BCH 4054 Chapter 20 Lecture Notes

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Chapter 20

The Tricarboxylic Acid Cycle

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TCA Cycle

aka **Krebs Cycle** and **Citric Acid Cycle**

- Occurs inside mitochondria (the matrix)
- Overall reaction:

$$\text{Acetyl-CoA} + 3\ NAD^+ + CoQ + GDP + P_i \rightarrow 2\ CO_2 + 3\ NADH + CoQH_2 + GTP + CoASH$$

- Bridging reaction required that converts pyruvate to acetyl-CoA.

Mitochondria have two membranes: inner and outer. The outer has pores and is permeable to many things. The inner is a permeability barrier which requires specific transporters for most compounds to move across it. The matrix is inside the inner membrane.

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Bridging Reaction: Pyruvate → Acetyl-CoA

- Catalyzed by **Pyruvate Dehydrogenase**
- Overall reaction:

$$\text{Pyruvate} + CoASH + NAD^+ \rightarrow \text{Acetyl-CoA} + NADH + CO_2$$

- Two **cosubstrate** coenzymes:
  - Coenzyme A and NAD^+
- Three **prosthetic group** coenzymes:
  - thiamine pyrophosphate, lipoic acid, FAD

The reaction is an oxidative decarboxylation of an alpha keto acid.

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Pyruvate Dehydrogenase

- Multienzyme complex (See Figure p.646)
- Multiple copies of three enzymes, each with a prosthetic group:

Pyruvate Dehydrogenase	PDH	Thiamine Pyrophosphate	24 dimers
Dihydrolipoyl Transacetylase	TA	Lipoic Acid	24 subunits (cubic core)
Dihydrolipoyl Dehydrogenase	DLD	FAD	12 subunits

Note the first subunit has the same name as the overall enzyme.

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Pyruvate Dehydrogenase, con't.

- Mechanism involves two covalent intermediates with the enzyme:
  - Addition of pyruvate to TPP and loss of CO_2 forms **hydroxyethyl TPP**.
    - (This same intermediate is formed by pyruvate decarboxylase in yeast alcoholic fermentation).
  - Transfer of hydroxyethyl group to lipoic acid to form acetyl lipoic acid.
    - (This is an internal oxidation-reduction: acetaldehyde is oxidized, lipoic acid is reduced.)
  - See mechanisms page 646.

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Pyruvate Dehydrogenase, con't.

- Note the role of lipoic acid as a **swinging arm** that can move to bind to three different active sites. It cycles through three chemical forms.
- The overall reaction is irreversible.
- The enzyme is found inside the mitochondria. Therefore pyruvate must cross the mitochondrial membrane.

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ABC's of the TCA Cycle

- A: two carbon unit (acetyl-CoA) is **Added** to four carbon **carrier** (oxaloacetate) to form six carbon intermediate (citrate)
- B: six carbon intermediate is **Broken Down** to four carbon succinate.
- C: **Carrier** (oxaloacetate) is regenerated from succinate.
  - See Figure 20.4

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Citrate Synthase

- **Perkin condensation** of acetyl-CoA with oxaloacetate. (Figure 20.5)
- Alpha hydrogen more labile than in an oxygen ester.
- Cleavage of thiolester contributes to overall negative ΔG.
  - Reaction is **removed from equilibrium.**
- Product is a **prochiral** compound.

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Aconitase

- Catalyzes equilibrium between **citrate**, **aconitate**, and **isocitrate.**
  - See Figure 20.7
- Elements of H_2O are removed and added back. Aconitate does not dissociate.
- Iron-sulfur cluster accepts the OH^- leaving group, and donates it back.
  - See Figure 20.8.
- The reaction operates **near equilibrium**.

In the inset of Figure 20.7, the iron sulfur cluster is shown associated with the substrate and with two cysteine residues.

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Fluoroacetate Inhibition

- Fluoroacetate forms fluorocitrate.
  - See Figure 20.9
- Fluorocitrate inhibits aconitase, thereby blocking the TCA cycle.
- Fluoroacetate is known as a **trojan horse inhibitor**.

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Isocitrate Dehydrogenase

- Oxidative decarboxylation of a beta-hydroxy acid.
- NAD^+ oxidizes the OH group to a carbonyl intermediate, forming a beta-keto acid, which undergoes decarboxylation.
  - (See Figure 20.10)
- The reaction is **removed from equilibrium**.

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Isocitrate Dehydrogenase

- First oxidative step of the cycle.
- First CO_2 produced.
- Mechanism similar to other beta-hydroxyacid oxidative decarboxylations:
  - Malic enzyme
  - 6-phosphogluconate dehydrogenase
- An **isozyme** of isocitrate dehydrogenase in both mitochondria and cytoplasm utilizes $NADP^+$ to form NADPH.

The $NADP^+$ isozyme was the first discovered. It is more easily measured because the NAD^+ enzyme requires ADP as an allosteric activator. In very old biochemistry textbooks, you may see NADPH as a product of this reaction. Malic enzyme and 6-phosphogluconate dehydrogenase, along with the NADPH form of isocitrate dehydrogenase, are the main sources of NADPH synthesis.

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α-Ketoglutarate Dehydrogenase

- The second oxidative step of the TCA cycle.
- Oxidative decarboxylation of an alpha-keto acid. (Figure 20.11)
- Mechanism identical to that of pyruvate dehydrogenase.
  - NAD^+ and CoASH cosubstrate coenzymes
  - TPP, lipoic acid, and FAD prosthetic group coenzymes.
- Reaction is **removed from equlibrium.**

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Succinyl-CoA Synthetase

- Preservation of the energy of a thiolester in the form of a phosphoanhydride (as GTP)
  - Figure 20.12
- Mechanism involves interemediate phosphorylation of the enzyme at a histidine residue.
  - Figure 20.13
- Reaction is **near equilibrium.**

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Succinate Dehydrogenase

- An integral membrane protein complex, in the inner mitochondrial membrane.
- Third oxidative step of the cycle. Contains FAD as a covalently bound prosthetic group.
  - See Figure 20.15
- Also contains an iron-sulfur protein.
  - See Figure 20.16
- Reaction is **near equilibrium.**

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Succinate Dehydrogenase, con't.

- Reaction often written with FAD as reactant and $FADH_2$ as product. (Figure 20.14)
- But FAD is a **prosthetic group coenzyme**, and is regenerated in the catalytic cycle.
- Overall reaction involves passing electrons to **coenzyme Q** (or **CoQ)**.

Succinate + CoQ $\rightleftarrows$ Fumarate + $CoQH_2$

Coenzyme Q is a lipid-soluble compound, and is dissolved in the inner mitochondrial membrane. We will see in Chapter 21 how it becomes re-oxidized by the electron transport chain.

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Fumarase and Malate Dehydrogenase

- Fumarase
  - Hydration of the double bond.
  - See Figure 20.17
- Malate Dehydrogenase
  - Fourth oxidative step of TCA cycle.
  - Regenerates oxaloacetate (OAA).
  - Reaction is **near equilibrium.** Equilibrium favors malate, therefore OAA concentration is kept low.

Oxaloacetate is a beta-ketoacid, and subject to chemical decarboxylation as a first order reaction. By keeping its concentration low, the rate of its decarboxylation will also be low.

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Tracing Carbon Atoms

- Radioactivity from 1-^{14}C-acetyl-CoA is not converted to CO_2 until the **second turn**. All of the radioactivity is lost in the second turn.
  - (See Figure 20.21a)
- Radioactivity from 2-^{14}C-acetyl-CoA is not converted to CO_2 until the **third turn**. The rest remains in the cycle intermediates, losing one-half on each subsequent turn.
  - (See Figure 20.21b)

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TCA Cycle as an Amphibolic Pathway

- TCA cycle intermediates are precursors of a number of compounds.
  - See Figure 20.22.
- Export of citrate to furnish acetyl-CoA to the cytoplasm.
  - See Figure 20.23

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Anaplerotic Reactions

"Filling Up" Reactions

- TCA cycle intermediates act **catalytically.**
- Reactions to replace them when their concentrations drop are called **anaplerotic** reactions.
- Four possible reactions: (Figure 20.24)
  - Pyruvate carboxylase
  - PEP carboxylase (primarily in plants)
  - Malic enzyme
  - PEP carboxykinase (Figure 20.25)

Pyruvate carboxylase is probably the most important anaplerotic reaction. The enzyme has biotin as a prosthetic group, and we will discuss the mechanism of this enzyme in more detail next term in connection with other biotin carboxylases.
PEP carboxykinase probably more important in working in reverse to form phosphoenolpyruvate.

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Pyruvate Carboxylase

- Contains biotin prosthetic group.
  - (See Figure 18.32)
- Two step reaction:

Biotin carboxylase:

$$\text{E-biotin} + \text{ATP} + CO_2 \rightarrow \text{E-biotin-}CO_2 + \text{ADP} + \text{Pi}$$

Transcarboxylase:

$$\text{E-biotin-}CO_2 + \text{pyruvate} \rightarrow \text{E-biotin} + \text{oxaloacetate}$$

- Biotin is the "swinging arm" communicating between the two active sites.

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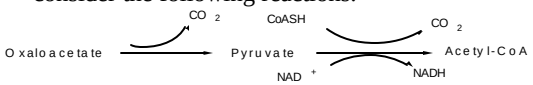
Net degradation of TCA intermediates

- Degradation of an intermediate, like oxaloacetate, involves more than just "running it through the cycle".
- For each OAA used in the cycle, one more is regenerated.
- Therefore for **stoichiometric** removal, one must consider the following reactions:



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Regulation of TCA Cycle

- Eight reactions, only three are **removed from equilibrium.**
  - Citrate synthase
  - Isocitrate dehydrogenase
  - Alpha-ketoglutarate dehydrogenase
- The "bridging reaction", pyruvate dehydrogenase, is also removed from equilibrium.
- These are primary control points.
  - See Figure 20.26.

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Regulation of Pyruvate Dehydrogenase

- TA inhibited by acetyl-CoA, activated by CoASH
- DLD inhibited by NADH, activated by NAD^+
- Mammalian enzyme inhibited by phosphorylation with PDH kinase (Figure 20.27)
  - PDH kinase activated by NADH and acetyl-CoA
  - PDH phosphatase activated by Ca^{2+}
- AMP activates, GTP inhibits

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Regulation of Citrate Synthase

- Inhibited by ATP
- Inhibited by NADH
- Inhibited by succinyl CoA

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Regulation of Isocitrate Dehydrogenase

- Activated by NAD^+ and ADP
- Inactivated by NADH and ATP
- E. coli enzyme inhibited by phosphorylation.
  - Kinase is inhibited by TCA cycle intermediates.
  - Kinase is activated when TCA cycle intermediates are low
    - Isocitrate is diverted to the **glyoxalate pathway.**

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Regulation of α-Ketoglutarate Dehydrogenase

- TA inhibited by succinyl-CoA
- DLD inhibited by NADH
- AMP activates
- (compare pyruvate dehydrogenase)

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Glyoxalate Cycle

- Found in plants and bacteria
- Two additional enzymes
  - **Isocitrate lyase** (Figure 20.29)

  Isocitrate $\rightarrow$ succinate + glyoxalate
  - **Malate synthase** (Figure 20.30)

  Acetyl-CoA + glyoxalate $\rightarrow$ malate + CoASH

Note that malate synthase is similar in mechanism to citrate synthase. Hydrolysis of the thiol ester bond provides the driving force for forming the C-C bond.

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Glyoxalate Cycle, con't.

- Overall cycle provides for **net** synthesis of a TCA cycle intermediate from 2 acetyl-CoA.
  - Figure 20.28
- Steps are compartmentalized between the **glyoxysome** and the **mitochondrion**.
  - Figure 20.31

Because of glyoxalate cycle, plants and bacteria can convert fatty acids (via acetyl-CoA) to carbohydrate (via oxaloacetate), where animals cannot carry out this conversion.