BB 422/622

OUTLINE:

Review of 421
Goals of 422
Review of chemical principles
- Thermodynamics
- C/O cycles
- Overview of Metabolism
- ATP cycles

Energy Coupling
Chemical Reactivity
Bioenergetics
Membrane Transport
- Review of membrane structure, dynamics, and proteins
- Mediated/non-mediated
- Energetics
- Facilitative Diffusion
  - ionophores
  - GLUT1
  - Aquaporins
  - Potassium channel
- Active Transport
  - Primary
    - Na/K pump
    - ABC transporters
  - Secondary
    - Glc import
    - Bicarbonate/Cl
    - Lactose/H+

Catabolism of Glucose
Glycogenolysis
- phosphorylase
- debranching enzyme
- phospho-gluco-mutase (PGM)

Glycolysis
- Introduction & overview;
- Phase I
  - hexokinase- phosphotransferase-coupling
  - phospho-gluco-isomerase (PGI)- endiol
  - phospho-fructo-kinase (PFK-1)-
  - Aldolase- Schiff base
    - (electron sink to stabilize a carbanion)
  - triose-phosphate isomerase (TPI)- endiol

Exam 1

- Phase II
  - **GAPDH**- oxidation
  - PG kinase- return on investment-
    - substrate-level phosphorylation
  - **PG mutase**- acid/base;
    - phospho-enzyme
  - **Enolase**- enolate
  - Pyruvate Kinase- phosphotransferase
- Summary: labeling studies, logic, energetics

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

Aerobic
- Pyruvate
  - pyruvate dehydrogenase complex
- Krebs' Cycle
  - How did he figure it out?
  - Overview
  - 8 Steps
    - Citrate Synthase
    - Aconitase
    - Isocitrate dehydrogenase
    - Ketoglutarate dehydrogenase

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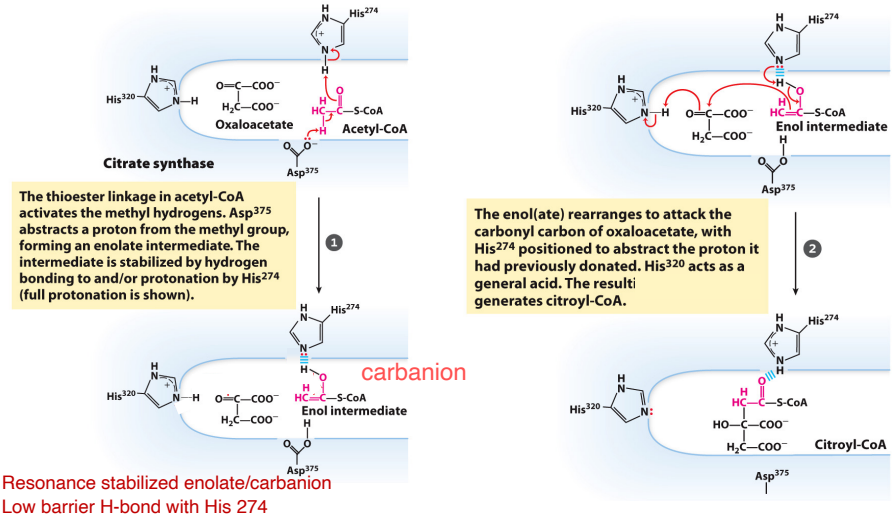
The Citric Acid Cycle: Citrate Synthase

Mechanism



- Resonance stabilized enolate/carbanion
- Low barrier H-bond with His 274

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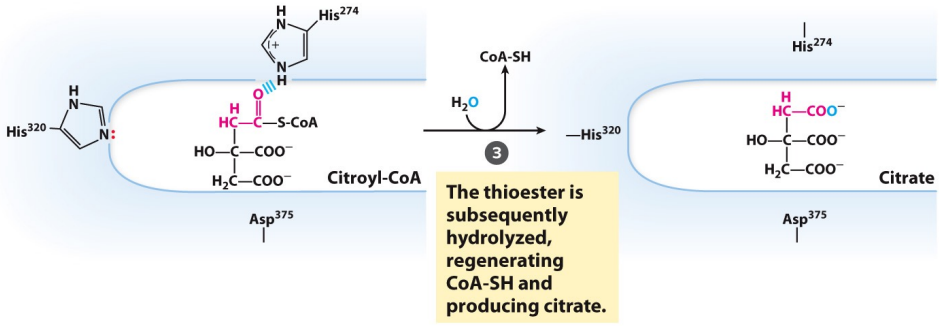
The Citric Acid Cycle: Citrate Synthase

Mechanism

Hydrolysis of Thioester; citroyl-CoA



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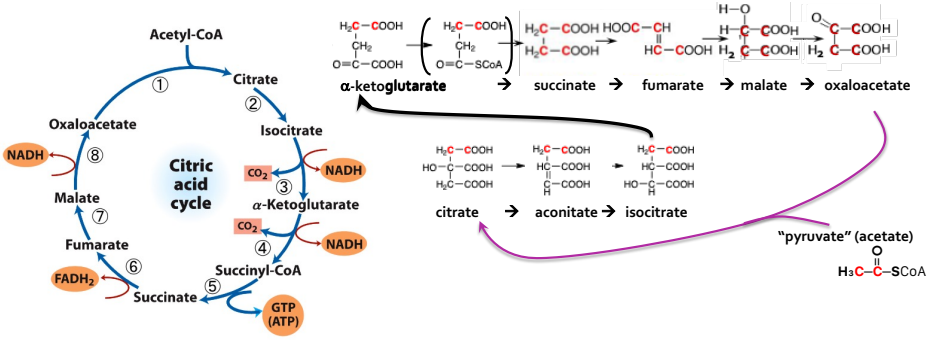
The Citric Acid Cycle

Citrate Synthase ✓

- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- 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



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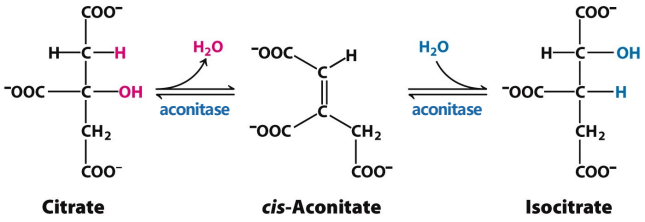
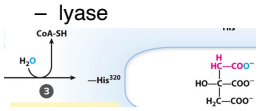
The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration



Citrate ***cis*-Aconitate** **Isocitrate**

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



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

- 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 and only one "acetyl-group" is involved.
  - 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 counterclockwise, not both)
  - Distinguished by three-point attachment to the active site

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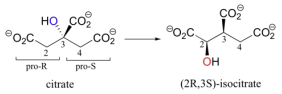
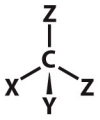
The Citric Acid Cycle: Aconitase

3-point attachment; prochirality

This binding protects the nascent acetate from chemistry





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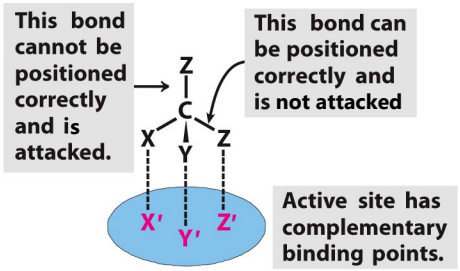

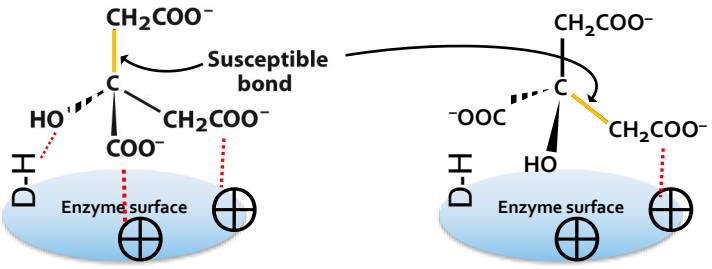

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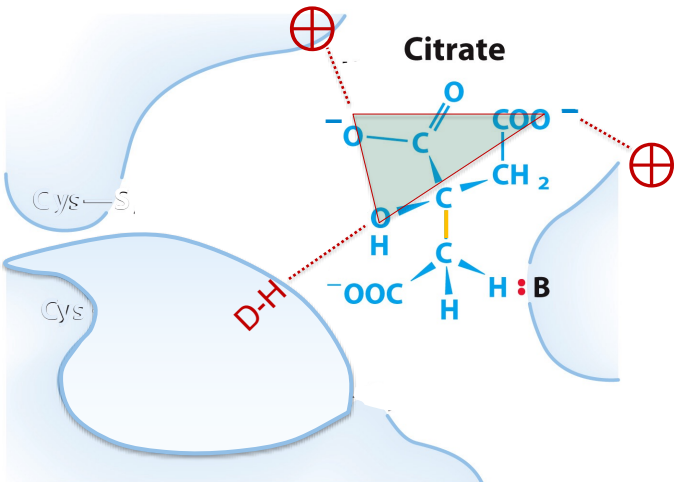
The Citric Acid Cycle: Aconitase

Iron-Sulfur Center in Aconitase



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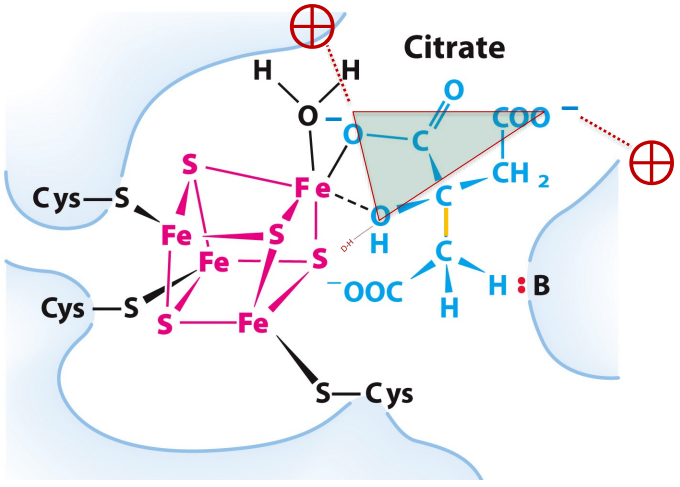
The Citric Acid Cycle: Aconitase

Iron-Sulfur Center in Aconitase



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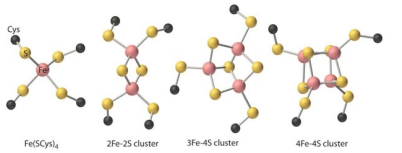
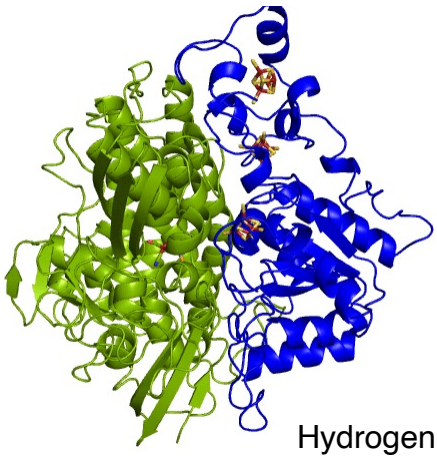
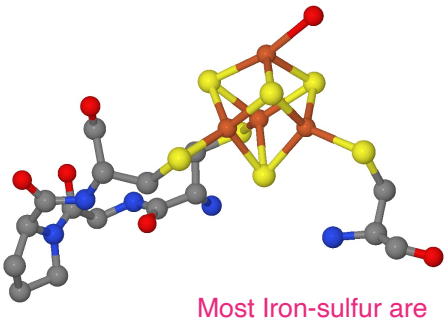
The Citric Acid Cycle: Aconitase

Iron-Sulfur Centers







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

Hydrogenase: $H_2 \rightarrow 2H^+$

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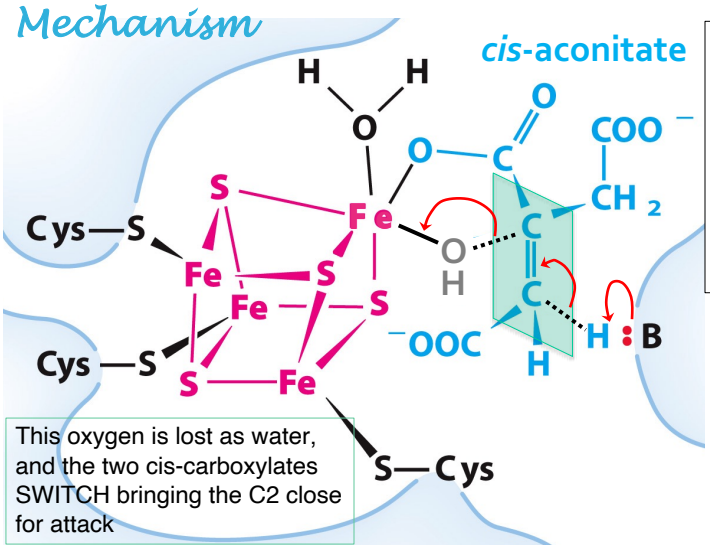
The Citric Acid Cycle: Aconitase

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



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

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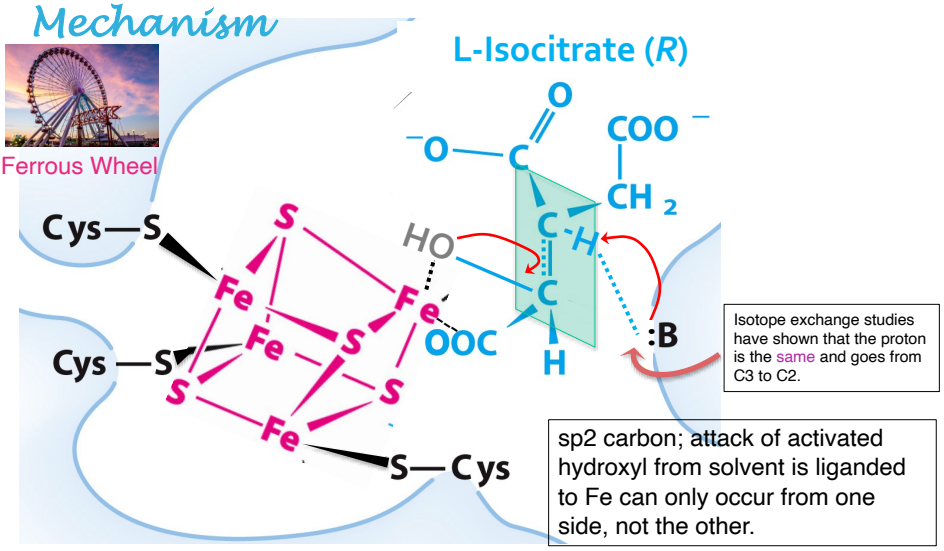
The Citric Acid Cycle: Aconitase

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



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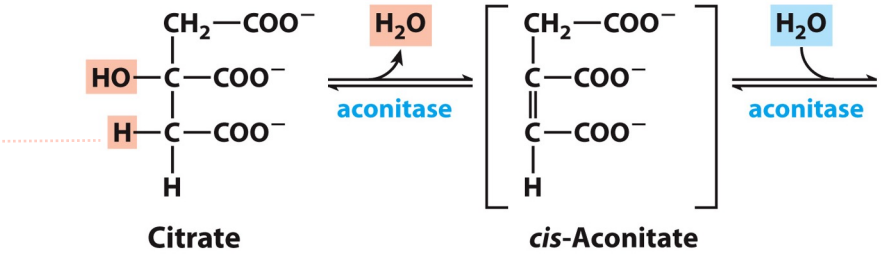
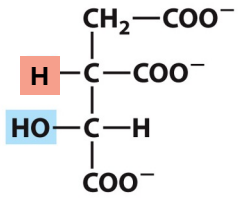
The Citric Acid Cycle: Aconitase

Isomerization by Dehydration/Rehydration



Citrate **cis-Aconitate**

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



Isocitrate

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

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The Citric Acid Cycle

Citrate Synthase ✓

Aconitase ✓

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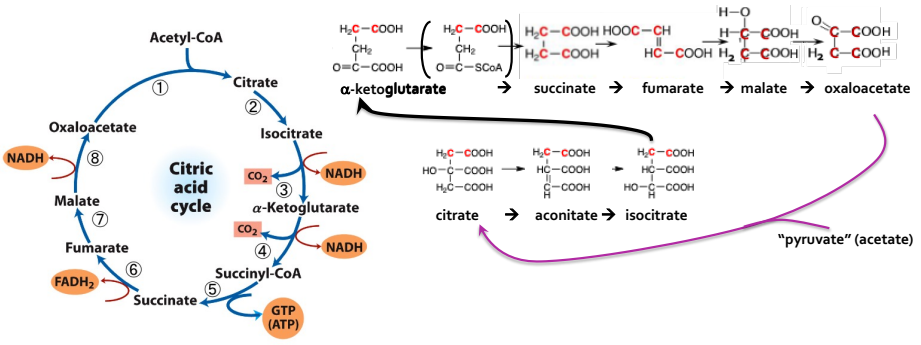
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH



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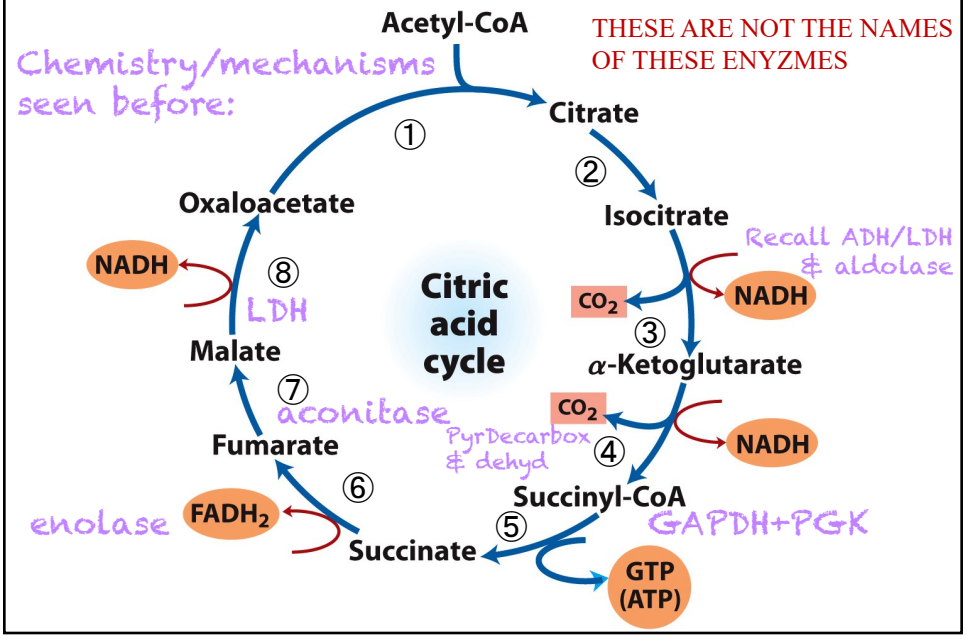
The Citric Acid Cycle



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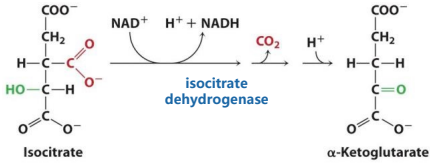
The Citric Acid Cycle: Isocitrate dehydrogenase



- 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 an 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)

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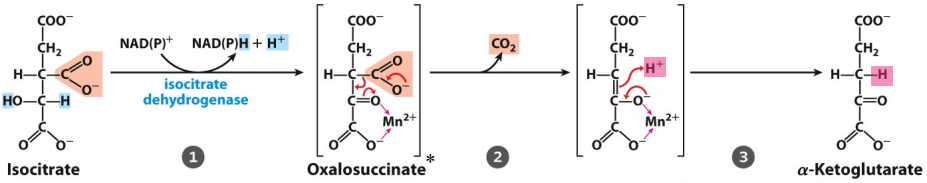
The Citric Acid Cycle: Isocitrate dehydrogenase

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

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

As in GAPDH, whereas we learned it in the opposite direction with LDH and ADH

*the "oxalo" is for the a-carbonyl to a carboxylate. Could call pyruvate, oxalomethane

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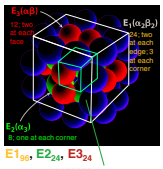
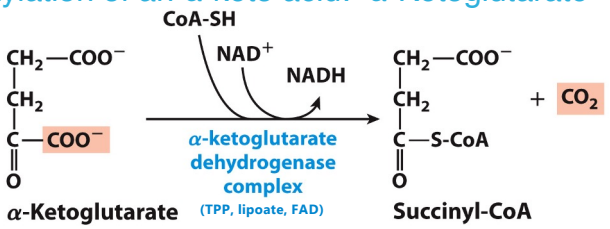
The Citric Acid Cycle: The α-Keto-Glutarate Dehydrogenase Complex

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?

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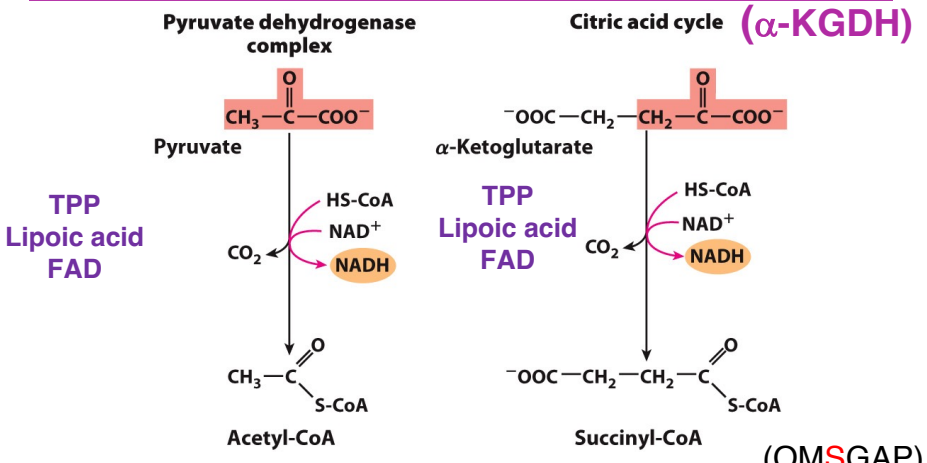
The Citric Acid Cycle: The α-Keto-Glutarate Dehydrogenase Complex



(OMSGAP)

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

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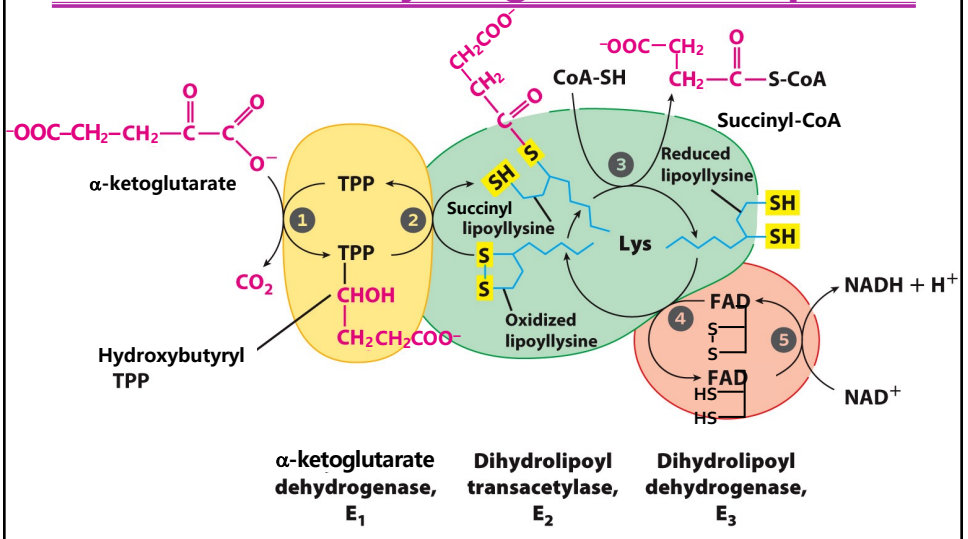
The Citric Acid Cycle: The α-Keto-Glutarate Dehydrogenase Complex



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The Citric Acid Cycle

Citrate Synthase ✓

Aconitase ✓
Isocitrate dehydrogenase ✓
α-ketoglutarate DHC

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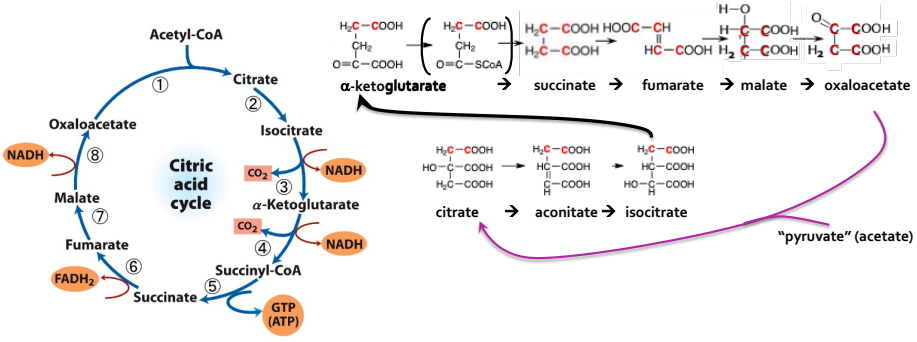

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Clinical Correlations Friday

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Clinical Correlations

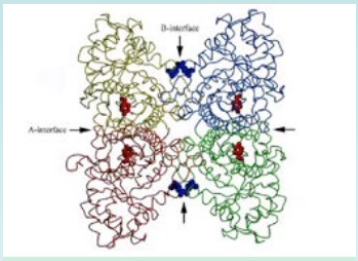
Glycolysis

Hemolytic Anemia: Deficiencies in Glycolytic Enzymes

Red-blood cells do not have nuclei or mitochondria. They still need to maintain their membrane potential using the Na/K pump and membrane shape using actin microfilaments. Both require ATP, which solely comes from glycolysis.

Any glycolytic enzyme that is impaired sufficiently will affect the efficiency of the entire pathway. Without ATP, the cells swell and lyse, which is a condition called **non-spherocytic hemolytic anemia**.

• For example, the precise defect in aldolase A that causes hemolytic anemia led to a deeper understanding of protein stability and cellular function.



The most common form is from a deficiency in pyruvate kinase. This form is more tolerable than a form from a deficiency of aldolase A. This is likely because there is a buildup of intermediates behind the block at PK, including 2,3-bisphosphoglycerate, the negative heterotropic allosteric effector of Hb. This causes Hb to release more O_2.

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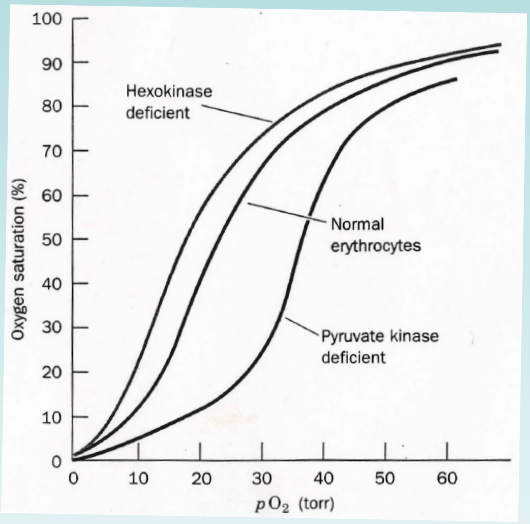
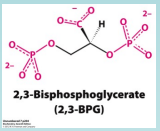
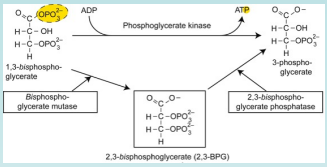
Clinical Correlations

Glycolysis

Hemoglobin Oxygen-binding curves







Recall that BPG is the key allosteric effector of Hb:

- If you do not make enough (HK deficiency), Hb behaves less cooperatively
- If you make too much (PK deficiency), HB behaves more cooperatively

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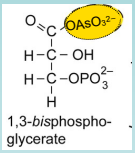
Clinical Correlations

Glycolysis

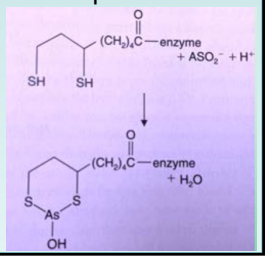


GAPDH: Arsenic poisoning and superoxide

Arsenate ($HAsO_4^{2-}$) can substitute for P_i in biochemical reactions. But the esters formed from arsenate are unstable and readily hydrolyze.
For the GAPDH reaction, the 1-arsenyl-2-phosphoglycerate degrades to 3-phosphoglycerate without production of ATP. Therefore, glycolysis does not net any ATP production.

Arsenite (AsO_2^-) is more toxic. It kills any enzyme that contains lipoic acid*:

Superoxide ($\bullet O_2^-$) overproduction can come from over-exposure to high blood glucose, especially in retina, kidney, and peripheral neurons.** This "reactive-oxygen species" (ROS) activates repair enzymes that end up modifying Cys residues in enzymes; thus, killing GAPDH.



*pyruvate dehydrogenase!
**early sites of damage from Type-2 diabetes

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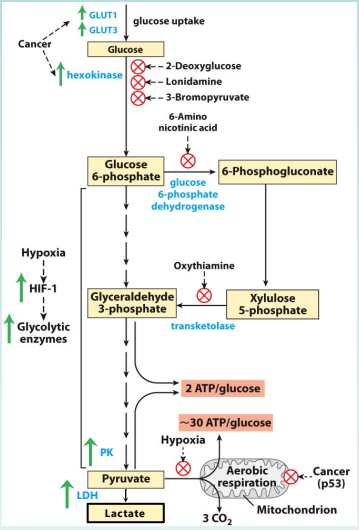
Clinical Correlations

Glycolysis Occurs at Elevated Rates
Warburg Effect in Tumor Cells



- Tumor cells often grow faster than angiogenesis allows growth of capillaries to aerate them, resulting in anaerobic metabolism (hypoxia).
- Tumor cells increased expression of LDH, and **many glycolytic enzymes (↑)**, for example an isozyme of pyruvate kinase (M2), a low activity embryonic form. Use to generate intermediates.
- Compounds that inhibit key steps in glycolysis (☒) can kill cancer cells by limiting energy and bio-mass production.
- In tumors, this Warburg effect is seen even without hypoxia.

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Clinical Correlations

Related to Fermentation – alcohol and aldehyde dehydrogenases

CLINICAL INSIGHT

Variations in K_M Can Have Physiological Consequences

Two enzymes play a key role in the metabolism of alcohol.

Humans' express alcohol dehydrogenase for ethanol metabolism, but is largely used in the reverse reaction, then *aldehyde dehydrogenase* takes it to acetate.

$$CH_3CH_2OH + NAD^+ \xrightleftharpoons{\text{Alcohol dehydrogenase}} CH_3CHO + NADH + H^+$$

Ethanol — Acetaldehyde

$$CH_3CHO + NAD^+ + H_2O \xrightleftharpoons{\text{Aldehyde dehydrogenase}} CH_3COO^- + NADH + 2H^+$$

- Some people respond to alcohol consumption with facial flushing and rapid heartbeat, symptoms caused by excessive amounts of acetaldehyde in the blood. There are two different acetaldehyde dehydrogenases in most people, one with a low K_M and one with a high K_M.
- The low K_M enzyme is genetically inactivated in some individuals. The enzyme with the high K_M cannot process all the acetaldehyde, and so some acetaldehyde persists in the blood causing vasodilation.

Knowing the values of the constants, K_m and V_{max}, for enzymes and their substrates is important.

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