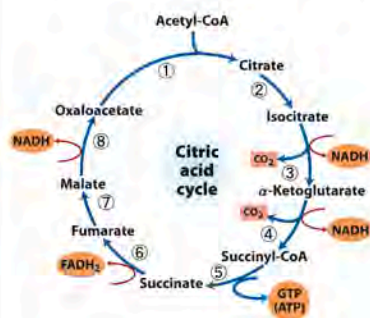
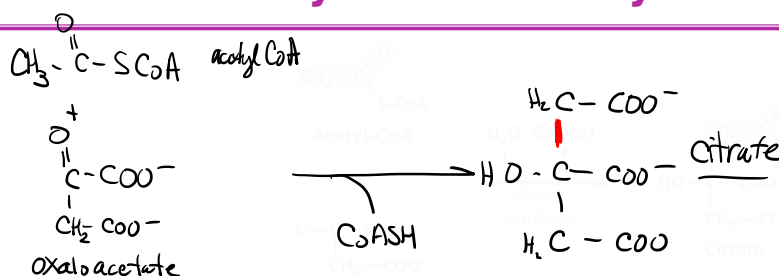


The Citric Acid Cycle

- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give FADH₂
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH



The Citric Acid Cycle: Citrate Synthase



- Condensation of acetyl-CoA and oxaloacetate
- The only reaction with C-C bond formation
- Highly thermodynamically favorable/irreversible ($\Delta G^\circ = -7.7 \text{ kcal/mol}$)
 - regulated by substrate availability and product inhibition
- Activity largely depends on [oxaloacetate].
- Rate-limiting step of CAC
- Uses acid/base catalysis
 - Carbonyl of oxaloacetate is a good electrophile.
 - Methyl of acetyl-CoA is not a good nucleophile...
 - ...unless activated by deprotonation.



The Citric Acid Cycle: Citrate Synthase

Mechanism

- Conformational change occurs upon binding oxaloacetate.
- Avoids unnecessary hydrolysis of thioester in acetyl-CoA

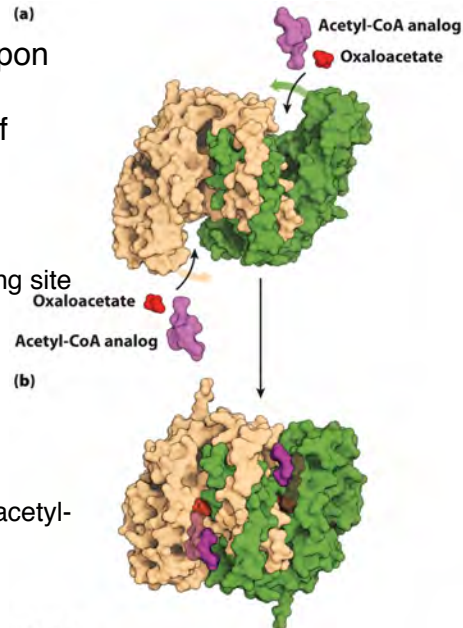
a) Open conformation:

Free enzyme does not have a binding site for acetyl-CoA. Ordered binding.

b) Closed conformation:

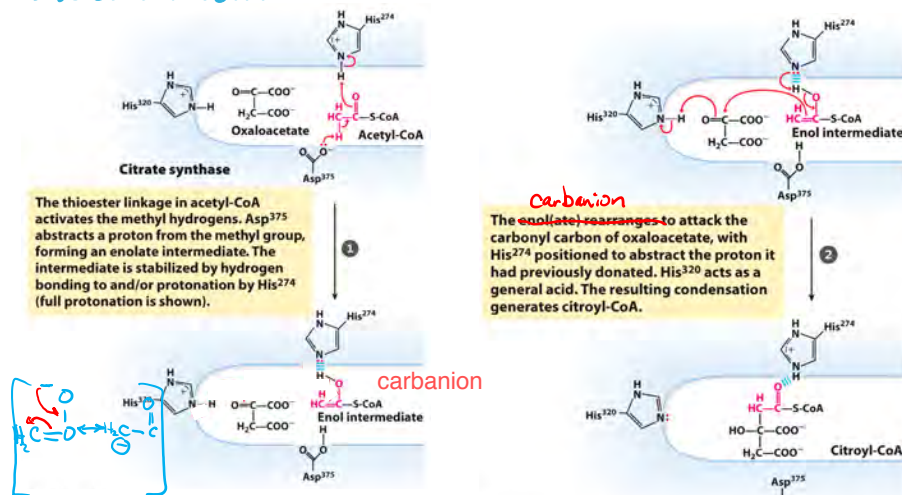
Binding of OAA creates binding for acetyl-CoA.

Reactive carbanion is protected.



The Citric Acid Cycle: Citrate Synthase

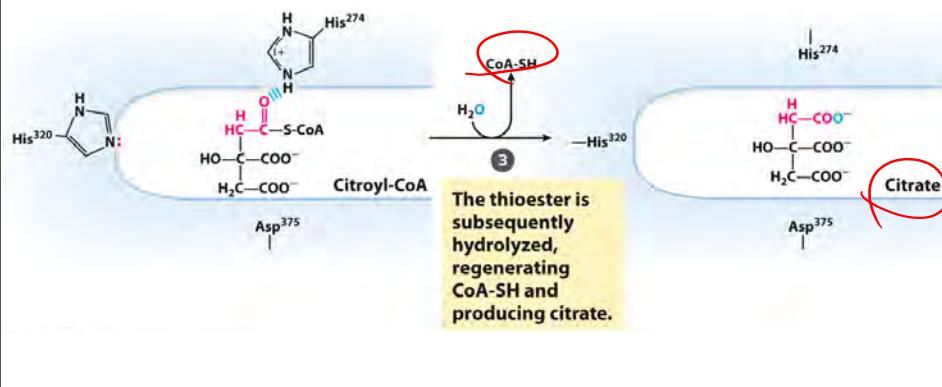
Mechanism



The Citric Acid Cycle: Citrate Synthase

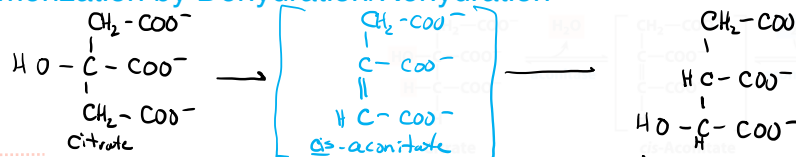
Mechanism

Hydrolysis of Thioester; citroyl-CoA



The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration

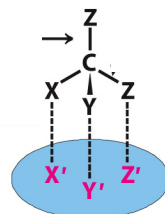
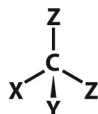
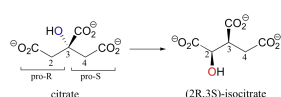


- Elimination of H₂O from the symmetrical molecule, citrate, gives a cis C=C bond.
 - lyase
- Rationale:
 - Citrate, a tertiary alcohol, is a poor substrate for oxidation.
 - Isocitrate, a secondary alcohol, is a good substrate for oxidation.
- Thermodynamically **unfavorable/reversible** ($\Delta G^\circ = +3.2 \text{ kcal/mol}$)
 - product concentration kept low to pull forward; citrate tends to "pool" with higher conc.
- Addition of H₂O to *cis*-aconitate is stereospecific.
 - This was initially very confusing to bio/organic chemists
 - Only R-isocitrate is produced by aconitase.
 - A biochemist names A.G. Ogston clarified the situation by realizing that the enzyme spatially templates this symmetrical molecule by binding in only one way (e.g., clockwise or counter clockwise, not both)
 - Distinguished by three-point attachment to the active site

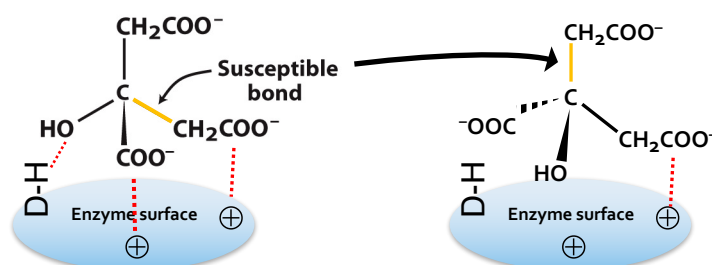
The Citric Acid Cycle: Aconitase

3-point attachment; prochirality

This binding protects the nascent acetate from chemistry

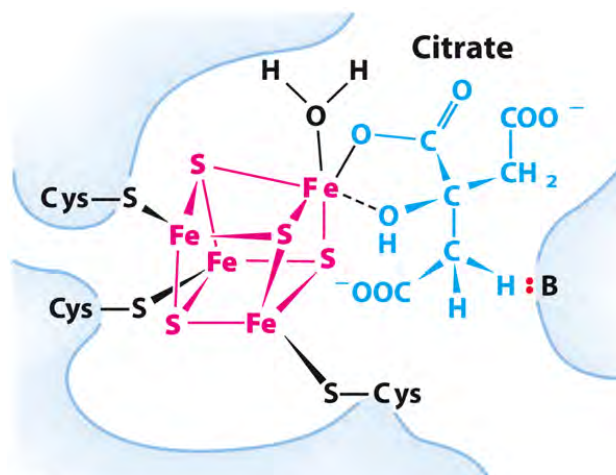


Active site has complementary binding points.



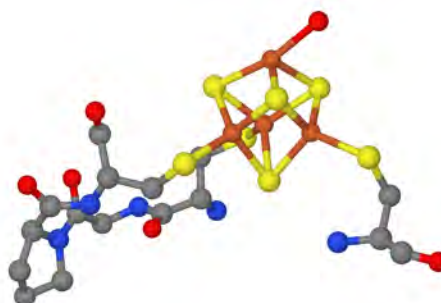
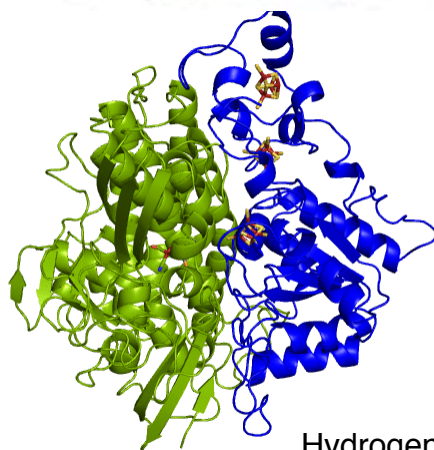
The Citric Acid Cycle: Aconitase

Iron-Sulfur Center in Aconitase



The Citric Acid Cycle: Aconitase

Iron-Sulfur Centers



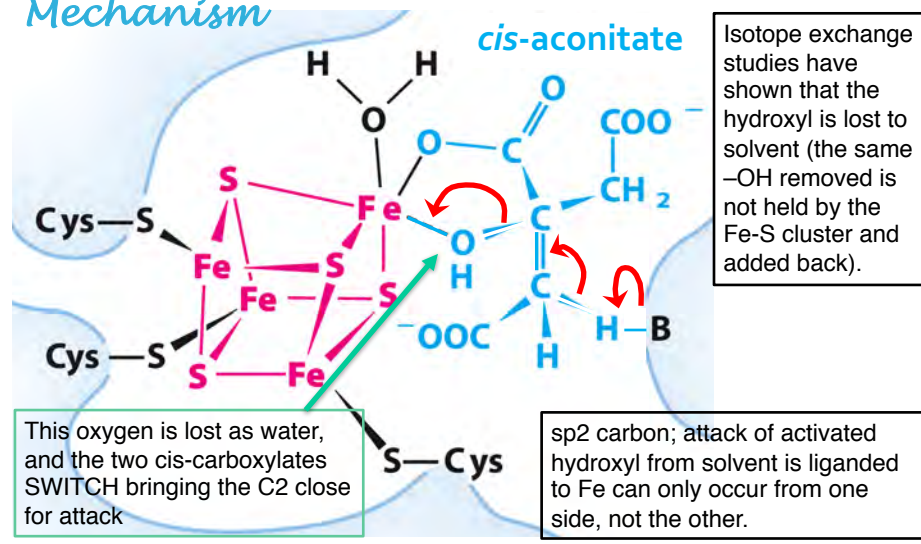
Most Iron-sulfur are involved in re-dox reactions (without proton transfers). In aconitase, the role is very different.

Hydrogenase: $\text{H}_2 \rightarrow 2\text{H}^+$

The Citric Acid Cycle: Aconitase

Water removal from **citrate** and subsequent addition to *cis*-aconitate are catalyzed by the **iron-sulfur center**: sensitive to oxidative stress.

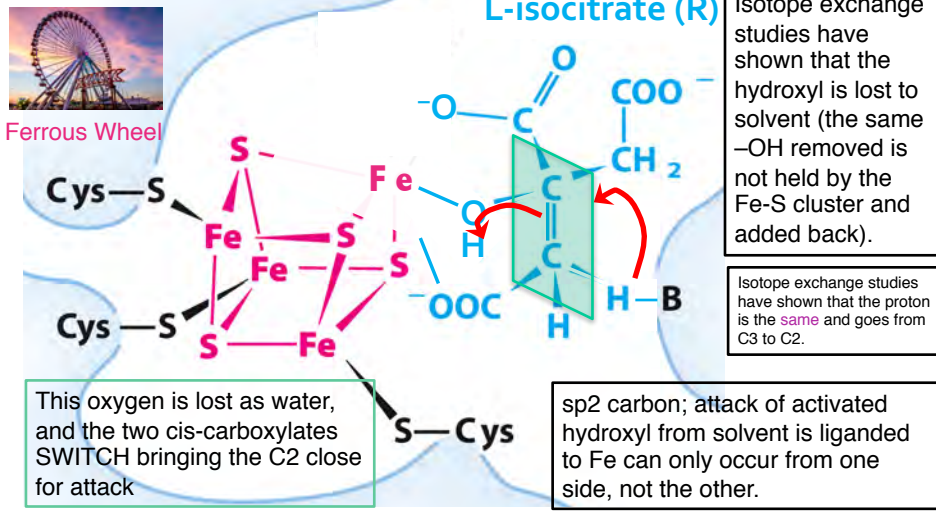
Mechanism



The Citric Acid Cycle: Aconitase

Water removal from **citrate** and subsequent addition to *cis*-aconitate are catalyzed by the **iron-sulfur center**: sensitive to oxidative stress.

Mechanism



The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration

