

The Wheels on the Bus Microbial Metabolism Part I

Microbial Metabolism

- Metabolism is the sum of the chemical reactions in an organism.
- Catabolism is the energy-releasing processes.
- Anabolism is the energy-using processes.
- Catabolism provides the building blocks and energy for anabolism.
- A metabolic pathway is a sequence of enzymatically catalyzed chemical reactions in a cell.
- Metabolic pathways are determined by enzymes.
- The collision theory states that chemical reactions can occur when atoms, ions, and molecules collide.
- Activation energy is needed to disrupt electronic configurations.
- Reaction rate is the frequency of collisions with enough energy to bring about a reaction.
- Reaction rate can be increased by enzymes or by increasing temperature or pressure.

Enzymes

- The turnover number is generally 1-10,000 molecules per second.
- Apoenzyme: protein
- Cofactor: Nonprotein component
 - Coenzyme: Organic cofactor
- Holoenzyme: Apoenzyme + cofactor

Enzyme-substrate interactions

- Substrates specifically bind to the active sites on the enzyme
 - “lock-and-key”
 - Induced fit
- Once the reaction is complete, the product is released and the enzyme reused

Cofactors: Metal Ions and Coenzymes

- Metallic cofactors
 - Iron, copper, magnesium, manganese, zinc, cobalt, selenium, etc.
 - Assist with precise functions between enzyme and substrate
 - Activate enzymes
 - Help bring the active site and substrate close together
 - Participate directly in chemical reactions
- Coenzymes
 - Organic compounds that work with the apoenzyme to alter the substrate
 - Remove a chemical group from one substrate and add it to another substrate
 - Carry and transfer hydrogen atoms, electrons, carbon dioxide, and amino groups
 - Vitamins are an important component of coenzymes

Important Coenzymes

- NAD⁺

- NADP+
- FAD
- Coenzyme A

Enzyme Classification

- Oxidoreductase
 - Oxidation-reduction reactions
- Transferase
 - Transfer functional groups
- Hydrolase
 - Hydrolysis
- Lyase
 - Removal of atoms without hydrolysis
- Isomerase
 - Rearrangement of atoms
- Ligase
 - Joining of molecules, uses ATP

Types of Reaction

- Condensation
- Hydrolysis
- Transfer reactions

Transfer reactions

- Transfer of electrons from one substrate to another
 - Oxidation and reduction
 - Oxidoreductase
- Transfer of functional groups from one molecule to another
 - Transferases
 - Aminotransferases

Location of Enzyme Action

Exoenzymes:

- Transported extracellularly
- Break down large food molecules or harmful chemicals

Endoenzymes:

- Retained intracellularly and function there
- Most enzymes of metabolic pathways

Factors Influencing Enzyme Activity

- Enzymes can be denatured by temperature and pH
- Temperature
- pH
- Substrate concentration
- Competitive inhibitors fill in the active site of an enzyme and competes with the enzyme's normal substrate.
- Noncompetitive inhibitors do not fill in or bind to the enzyme's active site.

- Feedback inhibition

Controls on Enzyme Synthesis

Repression

- Stops further synthesis of an enzyme somewhere along its pathway
 - If the end product of an enzymatic reaction reaches excess, the genetic apparatus for replacing enzymes is repressed
 - Response time is longer than for feedback inhibition, effects more enduring

Induction

- Enzymes appear (are induced) only when suitable substrates are present
 - Synthesis of an enzyme is induced by its substrate
 - Inverse of enzyme repression

Ribozymes

- RNA that cuts and splices RNA.
- They can function as catalysts.
- They have active sites that substrates can bind to.

Oxidation-Reduction

- Oxidation is the removal of electrons.
- Reduction is the gain of electrons.
- Redox reaction is an oxidation reaction paired with a reduction reaction.
- In biological systems, the electrons are often associated with hydrogen atoms. Biological oxidations are often dehydrogenations.

Electron carriers

- Coenzymes
 - Nicotinamide adenine dinucleotide (NAD)
- Respiratory chain carriers
 - Cytochromes (protein)

Adenosine Triphosphate (ATP)

- Temporary energy repository
- Breaking of phosphate bonds will release free energy
- Three-part molecule
 - Nitrogen base
 - 5-carbon sugar (ribose)
 - Chain of phosphates

Metabolic Role of ATP

- Primary energy currency of the cell
 - When it is used in a chemical reaction, it must be replaced
 - ATP utilization and replenishment is an ongoing cycle
- Substrate-level phosphorylation
 - Generation of ATP through a transfer of a phosphate group from a phosphorylated

compound directly to ADP

- Oxidative phosphorylation
 - A series of redox reactions occurring during the final phase of the respiratory pathway
- Photophosphorylation
 - ATP formed through a series of sunlight-driven reactions in phototrophs

Overview of the Three Main Pathways of Catabolism Pathways

- Catabolism
 - Embden-Meyerhof-Parnas (EMP) pathway or Glycolysis
 - Tricarboxylic acid cycle (TCA)
 - Respiratory chain
 - Aerobic
 - Anaerobic
 - Alternate pathways
 - Fermentation

Aerobic respiration

- Glycolysis
- Tricarboxylic acid (TCA)
- Electron transport

Glycolysis

- Oxidation of glucose
- Phosphorylation of some intermediates (Uses two ATPs)
- Splits a 6 carbon sugar into two 3 carbon molecules
- Coenzyme NAD is reduced to NADH
- Substrate-level-phosphorylation (Four ATPs are synthesized)
- Water is generated
- Net yield of 2 ATPs
- Final intermediates are two Pyruvic acid molecules

Six Carbon Stage

- 2 ATPs are used
- Glucose is split to form Glyceraldehyde-3-phosphate molecules.

Three Carbon Stage

- 2 Glyceraldehyde-3-phosphate molecules are oxidized to 2 pyruvic acid.
- 4 ATP produced
- 2 NADH produced

Glycolysis

- $\text{Glucose} + 2 \text{ ATP} + 2 \text{ ADP} + 2 \text{ PO}_4^- + 2 \text{ NAD}^+ \rightarrow$
 $2 \text{ pyruvic acid} + 4 \text{ ATP} + 2 \text{ NADH} + 2 \text{ H}^+$

Alternatives to Glycolysis

- Pentose phosphate pathway (PPP or HMS):

- Breaks down pentoses
- NADPH is produced
- Operates with glycolysis
- Produces key intermediates.
- Entner-Doudoroff pathway:
 - Produces NADPH and ATP
 - Generally not found in Gram positive bacteria
 - *Pseudomonas*, *Rhizobium*, *Agrobacterium* are representative organisms that use this pathway.

Cellular Respiration

- Oxidation of molecules liberates electrons for the electron transport chain.
- ATP generated by oxidative phosphorylation.

Intermediate Step

- Pyruvic acid (from glycolysis) is oxidized and decarboxylated

Krebs Cycle

- This cycle produces CO₂, H₂O, NADH and FADH₂
- Each pyruvic acid is processed to enter the TCA cycle (two complete cycles)
- CO₂ is generated
- Coenzymes NAD and FAD are reduced to NADH and FADH₂
- Net yield of two ATPs
- Critical intermediates are synthesized

Reactions of a Single Turn of the Krebs Cycle

- The 2C acetyl CoA molecule combines with oxaloacetic acid, forming 6C citrate, and releasing CoA.
- Citrate changes the arrangement of atoms to form isocitric acid.
- Isocitric acid is converted to 5C α-ketoglutaric acid, which yields NADH and CO₂.
- α-ketoglutaric acid loses the second CO₂ and generates another NADH+H⁺ plus 4C succinyl CoA.
- Succinyl CoA is converted to succinic acid and regenerates CoA. This releases energy that is captured in ATP.
- Succinic acid loses 2 H⁺ and 2 e⁻, yielding fumaric acid and generating FADH₂.
- Fumaric acid reacts with water to form malic acid.
- An additional NADH is formed when malic acid is converted to oxaloacetic acid, which is the final product to enter the cycle again, by reacting with acetyl CoA.

The Electron Transport Chain

- A series of carrier molecules that are, in turn, oxidized and reduced as electrons are passed down the chain.
- Energy released can be used to produce ATP by chemiosmosis.
- Mitochondria
 - eukaryotes
- Cytoplasmic membrane

– prokaryotes

Respiration

- Aerobic respiration: The final electron acceptor in the electron transport chain is molecular oxygen (O_2).
- Anaerobic respiration(?): The final electron acceptor in the electron transport chain is not O_2 .
- Yields less energy than aerobic respiration because only part of the Krebs cycle operates under anaerobic conditions.
- Energy produced from complete oxidation of 1 glucose using aerobic respiration
- ATP produced from complete oxidation of 1 glucose using aerobic respiration
- 36 ATPs are produced in eukaryotes.