Enzymology

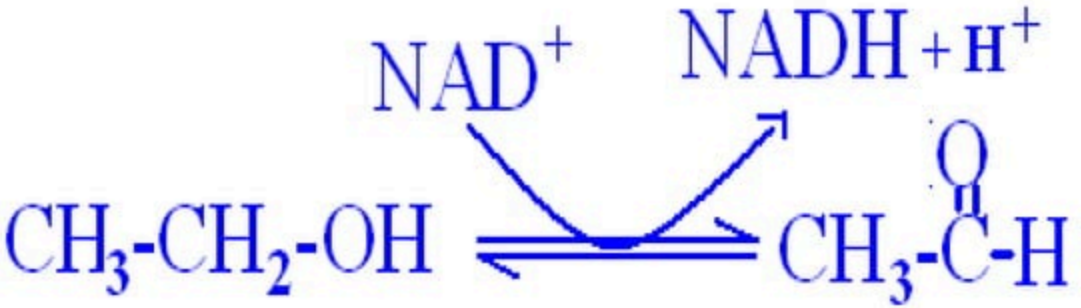
Enzyme classification

CLASSIFICATIONS OF ENZYMES

- Traditionally, enzymes were simply assigned names by the investigator who discovered the enzyme.
- As knowledge expanded, systems of enzyme classification became more comprehensive and complex.
- Currently enzymes are grouped into **six** functional classes by the **International Union of Biochemistry and Molecular Biology** (IUBMB)

EC1. Oxidoreductases

- Catalyze a variety of oxidation-reduction reactions.
- Common names include dehydrogenase, oxidase, reductase and catalase.
- Act on many chemical groups to add or remove hydrogen atoms (i.e. Involve transfer of electrons from one molecule to the other)



ethyl alcohol is oxidized to acetaldehyde and nicotinamide adenine dinucleotide is reduced

EC2. Transferases

- Catalyze transfers of groups (acetyl, methyl, phosphate, amine etc.).
- Common names include acetyltransferase, methylase, protein kinase, transaminases and polymerase.

- NOTE: The first three subclasses (in red) play major roles in the regulation of cellular processes.
  - The polymerase is essential for the synthesis of DNA and RNA.

Typical reactions of EC2

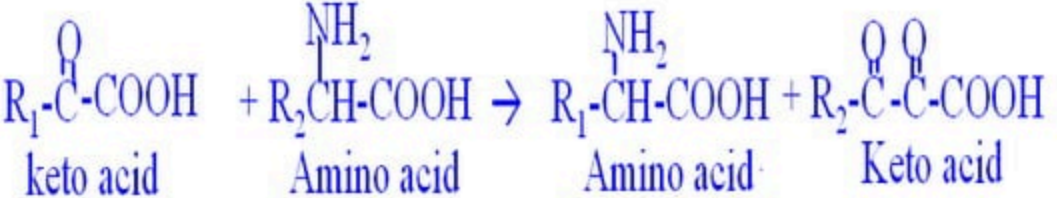
(a) Acetylation

(b) Methylation

(c) Phosphorylation

Typical reactions of EC2

Transamination



- This reaction occurs in presence of *pyridoxal phosphate* which is derived from Vitamin B6

EC3. Hydrolases: NBs

- Catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water.
  - The hydrolases catalyse the hydrolytic cleavage of C-O, C-N, C-C and some other bonds, including phosphoric anhydride bonds.
  - The E.C. classification for these enzymes generally classifies them firstly by the nature of the *bond* hydrolysed, then by the nature of the *substrate*, and lastly by the ***enzyme.***
    - E.g. ***Acetylpyruvate hydrolase***
  - Although the systematic name always includes *hydrolase*, the recommended name is, in many cases, formed by the name of the substrate with the suffix *-ase*. It is understood that the name of the substrate with this suffix means a hydrolytic enzyme.
    - Eg *esterase*

EC3. Hydrolases

- Common examples are;
  - **Proteases** splits protein molecules, e.g.
    - Amides → Acid + Ammonia
    - Polypeptides → smaller peptides
    - HIV protease is essential for HIV replication.
    - Caspase plays a major role in apoptosis.
  - **Nucleases** splits nucleic acids (DNA and RNA).
    - Based on the substrate type, they are divided into RNase and DNase. RNase catalyzes the hydrolysis of RNA and DNase acts on DNA.

EC3. Hydrolases

- They may also be divided into **exonuclease** and **endonuclease**. The exonuclease progressively splits off single nucleotides from one end of DNA or RNA. The endonuclease splits DNA or RNA at internal sites.

– Phosphatase catalyzes dephosphorylation (removal of phosphate groups).

- Example: calcineurin and many other phosphatases (refer CHO metabolism).
- The immunosuppressive drugs FK506 and Cyclosporin A are the inhibitors of calcineurin.

EC3. Hydrolases

– Esterases hydrolyze ester bonds

- Triglycerides → Glycerol + Fatty Acids

(here hydrolases (esterases) are called lipases)

Glucosidases: hydrolyzes fructose to glucose

EC4. Lyases

- Lyases refer to enzymes that catalyze addition to --C=O, -C=C- and -C=N- bonds and
- also cleavage of C-C, C-O, C-N by elimination forming double bonds
- They catalyze the cleavage of C-C, C-O, C-S and C-N bonds by means other than hydrolysis or oxidation.
- Common names include decarboxylase and dehydratases.

- Decarboxylases remove a molecule of CO_2 from keto acids or amino acids
  - $RCH_2\text{-}CO\text{-}COOH \rightarrow RCHO + CO_2$

    Keto acid Aldehyde
- Dehydratases remove a molecule of water

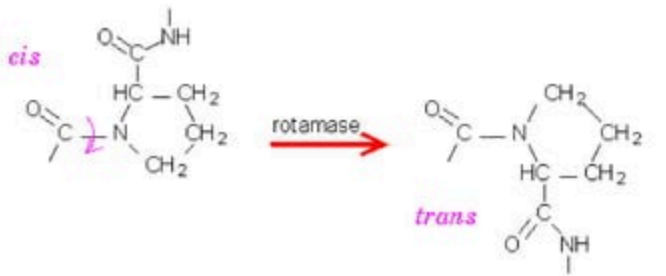
EC5. Isomerases

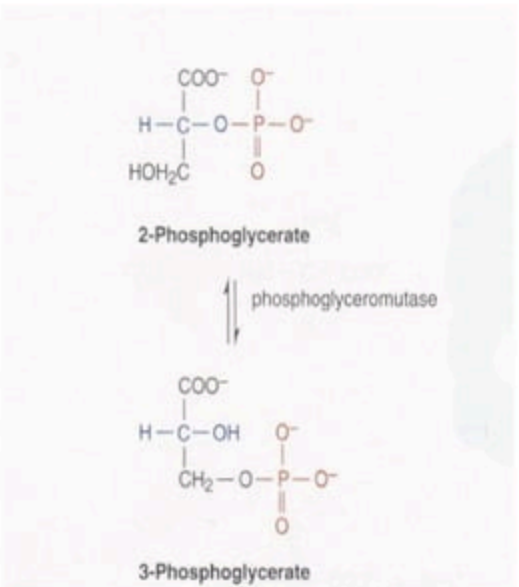
- Catalyze atomic rearrangements within a molecule.

- Examples include rotamase, protein disulfide isomerase (PDI), epimerase and racemase, phosphoglyceromutase etc etc.

EC5. Isomerases

Enzymes that catalyze change in geometrical or spatial configuration of the substrate

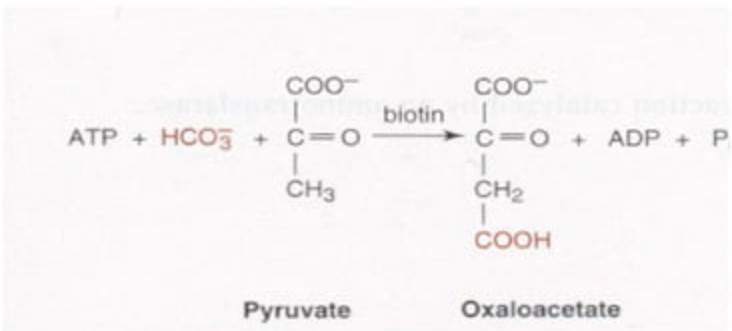




EC6. Ligases

- Enzymes that catalyze joining of two molecules at the expense of high energy bond of ATP; these are called synthetases

- Examples include peptide synthase, aminoacyl-tRNA synthetase, DNA ligase and RNA ligase.



Enzymology-Lecture 2

- *General properties of enzymes: conformation, stability*
- *Active site, specific activity, turnover number*
- *Coenzyme-cofactors-their origin and role*
- *Activation energy-how it is related to rates of enzymatic reactions*

Enzymes: General Ks

- Biological catalysts and enhance reaction rates
- Have high degree of catalytic power and specificity
- Most enzymes are proteins
- Contain an active site where a substrate binds and is converted to products
- One polypeptide chain has one active site
- Show stereochemical and structural specificity

ENZYME ACTIVE SITE

Active site is made of amino acid residue side chains from different regions of the enzyme, as a protein

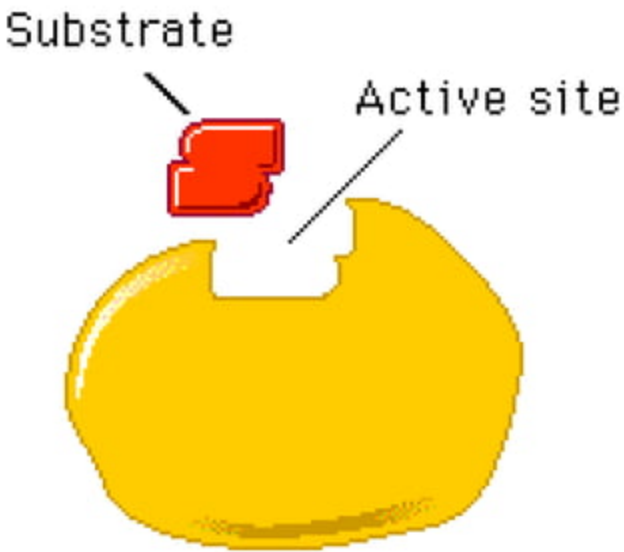
Active site: Active site is a crevice or pocket formed by a group of amino acid side chains belonging to residues forming the pocket.

Some or all of these side chains belong to residues separated (far away) from each other in the amino acid sequence.

ENZYME ACTIVE SITE

- A portion of this crevice or pocket defined by certain side chains is designated as substrate binding site (SBS) and an adjacent portion which contains side chains involved in catalysis is known as the catalytic site (CS).

- SBS may be hydrophobic or hydrophilic depending on the complementary structure of substrates, the CS is usually hydrophilic.



Schematic model
of an enzyme

Active sites of enzymes have some common features

- The active site of an enzyme is the region that binds the substrate (and the the prosthetic group if any) and contains the residues that makes or breaks the bonds
  - These groups are called the catalytic groups
-

Common features of enzyme active sites (AS)

1. It takes a small part of the total enzyme volume
   - Most of the rest of the enzyme does not take part in catalysis, that is the amino acid residues in the rest of the enzyme do not come into contact with the substrate
   - Qn: why are enzymes such big molecules?? Appr 100aa=10kDa!!!

AS

1. Is a 3D entity
   - Made of groups that come from different regions of the linear primary sequence of amino acid residues in the protein
   - Aa far apart may have stronger attrctions than adjacent ones
     - Eg in lysozyme, the important groups come from aa 35, 52, 62, 63 and 101 in the linear sequence of 129 aa residues

AS

3. Substrates are bound to enzymes by weak multiple attractions (H bonds, electrostaic interactions, van der Waals forces and hydrophobic interactions)

- ES complexes have weak electrostatic interactions: -3 to -12kcal/mol.
- Covalent bonds: -50kcal/mol to -110kcal/mol.

AS

4. ASs are crevisces or clefts in the enzyme, usually made by exclusion of a water molecule (unless is a reactant)-AS (catalytic sites)are thus non polar!

   NB the cleft may also contain some polar residues!!! esp at the substrate binding site

AS

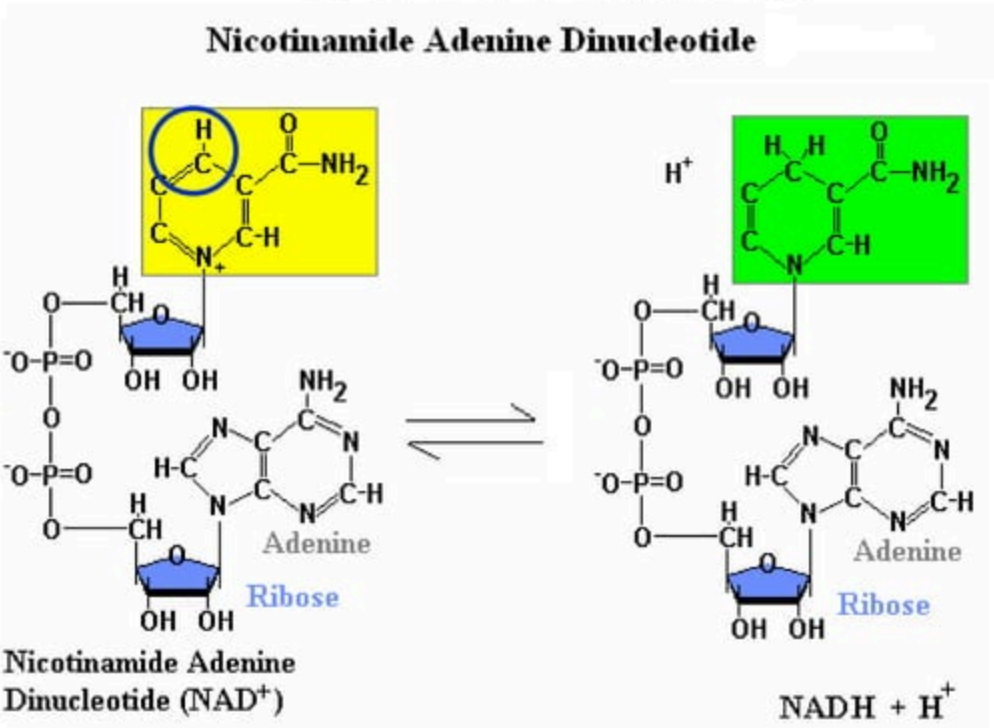
5. E Specificity depends on precise arrangement of atoms in the AS

- To fit in this site, the substrate must have a complementary shape (Emil Fischer's metamorphor of the **lock-and-key model**, 1890)
  - Now: Its known that the shape of AS is greatly modified by the binding substrate (Koshland, 1958 in his **induced-fit model**)

CO-ENZYMES

- Many enzymes require a coenzyme or cofactor for activity
  - Apoenzyme + Coenzyme → Holoenzyme
  - (inactive) (active)
- Coenzymes are derived from vitamins and act as co-substrates and are converted into products
- They take part in the reaction!
- Examples of co-enzymes include
  - FAD+ and NAD+

CO-ENZYMES


Nicotinamide Adenine Dinucleotide
Adenine
Ribose
Nicotinamide Adenine Dinucleotide (NAD+)
H+
Adenine
Ribose
NADH + H+

CO-FACTORS

- Cofactors are metal ions such as Cu, Mg, Mn, Fe:
- They not co-substrates in the reaction and are not usually converted to products
  - NB: they facilitate the reaction by presence alone!
- Coenzymes and cofactors alter the conformation around the active site of the enzyme

Enzyme properties

1. Denaturation
    1. Denaturation means breakdown of non covalent bonds like H-bonds and/or electrostatic and hydrophobic interactions.
    2. Are denatured at high pH and temperature

2. Specific Activity:
    1. Specific activity: = enzyme activity under specified conditions of substrate concentration, temperature, pH, ionic strength etc.
    2. It is defined as micromoles of substrate converted to product per minute per mg of the protein under specified conditions.

3. Turnover Number:
    1. Turnover number: This number represents the absolute catalytic efficiency of an enzyme.
    2. It is defined as μmoles of substrate converted to product per sec per mole of the active site of the enzyme.
    3. This latter part is important because a polymeric enzyme containing a number of subunits may have to be compared with another enzyme which may be monomeric.

pH effect

- **Optimum pH**: Enzymes show optimum activity in a certain pH range which varies with different enzymes.
  - At a pH one unit below or above this value the enzymes are only partially active. At pH values far removed from optimum, the enzymes can be denatured and lose their activity.
  - Disulfide formed by cysteines can be reduced by reducing agents like dithiothreitol (DTT).
  - While overall charge on the enzyme is important, denaturation of the active site structure results in loss of activity.

Effect of temperature

- Like non-enzymatic reactions, enzyme activity increases with increase in temperature usually doubling of rate with every 10 degree centigrade rise in temperature. (Kinetic theory)
- However, there is limit to this increase as enzyme active site is denatured as the temperatures rise above 37 degrees (for vertebrates).
- Some bacteria which survive in high temperature geysers have more temp. stable enzymes.
- The denaturation at elevated temperatures results from the breakdown of primarily H bonds.

- In a **holoenzymes** the protein component is known as the **apoenzyme**, while the non-protein component is known as the **coenzyme** or **prosthetic group.**

- A prosthetic group must be an organic molecule and must bind covalently to the apoenzyme.

- When the binding between the apoenzyme and non-protein components is non-covalent, the small organic molecule is called a **coenzyme**.

Mechanisms of enzyme Catalysis

Enzyme-Substrate Interactions

- The favoured model of enzyme substrate interaction is known as the **induced fit model (Daniel Koshland)**
- This model proposes that the initial interaction between enzyme and substrate is relatively weak, but that these weak interactions rapidly induce conformational changes in the enzyme that strengthen binding and bring catalytic sites close to substrate bonds to be altered.
- After binding takes place, one or more mechanisms of catalysis generates transition- state complexes and reaction products.
- The possible mechanisms of catalysis are four in number:

1. **Catalysis by Bond Strain:** In this form of catalysis, the induced structural rearrangements that take place with the binding of substrate and enzyme ultimately produce strained substrate bonds, which more easily attain the transition state.

The new conformation often forces substrate atoms and bulky catalytic groups, such as aspartate and glutamate, into conformations that strain existing substrate bonds.

2. Catalysis by Proximity and Orientation:

Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another.

In addition to inducing strain, groups such as aspartate are frequently chemically reactive as well, and their proximity and orientation toward the substrate thus favors their participation in catalysis.

3. Catalysis Involving Proton Donors (Acids) and Acceptors (Bases):

Other mechanisms also contribute significantly to the completion of catalytic events initiated by a strain mechanism, for example, the use of glutamate as a general acid catalyst (proton donor).

4. **Covalent Catalysis:** In catalysis that takes place by covalent mechanisms, the substrate is oriented to active sites on the enzymes in such a way that a covalent intermediate forms between the enzyme or coenzyme and the substrate.

One of the best-known examples of this mechanism is that involving proteolysis by **serine proteases**, which include both digestive enzymes (trypsin, chymotrypsin, and elastase) and several enzymes of the blood clotting cascade.

These proteases contain an active site 'serine' whose R group hydroxyl forms a covalent bond with a carbonyl carbon of a peptide bond, thereby causing hydrolysis of the peptide bond.

Lecture 3: enzyme kinetics

EFFECT OF SUBSTRATE

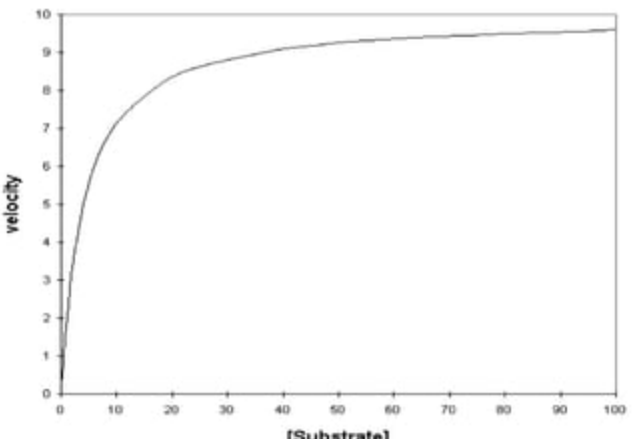
- In a normal, non-catalyzed chemical reaction we would expect that the *velocity* of the reaction would be directly proportional to the concentration of substrate.

- In other words, if you double the concentration of substrate the velocity should also double. The reason for this is purely a statistical one

- For a non-catalysed reaction, then a graph of velocity against substrate concentration would be a straight line.

EFFECT OF SUBSTRATE

- In an enzyme catalysed reaction the same type of experiment, measuring reaction velocity at various different concentrations, does not give a straight line but a curve.



EFFECT OF SUBSTRATE

- From the curve
  - Velocity V: the number of moles of product formed per second
  - V is proportional to S when S is small
  - At high S, V becomes independent of S
  - Michaeli and Menten (1913) proposed this to be due to the formation of an ES complex btwn S and Product

EFFECT OF SUBSTRATE

Enzyme and substrate form a complex

Eq 1

- Enzyme E combines with Substrate S to form an ES complex with a rate constant k1
- ES has 2 possible fates: can dissociate to E and S (with a rate constant k2) or can proceed to E and P (with a rate constant k3)
- NB: in the initial stages of reaction, reversion is not common!

EFFECT OF SUBSTRATE

- To relate the rate of catalysis and ES concentartion, we have
  - $V = k3[ES]$
- The rates of formation and breakdown of ES are given by
  - Rate of ES formation: $ES = k1[E][S]$
  - Rate of ES breakdown: $ES = (k2 + k3)[ES]$

EFFECT OF SUBSTRATE

Enzyme and substrate form a complex

Eq 1

- **In a steady state, [ES] stay the same while that of the substrate and product keep changing**

EFFECT OF SUBSTRATE

- From the 2 equations
- Rate of ES formation: $ES = k1[E][S]$
- Rate of ES breakdown: $ES = (k2 + k3)[ES]$

- We have:
  - $k1[E][S] = (k2 + k3)[ES]$

  OR

  - $[ES] = \frac{[E][S]}{(k2 + k3)/k1}$

$$-[ES] = \frac{[E][S]}{(k2 + k3)/k1}$$

$$\frac{(k2 + k3)}{k1}$$

Is called the Michaelis- Menten constant, Km

Thus $[ES] = \frac{[E][S]}{Km}$

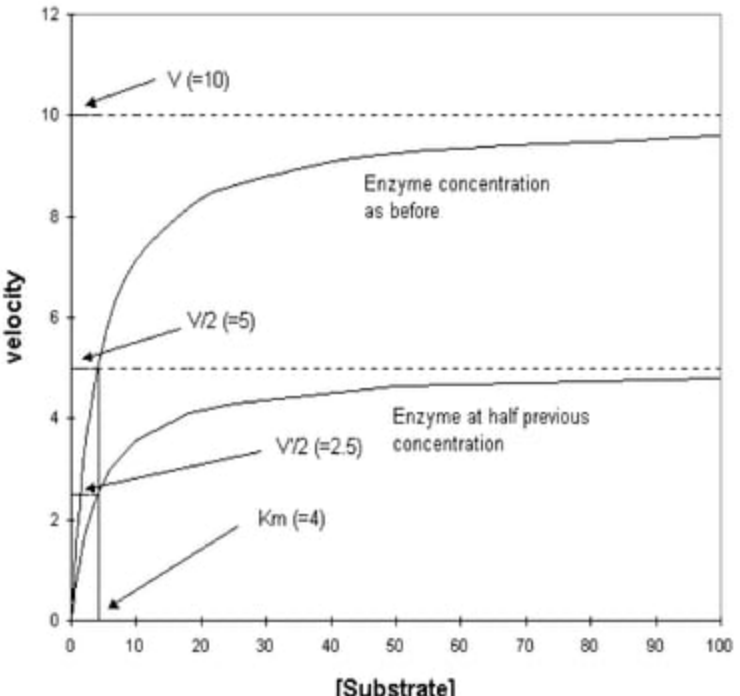
EFFECT OF ENZYME CONC

- The catalysis velocity is also dependent on the concentration of enzyme
-

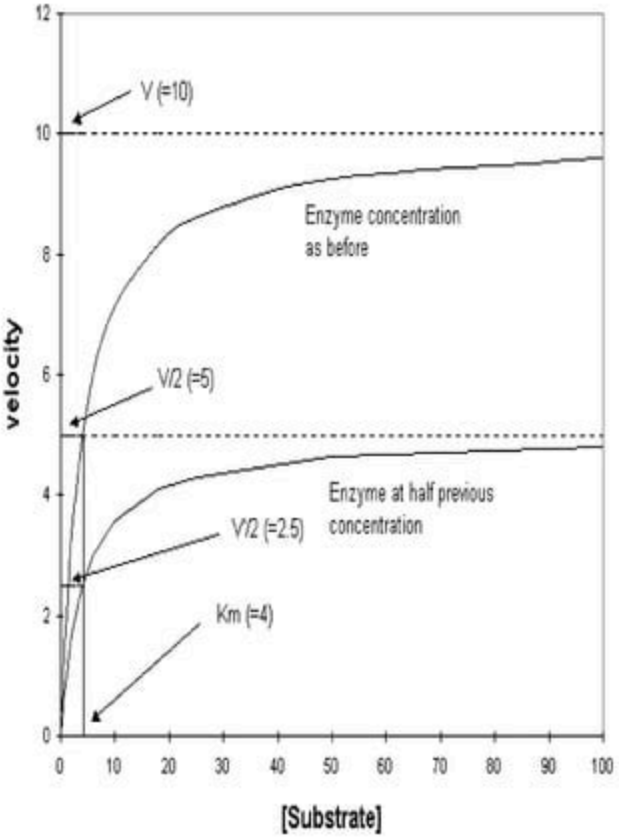
EFFECT OF ENZYME CONC

- Vmax is the reaction velocity at very high, saturating, concentrations of substrate.
- Remember under these conditions every enzyme molecule will have substrate attached to it and will be interacting with it to convert it to product as fast as it can.
- If we *double* the number of enzyme molecules we would have twice as many with substrate bound so you would expect the overall reaction rate to double.
- This is in fact the case. Vmax is directly *proportional* to the concentration of enzyme.

Km



- In this graph we note the effect of *halving* the concentration of enzyme the reaction velocity has reduced from 10 to 5 units.



•Km tells us about the affinity of the enzyme for its substrate. Changing the number of enzyme molecules doesn't alter their individual chemical characteristics

•Consequently Km is *independent* of enzyme concentration.

•Reducing the enzyme concentration by 50% has reduced Vmax by 50%, (Vmax/2), which is used for measuring Km

•The graph shows that the Vmax/2 line for the 50% enzyme cuts the curve at exactly the same point, relative to the horizontal axis, as the

- Km
  - Is a ratio of rate constants =k2 + k3/k1
  - Is equal to [S] when initial rate (v) is equal to ½ Vmax
  - Is a property of ES complex; does not depend on the concentration of E or S
- Vmax
  - Maximum velocity at a fixed E concentration
  - Directly proportional to the [E]

REGULATION OF ENZYME ACTIVITY

SUBSTRATE regulation

Substrate:

- A substance or compound that can binds to the active site of the enzyme and is converted to product (s).
- Enzymes that catalyze a biosynthetic pathway are usually inhibited by the ultimate end-product
  - This is called 'feed back inhibition'
  - E.g. Threonine is converted to isoleucine in 5 steps!
  - The first step is catalysed by threonine deaminase, inhibited by isoleucine at high levels by binding at a regulatory site, far from the catalytic site! Reversible.

ENZYME ACTIVITY CAN BE AFFECTED BY REGULATORY PROTEINS

- An Inhibitor: A compound that binds at or near the active site of the enzyme and thereby suppresses the rate of catalyzed reaction. Can be competitive, non-competitive or end product inhibitors
- A stimulator: A compound that binds at or near the active site of the enzyme and thereby increases the rate of catalyzed reaction

INHIBITORS

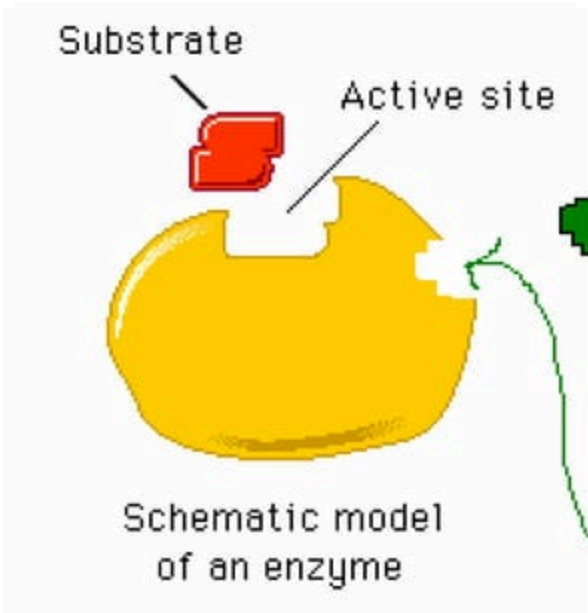
- Competitive inhibitors have structures similar to the substrate whereas structures of

- non-competitive inhibitors and end products inhibitors are generally different from structure of the substrate

ALLOSTERIC INHIBITORS AND ACTIVATORS

- Allosteric activators and inhibitors are compounds that alter the activity of **multimeric allosteric enzymes.**

- Their binding sites on the enzyme are different from the substrate binding site and their effect on the enzyme activity is through a distal conformational effect on the SBS

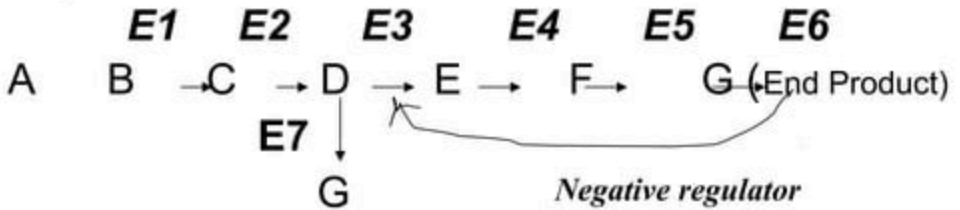

Substrate
Active site
an allosteric activator or inhibitor
Schematic model of an enzyme
allosteric inhibitor/activator site

REGULATION OF ENZYME ACTIVITY MECHANISMS

- **Regulation by modification**
  - *proteolytic cleavage*
  - *Covalent modification*
  - *Protein-protein interaction*
- **Allosteric regulation**
  - Properties of allosteric enzymes (important)
  - Sigmoid kinetics (what does Km mean in this case) (important)
  - Positive and negative modulators (where do they act and how do they modify activity at constant substrate concentration) (most important)
  - Models of allosteric transitions (important)
- **Induction and repression of enzyme synthesis**

Allosteric regulation!!!

These enzyme regulate a sequence of reaction at a single step:



- *In this sequence C can be converted to compound D or G but formation of D is a* ***committed step*** *i.e. once D is formed it must go to products. Enzyme catalyzing this reaction is called an* ***allosteric enzyme****. This enzyme can be up or down regulated. In the cell this reaction is essentially irreversible.*

Properties of Allosteric enzymes

1. *Catalyze irreversible reactions; are rate limiting*
2. *Generally contain more than one polypeptide chain*
3. *Do not follow Michaelis-Menten Kinetics*
4. *Can be upregulated by allosteric activators at constant [S]*
5. *Can be down regulated by allosteric inhibitors at constant [S]*
6. *Activators and Inhibitors need not have any structural resemblance to substrate structure*