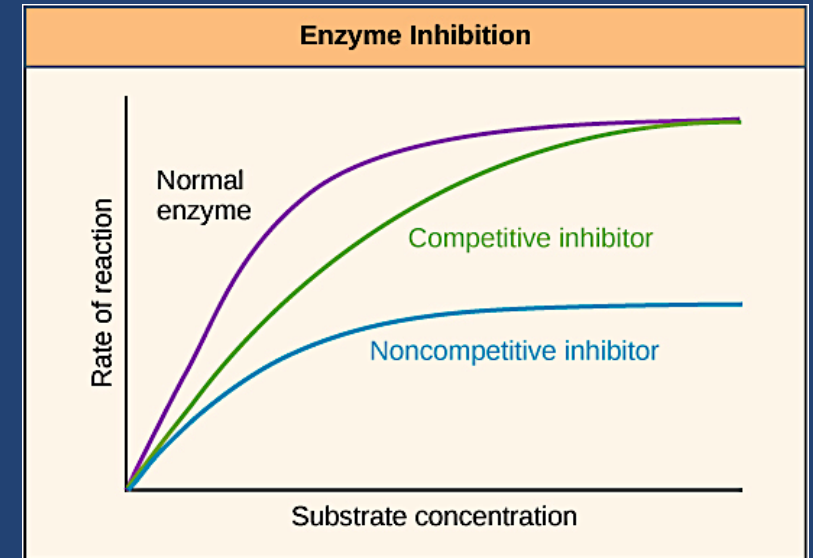


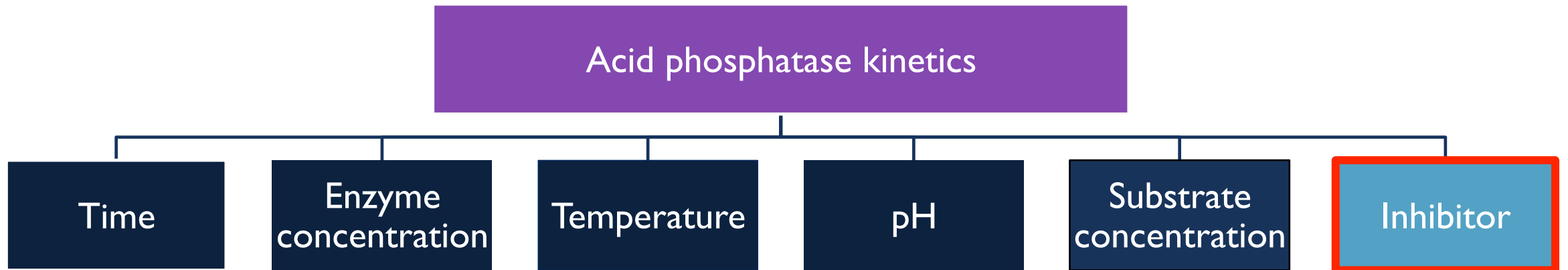
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EXP (8)

THE EFFECT OF INHIBITORS ON THE RATE OF AN ENZYME CATALYZED REACTION



In this experiment, we will continue to study acid phosphatase kinetics.



OBJECTIVES

- To study the effect of inhibitors on the rate of an enzymatic reaction.
- To determine the type of inhibition of acid phosphatase by K_2PO_4 and sodium fluoride (NaF).

INTRODUCTION

- Inhibitors are chemicals that reduce the rate of enzymatic reactions.
- They are usually specific and they work at low concentrations.
- They block the enzyme but they do not usually destroy it.
- Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors.

Types of inhibitors

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graph TD; A[Types of inhibitors] --> B[Irreversible inhibitors]; A --> C[Reversible inhibitors]; C --> D[Competitive]; C --> E[Noncompetitive]; C --> F[Uncompetitive];
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Irreversible inhibitors

Reversible inhibitors

Competitive

Noncompetitive

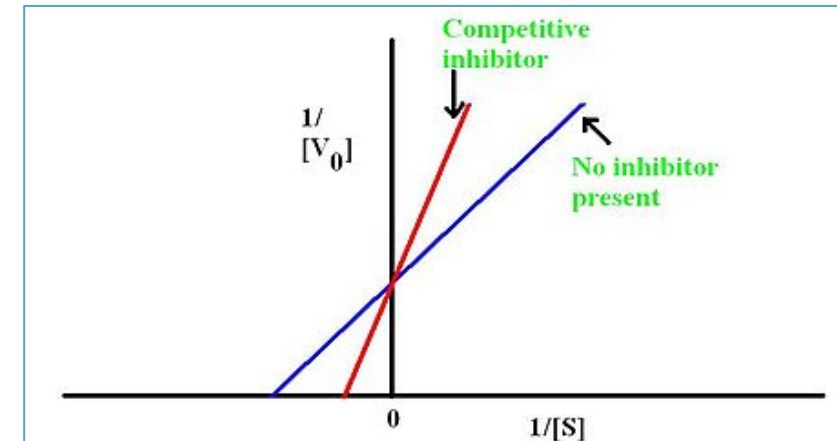
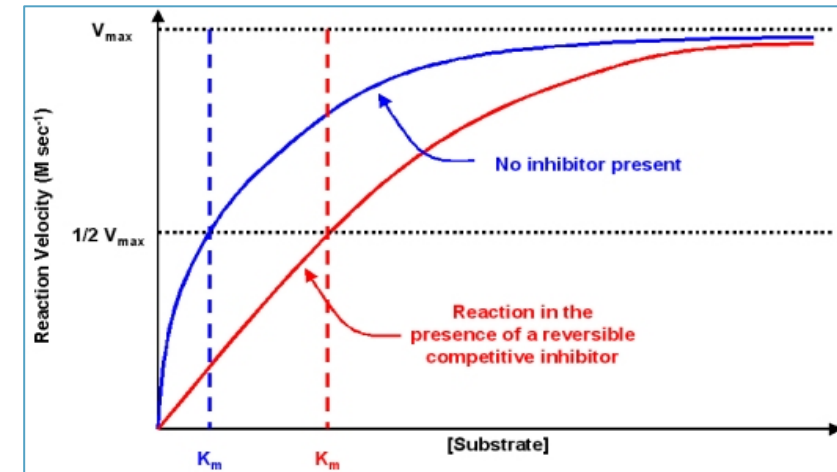
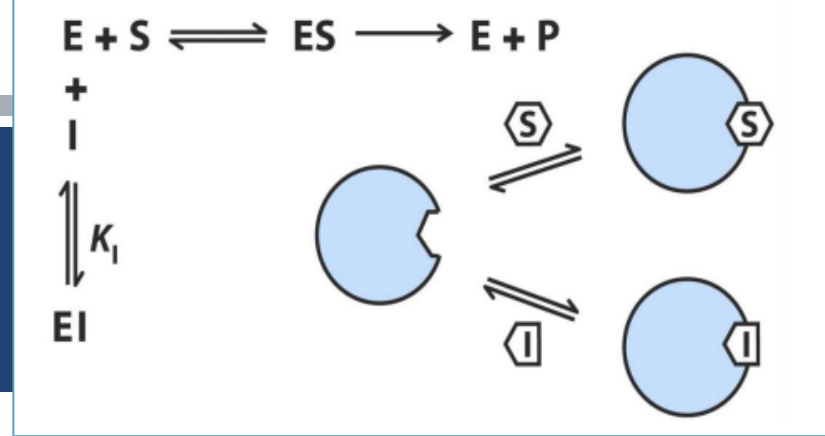
Uncompetitive

	Irreversible inhibitors	Reversible inhibitors
Type of bonds with E	Inhibitors bind covalently with enzyme	Inhibitors bind non-covalently with enzyme
Removal	Cannot be removed by dialysis or other way	Can be removed by dialysis
Activity Restoration	Permanently modify the active site residues(functional group) which the enzyme become inactive.	Removal of the inhibitor restores enzyme activity

It is relatively simple to distinguish the three types of reversible inhibition by comparing the Michaelis-Menten and Lineweaver-Burke kinetics (V_{\max} and K_m) in the presence and absence of the inhibitor.

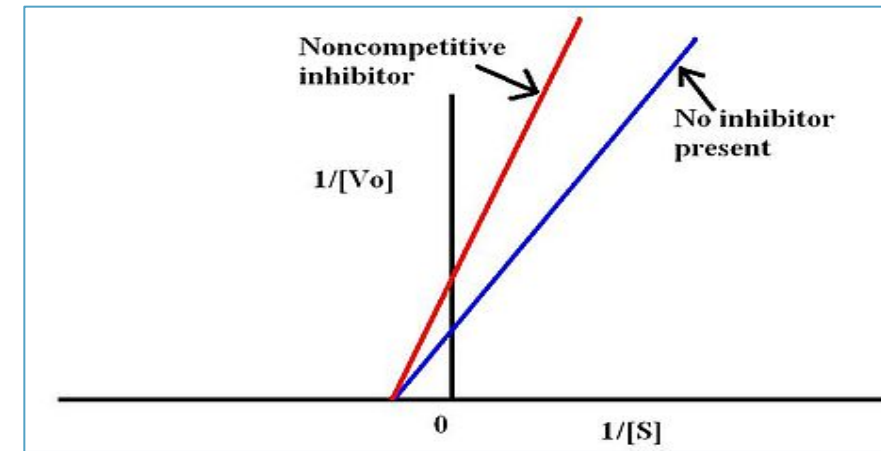
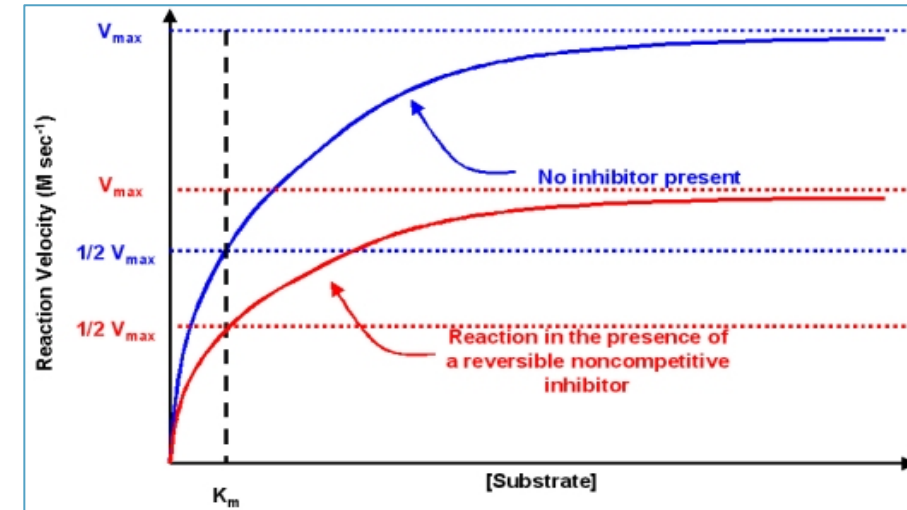
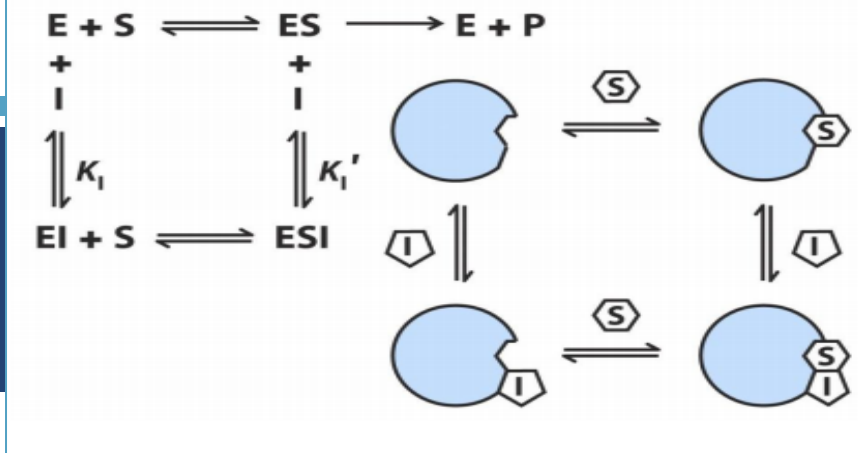
COMPETITIVE INHIBITORS

- As the name implies, the inhibitor compete with the substrate for active site of the enzyme.
- The substrate and inhibitors have resemble structures
- Have the same V_{max} (with I OR without I)
- K_m high and affinity low (with I)
- This type of inhibition can be overcome by sufficiently high concentrations of substrate by out-competing the inhibitor



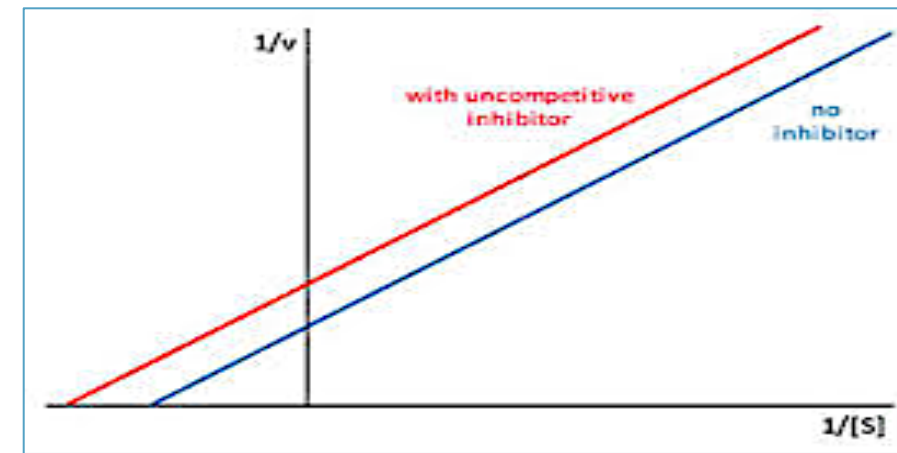
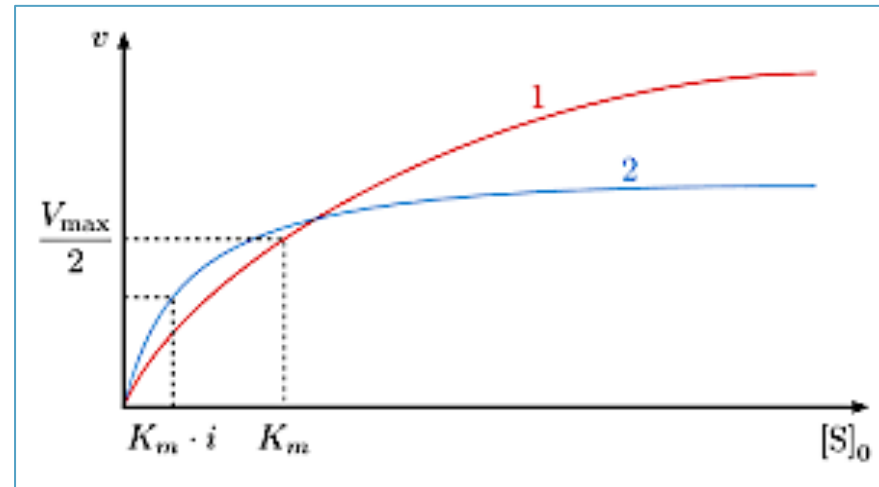
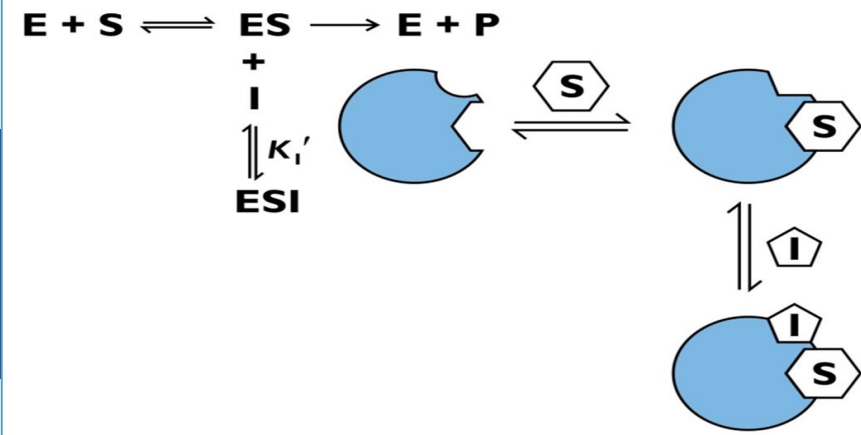
NONCOMPETITIVE INHIBITORS

- The inhibitors bind with site on the enzyme other than active site
- They can bind with E or ES complex.
- Have the same K_m (with I OR without I)
- low V_{max} (with I)



UNCOMPETITIVE INHIBITORS

- The inhibitor binds only to the substrate-enzyme complex
- Both V_{\max} and K_m are low (with I)



METHOD

- **K_2PO_4 and sodium fluoride** are inhibitors of acid phosphatase and it is your task to determine whether they are competitive, noncompetitive, or uncompetitive inhibitor.
- The setup is basically the same as in the experiment for the effect of substrate concentration on reaction velocity, except that a constant amount of inhibitor is added.
- The kinetics for the uninhibited reactions must be compared with those of reactions run in the presence of the inhibitor.
- Determinations of V_{max} and K_m will help you to determine the specific mode of inhibition

Method

Without I

- Prepare 8 tubes labeled as follows

Tube	A	B	C	D	E	F	G	H
[S] mM	0	0.5	1	2.5	5	10	25	50

- To each of these tubes add

Chemical	Volume (ml)
pH sodium acetate buffer	0.5
0.1M MgCl ₂	0.5
Corresponding p-nitrophenyl phosphate (pNPP)	0.5
Water	5

With I

- Prepare 8 tubes labeled as follows

Tube	A	B	C	D	E	F	G	H
[S] mM	0	0.5	1	2.5	5	10	25	50

- To each of these tubes add

Chemical	Volume (ml)
pH sodium acetate buffer	0.5
0.1M MgCl ₂	0.5
Corresponding p-nitrophenyl phosphate (pNPP)	0.5
Water	4
K ₂ HPO ₄ or Sodium fluoride (NaF)	1

- Place the tubes in a test tube rack situated in 37°C water bath and let stand for 5 min.

- Start the reaction by adding 0.5 ml enzyme and stop it by adding 0.5 ml KOH as in the following table:

Tube	Start the reaction	Stop the reaction
A	0 min	0 min
B	0 min	5 min
C	2 min	7 min
D	4 min	9min
E	6 min	11 min
F	8 min	13 min
G	10 min	15 min
H	12 min	17 min

- Determine the absorbance at 405 nm for each sample, using the first tube (0 mM of S) as the blank.

RESULTS

Tube	[S] (mM)	I/[S] (I/mM)	Abs at 405 nm		V=(A × 10 ⁶) / (18.8 × 10 ³ × time) (μmole of PNP/min)	I/V (I/ μmole of PNP/min)
			Without I	With I		
A	0					
B	0.5					
C	1					
D	2.5					
E	5					
F	10					
G	25					
H	50					

RESULTS

- Draw the curve using Michaelis-Menten, determine V_{max} and K_m for acid phosphatase of both inhibited and not inhibited reaction
- Prepare the double –reciprocal plot of Lineweaver-Burk and determine the K_m and V_{max} from the x and y intercepts of both inhibited and not inhibited reaction

DISCUSSION

- An introductory statement [**In this experiment, we studied the effect of inhibitors on the rate of acid phosphatase catalyzed reaction. The setup was basically the same as in the experiment for the effect of substrate concentration on reaction velocity, except that a constant amount of inhibitor (K_2PO_4 or *sodium fluoride*) was added**]
- Principle
- Compare the V_{max} and K_m obtained Michaelis-Menten and Lineweaver-Burk graphs of both inhibited and uninhibited reactions with each other to determine the type of inhibition.

QUESTIONS

- Determine the type of inhibition of K_2PO_4 and sodium fluoride. Justify your answer and discuss the difference you find

	K_2PO_4	NaF
Type of inhibition	Competitive inhibitor	Noncompetitive inhibitor
Resemblance to S structure	Resemble S structure	Does not resemble S structure
Binding with E	Bind with free E	Bind with free E or ES complex
Activity restore	High [S] remove I from the active site	High [S] <u>cannot</u> remove I from the active site
Vmax and Km	Same Vmax, high Km	Low Vmax, same Km