## FERMENTATION

**READING:** BOM-12

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5.7  NAD as a Redox Electron Carrier (Review)

5.8  Energy-Rich Compounds and Energy Storage (Review)

5.9  Energy Conservation (Review)

5.10 Glycolysis as an Example of Fermentation

Review the essential reactions of Glycolysis:

Glyceraldehyde-3-Phosphate Dehydrogenase

\[
\text{Glyceraldehyde-3-phosphate} + \text{NAD}^+ + \text{P}_i \xrightarrow{\text{glyceraldehyde-3-phosphate dehydrogenase}} \text{1,3-Bisphosphoglycerate} + \text{NADH} + \text{H}^+
\]

This is the only redox reaction in Glycolysis. Electrons are transferred to NAD\(^+\) and the free energy is conserved by the substrate-level phosphorylation.

Phosphoglycerate Kinase (Substrate-Level Phosphorylation)

\[
\text{1,3-Bisphosphoglycerate} + \text{ADP} \xrightarrow{\text{phosphoglycerate kinase}} \text{3-Phosphoglycerate} + \text{ATP}
\]

The free energy in the phosphoester bond of the substrate is used to phosphorylate an ADP.

Pasteur's usage, that "fermentation" means "life without air" required revision after the discovery of anaerobic respiration.

The current definition of fermentation, intended to distinguish fermentation from anaerobic respiration, is that it is a chemoorganotrophic metabolism in which the electrons released by the oxidation of the growth substrate reduce an electron acceptor that is internally generated as a result of the catabolic pathway itself.
This implies:

- fermentative organisms can inhabit ecological niches where external electron acceptors are not available (for many organisms fermentation is "facultative")
- no electron transport
- all ATP is generated by substrate-level phosphorylation.
- electrochemical gradient can be generated by reverse activity of $\text{H}^+$ translocating ATP synthase
- electrons released by oxidative reactions are a problem, not a benefit
- energetic inefficiency
- all fermentation pathways must maintain "redox balance".

The specific examples of fermentations that we cover are:

<table>
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<tr>
<td>HOMOLACTIC</td>
<td><em>Streptococcus, Lactobacillus</em></td>
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16.1.3 Lactic Acid Bacteria and Lactic Acid Fermentations

16.2.2 Clostridium
21.1 Fermentations: Energetic and Redox considerations

In this section you are interested specifically in the so-called "Phosphoroclastic Reaction" presented in Fig. 21.3.

This reaction is discussed below in the context of the Clostridial Acetone/Butanol Fermentation.

21.2 Fermentative Diversity: Lactic and Mixed Acid Fermentations

Ethanolic Fermentation

Under anoxic conditions, yeast down-regulate expression of pyruvate dehydrogenase and up-regulate expression of pyruvate decarboxylase.

In this reaction, the decarboxylation is not an oxidation, so NAD+ is not required as electron acceptor (no redox penalty).

Redox balance is restored by reducing acetaldehyde to ethanol.
Homolactic Fermentation

\[
\text{Glucose} + 6 \text{ O}_2 \rightarrow 6 \text{ CO}_2 + 6 \text{ H}_2\text{O} \quad \Delta G'' = -2,850 \text{ kJ mol}^{-1}
\]

\[
\text{Glucose} \rightarrow 2 \text{ Lactate} + 2 \text{ H}^+ \quad \Delta G'' = -196 \text{ kJ mol}^{-1}
\]

\[
2 \text{ ADP} + 2 \text{ Pi} \rightarrow 2 \text{ ATP} \quad \Delta G'' = +61 \text{ kJ mol}^{-1}
\]

Efficiency = \( \frac{61}{2,858} = 0.02 \) (about 2%)

Given this gross energetic inefficiency, how can we explain the widespread prevalence of Lactic Acid Bacteria in nature? Indeed, how did they evolve from ancestors which could metabolize glucose with much higher thermodynamic efficiency by using the TCA cycle and electron transport? And why do they often predominate, even in aerobic systems?

- Adaptation to environments rich in organic substrates but lacking O\textsubscript{2} or other external electron acceptors.
- pH change inhibits growth of competitors.
- Kinetic Efficiency vs Thermodynamic Efficiency (i.e. \#ATP/time rather than \#ATP/substrate molecule may be important)
- Can grow in environments with very low iron, since they don’t have cytochromes or other Fe-containing redox proteins.

The Lactic Acid fermentations have been important in human cultures as methods to prevent food spoilage without refrigeration.
21.3  Fermentative Diversity: Clostridial and Propionic Acid Fermentations

Here we are covering only the first 2 subsections (Sugar Fermentation by Clostridium Species and Solvent Production and Energetic Consequences)

BUTANOL/ACETONE FERMENTATION

Pyruvate decarboxylation in this fermentation involves an idiosyncratic "Phosphoroclastic Reaction" (pyruvate-ferredoxin oxidoreductase and hydrogenase) See Fig. 21.3.

The bioenergetic significance of this reaction is that it accomplishes oxidative decarboxylation of pyruvate without incurring the redox penalty of NAD\(^+\) reduction. The energy released in the oxidation is conserved by thioester bond formation in Acetyl-CoA. The lack avoidance of the ordinary redox penalty "liberates" the free energy stored in Acetyl-CoA so that it can drive, via Acetyl-P, an additional substrate-level phosphorylation. (See Fig. 21.3).

The balanced net reaction for the pyruvate-ferredoxin oxidoreductase reaction:

\[
\text{pyruvate} + \text{CoA} + 2 \text{FD}_{\text{ox}} + 2\text{H}^+ \rightarrow \Delta 2\text{e}^- \rightarrow \text{acetyl-CoA} + \text{CO}_2 + 2\text{FD}_{\text{ox}} + \text{H}_2
\]

Note that the iron-sulfur protein Ferredoxin (FD) merely shuttles electrons from the substrate to H\(^+\), and is regenerated in the process.

The Phosphoroclastic Reaction does not fit our strict definitions of "fermentation" and "respiration". It is not respiration because no electron transport chain is involved. It is not fermentation because an external electron acceptor (H\(^+\)) is used.

Clinical and Ecological significance:

*Clostridium perfringens*, the etiological agent of "Gas Gangrene' uses the Phosphoroclastic Reaction. The "gas" produced in infected tissue is a mixture of CO\(_2\) and H\(_2\).

*Clostridium botulinum* produces sufficient quantities of CO\(_2\) and H\(_2\) during growth in canned foods that the metal cans become noticeably distorted.

Methanogenic Archaea can shift the equilibrium of the Phosphoroclastic reaction toward completion by removing the products: CO\(_2\) + H\(_2\) --- \rightarrow CH\(_4\).

Note that in Fig. 21.6 each "2H" highlighted in brown represents the oxidation of one NADH. Also note that Fig. 21.6 does not show the 2 NADH produced by Glycolysis.
Fate of Acetyl-CoA Carbons:

The crux of it is that the pathway must re-oxidize 1 NADH for each Acetyl-CoA in order to maintain redox balance.

Pathway formatted for maximum ATP production:

\[
\begin{align*}
2 \text{ GLUCOSE} & \rightarrow 4 \text{ PYRUVATE} + 4 \text{ ATP} + 4 \text{ NADH} \\
4 \text{ PYRUVATE} & \rightarrow 4 \text{ ACETYL-CoA} + 4 \text{ CO}_2 + 4 \text{ H}_2 \\
1 \text{ ACETYL-CoA} + 2 \text{ Pi} & \rightarrow 1 \text{ ACETATE} + 1 \text{ ATP} \\
1 \text{ ACETYL-CoA} + 2 \text{ NADH} & \rightarrow 1 \text{ ETHANOL} \\
2 \text{ ACETYL-CoA} + 2 \text{ NADH} & \rightarrow \text{ BUTYRATE} + 1 \text{ ATP} \\
\hline
2 \text{ GLUCOSE} & \rightarrow \text{ ACETATE} + \text{ BUTYRATE} + \text{ ETHANOL} + 4 \text{ CO}_2 + 4 \text{ H}_2 + 6 \text{ ATP}
\end{align*}
\]

Pathway formatted for neutral products:

\[
\begin{align*}
2 \text{ GLUCOSE} & \rightarrow 4 \text{ PYRUVATE} + 4 \text{ ATP} + 4 \text{ NADH} \\
4 \text{ PYRUVATE} & \rightarrow 2 \text{ ACETOACETYL-CoA} + 4 \text{ CO}_2 + 4 \text{ H}_2 \\
1 \text{ ACETOACETYL-CoA} + 4 \text{ NADH} & \rightarrow \text{ BUTANOL} \\
1 \text{ ACETOACETYL-CoA} & \rightarrow \text{ ACETONE} + 1 \text{ CO}_2 \\
\hline
2 \text{ GLUCOSE} & \rightarrow \text{ ACETONE} + \text{ BUTANOL} + 5 \text{ CO}_2 + 4 \text{ H}_2 + 4 \text{ ATP}
\end{align*}
\]
COMPARATIVE SUMMARY OF What Happens to Pyruvate
DISCUSSION QUESTIONS:

BOM-11 Chap. 5: 11, 12, 13; Chap. 17: 18
BOM-12 Chap. 5: 11, 12, 13; Chap. 21: 1

1. The lactic acid fermentations are grossly inefficient from the standpoint of #ATP synthesized per glucose molecule. Yet, the Lactic Acid bacteria are widespread and predominate in many environments. How can this be explained?

2. Compare the energetic efficiency of the alcoholic fermentation to the homolactic fermentation in terms of #ATP per glucose.

3. What is the bioenergetic significance of the phosphoroclastic reaction (pyruvate -ferredoxin oxidoreductase) and why does it violate strict definitions of "fermentation" and "respiration"?

4. What role can methanogenic Archaea play in the Acetone-Butanol fermentation of Clostridia?

5. In what sense would it be reasonable to say that electron transport systems represent "making a virtue of necessity".

6. Explicitly define the difference between "respiration" and "fermentation".

7. Describe the concept of "redox balance".

8. Consider the fate of Acetyl-CoA carbons if Acetyl-CoA is formed by the phosphoroclastic reaction and if there is no condensation reaction to form acetoacetyl-CoA. In other words, there are only 2-carbon end products formed.

Write a balanced overall net reaction for metabolism of glucose under these circumstances. The reaction must represent a metabolism that is in redox balance. Show overall net yield of ATP.