

Chapter 16 Homework Assignment

- The following problems will be due once we finish the chapter:

1, 3, 7, 10, 16, 19, 20

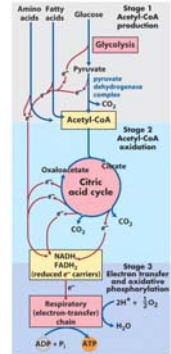
- Additional Problem:
 - Write out the eight reaction steps of the Citric Acid Cycle, using structures to describe the intermediates. Use the correct stoichiometry to show the final products derived from one Acetyl-CoA molecule. Identify the enzyme and any required cofactors for each step. Use arrows to show which reactions are irreversible and which are reversible.

Chapter 16

1

Aerobic Fate of Pyruvate

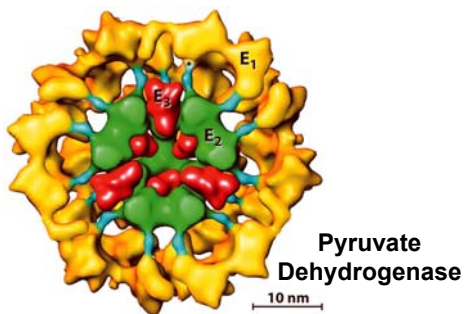
- Molecular processes by which cells use O_2 and produce CO_2 are termed **cellular respiration**
- Cellular respiration occurs in three major stages:
 - Organic fuel molecules are oxidized to yield two carbon fragments in the form of the acetyl group of Acetyl-Coenzyme A (Acetyl-CoA)
 - These acetyl groups are fed into the citric acid cycle (TCA) which enzymatically oxidized them to CO_2 and the energy released is conserved in NADH and $FADH_2$
 - These reduced coenzymes are themselves oxidized, giving up protons and electrons (e^- are transferred to O_2)



Chapter 16

3

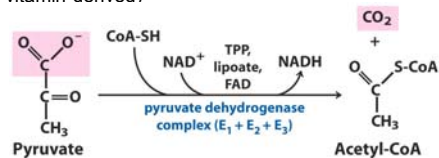
Chapter 16 The Citric Acid Cycle



Pyruvate Dehydrogenase

Production of Acetyl-CoA Oxidative Decarboxylation

- Conversion from a 3-carbon (Pyruvate) unit to a 2-carbon unit is achieved by **oxidative decarboxylation**
- This reaction is catalyzed by the **pyruvate dehydrogenase complex**
 - A cluster of 3 enzymes that requires 5 cofactors (4 of which are vitamin-derived)



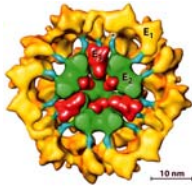
Chapter 16

$\Delta G'^{\circ} = -33.4 \text{ kJ/mol}$

4

Production of Acetyl-CoA Pyruvate Dehydrogenase Complex

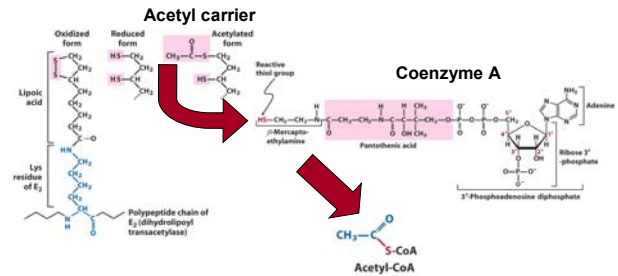
- The PD complex is a quintessential example of substrate channeling and quaternary structure
- All five steps occur with substrate/product "channeled" in transiting the 3 enzymes (E_1 , E_2 , and E_3)
- Each enzyme is present in **many** copies (12-60), making it several times the size of a ribosome
- In structure and mechanism, it is very similar to the α -ketoglutarate dehydrogenase (TCA cycle) and to α -keto-acid dehydrogenase (amino acid oxidation)
- It exploits the "swinging lipoyllysine arms" of E_2 that accept both the electrons and the acetate group of pyruvate (from E_1) and pass them to E_3



Chapter 16

5

Production of Acetyl-CoA The "Swinging Lipoyllysine Arms" in Action....



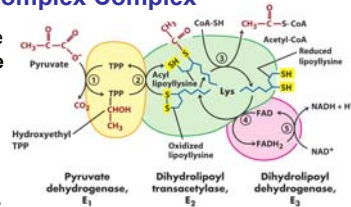
What do you remember about the acyl group transfer potential of thioesters?

Chapter 16

7

Production of Acetyl-CoA A VERY Complex Complex

- The five reaction sequence is an example of **substrate channelling**
- The intermediates of the multistep sequence never leave the complex and the local concentration of the substrate of E_2 is kept very high.
- This channelling prevents the theft of the activated acetyl group by other enzymes that use it as a substrate



Learn about "TPP" on your own:

What is it?

What group does it carry?

What role does it play, here and in fermentation of glucose to ethanol?

See p. 540-541, Fig. 14-13, Table 14-1

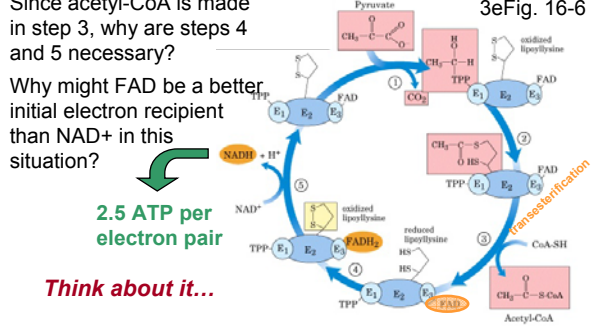
Chapter 16

Production of Acetyl-CoA ...in a Handoff to CoA and FAD

- Since acetyl-CoA is made in step 3, why are steps 4 and 5 necessary?
- Why might FAD be a better initial electron recipient than NAD^+ in this situation?

2.5 ATP per electron pair

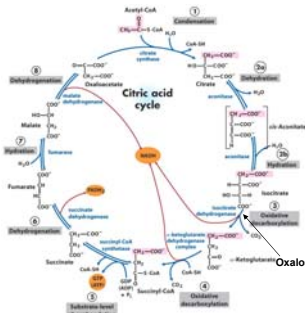
Think about it...



Chapter 16

8

The Citric Acid Cycle (TCA) A Satellite View



You simply have to learn:

- The eight steps and their characteristics (rxn type!)
- The structure of each reactant and product
- Enzyme names for each step
- Places where:
 - Oxidation occurs
 - CO₂ is released
 - GTP is produced
 - NADH or FADH₂ is produced

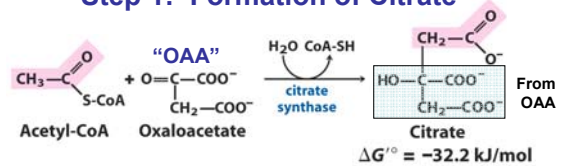
Piece of Cake!

Certain Actors Isolated Oxygen. Keeping Such Sucked For Many Others.

Chapter 16

9

The Citric Acid Cycle (TCA) Step 1: Formation of Citrate



- A 2 + 4 carbon condensation exploiting an “induced fit” conformational change in **Citrate Synthase** upon OAA binding
 - This change ensures productive regeneration of CoA-SH
- The reaction is driven by a large negative ΔG due to the hydrolysis of the high energy thioester of Acetyl-CoA
- This driving force is essential because the cellular concentration of OAA is μM or less

Chapter 16

11

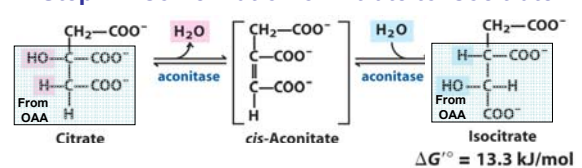
The Citric Acid Cycle (TCA) Overall, it's not so bad...

- A 2-carbon unit Acetyl-CoA is added to the cycle
- And two CO₂ molecules leave (but they are different carbons...)
- During the course of changes in the carbon skeleton and its oxidation state
- And the transfer of energy to form GTP (aka. the “Canadian \$”) and reducing power, as NADH and FADH₂
- It is at the hub of metabolism, because its intermediates can be end-products of catabolism as well as precursors for anabolism
- It is also coordinately regulated with other pathways (as we'll see later on)

Chapter 16

10

The Citric Acid Cycle (TCA) Step 2: Isomerization of Citrate to Isocitrate

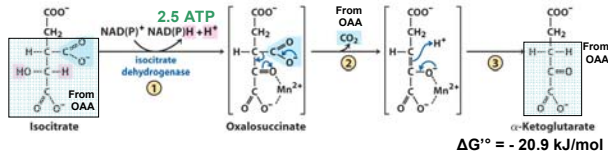


- A clever way to make an isomer!
- **Aconitase** contains an **iron-sulfur center**, which acts both in the binding of the substrate at the active site and in the catalytic addition and removal of H₂O
- Though energetically unfavorable, removal of product (in step 3) pulls the reaction along

Chapter 16

12

The Citric Acid Cycle (TCA) Step 3: Oxidation to α -Ketoglutarate

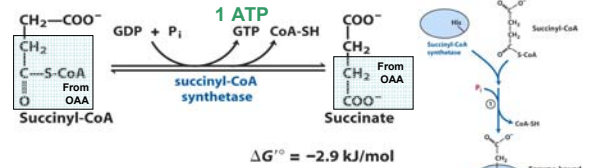


- **Isocitrate dehydrogenase** catalyzes the oxidation of a 2° alcohol to a ketone, followed by a decarboxylation
 - The reaction is dependent on NAD^+
- This reaction results in the loss of a carbon and of electrons, generating reducing power in an **irreversible** step of the cycle
- The enzyme utilizes a Mn^{2+} ion in the active site to interact with the transient carbonyl formed and to stabilize the transient enol formed during catalysis

Chapter 16

13

The Citric Acid Cycle (TCA) Step 5: Conversion of Succinyl-CoA to Succinate

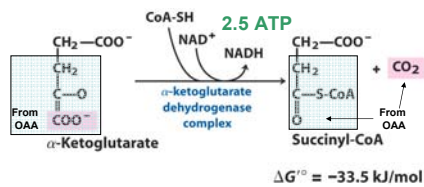


- Check out that ΔG° . Does that jibe with what you know about the energy of thioesters? What do you think happened?
- **Succinyl-CoA synthetase** carries out a substrate-level phosphorylation by replacing HS-CoA with P_i
- This P_i group is transferred to an adjacent His residue
- This His is located at the interface with the nucleotide-binding subunit allowing for transfer to GDP and regenerating the enzyme

Chapter 16

15

The Citric Acid Cycle (TCA) Step 4: Oxidation of α -KG to Succinyl-CoA

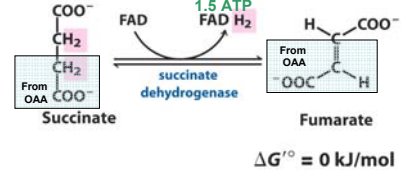


- **Does this look familiar?**
- **α -Ketoglutarate dehydrogenase complex** and its reaction are virtually identical to the pyruvate dehydrogenase complex
- The KDC has the same set of **five coenzymes** and is likewise irreversible

Chapter 16

14

The Citric Acid Cycle (TCA) Step 6: Oxidation of Succinate to Fumarate



- **Succinate dehydrogenase** is the only membrane-bound enzyme of the TCA cycle
- SD has 3 iron-sulfur centers that bring resulting electrons from FADH_2 to the chain of electron carriers in the membrane
- This transfer to FADH_2 ultimately yields 1.5 ATPs/electron pair
- The reaction can be strongly inhibited by the succinate analog **malonate**

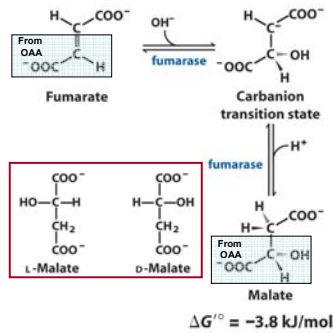
Chapter 16

16

The Citric Acid Cycle (TCA)

Step 7: Hydration of Fumarate to Malate

- Fumarase** catalyzes the reversible stereospecific hydration of fumarate to L-malate
- This reaction is highly stereospecific in that it will catalyze the hydration of the trans double bond of fumarate but not the cis form
- In the reverse direction, the enzyme is equally specific in that it will only dehydrate L-Malate, not D-Malate.



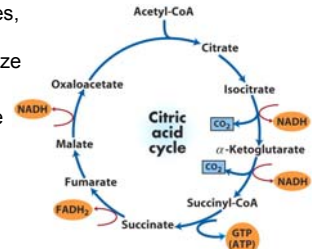
Chapter 16

17

The Citric Acid Cycle (TCA)

Show Me the Money!!

- Remember, each glucose molecule yields **two** pyruvates, so that means we need two turns of the TCA to fully oxidize a single glucose.
- So at the end of two turns we have:
 - 2 ATP (from GTP)
 - 6 NADH (~ 15 ATP)
 - 2 FADH₂ (~ 3 ATP)



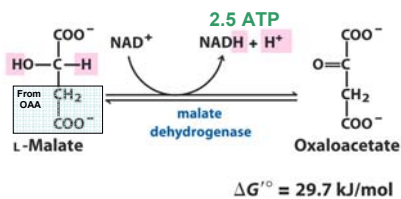
Total of ~ 20 ATP for each Glucose just from the TCA!

Chapter 16

19

The Citric Acid Cycle (TCA)

Step 8: Oxidation of Malate to Oxaloacetate (OAA)



- How in the world does this reaction run? Look at that ΔG° .
- What do you think drives this reaction? Think about the rest of the cycle....

Chapter 16

18

The Citric Acid Cycle (TCA)

What about \$\$ from Glycolysis?

Glycolysis – per glucose TCA Cycle – per Glucose

2 ATP	2 GTP (2 ATP)
2 NADH (~ 5 ATP)	6 NADH (15 ATP)
	2 FADH ₂ (3 ATP)

Total of ~ 27 ATP for each Glucose

Are we missing anything?

Overall, about 65% of the total 2840 kJ/mole of glucose becomes available to the cell (see Box 13-1).

Next Question:

How is energy generated from NADH and FADH₂?

Chapter 16

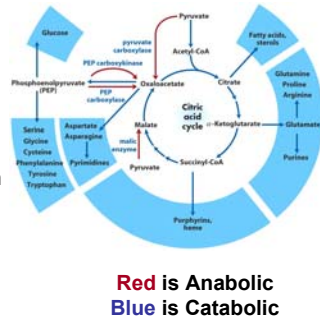
20

The Citric Acid Cycle (TCA)

The TCA Cycle is Amphibolic

- The TCA cycle is an **amphibolic pathway** meaning it serves in both catabolic and anabolic processes
- This cycle also provides precursors for many biosynthetic pathways, such as:

- Amino Acids
- Porphyrin Ring of Heme
- Fatty Acids and Sterols
- Glucose



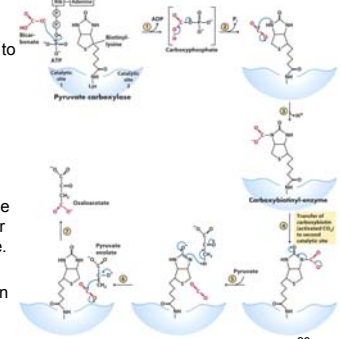
Chapter 16

21

The Citric Acid Cycle (TCA)

Pyruvate Carboxylase

- The most important anaplerotic reaction is the reversible carboxylation of pyruvate by CO_2 to form oxaloacetate
- The enzymatic addition requires energy which is supplied by ATP
- The reaction also requires the vitamin **biotin** which is the prosthetic group of the enzyme
- There are three catalytic sites, one for ATP/Bicarbonate, a second for the biotin, and a third for pyruvate.
- This enzyme is a regulatory enzyme that is basically inactive in the **absence** of Acetyl-CoA



Chapter 16

23

The Citric Acid Cycle (TCA)

How does the cycle run if intermediates leave?

- As intermediates of the cycle are removed for other uses, they are replenished by **anaplerotic reactions**
- Under normal circumstances, the rate at which intermediates are siphoned off and replenished are in balance so that the concentrations of these intermediates remains almost constant.

TABLE 16-2 Anaplerotic Reactions

Reaction	Enzyme	Tissue(s)/organism(s)
$\text{Pyruvate} + \text{HCO}_3^- + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i$	pyruvate carboxylase	Liver, kidney
$\text{Phosphoenolpyruvate} + \text{CO}_2 + \text{GDP} \rightarrow \text{oxaloacetate} + \text{GTP}$	PEP carboxylase	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + \text{HCO}_3^- \rightarrow \text{oxaloacetate} + \text{P}_i$	PEP carboxykinase	Higher plants, yeast, bacteria
$\text{Pyruvate} + \text{HCO}_3^- + \text{NAD(P)}^+ \rightarrow \text{malate} + \text{NAD(P)}^+$	malic enzyme	Widely distributed in eukaryotes and prokaryotes

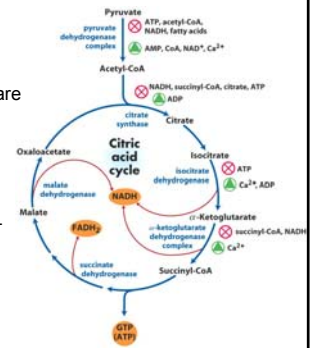
- Pyruvate carboxylase** – a key enzyme
 - Requires biotin (attached to lysine!) and is positively activated by acetyl-CoA
- PEP carboxylase** - Activated by fructose 1,6 bisphosphate

Chapter 16

22

The Citric Acid Cycle - Regulation

- The PDH complex is controlled by both allosteric and covalent mechanisms
- The 3 TCA cycle exergonic steps are also sites of regulation:
 - Citrate synthase
 - Isocitrate dehydrogenase
 - α -ketoglutarate dehydrogenase
- Inhibition by ATP, NADH, succinyl-CoA
- Activation by ADP and Ca^{2+}
- Regulation similar to that seen for Glycolysis. Is that logical?

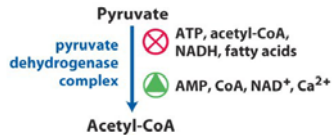


Chapter 16

24

The Citric Acid Cycle - Regulation Pyruvate Dehydrogenase

- Acetyl-CoA and NADH are reaction products; ATP is the ultimate product (**feedback inhibition**)
- Fatty acids are good fuel alternative, and enhance **allosteric inhibition**
- The activators (AMP, etc.) indicate an energy-poor state, and that higher flux is needed
- Compare to pyruvate kinase!

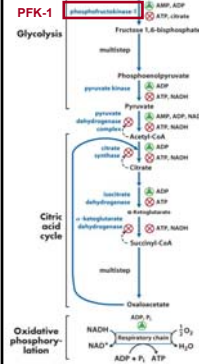


- On a second level, a **kinase**, allosterically activated by high [ATP], inactivates an E₁ subunit (**covalent regulation**) by serine phosphorylation
- With low [ATP], phosphatase reverses the phosphorylation and E₁ is reactivated

Chapter 16

25

“Coordinate Regulation” of ATP-producing Pathways

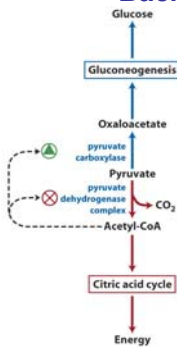


- The ATP/ADP ratio is an important measure of cellular energy status... And thus affects a number of reactions in glycolysis and the TCA
- For example, high ATP inhibits PFK-1 (glycolysis), which can be further shut down by citrate (indicating that the TCA cycle is slow)
- Conversely, glycolysis, pyruvate oxidation, the TCA cycle, and oxidative phosphorylation all accelerate if ADP, AMP, and/or Pi levels rise.

Chapter 16

27

The Citric Acid Cycle - Regulation Back to the Fate of Pyruvate



- What governs its fate?
- When energy needs are met, acetyl-CoA accumulates, and inhibits its own synthesis from pyruvate
- This inhibition stimulates **pyruvate carboxylase**, which shunts extra pyruvate into glucose synthesis
- Bypass 2 of Gluconeogenesis is similarly controlled by AMP inhibition
- Therefore, high [ATP], [acetyl-CoA], or [citrate] favor making glucose
- As does the hormone **glucagon**...

Chapter 16

26

Can You Now Answer These Questions?

- What general metabolic states will inhibit, or enhance, glycolysis and the TCA cycle?
- What molecules have effects on multiple steps in either or both pathways?
- Which steps are directly inhibited by their immediate products?
- What 3 general factors govern flux through the TCA cycle?
- Why are anaplerotic reactions important?

Chapter 16

28