Department of Chemistry and Biochemistry University of Lethbridge

#### **Biochemistry 3300**







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#### The Eight Steps of the Citric Acid Cycle



#### **Enzymes:**

- 4 dehydrogenases (2 decarboxylation)
- 3 hydration/dehydration
- 1 substrate level phosphorylation

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### **Overall Reaction (TCA cycle)**

**Overall reaction** 



**Citric acid cycle** is central to the energy-yielding metabolism, but it <u>also produces 4- and 5-carbon precursors</u> for other metabolic pathways.

Replenishing (anaplerotic) reactions are needed to keep the cycle going!

### **TCA Cycle – Citrate Synthase**



**Rxn 1** Formation of Citrate by condensation of oxaloacetate and acetyl-CoA, catalyzed by citrate synthase.



Citroyl-CoA is formed as an intermediate

 $\Delta$ G'° has to be large to overcome the low oxaloacetate concentration

#### **TCA Cycle – Citrate Synthase**

#### **Rxn 1** Structure of citrate synthase from *G. galus* mitochondria



 $\rightarrow$  creates binding site for Acetyl-CoA

#### **Structure of Citrate Synthase**



Ordered sequential mechanism



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**Rxn 1** Mechanism of citrate synthase reaction (1<sup>st</sup> step)

**Formation of Enol Intermediate** 



The thioester linkage in acetyl-CoA activates the methyl hydrogens, and Asp<sup>375</sup> abstracts a proton from the methyl group, forming an enolate intermediate.

The intermediate is stabilized by hydrogen bonding to and/or protonation by His<sup>274</sup> (full protonation is shown).





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**Rxn 1** Mechanism of citrate synthase reaction (2<sup>nd</sup> step)

α-keto addition (condensation)





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**Rxn 1** Mechanism of citrate synthase reaction (3<sup>rd</sup> step)

#### **Thioester hydrolysis**



CoA-SH and producing citrate.

**Rxn 1** Mechanism of citrate synthase reaction



The thioester linkage in acetyl-CoA activates the methyl hydrogens, and Asp<sup>375</sup> abstracts a proton from the methyl group, forming an enolate intermediate.

The intermediate is stabilized by hydrogen bonding to and/or protonation by His<sup>274</sup> (full protonation is shown).



oxaloacetate, with His<sup>274</sup> positioned to abstract the proton it had previously donated. His<sup>320</sup> acts as a general acid.

"Stabilized" enol intermediate of acetyl CoA attacks  $\alpha$ -keto group of oxaloacetate.

Hydrolysis of citroyl-CoA intermediate drives reaction



The thioester is subsequently hydrolyzed, regenerating CoA-SH and producing citrate.



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#### **TCA Cycle – Aconitase reaction**

#### **Rxn 2** Formation Isocitrate via cis-Aconitate

Aconitase dehydrates citrate to cis-aconitate ... then hydrates cis-aconitate to isocitrate



Hydroxyl moved from C3 to C2





### TCA Cycle – Aconitase mechanism

#### **Rxn 2** Mechanism of aconitase

## Aconitase contains an iron-sulfur center

4Fe:4S center aids substrate binding and is required for catalytic addition / removal of  $H_2O$ .

*Cis*-aconitate intermediate does not (typically) dissociate from enzyme.



### TCA Cycle – isocitrate dehyrogenase



#### **Rxn 3** Oxidation decarboxylation of Isocitrate to $\alpha$ -ketoglutarate



**Mn<sup>2+</sup>** in the active site interacts with the carbonyl group of intermediate oxalosuccinate and stabilizes the transiently formed enol.

Two different isocitrate DH<sub>2</sub>ases : a NAD<sup>+</sup> and a NADP<sup>+</sup> dependent form. An NAD<sup>+</sup> dependent form in the mitochondrial matrix An NADP<sup>+</sup> dependent form in both the mitochondria and cytosol

### TCA Cycle –

#### α-ketoglutarate dehyrogenase complex



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#### **Rxn 4** Oxidation of $\alpha$ -ketoglutarate to succinyl-CoA and CO<sub>2</sub>



The mechanism is identical to the pyruvate dehydrogenase reaction.

 $\begin{array}{l} \alpha \text{-ketoglutarate dehydrogenase complex is very similar to the} \\ \textbf{pyruvate dehydrogenase complex (homologs of E_1, E_2, and E_3).} \\ \text{It also contains TPP, E_2 bound lipoate, FAD, NAD and CoA.} \\ \text{E_3 is identical in both complexes.} \\ \hline \textbf{Specificity due to E1} \end{array}$ 

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#### **Rxn 5** Conversion of Succinyl-CoA to Succinate



 $\Delta G'^{\circ}$  = -2.9 kJ/mol

Energy of the thioester bond cleavage drives the formation of a phosphoanhydride bond in GTP. - (another) substrate level phosphorylation

- conversion of one high energy bond to another



<u>Step 1</u> Succinyl-CoA binds to the enzyme and a phosphoryl group replaces the CoA of succinyl-CoA

(substrate level phosphorylation)

Consume a high-energy thioester and create a high-energy acyl phosphate.



*Rxn* **5** Mechanism of succinyl-CoA synthetase

#### <u>Step 2</u>

Succinyl phosphate transfers phosphoryl group to His residue on the enzyme.

Consume a high-energy acyl phosphate and create a high-energy phosphohistidyl.

<u>Step 3</u> Phosphohistidyl enzyme transfers phosphoryl group to GDP

Consume a high-energy phosphohistidyl and create a high-energy phosphoanhydride.

#### GTP can be:

- 1) converted to ATP by nucleoside diphosphate kinase.
- 2) utilized by G-proteins and other GTPases



#### *Rxn* **5** Mechanism of succinyl-CoA synthetase



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#### **Succinyl-CoA synthetase Structure**

**Rxn 5** Structure of succinyl-CoA synthetase

Two subunits: α Subunit (32 kDa) His<sup>246</sup> is phosphorylated

β Subunit (42 kDa) confers ATP/GTP specificity

Active site is at the Subunit interface → "power helices" facilitate phosphoryl group transfers



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PDBid 1SCU E.coli Slide 19

### TCA Cycle – Succinate dehydrogenase (a.k.a Complex II)



#### **Rxn 6** Oxidation of Succinate to Fumarate – Succinate dehydrogenase



Eukaryotic succinate dehydrogenase is tightly bound to the inner mitochondrial membrane; prokaryotes  $\rightarrow$  plasma membrane.

Succinate dehydrogenase is Complex II of Electron Transfer Chain

### TCA Cycle – Succinate dehydrogenase (a.k.a Complex II)



**Rxn 6** Oxidation of Succinate to Fumarate – Succinate dehydrogenase

Succinate dehydrogenase (or Complex II) contains several iron-sulfur centers that mediate the flow of electrons from succinate (via a covalently bound FAD to enzyme) to the electron transfer chain and finally to  $O_2$ .



Malonate, an analog of succinate strongly inhibits succinate dehydrogenase

 $\rightarrow$  not normally present in cells

#### **TCA Cycle - Fumarase**

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#### **Step 7** Hydration of Fumarate to Malate – Fumarase



### TCA Cycle -Malate Dehydrogenase



Step 8 Oxidation of Malate to Oxaloacetate – L-malate dehydrogen



 $\Delta G'^{\circ}$  = 29.7 kJ/mol

#### L-malate is oxidized to oxaloacetate

 $\rightarrow$  equilibrium strongly favours substrate (L-malate)

Couple reaction with strongly favourable Citrate Synthase reaction to overcome unfavourable energetics

### **Energy Yields**





#### Yield = ~32 ATP / glucose

#### Standard Conditions: 32 x 30.5 kJ/mol = 976 kJ/mol Combustion of glucose = 2,840 kJ/mol

Succinate	Number of ATP or reduced	Number of ATP
React	coenzyme directly formed	ultimately formed*
Glucose —→ glucose 6-phosphate	-1 ATP	-1
ructose 6-phosphate $\longrightarrow$ fructose 1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde 3-phosphate $\longrightarrow$ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate $\longrightarrow$ 2 3-phosphoglycerate	2 ATP	-2
2 Phosphoenolpyruvate $\longrightarrow$ 2 pyruvate	-2 ATP	-2
2 Pyruvate $\longrightarrow$ 2 acetyl-CoA	-2 NADH	-5
2 Isocitrate $\longrightarrow$ 2 $\alpha$ -ketoglutarate	2 NADH	-5
$2 \alpha$ -Ketoglutarate $\longrightarrow 2$ succinyl-CoA	2 NADH	-5
2 Succinyl-CoA $\longrightarrow$ 2 succinate	-2 ATP (or 2 GTP)	-2
2 Succinate $\longrightarrow$ 2 fumarate	-2 FADH <sub>2</sub>	-3
2 Malate $\longrightarrow$ 2 oxaloacetate	2 NADH	- 5
otal		30-32

\* This is calculated as 2.5 ATP per NADH and 1.5 ATP per FADH<sub>2</sub>. A negative value indicates consumption.

<sup>†</sup> This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–27 and 19–28.



### **Summary of Enzyme Properties**

Enzyme	EC Class	Mechanism	Inter	$\Delta G^{\prime o}$	Products
Pyruvate Dehydrogenase Complex	Oxidoreductase(E1)	TPP; $\alpha$ -keto deCO <sub>2</sub>	ylid; enol	-33	Acetyl CoA; NADH; CO <sub>2</sub>
	Transferase (E2)	Lipoate	Acyl lipoate		
	Oxidoreductase(E3)	$NAD+\toNADH$			
Citrate Synthase	Transferase	Condensation	enol; citroyl-CoA	-32	2C + 4C sugars $\rightarrow$ 6C sugar
Aconitase	Lyase	Dehydration; Hydration	cis-aconitate; 4Fe●4S	+13	
Isocitrate Dehydrogenase	Oxidoreductase	Mn <sup>2+</sup> ; oxidative deCO <sub>2</sub>	$\beta$ -keto CO <sub>2</sub> ; enol	-8	NADH; CO <sub>2</sub>
$\alpha$ -ketoglutarate Dehydrogenase Complex	See PDC.	See PDC.	See PDC	-34	Succinyl CoA; NADH; CO <sub>2</sub>
Succinyl-CoA synthetase	Ligase	Phosphoryl transfer; SLP	Succinyl phosphate; phospho-His	-3	GTP(ATP)
Succinate Dehydrogenase	Oxidoreductase	$FAD\toFADH_2$	4Fe●4S	0	FADH <sub>2</sub>
Fumarase	Lyase	Hydration	carbanion; ???	-4	
Malate Dehydrogenase	Oxidoreductase	$NAD+\toNADH$		+30	NADH

# Why is Oxidation of Acetate so Complicated?





It is a hub of intermediary metabolism; In aerobic organisms it serves in catabolic and anabolic processes. →amphibolic pathway

-oxidative catabolism -production of biosynthetic precursers.

Intermediates removed from the Cycle are replenished by anaplerotic reactions.

Under stady state conditions (normal) intermediate concentrations remain constant

Anaerobic bacteria (above) have an 'incomplete' TCA  $\rightarrow$  lack  $\alpha$ -ketoglutarate dehydrogenase complex



### **Citric Acid Cycle in Anabolism**





### **Regulation of the Citric Acid Cycle**



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Mammals, allosteric regulation is complemented by covalent protein modification.

E<sub>1</sub> of PDH complex can be inactivated by phosphorylation
kinase that inactivates E1 is a subunit of the mammalian PDH complex

PDH kinase is allosterically activated by ATP

TCA Cycle is regulated at its 3 exergonic steps. Citrate synthase, isocitrate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase complex

### **Related Pathway - The Glyoxylate Cycle**



In many organisms other than vertebrates, the glyoxylate cycle serves as mechanism for converting acetate to carbohydrate.

The glyoxylate cycle produces four-carbon compounds from acetate.

Lipid body



In plants, glyoxylate cycle enzymes are found in organelles → Glyoxysomes

Found in lipid rich seeds during germination, before glucose from photosynthesis is available.

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### Relationship Between the Glyoxylate And 1 Citric Acid Cycle



### **Coordinated Regulation**

Sharing common intermediates requires coordinated regulation.

*Isocitrate* is at the branch point between the glyoxylate and TCA cycle

Isocitrate DH is also regulated by covalent modification (specific protein kinase)

E. coli has the full complement of enzymes and therefore grows on acetate as the sole carbon source.





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