Notes 11/26

Monday, November 26, 2007
10:00 AM

Heather Graehl

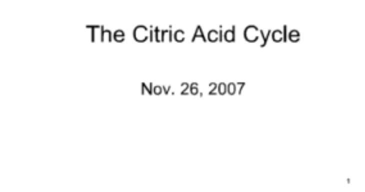
Notes 1126

Audio recording started: 10:01 AM Monday, November 26, 2007

FINAL SLIDES:

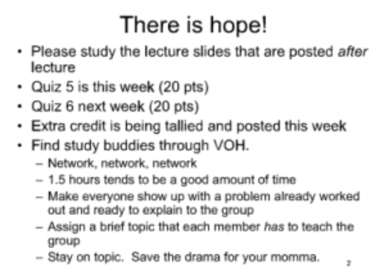
The Citric Acid Cycle

Nov. 26, 2007



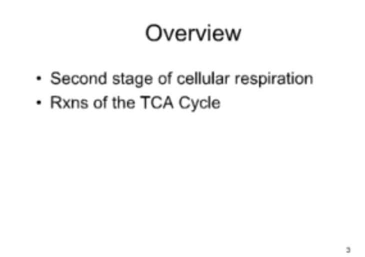
There is hope!

- Please study the lecture slides that are posted *after* lecture
- Quiz 5 is this week (20 pts)
- Quiz 6 next week (20 pts)
- Extra credit is being tallied and posted this week
- Find study buddies through VOH.
  - Network, network, network
  - 1.5 hours tends to be a good amount of time
  - Make everyone show up with a problem already worked out and ready to explain to the group
  - Assign a brief topic that each member *has* to teach the group
  - Stay on topic. Save the drama for your momma.

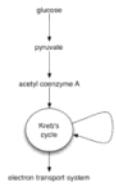


Overview

- Second stage of cellular respiration
- RXNS of TCA Cycle (AKA Cytric and Kreb Cycle)



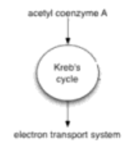
3 Stages of cellular respiration



1. Acetyl-CoA production
2. Acetyl-CoA oxidation
3. Electron transfer and oxidative phosphorylation

Get acetyl-CoA from pyruvate (from fatty acids, carbs, or proteins)

3 Stages of cellular respiration



1. Acetyl-CoA production
2. **Acetyl-CoA oxidation**
3. Electron transfer and oxidative phosphorylation

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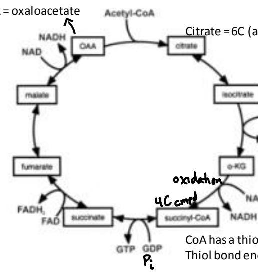
Reactions of the Citric Acid Cycle

Function of cycle is to oxidize acetyl-CoA

OAA = oxaloacetate



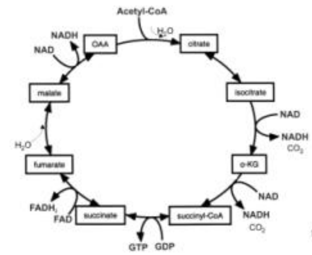
Citrate = 6C (aka citric acid)

6C compound

Alpha-ketoglutarate (5 C compound... loss CO_2)

CoA has a thiol group that acts to transfer acyl groups.
Thiol bond energy is large and has enough energy to make GTP.

Reactions of the Citric Acid Cycle



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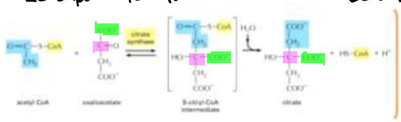
Step 1 is catalyzed by citrate synthase

1. Acetyl-CoA + oxaloacetate + H_2O ⇌ CoASH + citrate

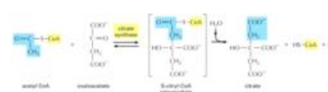
2C cmpd 4C cmpd 6C cmpd



Citrate synthase = enzyme

Step 1 is catalyzed by citrate synthase

1. Acetyl-CoA + oxaloacetate + H_2O ⇌ CoASH + citrate



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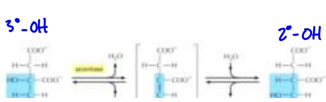
Step 2 is catalyzed by aconitase

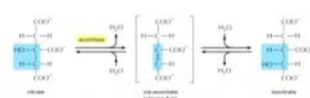
2. Citrate ⇌ isocitrate



Why would our cell bother with rearranging this:
- 3^0 alcohols cannot be reduced without breaking C-C bond.
- 2^0 alcohols can be reduced more easily

Step 2 is catalyzed by aconitase

2. Citrate ⇌ isocitrate



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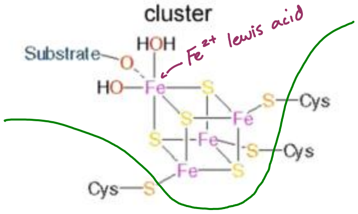
$\Delta G^{0'}$ = +6.7kJ/mol
At equilibrium citrate is favored

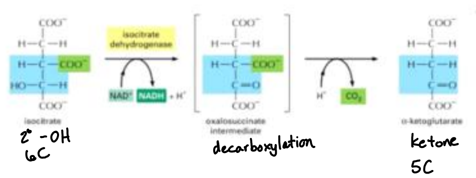
Enzyme = aconitase

Aconitase utilizes an Fe-S cluster



Step 3 is catalyzed by isocitrate dehydrogenase

$$3.\ \text{Isocitrate} + NAD^+ \rightleftharpoons \alpha\text{-ketoglutarate} + NADH + CO_2 + H^+$$



C oxidized into CO_2
NAD^+ reduced to NADH

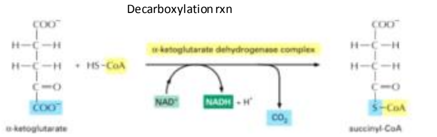
Missing Slide Figure 21-21
- Mechanism of rxn 3

Step 4 is catalyzed by α-ketoglutarate dehydrogenase complex

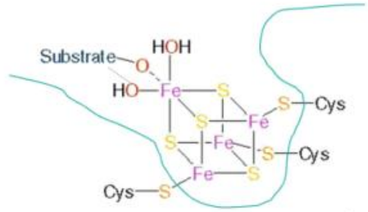
Enzyme = alpha-ketoglutarate dehydrogenase complex

$$4.\ \alpha\text{-Ketoglutarate} + CoASH + NAD^+ \rightleftharpoons \text{succinyl-CoA} + NADH + CO_2 + H^+$$

Decarboxylation rxn



Aconitase utilizes an Fe-S cluster



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Step 3 is catalyzed by isocitrate dehydrogenase

$$3.\ \text{Isocitrate} + NAD^+ \rightleftharpoons \alpha\text{-ketoglutarate} + NADH + CO_2 + H^+$$

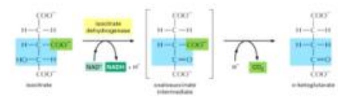

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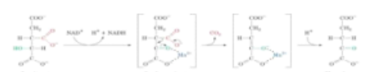
Figure 21-21 Probable reaction mechanism of isocitrate dehydrogenase.



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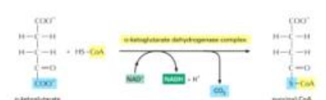
Step 4 is catalyzed by α-ketoglutarate dehydrogenase complex

$$4.\ \alpha\text{-Ketoglutarate} + CoASH + NAD^+ \rightleftharpoons \text{succinyl-CoA} + NADH + CO_2 + H^+$$



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αKG DH Complex

Complex has 3 enzymes
(looks very similar to pyruvate dehydrogenase complex)

Probably evolutionarily related (homologous)

Enzyme	Coenzyme
α-ketoglutarate dehydrogenase	Thiamine pyrophosphate
Dihydrolipoyl transsuccinylase	Lipoic acid, CoASH
Dihydrolipoyl dehydrogenase	FAD, NAD^+

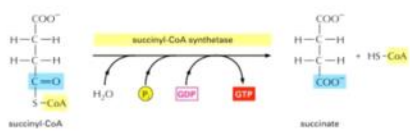
Same name as in pyruvate dehydrogenase

Step 5 is catalyzed by Succinyl-CoA synthetase

Named for reverse reaction.

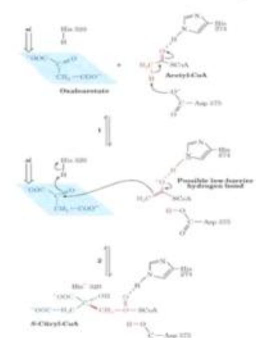
5. Succinyl-CoA + GDP + P_i ⇌ succinate + GTP + CoASH

GTP has guanosine
ATP has adenosine



High energy thiol-ester linkage used to make high energy compound GTP

Figure 21-19 Mechanism and stereochemistry of citrate synthase rxn



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αKG DH Complex

Enzyme	Coenzyme
α-ketoglutarate dehydrogenase	Thiamine pyrophosphate
Dihydrolipoyl transsuccinylase	Lipoic acid, CoASH
Dihydrolipoyl dehydrogenase	FAD, NAD^+

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Step 5 is catalyzed by Succinyl-CoA synthetase

5. Succinyl-CoA + GDP + P_i ⇌ succinate + GTP + CoASH

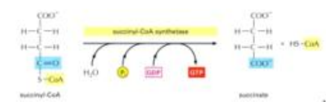

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Figure 21-22a
Formation of succinyl phosphate, a "high-energy" mixed anhydride

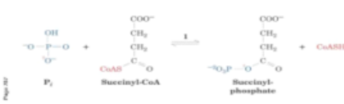

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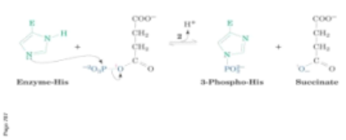
Figure 21-22b Formation of phosphoryl–His, a "high-energy" intermediate



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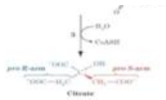

Figure 21-21 Probable reaction mechanism of isocitrate dehydrogenase.

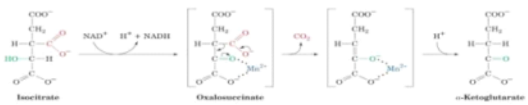

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Figure 21-22a
Formation of succinyl phosphate, a "high-energy" mixed anhydride

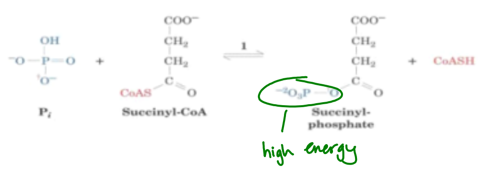

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Figure 21-22b Formation of phosphoryl–His, a "high-energy" intermediate

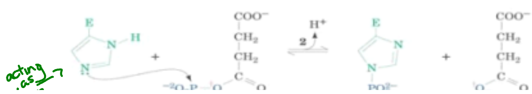
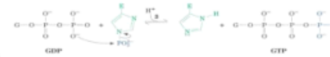


Figure 21-22c
Transfer of the phosphoryl group to GDP, forming GTP



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The first five steps of the TCA cycle produce NADH, CO_2, GTP (ATP), & succinate

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Step 6 is catalyzed by succinate dehydrogenase

6. Succinate + [FAD] ⇌ fumarate + [$FADH_2$]

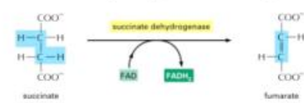

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Figure 21-23
Covalent attachment of FAD to a His residue of succinate dehydrogenase.

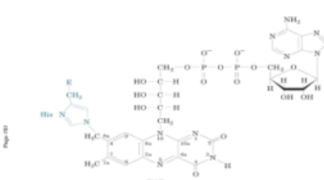

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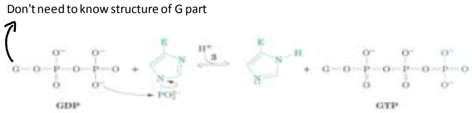
Figure 21-22c

Transfer of the phosphoryl group to GDP, forming GTP

Don't need to know structure of G part



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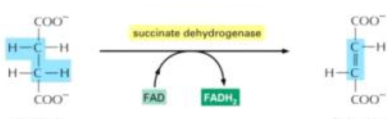
The first five steps of the TCA cycle produce NADH, CO_2, GTP (ATP), & succinate

- First 5 steps:
  - Producing NADH
  - Producing CO_2
  - Produce GTP or ATP
  - Produce succinate

Step 6 is catalyzed by succinate dehydrogenase

FAD has brackets because it is attached covalently to succinate dehydrogenase

6. Succinate + [FAD] $\rightleftharpoons$ fumarate + [$FADH_2$]

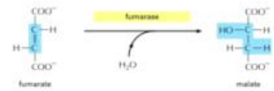


Succinate dehydrogenase is only enzyme bound to inner mitochondria membrane

Stereospecific but won't go into details of this.

Step 7 is catalyzed by fumarase

7. Fumarate + H_2O $\rightleftharpoons$ L-malate



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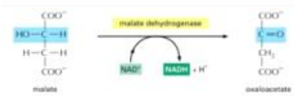
Step 8 is catalyzed by malate dehydrogenase

8. L-Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+



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Net for rxns 1 to 8

Acetyl-CoA + 3 NAD^+ + [FAD] + ADP + P_i + 2 H_2O $\rightleftharpoons$ 2 CO_2 + 3 NADH + 3 H^+ + [$FADH_2$] + ATP + CoASH

$\Delta G^{\circ\prime} = -40$ kJ/mol

Net for glycolysis and TCA cycle

Glucose + 2 H_2O + 10 NAD^+ + 2 [FAD] + 4 ADP + 4 P_i $\rightleftharpoons$ 6 CO_2 + 10 NADH + 10 H^+ + 2 [$FADH_2$] + 4 ATP

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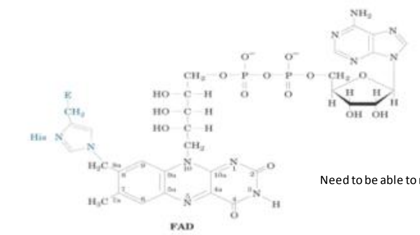
Covalent attachment of FAD to a His residue of succinate dehydrogenase

- Part of reasoning that succinate dehydrogenase is stuck to mitochondria
- Coenzyme Q (found in inner membrame space of mitochondria) is involved into oxidazing $FADH_2$ back into FAD

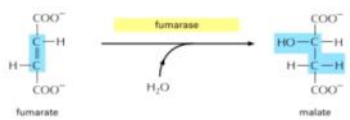
Figure 21-23



Need to be able to recognize

Step 7 is catalyzed by fumarase

7. Fumarate + H_2O $\rightleftharpoons$ L-malate

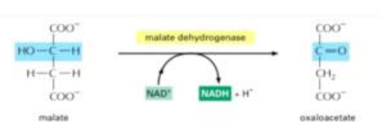


2° -OH

Step 8 is catalyzed by malate dehydrogenase

FINAL STEP

8. L-Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+



2° -OH   oxidize   ketone

NAD^+ reduced → NADH

Net for rxns 1 to 8

$$\text{Acetyl-CoA} + 3\,NAD^+ + [FAD] + ADP + P_i + 2\,H_2O \rightleftharpoons$$
$$2\,CO_2 + 3\,NADH + 3\,H^+ + [FADH_2] + ATP + CoASH$$
$$\Delta G^{\circ\prime} = -40 \text{ kJ/mol}$$

2 C come from Acetyl-CoA

Net for glycolysis and TCA cycle

$$\text{Glucose} + 2\,H_2O + 10\,NAD^+ + 2\,[FAD] + 4\,ADP + 4\,P_i \rightleftharpoons$$
$$6\,CO_2 + 10\,NADH + 10\,H^+ + 2\,[FADH_2] + 4\,ATP$$

Table 21-2

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})	ΔG (kJ · mol^{-1})
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase multienzyme complex	−33	Negative
5	Succinyl-CoA synthetase	−2.1	~0
6	Succinate dehydrogenase	+6	~0
7	Fumarase	−3.4	~0
8	Malate dehydrogenase	+29.7	~0

Do on own.

A typical intramitochondrial concentration of malate is 0.22 mM. If the [NAD+]/[NADH] ratio in mitochondria is 20 and if the malate dehydrogenase reaction is at equilibrium, calculate the intramitochondrial concentration of oxaloacetate at 25°C.

$$\text{Malate} + \text{NAD}^+ \rightleftharpoons \text{oxaloacetate} + \text{NADH} + \text{H}^+$$

$$\Delta G^{\circ\prime} = -RT\ln K_{eq}$$

$$= -(8.314\ \text{J/mol}\cdot\text{K})(298)\ln\left(\frac{[1]x}{[20]\left[2.2\times10^{-4}\right]}\right)$$

$$\frac{-30{,}000\ \text{J/mol}}{2478\ \text{J/mol}} = \ln\left(x/4.4\times10^{-3}\right)$$

$$-12.1 = \ln\left(x/4.4\times10^{-3}\right)$$

$$x = (5.6\times10^{-6})\,(4.4\times10^{-3})$$

$$x = [\text{oxaloacetate}] = 0.024\,\mu M$$