

Slide : Enzymes 2

Dr. Name : Nayef Karadsheh

Sections : 4, 5, 6

■ Slide □ Sheet



Medical Committee
The University of Jordan

Biochemistry

cybernetics
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astrobiology
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electrobiology
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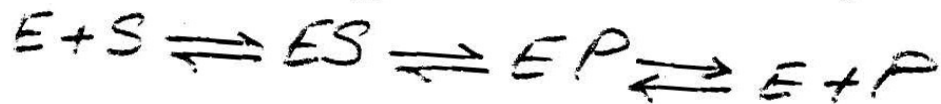


Mousa Suboh

Enzymes

- Historical Background

I. Enzyme Catalyzed Reaction



- Enzymes are Proteins
(Exception Ribozymes)
- High Catalytic Power
increase rate by 10^6 to 10^{14} fold
- High Specificity
- Enzymes are Regulated

A. The Active site

- 3-dimensional structure
- Role of functional groups, Cofactors
- Transition state

B. Substrate Binding site

1. Lock-and-key Model

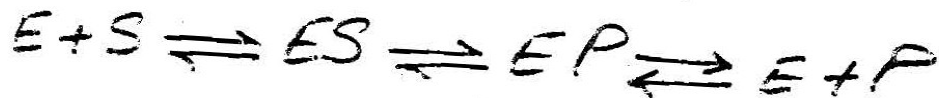
2. Induced-fit Model

C. The Transition State Complex

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Active Sites of Enzymes Have Some Common Features :-

- The Catalytic groups

- The active site takes up a relatively small part of the total volume of an enzyme.

- The active site is a three-dimensional entity formed by groups that come from different parts of the linear amino-acid sequence.

- Substrates are bound to enzymes by multiple weak interactions.

- Active sites are clefts or crevices

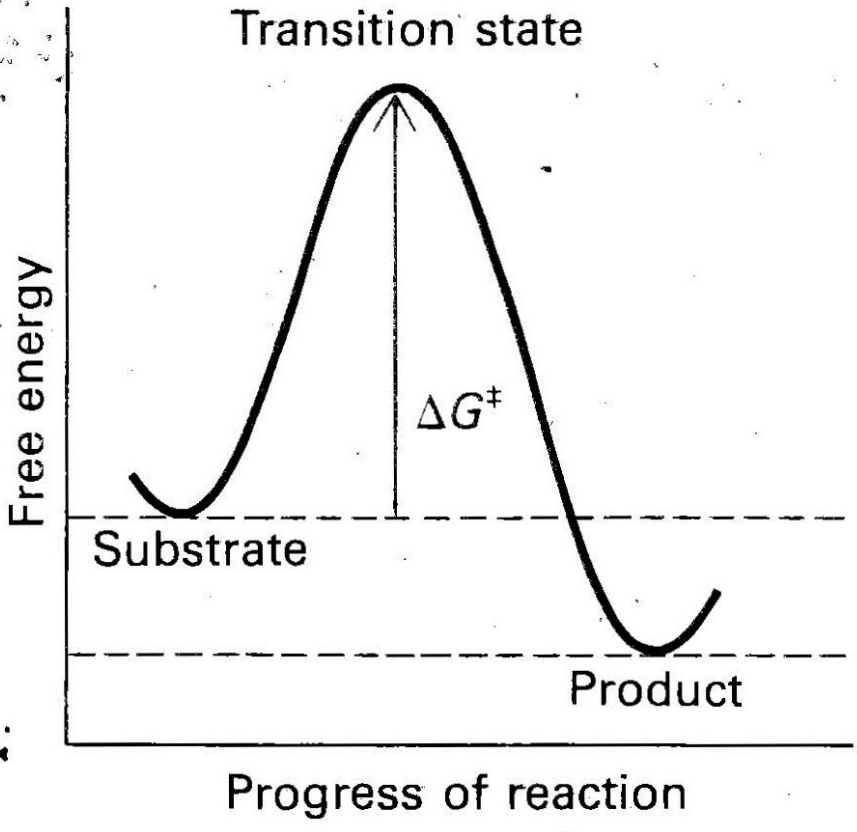
- The specificity of binding depends on the precisely defined arrangements of atoms in an active site.

Emil Fischer's Lock & Key model

Koshland's Induced Fit model

Transition state

74
④a



Transition state:

• Bonds in substrate are x. strained

In others

Electronic configuration in substrate A

comes very strained and unstable

energy

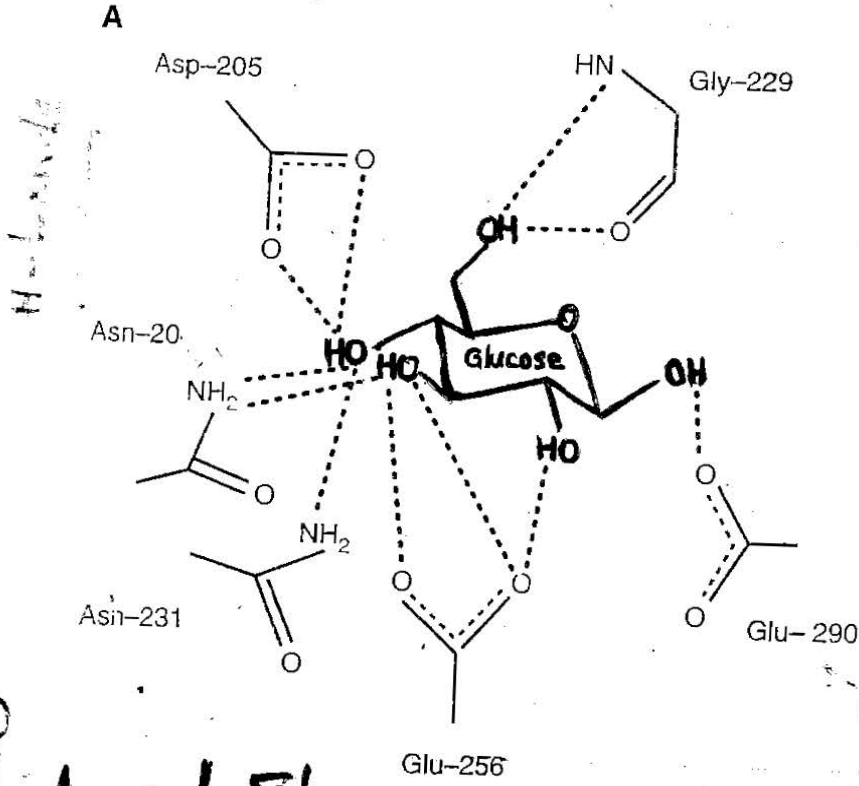


Substrate Binding sites

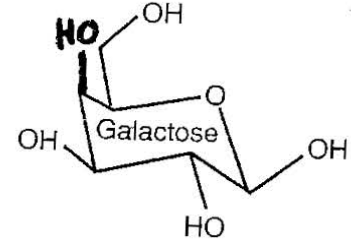
S binds E through

- hydrophobic
- Electrostatic
- H-bonds

3



B

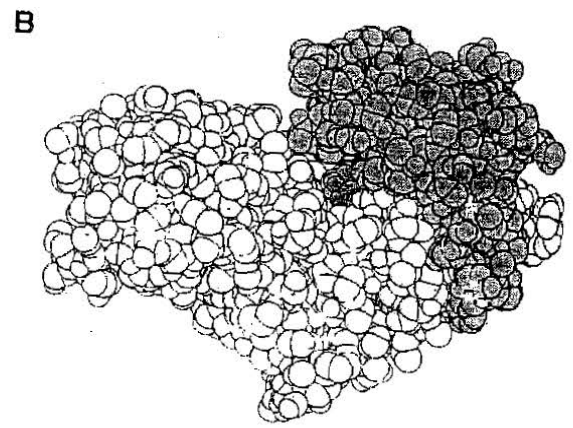
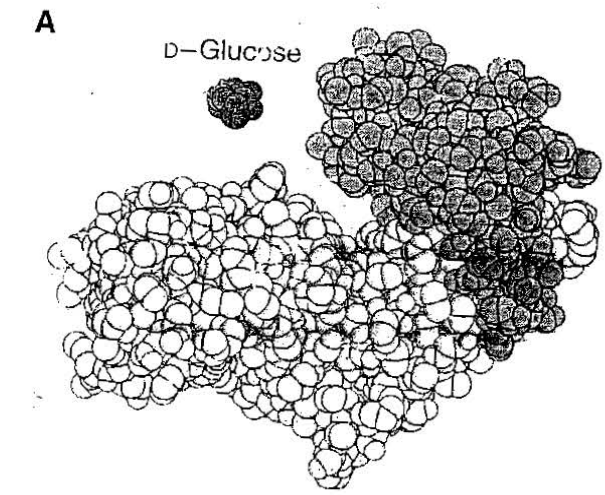


① Lock-and-key model for substrate binding

D

Induced Fit Model

Yeast HK
or human GK



The energy diagram for the decomposition of H_2O_2

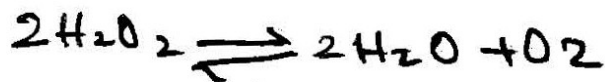
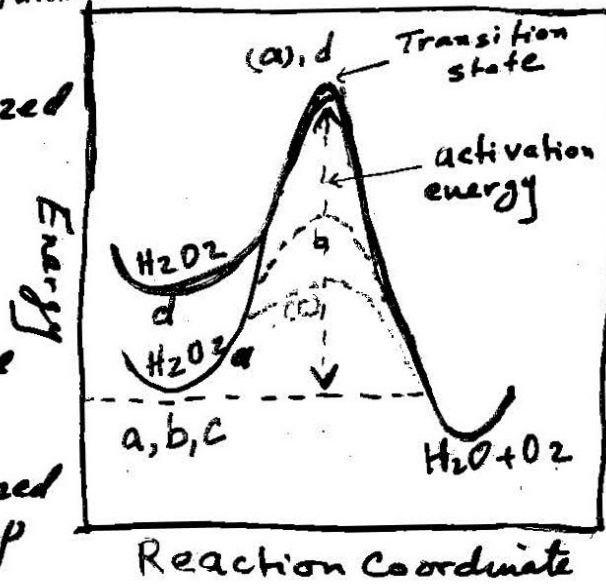
$$2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2$$

Curve a :- uncatalyzed

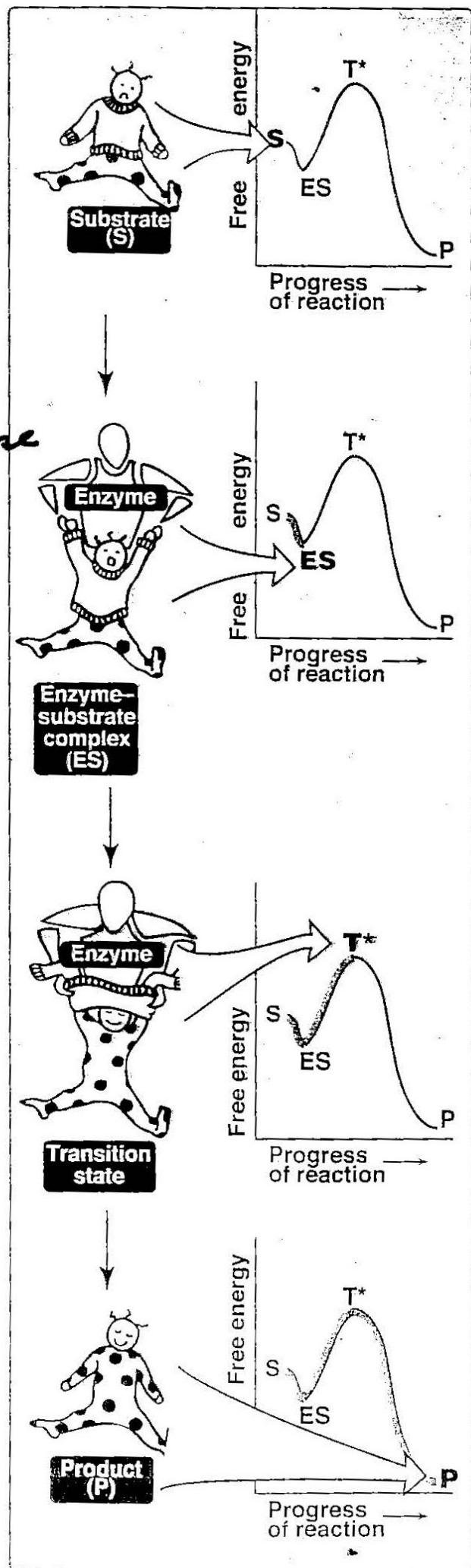
Curve b :- + iron catalyst
↑ 30,000

Curve c :- + Catalase
↑ 100,000,000

Curve d :- uncatalyzed
but at elevated temp

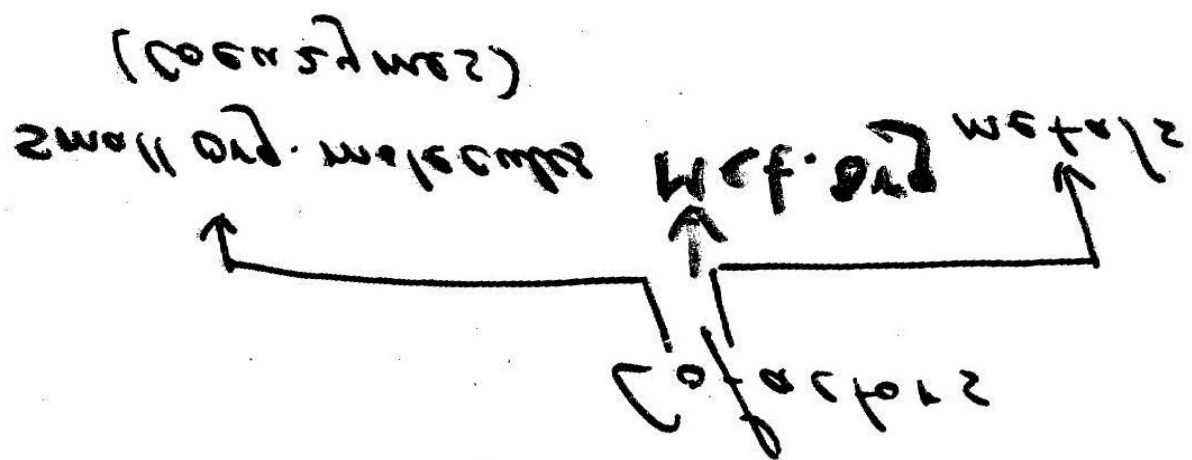
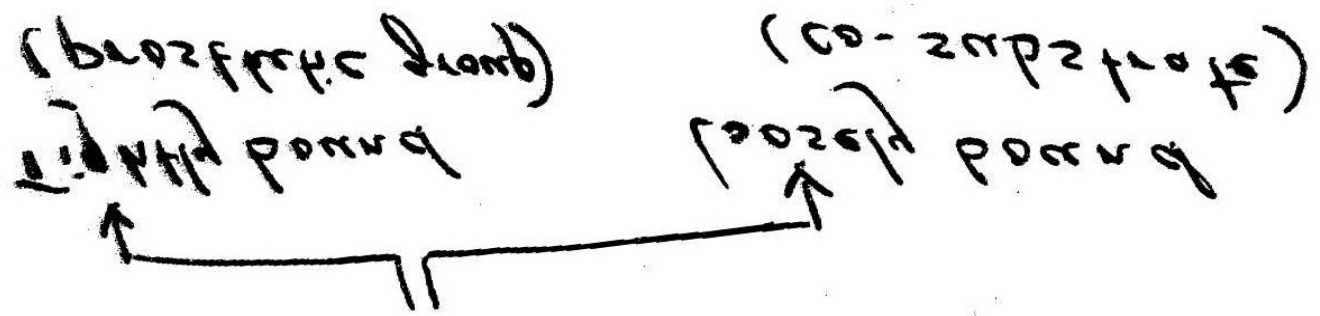


A dip occur because initial multiple weak bonds between E and S



B12
 5' deoxythymine
 Biotin
 Co A
 5' deoxyuracil
 SAM
 SAM
 [Methyltransferase]

K
 W_u⁺
 Z_u⁺
 W₀
 W_i
 W_u⁺
 W_u⁺
 W_u⁺
 W_u⁺



SAM + Cofactor = SAM + Cofactor

Definition

SAM - Cofactor

II Functional Groups in Catalysis

Mechanism of Enzyme Action Involves

- Proximity and Orientation - All
- Electrostatic Interaction to stabilize transition state - All
- Covalent intermediates - some

Enzymes Employ:-

A. Functional groups of Amino Acid side chains

All polar amino acids are involved
e.g. Ser, Cys, Lys, His

B. Coenzymes in Catalysis

→ Provides functional groups

→ Made from vitamins

1. Activation-Transfer-Coenzymes

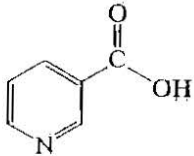
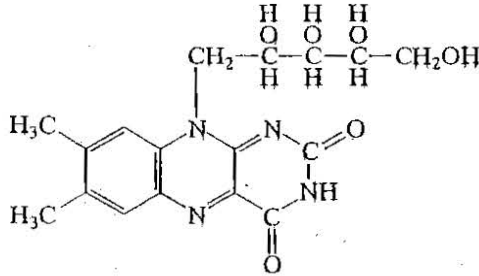
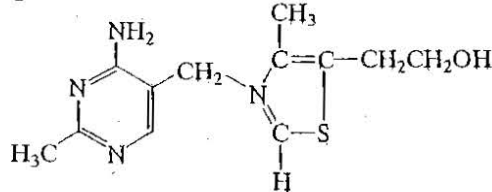
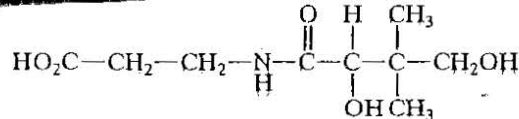
Functional gr. of Coenzyme binds covalently to [S], another portion binds tightly to [E]

2. Oxidation-Reduction Coenzymes

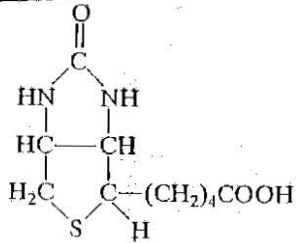
TABLE 19.1 The Water-Soluble Vitamins and the Coenzymes of Which They Are Structural Components

Vitamin	Coenzyme	Function
Thiamine (B ₁)	Thiamine pyrophosphate	Decarboxylation reactions
Riboflavin (B ₂)	Flavin mononucleotide (FMN)	Carrier of H atoms
	Flavin adenine dinucleotide (FAD)	
Niacin (B ₃)	Nicotinamide adenine dinucleotide (NAD ⁺)	Carrier of hydride ions
	Nicotinamide adenine dinucleotide phosphate (NADP ⁺)	
Pyridoxine (B ₆)	Pyridoxal phosphate	Carriers of amino and carboxyl groups
	Pyridoxamine phosphate	
Cyanocobalamin (B ₁₂)	Deoxyadenosyl cobalamin	Coenzyme in amino acid metabolism
Folic acid	Tetrahydrofolic acid	Coenzyme for 1-C transfer
Pantothenic acid	Coenzyme A	Acyl group carrier
Biotin	Biocytin	Coenzyme in CO ₂ fixation
Ascorbic acid	Unknown	Hydroxylation of proline and lysine in collagen

Table 6.1
Characteristics of vitamins and coenzymes

Name/Structure of Vitamin	Related Coenzyme	Reaction type (page numbers ^a)	Deficiency Disease
Water-Soluble Vitamins			
Niacin 	NAD⁺, NADP⁺	Oxidation-reduction (515-524)	Pellagra
Riboflavin (vitamin B₂) 	FAD, FMN	Oxidation-reduction (405-411)	Growth retardation
Thiamine (vitamin B₁) 	Thiamine pyrophosphate	Decarboxylation (16-18)	Beriberi
Pantothenic acid (vitamin B₃) 	Coenzyme A	Acyl group activation and transfer (pp. 440-441, 442-443)	Dermatitis (chickens)

Biotin

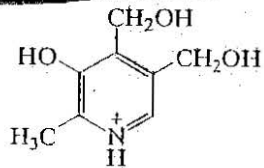


Biotinylated enzymes

CO₂ activation and transfer

Dermatitis (humans)

Pyridoxine (vitamin B₆)

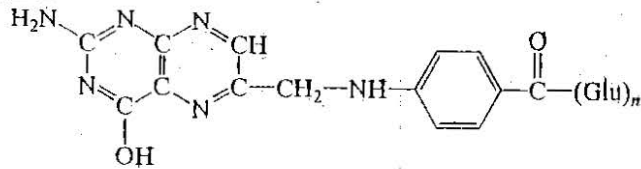


Pyridoxal phosphate

Amino group transfer
(pp. 605-606)

Dermatitis (rats):
neurological symptoms

Folic acid

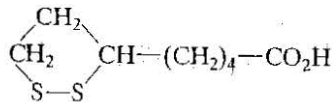


Tetrahydrofolate

Transfer of one carbon unit
(pp. 600-601)

Anemias

Lipoic acid (may not be a vitamin)

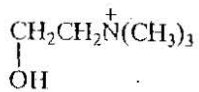


Attached to ε-NH₂ group
of Lys in protein

Acyl group activation
and transfer (pp. 485-493)

Growth deficiencies

Choline



?

(pp. 163, 171, 242-243)

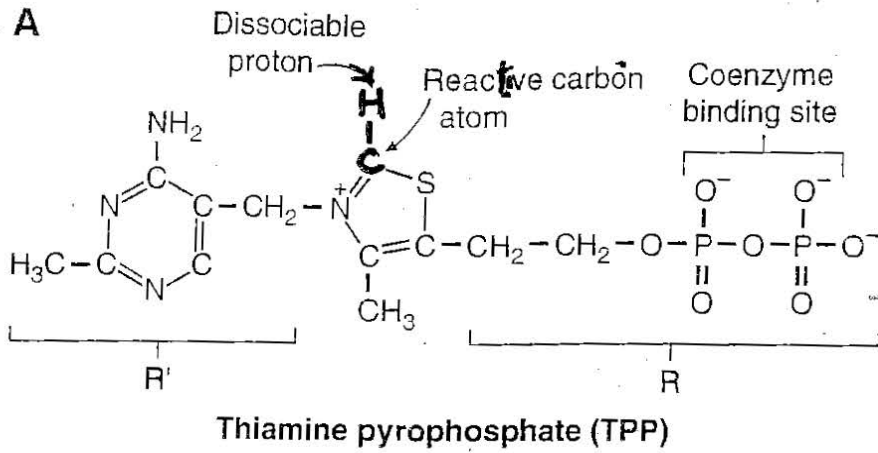
Impaired brain development

may not be Vit.

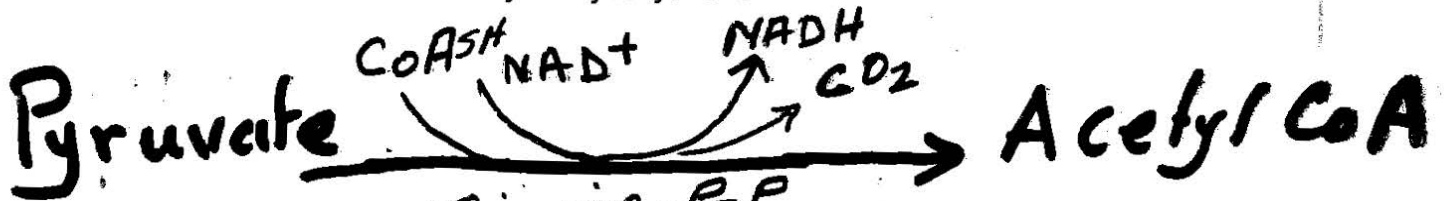
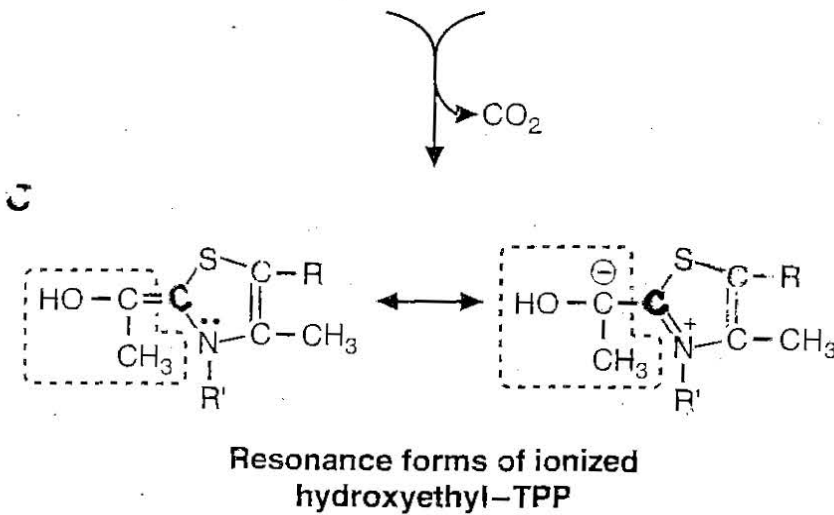
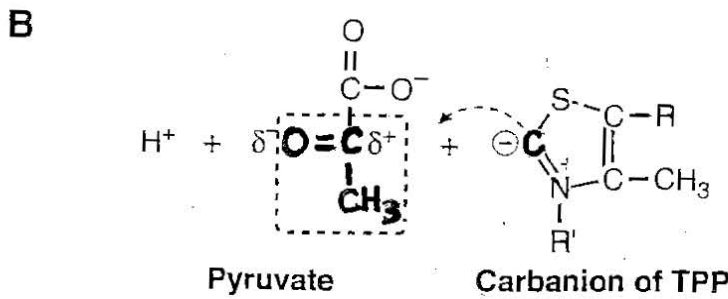
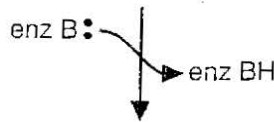
Table 8.1. Some Functional Groups in the Active Site

Function of Amino Acid	Enzyme Example
<i>Covalent intermediates</i>	
Cysteine-SH	Glyceraldehyde 3-phosphate dehydrogenase
Serine-OH	Acetylcholinesterase
Lysine-NH ₂	Aldolase
Histidine-NH	Phosphoglucomutase
<i>Acid-base catalysis</i>	
Histidine-NH	Chymotrypsin
Aspartate-COOH	Pepsin
<i>Stabilization of anion formed during the reaction</i>	
Peptide backbone-NH	Chymotrypsin
Arginine-NH	Carboxypeptidase A
Serine-OH	Alcohol dehydrogenase
<i>Stabilization of cation formed during the reaction</i>	
Aspartate-COO ⁻	Lysozyme

Thiamine Pyrophosphate (TPP)

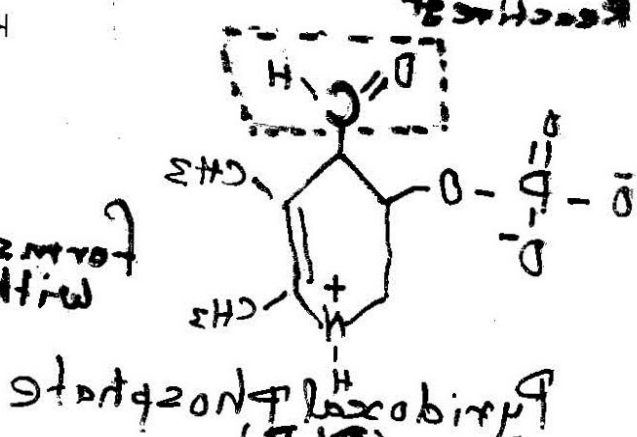
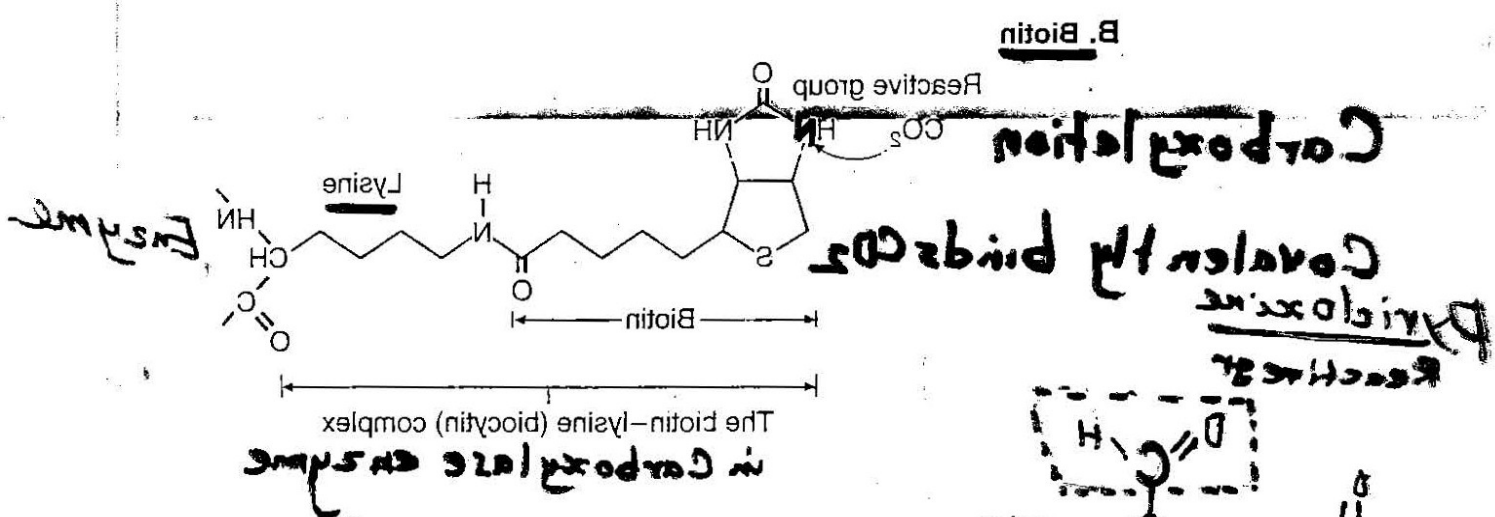
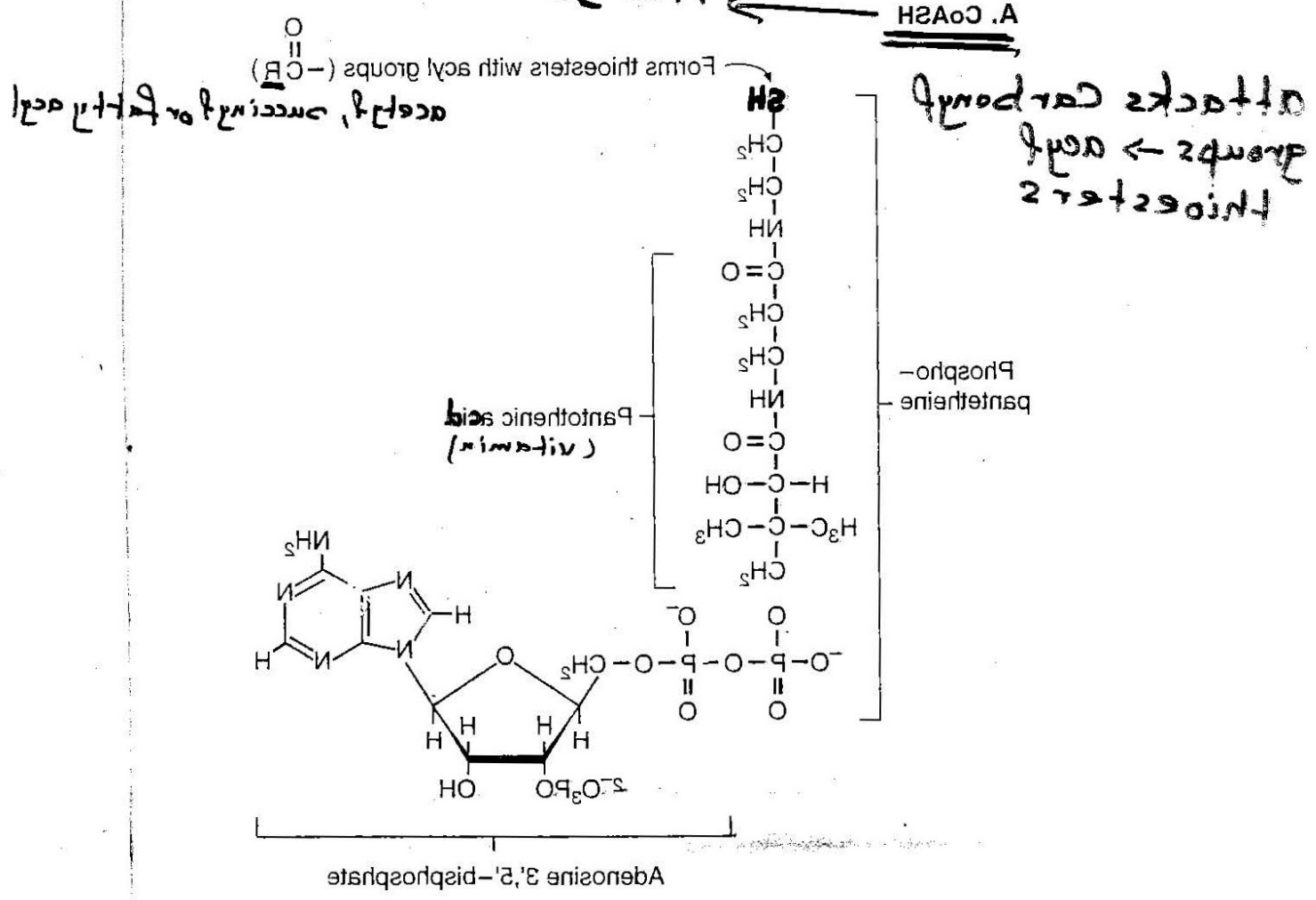


Chelate Mg^{2+}
which binds
enzyme
tightly



Pyruvate Dehydrogenase Complex

2
 A standard for the acyl
 group that forms
 a thioester
CoA
 Acetyl CoA

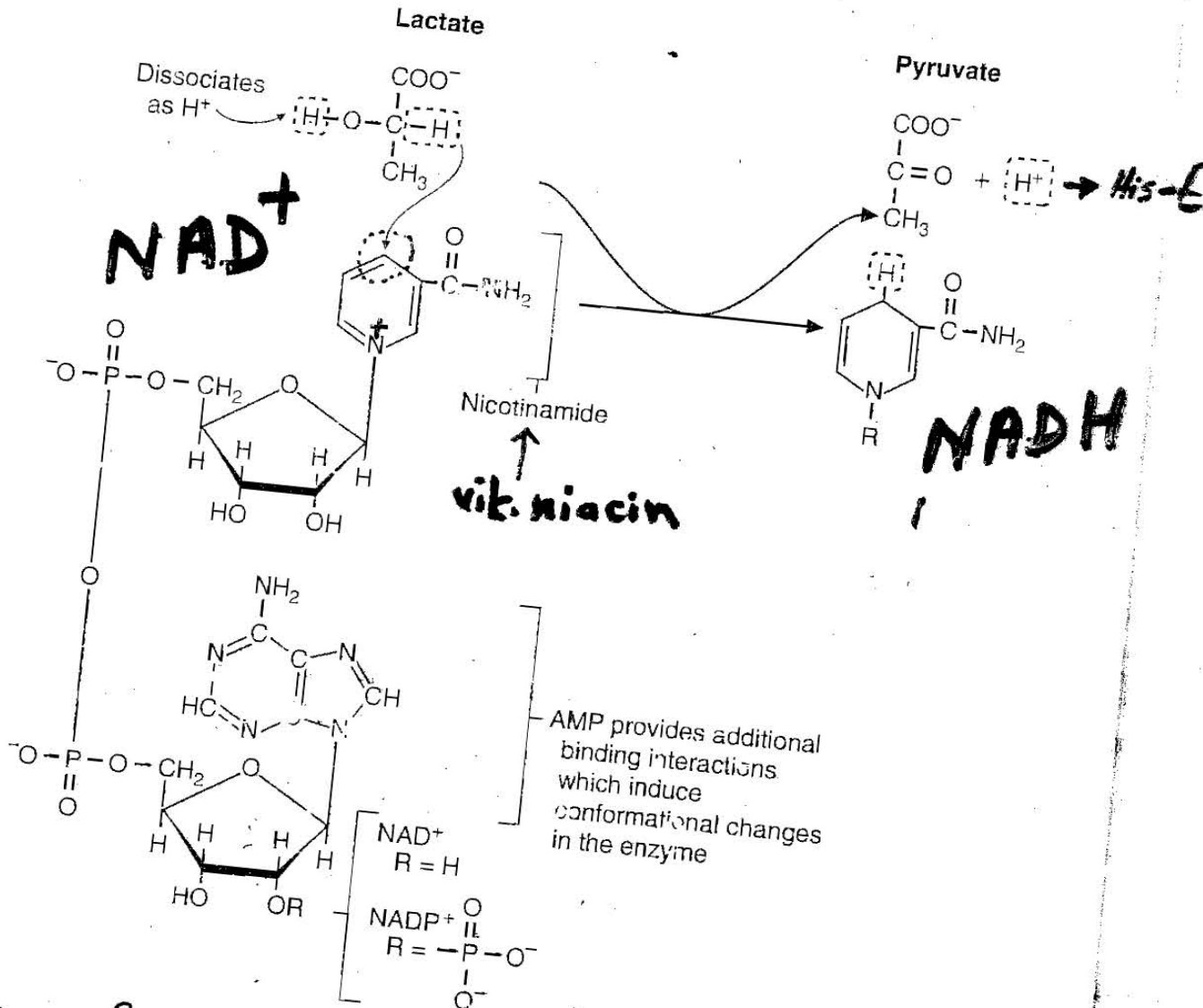


Oxidation-Reduction Coenzymes

NAD⁺ (NADP⁺)

NAD & NADH

are loosely dissociated coenzymes called coenzymes rather than substrates as they are common to so many reactions



- Transfers Hydride ions, hydrogen atoms, oxygen
- No covalent intermediates
- Require participation of amino groups of [E]
- Unique roles in generation of ATP
- Some work with metal to transfer e⁻

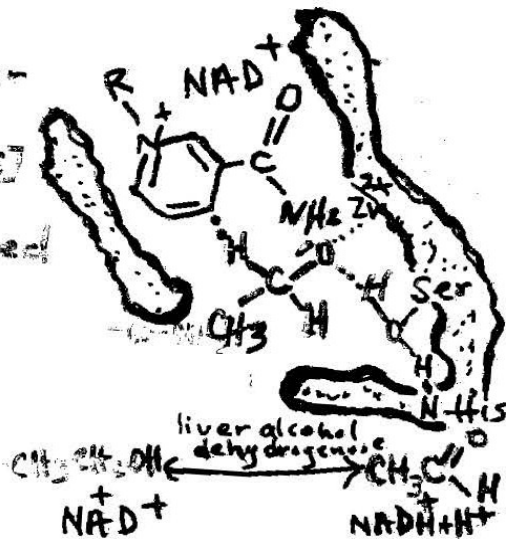
Other coenzymes

Vit E + C are ox-red coenzymes - Antioxidants



C. Metal Ions in Catalysis

- His pulls H^+ from ser
- Ser O pulls H^+ from substrate $[S]$
- O^- of $[S]$ is stabilized by Zn^{2+}



Metal ions act as electrophiles

- they assist in binding of substrates
- stabilize developing anions

e.g. Mg^{2+} in binding ATP, TPP

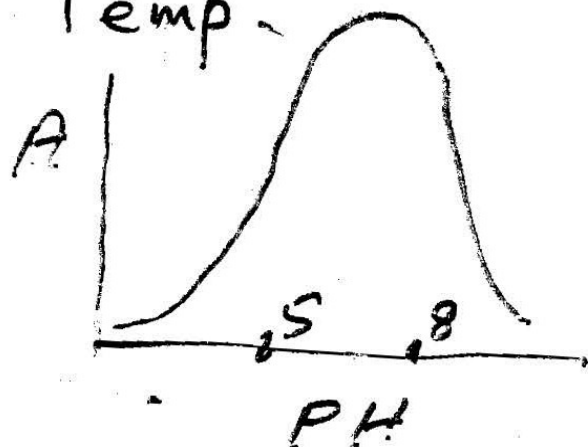
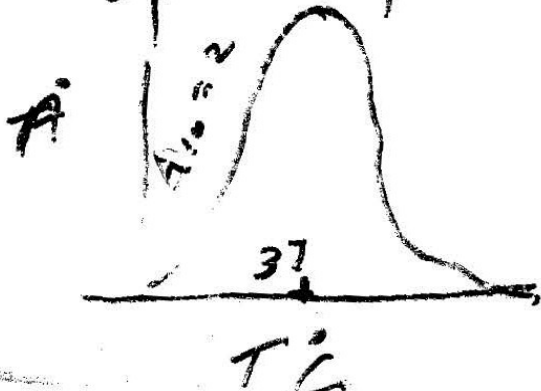
Zn^{2+} in ADH

• electron transport in Ox-Red. reactions e.g. cyt.

D. Non Catalytic Roles of Cofactors

- binding different regions \rightarrow tert. structural
- serve as $[S]$, cleaved during reaction

III Optimal pH and Temp.



Mechanism-Based Inhibitors

116

- Irreversible Inhibitors

e.g. DIFP

Aspirin (Acetylsalicylic acid)

- Suicide Inhibitors

Allopurinol

Penicillin

- Transition state analogues

- Abzymes

Abzymes:-

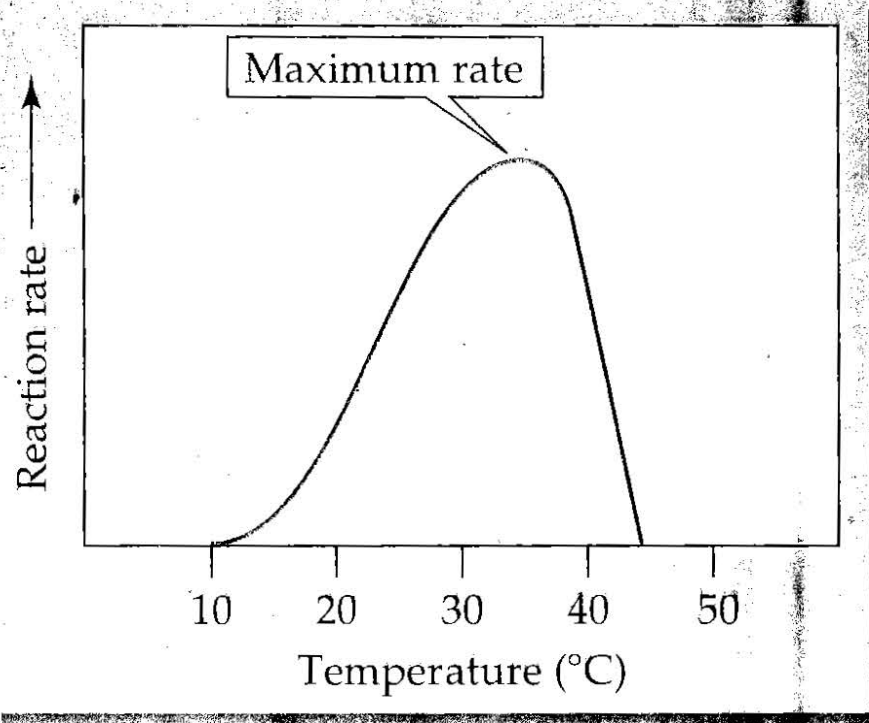
Antibodies raised against Analogs of transition-state complex

• Have an arrangement of amino acids in the variable region similar to the active site of the enzyme in the transition state

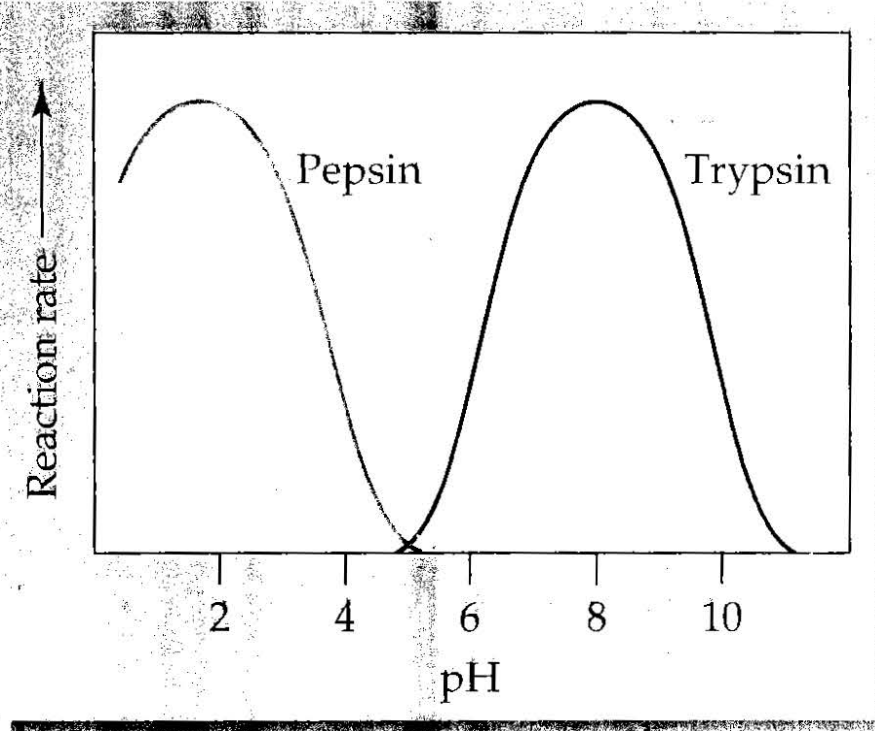
2.1. Abzyme for cocaine esterase transition state analogue → therapeutic use

T-169

Figure 19.7 Effect of temperature and pH on reaction rate



(a)

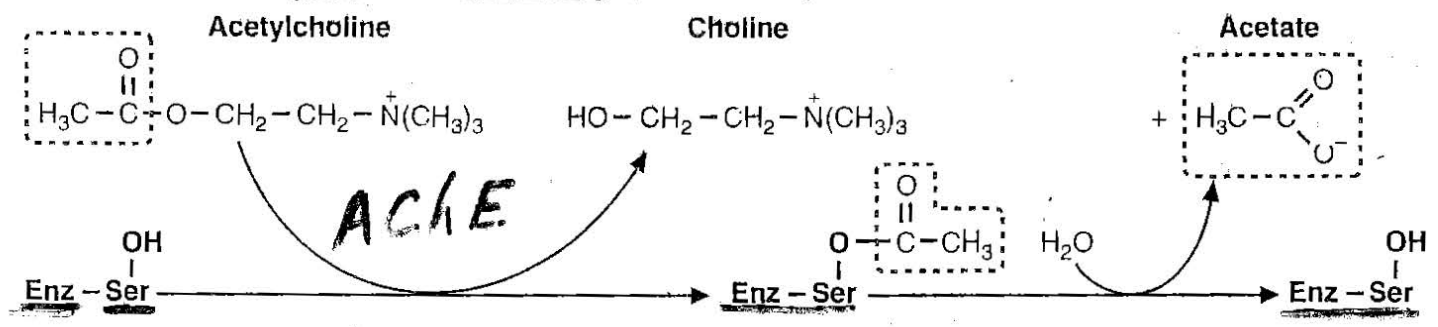


(b)

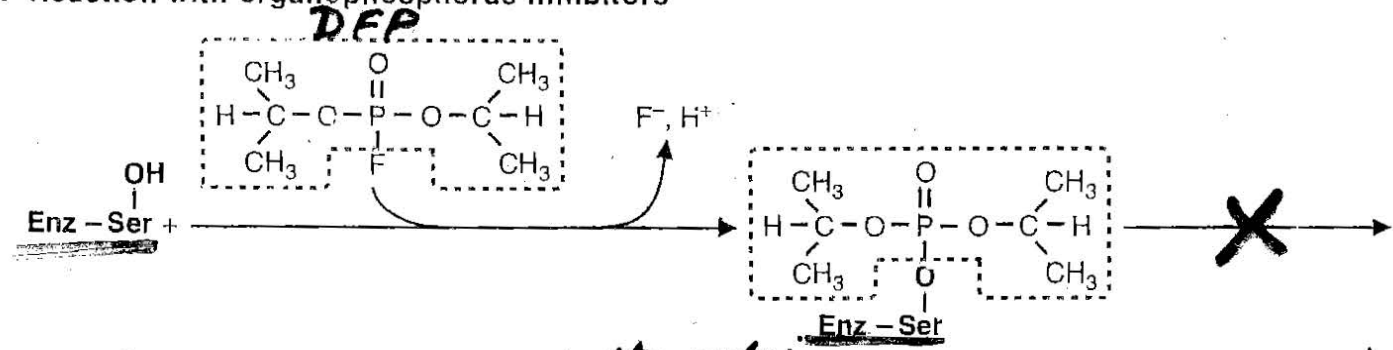
MECHANISM-BASED INHIBITORS

A- React Irreversibly with functional groups - COVALENT INHIBITORS

A. Normal reaction of acetylcholinesterase (ACHE)



B. Reaction with organophosphorus inhibitors



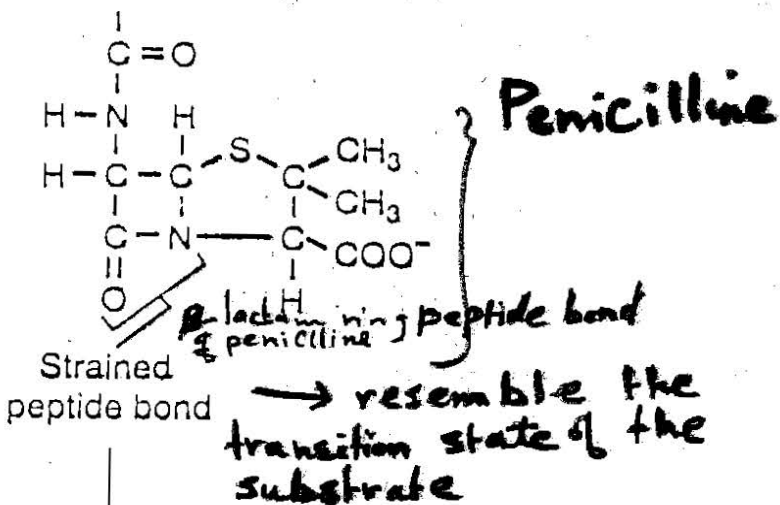
Irreversible Inhibition

of all the serines in the protein, DFP reacts only with the catalytically active serine → phosphate ester, as the functional gr. is activated by other residues in the active site.

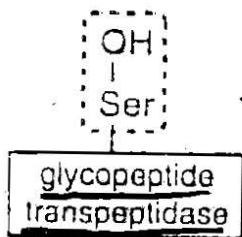
Aspirin inhibit the prostaglandin endoperoxidase synthase (cyclo-oxygenase)

B. Transition State Analogues and substrate analogues

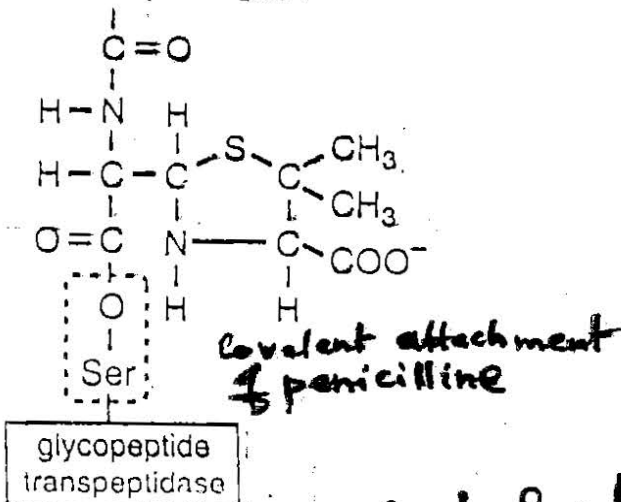
Bacterial Enzyme Glycopeptide transpeptidase



bacterial serine proteases :- cleaves peptide bond between 2 D-alanine residues. (for cross-linking bacterial cell wall)



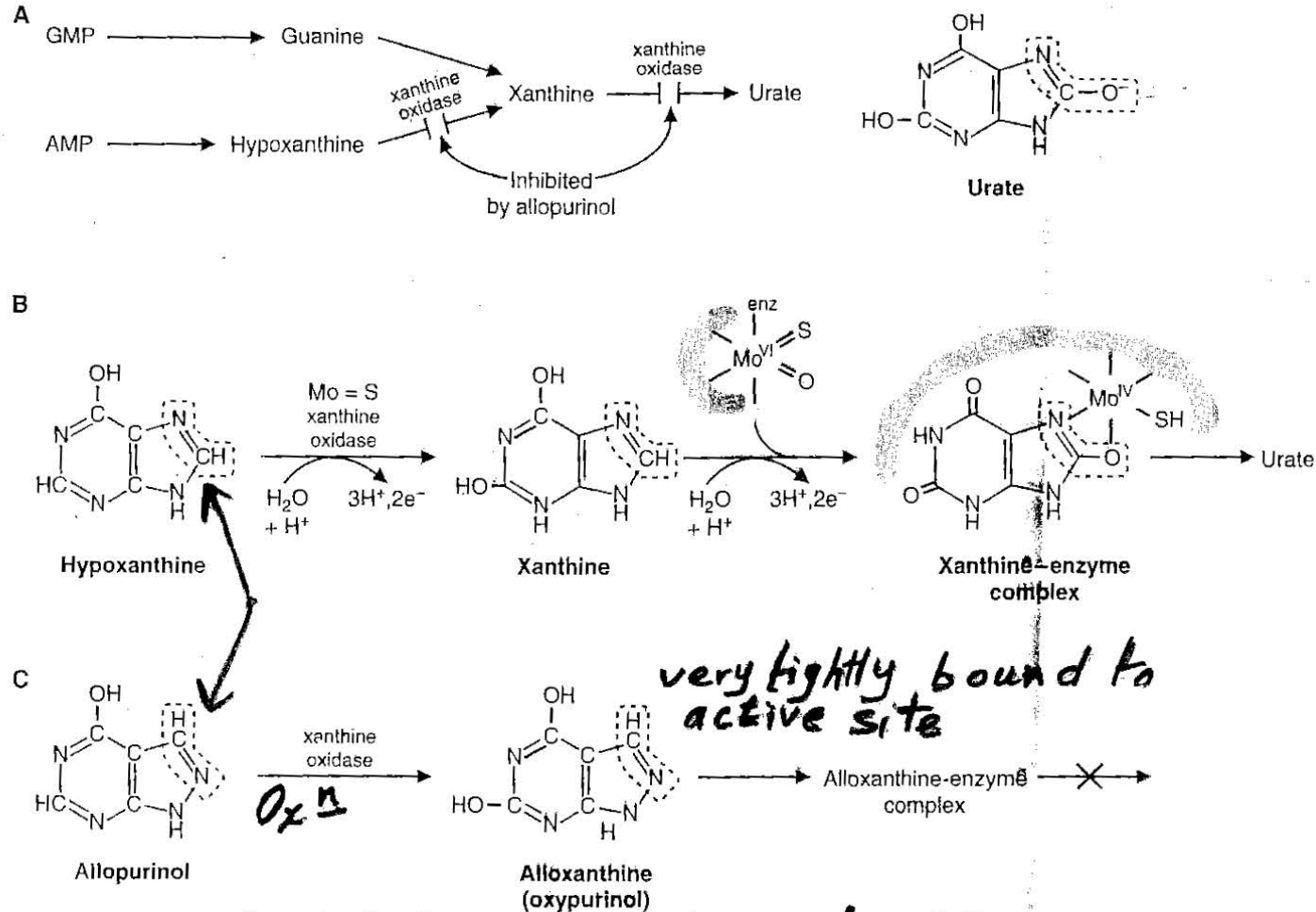
(Partial reaction to form permanent irreversible inhibitor in the active site)



suicide inhibition

Allopurinol is a suicide inhibitor of Xanthine Oxidase

2)



C - Heavy Metals Inhibition :- Hg, Pb, Al, Fe

Bind functional groups, non-specific for enzymes
 e.g. Hg binds many enzymes particularly at -SH gr.
 Lead (Pb) inhibits by replacing normal functioning metals as Ca, Fe or Zn
 - Inhibiting Ca^{2+} : Ca-Calmodulin + Protein Kinase C