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 O₂ low
- Anaerobic or microaerophilic organisms
  - Do not require O₂ for survival and multiplication
  - O₂ is not the terminal electron acceptor
  - Can tolerate low O₂ conc., growth is inhibited under higher O₂ conc. (aerotolerant anaerobes)
- Fermentative metabolism
  - In the presence or absence of O₂
  - Different end products
- Metabolic oddities
  - PPi-linked enzymes (instead of ATP)
  - Fe-S based carbohydrate catabolism
### Synthesis of ATP


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

### Glycolysis

- 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

These reactions are NOT in redox balance! Fd recycling?

### PPi vs. ATP

- **Inorganic pyrophosphate**
- **Adenosine Triphosphate**

<table>
<thead>
<tr>
<th>Inorganic pyrophosphate</th>
<th>Adenosine Triphosphate</th>
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<tbody>
<tr>
<td><img src="image1" alt="PPi" /></td>
<td><img src="image2" alt="ATP" /></td>
</tr>
<tr>
<td>'High Energy' Phosphoanhydride bond</td>
<td><img src="image3" alt="Structure" /></td>
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<tr>
<td>O - P - O - P - O'</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>O'</td>
<td>ATP</td>
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</tbody>
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Phosphofructokinase

Pyruvate phosphate dikinase

Phosphofructokinase

Ferredoxin (2Fe-4S)

PPPi vs. ATP
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
- Hydrogenases
- Acetyl-CoA
- Succinate
- Malonyl-CoA
- ADP
- ATP
### Important Enzymes

- **PFO** - pyruvate ferredoxin oxidoreductase
  - pyruvate + CoA + (ox)ferredoxin = acetyl-CoA + CO₂ + (red) ferredoxin
- **NFO** - NAD(P)H ferredoxin oxidoreductase
  - 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

### 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 Activation

**Prodrug requiring a reductive environment**

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

**Little toxicity to humans due to semicarbazide.
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

Carbohydrate Metabolism in Eukaryotes

Hexose → Pyruvate → $\text{CO}_2 + \text{Acetate}$
- No compartmentalization

Hexose → Pyruvate → $\text{CO}_2 + \text{H}_2 \text{O}$
- Mitochondrial compartmentalization

Hexose → Pyruvate → $\text{CO}_2 + \text{H}_2 \text{Acetate}$
- Hydrogenosomal compartmentalization

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