Zymogens: inactive precursor of the enzyme (السلسلة البروتينية اللازمة)

becomes → activated by breaking peptide (covalent) bonds

(Irreversible / covalent not allosteric)

Ex1: Digestive Enzymes

Trypsinogen —Enteropeptidase (في الأمعاء)→ Trypsin

Trypsin (one way only) → Chemotrypsinogen (from Zymogen)

Chemotrypsinogen —Trypsin→ Chemotrypsin

Chemotrypsinogen: Zymogens, inactive, from pancreas

Chemotrypsin: active enzymes, proteolytic (تهضم البروتينات في الأمعاء)

Ex2: Hormons

Pre-insulin (Inactive) → Insulin (active)

Ex3: Clotting Factors

Prothrombin —Vitamin K, Clotting Factors, Ca+2→ Thrombin

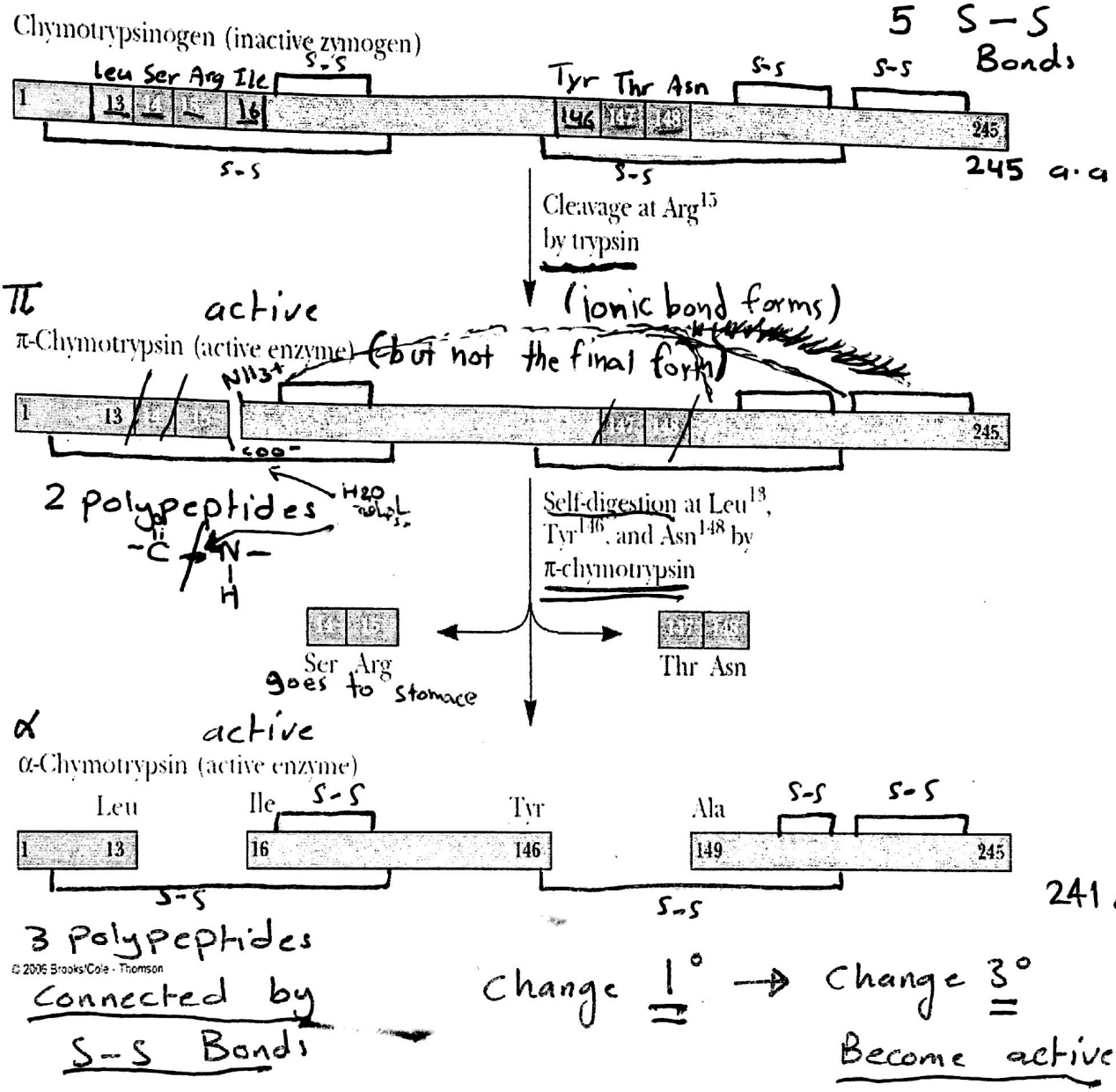
Thrombin → Fibrinogen (Soluble) (ذائب في الدم) —Break 4 peptide Bonds→ Fibrin (insoluble)

Fibrinogen: Zymogen, inactive

Fibrin: active

* Hemophilia: Genetic disease affecting clotting

activation of Chemotrypsinogen



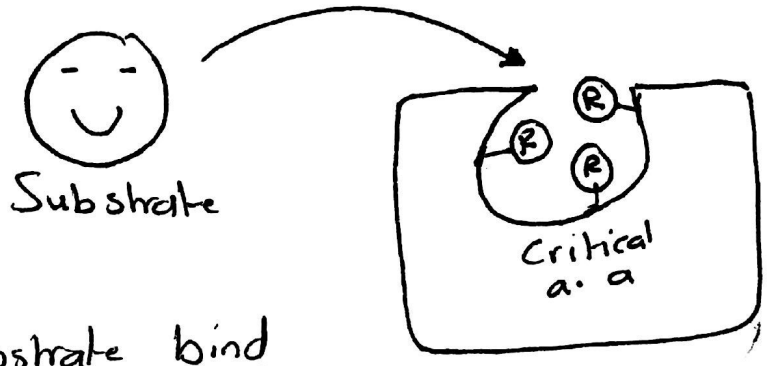
* From X-Ray Crystalography

The amino-end of Isoleucine 16 after First NH_3^+ Cleavage make ionic bond with $\overline{Asp}$ 194

Nessecary for activity

(Chemotrypsinogen Lack this ionic bond)

Active Site الموقع النشط



* the substrate bind to R-groups in the active site

these R-groups are essential and play Catalytic role

* the most common R-groups that play Catalytic role in Enzymes

1- Imidazole of Histidine
2- OH of Serine
3- COO^- of Aspartic acid and Glutamic acid
4- SH of Cystine
5- amino group of Lysine (just understand them)
6- phenol group of Tyrosine
7- α-amino-end
α-Carboxyl end

which group of amino-acids are likely to be found in the active site of an enzyme ?

a. leucine , lysine , alanine nonpolar

b. Cystein , isoleucine , phenylalanine nonpolar

c. tyrosine , threonine , leucine nonpolar

d. Serine , histidine , aspartate

Q: The amino-acids (R-Groups) in the active site can be involved in all of these processes , except :-

a. Binding of the substrate

b. Becoming part of the product of the reaction

c. The actual chemical mechanism for the reaction

d. Binding of some necessary cofactor for the reaction

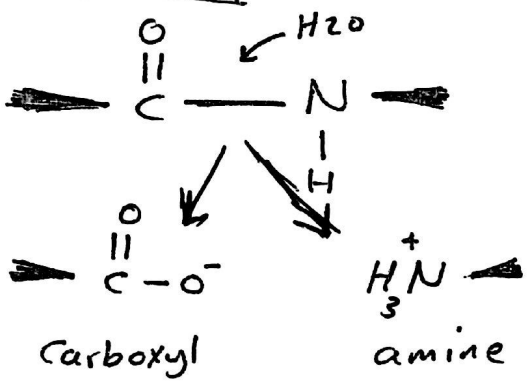
e. all of these can be functions of the amino-acids in the active site

الأنزيم المسروح بستالكمحص

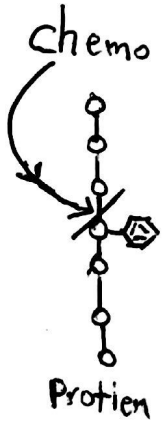
Chemotrypsin :-

Secreted by pancrease to the intestine

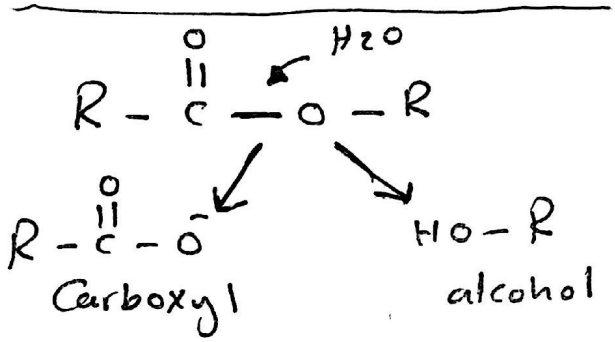
* Catalyze the hydrolysis of peptide bond at aromatic amino-acids



صم طعن البروتينات

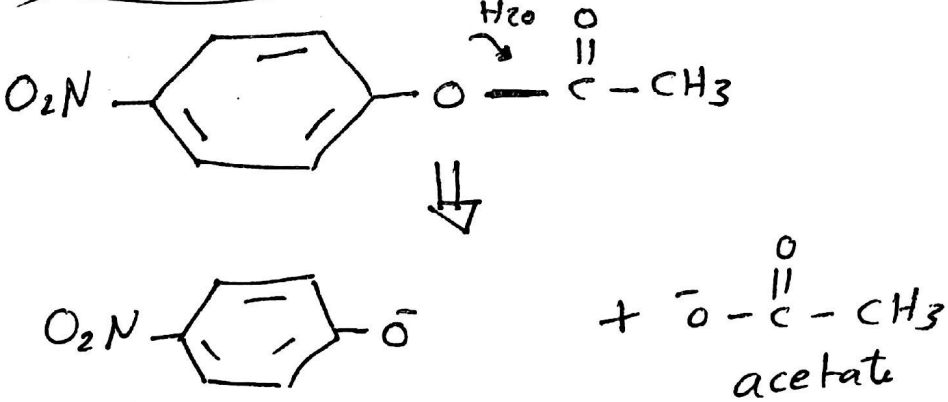


* also, break Ester bond



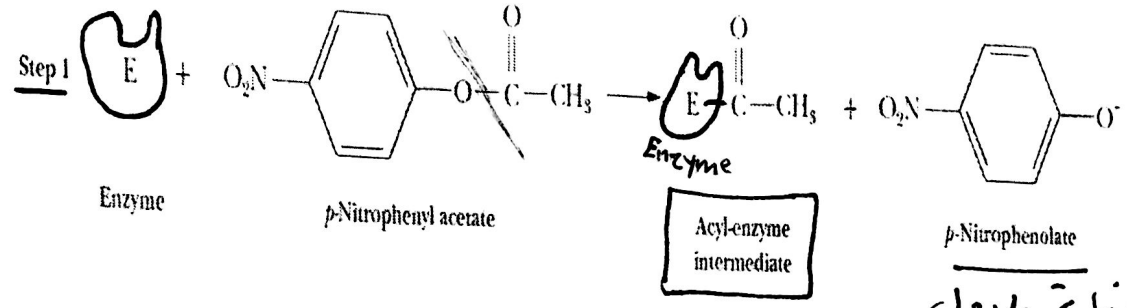
* المادة المستخدمة لدراسة هذا الأنزيم هي (في المختبر)

P- Nitro-phenyl acetate



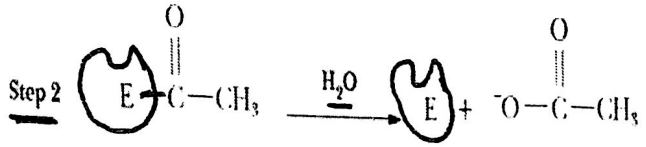
Chemotrypsin works in 2 steps

Step 1

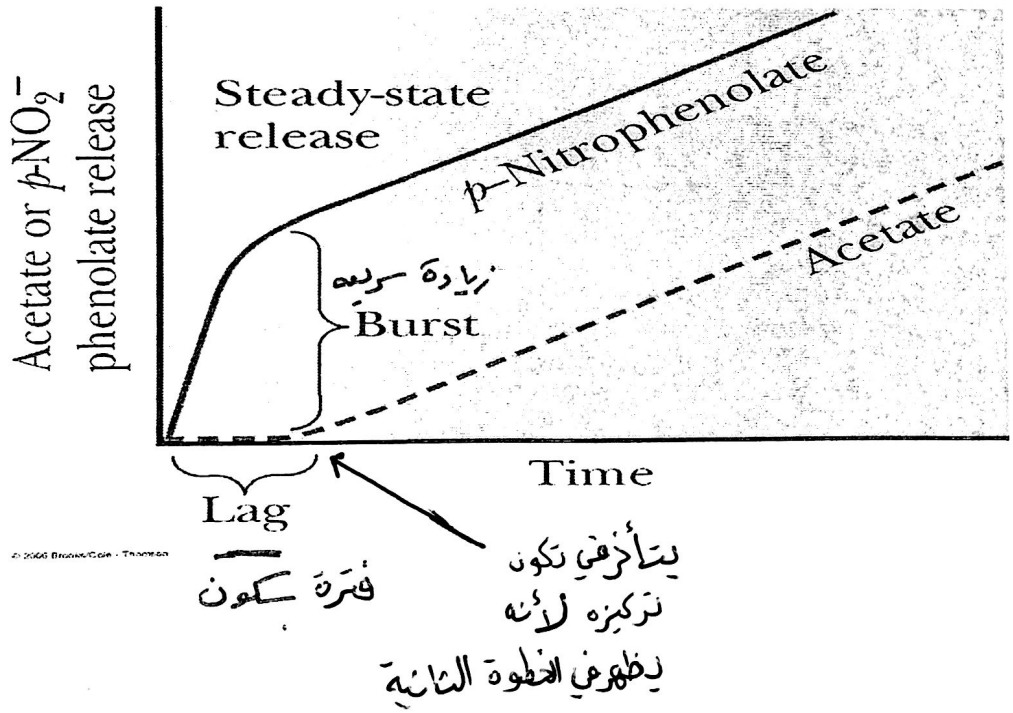


الناتج الاول يحرر بسرعة
Burst

Step 2



الناتج الثاني
Lag



ctive Site of chemotrypsin

it was found that 2 amino-acids in the active Site of chemotrypsin are essential (critical for) activity

Serine 195 → So it belongs to Serine-protease Family

إنزيمات مكسرة للبروتين

(-Trypsin
-Thrombin
-elastase
-chemotrypsin) تشاطوهم بيتعمد على Serine

Histidine 57

But how do we know that?

Labeling:- binding a substance by covalent Bond to a specific Side chain (R)

Covalent modification

Serine 195 labeled by DIPF → added when: Chemotrypsin becomes inactive.

ضيفت إلى جميع المواد وحدد = R groups المسؤولة في التفاعل

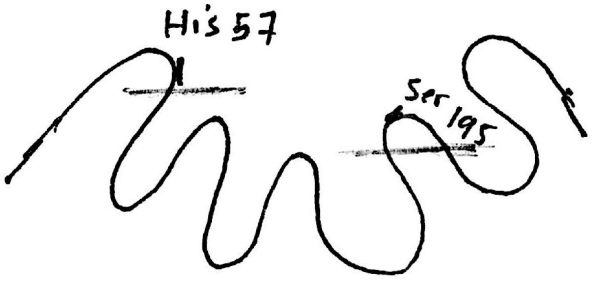
Histidine 57 labeled by TPCK → chemotrypsin becomes inactive

Q: Inhibitors (Ex. DIPF, TPCK) which bind covalently to specific amino-acids are useful in determining which amino-acids are in the active site of the enzyme

A. True
B. False

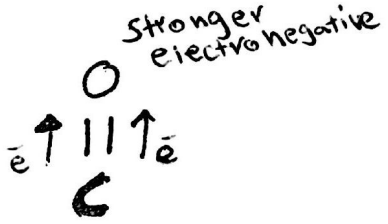
Site !!

X-ray Crystalography Show that folding of chemotrypsin in an anti-parallel β-sheets positioned them in the active site pocket



Chemotrypsin Mechanism of action

Electrophile → ē-poor

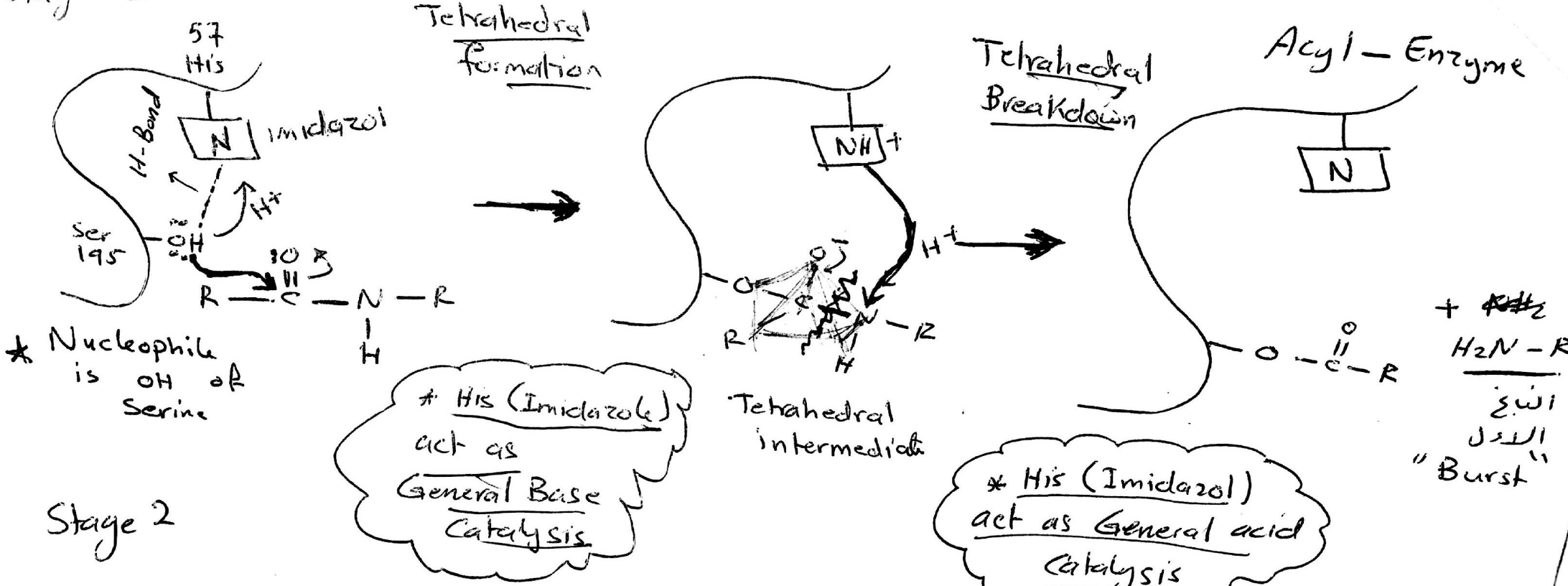


Nucleophile → ē-rich $-\ddot{O}H$

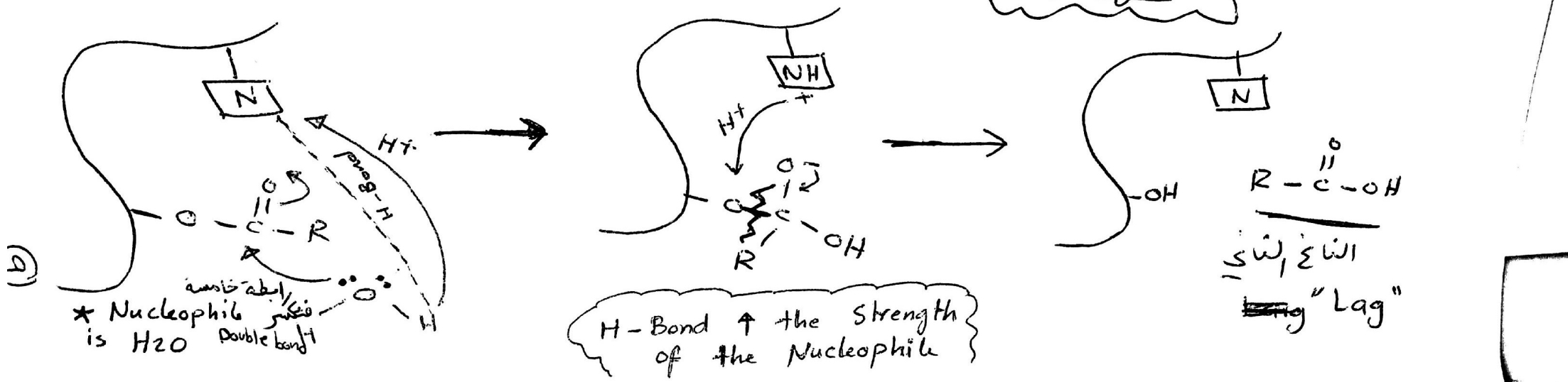
* Usually Nucleophile attack and bind to Electrophile

* Chemotrypsin Mechanism of action

Stage 1



Stage 2



Q: The initial bond formation in the covalent intermediate in the reaction catalyzed by chemotrypsin is between:-

a. Serine and the carbonyl carbon in the peptide backbone

b. Serine and the nitrogen in the peptide backbone

c. histidine and the carbonyl carbon in the peptide backbone

d. histidine and the nitrogen in the peptide backbone

Q: An important step in elucidating the behavior of an enzyme is:-

a. Obtaining a crystalline sample of the enzyme

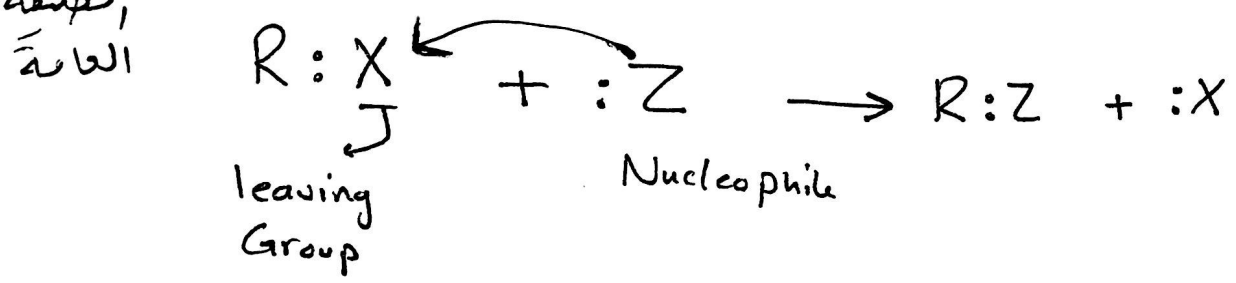
b. insuring that metal ions are always excluded from the enzyme sample

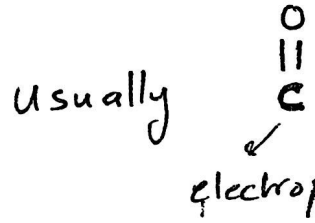
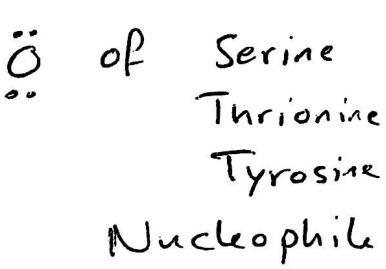
c. determing the active site residues

d. none-of-these.

Common Reaction in Enzyme mechanism

1 Nucleophilic Substitution Reaction (S_N1, S_N2)



Usually  / 

electrophile

of Serine, Thrionine, Tyrosine

Nucleophile

* If the rate of the reaction depends on [R:X] Only, then the reaction is

S_N1 → First order

Here :X leave then :Z attack quickly

rate limiting step

↳ Can attack from any direction

2 sterioisomers produced

S_N1 Not steriospecific (does not give 1 sterioisomer)

* If the reaction rate depends on both [R:X] and [:Z]

then the reaction is S_N2 → Second Order

Here :Z attack while :X still bound

↳ attack from one direction produce one sterioisomer

R:X ← Z

S_N2 steriospecific

because C (Achiral) return C (Carbonyle) → Not chiral

No sterioisomers

At the end of reaction

2 Acid- Base Catalysis

→ according to Bronsted- lowry

acid → H+ donor

Base → H+ acceptor

] General acid- Base Catalysis

إذا عملوا على Bronsted lowry

If the amino-acid act as H+ donor → General acid Catalysis

If the amino-acid act as H+ acceptor → General. Base Catalysis

Imidazol, OH, COOH, SH, NH, phenol

General acid - Base Catalysis

Lewis acid- Base Catalysis

according to lewis ↙

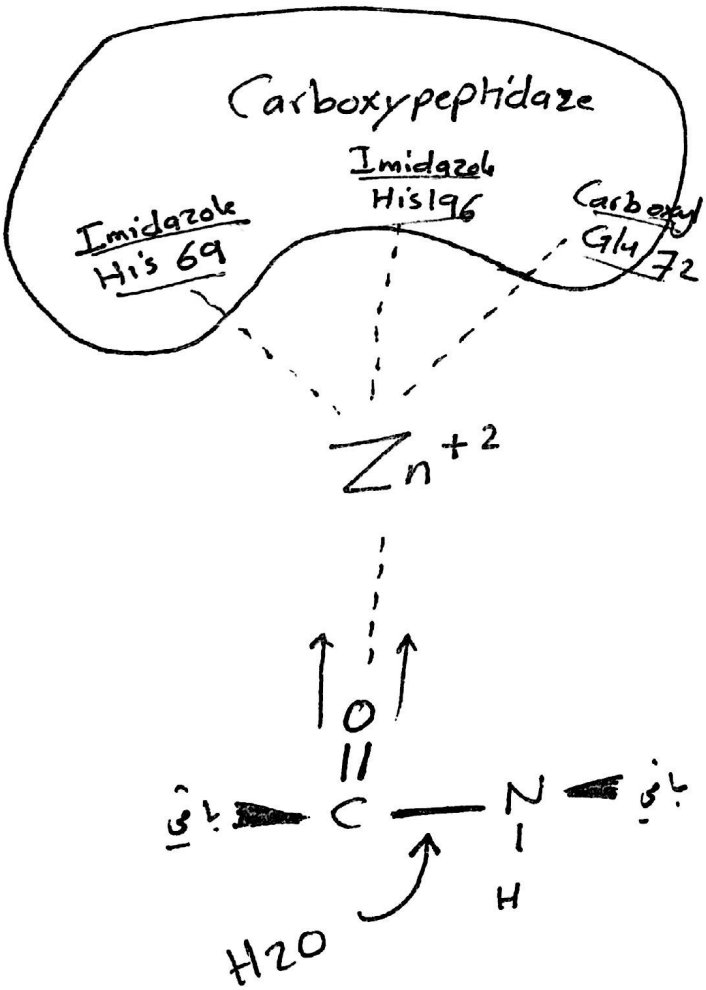
acid : ē-pair acceptor (ē-poor)

base : ē-pair donor (ē-rich)

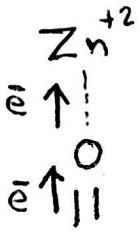
Metal ions Mn^{+2}, Mg^{+2}, Zn^{+2} in Enzymes are lewis acids

Ex:- Zn^{+2} in Carboxypeptidase انزيم مهم لطحن البروتينات

Hydrolyze the C-terminal تبدأ من نهاية Carboxyle



coodination Bond



تجمع كثافة الإلكترونات

معرضة أكثر

→ More Succiptable for water attack

pecificity of the active site

a: absolute specificity : One and only one Substrate bind
One reaction
One product
→ Rigid active site
→ lock and Key

b. Relative specificity :- Similar Substances Can bind
→ Flixable active site
→ Induced Fit model

Steriospecific Enzymes:-

Enzymes bind to specific Sterioisomer either L or D,

Here the active site is chiral, and the active site must be as the substrate not the mirror image

L binds with L
D - D

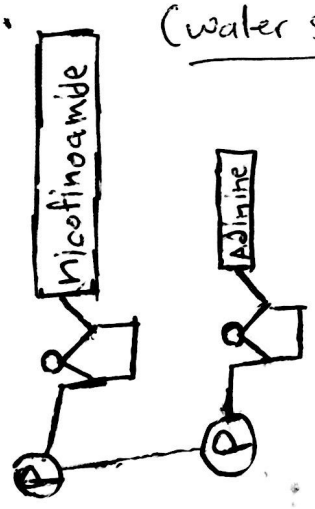
Factors العوامل المساعدة

Cofactors are Non-protein substances, take part in enzymatic reaction and regenerated at the end of the reaction

* Cofactors may bind to the enzyme by covalent or non-covalent bond.

Ex:-
- Metal ions ; act as lewis acids
- Coenzymes → Organic Compounds, many of them are Vitamins or related to Vitamins.

* B vitamins (water soluble) important in → oxidation-reduction reaction
or
→ Group transfer reaction



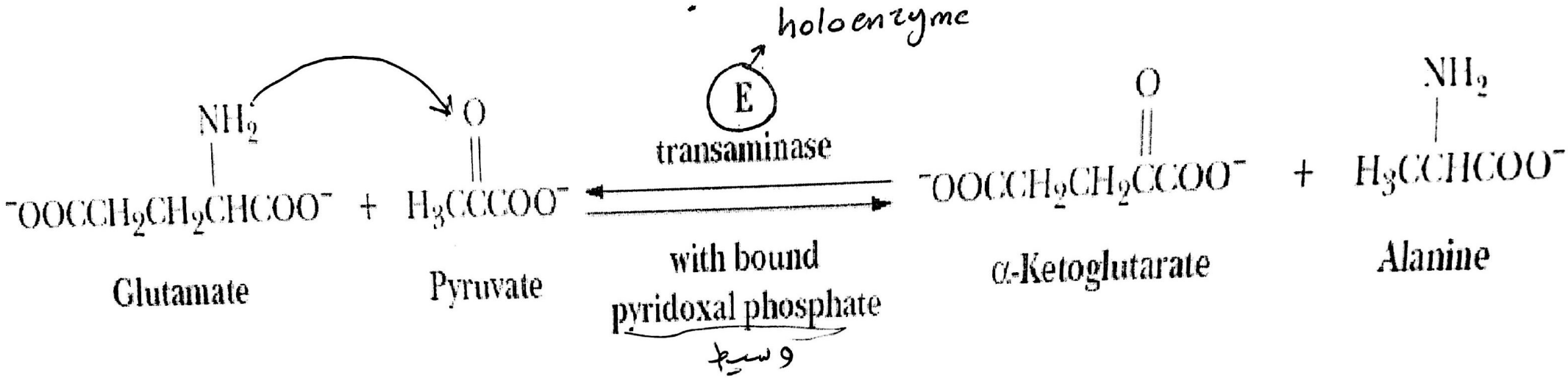
Ex1:- NAD^+ (DNA, RNA يكون)
Nicotinamide - Adinine - Dinucleotide
derived from Vitamin Niacin (Nicotinic acid)
Nicotinamide Ring is the active site of redox

Ex2: Vitamin B_6

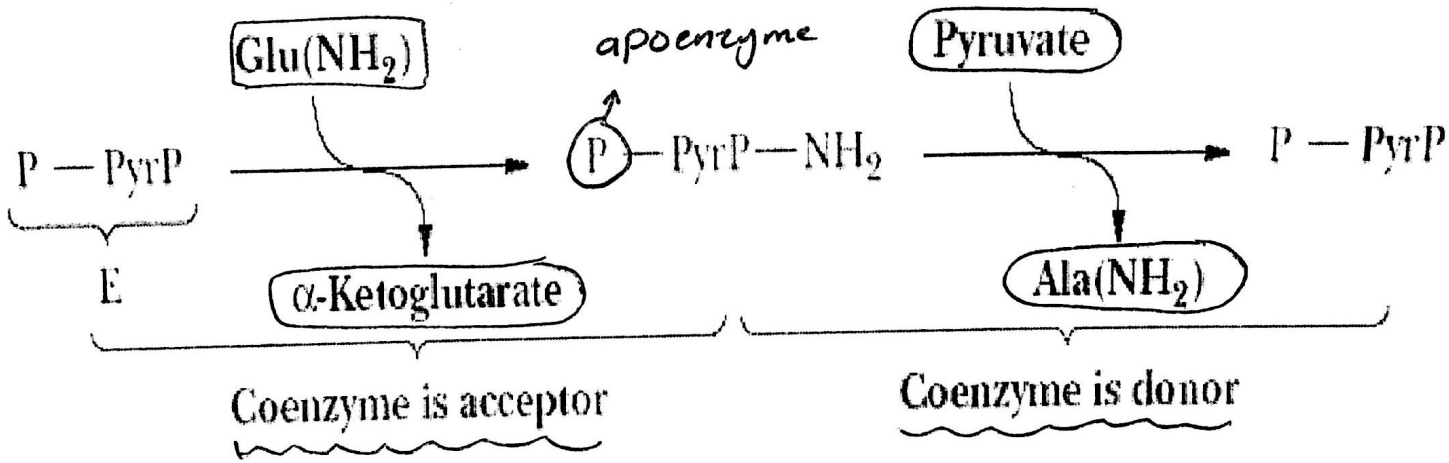
1- Pyridoxal 2- Pyridoxamine 3- Pyridoxin

active forms: 1- Pyridoxal phosphate 2- pyridoxamine phosphate

transfer amino-group from one substance to another (transamination)



This amino (NH_2) group transfer reaction occurs in two stages:



The role of pyridoxal phosphate as a coenzyme in a transamination reaction.

PyrP is pyridoxal phosphate, P is the apoenzyme (the polypeptide chain alone), and E is the active holoenzyme (polypeptide plus coenzyme).

Table 7.1

Coenzymes, Their Reactions, and Their Vitamin Precursors

Coenzyme	Reaction Type	Vitamin Precursor	See Section
Biotin	Carboxylation	Biotin	~~18.2, 21.6~~
Coenzyme A	Acyl transfer	Pantothenic acid	~~15.7, 19.3, 21.6~~
Flavin coenzymes (FAD, FMN)	Oxidation–reduction	Riboflavin (B_2)	~~15.7, 19.3~~
Lipoic acid	Acyl transfer	—	~~19.3~~
Nicotinamide adenine coenzymes (NAD^+, $NADP^+$)	Oxidation–reduction	Niacin (B_3)	~~15.7, 17.3, 19.3~~
Pyridoxal phosphate	Transamination	Pyridoxine (B_6)	~~23.1~~
Tetrahydrofolic acid	Transfer of one-carbon units	Folic acid (B_9)	~~23.1~~
Thiamine pyrophosphate (TPP)	Aldehyde transfer (Decarboxylation)	Thiamine (B_1)	~~17.1, 18.1~~

wish you all Luck
Dr. Tariq . Jibril
0790 979 188