Slide : Enzymes 5

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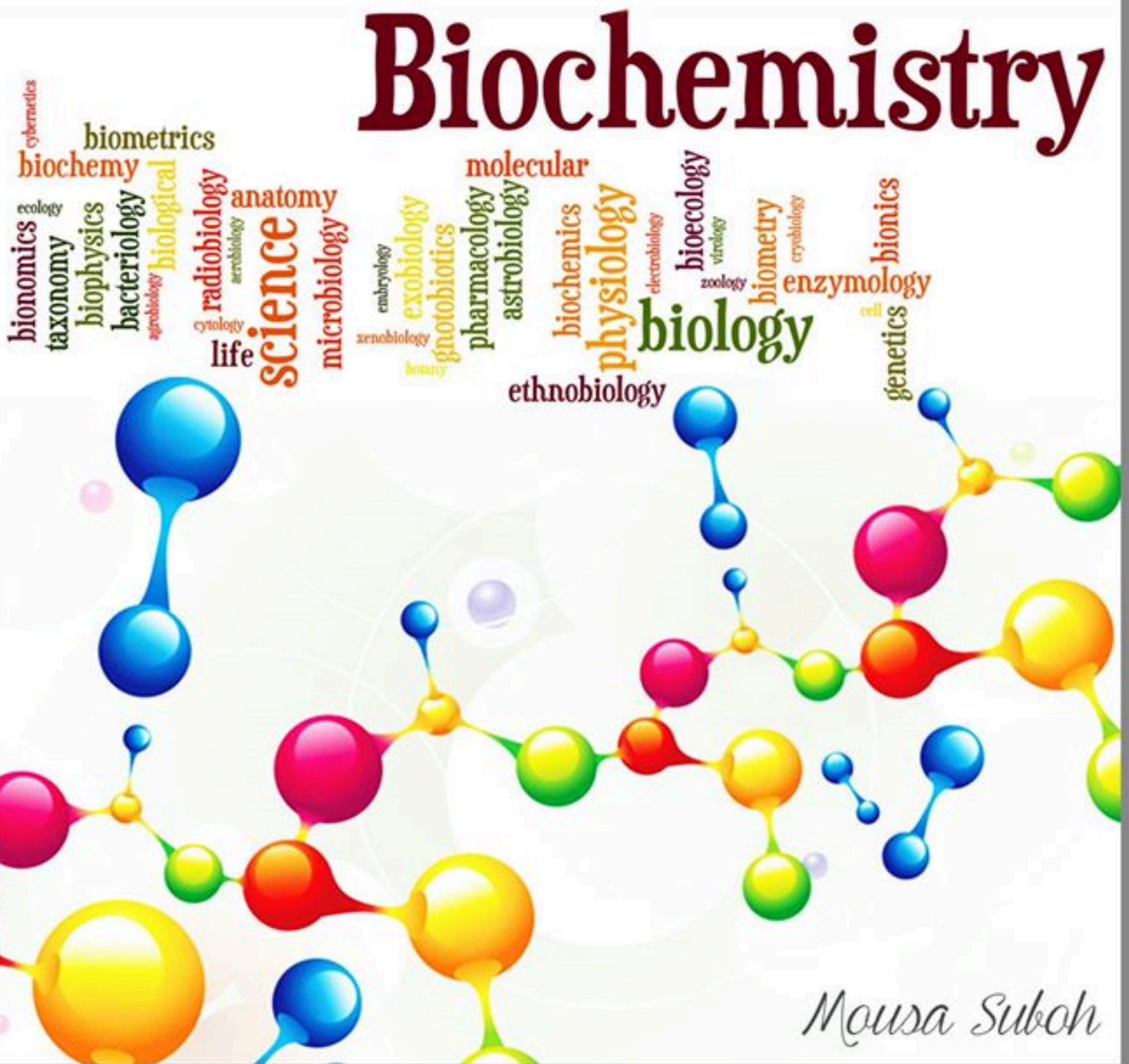
Sections : 4,5,6

■ Slide □ Sheet

Medical Committee
The University of Jordan

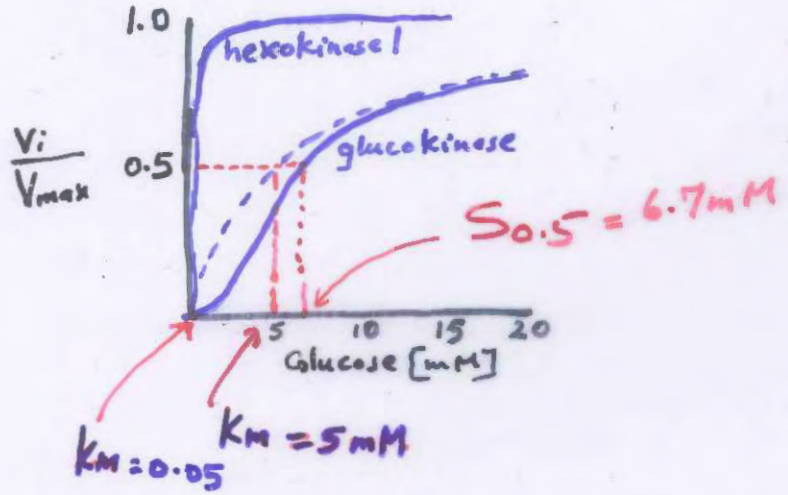


Hexokinase Isozymes have different Km values for glucose

HK-I (110 kD) in erythrocytes, muscle, brain & most other tissues
Km 0.02 — 0.13 mM

GK isozyme in liver: 5 to 6 mM (Km)
(55 kD)

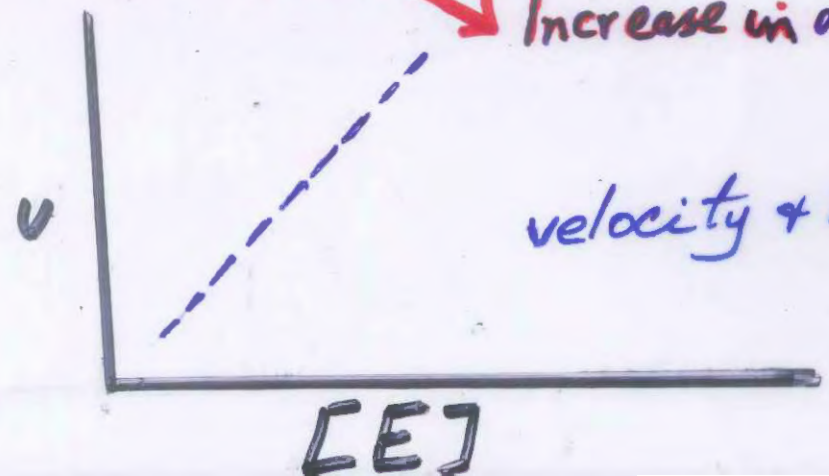
Km for GK as calculated from Michaelis-Menten Eq.

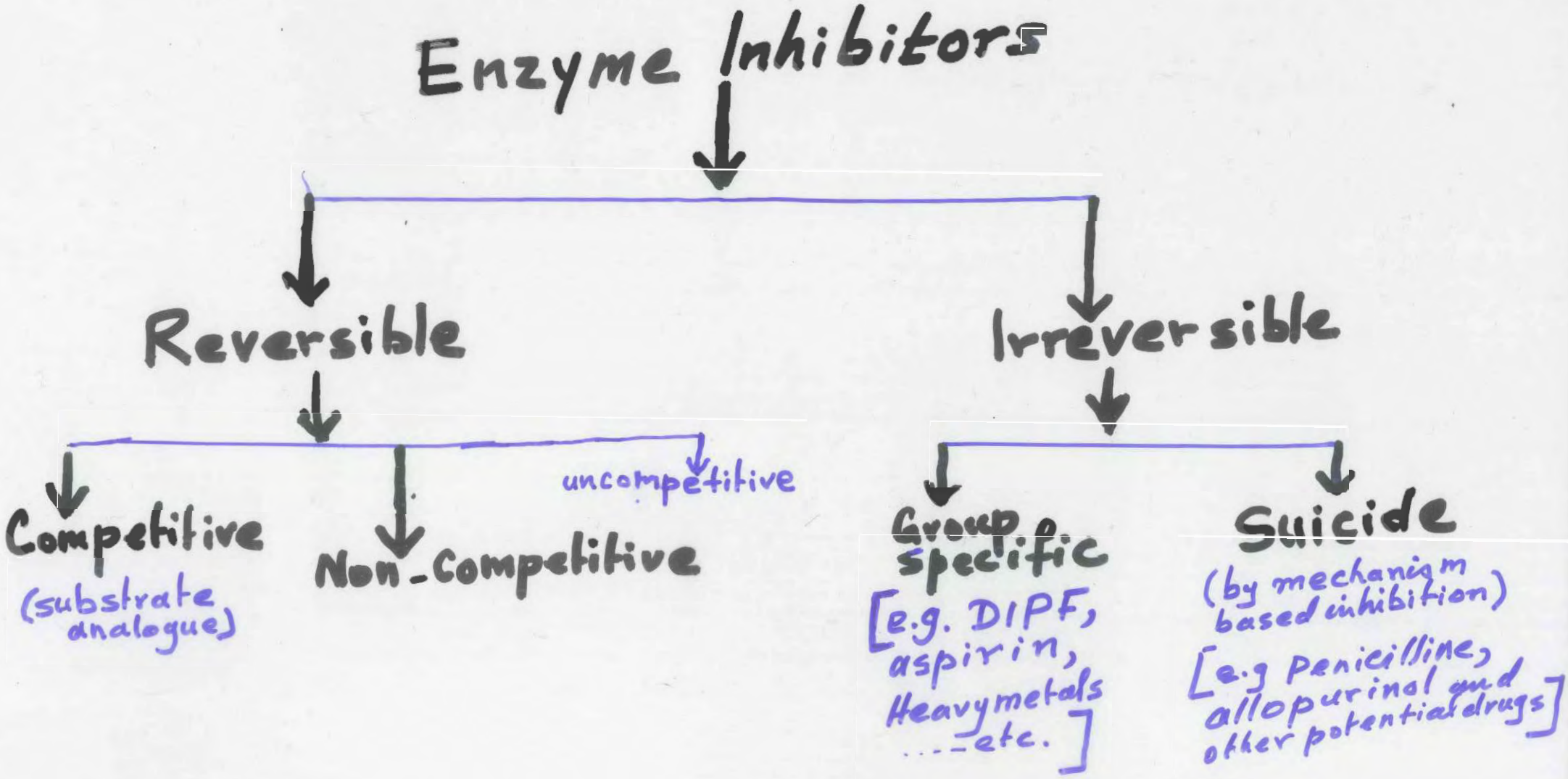


- Which enzyme activity increases after a high carbohydrate meal

- $V_{max} = k_3 [E_T]$

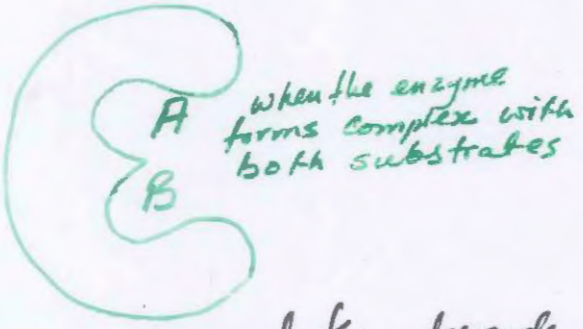
Increase in V_{max} → Increase in Catalytic Power/Kat and/or Increase in amount of enzyme




Enzyme Inhibitors
Reversible
Irreversible
Competitive
(substrate analogue)
Non-Competitive
uncompetitive
Group specific
[e.g. DIPF, aspirin, Heavy metals ... etc.]
Suicide
(by mechanism based inhibition)
[e.g Penicilline, allopurinol and other potential drugs]

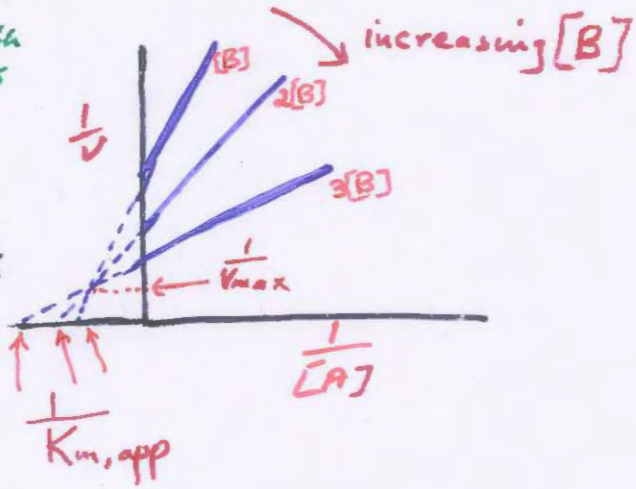
Multi Substrate Reactions :-

$$A + B \longrightarrow C + D$$



when the enzyme forms complex with both substrates

an apparent Km depends on conc. of the other substrate



II Reg. by Rev. Inhibition

→ 1.- Competitive

Increases Km

→ 2. - Noncompetitive

I binds E inpresecnce or absence of S

decreases Vmax

X 3. - Uncompetitive

I binds only to ES complex. Occurs with enzyme of a multi substrate having an ordered sequence of substrate binding.

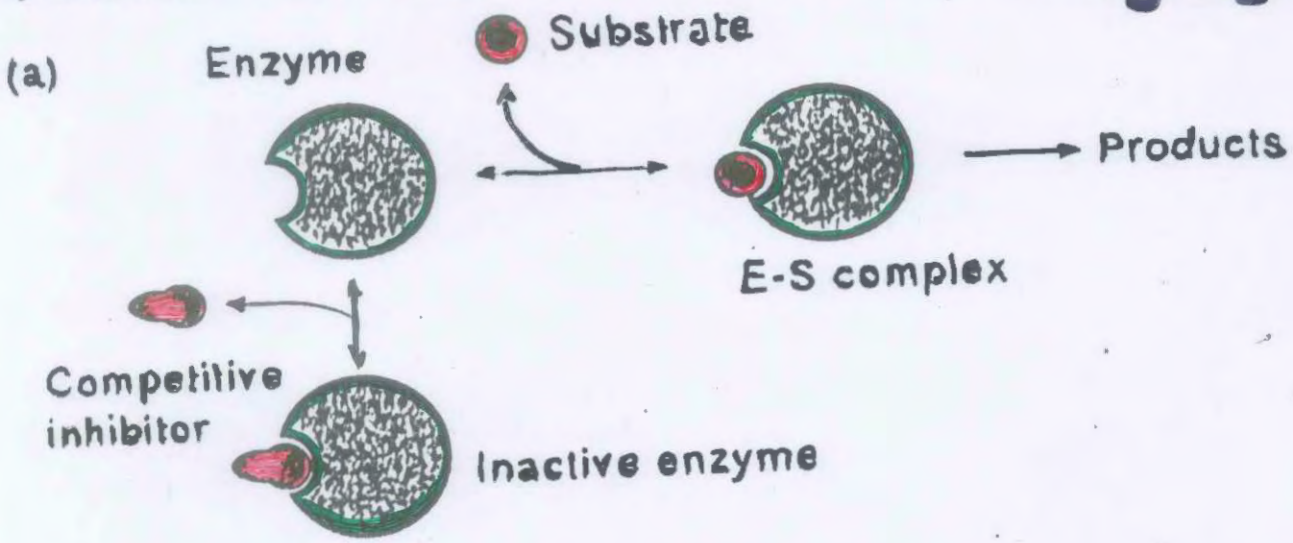
Decreases both Vmax and Km - Not encountered in medicine. Rare with single substrate reactio

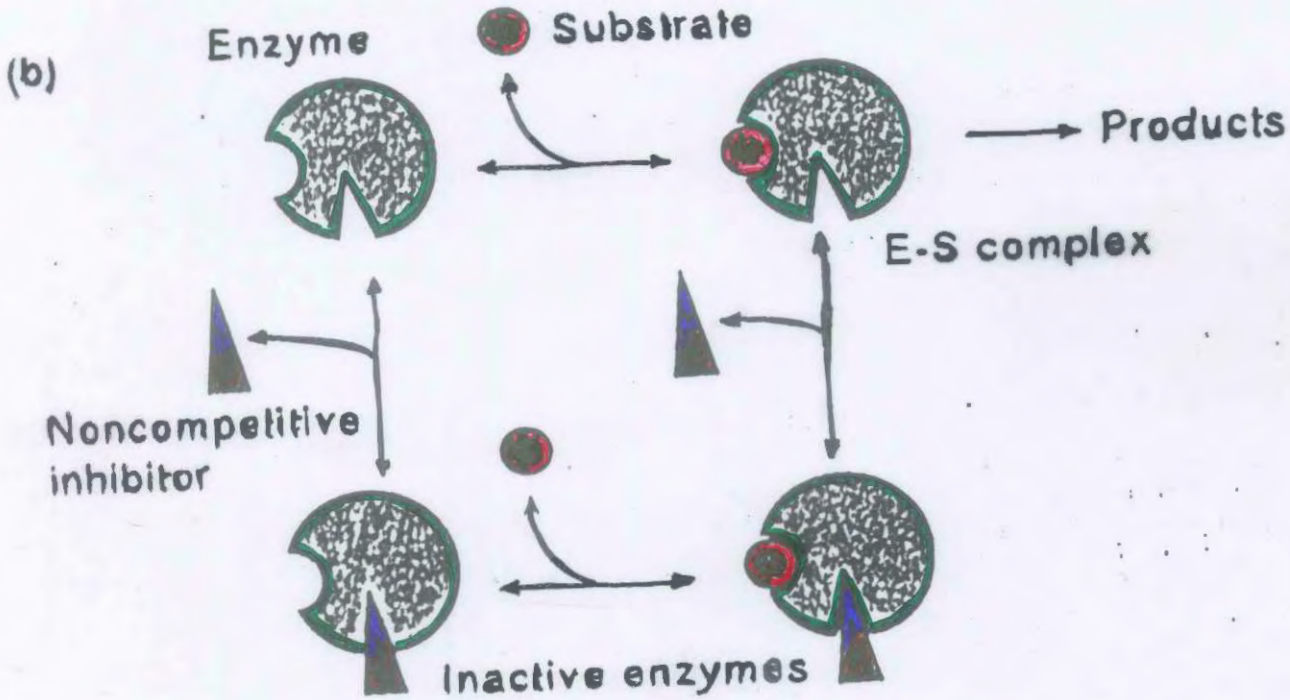
→ Product inhibition

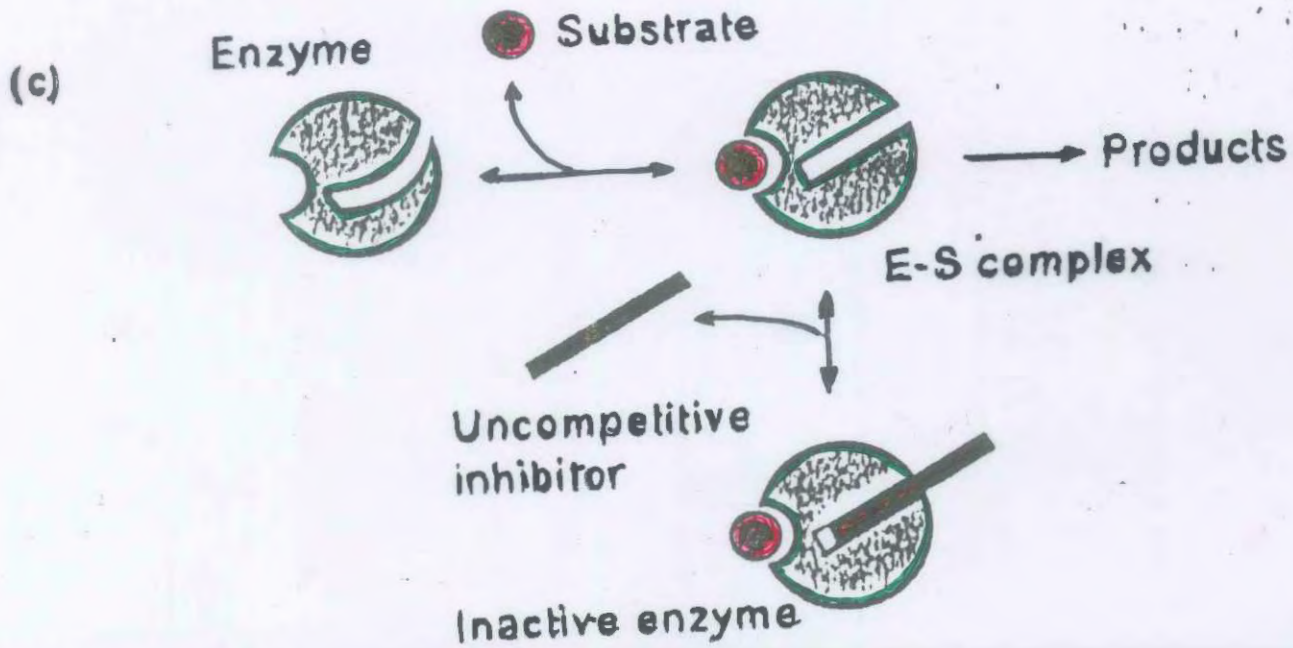
Most products are reversible inhibitor for the enzymes that produce them — to prevent their accumelation

e.g. Glu-6-P ↑ inhibition of hexokinas

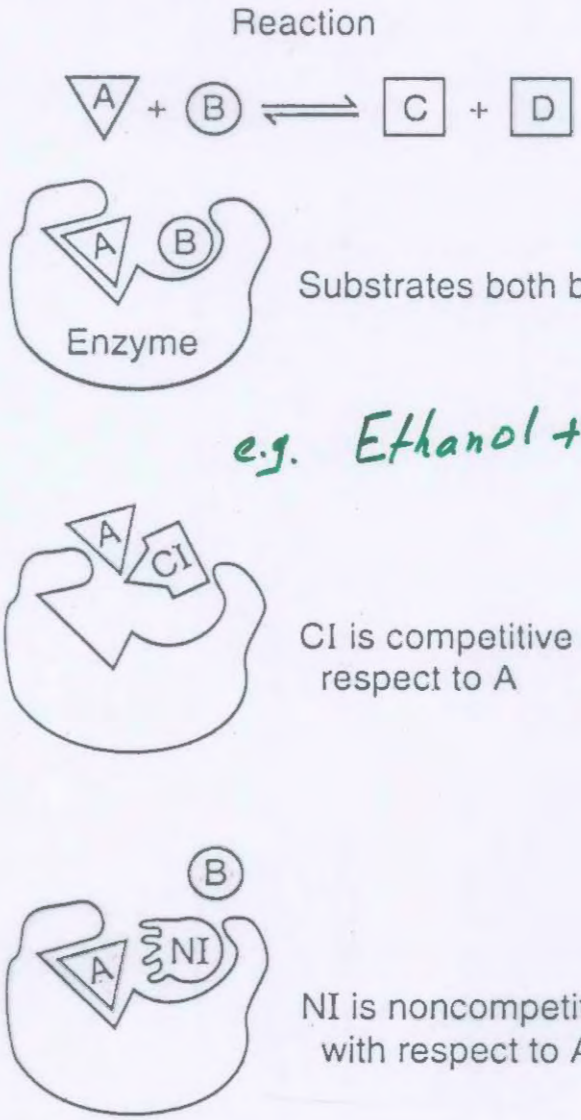
Reversible Inhibition – Single[S] 76







-Reversible Inhibition at the Active Site



e.g. Ethanol + NAD^+ A.D. → Acetaldehyde + NADH + H^+

NADH is a CI

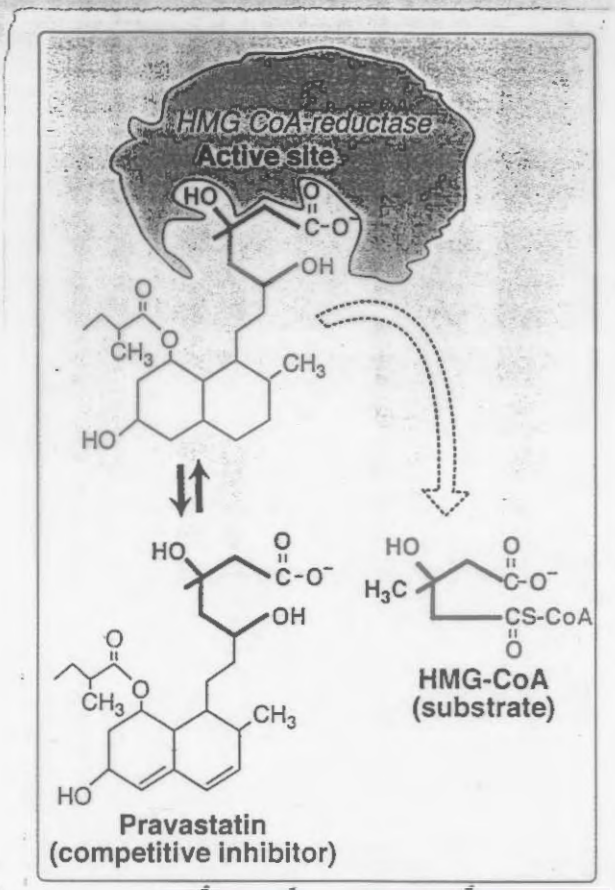
Fig. 9.27. Competitive or noncompetitive inhibition with respect to a given substrate. A and B are substrates for the reaction. The enzyme has separate binding sites for each substrate, which overlap in the active site. The competitive inhibitor (CI) competes for the binding site of A, the substrate it most closely resembles. NI is a noncompetitive inhibitor with respect to the substrate A, and A can still bind to its binding site in the presence of NI. However, NI is competitive with respect to B because it binds to the B binding site. In contrast, an inhibitor that is uncompetitive with respect to A might resemble NI, but it could only bind to the B site after A is bound.

Competitive

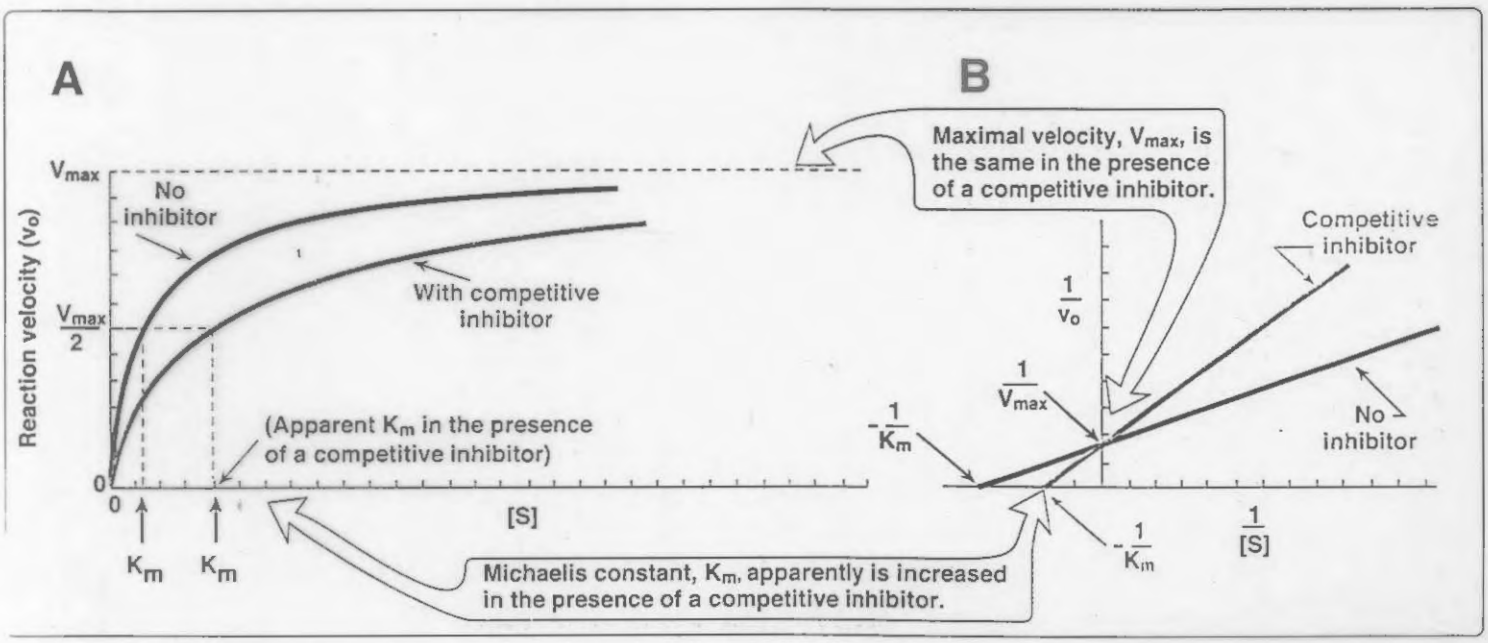
non-Competitive

Uncompetitive

8b



Competitive Inhibitor



$$E + S \rightleftharpoons E\text{-}S \rightarrow P$$

$$I \uparrow\downarrow$$

$$E\text{-}I$$

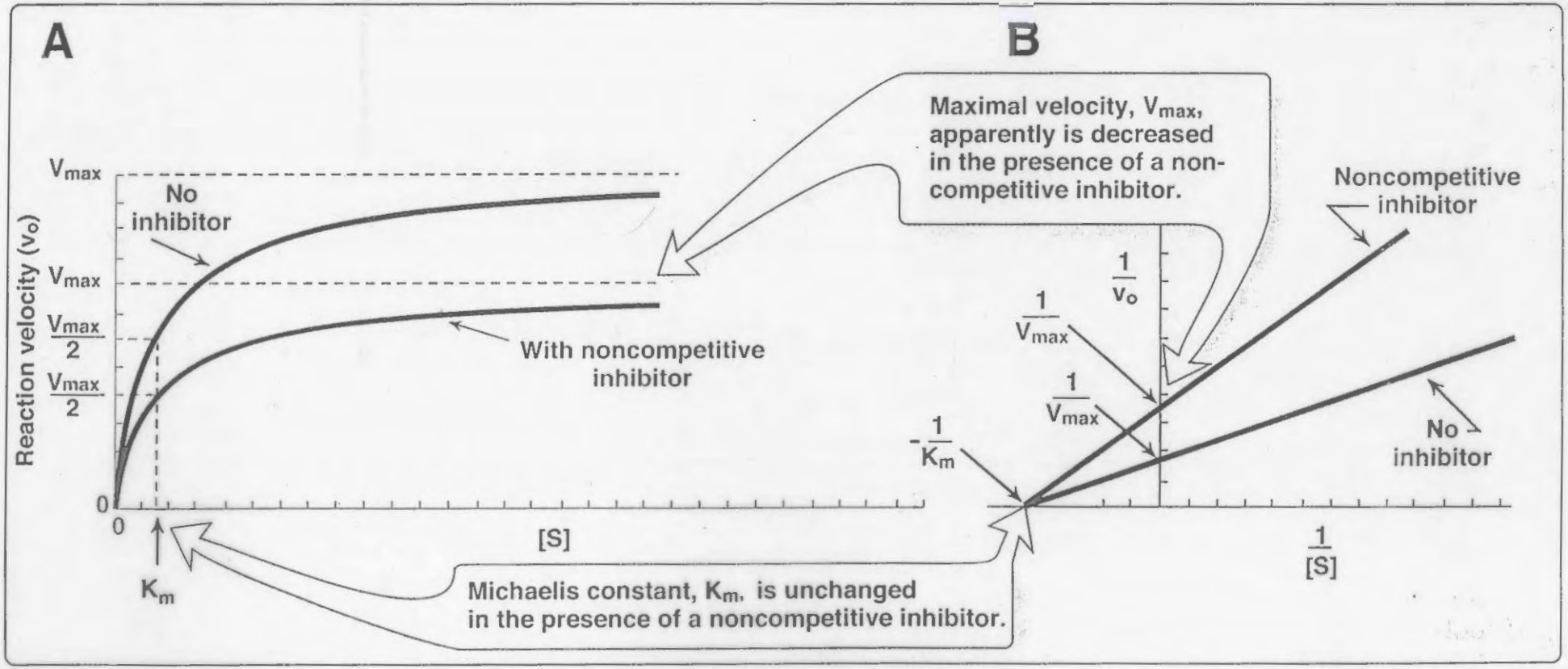
Noncompetitive Inhibition

$E + S \rightleftharpoons E\text{-}S \rightarrow P$

$E\text{-}S + I \rightleftharpoons E\text{-}S\text{-}I$

$E + I \rightleftharpoons E\text{-}I$

I has similar affinity to E + ES as below. If I has different affinity to E + ES, the lines will intersect above or below the abscissa — change both K_m & V_{max}



III Regulation through Conformational change

A- Conformational Changes in Allosteric Enzymes

- Allosteric Enzymes
- Allosteric sites
- Allosteric Effectors
- Multi subunit
- Positive cooperativity
- Feedback Inhibition

B- Conformational changes from Covalent Modification

- phosphorylation - dephosphorylation
- other covalent modification e.g. Acetyl ; ADP-ribose or lipid moieties

C- Modulator PROTEINS

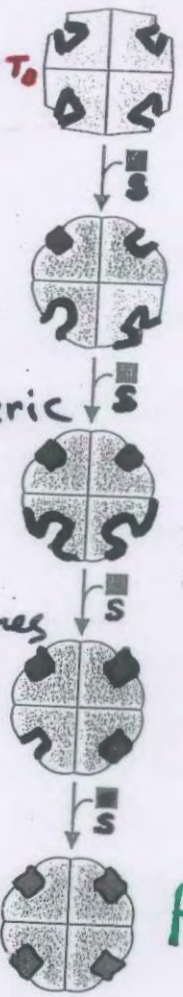
e.g. Ca^{2+} - Calmodulin

G- Proteins

D- PRECURSOR CLEAVAGE :- Proteolytic cleavage

IV- AMOUNT OF ENZ. PRESENT

A sequential model for Allosteric Enzymes



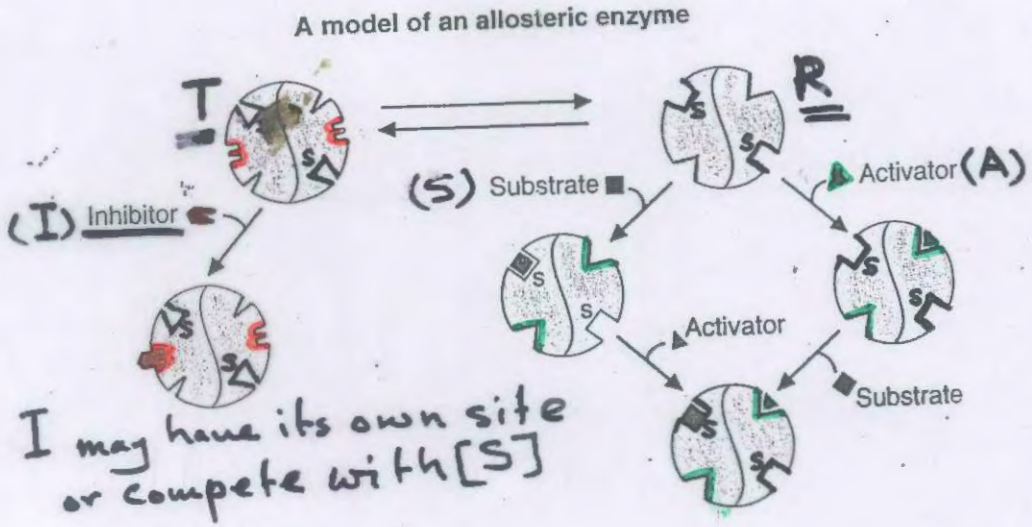
T (taut)

R (relaxed)

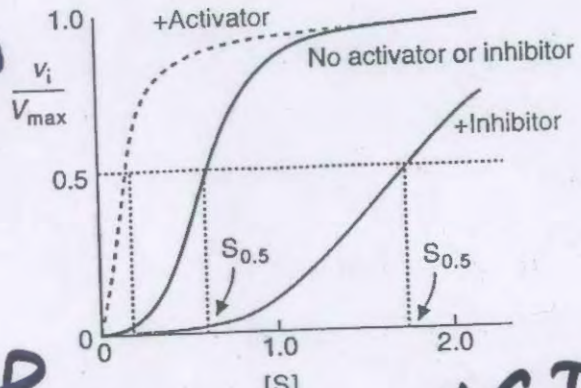
1- Cooperativity in substrate binding to allosteric enzymes

. Most allosteric enzymes follow sequential models

2- Allosteric Activators and Inhibitors



e.g. Activation of PFK and GP, ICD by ADP and AMP when ATP↓

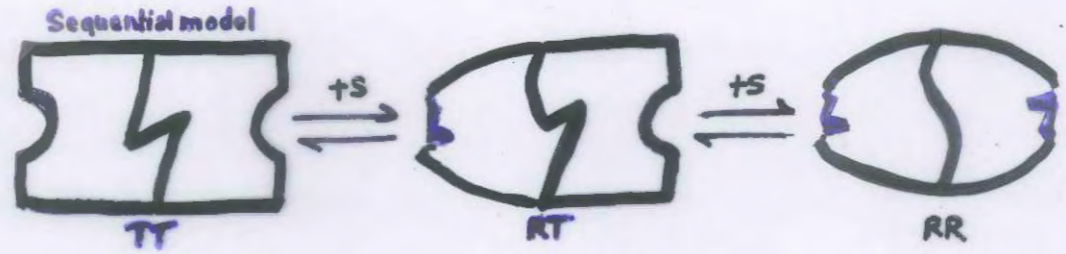


PFK + GP → ↑ AMP

ICD → ↑ ADP

Allosteric Models :-

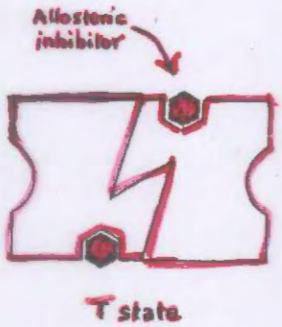
Most enzymes → A



e.g Hb → B

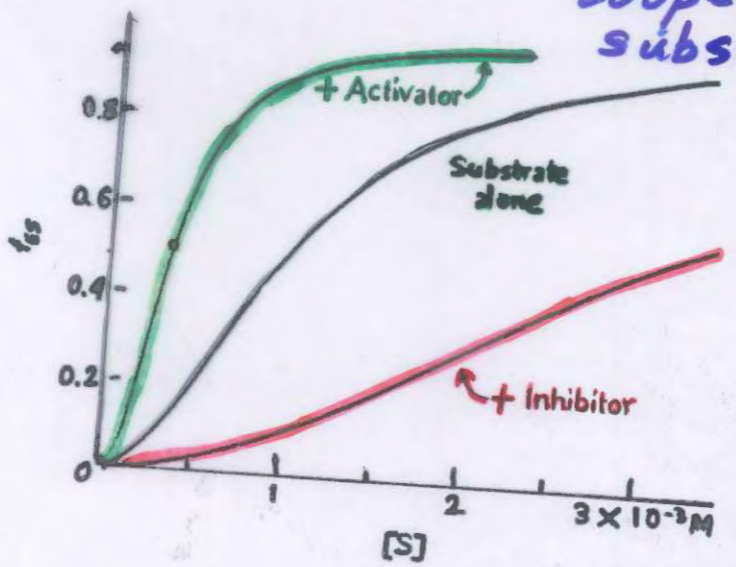
Allosteric Enzymes

- multi subunits
- Allosteric site
- Reg. + catalytic subunits e.g. AcTase
- Cooperativity in substrate binding



REG. THROUGH CONFORMATIONAL CHANGES :-

Cooperativity in substrate Binding



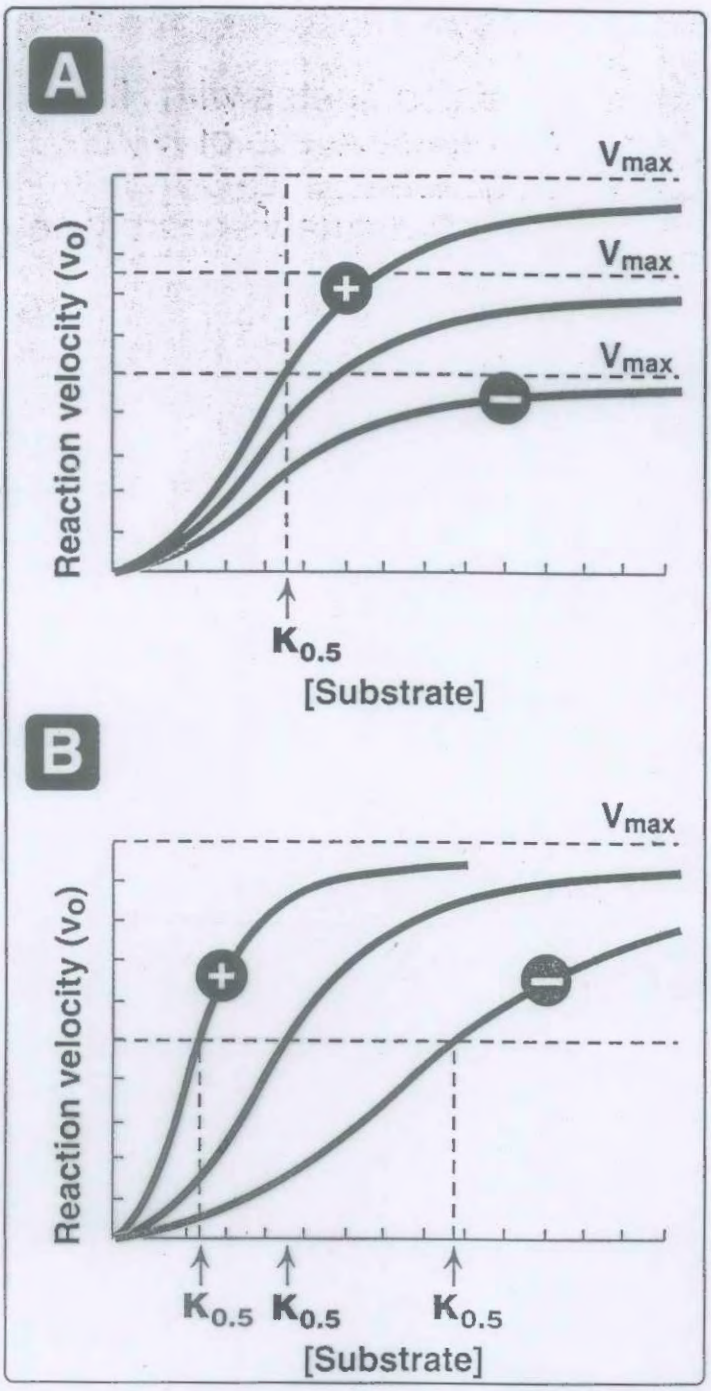
Advatages of Allosteric Regulation

- stronger effect than comp. & non-comp. I at the active site

- Allosteric effectors do not occupy active site can be activators

- Effector need not bear resemblance to [S] or [P]

- Rapid as conc. changes e.g. feed back or signal molecules



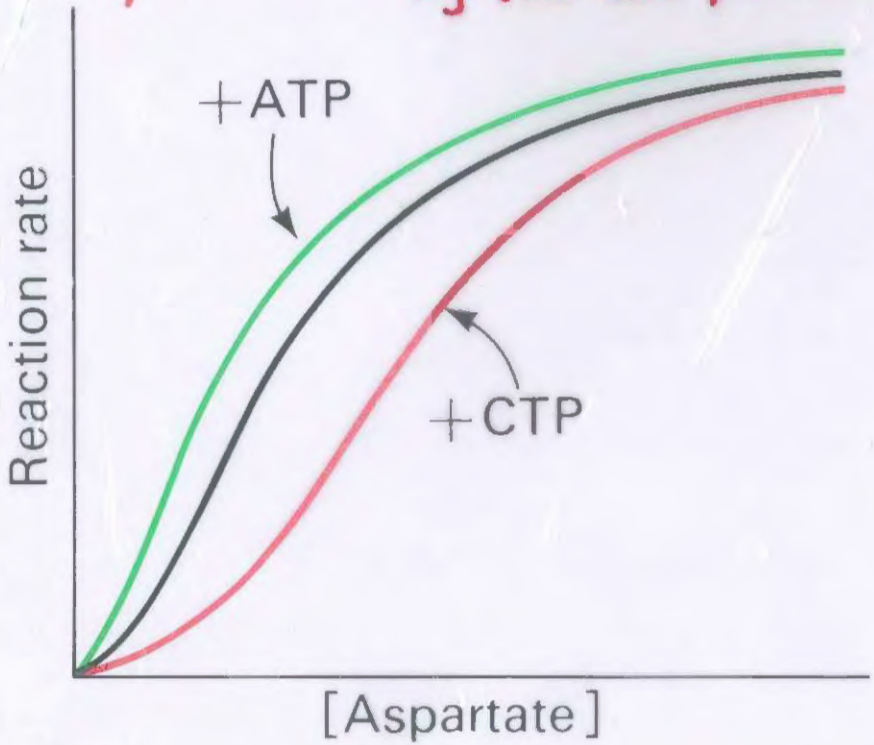
Affecting Vmax or Vmax & Km ($K_{0.5}$ or $S_{0.5}$)

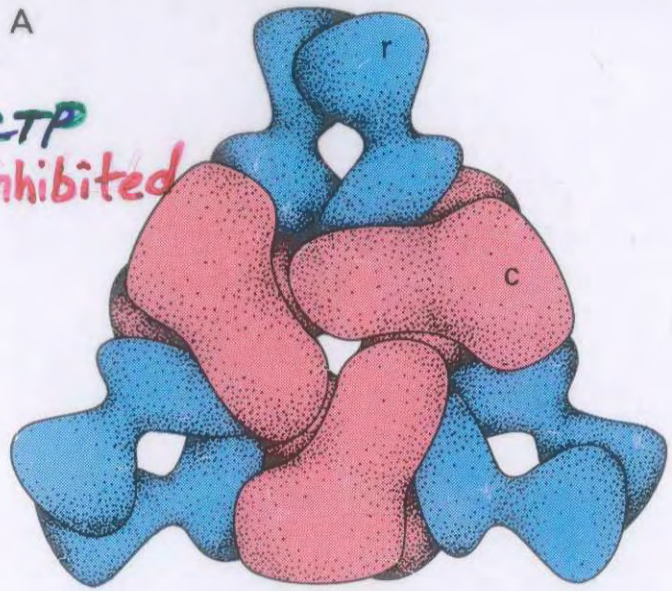
K effectors

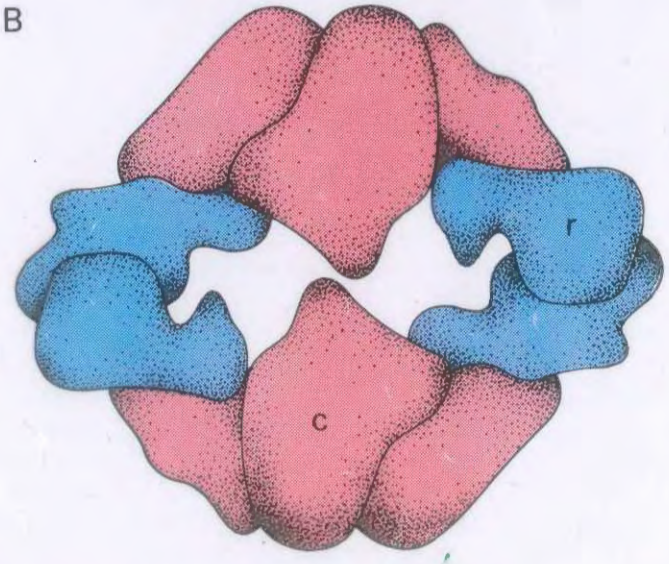
Asp + Carbamoyl phosphate —ATCase→

N-Carbamoyl-aspartate →→→ CTP

Aspartate Transcarbamoylase is Allosterically is inhibited by the end product of its pathway







. Feed back regulation e.g. CTP

. Feed Forward Regulation

e.g. disposal of toxic compounds

storage pathway e.g glycogen synthesis

G6P is activator for glycogen synthase

Figures 10-2 and 10-5

Stryer: *Biochemistry*, Third Edition