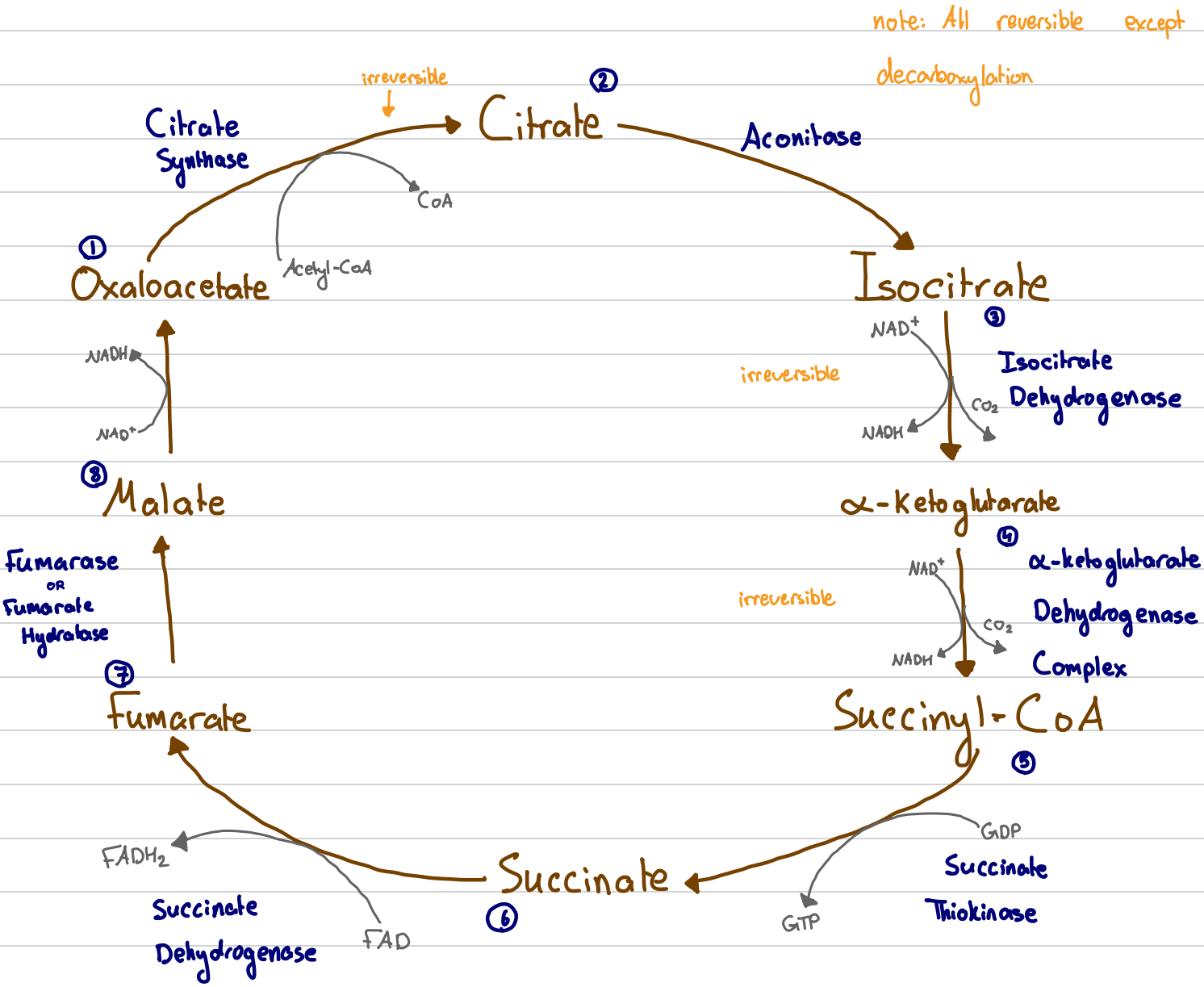
Krebs Cycle

General:

Mnemonic → Citrate Is Krebs Starting Substrate For Making Oxaloacetate

Products → 3 NADH + 1 GTP (GTP → ATP) + 1 $FADH_2$ + 2 CO_2

Total ATP = 3×(2.5) + 1×1 + 1×1.5 = 10



FAD vs NAD:

① → FAD gains e^- one by one, sequential. By different sources. form of H•

→ NAD gains pair of electrons at once by same source. form of hydride $\ddot{H}^-$

② → FAD used in succinate dehydrogenase.

→ FAD used in α-keto glutarate dehydrogenase complex, FAD oxidized disulfide bridge in Lipoic acid in Transacylase to form $FADH_2$ then transfers electrons to NAD^+

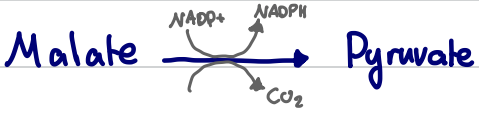
→ NAD used in most dehydrogenases. Oxidizes alcohol to ketones

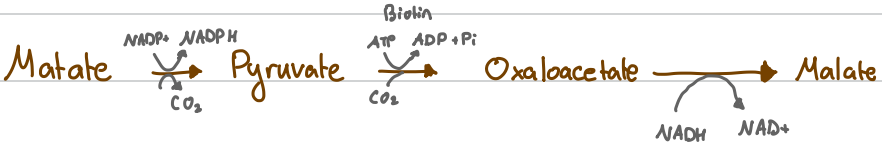
③ → Since FAD forms free radicals, if forms tight covalent bonds to enzymes. This changes the $E^\ominus$

→ NAD can be free

④ → NADH used in regulating krebs cycle, acting as inhibitor. While $FADH_2$ not used

⑤ → $NADP^+$ used to convert malate to pyruvate





Details of steps:

Step 1 :

Reaction: Oxaloacetate + Acetyl CoA ⟶ Citrate + CoASH

Enzyme: Citrate synthase

Regulation: Activation / Inhibition of enzyme

→ Activated by oxaloacetate which results in conformational shape change allowing Acetyl - CoA to bind to enzyme.

→ Inhibited by citrate

→ Inhibited by ATP allosterically

→ Inhibited by NADH

→ Inhibited by succinyl - CoA

Other regulations using citrate:

→ Inhibiting rate-limiting step of glycolysis. Inhibiting phosphofructokinase

→ In gluconeogenisis, it stimulates fructose-1,6-Bisphosphatase

→ Plays role in fatty acid synthesis

↳ Provides Acetyl CoA source

↳ Activates acetyl CoA carboxylase

↳ Inside mitochondria, fatty acid oxidized. Outside mitochondria fatty acid synthesized

↳ Citrate breaks down to Acetyl CoA + oxaloacetate. Oxaloacetate reduced to malate to cross mitochondrial membrane then oxidized again to oxaloacetate

Step 2:

Reaction: Citrate ⇌ Cis-aconitate ⇌ Iso-citrate

3° alcohol — 2° alcohol - isomerization

Enzyme: Aconitase

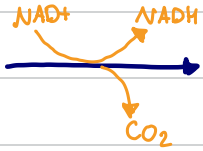
Regulation:

→ Inhibited by Fluoroacetate. Toxic, stops Krebs cycle. Non-competitive

→ Aconitase enzyme contains Fe-S clusters

→ isomerization RXN

Step 3:

Reaction: Isocitrate 

α-ketoglutarate

Enzyme: Isocitrate dehydrogenase

Regulation:

→ Dehydration + decarboxylation.

→ Rate-Limiting Step !! Best regulation

→ Inhibited by NADH

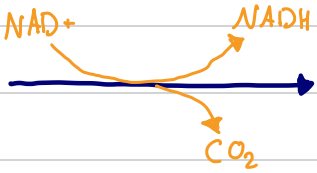
→ Inhibited by ATP

→ Activated allosterically by ADP

→ Activated allosterically by Ca^{2+} ⇒ shift to left. Lower km. Higher affinity

↳ muscle contraction ⇒ active ⇒ more ATP

Step 4:

Reaction: α-keto glutarate + CoA 

Succinyl CoA

Enzyme: α-ketoglutarate complex

Mechanism of α-keto acid dehydrogenase complexes:

→ Include α-ketoglutarate / pyruvate / branched chain keto acid DH complexes
(↳ alanine keto acid)

→ Three enzymes. 5 cofactors → (TPP) Take (Lipoic acid) Loving (CoA) Care (FAD) for (NAD) Nancy. TLCFN

① Decarboxylase (E1 /E2 /E3). Cofactors: Thiamin Pyrophosphate TPP

② Transacylase. Adds CoA and uses -S-S- to transfer H to FAD. Cofactors: Lipoic acid

③ Dehydrogenase. FAD takes H from Lipoic acid on transacylase then it transfers them to NAD+. NADH formed.

→ Mechanism:

1: CO_2 removed from α-kG. TPP used. Decarboxylase

2: -S-S- oxidize α-kG to form -SH + -SH. Lipoic acid used. Transacylase

3: CoA is added & Succinyl CoA released. Transacylase

4: FAD takes H from -SH & forms -S-S- again

5: FAD transfers H to NAD+ forming NADH

6: Energy conserved in NADH & thioester of CoA

Regulation:

→ Activated by Ca^{2+}

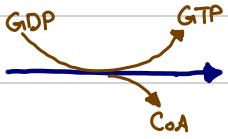
→ Inhibited by NADH

→ Inhibited by GTP

→ Inhibited by Succinyl - CoA

→ Inhibited by Arsenite. Toxic. Non-competitive

Step 5:

Reaction: Succinyl CoA 

Succinate

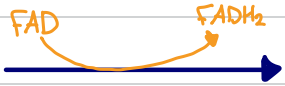
Enzyme: Succinate thiokinase

Mechanism:

* Cleavage of high energy thioester bond & using energy to add P_i to GDP via substrate level phosphorylation

* GTP + ADP ⇌ ATP + GDP via nucleoside (to working with sugar + base) diphosphate (to transfer of two P_i) kinase (to transfer of P_i)

Step 6:

Reaction: Succinate 

Fumarate

Enzyme: Succinate Dehydrogenase

Mechanism

→ Succinate Dehydrogenase only enzyme in inner membrane in Krebs cycle

→ FAD is used rather than NAD+ because FAD gets reduced more easily than NAD+. FAD larger E° value. NAD+ not enough to oxidize succinate.

→ Succinate Dehydrogenase = Complex II of ETC

Regulation:

* Inhibited by Malonate. Toxic. Competitive

Step 7:

Reaction: Fumarate $\xrightarrow{H_2O}$ Malate

Enzyme: Fumarase

Step 8:

Reaction: Malate $\xrightarrow{NAD^+ \rightarrow NADH}$ Oxaloacetate

Enzyme: Malate Dehydrogenase

Mechanism:

→ -OH on alcohol oxidized to keto group

→ ΔG° = positive. Reaction driven by step 1. Citric synthase. Which has ΔG° = -ve

Oxaloacetate Junctions:

* Glucose → Pyruvate
  Pyruvate + Carboxylase + Biotin
  Oxaloacetate

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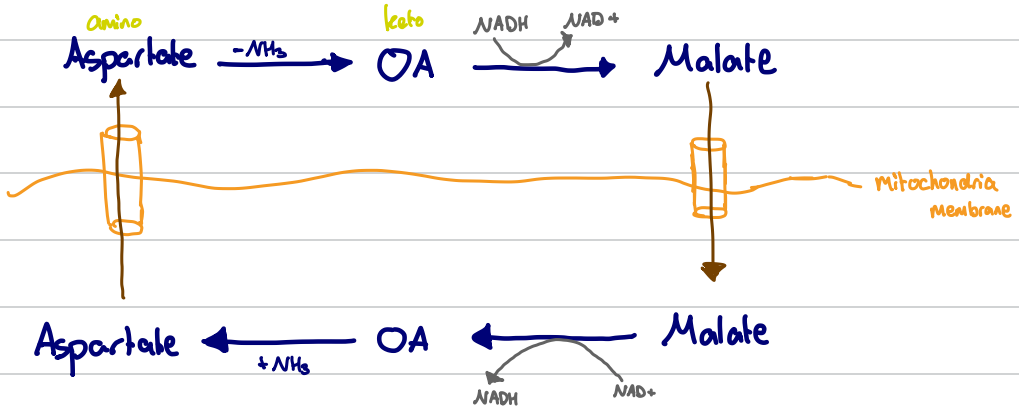
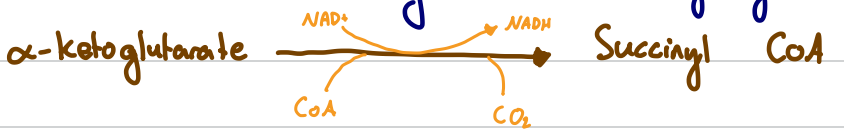


Toxins on the Krebs cycle:

* Flouroacetate: Aconitase non-competitive inhibitor

Citrate ⟶ Isocitrate

* Arsenite: α-ketoglutarate dehydrogenase non-competitive inhibitor



* Malonate: Succinate Dehydrogenase competitive inhibitor

Krebs Cycle intermediates used in other pathways:

* Citrate:

↳ fatty acid synthesis in liver

↳ Activates Acetyl CoA carboxylase

↳ Breaks down to form Acetyl CoA, building blood of fatty acids.

↳ Occurs in cytosol NOT mitochondria

* α-ketoglutarate:

↳ Converted to glutamate

↳ Forming GABA

↳ Neurotransmitter on NS

* α-ketoglutarate:

↳ Converted to glutamine

↳ Skeletal muscle & other tissues

↳ Protein synthesis

* Succinyl - CoA:
↳ Heme synthesis in bone marrow

* Malate:
↳ Increase blood glucose when fasting via gluconeogenisis
↳ In liver

* Oxaloacetate:
↳ Amino acid synthesis

Anaplerotic Reactions:

→ Since intermediates can be used, we need reactions that replenish those intermediates called anaplerotic reactions.

* Aspartate ⟶ Oxaloacetate

* Alanine ⟶ Pyruvate + HCO_3^- ⟶ Oxaloacetate !! imp

* Glutamate ⟶ α-Ketoglutarate

* Amino Acids ⟶ Propionyl CoA ⟶ Succinyl CoA

* Amino Acids ⟶ Fumarate

→ All anaplerotic reactions use amino acid except pyruvate to oxaloacetate

Pyruvate Carboxylase:

- → Carboxylation of pyruvate to form oxaloacetate. Anaplerotic reaction
- → Needs biotin Coenzyme
- → Activated by Co A
- → Found in kidneys + liver + brain + adipocytes + fibroblasts
- → High conc. in liver & kidney