Notes 11/27

Tuesday, November 27, 2007
10:02 AM

Notes 1127

Audio recording started: 10:02 AM Tuesday, November 27, 2007

The Citric Acid Cycle Part II

Nov. 27, 2007

Overview

- Frequently asked questions
- Regulation of TCA Cycle Enzymes
- Carbon tracing (keeping track of carbons)

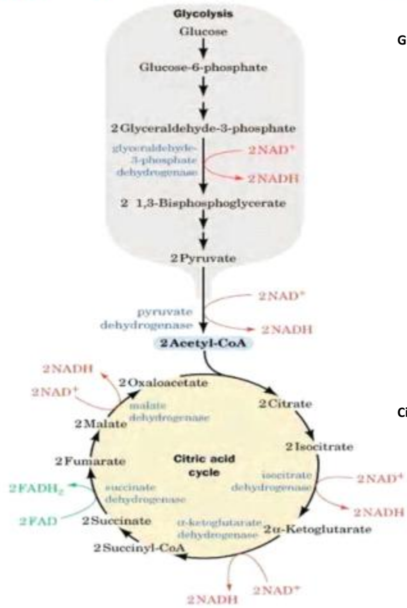
Do I need to know…?

- Understand $\Delta G^{o\prime}$ and ΔG values and what they mean Generally large negative delta G reacton will be regulated
- Understand mechanisms including the intermediates
- Understand arrow pushing

Why is Fructose-1,6-bisphosphate a deinhibitor of phosphofructokinase?

- The positive effect of F1,6B on PFK activity is well documented, but I don't know how it induces activity.

How is glycolysis and the TCA cycle linked?



Glycolysis:

- Anaerobic Pathway
- Creates Pyruvate from Glucose
  Pyruvate passed from cytosol into mitochondria.
- Glycolysis Mnemonic: "oodness Gracious, Friendly Franklin Did Great By Picking Pumpkins (to) Prepare Pies"
  - Glucose
  - Glucose-6-phosphate
  - Fructose-6-phosphate
  - Fructose-1,6-bisphosphate
  - DHAP
  - Glyceraldehyde 3-P
  - 1,3-Bisphospholycerate
  - 3-Phosphoglycerate
  - 2-Phosphoglycerate (to)
  - Phosphoenolpyruvate (PEP)
  - Pyruvate

Citric Acid Cycle

- Reduces carbons
- Pyruvate converted to acetyl-CoA to start citric acid cycle.
- Mighty OAK = Mitochondria Oxaloacetate and Acetyl-CoA

NAD+ and FAD

- Why is one charged and the other isn't?
- $NAD^+ + H^+ + 2e^-$ <--> NADH
- $FAD + 2H^+ + 2e^-$ <--> $FADH_2$

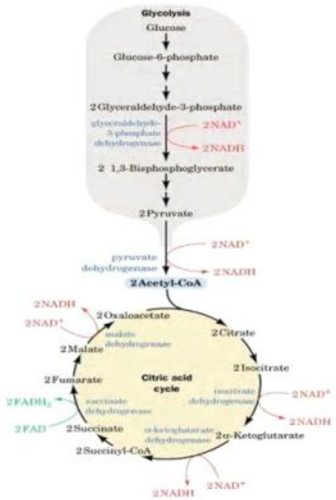
NAD^+ is oxidized state
FAD is oxidized state
  Where is the charge for FAD? FAD**H_2** balances charge

Net for glycolysis and TCA cycle

$$\text{Glucose} + 2\,H_2O + 10\,NAD^+ + 2\,[FAD] + 4\,ADP + 4\,P_i \rightleftharpoons 6\,CO_2 + 10\,NADH + 10\,H^+ + 2\,[FADH_2] + 4\,ATP$$

- Why is there a net of 10 NAD+?

2 NADH from glycolysis
2x4 from Citric Acid Cycle
Pyruvate to acetyl CoA = 2 more
Total: 10 NADH



Why are some kinases named after the reverse reaction?

a) Biochemists around the world have plotted against intro biochem students
b) Biochemists first discovered the reverse reaction
c) Kinase is so much fun to say

Kinases

enzymes that transfer a phosphoryl group from a nucleoside triphosphate to an acceptor molecule

catalyze a phosphorylation rxn

E.g. Pyruvate kinase
PEP + ADP --> pyruvate + ATP

How do we classify all these new enzymes we have learned?

Class	Subclass	Reaction
Oxidoreductase	dehydrogenase	Transfer of a hydride ion
	oxidase	O_2 is the electron acceptor
	oxygenase	O_2 is directly incorporated into the substrate
	reductase	Electron transfer between any 2 compounds
Transferase	kinase	Transfer of phosphoryl group(s) between a nucleoside triphosphate and another compound
	aminotransferase, transaminase	Transfer of an amino group between compounds
Hydrolase	phosphatase	Hydrolysis of a single bond between a compound and a phosphoryl group to generate P_i
	peptidase, protease, proteinase	Hydrolysis of a peptide bond
	glycosidase	Hydrolysis of a glycosidic bond
Lyase	synthase	A compound with a double bond combines with another compound to make a new compound that has a single bond
Isomerase	mutase	Intramolecular rearrangement of a functional group from one position to another
Ligase	synthetase	Formation of a new bond at the expense of ATP (GTP) hydrolysis

Anotehr mnemoic
- Cofactors of pyruvate DH and a-KG DH complexes
  - Tender loving Care for Nobody
  - Tiamine pyrophosphate, lipoic acid, CoA, FAD, NAD

End of FAQ

- Now it's time for a deeper

- Cofactors of pyruvate DH and a-KG DH complexes
  - Tender loving Care for Nobody
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End of FAQ

- Now it's time for a deeper understanding of the TCA cycle.
  - More detail about some of steps
  - Regulation of the cycle

More Detail About Some of the TCA Cycle Steps

Reaction	Enzyme	$\Delta G^{\circ\prime}$ $(kJ \cdot mol^{-1})$	ΔG $(kJ \cdot mol^{-1})$
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase multienzyme complex	−33	Negative
5	Succinyl-CoA synthetase	−2.1	~0
6	Succinate dehydrogenase	+6	~0
7	Fumarase	−3.4	~0
8	Malate dehydrogenase	+29.7	~0

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- Why does succinate DH use FAD instead of NAD+?

FAD is oxidizes alkanes ot alkenes, wheras NAD^+ oxidizes alcohols to aldehydes or ketons. Oxidation of alkane to alkene is sifficiently exergonic to reduce FAD to $FADH_2$ but not to reduce NAD^+ to NADH.

- Why is the malate DH rxn reversible but its DGº' value is a large positive number?

Because it forms oxaloacetate which combines with acetyl-CoA with a more exergonic reaction at -31.5kJ/mol

- What happens to the NADHs and $FADH_2$s?

Go onto electron transport chain and oxidative phosphorylation.

Regulation of TCA Cycle Enzymes

Reaction	Enzyme	$\Delta G^{\circ\prime}$ ($kJ \cdot mol^{-1}$)	ΔG ($kJ \cdot mol^{-1}$)
1	Citrate synthase	−31.5	Negative
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- What enzymes are regulated?
- What is the RDS?
- Positive and negative effectors?

3 Regulated Steps in TCA:
- Steps 1,3,4

Reaction 1:
- RDS
- Regulated step

Rxn 1: First Regulated Step

Reaction	Enzyme	$\Delta G^{\circ\prime}$ ($kJ \cdot mol^{-1}$)	ΔG ($kJ \cdot mol^{-1}$)
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
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- Rxn 1
- RDS
- Highly spontaneous
- Enough energy to make ATP but it doesn't
- Citrate is symmetric but also prochiral

  Citric synthase - oxalacetate and acetyl-CoA to citrate
  Prochiral - can distinguish one end of molecule from the other
- Positive effectors:
  - ADP
- Negative effectors
  - ATP, NADH, Citrate, Succinyl-CoA
- Acetyl CoA and OAA availability also effect the rxn

Aconitase:
Citrate becomes isocitrate

Rxn 2

Reaction	Enzyme	$\Delta G^{\circ\prime}$ ($kJ \cdot mol^{-1}$)	ΔG ($kJ \cdot mol^{-1}$)
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase multienzyme complex	−33	Negative
5	Succinyl-CoA synthetase	−2.1	~0
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- Rxn 2
- Reversible
- Not significantly regulated
- At equilibrium 91% citrate, 6% cis-aconitate, 3% isocitrate
- Stereospecific rxn
- Isocitrate is chiral

Rxn 3: Second regulated step

Reaction	Enzyme	$\Delta G^{\circ\prime}$ ($kJ \cdot mol^{-1}$)	ΔG ($kJ \cdot mol^{-1}$)
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase multienzyme complex	−33	Negative
5	Succinyl-CoA synthetase	−2.1	~0
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- Rxn 3
- Regulated rxn
- Positive effectors
  - ADP, NAD+, Ca^{2+}
- Negative effectors
  - ATP, NADH
- In *E. coli*, reversible phosphorylation inhibits isocitrate binding

Ca^{2+} stimulates glycogen breakdown. Glycogen is storage molecule of glucose in animals.

Rxn 4: Last regulated step

Alpha-ketogluterate dehydrogenase complex

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})	ΔG (kJ · mol^{-1})
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase multienzyme complex	−33	Negative
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- Rxn 4
- E_3 is identical between PDH complex and α-KG DH complex
- Last regulated step of cycle
- Positive effectors
  - Ca^{2+}
- Negative effectors
  - ATP, NADH, Succinyl-CoA
- There rest of the rxns are not significantly regulated

Rxn 6: Why is a covalently linked FAD used?

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})	ΔG (kJ · mol^{-1})
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase multienzyme complex	−33	Negative
5	Succinyl-CoA synthetase	−2.1	~0
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- The oxidation of an alkane to alkene is exergonic enough to reduce FAD, but not NAD+
- Covalent linkage of FAD to s~~uccinyl-CoA~~ succinate dehydrogenase ~~synthetase~~ provides a more favorable microenvironment for its reduction
- NAD+ funx to oxidize alcohols to aldehydes or ketones

Rxn 8: How does it go in the forward direction despite a large positive ΔG°'?

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Reaction	Enzyme	$\Delta G^{\circ\prime}$ ($kJ \cdot mol^{-1}$)	ΔG ($kJ \cdot mol^{-1}$)
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase multienzyme complex	−33	Negative
5	Succinyl-CoA synthetase	−2.1	~0
6	Succinate dehydrogenase	+6	~0
7	Fumarase	−3.4	~0
8	Malate dehydrogenase	+29.7	~0

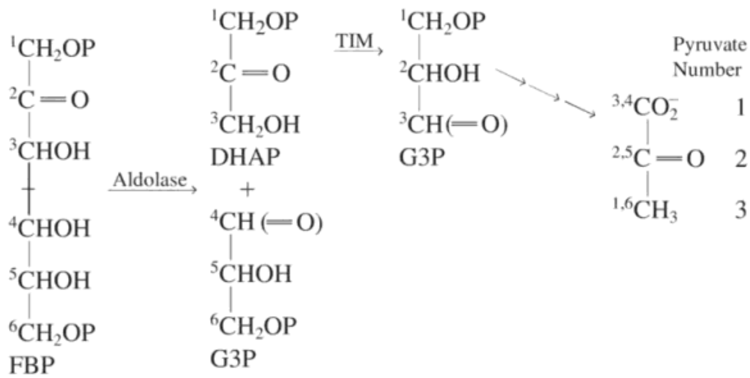
- The energy of rxn 1 is used to drive rxn 8

Carbon Tracing

GLUCOSE TO PYRUVATE

Glucose
(O═)CH–CH(OH)–CH(OH)– | –CH(OH)–CH(OH)–CH_2(OH)
1 2 3 4 5 6

	1,6 2,5 3,4	Glucose numbers
Pyruvate	CH_3—C(═O)—CO_2^-	
	3 2 1	Pyruvate numbers
	1,6 2,5 3,4	Glucose numbers
Acetyl-CoA	CH_3—C(═O)—ScoA + CO_2	
	2 1	Acetyl-CoA numbers



TCA CYCLE

The two carbons entering from acetyl-CoA do not leave as CO_2 on the first cycle.

Carbons 2 and 3 of succinate are equivalent.

Carbons 1 and 4 of succinate are equivalent.