BI/CH 422/622

Announcements:

Exam 1 is posted. Grades will be done by Monday; Gradescope has you ID# so that you can look at whole class on website.

You have one week from return of grades, as usual.

OUTLINE:

Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations

Exam-1 material

Pyruvate

pyruvate dehydrogenase

Exam-2 material

Krebs' Cycle

How did he figure it out?
Overview
8 Steps

Citrate Synthase
Aconitase
Isocitrate dehydrogenase
Ketoglutarate dehydrogenase
Succinyl-CoA synthetase
Succinate dehydrogenase
Fumarase
Malate dehydrogenase

The Citric Acid Cycle

Citrate Synthase ✓

- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
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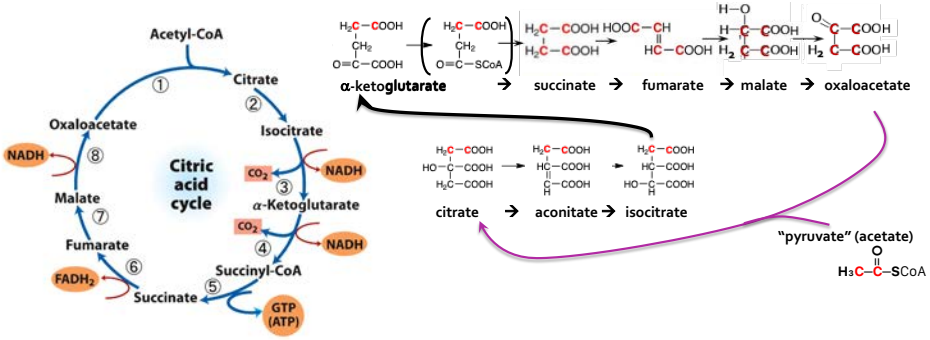
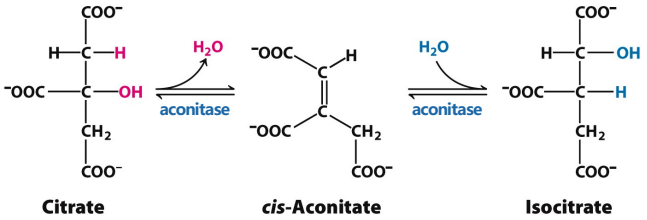
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give $FADH_2$
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH



The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration



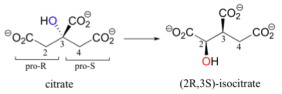
- Elimination of H_2O from the symmetrical molecule, citrate, gives a cis C=C bond.
  - lyase

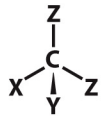
- Rationale:
  - Citrate, a tertiary alcohol, is a poor substrate for oxidation.
  - Isocitrate, a secondary alcohol, is a good substrate for oxidation.
- Thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +3.2 kcal/mol)
  - product concentration kept low to pull forward; citrate tends to "pool" with higher conc.
- Dehydration & Addition of H_2O 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

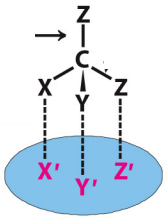
3-point attachment; prochirality

This binding protects the nascent acetate from chemistry

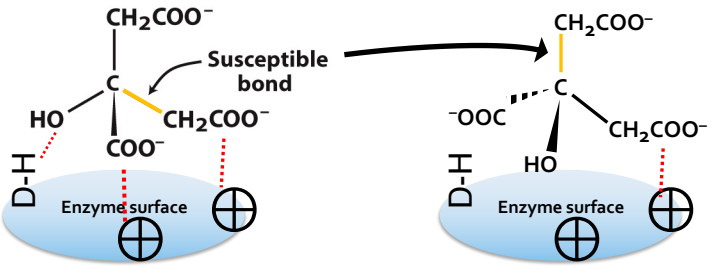




(b)

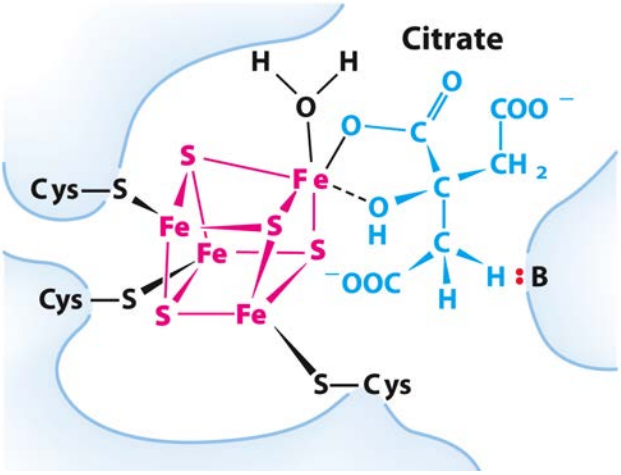


Active site has complementary binding points.



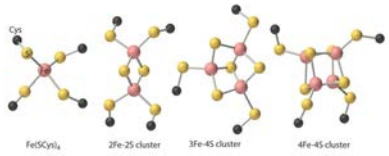
The Citric Acid Cycle: Aconitase

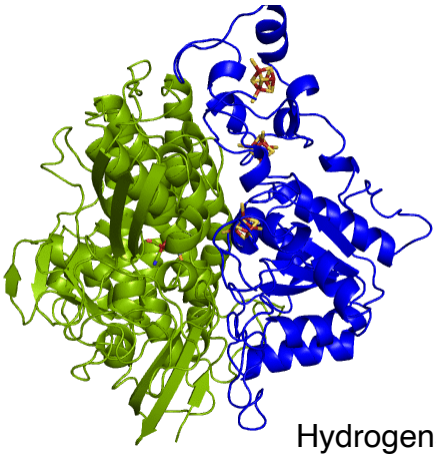
Iron-Sulfur Center in Aconitase

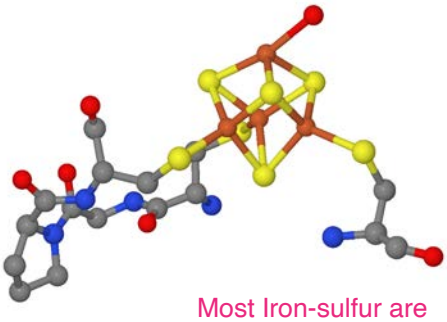


The Citric Acid Cycle: Aconitase

Iron-Sulfur Centers







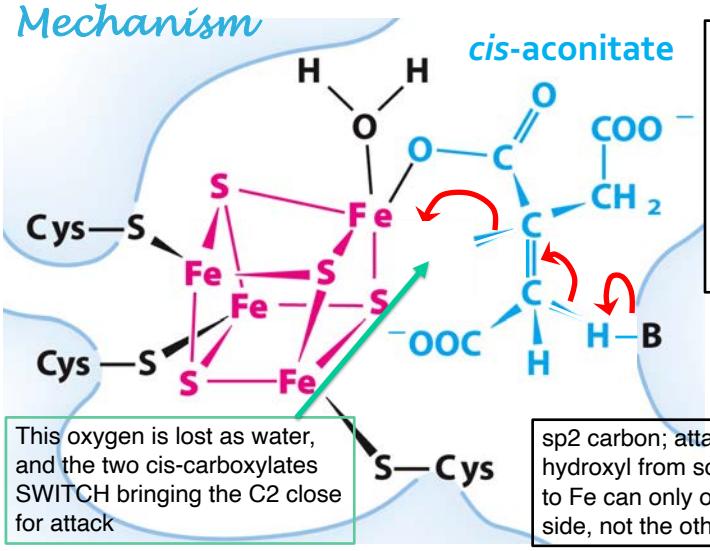
Most Iron-sulfur are involved in re-dox reactions (without proton transfers). In aconitase, the role is very different.

Hydrogenase: $H_2 \rightarrow 2H^+$

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



Isotope exchange studies have shown that the hydroxyl is lost to solvent (the same –OH removed is not held by the Fe-S cluster and added back).

This oxygen is lost as water, and the two cis-carboxylates SWITCH bringing the C2 close for attack

sp2 carbon; attack of activated hydroxyl from solvent is liganded to Fe can only occur from one side, not the other.

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

Ferrous Wheel

Isotope exchange studies have shown that the hydroxyl is lost to solvent (the same –OH removed is not held by the Fe-S cluster and added back).

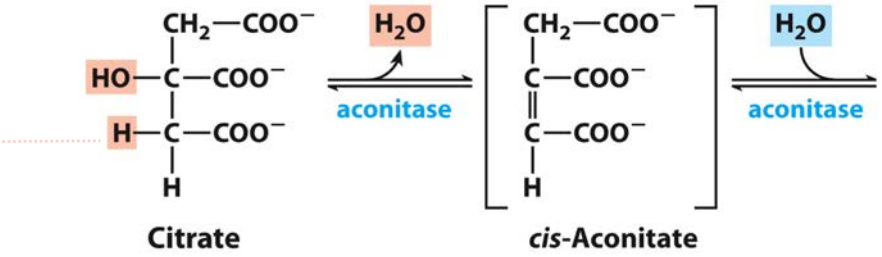
Isotope exchange studies have shown that the proton is the same and goes from C3 to C2.

This oxygen is lost as water, and the two cis-carboxylates SWITCH bringing the C2 close for attack

sp2 carbon; attack of activated hydroxyl from solvent is liganded to Fe can only occur from one side, not the other.

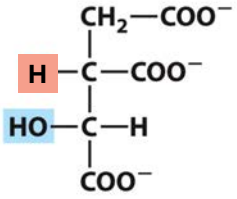
The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration



Citrate — *cis*-Aconitate

- Dehydration of H_2O from the symmetrical molecule, citrate, gives a *cis* C=C bond.
- Rehydration of H_2O to *cis*-aconitate is stereospecific.



Isocitrate

$\Delta G'^{\circ} = 13.3$ kJ/mol

The Citric Acid Cycle

Citrate Synthase ✓

Aconitase ✓

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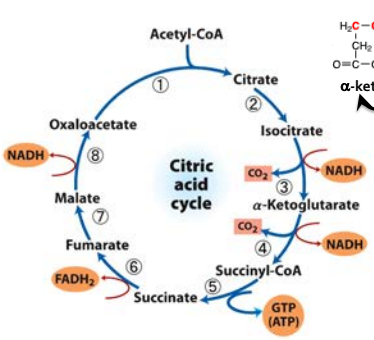
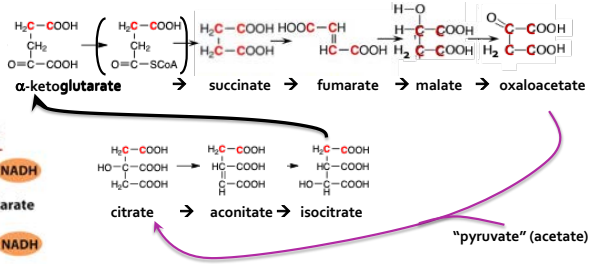
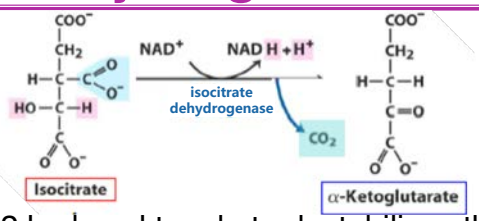




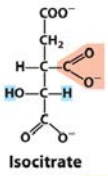
The Citric Acid Cycle: Isocitrate dehydrogenase



- 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 a alcohol-to-keto dehydrogenation at C2 using NAD^+
  - –Second, allow for decarboxylation (the oxidation of the carboxylate to CO_2, with the reduction of C3.
  - –C2 is oxidized, C3 is reduced, Carboxylate is oxidized: Net oxidation is $2e^-$
- Isozymes are specific for $NADP^+$ (cytosolic) or NAD^+ (mitochondrial).
- Favorable but irreversible due to loss of CO_2 ($\Delta G^{\circ\prime} = -2.0$ kcal/mol)
- Regulated by [ATP] (OMSGAP)

The Citric Acid Cycle: Isocitrate dehydrogenase

Mechanism



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

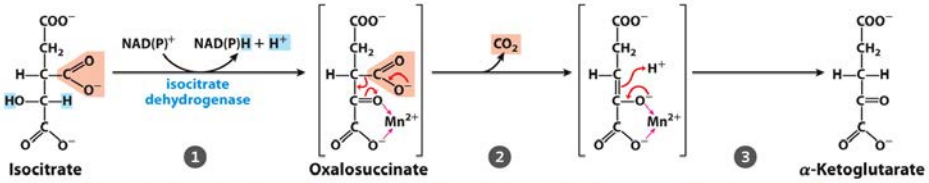
This mechanism is just like that of LDH or other dehydrogenases (Base abstracts –OH proton, carbonyl forms, elimination of :H–)

The Mn^{++} cofactor stabilizes the α-keto acid, which destabilizes the middle carboxylate

This is an oxidative decarboxylation at carboxylate β-to carbonyl, so it does not need TPP!! Uses Mn & α-carbonyl

The Citric Acid Cycle: Isocitrate dehydrogenase

Mechanism



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

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

Rearrangement of the enol intermediate generates α-ketoglutarate. (protonation of the carbanion)

This mechanism is just like that of LDH or other dehydrogenases (Base abstracts –OH proton, carbonyl forms, elimination of :H–)

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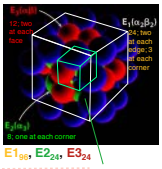
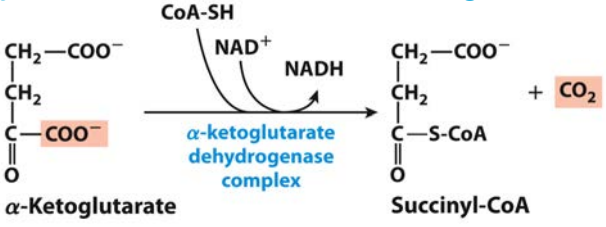
The Citric Acid Cycle: The α-Keto-Glutarate Dehydrogenase Complex

Oxidative Decarboxylation of an α-keto acid: α-Ketoglutarate Dehydrogenase



$E1_{\beta 12}$, $E2_{24}$, $E3_{24}$

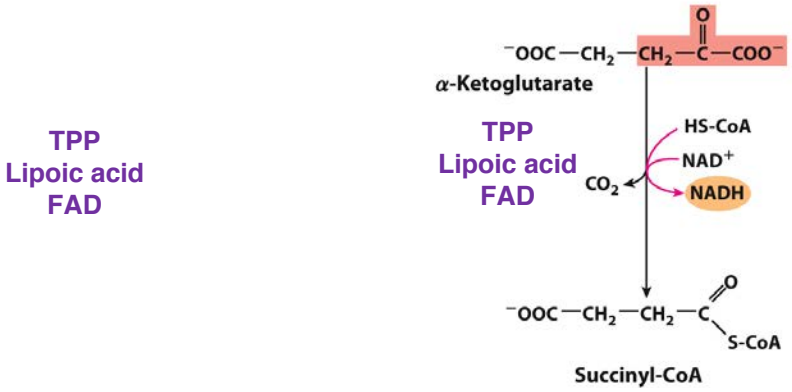


- Last oxidative decarboxylation in TCA cycle
  - full oxidation of all carbons of glucose:
    - Takes two turns of the cycle
    - The carbons oxidized are not directly from glucose because the carbons came from oxaloacetate, not acetyl-CoA
- Requires TPP, FAD, Lipoic acid cofactors
- Succinyl-CoA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

Where have we seen this before?

The Citric Acid Cycle: The α-Keto-Glutarate Dehydrogenase Complex

Citric acid cycle (α-KGDH)

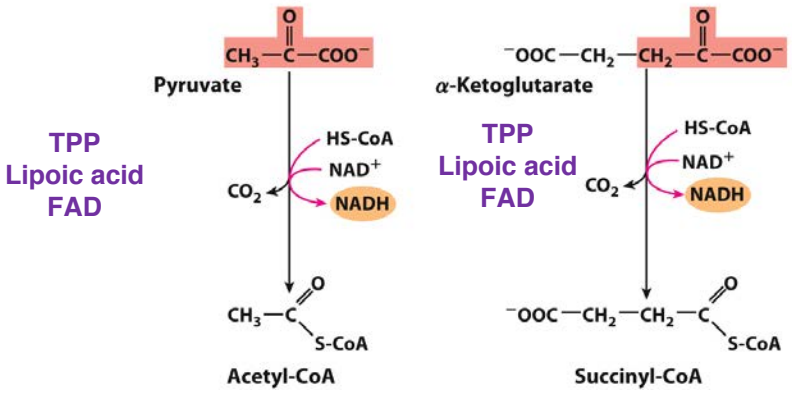


- Complex similar to pyruvate dehydrogenase
  - same coenzymes, identical mechanisms, E2 & E3 are identical
  - active site of E1 different to accommodate different-sized substrates

The Citric Acid Cycle: The α-Keto-Glutarate Dehydrogenase Complex

Pyruvate dehydrogenase complex

Citric acid cycle (α-KGDH)



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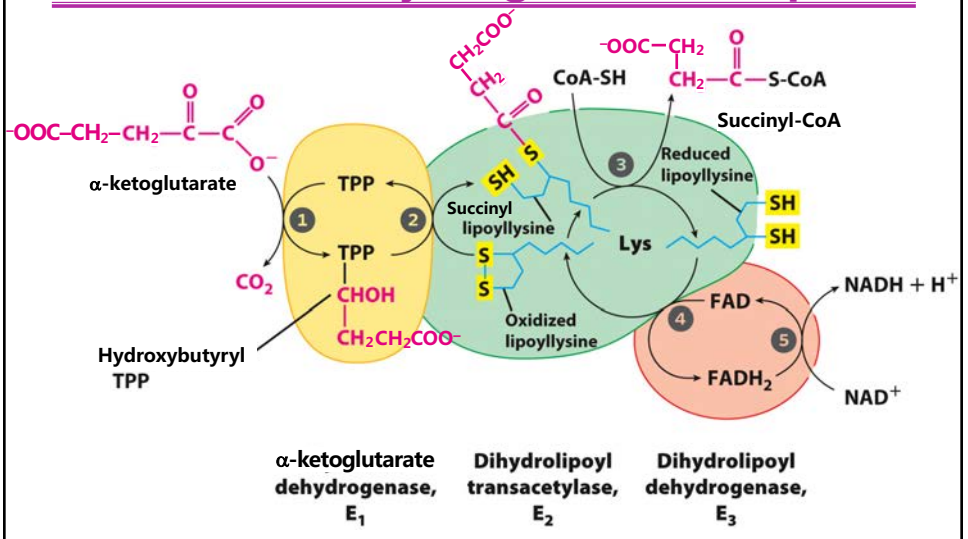

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α-ketoglutarate dehydrogenase complex

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