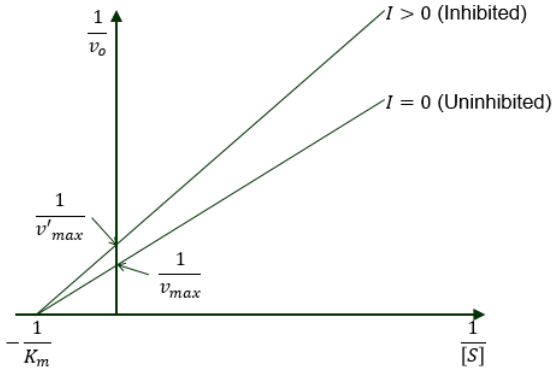

Figure 5: Lineweaver-Burke plot for reversible Noncompetitive inhibition

Mixed Inhibition

- The action is termed mixed inhibition if the inhibitor constant for both the steps is different.

  The mechanism of the reaction is:

$$E + I \rightleftharpoons EI \qquad (\text{inhibitor constant} = K_I)$$

$$ES + I \rightleftharpoons ESI \qquad (\text{inhibitor constant} = \alpha K_I)$$

- In the Lineweaver-Burke plot, the lines will not intersect at the $\frac{1}{v_o}$-axis or the $\frac{1}{[S]}$-axis.

$$K_I = \frac{[E][I]}{[EI]} \tag{42}$$

$$\alpha K_I = \frac{[ES][I]}{[ESI]} \tag{43}$$

Mixed Inhibition (2)

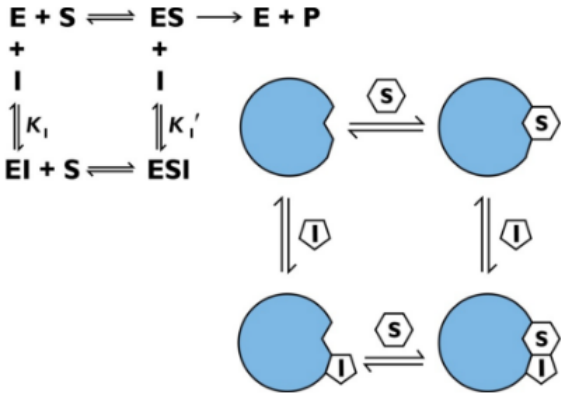

Figure 6: Mixed inhibition

Mixed Inhibition (3)

$$\frac{[E]_o[S]}{[ES]} = K_M\Big(1+\frac{[I]}{K_I}\Big)+\Big(1+\frac{[I]}{\alpha K_I}\Big)[S] \tag{44}$$

$$v_o = \frac{v_{max}[S]}{K_M\Big(1+\frac{[I]}{K_I}\Big)+\Big(1+\frac{[I]}{\alpha K_I}\Big)[S]} \tag{45}$$

Taking the inverse of Eq. (45), we have

$$\frac{1}{v_o} = \frac{K_M}{v_{max}}\frac{1}{[S]}\Big(1+\frac{[I]}{K_I}\Big)+\frac{1}{v_{max}}\Big(1+\frac{[I]}{\alpha K_I}\Big) \tag{46}$$

Fig. 7 shows the Lineweaver-Burke plot

Mixed Inhibition (4)

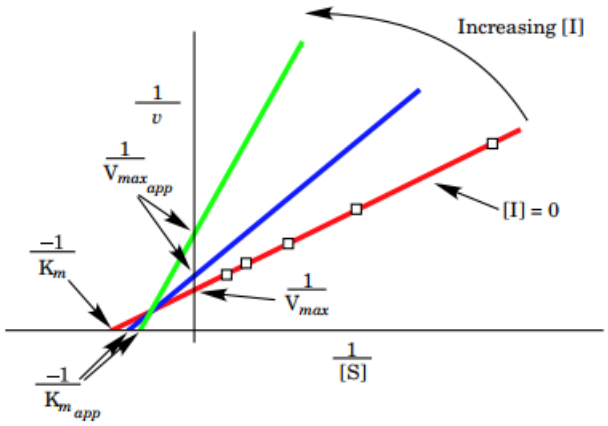

Figure 7: Lineweaver-Burke plot for Mixed inhibition

Substrate Inhibition

- Usually increase in substrate concentration increases the velocity of enzyme reaction.
- Some enzyme display the phenomenon of substrate inhibition which implies that large amount of substrate can influence and slow down the reaction.
  - For example, invertase or β-fructofuranosidase responsible for hydrolysing the disaccharide sucrose into glucose and fructose.
  - It is also observed that substrate inhibition occurs with this enzyme when two substrates molecules bind to the active site at the same time.
  - It is observed during the hydrolysis of ethyl butyrate by sheep liver carboxylesterase and during cholinester splitting process.

The kinetic model for substrate inhibition is:

$$v_o = \frac{v_{max}[S]}{K_M + [S] + \frac{[S]^2}{K_I}} \tag{47}$$

Working Session 4

Determination of type enzyme inhibition

The initial rate without and with an inhibitor is given below. Determine the nature of inhibition.

Initial Substrate concentration ($mmol/L$)	Initial rate ($mmol/L/min$)	Initial rate in the presence of 0.2 $mmol/L$ of inhibitor
1	0.00393	0.0000859
4	0.0005	0.000103
6	0.000516	0.000105
8	0.000524	0.000106
12	0.000532	0.000107
15	0.000536	0.000108
20	0.000539	0.000108

- Estimate K_M, v_{max} and K_I.
- Determine the nature of inhibition.

Solution to working session 4

The Lineweaver-Burke plot for uninhibited and inhibited reaction will be used to determine the nature of inhibition.

$1/[S]$	$1/v_0$ $[I] = 0$	$1/v_0$ $[I] = 0.2$
1.000	2544.529	11641.444
0.250	2000.000	9708.738
0.167	1937.984	9523.810
0.125	1908.397	9433.962
0.083	1879.699	9345.794
0.067	1865.672	9259.259
0.050	1855.288	9259.259

- From the plot (Fig. 8), the inhibition type is Noncompetitive.

Solution to working session 4 (2)

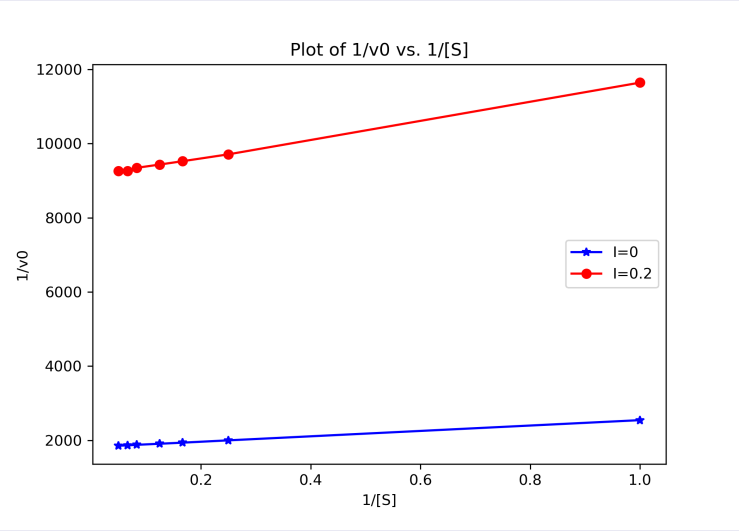

Figure 8: Lineweaver-Burke plot for solution 4

Solution to working session 4 (3)

For Noncompetitive inhibition, the Michaelis-Menten equation is

$$\frac{1}{v_o} = \frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right) + \frac{K_M}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{[S]}$$

the slope of the inhibited reaction becomes:

$$\text{slope} = \frac{K_M}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

the intercept of the inhibited reaction is:

$$\text{intercept} = \frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)$$

Therefore, dividing the slope by the intercept i.e. $slope/intercept$, we get

$$\frac{\text{slope}}{\text{intercept}} = \frac{\frac{K_M}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)}{\frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)} = K_M$$

$$K_M = 0.276985\ mmol/L$$

Solution to working session 4 (4)

The intercept of the plot of the uninhibited reaction gives $\frac{1}{v_{max}}$

$$v_{max} = \frac{1}{intercept}$$
$$v_{max} = 0.00055\ Lmin/mmol$$

The K_I is obtained using the value of intercept of inhibited reaction

$$\text{intercept} = \frac{1}{v_{max}}\left(1 + \frac{[I]}{K_I}\right)$$
$$K_I = \frac{[I]}{\text{intercept}_{inhibited} * v_{max} - 1}$$
$$K_I = \frac{0.2}{(9110.99665 \times 0.00055004) - 1}$$
$$K_I = 0.04986\ mmol/L$$

Problems

Problem 1

The initial rate of reaction for the enzymatic cleavage of deoxyguanosine triphosphate was measured as a function of initial substrate concentration as follows:

Substrate Concentration $\mu mol/L$	Initial Reaction Rate $\mu mol/L\ min$
6.7	0.30
3.5	0.25
1.7	0.16

a. Calculate the Michaelis-Menten constants of the reaction.

When the inhibitor was added, the initial reaction rate was decreased as follows:

Substrate $\mu mol/L$	Inhibitor $\mu mol/L$	Initial Reaction Rate $\mu mol/L\ min$
6.7	146	0.11
3.5	146	0.08
1.7	146	0.06

Is this competitive inhibition or noncompetitive inhibition? Justify your answer by showing the effect of the inhibitor graphically.

Problem 2

Eadie (1942) measured the initial reaction rate of hydrolysis of acetylcholme (substrate) by dog serum (source of enzyme) in the absence and presence of prostigmine (inhibitor), $1.5x10^{-7}$ mol/L and obtained the following data:

	Initial Reaction Rate ($mol/L\ min$)	
Substrate Conc. (mol/L)	Absence of Prostigmine	Presence of Prostigmine
0.0032	0.111	0.059
0.0049	0.148	0.071
0.0062	0.143	0.091
0.0080	0.166	0.111
0.0095	0.200	0.125

(a) Determine the nature of the inhibition.

(b) Evaluate K_M, K_I and v_{max}.

Problem 3

An enzyme (cathepsin) hydrolyzes L-glutamyl-L-tyrosine to carbobenzoxy-L-glutamic acid and L-tyrosine. It has been found that the glutamic acid formed in the hydrolysis, inhibits the progress of the reaction by forming a complex with cathepsin. The course of the reaction is followed by adding tyrosme decarboxylase which evolves CO_2.

Substrate $\mu mol/mL$	Inhibitor $\mu mol/mL$	Initial Reaction Rate $\mu mol/mL\ min$
4.7	0	0.0434
4.7	7.57	0.0285
4.7	30.30	0.0133
10.8	0	0.0713
10.8	7.57	0.0512
10.8	30.30	0.0266
30.3	0	0.1111
30.3	7.57	0.0909
30.3	30.30	0.0581

Calculate

(a) the value of Michaelis-Menten constants of the enzyme, and

(b) the dissociation constant of enzyme-inhibitor complex, K_I.

(c) What is type of inhibition does the glutamic acid formed exhibit?