Programme: Bachelor of Science (First Year)

Subject: Microbiology

Course Code: MIC 101

Course Title: Microbiology and Biochemistry I

Unit 7: Enzymes

Module Name: Mechanism of enzyme action and multi enzyme complexes

Name of the Presenter: Ms. Rajani Prabhu

An Introduction to Enzymes

- Enzymes are the reaction catalysts of biological systems. They have extraordinary catalytic power, often far greater than that of synthetic catalysts.
- They have a high degree of specificity for their substrates, they accelerate specific chemical reactions, and they function in aqueous solutions under very mild conditions of temperature and pH. Few nonbiological catalysts show all these properties.
- The term enzyme was coined in 1878 by Friedrich Wilhelm Kuhne
- Some of the many enzymes participating in metabolism are regulatory enzymes, which can respond to various metabolic signals by changing their catalytic activity accordingly. Through the action of regulatory enzymes, enzyme systems are highly coordinated to yield a harmonious interplay among the many different metabolic activities necessary to sustain life.

MECHANISM OF ENZYME ACTION

- Catalysis is the prime function of enzymes. The nature of catalysis taking place in the biological system is similar to that of nonbiological catalysis.
- For any chemical reaction to occur, the reactants have to be in an activated state or transition state.

Enzymes lower activation energy:

- The energy required by the reactants to undergo the reaction is known as **activation energy.** The reactants when heated attain the activation energy.
- The catalyst (or the enzyme in the biological system) reduces the activation energy and this causes the reaction to proceed at a lower temperature.
- Enzymes **do not alter the equilibrium constants**; they only enhance the velocity of the reaction.
- The role of catalyst or enzyme is comparable with a tunnel made in a mountain to reduce the barrier as illustrated in fig 1

- The enzyme lowers energy barrier of reactants, thereby making the reaction go faster. The enzymes reduce the activation energy of the reactants in such a way that all the biological systems occur at body temperature (below 40"C).

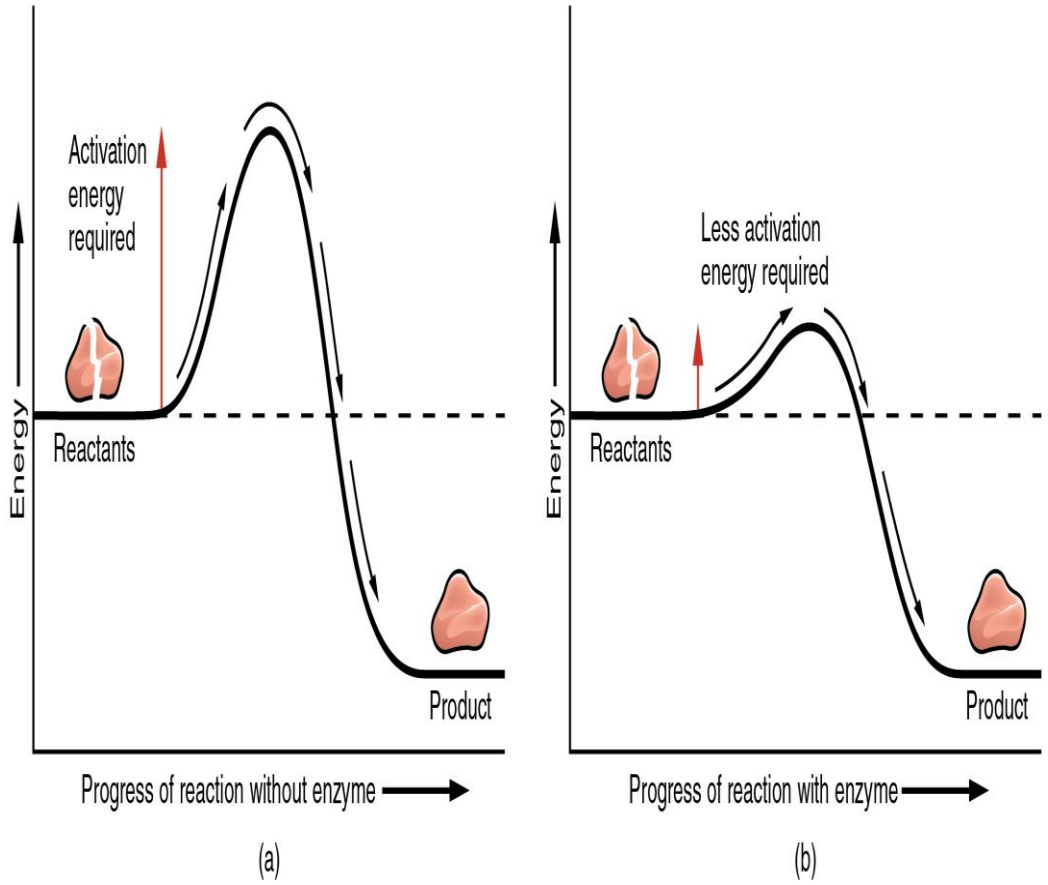

Fig 1

Attributes:

File:212 Enzymes-01.jpg

OpenStax College

Anatomy & Physiology, Connexions Web site. http://cnx.org/content/col11496/1.6/

Enzyme-substrate complex formation

- The prime requisite for enzyme catalysis is that the substrate (S) must combine with the enzyme (E) at the active site to form enzyme-substrate complex (ES) which ultimately results in the product formation (P)
  A few theories have been put forth to explain mechanism of enzyme-substrate complex formation.

Lock and key model or Fischer's template theory

- This theory was proposed by a German biochemist**, Emil Fischer**.
- This is in fact the very first model proposed to explain an enzyme catalysed reaction.

- According to this model, the structure or conformation of the enzyme is rigid. The substrate fits to the binding site (now active site) just as a key fit into the proper lock
- Thus, the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind.
- This model does not give any scope for the flexible nature of enzymes; hence the model totally fails to explain many facts of enzymatic reactions, the most important being the effect of allosteric modulators.

Induced fit theory or Koshland's model

- Koshland in 1958, proposed a more acceptable and realistic model for enzymes substrate complex formation. As per this model, the active site is not rigid and pre-shaped.
- The essential features of the substrate binding site are present at the nascent active site. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate binding site.
- due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis Fig 2
- Induced fit model has sufficient experimental evidence from the X-ray diffraction studies.
- Koshland's model also explains the action of allosteric modulators and competitive inhibition on enzymes.

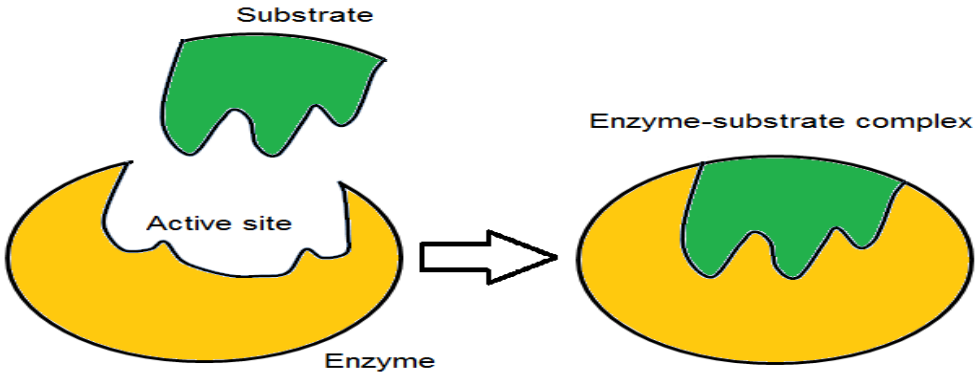

Fig 2

Attributes:

File:Inducedfit080.png

Hottuna080

Paint

GNU Free Documentation License

Substrate strain theory

- In this model, the substrate is strained due to the induced conformation change in the enzyme.
- It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product.

- In fact***, a combination of the induced fit model with the substrate strain is considered to be operative in the enzymatic action***

Multienzyme complexes

- Multienzyme complexes are stable assemblies of more than one enzyme, that carries out a single or a series of biochemical reactions taking place in the cells.
- These are distinct from a multienzyme polypeptide, in which multiple catalytic domains are found in a single polypeptide chain.
- Multienzyme complex: have multiple enzyme unit to carry out different reaction in sequence
  Only the native intact multi enzyme complex is functionally active and not the individual units, if they are separated
- Examples include pyruvate dehydrogenase, fatty acid synthetase, glutamine synthetase, proteasome, rubisco.
- Three levels of organisation have been recognised:
  -First level: subunits not physically associated—important in biosynthetic sequence– shows feedback inhibition
  -Second level: subunits are clustered together and are inactive if separated
  -Third level: enzymes are associated with cellular membranes

Example of multienzyme complex: Pyruvate dehydrogenase complex

- Pyruvate dehydrogenase complex (PDH complex) is a multienzyme complex containing: 3 enzymes + 5 coenzymes + other proteins (+ ATP coenzyme as a regulator)
- 5 conenzymes areThiamine pyrophosphate (TPP) , Co-enzyme A (CoA) , FAD , NAD+ and Lipoamide
- It is a Multienzyme Complex made up of 36 subunits
- E1 = pyruvate dehydrogenase E2 = dihydrolipoamide acetyltransferase E3 = dihydrolipoamide dehydrogenase
- Pyruvate dehydrogenase is an enzyme that catalyzes the reaction of pyruvate and a lipoamide to give the acetylated dihydrolipoamide and carbon dioxide. The conversion requires the coenzyme thiamine pyrophosphate
- Overall reaction of pyruvate dehydrogenase complex

pyruvate + CoASH + NAD+ → acetyl-CoA + CO_2 + NADH + H+

Pyruvate Dehydrogenase (Enzyme 1)

- It catalyses oxidative decarboxylation.
- Cofactors: TPP, Thiamine (B1), a B-complex group vitamin

Dihydro Lipoyl Trans Acetylase (Enzyme 2)

- Catalyses transacylation reaction- transfers the acetyl from lipoyl to the thiol of coenzyme A producing acetyl-CoA.
- E2 can also be known as lipoamide reductase-transacetylase.

Dihydro Lipoyl Dehydrogenase (Enzyme 3)

- The last step is the oxidation of lipoamide.

- the cofactors, namely TPP, Lipoamide & FAD are regenerated.
- FADH2 transfers the reducing equivalents to NAD+ to give NADH + H+, which can pass through the ETC to give 3 ATP (6 ATP from 2 moles of pyruvate formed from glucose) by oxidative phosphorylation.

Advantages of Multienzyme complex

- Multienzyme complexes can be considered a step forward in the evolution of catalytic efficiency as they provide advantages that individual enzymes, even those that have achieved catalytic perfection, would not have alone.
- The rate at which an enzymatic reaction proceeds is partly determined by the frequency with which enzymes and their substrates collide. Hence, increasing the concentrations of enzymes and substrates increases reaction rate.
- However, due to enormous number of different reactions that occur within the cell, concentrations cannot be increased. And in fact in the cells most reactants are present in micromolar concentrations, whereas most enzymes are present in much lower concentrations.
- So to increase the reaction rate, one route is to optimize the spatial organization of enzymes with the formation of multienzyme complexes and multifunctional enzymes, that is, structures that allow minimizing the distance between the active site that catalyses reactions in subsequent step in the sequence, by having active sites close to each other.