BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
- Introduction & overview; 2 phases
- Phase I
- Phase II
- Summary: logic, energetics, labeling studies

Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations
- Lactate-lactate dehydrogenase
- Acetoacetate decarboxylase
- Ethanol-pyruvate decarboxylase & alcohol dehydrogenase

Exam-1 material

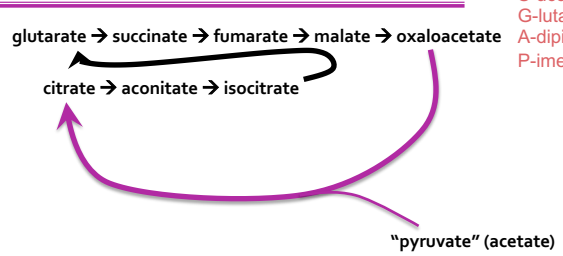
Exam-2 material

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

Time B.C. (Before the Cycle)

O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

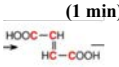
Hans Krebs
1900-1981



Krebs confirmed that the pathway was consistent with succinate, fumarate, and malate proved to be useful because all these molecules increased oxygen consumption in the pigeon breast muscle.

The first clue came from an experiment with fumarate. Krebs did careful measurements using the Warburg manometer. Fumarate gave greater than expected oxygen consumption in the pigeon breast muscle.

Dr. Kornberg: **Lecture 02.08.17**
(19:54-20:39)
(1 min)



$3O_2 + C_4H_4O_4 \rightarrow 4CO_2 + 2H_2O$

∴1 µmole fumarate would consume 3 µmole O_2

1) Malonic acid inhibition of the succinate → fumarate step prevented this increase…. BUT, succinate **accumulated!**
2) How can fumarate give rise to succinate? There must be a cycle
3) Tested by showing that using succinate or fumarate you could detect the formation of citrate.

Later in 1937, he proposed that pyruvate would combine with oxaloacetate to make citrate in a cycle he called the Citric Acid Cycle. Later, Fritz Lipmann showed that it was acetyl-CoA and not pyruvate.

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
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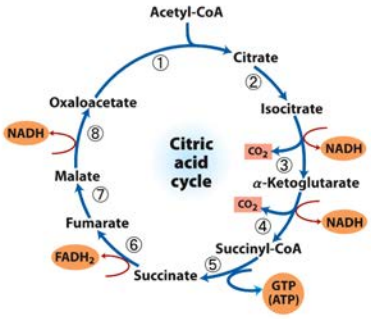
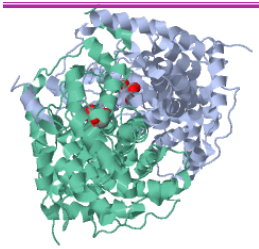
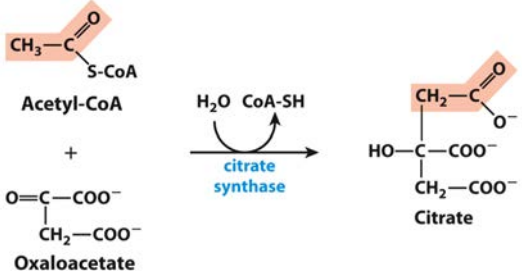
- Step 8: Dehydrogenation to give NADH

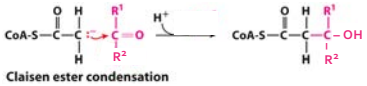


The Citric Acid Cycle: Citrate Synthase





- Joining of acetyl-CoA and oxaloacetate with C-C bond formation
- Highly thermodynamically favorable/irreversible ($\Delta G^{\circ\prime} = -7.7$ 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 to form a carbanion.

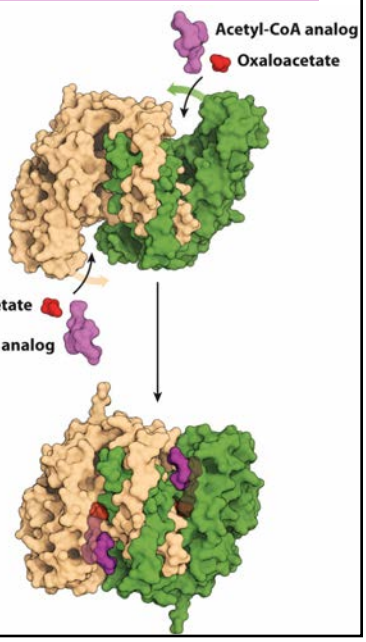


Claisen ester condensation

The Citric Acid Cycle: Citrate Synthase

Mechanism

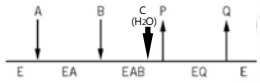
- Conformational change occurs upon binding oxaloacetate.
- Avoids unnecessary hydrolysis of thioester in acetyl-CoA



a) Open conformation:

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

Sequential ordered ter bi



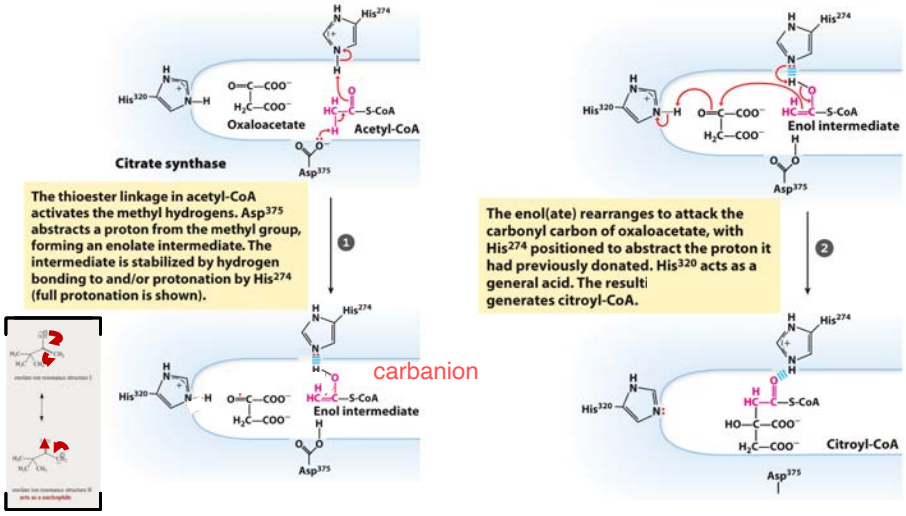
b) Closed conformation:

Binding of OAA creates binding for acetyl-CoA.

Reactive carbanion is protected.

The Citric Acid Cycle: Citrate Synthase

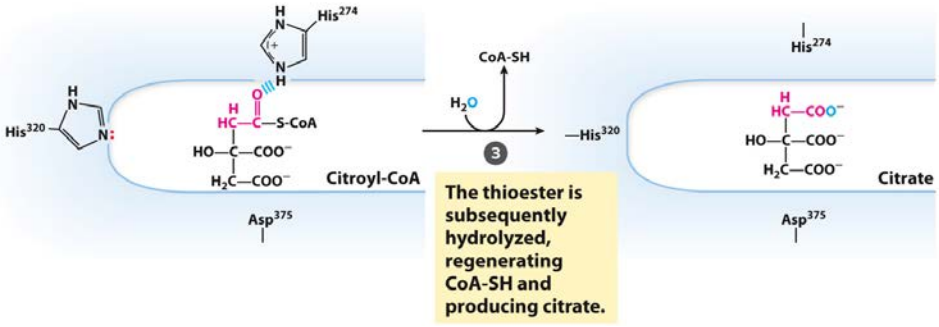
Mechanism



The Citric Acid Cycle: Citrate Synthase

Mechanism

Hydrolysis of Thioester; citroyl-CoA



The Citric Acid Cycle

Citrate Synthase ✓

- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
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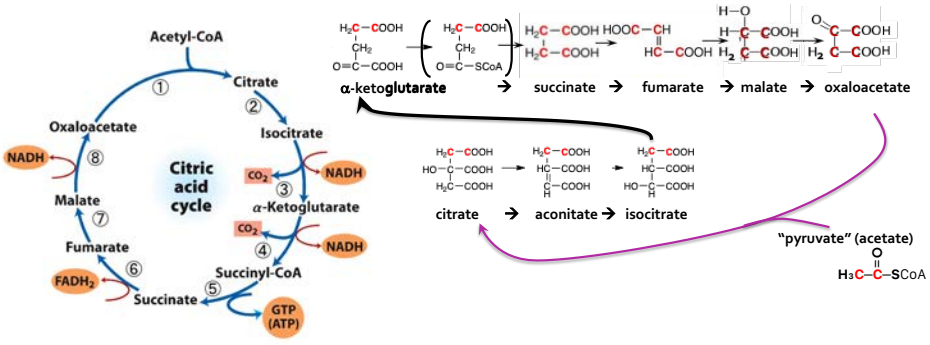
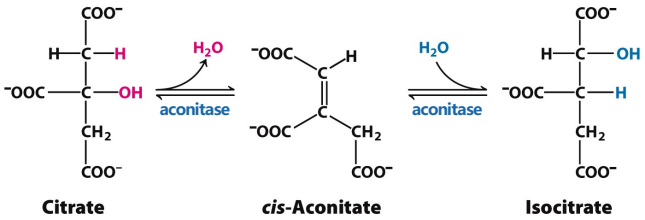


The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration



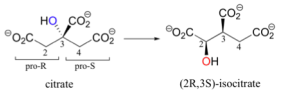
- Elimination of H_2O from the symmetrical molecule, citrate, gives a cis C=C bond.
  - lyase

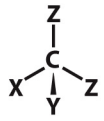
- Rationale:
  - Citrate, a tertiary alcohol, is a poor substrate for oxidation.
  - Isocitrate, a secondary alcohol, is a good substrate for oxidation.
- Thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +3.2 kcal/mol)
  - product concentration kept low to pull forward; citrate tends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

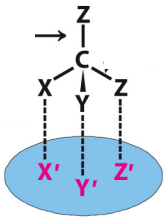
The Citric Acid Cycle: Aconitase

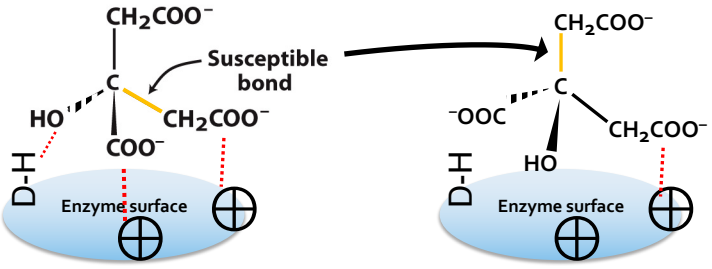
3-point attachment; prochirality

This binding protects the nascent acetate from chemistry



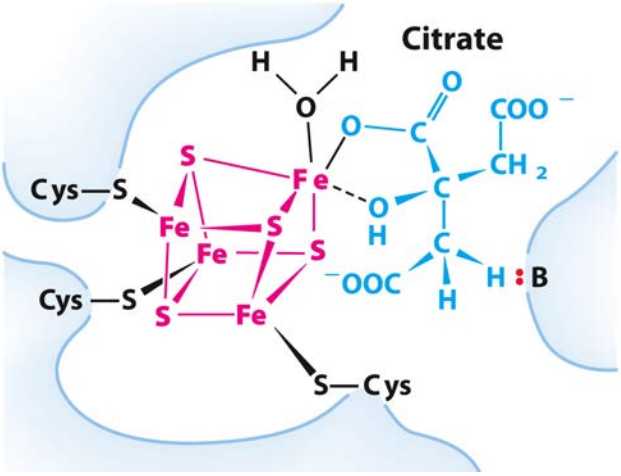






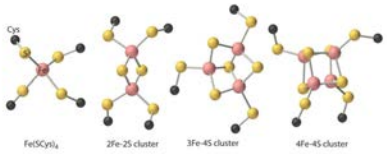
The Citric Acid Cycle: Aconitase

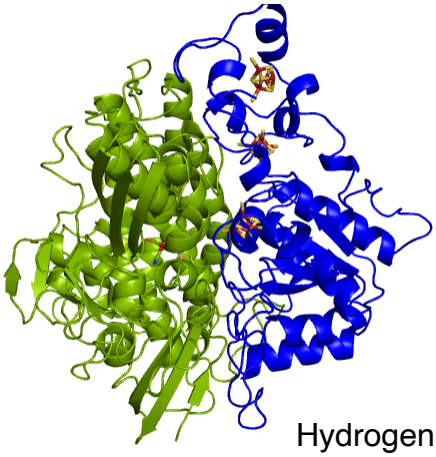
Iron-Sulfur Center in Aconitase

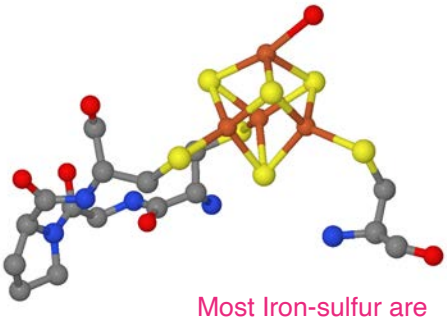


The Citric Acid Cycle: Aconitase

Iron-Sulfur Centers







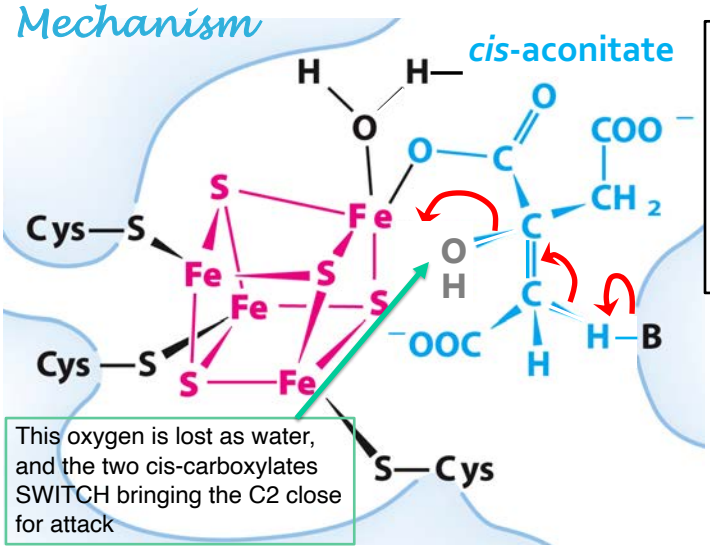
Most Iron-sulfur are involved in re-dox reactions (without proton transfers). In aconitase, the role is very different.

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).

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

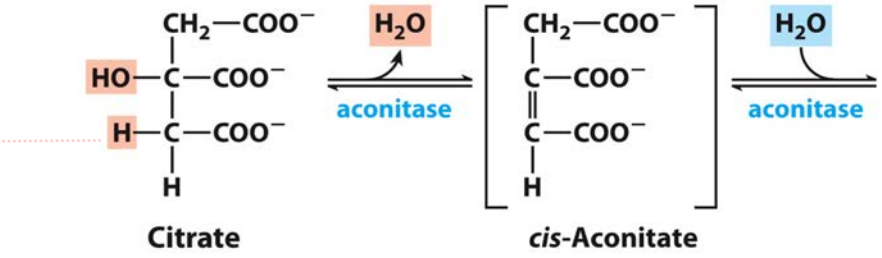
Isotope exchange studies have shown that the hydroxyl is lost to solvent (the same –OH removed is not held by the Fe-S cluster and added back).

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

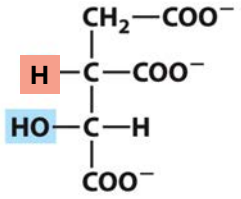
sp2 carbon; attack of activated hydroxyl from solvent is liganded to Fe can only occur from one side, not the other.

The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration



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



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

The Citric Acid Cycle

Citrate Synthase ✓

Aconitase ✓

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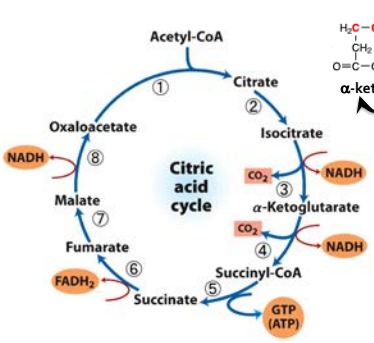
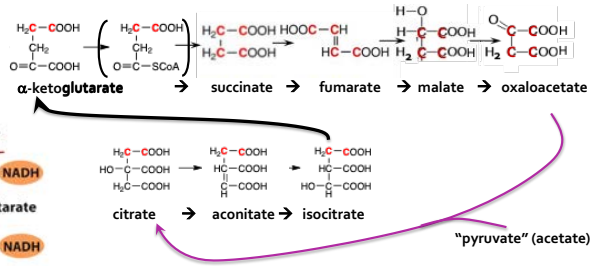
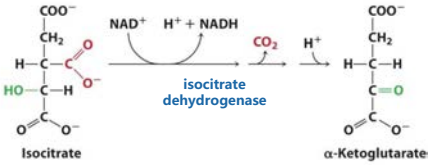
- Step 8: Dehydrogenation to give NADH





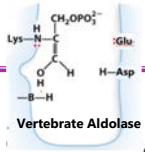
The Citric Acid Cycle: Isocitrate dehydrogenase

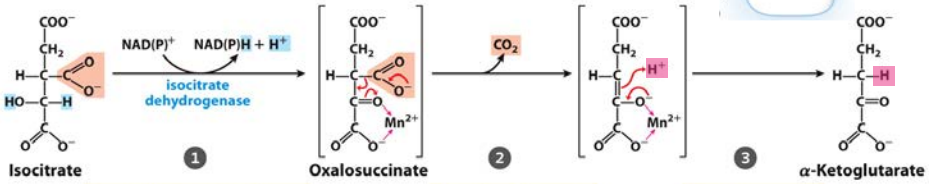


- Converting the C2 hydroxyl to a keto destabilizes the C-C bond to the carboxylate at C3 (C-C bond β-to carbonyl).
- This requires a 2-step process:
  - –First perform an alcohol-to-keto dehydrogenation at C2 using NAD^+
  - –Second, using carbonyl, allow for decarboxylation (the oxidation of the carboxylate 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).

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)

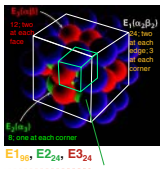
This mechanism is just like that of LDH or other dehydrogenases (Base abstracts –OH proton, carbonyl forms, elimination of :H–)

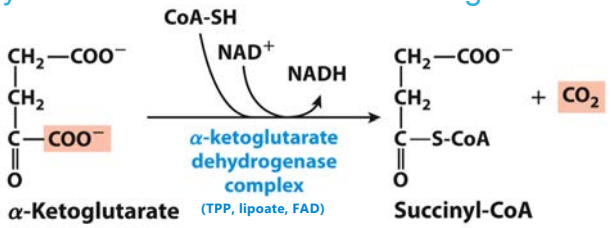
The Mn^{++} cofactor stabilizes the α-keto acid, which destabilizes the middle carboxylate (electron sink)

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

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

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

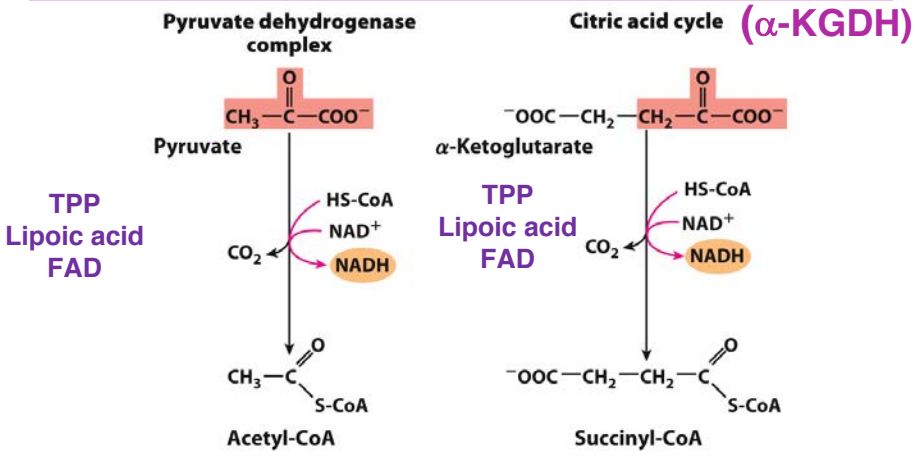




- Last oxidative decarboxylation in TCA cycle
  - full oxidation of all carbons of glucose:
    - Takes two turns of the cycle
    - The carbons oxidized are not directly from glucose because the carbons came from oxaloacetate, not acetyl-CoA
- Requires TPP, FAD, Lipoic acid cofactors
- Succinyl-CoA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

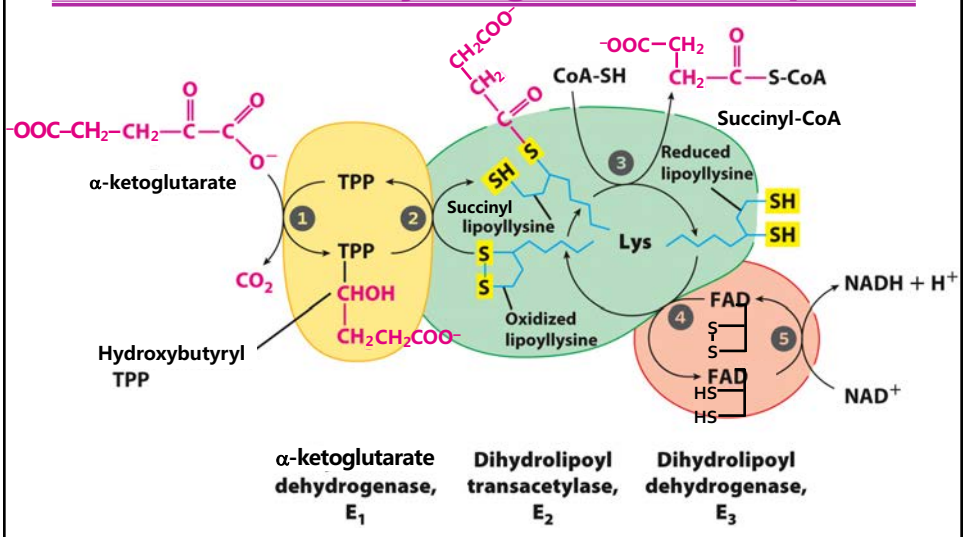
Where have we seen this before?

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



- 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



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Aconitase ✓

Isocitrate dehydrogenase ✓

α-ketoglutarate dehydrogenase complex

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