

CHAPTER 5 – MAJOR METABOLIC PATHWAYS

Introduction (p.133) –

1. Metabolic Engineering (remove and/or add genes)
2. Catabolism – process of degrading into smaller/simpler products & produces energy
3. Anabolism – synthesis of more complex compounds & requires energy

5.2-Bioenergetics (p. 134)

1. Metabolic reactions in a bacterial cell (**Fig. 5.1**)

(1) Fueling (Class I), (2) Biosynthesis (Class II), (3) Polymerization (Class III)



4. Formation of ATP:

1. Substrate level phosphorylation - organic compounds as energy source
2. Oxidative phosphorylation - electron transport
3. Photophosphorylation - electron transport

5. **Fig. 5.2** PEP and 1,3-DPG are high energy P cpds & P donors, while

6. H atoms – released in biological redox reactions carried by $\text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+$ (**Fig.5.3**)

7. (p. 36) $\text{NADH} + \text{H}^+$ – two major functions:

(1) Reducing Power - supply H in biosynthetic reactions



(2) ATP formation in Respiration Metabolism - H transferred to O_2

Results in formation of up to 3 ATP molecules.

ATP can be formed if alternate electron acceptor (AEA) is present (NO_3^-)

5.3 Glucose Metabolism: Glycolysis & TCA Cycle (Embden-Meyerhof-Parnas) pathway

1. (p.137) Aerobic catabolism of organic compounds – 3 phases:

(1) EMP pathway for fermentation of glucose to pyruvate

(2) Krebs, tricarboxylic acid (TCA), or citric acid cycle for conversion of pyruvate to NADH and CO_2

(3) Respiratory or electron transport chain for formation of ATP by transferring e^- from NADH to an electron acceptor (EA).

(a) aerobic respiration - O_2 as EA

(2) anaerobic respiration – NO_3^- , SO_4^{2-} , Fe^{2+} , S^0

2. Glycolysis – **Fig. 5.4 (p.138)**

(1) Energy (ATP) used in Steps 1 and 3 for phosphorylation

(2) Energy (ATP) gained by substrate-level P : $1,3\text{-DP-GA} \rightarrow 3\text{P-GA}$
: $\text{PEP} \rightarrow \text{pyruvate}$

Question: What is the net ATP yield for EMP pathway?

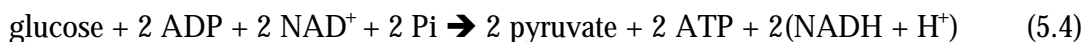
3. Pyruvate as key metabolite:

(1) anaerobic conditions – lactic acid, ethanol, acetone, acetic acid

(2) aerobic conditions – converted to NADH and CO_2 through TCA cycle

(3) Glycolysis reaction:

Occurs in CYTOPLASM



4. Bioenergetics of Glycolysis: Efficiency of energy conversion

Glycolysis: $\Delta G = - 52,000$ cal/mol glucose
2 ADP \rightarrow 2 ATP $\Delta G = + 14,600$ cal/mol glucose
 $\Delta G = - 37,400$ cal/mole glucose

(1) Efficiency = $\frac{14,600 \text{ cal}}{52,000 \text{ cal}} = 28\%$ for lactic acid fermentation

(2) Efficiency in utilizing total energy potentially available from glucose:

E = $\frac{14,600 \text{ cal}}{686,000 \text{ cal}} = 2\%$ (typical of fermentations)

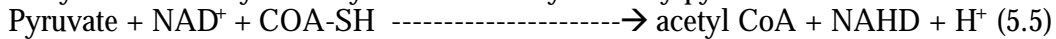
1. (p. 139) TCA cycle main functions:

1. provide e (NADH) for electron transport chain and biosynthesis
2. supply C skeletons for AMINO ACID synthesis
3. generate energy

TCA Cycle occurs in matrix of Mitochondria in Eucaryotes

TCA Cycle occurs in membrane-bound enzymes in Procaryotes

Entry into Krebs Cycle – acylation of coenzyme-A by pyruvate



Pyruvate dehydrogenase

Note: Acetyl CoA is transferred through the mitochondrial membrane at expense of conversion of 2 NADHs produced in glycolysis to 2 FADH.

Acetyl CoA = key intermediate in metabolism of **AMINO ACIDS** & **FATTY ACIDS**

6. TCA Cycle – **Fig. 5.5** (p. 140)

Question: Show where Reducing Power is generated and energy is made in TCA

Precursors for certain AMINO ACIDS: succinate and α -ketoglutarate

7. Heterotrophic CO₂ fixation!

(1) TCA intermediates used for biosynthesis \rightarrow “short-circuit” of TCA cycle

(2) Heterotrophic CO₂ fixation by cell to maintain functioning TCA cycle

(a) PCP + CO₂ = oxalacetate

(b) pyruvate + CO₂ = oxalacetate

(c) pyruvate + CO₂ = malate

(3) Can be important factor in culturing microbes. Growth can be limited by the

RATE OF CO₂ FIXATION TO MAINTAIN THE TCA CYCLE! –

(a) when culture is initiated at low density with little accumulation of intracellular CO₂, or

(b) when a gas sparge rate into a fermentation tank is high

5.4 Respiration (p. 141)

Question: How many (max) ATPs are produced through EMP and TCA cycle with O₂ as terminal electron acceptor? Show calculations using spreadsheet and connect biochemical reaction to the production of reducing power. (See Table 5.1, p.141)

1. Oxidative phosphorylation - forming ATP from electron transport chain
 - a. 3 ATPs for each NAHD + H
 - b. 2 ATPs for each FADH₂ in eucaryotes
2. Figure 5.6 – Respiratory (cytochrome) chain
 - a. regenerate NADs for glycolysis
 - b. generate ATPs for synthesis

3. Overall reaction of glucose metabolism in eucaryotes:



P/O = 36 ATP/ 12 O = 3 ATP/O for NADH

P/O = 4 ATP/ 2 O = 2 ATP/O for FADH₂

Energy Balance for Efficiency:

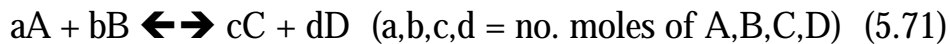
36 ATPs x 7.3 kcal/mole ATP = 263 kcal captured

$\Delta G^\circ = 686$ kcal for oxidation of glucose according to equation (5.7)

Efficiency = 263 kcal captured/ 686 kcal released = 38%

Now consider the relationship between standard free energy change (ΔG°) and the equilibrium constant for a chemical reaction:

Consider:



Free Energy Change @ constant Temp and Press= f (standard free energy, concentration of reactants and products of the reaction)

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\text{C}]^c [\text{D}]^d}{[\text{A}]^a [\text{B}]^b} \quad (5.7.2)$$

So the standard free energy change for glucose oxidation is 686 kcal/mole.

$\Delta G = \Delta G^\circ$ only when all reactants and products are present at 1.0 M.

Now, how about the concentrations of reactants and products in a human cell? Human erythrocyte has been evaluation of steady-state concentrations of intermediates in glycolysis. When these concentrations of reactants and products are considered, the energy recovery efficiency is about 53%.



Efficiency = 14.6 kcal captured/ 52 kcal released = 28%

In muscle tissue for glycolysis efficiency - the actual efficiency is higher than 53% (close to 60%).

Back to p. 141. For Prokaryotes – $ATP/NADH + H < 2$; $ATP/FADH_s = 1$
5.5 (p.142) CONTROL SITES IN AEROBIC GLUCOSE METABOLISM

I Glycolysis:

1. phosphofructokinase (active) $\frac{ATP}{ADP}$ phosphofructokinase (inactive)

Phosphofructokinase is ALLOSTERIC enzyme activated by ADP & Pi, and inactivated by ATP.

2. O₂ concentration – regulatory effect called Pasteur Effect. Glycolysis Rate higher under anaerobic conditions. Why does aerobic conditions slow down rate of glycolysis? Explain

3. High NADH/NAD⁺ reduces the rate of glycolysis.

II. Krebs Cycle: High ATP/ADP and NADH/NAD⁺ ratios reduce the processing rate of the TCA cycle.

III. Electron Transport Chain inhibition

1. Block e⁻ transport in span between NADH and ubiquinone (act on NADH dehydrogenase)

Rotenone (plant substance used by South American Indians as fish poison)

Amytal (barbiturate drug)

Piercidin (antibiotic that resembles ubiquinone and can compete with it)

2. Block e⁻ transport in span between cytochrome b to c

Antimycin A (isolated from Streptomyces griseus)

3. Block e⁻ transport from cytochrome aa₃ to oxygen

Hydrogen cyanide (HCN)

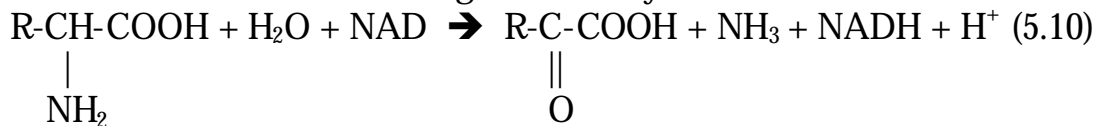
Hydrogen sulfide (H₂S)

Carbon monoxide (CO)

5.6 (p. 143) METABOLISM OF NITROGENOUS COMPOUNDS

Used by the cell as carbon, nitrogen, and energy sources

1. Amino Acids converted to organic acids by deamination



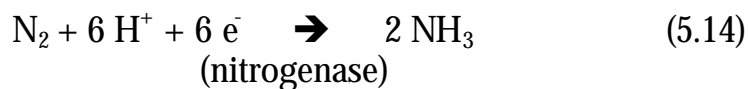
a) Organic acids – oxidized for energy production (ATP)

b) NH₃ used in protein and nucleic acid synthesis

2. Transamination–change amino acids to organic acids & other amino acids amino group exchanged for keto group of 2-keto acid

4. Nucleic Acids–purines/ymidines→ urea + acetic acid → NH₃ + CO₂

5.7 (p. 144) NITROGEN FIXATION



- Need reducing conditions (no oxygen)

- **Rhizobium and Azospirillum** – used for agricultural purposes – fix N and provide ammonium to plants. **They are bioprocess products.**

5.8 (p. 144-145) METABOLISM OF HYDROCARBONS

1. Aliphatic HCs – Bioremediation – requires oxygen (Pseudomonas, Mycobacteria).

a. Low water solubility is a barrier to rapid metabolism.

Refer to article “Transfer of Polycyclic Aromatic Hydrocarbons Between Model Membranes: Relation to Carcinogenicity.” Authors: Plant, A.L., H.J. Pownall, and L. C. Smith. Journal: Chem.-Biol. Interactions, 44: 237-246 (1983).

b. Metabolism–first step–oxygenation by oxygenases (alcohol, aldehyde, acid)

2. Aromatic Hydrocarbons –Metabolism – aerobic, oxygenases.

See example for Benzene (p. 145)

Benzene → catechol → cis-cis-muconate → B-keto adipate → acetyl CoA + succinate

Anaerobic metabolism – alternate electron acceptors (NO_3^- , SO_4^{-2} , Mn^{+4} , Fe^{+3})

5.9 (p. 145) OVERVIEW OF BIOSYNTHESIS

1. Pentose-phosphate pathway (Hexose-monophosphate pathway) (Fig. 5.7)
 - a. Reducing Power produced (NADPH) for anabolism
 - b. Carbon skeletons (3, 4, 5, & 7 C) for biosynthesis using NADPH
 - (1) ribose, purines, coenzymes, aromatic amino acids)
 - (2) amino acid – commercial products - families – Fig. 5.8 (p. 147)
 - c. Fatty Acid synthesis – stepwise buildup of acetyl-CoA.
 - (1) acetyl-CoA + CO₂ → malonyl-CoA intermediate of F.A. synthesis
 - (2) CO₂ requirement – can lengthen start-up phase for commercial prod. (supply key lipids, e.g., oleic acid)
 - d. Polysaccharide synthesis – from hexose (e.g., glucose) is common
If < 6 carbons, **EMP** pathway can operate in reverse to produce glucose = GLUCONEOGENESIS
 - (a) start with pyruvate, but pyruvate-PEP is irreversible in glycolysis (EMP)
 - (b) pyruvate + CO₂ + ATP + H₂O → oxalacetate + ADP + Pi + 2H⁺
oxalacetate + ATP → PEP + ADP + CO₂ (5.17)
 - (c) after PEP, glycolysis reversible up to fructose-1,6-diphosphate
need two enzymes NOT in EMP
 1. fructose-1,6-diphosphatase
 2. glucose-6-phosphatase

5.10 (p. 148) ANAEROBIC METABOLISM

1. Anaerobic Respiration (Section 5.2) -
 - a. same pathways as aerobic metabolism
 - b. different terminal electron acceptor (Alternate Electron Acceptor)
e.g., Denitrification (Dissimilatory) NO₃⁻ → N₂
 - c. Fermentation – generation of energy without electron transport chain
 1. Fig. 5.9 – lactic acid & alcoholic fermentations
 2. Fig. 5.10- Fermentation of sugars from lactic acid
 - d. Fig. 5.11 – Entner-Doudoroff Pathway –
 1. fermentation of glucose by Zymomonas.
 2. production of 1 mole ATP per mole glucose (not 2)
 - (a). low energy yield forces more glucose into ethanol and less into cell mass than for yeast, which uses glycolysis to produce pyruvate (yields 2 moles ATP/mole glucose)

1. Fix CO₂ by enzyme ribulose biphosphate carboxylase

a. Key step in Calvin Cycle

Ribulose-1,5-diphosphate + CO₂ → 2 glyceric acid-3-phosphate

b. See Fig. 5.12 (p. 151):

(1) CO₂ fixation

(2) Reduction of fixed CO₂

(3) Regeneration of CO₂ acceptor

2. Photosynthesis – 6 CO₂ + H₂O → C₆H₁₂O₆ + 6 O₂ (5.19)
light

(1) Phase 1 = Light Phase

H₂O + NADP + Pi + ADP → oxygen + NADPH + H + ATP (5.20)
light

(2) Phase 2 = Dark Phase

CO₂ + NADPH + H⁺ + ATP → 1/6 glucose + NADP⁺ + ADP + Pi

3. Photophosphorylation – ATP generation in photosynthesis, whereby light absorbed by chlorophyll results in electron excitation, and return to normal state by emitting light quanta (fluorescence) and electron that travels through electron transport chain.

5.12 (p. 152) SUMMARY – class read on you own

End Chapter 5