THE KREBS CITRIC ACID CYCLE

Ppt presentation is on the web at: http://www.medschool.lsumc.edu/bioc/courses/Dental/

Cellular Respiration can be divided into 3 stages:
Stage 1: Oxidation of organic fuel polymers to generate and absorb monomers. FATS, PROTEINS, POLYSACCHARIDES are broken-down into monomeric components—fatty acids, amino acids, and monosaccharides.
Stage 2: Fatty acids, amino acids, and monosaccharides are oxidized to form acetyl CoA.
Stage 3: Aerobic Metabolism: Acetyl CoA (2C fragments) is fed into the citric acid cycle (Krebs cycle or TCA [**tri**<u>**c**</u>arboxcylic **a**cid cycle]) and oxidized. (GTP / ATP), NADH $FADH_2$ and CO_2 are produced. The second step of stage 3 metabolism includes oxidative phosphorylation; electrons from H atoms carried by NADH & $FADH_2$ enter the electron transport chain and generate ATP.

Transition Rxn: Oxidation of Pyruvate to Acetyl-CoA and CO_2 (Fig. 8-2, Fig. 8-3) **by the Pyruvate Dehydrogenase Complex:**
-oxidative decarboxylation, irreversible, exergonic (Principles of Bioenergetics, #6)
-multienzyme complex catalyzes a series of interrelated reactions; complex keeps all necessary cofactors and intermediates in close proximity
-complex contains 3 distinct 3 enzymes (E_1, E_2 & E_3), 5 different cofactors (TPP, FAD, CoA, NAD^+ & lipoic acid) & 2 major regulatory sites/enzymes.
E_1 is Pyruvate decarboxylase
E_2 is dihydrolipoyl transacetylase
E_3 is dihydrolipoyl dehydrogenase
-Oxidation-Reduction Reactions:
1. decarboxylation: the keto-carbon of pyruvate reacts with TPP on E_1 resulting in the loss of CO_2 & the formation of hydroxyethyl-TPP (exergonic)
a. The reactive site of TPP is a thiazolium ring.
2. TPP of E_1 transfers the acetyl group to the oxidized disulfide of lipoamide on E_2, forming a high energy acetyl thioester on E_2.
a. Lipoic acid is attached through an amide bond to a lysine sidechain of E2. This lipoyl group acts as a carrier of both Hydrogen and Acetyl groups.
3. The acetyl group is transferred from the thiol of lipoamide to the thiol of Coenzyme A to form acetyl-CoA in a transthiolation that is isoergonic and leaves lipoamide in a fully reduced state.
4. Reduced lipoate on E_2 is oxidized to a disulfide by E_3 and two hydrogen atoms (electrons) are transferred to FAD to make $FADH_2$.
5. E_3-$FADH_2$ is oxidized by NAD to yield 1 molecule of $NADH^+ + H^+ + E_3$-FAD

Regulation of E_1 (Pyruvate Decarboxylase part of the Pyruvate Dehydrogenase Complex (PDH)):
-phosphorylation/dephosphorylation: Phosphorylation of E_1 by PDH kinase inhibits the rxn. This kinase is activated by NADH and acetyl CoA.
-Free Ca^{2+} acts as a messenger. When ATP concentrations are low, Ca^{2+} activates the dephosphorylation of E_1 by phosphopyruvate dehydrogenase phosphatase. This activates PDH.
-Both of these enzymes (PDH kinase & PDH phosphatase) reside in the PDH multi-enzyme complex
-ATP, NADH and acetyl CoA also serve as allosteric inhibitors enzymes in the PDH complex

KREBS/TCA CYCLE:
-Pyruvate generated in cytosol enters mitochondria & is metabolized by the enzymes that make up the cycle. These enzymes are located in the mitochondrial matrix (Fig 9.1, p121)
-Pathway is amphibolic meaning that it is both catabolic and anabolic
-Krebs cycle intermediates all are either di- or tri- carboxylic acids.

Net Reaction of the Krebs cycle:
$$\text{Acetyl-CoA} + 3\ NAD^+ + FAD + GDP + P_i + 2\ H_20 \rightarrow 2\ CO_2 + 3\ NADH + H^+ + FADH_2 + GTP + CoA$$

ENZYMES & REACTIONS OF THE KREBS CYCLE- oxidation of acetyl CoA (2C) has 8 distinct enzymatic steps:
1. Citrate synthase
-formation of citrate from acetyl CoA & oxaloacetate via condensation reaction

-irreversible, exergonic
-hydrolysis of thioester following condensation drives the reaction forward.

2. Aconitase
   -reversible isomerization reaction, isoergonic conversion of citrate to isocitrate
3. Isocitrate dehydrogenase-regulated step
   -oxidative decarboxylation; isocitrate (6C) → α-ketoglutarate (5C) + CO_2 + NADH
   -endergonic oxidation of alcohol to a ketone by NAD+; exergonic decarboxylation
   -forward reaction favored under physiological conditions but is reversible *in vitro*
   -allosteric activator: ADP
4. α-ketoglutarate dehydrogenase
   -unidirectional, exergonic oxidative decarboxylation, produces CO2, NADH
   -Succinyl-CoA product is a thioester
   -multienzyme complex similar to PDH (3 proteins, 5 cofactors)
5. Succinate thiokinase
   -substrate level phosphorylation, high energy bond is conserved as GTP,
   -functionally isoergonic
6. Succinate Dehydrogenase
   -integral inner mitochondrial membrane protein, unique to Krebs, others in matrix
   -bidirectional oxidation-reduction reaction
   -inhibited by malonate, 3C analog of succinate
7. Fumarate
   -reversible hydration of fumarate to malate
   -isoergonic lyase reaction
8. Malate dehydrogenase
   -simple oxidation reduction to regenerate oxaloacetate
   -bidirectional

Net Reaction:
Acetyl-CoA + 3 NAD^+ + FAD + GDP + P_i + 2 H_20 → 2 CO_2 + 3 NADH + H^+ + $FADH_2$ + GTP + CoA

NADH & $FADH_2$ carry reducing equivalents to the electron transport chain where they are used to generate ATP.

From the electrons supplied by each molecule of NADH, the ETC generates ~2.5 molecules of ATP/NADH
From the electrons supplied by each molecule of $FADH_2$, the ETC generates ~1.5 molecules of ATP/$FADH_2$
The GTP is used to make a single molecule of ATP

Therefore, the yield of ATP from the oxidation of single molecule of Acetyl-CoA is:

3 NADH	x 2.5 = 7.5	From Krebs Cycle
1 $FADH_2$	x 1.5 = 1.5	From Krebs Cycle
1 GTP	x 1.0 = 1.0	From Krebs Cycle
TOTAL	10.0	

The oxidation of pyruvate by PDH yields 1 NADH which translates to 2.5 ATP for a GRAND TOTAL of 12.5 ATP

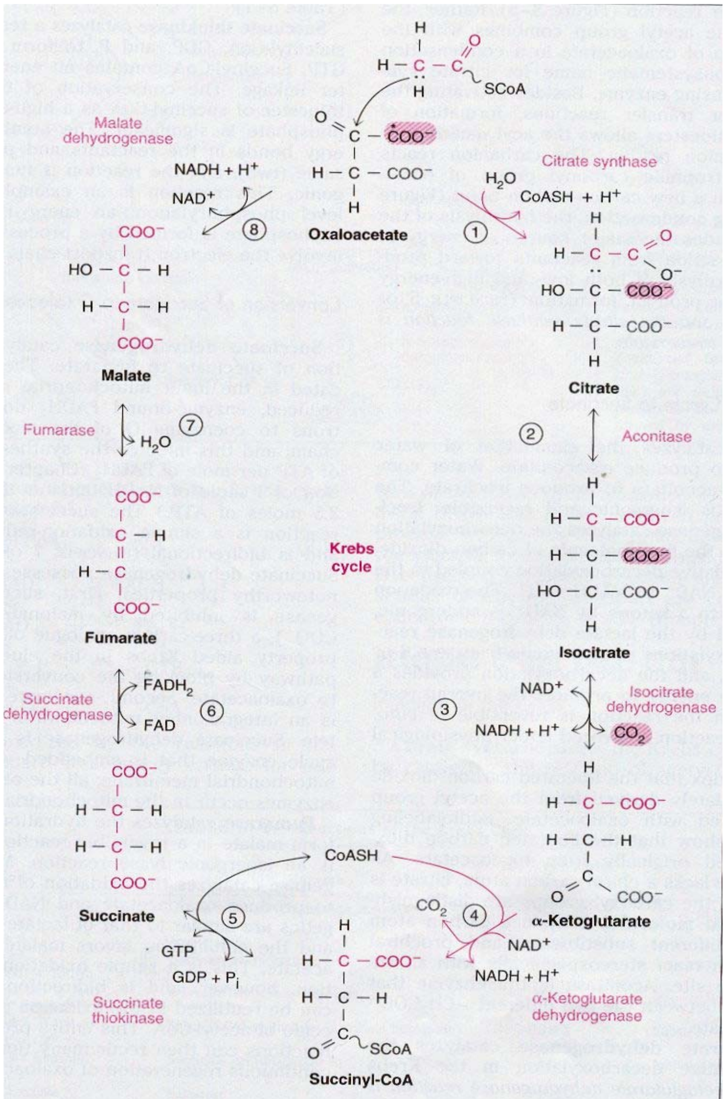
Pyruvate Oxidation:	2.5
Krebs Cycle	10.0
GRAND TOTAL	12.5

The Amphibolic Nature of the Krebs Cycle

Reaction intermediates of the Krebs cycle can be completely oxidized to CO_2 (catabolic) or used to synthesize other compounds (anabolic).

-catabolic function: oxidation of acetyl CoA to CO_2.
-anabolic function: intermediates in the cycle can be precursors in biosynthetic rxns. α-ketoglutarate, succinate and oxaloacetate-precursors for amino acids
-mechanisms to replenish intermediates called anapleurotic (to fill up) reactions
-most important: carboxylation of pyruvate → oxaloacetate by pyruvate carboxylase (pp 116-117)
   -reversible reaction

-when deficient in oxaloacetate or other intermediates, the rxn occurs. It uses one ATP.
-Pyruvate carboxylase complex enzyme w/ 4 biotin-containing prosthetic groups similar reaction carried out by Malic enzyme (reverse will generate NADPH). Malic enzyme catalyzes the following reaction:



pyruvate + CO_2 + NADPH ←→ Malate + $NADP^+$

TABLE 8–2. Reactions of the Krebs Cycle

Reaction	Enzyme	ΔG°′ per mole* (kJ)	Comments
1. Acetyl-CoA + oxaloacetate^{-2} + H_2O → citrate^{-3} + CoASH + H^+	Citrate synthase (condensing enzyme)	−38.0	Irreversible
2. Citrate^{-3} ⇄ isocitrate^{-3}	Aconitase	6.66	
3. Isocitrate^{-3} + NAD^+ ⇄ α-ketoglutarate^{-2} + CO_2 + NADH	Isocitrate dehydrogenase	−7.11	Regulatory enzyme
4. α-Ketoglutarate^{-2} + CoASH + NAD^+ → succinyl-CoA^{-1} + CO_2 + NADH	α-Ketoglutarate dehydrogenase	−24.0†	Irreversible
5. Succinyl-CoA^{-1} + GDP^{-3} + P_i^{-2} ⇄ succinate^{-2} + CoASH + GTP^{-4}	Succinyl CoA-synthetase (succinate thiokinase)	−8.96‡	Substrate level phosphorylation
6. Succinate^{-2} + (FAD) ⇄ fumarate^{-2} + ($FADH_2$)	Succinate dehydrogenase	0.0	Inhibited by malonate
7. Fumarate^{-2} + H_2O ⇄ malate^{-2}	Fumarase	−3.68	
8. Malate^{-2} + NAD^+ ⇄ oxaloacetate^{-2} + NADH + H^+	Malate dehydrogenase	28.0	
		−47.09 *total*	

*Based on the data of M.J. Johnson at 25°C, pH 7.0. *In* P.D. Boyer, H. Lardy, and K. Myrbäck (eds.). *The Enzymes*, 2nd ed. New York, Academic Press, 1960, vol. 3, p. 407.
†Based on data in Tables 5–3 and 6–3.
‡Based on data in Table 6–3.