

Pyruvate Dehydrogenase Complex and Acetyl CoA

- Uses lots of when you cluster them together substrate channeling.
- Metabolism converges on acetyl-CoA.
- Steadily strip away fuel source.
- pyruvate → acetyl coa → fed into 2nd more electron stripped away citric acid cycle → electron carrier make more ATPs → oxidative phosphorylation stage 1 are anaerobic 2 and 3 aerobic ultimate electron acceptor is oxygen needed to complete reaction.
- 3 carbon pyruvate 2 carbon acetyl groups produce acetyl coa by many pathways (fatty acids) (amino acid) acetyl coa conversion point. Many pathways can be fed into this. Only acetyl coa can go into kreb cycle
- S
- Pyruvate metabolism occurs in the mitochondrial matrix.
- Take pyruvate takes it to pyruvate access it can access PDH
- Transportator recognizing pyruvate (MPC) mitochondrial pyruvate carrier
 - From cytosol → mitochondria
- Pyruvate is oxida
- Take carbon 1 remove it lose as CO₂ decarboxylation pyruvate oxidized to acetyl group covalent bound to CoA coenzyme A
- Overall delta G -33.4 exergonic irreversible
- Catalyzed by multiple enzymes but all part of the same protein complex
 - quaternary- many enzymes.
- Pyruvate dehy
 - Series of intermediate many coenzyme
 - FAD typically bound to enzyme during the course
 - NAD → nadh
 - Tpp
- Coenzyme A form
 - Adenine base ribose sugar
 - Additional phosphate group
 - Reactive thiol group
 - Whole time is inert other than reactive thiol group
 - Can form thiol ester group
- Reason why it is good at carrying thioesters formed with coa have high acyl transfer potential good substrate. High negative g
- S
- Lipoate undergoes reversible disulfide bond formation
- One end internal disulfide bond
- In enzyme covalently bound to end of lysine residue to form lipoamide
 - Disulfide reactive group
- Oxidized - cyclic

- Many forms reduced 2 free thiol group
- Acetylated form (transferred from lipoate)
- By having the lysine sidechain, create an arm (flexibility)
 - Similar to biotin.
- S
- Pyruvate dehydrogenase (complex) contains three enzymes
 - E1:
 - E2
 - E3
- Multiple copies associate together huge multisubunit protein complex under fairly low
- 500 angstrom
- Form this core 100 E1 in outer shell
- Core 20-30 E2/E3 enzymes
- E1 first reaction first interaction pass on to internal where it interact with the other 2 enzymes
- S
- Step 1: Decarboxylation of pyruvate involving TPP
- Break c-c bond between c1 and c2 → carbanion → stabilized by TPP → you get hydroxyethyl-tpp-e1 (end product of this step)
 - Covalent bounded to c2 carbon to tpp cofactor c1 lost as CO₂.
- s
- Step 2: oxidation of
 - Oxidize to acetyl group transferred from tpp to lipoamide
 - C1 attacks on of the sulfur and forms a covalent bond between c2 and sulfur → oxidation of hydroxyl group to ketone breaks tpp covalent bond.
 - Left with acetyl group covalent bonded to lipoamide
 - Happens Interface at e1(tpp) and e2 (lipoamide)
 - One hand to the other
- In addition to the transfer of group, hydroxyethyl group was oxidized to form acetyl group and the electron lost in oxidation is transferred to lipoamide acetyl for reduction
- S
- Step 3: transfer of acetyl group to Coa
 - To produce acetyl coa happens in e2
 - Coa group comes thiol attach thioester (with lipoamide) for thiol acetyl coa
 - Transesterification moving thioester from lipoamide to acetyl coa
 - Result in full reduction of lipoamide both are thiol form
- S
- Step 4 regeneration of lipoamide group by e3
- Reoxidize it so it can be recycle for other reactions. Step 2 most important
- Regeneration of lipoamide
- Transfer of electrons into carrier more useful to use: NADH
- Oxidize substrate to for lipoamide. In action site of e3- disulfide bond and FAD molecule irreversibly bound to enzyme.

- Disulfide in e3 exchange with lipoamide. Oxidation of lipoamide reduction of E3 disulfide
- Now we have regenerated lipoamide
 - Transfer of electron from e2 cofactor to e3 enzyme
- S
- Step 5: Transfer of electrons to NADH
- Want to something that can diffuse away.
- Start with thiol group, it donates the hydrogen atoms to FAD oxidized
 - So FAD⁺ becomes FADH transient
- FADH transfers its electrons to NAD⁺ reduction of of NAD⁺ and FADH to be reduced.
- S
- Disulfide bonds on E3 of cysteine
- The free thiol group can pass its electrons to FAD bc it is right it and FAD⁺ can pass its electrons to NAD⁺ also right there to produce NADH
- NAD sits on towards outside of the enzyme.

- Clicker
- C1 of pyruvate will be diffused away

- Lipoyllysine arm bridges E2→ E3
- Several enzymes to convert e2→ e3 have to get intermediate
- Lipoamide cofactor covalent bound to lysine sidechain.
- Same cofactor with E1 and E3
 - Flexibility of lipoamide arm that allows it to happen for it to complete its cycle. 50 Å movement. Same sets of groups.

- PDH complex is an example of substrate channeling
- Having enzyme cluster together problem. Multi enzyme complex
- One feed into another if you cluster than more efficient process rather than diffuse it out of cell blah blah local concentration high. Quick reactivity rate.
- You don't actually lose high energy intermediate.
- You don't want high energy intermediate to be dispersed.
 - Substrate channeling
 - One to the other active site
 - Formation of large complex
 - Permanent complex that dissociate together
 - *Some are transient complexes when function is not needed.*
 - *Many more transient complexes form dispersed than thought*
 - *Important*
 - *Driving force to cluster together is controlled by hydrophobic effect and phase separation by tweaking physical properties → formation of transient complex.*

Kreb Cycle

- Extract energy in acetyl coa during respiration as energy as possible
- Kreb cycle series of sequential oxidation reaction stripping electrons off of 2 carbon acetyl groups and they will be provided in electron carriers.
- Significant thermodynamic energy remain in acetyl-coa enter krebs cycle
- 8 different reactions. Not arbitrary. How some protein complex are similar to
 - Related by divergence
- Glycolysis captures a small amount of available energy
- Glycolysis liberate 146 energy
- Max energy extracted by
 - When glucose is converted to CO₂ most oxidized form
- Almost 2k amount of energy trapped in it.
- So far we haven't extract most of it.
- That's why other cycles exist.
- Second cycle (series of chemical transformation and they will go thru sequential oxidation and release electrons and passed on to a pool of reduced carriers (NAD or FAD) and we will use reduced carrier to produce atp at 3rd stage
- We will produce CO₂ at some point.
 - CO₂- Excrete most oxidized form so might as well bye bye
- End product- formation of oxaloacetate is the end product.
- Cycle- Oxaloacetate and be combined with acetyl-coa to form citrate.
- Chemical logic of Krebs Cycle
- Allows us to produce energy at a later state.
- There are a series of problems with the molecule and the solution is what is done to the molecule by various reaction
- Reaction 1:
- Problem unreactive methyl groups
- Solution combine acetyl with oxaloacetate to form methylene
- 2 carbon frag react with 4 carbon frag (oxaloacetate)
 - The only reaction that resolves in the formation of C-C bond
 - Results in hydrolysis of thioester
- Nucleophilic attack by methyl group of acetyl coa
 - How? By deprotonate methyl group → carbanion → attack the partial positive of ox covalent bond
- Really exergonic reaction and not all are energetically favored.
- Position of carbonyl group is crucial
- Merry go round parents pushing highly exergonic are the parents
- **Citrate synthase** exhibits induced fit binding

- 2 substrate acetyl coa and oxaloacetate
- Binds in obligated order
 - Oxa first then acetyl coa
 - Binding large conformational change to create binding site for acetyl coa through restructure of binding to ox
- Example of Uncompetitive inhibition → obligated order binding
- Measure of km
 - Competitive inhibitor to acetyl coa: if respect to acetyl coa then competitive if oxaloacetate then uncompetitive
- Reaction 2: Conversion of citrate to isocitrate
- Isomerization- reshuffle of electrons in citrate so the hydroxyl group can be readily oxidized
 - Problem: tertiary alcohol are not reactive as primary or secondary
 - Solution: Converted 3 → 2 order alcohol easier to be oxidized
- Performed by aconitase catalyze dehydration rehydration take off hydroxyl group from citrate and proton from adj carbon can lost as water molecule forms transition state cis-aconitase(double bonds) another water comes and added in the reverse position.
- Delta standard not energetically favorable (13.3)
- To happen keep isocitrate concentration low convert it to another reaction so it can be spontaneous
- **Aconitase** uses an Fe-S cluster
- 4 iron and 4 sulfur atom box
- Involved in isomerization
- Stabilize substrate they can interact with the negative charge in citrate hold it in place for it to be converted
- Good at accepting and giving back electron by altering oxidative state for it to be achieved more readily
- sensitive to oxidative stress
 - If Oxygen radicals accumulate and not neutralize then,
 - They will irreversibly reduce themselves → no longer bind to citrate
 - Absence of Aconitase activity good biomarker oxidative stress
- **Reaction 3: Oxidation of isocitrate by isocitrate DH→ alpha keto**
- Problem: Conserve energy from oxidation
- Solution: Reduce NADH or DADPH
- Oxidative decarboxylation both
- Carboxylation lost as co2 and hydrogen atoms when alcohol is converted into ketone is transfered to electron carrier (NAD(P) → nad(p)h) NAD+ usually in kreb
- Oxidizes alcohol from this to another alcohol move from this carbon position to this other last step so it would be more readily.

- Reaction 4: Oxidation of alpha ketoglutarate to succinyl-coa
- Problem: conserve energy from oxidation
- Solution: Reduce NADH or NADPH
- Oxidative decarboxylation reaction formation of high energy thioester succinyl group covalently bonded to the carbonyl carbon.
- 3 enzyme complex- uses 3 enzyme 5 cofactors
 - **It's like the PDH complex**
- Alpha ketog dehydrogen complex organization and cofactor identical E3 is the same enzyme catalyzing the same enzyme.

- Divergent evolution in oxidative decarboxylation
 - Similar enzyme
 - Same reacting group (shorter or longer with different number of carbon)
 - Evolve from common ancestor
 - few mutation to allow better fit (e.g. pyruvate to fit best in PDH)

- Reaction 5: Conversion of succinyl-coa to succinate
- Take high energy thioester take energy out and put it to GTP
- Breaking down the s-coa
- Substrate level phosphorylation (mechanism)
 - Succinyl-coa synthetase has a conserved His sidechain
 - Succinyl-coa bind to active site
 - Interact with inorganic phosphate→ attack and replace the coa
 - Retain the energy by broken down thioester bc replacement
 - Phosphorylated intermediate
 - Give phosphorylate group to His
 - succinate will bye bye high energy is transferred to His
 - His phosphorylated and give it to GDP to make GTP.
 - GTP can be converted to ATP→ Only atp generated.

- Reaction 6: Oxidation of succinate to fumarate
 - Challenge: Conserve energy from oxidation
 - Solution: Reduce FAD
 - Remove the hydrogens of succinate to fumarate
 - By succinate DH
 - Electron given to FAD→ FADH₂
 - In this this FAD covalently bound to the enzyme after it is transferred those hydrogen remain covalently bound. SDH inserted in mitochondrial in membrane.
 - Plug its electron to electron transport.
 - Extremely potent inhibit: malonate competitive inhibitor of succinate
 - Both have negative carboxylate groups

- CDH recognize by the 2 negative with it positive sidechain
- Stuck there → stop reaction

- Hydration of fumarate to malate
 - Problem: C=C double bond resists further oxidation
 - We want to remove double bond and add something more accessible
 - Solution: Add water to double bond.
 - There's a carbanion intermediate
 - Enzyme fumarase → reversible maintain concentration balance
 - Known for its stereospecificity.
 - The active site is tailor will interact with fumarate in the trans position and not maleate in the cis conformation
- Reaction 8: oxidation of malate to oxaloacetate
- Challenge: Conserve energy from oxidation
- Solution: Reduce NAD^+
- Another dehydration
 - Previously added alcohol forms a ketone
- Hydrogen → electron carrier NADH
- Enzyme: **Malate dehydrogenase**
- Oxaloacetate participates in the next kreb cycle. Cycle
- Delta g 30 Highly endergonic reaction
- Go through a lot of effort for it to be forward direction. The only way for it to be spontaneous is for oxaloacetate to be extremely low.
 - You get large gradient to have an effect on delta G make it endergonic

Kreb Cycle II

- In place to provide advantage they can provide energy and intermediate.
- Source of biomolecules to go into other pathways.

- 2) Citrate 6 carbon citrate go thru transform to strip away electrons from acetyl group
 - Not go to be oxidized.
- 3) Ox decarboxylation NADH and CO₂
- 4) Followed by another one → succinyl coa
- 5) Create gtp → atp when succinyl → succinate (4c)
- 6) succinate → fumarate reduced FADH
- 7) Hydrate fumarate convert to malate
- 8) Malate → oxalate another NADH
- Ox fed into another
- Reduced electron carrier
- Shoot electron out taken by electron carrier
- Net gain 2CO₂+3NADH+FADH₂+GTP(ATP)+COA+3H⁺
 - Single turn of kreb cycle
 - 6 carbon glucose → 2 pyruvate → 2 acetyl coa
 - Per glucose double net gain

- Where carbon come from 2 carbon unit a series
 - 2 carbon acetyl coa form carbon 1 and 2 of citrate
 - Stayed as carbon 1 and 2 throughout the kreb cycle.
 - A take a second cycle for it to be lost will turn into 4 carbon oxaloacetate
 - You need two turns.
- How much energy is being reduced.

- Reduced electron carrier will create most of the ATP
- Glycolysis 150 ish
- Total 3000 kreb cycle 1000 liberated by glucose and glycolysis.
- You pay for this and that you only get so many spare money for yourself opposed to someone who is single.

- Acetyl-coa oxidation is regulated at multiple points
- Point of regulation
- In order to initiate kreb you have to transporter to mitochondria
 - MPC-bottleneck for pyruvate to pass
 - You can control the flux of pyruvate
 - Formed of different subunits either subunit 1+2/1+3.
- Under process fermentation and not aerobic
 - Expression that express 1+2 is turned on (2 is inactive mitochondria carrier)
 - Turn on 2 assembly transport is inactive pyruvate can no longer pass or diminish
 - Want to turn on aerobic 3 is turned on very active pyruvate transport channel.

- Regulated by gene expression.
- Regulation at PDH complex.
 - Pyruvate to acetyl coa regulated by phosphorylation.
 - E1: 1st step it can exist in 2 form
 - Serine sidechain
 - serine-(hydroxyl) dephosphorylated, this enzyme is active pyruvate can be converted to acetyl coa
 - Phosphorylated by PD kinase phosphorylate the serine sidechain shut off conversion of
 - PD phosphatase balance
 - How much flux goes to PD allosteric regulated by products or substrate of the PDH complex. A
 - Accumulate acetyl coa activate
 - Accumulate pyruvate - neg regulator of PD kinase more act phospho dephosp active e1 enzyme
 - **If you want more acetyl coa you want no PD kinase because PD kinase is phosphorylated PDH complex and turns it off.**
 - **How to turn on pyruvate → acetyl coa, pyruvate negative inhibitor of PD kinase, why? Because a negative (inhibitor to PD kinase) and negative (enzyme inhibits reaction) is positive (yes reaction!).**
 - **Phosphorylated PD kinase NO acetyl coa**
 - **Dephosphorylated PD kinase YES acetyl coa**
- Regulation of krebs cycle
- Substrate available
 - Maintain acetyl coa low (no glucose) citrate activity decrease decrease citrate
- Product inhibition
 - A bunch of NADH equilibrium towards reactants limit reaction rate
- Allosteric modulation
 - Green are product activator
 - Red are inhibitor
 - Inhibit earlier step
 - Citrate synthase upregulated by presence of ADP bind to citrate make it more active enzyme ADP accumulate you want more ATP turn on flux of the Krebs Cycle.
- PFK-1 has multiple allosteric regulators
 - High citrate you don't need funnel more glucose.
 - Negative feedback loop
- Krebs Cycle is an amphibolic pathway
 - Able of anabolic and catabolic generating intermediate can be fed into other pathways.
 - Biosynthetic
 - Inject intermediate into krebs cycle
 - Cataplerotic: Take intermediate from krebs cycle

- Taking oxaloacetate and plug it to get PEP (gluconeogenesis)
- Taking intermediate and funneling it elsewhere
- Aspartyl aminotransferase
- Taking pyruvate and aspartate to get alanine and oxaloacetate

- Anaplerotic

- Blue cataplerotic
- Red anaplerotic
- Replenish kreb cycle away
 - Reaction that feed in extra intermediate
 - Take 4 carbon molecule cost some energy to perform these reaction
 - Pyruvate carboxylase react with bicarbonate +ATP → oxaloacetate

- A mutation in isocitrate DH leads to gliomass