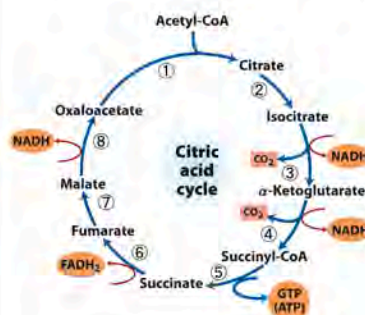
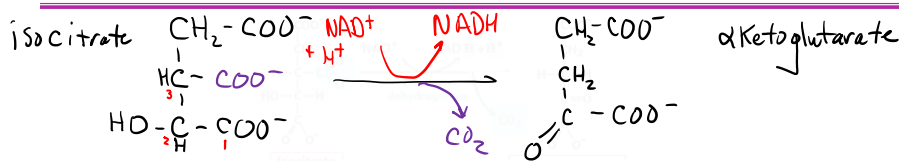


# The Citric Acid Cycle

- Citrate Synthase** ✓ • Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
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  - Step 8: Dehydrogenation to give NADH



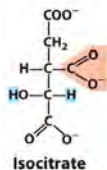
## The Citric Acid Cycle: Isocitrate dehydrogenase



- Converting the C2 hydroxyl to a keto destabilizes the C-C bond to the carboxylate at C3.
- This requires a 2-step process:
  - First perform an alcohol-to-keto dehydrogenation at C2 using NAD<sup>+</sup>
  - Second, allow for decarboxylation (the oxidation of the carboxylate to CO<sub>2</sub>, with the reduction of C3).
  - C2 is oxidized, C3 is reduced, Carboxylate is oxidized: Net oxidation is 2e<sup>-</sup>
- Isozymes are specific for NADP<sup>+</sup> (cytosolic) or NAD<sup>+</sup> (mitochondrial).
- Favorable but irreversible due to loss of CO<sub>2</sub> ( $\Delta G^\circ = -2.0 \text{ kcal/mol}$ )
- Regulated by [ATP] (OMSGAP)

# The Citric Acid Cycle: Isocitrate dehydrogenase

## Mechanism



Isocitrate

Isocitrate is oxidized by hydride transfer to  $\text{NAD}^+$  or  $\text{NADP}^+$  (depending on the isocitrate dehydrogenase isozyme).

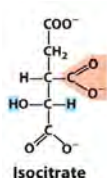
This mechanism is just like that of LDH or other dehydrogenases (Base abstracts  $-\text{OH}$  proton, carbonyl forms, elimination of  $:\text{H}-$ )

The  $\text{Mn}^{++}$  cofactor stabilizes the  $\alpha$ -keto acid, which destabilizes the middle carboxylate

Though this is an oxidative decarboxylation at carboxylate  $\alpha$ -to carbonyl, it does not need TPP!!

# The Citric Acid Cycle: Isocitrate dehydrogenase

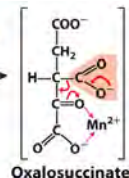
## Mechanism



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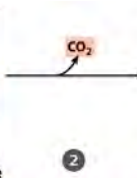


Oxalosuccinate

Decarboxylation is facilitated by electron withdrawal by the adjacent carbonyl and coordinated  $\text{Mn}^{2+}$ .

The  $\text{Mn}^{++}$  cofactor stabilizes the  $\alpha$ -keto acid, which destabilizes the middle carboxylate

Though this is an oxidative decarboxylation at carboxylate  $\alpha$ -to carbonyl, it does not need TPP!!

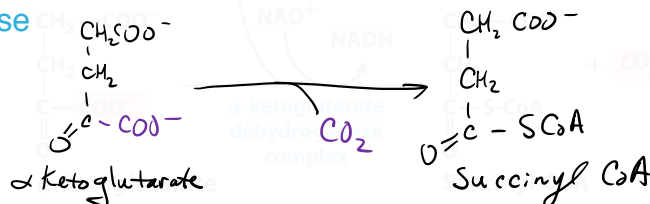
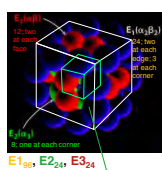


$\alpha$ -Ketoglutarate

Rearrangement of the enol intermediate generates  $\alpha$ -ketoglutarate. (protonation of the carbanion)

## The Citric Acid Cycle: The $\alpha$ -Keto-Glutarate DeHydrogenase Complex

Oxidative Decarboxylation of an  $\alpha$ -keto acid:  $\alpha$ -Ketoglutarate Dehydrogenase



$\Delta G'^{\circ} = -33.5 \text{ kJ/mol}$

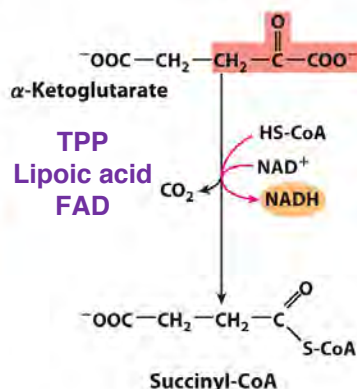
- Last oxidative decarboxylation in TCA cycle
  - full oxidation of all carbons of glucose:
    - Takes two turns of the cycle
    - The carbons oxidized are not directly from glucose because the carbons came from oxaloacetate, not acetyl-CoA
- Requires TPP, FAD, Lipoic acid cofactors
- Succinyl-CoA is another higher-energy thioester bond.
- Highly thermodynamically **favorable/irreversible** ( $\Delta G'^{\circ} = -8.0 \text{ kcal/mol}$ )
  - regulated by product inhibition

Where have we seen this before?

## The Citric Acid Cycle: The $\alpha$ -Keto-Glutarate DeHydrogenase Complex

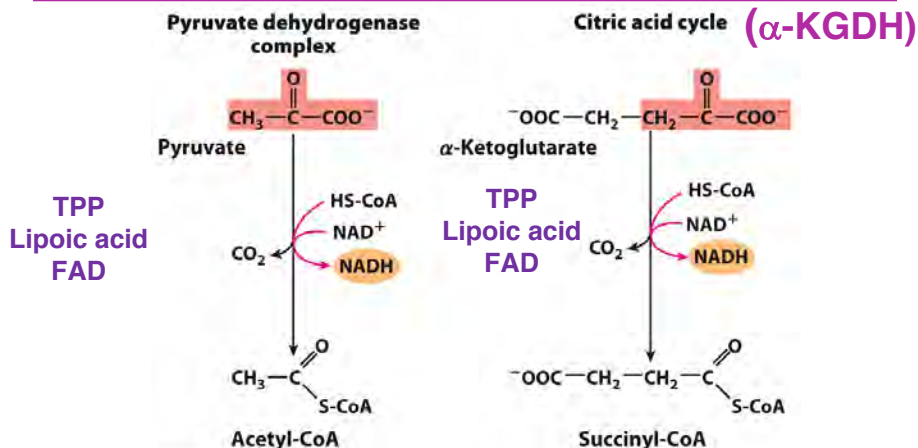
Citric acid cycle ( $\alpha$ -KGDH)

TPP  
Lipoic acid  
FAD



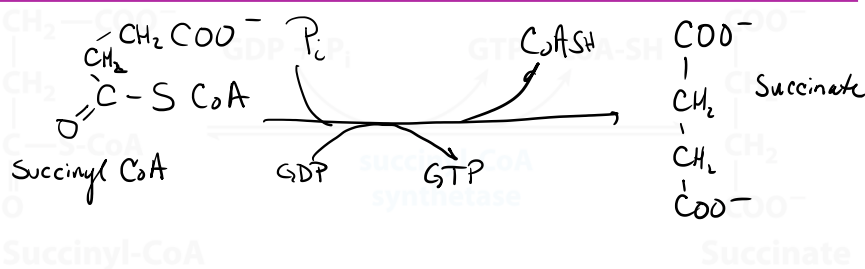
- Complex similar to pyruvate dehydrogenase
  - same coenzymes, identical mechanisms, E2 & E3 are identical
  - active site of E1 different to accommodate different-sized substrates

## The Citric Acid Cycle: The $\alpha$ -Keto-Glutarate DeHydrogenase Complex



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  - active site of E1 different to accommodate different-sized substrates

## The Citric Acid Cycle: Succinyl-CoA Synthetase



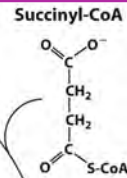
- This step was not appreciated in original cycle until discover of CoA and its role (Fritz Lipmann). Named for the reverse reaction.
  - Substrate-level phosphorylation (like GAPDH + 1,3-BPG kinase)
  - The energy of thioester allows for incorporation of inorganic phosphate.
  - Goes through a phospho-enzyme intermediate
  - Produces GTP, which can be converted to ATP
  - Slightly thermodynamically **favorable/reversible** ( $\Delta G^\circ = -0.7 \text{ kcal/mol}$ ).
    - product concentration kept low to pull forward
- (OMSGAP)

## The Citric Acid Cycle: Succinyl-CoA Synthetase

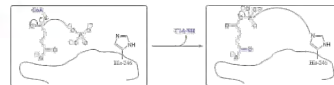
### Mechanism

Phospho-His  
transfer to NDP;  
slightly downhill  
energetically

Succinyl-CoA  
synthetase  
His—



Thioester to mixed  
anhydride;  
uphill energetically



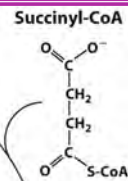
Phosphoryl transfer to His;  
downhill energetically

## The Citric Acid Cycle: Succinyl-CoA Synthetase

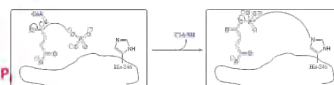
### Mechanism

Phospho-His  
transfer to NDP;  
slightly downhill  
energetically

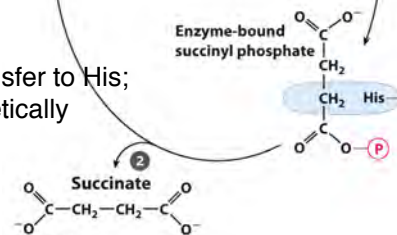
Succinyl-CoA  
synthetase  
His—



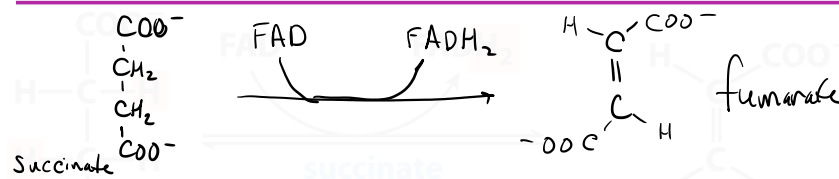
Thioester to mixed  
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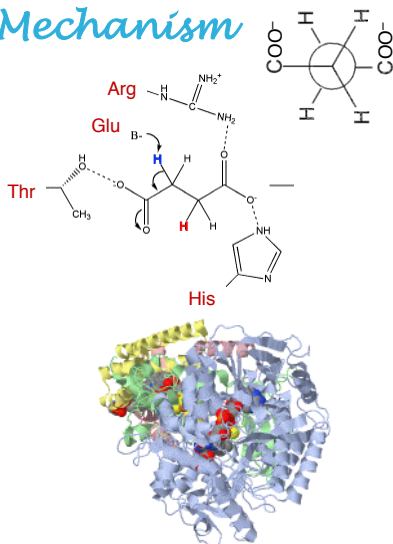
## The Citric Acid Cycle: Succinate Dehydrogenase



- Have not seen this cofactor chemistry yet: use in the alkane oxidation  $\rightarrow$  alkene.
- But we have seen these next 3 steps. If it worked once it will work again: Aconitase and ICDH
- Famous competitive inhibitor: malonate (OMSGAP)
- Reduction of the alkane to alkene requires  $\text{FADH}_2$ .
  - Reduction potential of carbon-hydrogen bond is too low for production of NADH.
  - The 2 hydrogens are removed stereospecifically.
  - FAD is covalently bound at His, unusual
  - Series of 3 iron-sulfur clusters to transfer electrons from  $\text{FADH}_2$  to electron transport chain
- Bound to mitochondrial inner membrane
  - acts as Complex II in the electron-transport chain
- Near equilibrium/**reversible** ( $\Delta G^\circ = -0.5 \text{ kcal/mol}$ ); [fumarate] kept low

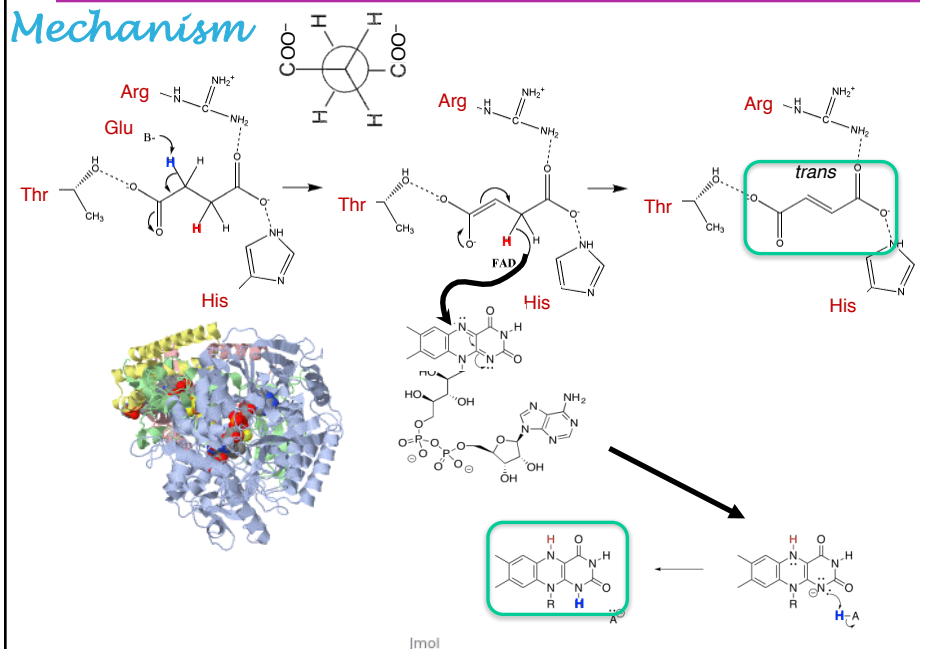
## The Citric Acid Cycle: Succinate Dehydrogenase

### Mechanism

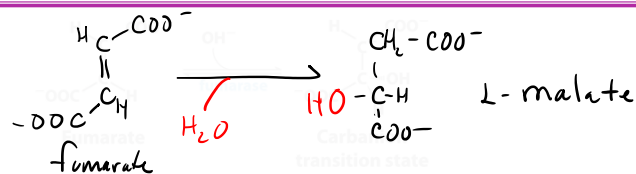


## The Citric Acid Cycle: Succinate Dehydrogenase

### Mechanism



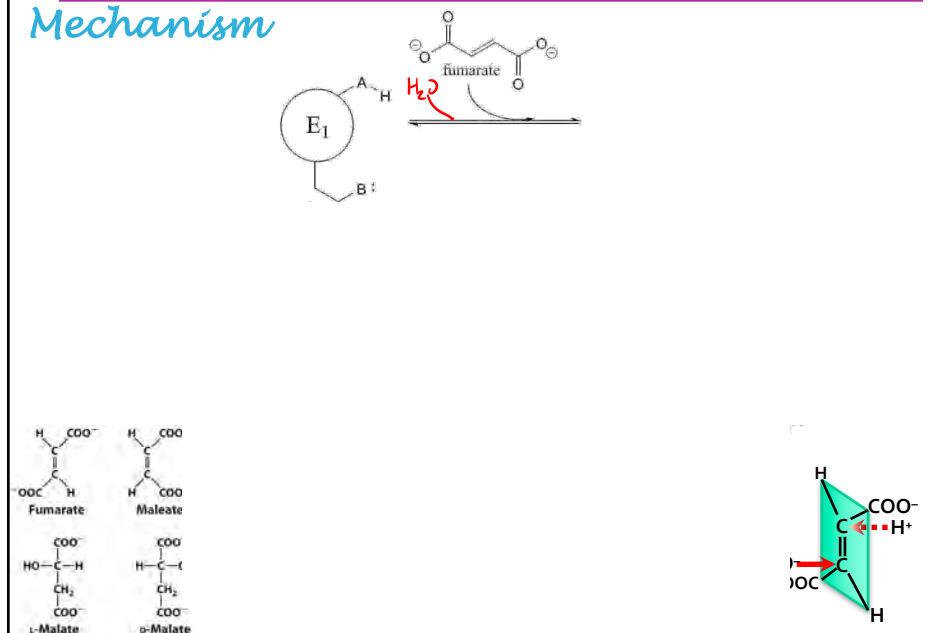
## The Citric Acid Cycle: Fumarase



- Stereospecific
  - Addition of water is always trans and forms L-malate.
  - OH<sup>-</sup> adds to fumarate... then H<sup>+</sup> adds to the carbanion.
  - Cannot distinguish between inner carbons, so either can gain -OH
- Slightly thermodynamically favorable/reversible ( $\Delta G^{\circ} = -0.9$  kcal/mol).
  - product concentration kept low to pull reaction forward

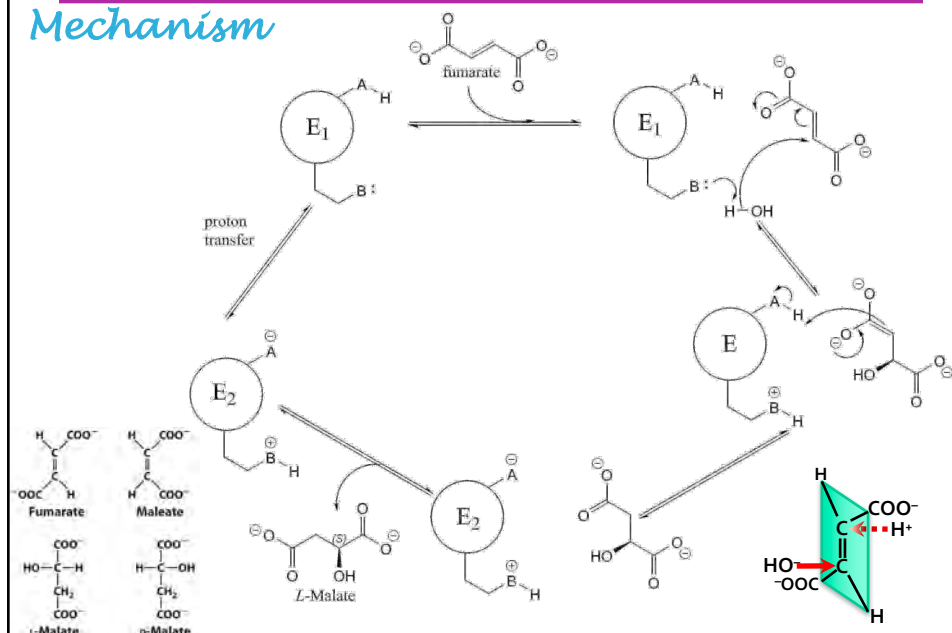
# The Citric Acid Cycle: Fumarase

## Mechanism



# The Citric Acid Cycle: Fumarase

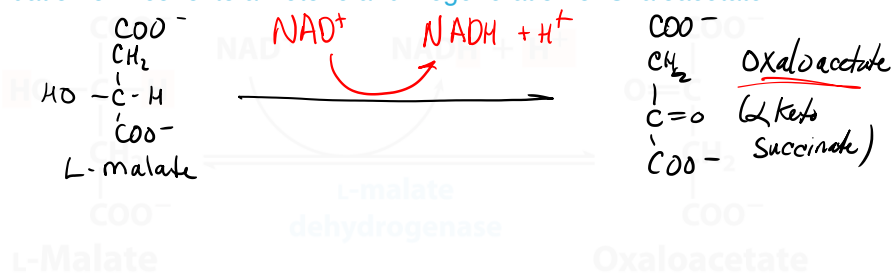
## Mechanism





## The Citric Acid Cycle: Malate Dehydrogenase

Oxidation of Alcohol to a Ketone and Regeneration of Oxaloacetate



- Final step of the cycle
- Regenerates oxaloacetate for citrate synthase
- Highly thermodynamically **UNfavorable** ( $\Delta G^\circ = +7.1 \text{ kcal/mol}$ ).
  - Reversible
  - oxaloacetate concentration kept VERY low by citrate synthase
    - pulls the reaction forward ( $-7.7 + 7.1 = -0.6$ )

## The Citric Acid Cycle

Citrate Synthase

Aconitase

ICDH&aKGDH

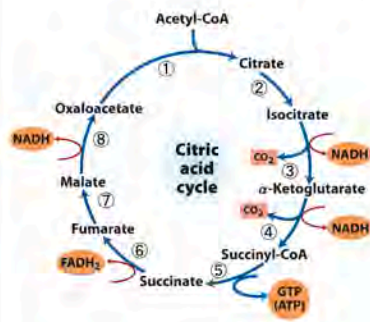
Suc-CoA Synthetase

SucDH

Fumarase

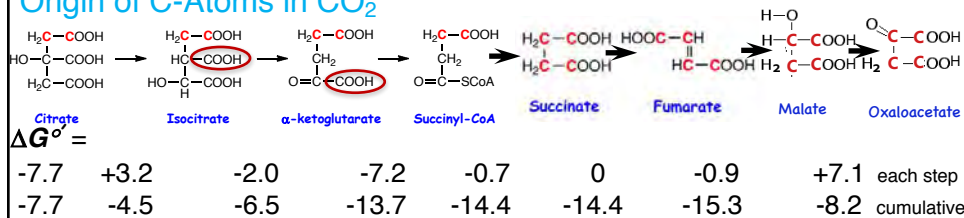
Malate DH

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- ✓ Step 5: Substrate-level phosphorylation to give GTP
- ✓ Step 6: Dehydrogenation to give FADH<sub>2</sub>
- ✓ Step 7: Hydration
- ✓ Step 8: Dehydrogenation to give NADH



# The Citric Acid Cycle

## Origin of C-Atoms in CO<sub>2</sub>



## Energetics: why the cycle only turns in one direction

- Carbons from acetate are red.
- All CO<sub>2</sub> generated during the citric acid cycle is produced before succinyl-CoA is made.
- In one turn of the citric acid cycle, neither of the acetyl-CoA carbons is lost.
- Both CO<sub>2</sub> molecules lost were present on the oxaloacetate used to begin the cycle.
- **Overall energetics makes cycle irreversible:**
  - PDH, citrate synthase, IDH, and KDH
  - what about kinetics?
- **Nature of a cycle; acts as a unit and goes as fast as the available carbon**