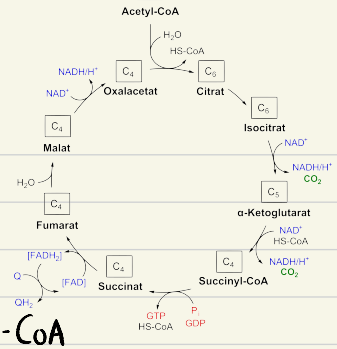
Krebs cycle



useful resources !!

https://youtu.be/rr7IRYLqleg?si=GmLP14ItG6ALltd3

https://www.khanacademy.org/science/biology/cellular-respiration-and-fermentation/pyruvate-oxidation-and-the-citric-acid-cycle/a/the-citric-acid-cycle

① Sources & uses of Acetyl-CoA

* TCA cycle starts with acetyl-CoA that react with last intermediate [oxaloacetate]

* Krebs cycle is a universal pathway

Sources : ① oxidation of carbohydrates ——→ Pyruvate ——→ Acetyl-CoA (Pyruvate dehydrogenase complex)

② degredation of protiens ——→ amino acids ——→ Acetyl-CoA

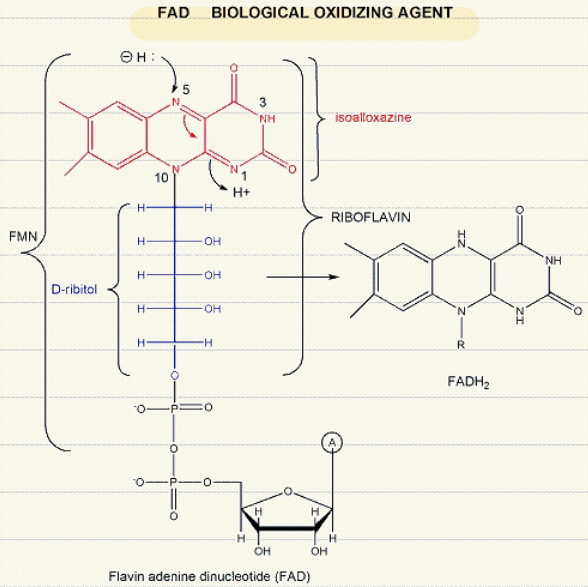
↓ Ketogenic : used to produce Ketone bodies

③ fatty acid oxidation: fatty acids are stored in adipose tissue as triacylglycerol → break down of triacylglycerol produces energy by hydrolyzing ester bonds between glycerol & fatty acids → fatty acids released in blood stream → oxidation of fatty acids produces acetyl-CoA

Uses of Acetyl CoA :

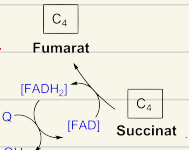
1. Oxidation in citric acid cycle
2. Produce fatty acid — note : synthesis & break down of fatty acids does not occur simultaneously!
3. Cholestrol & steriod compounds synthesis
4. Ketone body synthesis

FAD



* Single electrons (H·), different sources - one by one

* • Succinate to fumarate during krebs cycle
  • lipolate to lipolate disulfide



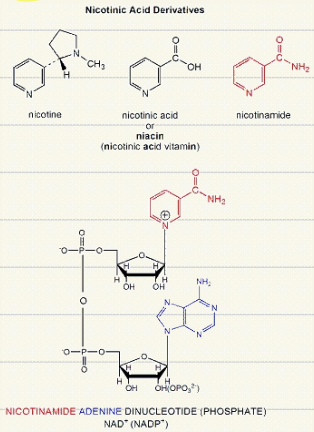
* lipoate to lipoate disulfide in α-keto gluterate

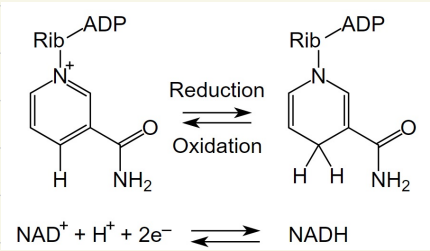
Explaination : lipoate a co-factor in α-KG dehydrogenase complex undergoes oxidation, FAD accepts electrons during this process → occurs when α-KG is oxidized to Succinyl-CoA

* FAD remains tightly bound to enzymes, sometimes even covalently

* E° for enzyme bound FAD varies

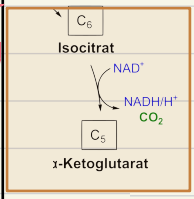
NAD



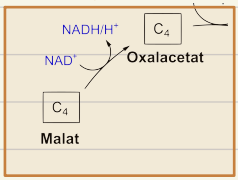


* pair of electrons (H-), same source

* Alcohols to ketons



isocitrate dehydrogenase

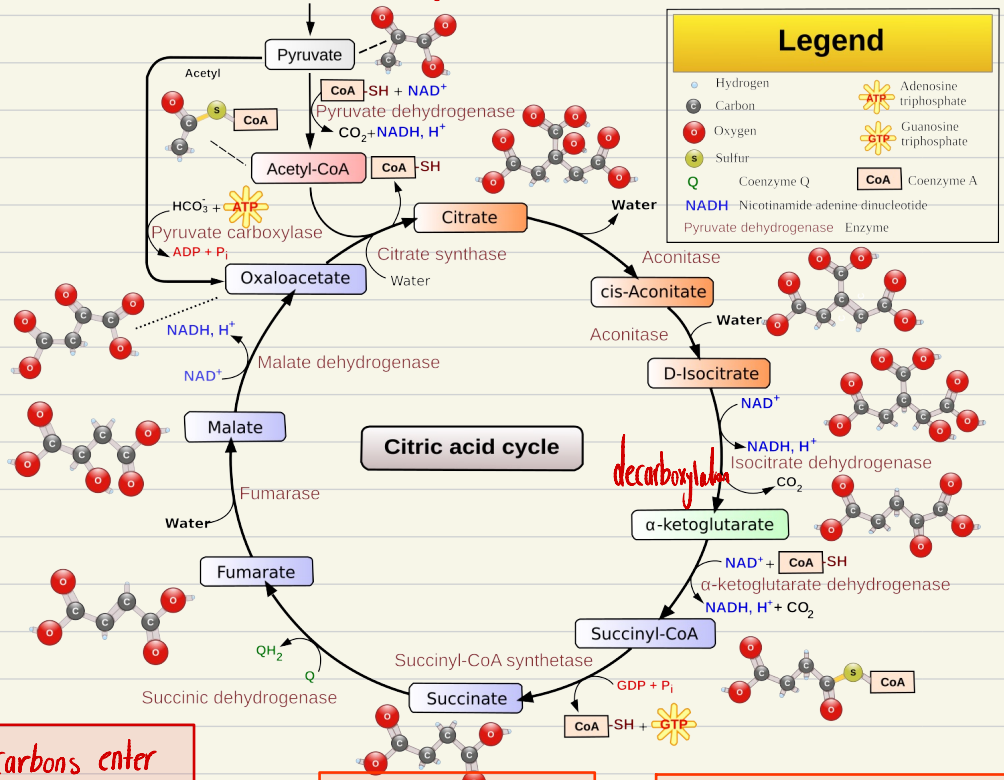


malate dehydrogenase

* NADH plays a regulatory role in balancing energy metabolism

Stepwise reaction

in TCA cycle, oxidation of main substrate & reduction of co-enzyme



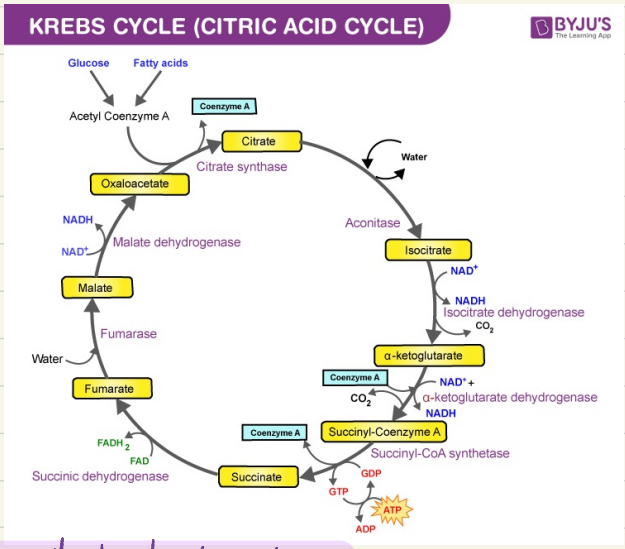
2 carbons enter as acetyl & 2 carbons leave as oxygen → by isocitrate dehydrogenase & α-ketoglutarate dehydrogenase

Krebs cycle contains catabolic & anabolic pathways

quick mnemonic for substrates

'Citrate (citrate) is (isocitrate) Krebs (α-ketoglutarate) Starting (Succinyl-CoA) substrate (succinate) For (fumarate) making (malate) oxaloacetate

Enzymes of TCA Cycle

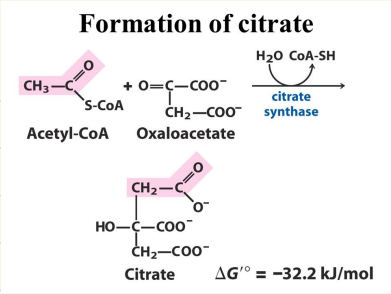


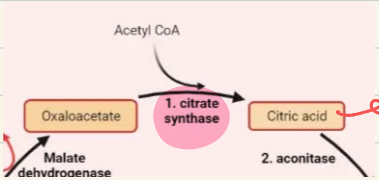
* Citrate Synthase - not an allosteric enzyme
* Aconitase
* Isocitrate dehydrogenase
* α-Ketogluterate dehydrogenase
* Succinate thioKinase / Succinyl CoA Synthetase
* Succinate dehydrogenase → found in inner mitochonderial membrane!!
* Fumrase
* Malate dehydrogenase

Note:

whenever there's dehydrogenase → There's a redox reaction

Step 1 : formation of citrate





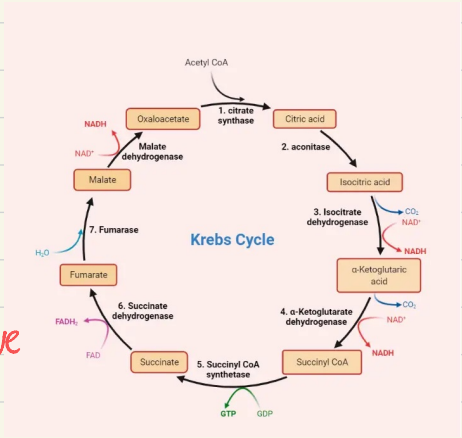
feedback ⊖

1. citrate synthase is inhibited by its product, citrate

2. substrate availability (oxaloacetate) is another way of regulation

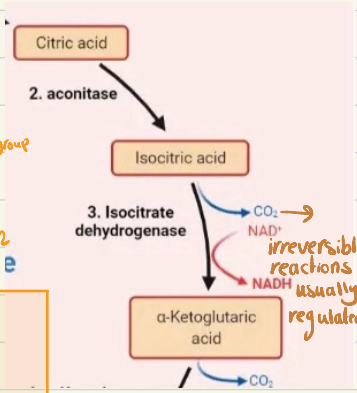
3. ↑↑ citrate ⟶ inhibits phosphofructokinase → we have alot of ATP no need for glucose degredation

4. binding of oxaloacetate causes a conformational change in co-enzyme that generates a binding site for acetyl-CoA



Note : Citrate can exit to cytosol & activate fatty acid synthesis by reproducing acetyl CoA

Step 2 & 3 : formation & oxidation of isocitrate



Step 2: isomerization of citrate into isocitrate (differs in location of hydroxyl group)

enzyme responsible is aconitase → fe-s protien

Note: Aconitase is inhibited by fluoroacetate that is used as a rat poison

irreversible reactions usually regulated

Step 3: conversion of isocitrate to α-ketoglutarate (3° to 2° alcohol)

enzyme: isocitrate dehydrogenase

Oxidative decarboxylation ⟶ irreversible !!!

Regulated

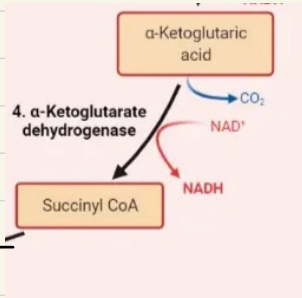
- Ca^{++} ⊕
- ADP ⊕
- ATP ⊖ NADH ⊖

Step 4: α-Ketogluterate to Succinyl CoA

Oxidative decarboxylation by α-Ketogluterate dehydrogenase complex → multimolecular aggregates of 3 enzymes

Coenzymes: Thiamin pyrophosphate (TPP), lipoic acid, FAD, NAD^+ & CoA

Energy conserved as NADH, thioester bond



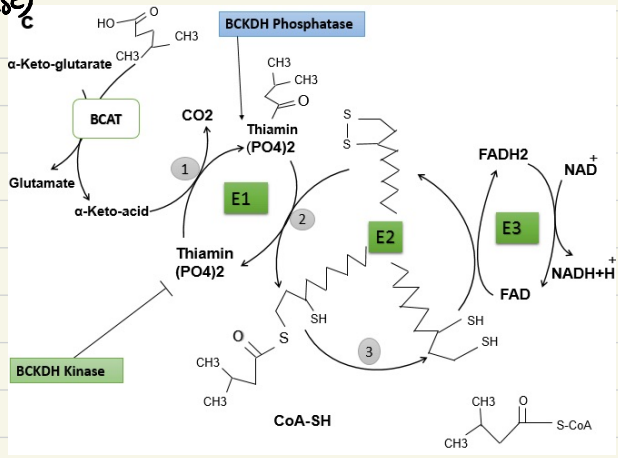
α-Ketoacid dehydrogenase complex

* α-ketogluterate, Pyruvate & branched chain α-Keto acid dehydrogenase Complexes
  - all of these complexes act on α-keto acids, which are molecules that have a keto group (C=O) next to a carboxyl group (COOH)
* Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E_2 & E_3 remain bound → higher rate)

* E_1 → α-Ketodehydrogenase → Catalyzes the initial decarboxylation (removal of CO_2) of the α-keto acid with the help of TPP
* E_2 → Dihydrolipoyl Transacylase → Transfers acyl group from the decarboxylated α-keto acid to (coenzyme A) forming a CoA derivative
* E_3 → Dihydrolipoyl dehydrogenase → Regenerates oxidized form of lipoic acid using FAD & transfers electrons to NAD^+ → NADH

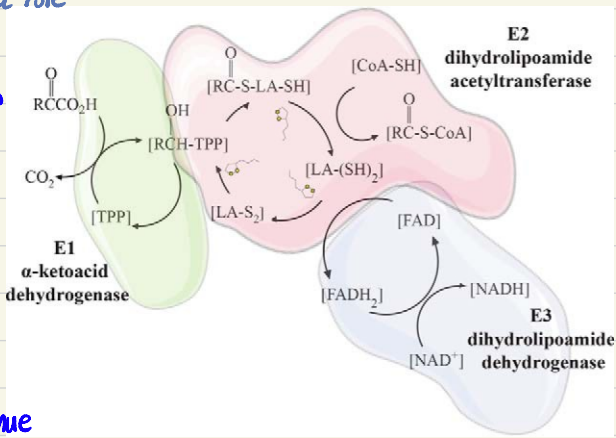
E1 (α-keto acid dehydrogenase) (decarboxylase)

decarboxylative of α-keto acid, utilizes cofactor TTP



E2 (dihydrolipoyl Transacylase)

E2 accepts the remaining acyl group after decarboxylation. it contains lipoic acid, prosthetic group that plays a central role in transfer of acyl group, acyl group transferred to co-enzyme A → forming acyl CoA derivatives ((Succinyl-CoA))



E3 (dihydrolipoyl dehydrogenase)

this enzyme regenrates the oxidized form of lipoic acid to ensure that reaction can continue

FAD is reduced to $FADH_2$ & electrons are passed to NAD^+ forming NADH

https://youtu.be/RYAl2dTjrQU?si=D7VVJFpk-sQO3nLh ← useful video!!

Thiamine pyrophosphate : Thiamine (vit B_1) deficiency → α-Ketoglutarate, Pyruvate & branch chain α-keto acid accumulate in the blood

Step 5: cleavage of Succinyl CoA & Generation of ATP

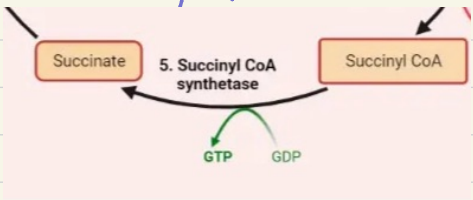
Succinate thiokinase (Succinyl CoA Synthetase) → Reversible step

Cleaves the high energy thioester bond of Succinyl COA

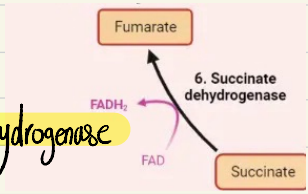
GTP is produced by Substrate level phosphorylation

GTP & ATP are energetically interconvertible by nucleoside diphosphate

$GTP + ADP \rightleftharpoons GDP + ATP$



Step 6 : Oxidation of Succinate



Succinate is oxidized to fumerate by succinate dehydrogenase

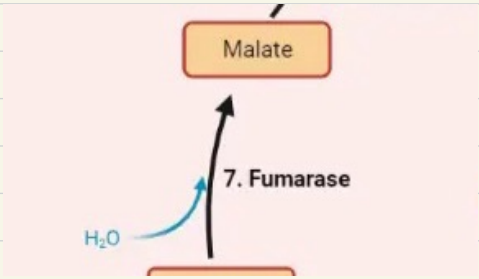
FAD —reduced→ $FADH_2$

reducing power of Succinate is not sufficient to reduce NAD^+

Note!! Succinate dehydrogenase is the only enzyme of TCA embeded in inner mitochondrial membrane

*S·D·H functions as complex II of ETC

Step 7 : Hydration of fumrate



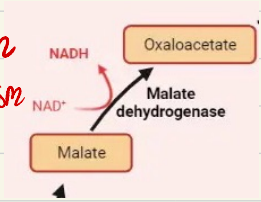
Fumrate is hydrated to malate by fumarate

Fumarate is hydrated to malate by fumarase

Fumarate hydratase

Reversible reaction ✓✓

Step 8: Oxidation of malate : important junction point in metabolism



Malate is oxidized to oxaloacetate by malate dehydrogenase

Alcohol group of malate oxidized to Keto group

* This reaction produces 3rd & final NADH of the cycle

* ΔG° of reaction is +ve. reaction is driven by highly exergonic citrate synthase reaction

D·H = dehydrogenase

Overall Summary

Step	Enzyme	Substrate	Product	Brief description	Reversible?
①	Citrate Synthase	Acetyl CoA + Oxaloacetate	Citrate	Acetyl-CoA combines with oxaloacetate to form citrate releasing CoA-SH	✗
②	Aconitase	Citrate	Isocitrate	Isomerization	✓
③	Isocitrate · D·H	Isocitrate	α-ketoglutarate + CO_2	decarboxylation	✗
④	α-Ketoglutarate D·H	α-Ketogluterate + CoA	Succinyl-CoA	decarboxylation	✗
⑤	Succinyl-CoA Synthetase	Succinyl CoA	Succinate + CoA + GTP	Substrate level Phosphorylation	✓
⑥	Succinate D·H	Succinate	Fumarate	Oxidation	✓
⑦	Fumarase	Fumarate	Malate	Hydration	✓
⑧	Malate · D·H	Malate	Oxaloacetate	Oxidation	✓