Anaerobic Protist Metabolism

*Giardia, Entamoeba* and Trichomonads

Review glycolysis and oxidative decarboxylation

Parasite Biochemistry (in general)

- Parasitic life style = ADAPTATIONS
- Specific niche = diversity in adaptations
  - Anaerobic vs. aerobic
- All parasites still require a supply of energy for biosynthesis of macromolecules, growth, mechanical activity, reproduction etc.
- A major nutritional requirement is supplied by the host.
- Limited range of biosynthetic pathways - evolution of salvage pathways - no purine (A, G) biosynthetic pathway in any parasites - only salvage pathways.
## Amitochondriate Biochemistry

- Lumen dwelling - environment is $O_2$ low
- Anaerobic or *microaerophilic* organisms
  - Do not require $O_2$ for survival and multiplication
  - $O_2$ is not the terminal electron acceptor
  - Can tolerate low $O_2$ conc., growth is inhibited under higher $O_2$ conc. (aerotolerant anaerobes)
- Fermentative metabolism
  - In the presence or absence of $O_2$
  - Different end products
- Metabolic oddities
  - PPI-linked enzymes (instead of ATP)
  - Fe-S based carbohydrate catabolism

## Synthesis of ATP

- **Oxidative phosphorylation** - coupling of ATP formation to the respiratory chain (electron transport, membrane associated, $O_2$ as final e- acceptor). As electrons move through complexes, a proton gradient is generated which drives ATP formation. **Chemiosmotic theory - P. Mitchell, 1978.**
- **Substrate level phosphorylation** - direct phosphorylation of ADP via the transfer from a high-energy intermediate.
Why dislike biochemistry?

Eukaryotic Central Energy Metabolism

Figure 2. A simple 'stoichiometric' model for central energy metabolism in eukaryotes. Dotted lines indicate intermedia-
diary metabolism and multiple enzyme-catalyzed reactions. Abbreviations: I, II, III, IV, V, mitochondrial complexes I, II, III, IV, and V, respectively; lact, alternative mitochondrial NADH-ubiquinone oxidoreductase; c, mitochondrial cytochrome c; UQ, ubiquinone.
Basic Regulation of Glycolysis

- **Phosphofructokinase** - tightly regulated by ATP/ADP/AMP ratios. Inhibited by ↑ ATP, activated by ↑ AMP, activated by ↑ ADP. Energy status of the cell helps regulate glycolysis.

- **Substrate level phosphorylation** - direct phosphorylation of ADP via the transfer from a high-energy intermediate.
Glycolysis I

- Overall scheme is similar to aerobic eukaryotes
- Enzymes that utilize PPi instead of ATP
- Glycolytic control points are lacking - high flux through glycolysis
- 2 routes to produce pyruvate: direct + bypass
- Fe-S proteins for pyruvate decarboxylation
- Fd more like anaerobic bacteria

PPi vs. ATP

Inorganic pyrophosphate

Adenosine Triphosphate

‘High Energy’ Phosphoanhydride bond

Phosphoanhydride bond

Adenosine

AMP

ADP

ATP

PPi
Glycolysis II

- PPI phosphofructokinase
  - No regulation
- 2 routes to produce pyruvate: direct + bypass
  - Slightly different
- PEP is a key metabolite instead of acetyl-CoA
- Pyruvate catabolism is compartmentalized
- Fe-S proteins for pyruvate decarboxylation
- Fd more like mitochondrial

Differences in ferredoxins

- 2 [4Fe-4S]
- [2Fe-2S]
Hydrogenosomal Metabolism

Glycolysis

pyruvate

pyruvate → CO₂

NADH → NAD⁺

NAD⁺ → NADH

Hydrogenase

Hydrogenase

2H⁺ → H₂

Acetyl-CoA

Acetyl-CoA + SUCCINYL-CoA → ACETATE + SUCCINATE

STK

ATP

ADP

PFO

Pyruvate ferredoxin oxidoreductase

pyruvate + CoA + (ox)ferredoxin = acetyl-CoA + CO₂ + (red) ferredoxin

NFO - NAD(P)H ferredoxin oxidoreductase

NAD⁺ → NADH + H⁺

reduced ferredoxin + NAD⁺ = oxidized ferredoxin + NADH + H⁺

Hydrogenase

reduced ferredoxin + 2 H⁺ = oxidized ferredoxin + H₂

Acetate: succinate CoA transferase

Acetyl-CoA + succinate = succinyl-CoA + acetate

Succinate thiokinase (succinyl-CoA synthetase)

ADP + phosphate + succinyl-CoA = succinate + CoA + ATP

Important Enzymes
**Metronidazole**

- Rare example of a drug developed for parasitic disease that gained broader use as an antibiotic.
- Extracts of *Streptomyces* were screened for activity against *T. vaginalis*. Azomycin was identified (nitroimidazole).
- Synthetic derivative, Metronidazole, became major drug used for treatment (Flagyl).
- Soon after, was also found effective against *Giardia* and *Entamoeba*.
- 1970’s: became useful for treatment against anaerobic bacteria.

![Metronidazole structures]

**Metronidazole Activation**

**Prodrug** requiring a reductive environment

![Metronidazole activation diagram]

**Mode of action:**
Reduced in anaerobic organisms to reactive metabolites which release superoxide anions.

Little toxicity to human - due to aerobic respiration.
Review

- Glycolysis
  - Compare/contrast mammalian with parasitic

- Mitochondrial respiration
  - Compare/contrast aerobic respiration with anaerobic protist metabolism
  - Future lectures will include variations on the “aerobic” theme.

KEY TO REMEMBER
- Adaptations for specific niche
- Unique biological properties

Comparison of Pyruvate Decarboxylation

PFO complex?

PDC complex
How to Find New Drugs

1. Random screening
   no design or biological insight
2. Analogues of known drugs
   not a new target
3. Rational lead discovery
   long time & expensive

If you need any of the pathways
enlarged please email me