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: anaerobic fates of pyruvate
- Lactate-lactate dehydrogenase
- Acetoacetate decarboxylase
- Ethanol-pyruvate decarboxylase & alcohol dehydrogenase

Exam-1 material

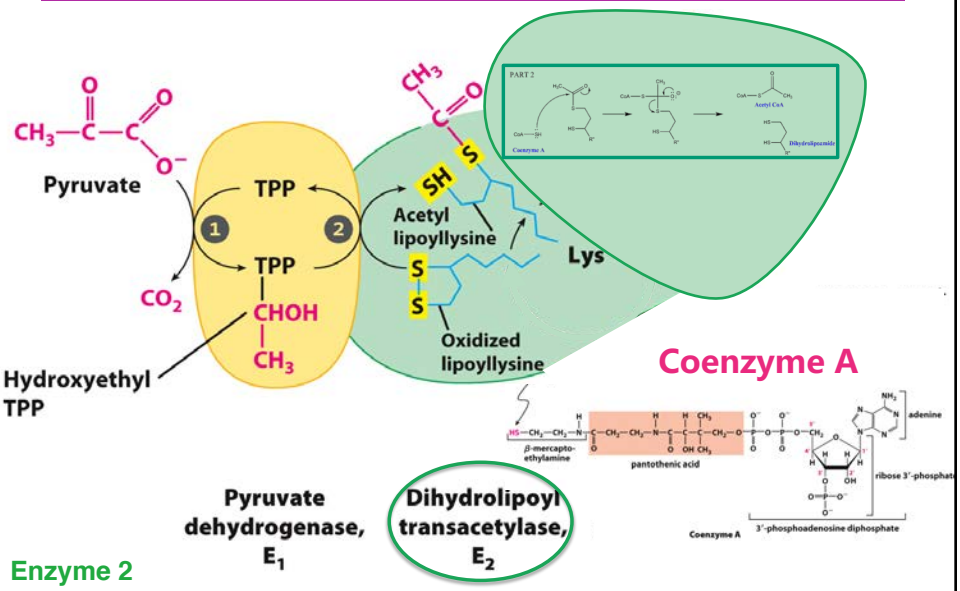
Exam-2 material

Pyruvate oxidation: aerobic fates of pyruvate
- pyruvate dehydrogenase complex

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
- Energetics; Regulation
- Summary

Oxidative Phosphorylation
- Electron Transport
- Chemiosmotic theory
- ATP synthesis

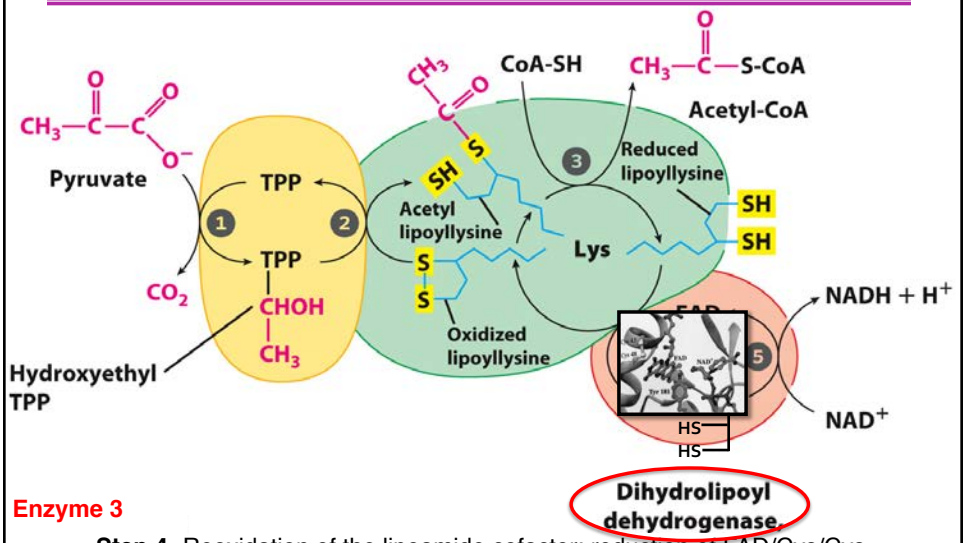
Pyruvate Oxidation



Enzyme 2

- Formation of acetyl-CoA: simple thio-ester exchange

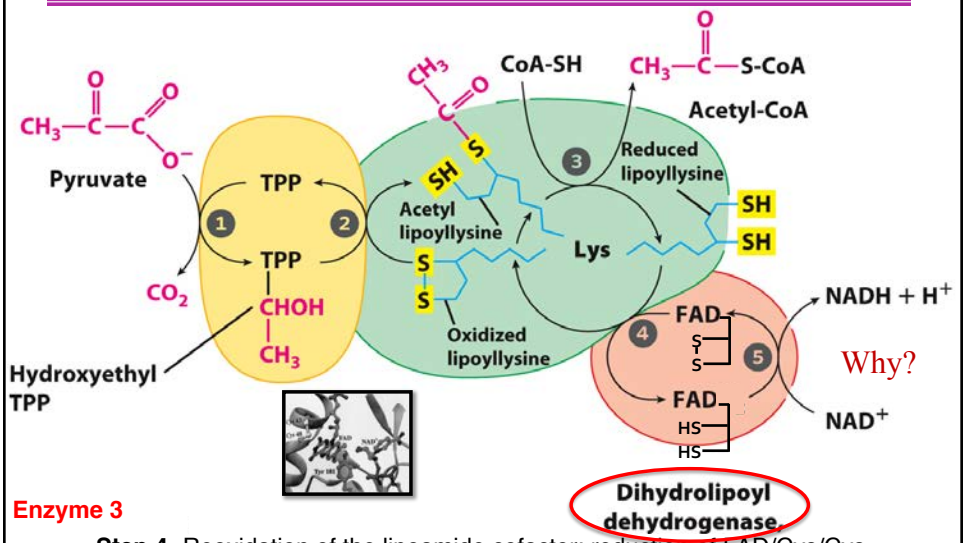
Pyruvate Oxidation



Enzyme 3

- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
  - Disulfide interchange with dihydrolipoyllysine and 2 adjacent Cys in disulfide bond
  - The reduced Cys reform disulfide using FAD, which funnels electrons to NAD^+
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

Pyruvate Oxidation



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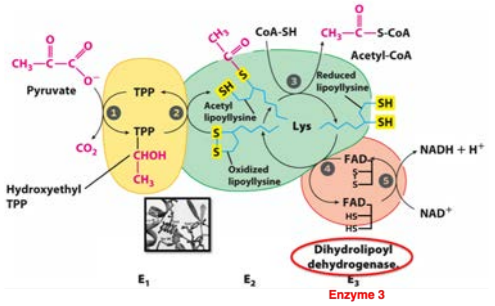
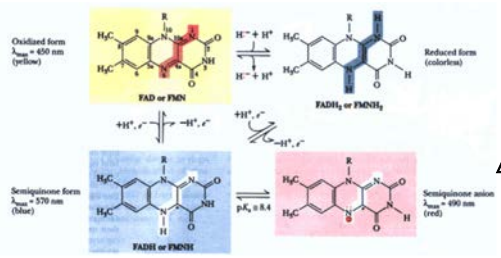
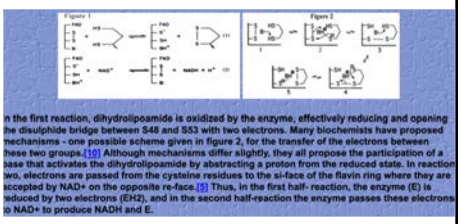





n the first reaction, dihydrolipoamide is oxidized by the enzyme, effectively reducing and opening he disulphide bridge between S48 and S53 with two electrons. Many biochemists have proposed nechanisms - one possible scheme given in figure 2, for the transfer of the electrons between hese two groups.[10] Although mechanisms differ slightly, they all propose the participation of a ase that activates the dihydrolipoamide by abstracting a proton from the reduced state. In reaction wo, electrons are passed from the cysteine residues to the si-face of the flavin ring where they are ccepted by NAD+ on the opposite re-face.[9] Thus, in the first half- reaction, the enzyme (E) is educed by two electrons (EH2), and in the second half-reaction the enzyme passes these electrons o NAD+ to produce NADH and E.

$$\Delta E^{o'} = E^{o'}_{(reduction)} - E^{o'}_{(oxidation)}$$

$$\Delta E^{o'} = E^{o'}_{(NAD^+)} - E^{o'}_{(FAD)}$$

$$= -0.320\ V - (+0.031\ V^*)$$

$$= -0.351\ V$$

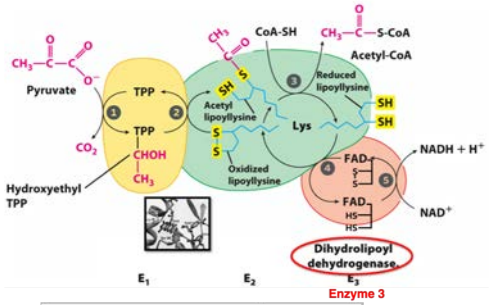
$$\Delta G^{o'} = -n\,\mathcal{F}\Delta E^{o'}$$

$$= -(2)(23.06 kcal V^{-1} mol^{-1})(-0.351\ V)$$

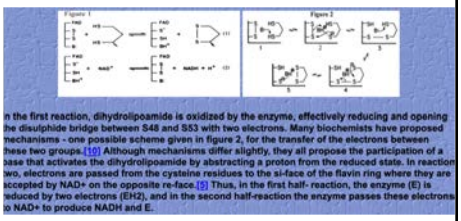
$$= +16\ kcal\ mol^{-1}$$

*from Maeda-Yorita et al., (1994) *Biochem.* **33**, 6213

Pyruvate Oxidation



Standard Redox Potentials E_0			
Half reaction		Half reaction	E_0(V)
Succinate + CO_2 + $2H^+$ +2e$^-$	↔	α-ketoglutarate + H_2O	-0.670
Acetate + $2H^+$ +2e$^-$	↔	acetaldehyde	-0.581
$2H^+$ +2e$^-$	↔	H_2	-0.421
α-ketoglutarate + CO_2 + $2H^+$ +2e$^-$	↔	citrate	-0.380
Cystine + $2H^+$ +2e$^-$	↔	2 cysteine	-0.340
NAD^+ + $2H^+$ +2e$^-$	↔	NADH + H^+	-0.320
$NADP^+$ + $2H^+$ +2e$^-$	↔	NADPH + H^+	-0.324
Acetaldehyde + $2H^+$ +2e$^-$	↔	ethanol	-0.197
Pyruvate + $2H^+$ +2e$^-$	↔	lactate	-0.185
Oxaloacetate + $2H^+$ +2e$^-$	↔	malate	-0.166
FAD + $2H^+$ +2e$^-$	↔	$FADH_2$	0.031
Fumarate + $2H^+$ +2e$^-$	↔	succinate	0.031
Ubiquinone + $2H^+$ +2e$^-$	↔	ubiquinol	0.045
2 cytochrome $b_{(ox)}$ +2e$^-$	↔	2 cytochrome $b_{(red)}$	0.070
2 cytochrome $c_{1(ox)}$ +2e$^-$	↔	2 cytochrome $c_{1(red)}$	0.254
2 cytochrome $a_{3(ox)}$ +2e$^-$	↔	2 cytochrome $a_{3(red)}$	0.385
1/2 O_2 + $2H^+$ +2e$^-$	↔	H_2O	0.816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$$\Delta G^{o'} = -n\,\mathcal{F}\Delta E^{o'}$$

$$= -(2)(23.06 kcal V^{-1} mol^{-1})(-0.351\ V)$$

$$= +16\ kcal\ mol^{-1}$$

If use S-S, with $E^{o'}_{(S-S)} = -0.34$ V, $\Delta G^{o'} = -0.92$ kcal/mol

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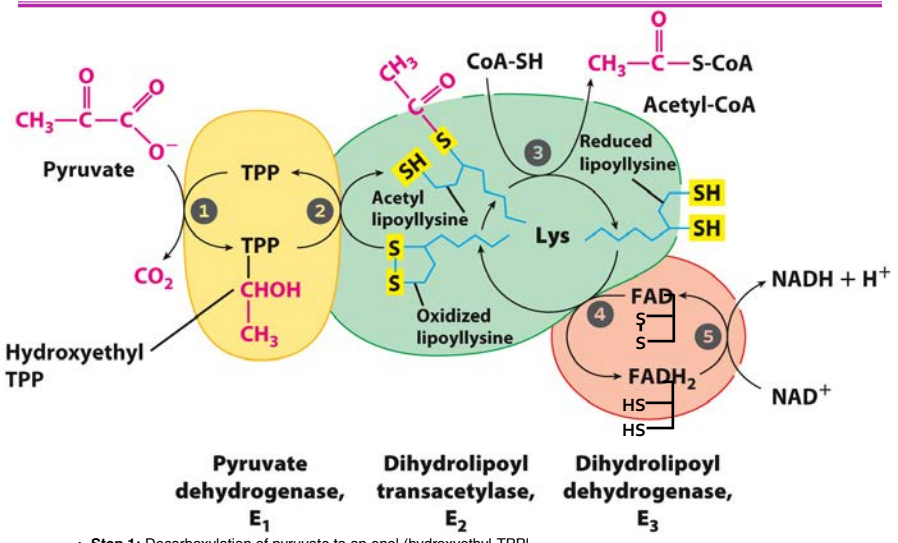
Pyruvate Oxidation

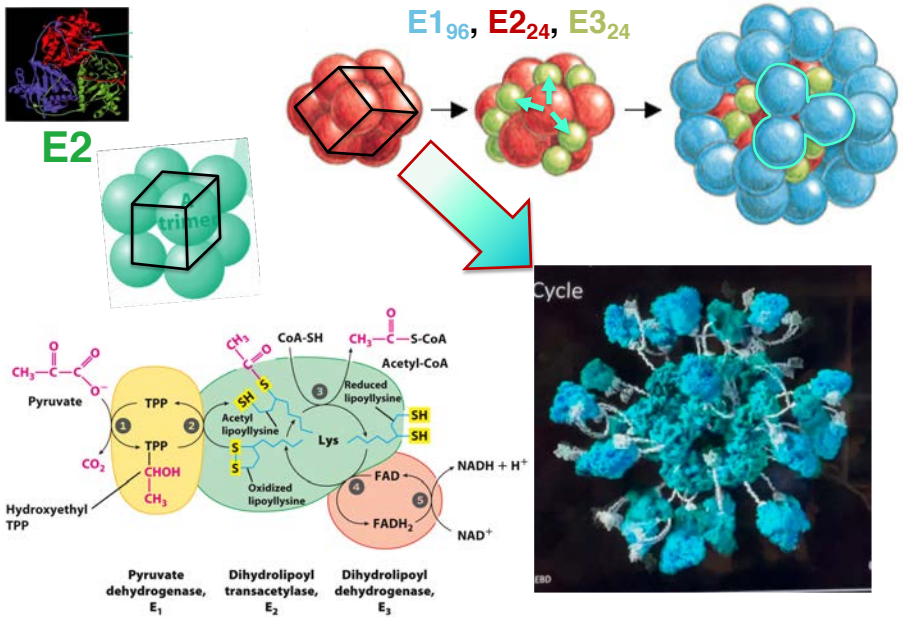


Enzyme 1
- **Step 1:** Decarboxylation of pyruvate to an enol (hydroxyethyl-TPPl
- **Step 2:** Acylation of enol to a thioester on lipoic acid.

Enzyme 2
- **Step 3:** Formation of acetyl-CoA: simple thio-ester exchange

Enzyme 3
- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

Pyruvate Oxidation



Pyruvate Oxidation

Overall Reaction of PDC

Pyruvate + Coenzyme-A (CoASH) + NAD^+

PDC (TPP, lipoic acid, FAD) ⇅ $\Delta G^{\circ\prime} = -8$ kcal/mol

CO_2 + **Acetyl-Coenzyme-A** (Ac-CoA) + NADH + H^+

Pyruvate Oxidation

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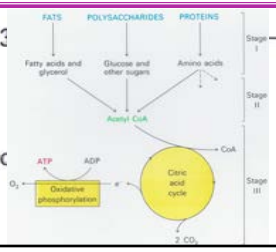
Fates of Acetyl CoA

(HMG-CoA)



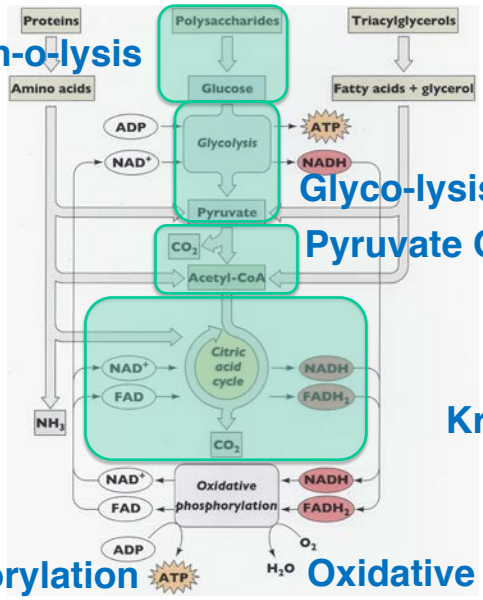
Acetyl CoA ⟹ Fatty acids

Amino acids

CO_2

1. Oxidation
2. Lipid metabolism
3. Amino acid metabolism

The Citric Acid Cycle



Glycogen-o-lysis

Glyco-lysis

Pyruvate Oxidation

Krebs' Cycle

Phosphorylation

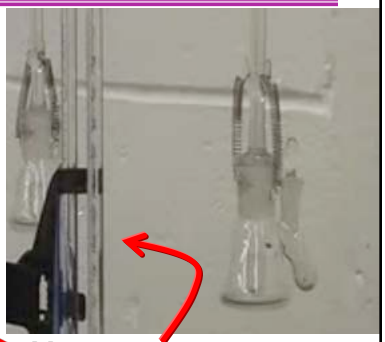
Oxidative

The Citric Acid Cycle

a.k.a. Krebs Cycle,
a.k.a. Tricarboxylic Acid Cycle (TCA)

Time B.C. (Before the Cycle)

Otto Warburg
1883-1970



Manometer

Substrates
(e.g., glucose)

CO_2 trap
(KOH)

Tissues

Warburg Apparatus

-respiration

-Measure rates of O_2 consumption

UTube instructions
(http://youtu.be/M-HYbZwN43o)

$CO_2 \rightarrow H_2CO_3 \rightarrow 2H^+ + CO_3^{-2} \rightarrow K_2CO_3 + H_2O$

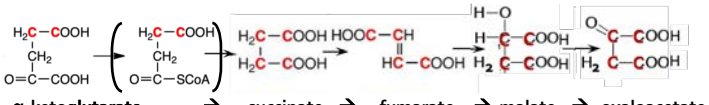
Time B.C. (Before the Cycle)

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

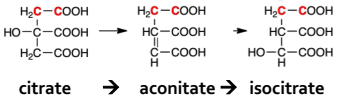
In 1920 BC, what was known about respiration?

1) Glycolysis gives rise to pyruvate
2) Adding 1 mole pyruvate to respiring tissues in a Warburg apparatus, there are 2.5 moles O_2 consumed: $2^{1/2}O_2 + C_3H_4O_3 \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow 3CO_2 + 2H_2O$
3) Any intermediate in the process will be oxidized at a rate ≥pyruvate
4) Many intermediates were tried, but few met this criteria, they were: succinate, fumarate, malate, alpha-ketoglutarate, etc.

Albert Szent-Györgyi
1893-1986



α-ketoglutarate → succinate → fumarate → malate → oxaloacetate



citrate → aconitate → isocitrate

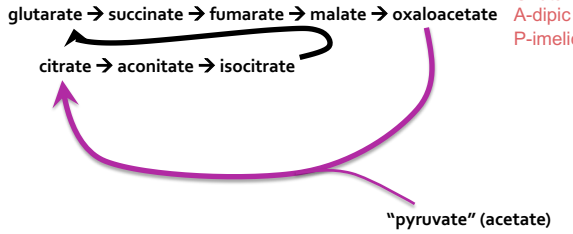
5) Others had already worked out several compounds and their interconversion. Specifically, Albert Szent-Györgi had worked out the interconversion of the dicarboxylic acids. Carl Martinus worked out the interconversion of the tricarboxylic acids

6) In 1937, with help of German biochemist Franz Koop, Carl Martinus, demonstrated a series of reactions using citrate that produced α–ketoglutarate. Thus tricarboxylic acid and dicarboxylic acids would be interconverted with loss of CO_2, but also support respiration.

Time B.C. (Before the Cycle)

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

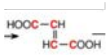
Hans Krebs
1900-1981



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.

He observed much more that 3:1!!

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.



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

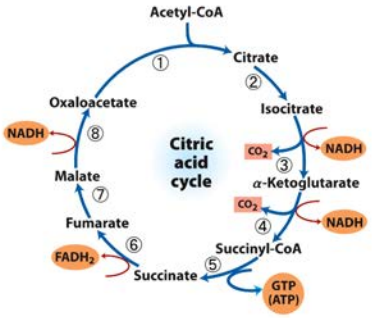
$\therefore$1 µmole fumarate would consume 3 µmole O_2

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.

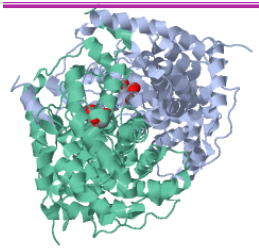
Later in 1937, he proposed that pyruvate would combine with oxaloacetate to make citrate in a cycle he called the Citric Acid Cycle. Later, Fritz Lipmann showed that it was acetyl-CoA and not pyruvate.

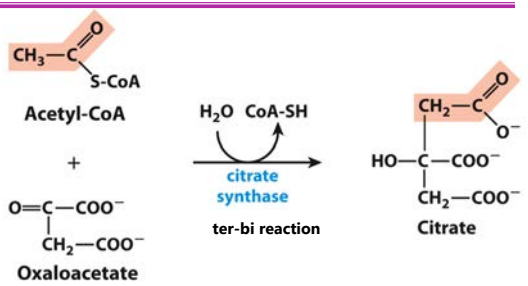
The Citric Acid Cycle

- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give $FADH_2$
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH

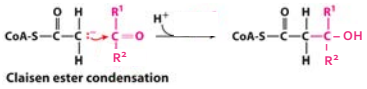


The Citric Acid Cycle: Citrate Synthase





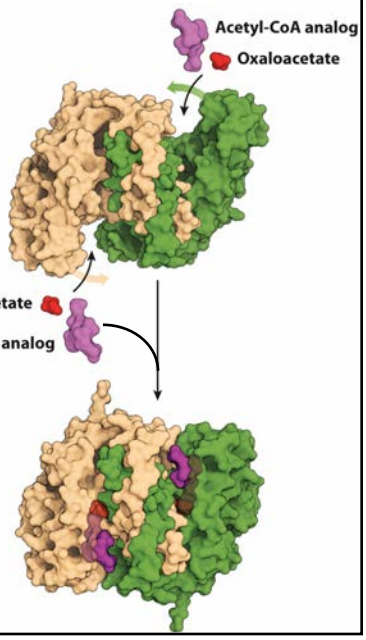
- Joining of acetyl-CoA and oxaloacetate with C-C bond formation
- Highly thermodynamically favorable/irreversible ($\Delta G^{\circ\prime} = -7.7$ kcal/mol)
  - regulated by substrate availability and product inhibition
- Activity largely depends on [oxaloacetate].
- Rate-limiting step of CAC
- Uses 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.



The Citric Acid Cycle: Citrate Synthase

Mechanism

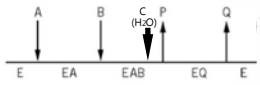
- Conformational change occurs upon binding oxaloacetate.
- Avoids unnecessary hydrolysis of thioester in acetyl-CoA



a) Open conformation:

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

Sequential ordered ter bi



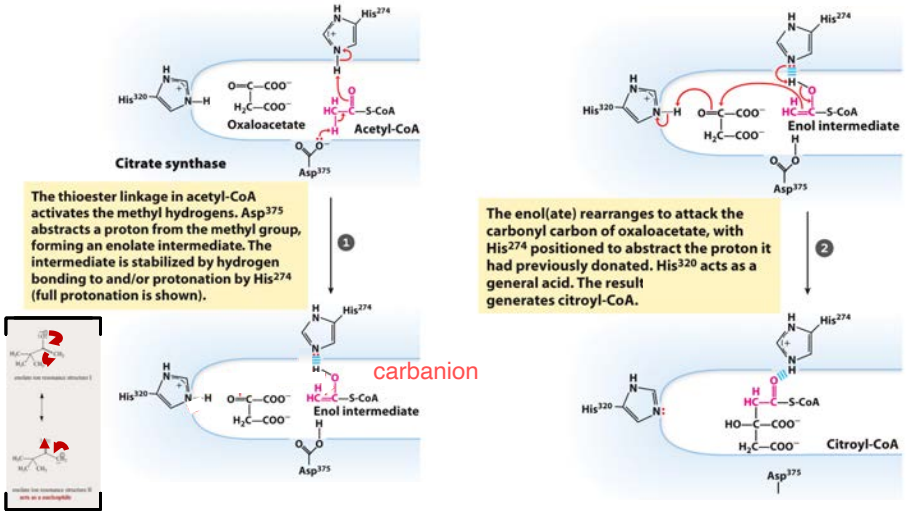
b) Closed conformation:

Binding of OAA creates binding for acetyl-CoA.

Reactive carbanion is protected.

The Citric Acid Cycle: Citrate Synthase

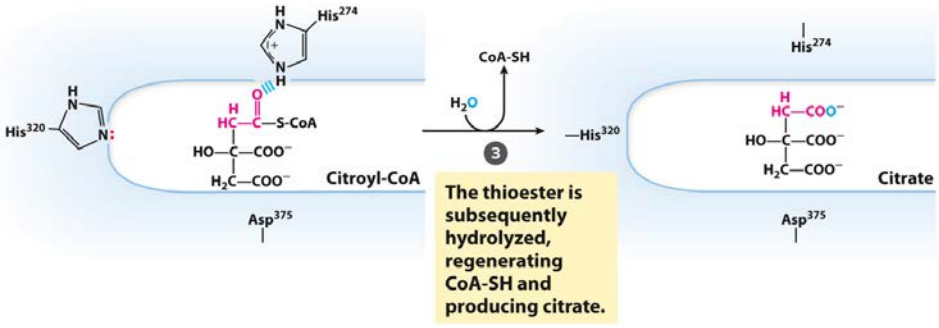
Mechanism



The Citric Acid Cycle: Citrate Synthase

Mechanism

Hydrolysis of Thioester; citroyl-CoA



The Citric Acid Cycle

Citrate Synthase ✓

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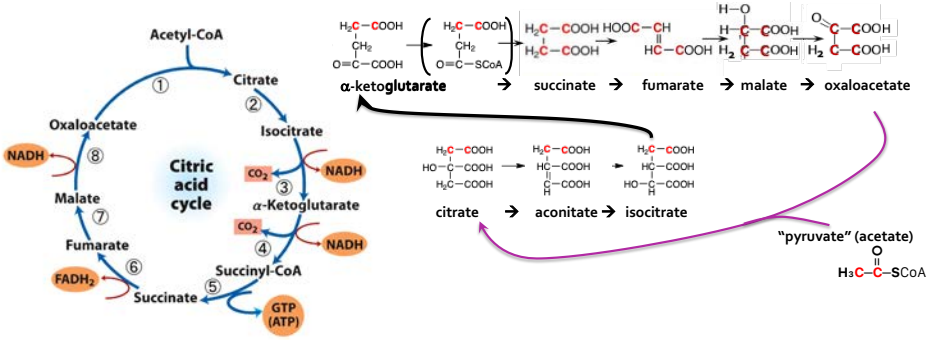
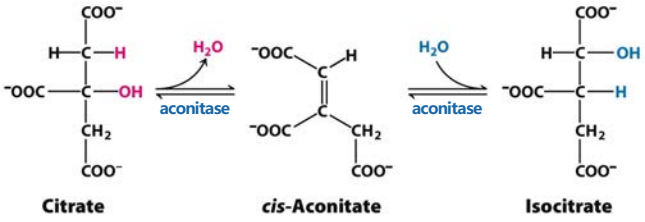


The Citric Acid Cycle: Aconitase

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.

Realize that biochemists could distinguish the two acetates by running the citrate synthase reaction with $H_2{}^{18}O$.

- 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