

Chapter 5 Microbial Metabolism

Introduction

In living systems, thousands of chemical reactions take place, many of them at the same time. These reactions are referred to as metabolic reactions or metabolism. Metabolic reactions follow the Laws of Thermodynamic: 1) Energy can be neither destroyed nor created; 2) Energy can be converted from a higher to lower grade. Each Metabolic reaction either requires energy input or liberates energy. This energy can be measured in terms of heat units - the calorie. Essentially, all of these metabolic chemical reactions involve substances called enzymes. Enzymes are protein molecules that can accelerate chemical reactions but are not themselves changed by the reaction. Such substances are called biological catalysts. The second focus of this week's work is an examination of these enzymes and their functioning.

Visit these sites for Enzymes and how they function

The section titled Activation Energy and Enzymes is the portion of this page to focus on. Pretty neat ways for molecules to interact I think. Be sure you study this material.

The University of Arizona offers a tutorial site with question for those willing to master a part of chapter 5.

http://www.biology.arizona.edu/biochemistry/problem_sets/energy_enzymes_catalysis/energy_enzymes_catalysis.html

Enzymes allow reactions to proceed at rates which are compatible with the state of some chemical-physical systems which we define as "living"

1. Proteins which lower the energy barrier of reactions - act as "catalysts"
2. Influence rates only - do not affect the equilibrium state
3. Small amounts present - concentration dependency
4. Form reversible complex with substrate
5. Not consumed in the reaction - used over and over
6. Show specificity for a reaction - varies some for different enzymes
7. Fooled by inhibitors - forms the basis for some forms of chemotherapy
8. Each enzyme shows optimal conditions for activity - temperature, pH, etc.
9. Many enzymes require co-factors such as vitamins
10. 2,000 to 3,000 different enzymes in each cell- different cell types have different enzyme sets
11. Often involved in multi-step biochemical pathways
12. Subject to regulation by feedback control and other mechanisms
13. Production directly controlled by genes

What can an Enzyme do?

- = Enzymes increase the likelihood of substrate product reactions
- = Energy of Activation lowered
- = Same ΔG as uncatalyzed reaction

Characteristic of Enzymes

- Enzymes are proteins that bind to a reactant (substrate) to form a:

enzyme-substrate complex

Enzyme + Substrate ----> enzyme-substrate complex ----> enzyme + transformed substrate (product)

- Some enzymes require inorganic ions, or cofactors. An enzyme complex consisting of the protein portion (apoenzyme) and non-protein portion (cofactor) is called a holoenzyme
- This binding is the interactions of relatively weak chemical bonds such as Hydrogen bonds.
- The formation of the enzyme-substrate complex is 3d in design taking place within an "active site" of the enzyme encouraging a chemical reaction to take place.

* Lock and key theory

- One idea holds that the active site of the enzyme match. the shape of the substrate much like a key fits a lock:

* Induced-fit theory

- Another idea holds that the substrate induces a change in the shape of the enzyme resulting in a very close fit of enzyme and substrate:

Although some enzyme function by the lock and key design, most enzyme function more like the induce-fit model.

* Inhibition of Enzymes Action

- Each chemical reaction within a cell has to be carefully regulated.

= Competitive inhibition

This type of inhibition is reversible and is used by the cell for feed back processes.

- Slow Down -

The product of the enzyme reaction resembles the substrate that the product competes for the binding site within the enzyme.

Binding of the product to the active site limits substrate access to the site reducing the overall reaction rate.

= Noncompetitive Inhibition

Inhibition may result from binding of molecule to a separate site not too far from the active site. The molecular arrangement may block the substrate preventing the formation of an enzyme-substrate complex.

Example: Carbon monoxide blocks the active site of the heme-molecule preventing oxygen up-take. Or, penicillin block the active site of an enzyme necessary for the synthesis of bacterial cell walls.

Noncompetivite inhibitors tend to bind to the enzyme irreversibly.

Example: Carbamate insecticides to the colinesterase enzyme

=Allosteric Regulation

Both positive and negative feed-back inhibition.

Binding of molecules to the allosteric type enzyme results in a shape change of the enzyme. This shape change may or may not enhance the active site responsiveness to the substrate.

Factors Influencing Enzymatic Activity

*** Temperature**

Increases in temperature will speed up the rate of nonenzyme mediated reactions, and so temperature increase speeds up enzyme mediated reactions, but only to a point. When heated too much, enzymes (since they are proteins dependent on their shape) become denatured. When the temperature drops, the enzyme regains its shape.

Thermolabile enzymes, such as those responsible for the color distribution in Siamese cats and color camouflage of the Arctic fox, work better (or work at all) at lower temperatures.

= Depending on the environmental adaption of a microbe too high of temperature causes denaturation

* pH

= Drastic changes in pH can lead to the loose in the three dimensional structure of an enzyme resulting in denaturation. Enzymes are adapted to operate at a specific pH or pH range

* Salinity

= Most bacteria that inhabit the human system are not high salt tolerant. Salt (NaCl) interferes with Hydrogen binding sites. within the active site of enzyme

* Substrate Concentration

= Under high substrate concentration enzymes become saturated leveling out the rate of substrate to product turn over.

Cellular Respiration

Prokaryotic microbes can be classified as :

aerobic (requiring O₂ Example: *B. subtilis*)

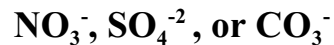
facultative anaerobes (microbes that can grow with or without O₂ - Example: streptococci or yeast)

anaerobic (microbes that grow in the absence of O₂ Example: *Thiobacillus denitrificans*) organisms.

The process of aerobic and anaerobic respiration takes place in the cytoplasm of prokaryotic cells across the cell membrane. A carbon compound like -glucose- can be broken down into 2 three carbon organic acids. This step yielding a net of 2 ATP's and some NADH. If the microbe is an anaerobe then it will need to degrade the NADH down to NAD⁺ to continue the cycle of Glycolysis. The most common by-product of fermentation processes can either be ethanol or lactic acid.

Some bacteria are capable of carrying aerobic respiration using oxygen as its final electron accept: Electrons stripped from carbon during the first and second stages of the respiratory process are channeled into a series of trans-spanning membranes (commonly referred to as the electron transport system) located within the membrane. Here the electrons do work: pumping H⁺ ions against a concentration gradient. Finally, these electrons are absorbed by O₂ forming H₂O as H⁺ stream through a ATPase channel protein.

Anaerobic forms of bacteria will use other types of ionic compounds like:



as a final acceptor of electrons, which are stripped from a carbon source, instead of oxygen.

Go to the following Web site:

Study this page to augment your information and understanding of cellular respiration.

<http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookGlyc.html>

Great non-technical web site with diagrams and hot links to side bars for information amplification.

http://faculty.nl.edu/jste/cellular_respiration.htm

I. GLYCOLYSIS The first stage of the energy-releasing pathway

- A. Enzymes in the cytoplasm catalyze several steps in glucose breakdown
 - 1. Glucose is the first phosphorylated in energy-requiring steps, then the six-carbon intermediate is split to form two molecules of PGAL
 - 2. Enzymes remove H^+ and electrons from PGAL and transfer them to NAD^+ which becomes NADH (used later in the electron transport system)
 - 3. By substrate-level phosphorylation - four ATP are produced but remember two ATPs are used to initiate the catabolism process.
- B. The end products of glycolysis are: 2 pyruvates, 2 ATP (net gain) and 2 NADH for each molecule of glucose degraded

II. Completing the Aerobic Pathway

A. preparatory steps and the Krebs cycle

1. Pyruvate enters the mitochondria and is converted to acetyl-CoA

a) The first of the 3 CO₂ is lost

b) Acetyl-CoA then combines with oxaloacetate present in the mitochondria from the previous turn of the cycle to form Citric acid

2. During each turn of the Krebs cycle, the acetyl-CoA is further degraded leaving as two carbon dioxide (CO₂)

B. Functions of the second stage

1. H⁺ and e⁻ are transferred to NAD⁺ and FAD

2. Two molecules of ATP are produced by substrate-level phosphorylation

C. Third stage of aerobic pathway -- Electron transport phosphorylation

1. NADH and FADH₂ give up their electrons to transport (enzyme) systems embedded in the mitochondrial inner membrane.

2. According to the chemiosmotic theory, energy is released in the passage of electrons through components of the transport series.

a. The energy is used to pump hydrogen ions out the cytoplasm

b. When hydrogen ions flow back through the ATP synthase in the channels, the coupling phosphate (Pi) to ADP yields ATP

D. Summary of the energy harvest

- 1. Electron transport yields - 22 ATP;
Krebs yields 2 ATP by GTP transfer;
Preparatory steps - 6 ATP,
glycolysis yields 8 ATP;

For a grand total of **38 ATP per glucose molecule** vs. **36 for animal muscle cells**

E. Alternates to glycolysis

1. Entner-Doudoroff pathway.

a. Second important pathway

b. Glucose is used as beginning substrate resulting in 3-phosphoglyceraldehyde and pyruvate.

2. Pentose phosphate pathway.
 - a. Allows for oxidation of glucose without use of glycolysis
 - b. Glucose is used as starting resulting in the production of 3CO_2
 - 3 ribulose-5-phosphate (ribulose-5-P)
 - 1) three ribulose-5-P are combined producing two (2) fructose-6-P one (1) glyceraldehyde-3-P
 - 2) the 3-carbon aldehyde can be oxidized to acetic acid by *Gluconobacter species*

III. Fermentation

A. Redox occurs in the absence of any added electron acceptor; glucose is only partially broken down

B. Organic compounds serve as both electron acceptors and electron donors.

1. Only 2 ATP produced by this bio-chemical process

2. NADH/NAD⁺ cycle

- a. A pool of electron donor to acceptor must be maintained

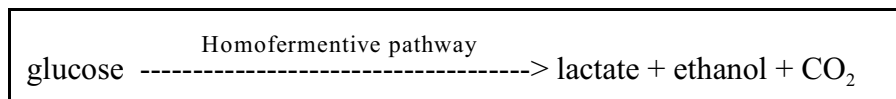
C. Types of fermentation

1. Alcoholic fermentation

- a. acetaldehyde reduced to ethanol using NADH as electron source.

2. Homolactic fermentation

- a. uses pentose phosphate pathway to produce either ethanol or lactic acid



3. Lactic acid fermentation

- a. pyruvic acid is reduced to lactic acid

IV. Photosynthesis

A. Photosynthesis is the unitization of light energy from the sun to synthesize carbohydrates from CO₂.

1. Light energy is converted to chemical energy

2. Photosynthesis takes place in two different sets of reactions

- a. light reaction

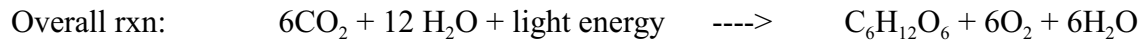
- 1) ATP generated by cyclic photophosphorylation method using

bacterial chlorophyll

a) electrons derived from either H₂O or H_sS

b. dark reaction

1) Dark reaction - CO₂ is reduced by chemical energy generated in light reaction to synthesize carbohydrates



B. Different types of photosynthetic modes

1. Autotrophs: inorganic carbon (CO₂) used as a sole carbon source.

a. Photo-autotrophs - sun used as energy source

Oxygenic - producing O₂

Example: Cyanobacteria and algae

Anoxygenic - non O₂ producer

Example: Green and purple bacteria

b. Chemo-autotrophs (Chemolithotrophs) - those prokaryotes that use inorganic compounds such as hydrogen sulfide, ammonia, or nitrite and/or other inorganic compounds as their electron source.

Example: *Pseudomonas saccharophila* or *Nitrosomonas*, *Nitrobacter*