BB 422/622

OUTLINE:

Review of 421
Goals of 422
Review of chemical principles
- Thermodynamics
- C/O cycles
- Overview of Metabolism
- ATP cycles

Energy Coupling
Chemical Reactivity
Bioenergetics
Membrane Transport
- Review of membrane structure, dynamics, and proteins
- Mediated/non-mediated
- Energetics
- Facilitative Diffusion
  - ionophores
  - GLUT1
  - Aquaporins
  - Potassium channel
- Active Transport
  - Primary
    - Na/K pump
    - ABC transporters
  - Secondary
    - Glc import
    - Bicarbonate/Cl
    - Lactose/H+

Catabolism of Glucose
Glycogenolysis
- phosphorylase
- debranching enzyme
- phospho-gluco-mutase (PGM)

Glycolysis
- Introduction & overview;
- Phase I
  - hexokinase- phosphotransferase-coupling
  - phospho-gluco-isomerase (PGI)- endiol
  - phospho-fructo-kinase (PFK-1)-
  - Aldolase- Schiff base (electron sink to stabilize a carbanion)
  - triose-phosphate isomerase (TPI)- endiol

Exam 1

- Phase II
  - **GAPDH**- oxidation
  - PG kinase- return on investment- substrate-level phosphorylation
  - **PG mutase**- acid/base; phospho-enzyme
  - **Enolase**- enolate
  - Pyruvate Kinase- phosphotransferase
- Summary: labeling studies, logic, energetics

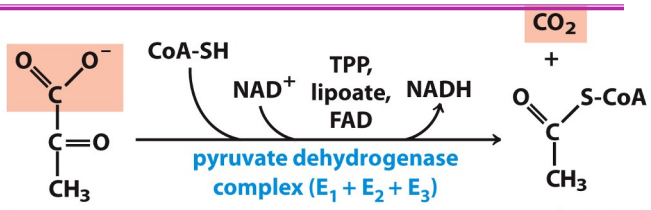
Catabolism of Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations- Anaerobic
- Lactate
  - lactate dehydrogenase
- Acetoacetate decarboxylase
- Ethanol
  - pyruvate decarboxylase
  - alcohol dehydrogenase

Aerobic
- Pyruvate
  - pyruvate dehydrogenase complex
- Krebs' Cycle
  - How did he figure it out?
  - Overview
  - 8 Steps
  - Citrate Synthase

8

Pyruvate Oxidation

Conversion of Pyruvate to Acetyl-CoA



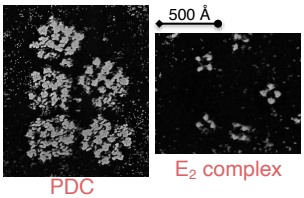
- Net reaction:
  - oxidative decarboxylation of pyruvate
    - Means pyruvate will get oxidized as the carboxylate leaves (as CO_2)
  - first carbons of glucose to be fully oxidized (C3 & C4)
- Fairly simple reaction done by a complicated process.
- Highly thermodynamically favorable/irreversible ($\Delta G^{\circ\prime} = -8$ kcal/mol); mostly due to the loss of CO_2
- Catalyzed by the **Pyruvate Dehydrogenase Complex (PDC)**
  - Three main enzyme, each with multiple subunits: E1, E2, E3
  - Regulatory subunits: PD kinase & PD phosphatase
  - Overall structure of $E1_{96}$, $E2_{24}$, $E3_{24}$
  - requires 5 coenzymes
  - TPP, lipoic acid, and FAD are prosthetic groups.
  - NAD^+ and CoA-SH are co-substrates.

10

Pyruvate Oxidation

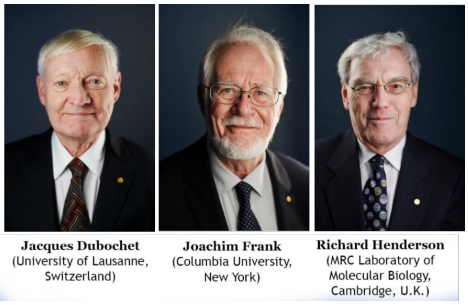
PDC is a large (up to **10 MDa**) multienzyme complex.

- –**pyruvate dehydrogenase (E_1)**
- –**dihydrolipoyl transacetylase (E_2)**
- –**dihydrolipoyl dehydrogenase (E_3)**



Cryo-Electron Microscopy

Nobel Prize for Chemistry in 2017

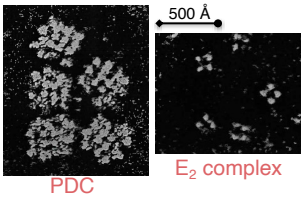


11

Pyruvate Oxidation

PDC is a large (up to **10 MDa**) multienzyme complex.

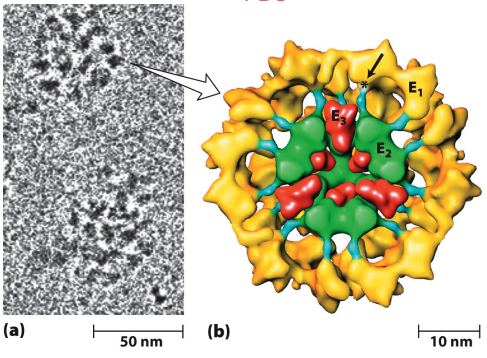
- –**pyruvate dehydrogenase (E_1)**
- –**dihydrolipoyl transacetylase (E_2)**
- –**dihydrolipoyl dehydrogenase (E_3)**



Cryoelectronmicroscopy

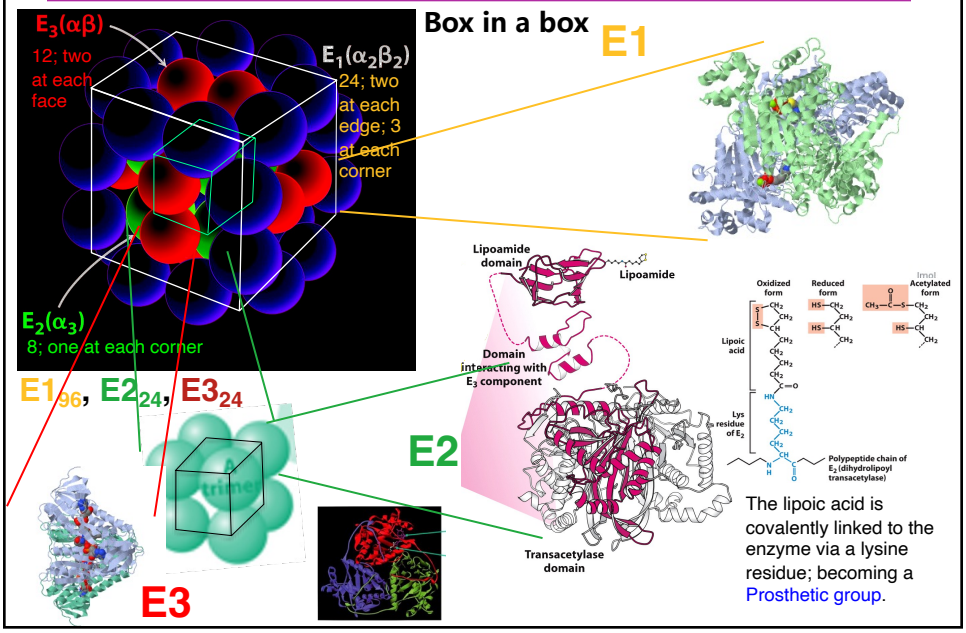
:

- Samples are in a near-native frozen hydrated state.
- Low temperature protects biological specimens against radiation damage.
- Electrons have a smaller wavelength and produce much higher-resolution images than light.
- No need for a crystal.



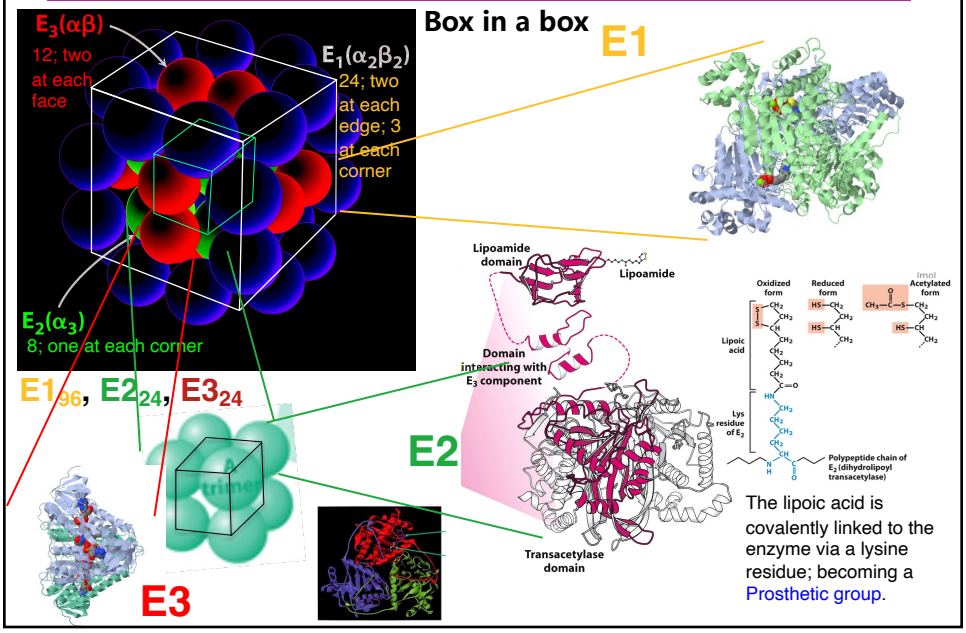
12

Pyruvate Oxidation



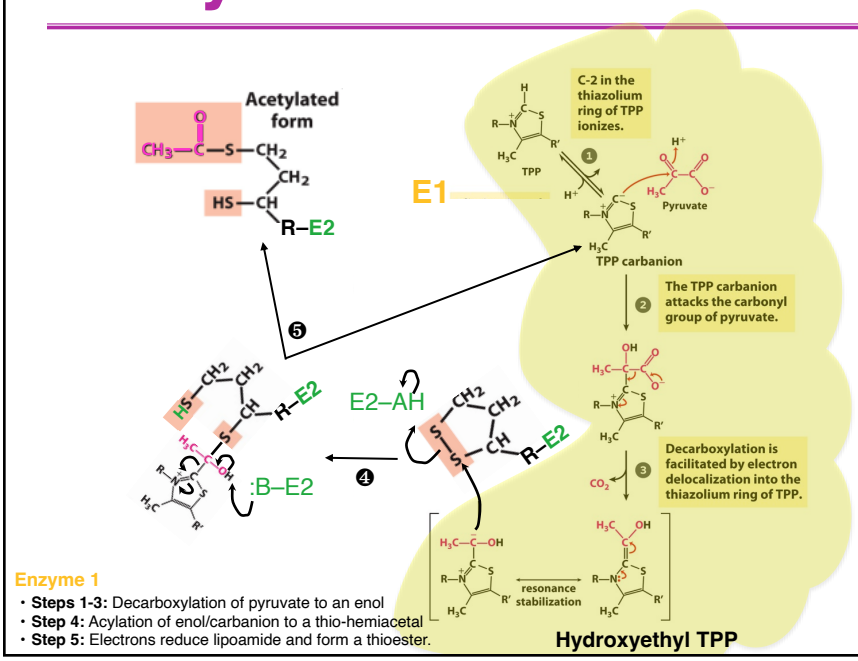
13

Pyruvate Oxidation



14

Pyruvate Oxidation

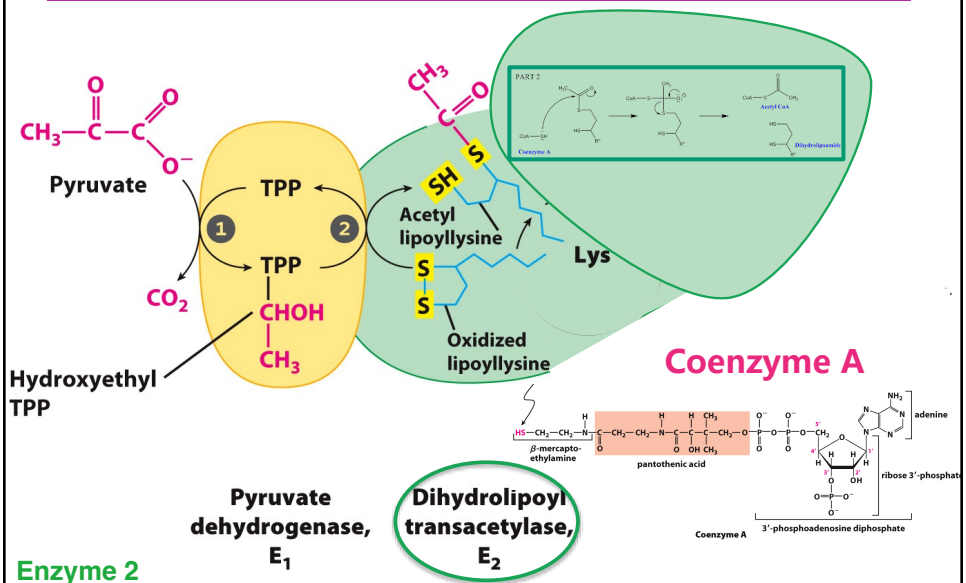


Enzyme 1

- **Steps 1-3:** Decarboxylation of pyruvate to an enol
- **Step 4:** Acylation of enol/carbanion to a thio-hemiacetal
- **Step 5:** Electrons reduce lipoamide and form a thioester.

16

Pyruvate Oxidation

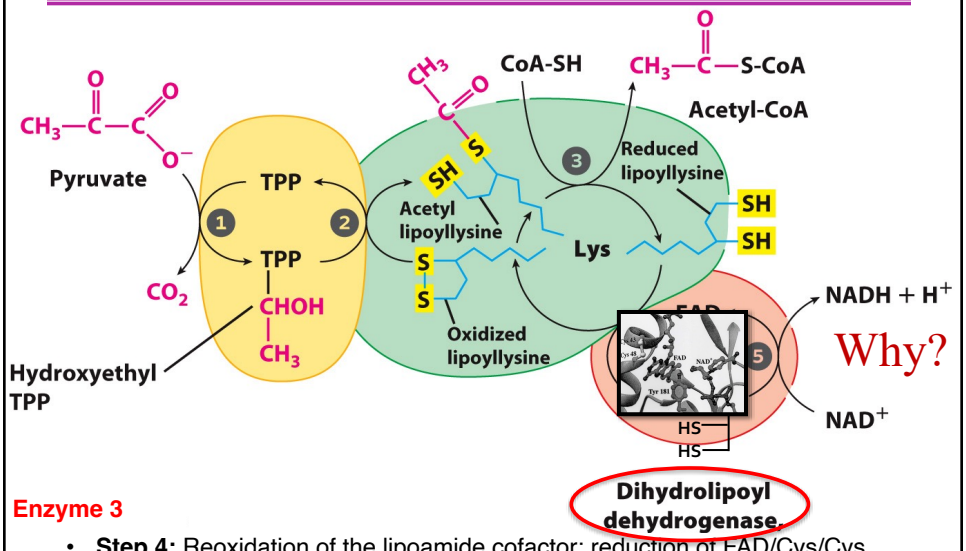


Enzyme 2

- Formation of acetyl-CoA: simple thio-ester exchange

17

Pyruvate Oxidation

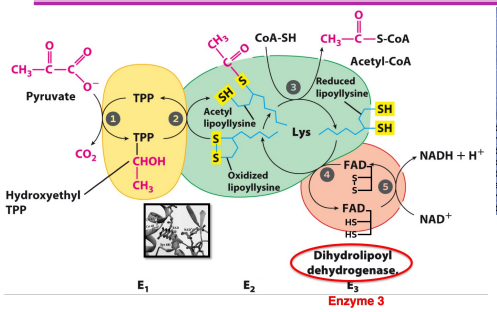


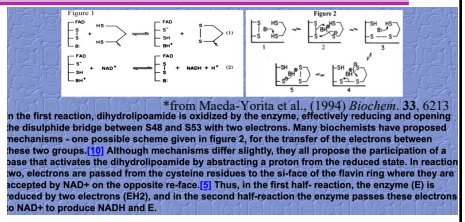
Enzyme 3

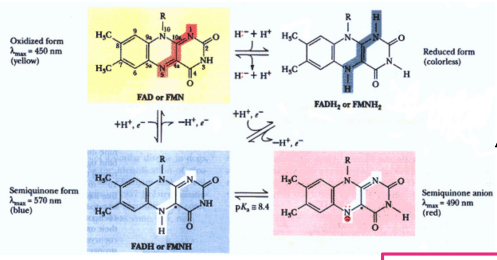
- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
  - Disulfide interchange with dihydrolipoyllysine and 2 adjacent Cys in disulfide bond
  - The reduced Cys reform disulfide using FAD, which funnels electrons to NAD^+
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

18

Pyruvate Oxidation







$$E\text{-}FADH_2 + NADH + H^+ \leftrightarrow NADH + H^+ + E\text{-}FAD$$

$$\Delta E^{o'} = E^{o'}_{(reduction)} - E^{o'}_{(oxidation)}$$

$$\Delta E^{o'} = E^{o'}_{(NAD^+)} - E^{o'}_{(FAD)}$$

$$= -0.320\ V - (+0.031\ V^*)$$

$$= -0.351\ V$$

$$\Delta G^{o'} = -n\,\mathcal{F}\,\Delta E^{o'}$$

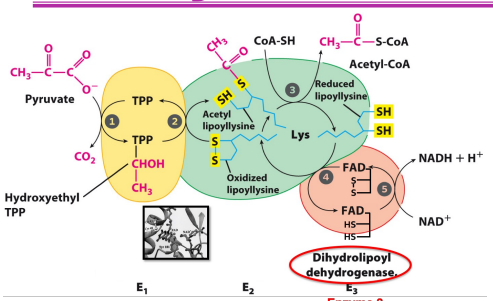
$$= -(2)(23.06 kcal V^{-1} mol^{-1})(-0.351\ V)$$

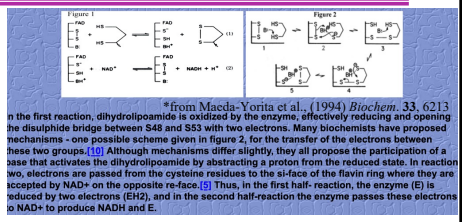
$$= +16\ kcal\ mol^{-1}$$

If use S-S, with $E^{o'}_{(S\text{–}S)} = -0.34$ V, $\Delta G^{o'} = -0.92$ kcal/mol

21

Pyruvate Oxidation





*from Maeda-Yorita et al., (1994) *Biochem.* 33, 6213

In the first reaction, dihydrolipoamide is oxidized by the enzyme, effectively reducing and opening the disulphide bridge between S48 and S53 with two electrons. Many biochemists have proposed mechanisms - one possible scheme given in figure 2, for the transfer of the electrons between these two groups.[10] Although mechanisms differ slightly, they all propose the participation of a base that activates the dihydrolipoamide by abstracting a proton from the reduced state. In reaction two, electrons are passed from the cysteine residues to the si-face of the flavin ring where they are accepted by NAD+ on the opposite re-face.[5] Thus, in the first half- reaction, the enzyme (E) is reduced by two electrons (EH2), and in the second half-reaction the enzyme passes these electrons to NAD+ to produce NADH and E.

Standard Redox Potentials E'_0			
Half reaction		Half reaction	E'_0(V)
Succinate + CO_2 + $2H^+$ +2e⁻	↔	α-ketoglutarate + H_2O	-0.670
Acetate + $2H^+$ +2e⁻	↔	acetaldehyde	-0.581
$2H^+$ +2e⁻	↔	H_2	-0.421
α-ketoglutarate + CO_2 + $2H^+$ +2e⁻	↔	citrate	-0.380
Cystine + $2H^+$ +2e⁻	↔	2 cysteine	-0.340
NAD^+ + $2H^+$ +2e⁻	↔	NADH + H^+	-0.320
$NADP^+$ + $2H^+$ +2e⁻	↔	NADPH + H^+	-0.324
Acetaldehyde + $2H^+$ +2e⁻	↔	ethanol	-0.197
Pyruvate + $2H^+$ +2e⁻	↔	lactate	-0.185
Oxaloacetate + $2H^+$ +2e⁻	↔	malate	-0.166
FAD + $2H^+$ +2e⁻	↔	$FADH_2$	0.031
Fumarate + $2H^+$ +2e⁻	↔	succinate	0.031
Ubiquinone + $2H^+$ +2e⁻	↔	ubiquinol	0.045
2 cytochrome $b_{(ox)}$ +2e⁻	↔	2 cytochrome $b_{(red)}$	0.070
2 cytochrome $c_{1(ox)}$ +2e⁻	↔	2 cytochrome $c_{1(red)}$	0.254
2 cytochrome $a_{3(ox)}$ +2e⁻	↔	2 cytochrome $a_{3(red)}$	0.385
1/2 O_2 + $2H^+$ +2e⁻	↔	H_2O	0.816

$E\text{-}FADH_2 + NADH + H^+ \leftrightarrow NADH + H^+ + E\text{-}FAD$

$$\Delta E^{o'} = E^{o'}_{(reduction)} - E^{o'}_{(oxidation)}$$

$$\Delta E^{o'} = E^{o'}_{(NAD^+)} - E^{o'}_{(FAD)}$$

$$= -0.320\ V - (+0.031\ V^*)$$

$$= -0.351\ V$$

$$\Delta G^{o'} = -n\mathcal{F}\Delta E^{o'}$$

$$= -(2)(23.06 kcal V^{-1} mol^{-1})(-0.351\ V)$$

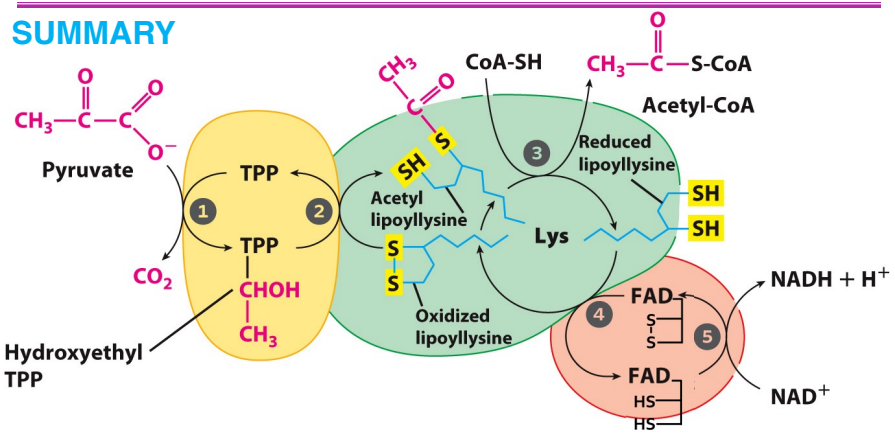
$$= +16\ kcal\ mol^{-1}$$

If use S-S, with $E^{o'}_{(S\text{–}S)} = -0.34$ V, $\Delta G^{o'} = -0.92$ kcal/mol

22

Pyruvate Oxidation

SUMMARY



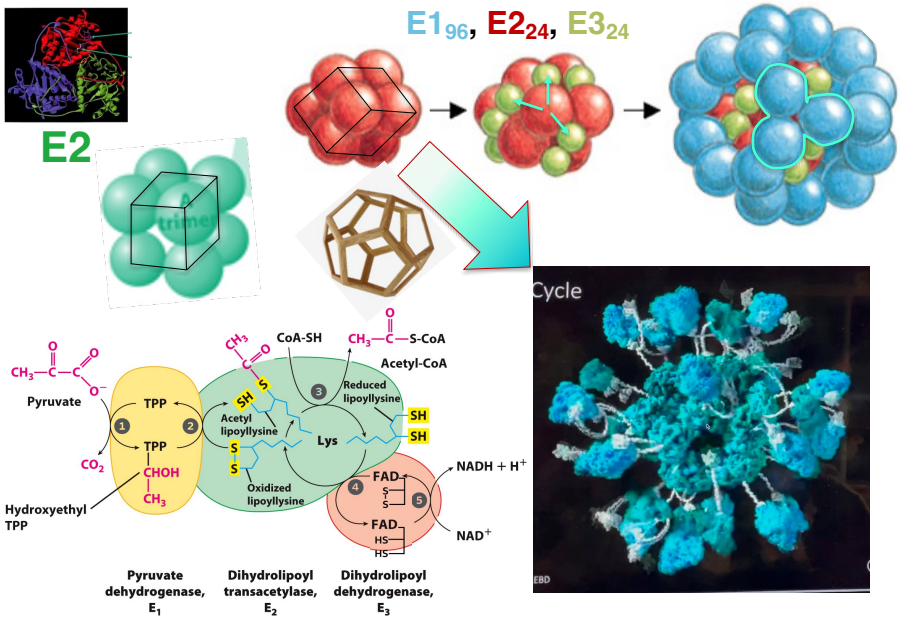
Enzyme 1
- **Step 1:** Decarboxylation of pyruvate to an enol (hydroxyethyl-TPP)
- **Step 2:** Acylation of enol to a thioester on lipoic acid.

Enzyme 2
- **Step 3:** Formation of acetyl-CoA: simple thio-ester exchange

Enzyme 3
- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

23

Pyruvate Oxidation



25

Pyruvate Oxidation

Overall Reaction of PDC

Pyruvate + Coenzyme-A (CoASH) + NAD^+

PDC (TPP, lipoic acid, FAD) ⇅ $\Delta G^{\circ\prime} = -8$ kcal/mol

CO_2 + **Acetyl-Coenzyme-A** (Ac-CoA) + NADH + H^+

26

Pyruvate Oxidation

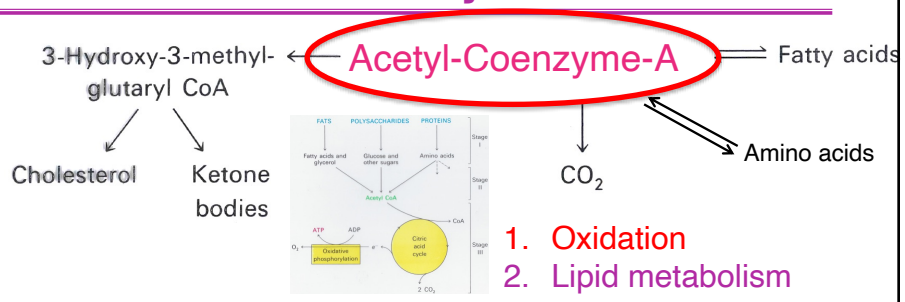
Overall Reaction of PDC

Pyruvate + Coenzyme-A (CoASH) + NAD^+

PDC (TPP, lipoic acid, FAD) ⇅ $\Delta G^{\circ\prime} = -8$ kcal/mol

CO_2 + Acetyl-Coenzyme-A (Ac-CoA) + NADH + H^+

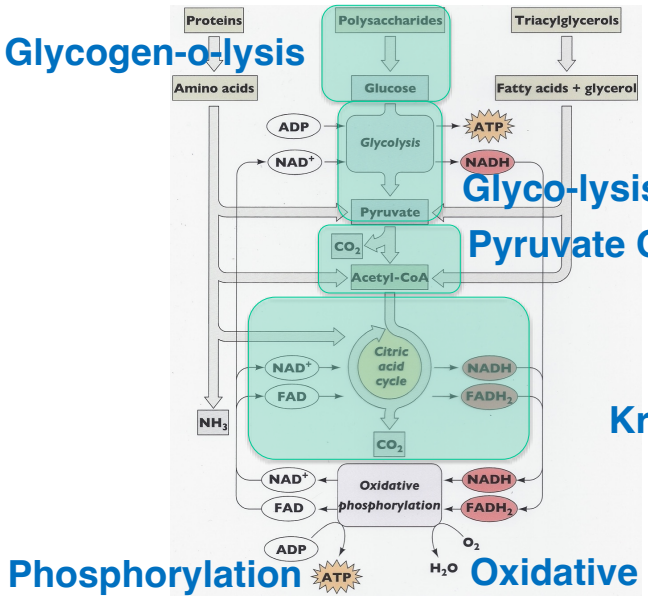
Fates of Acetyl CoA



1. Oxidation
2. Lipid metabolism
3. Amino acid metabolism

28

The Citric Acid Cycle



Glycogen-o-lysis

Glyco-lysis

Pyruvate Oxidation

Krebs' Cycle

Phosphorylation

Oxidative

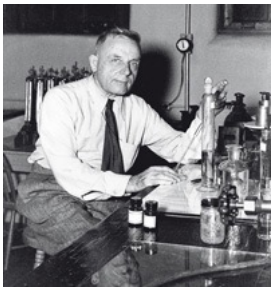
29

The Citric Acid Cycle

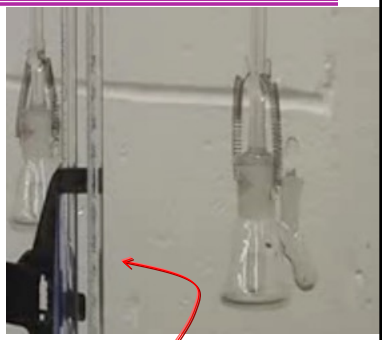
a.k.a. Krebs Cycle,
a.k.a. Tricarboxylic Acid Cycle (TCA)

30

Time B.C. (Before the Cycle)



Otto Warburg
1883-1970



Manometer

Warburg Apparatus
-respiration
-Measure rates of O_2 consumption

Substrates
(e.g., glucose)

Tissues

CO_2 trap

$CO_2 \rightarrow H_2CO_3 \rightarrow 2H^+ + CO_3^{-2}$
$\xrightarrow[KOH]{} K_2CO_3 + H_2O$

UTube instructions
(http://youtu.be/M-HYbZwN43o)

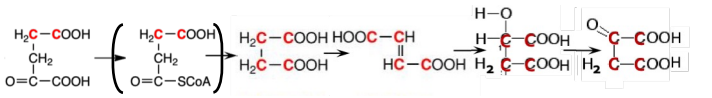
31

Time B.C. (Before the Cycle)

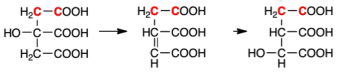
O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

In 1920 BC, what was known about respiration?

1) Glycolysis gives rise to pyruvate
2) Adding pyruvate to respiring tissues in a Warburg apparatus, there are 2.5 O_2 consumed: $2^{1/2}O_2 + C_3H_4O_3 \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow 3CO_2 + 2H_2O$
3) Any intermediate in the process will be oxidized at a rate ≥pyruvate
4) Many intermediates were tried, but few met this criteria, they were: succinate, fumarate, malate, alpha-ketoglutarate, etc.



α-ketoglutarate → succinate → fumarate → malate → oxaloacetate



citrate → aconitate → isocitrate

Albert Szent-Györgyi
1893-1986

5) Others had already worked out several compounds and their interconversion. Specifically, Albert Szent-Györgi had worked out the interconversion of the dicarboxylic acids. Carl Martinus worked out the interconversion of the tricarboxylic acids

6) In 1937, with help of German biochemist Franz Koop, Carl Martinus, demonstrated a series of reactions using citrate that produced α–ketoglutarate. Thus, tricarboxylic acid and dicarboxylic acids would be interconverted with loss of CO2 but also support respiration.

32

Time B.C. (Before the Cycle)

O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

Hans Krebs
1900-1981

glutarate → succinate → fumarate → malate → oxaloacetate

citrate → aconitate → isocitrate

Krebs confirmed that the pathway was consistent with succinate, fumarate, and malate proved to be useful because all these molecules increased oxygen consumption in the pigeon breast muscle.

The first clue came from an experiment with fumarate. Krebs did careful measurements using the Warburg manometer. Fumarate gave greater than expected oxygen consumption in the pigeon breast muscle.

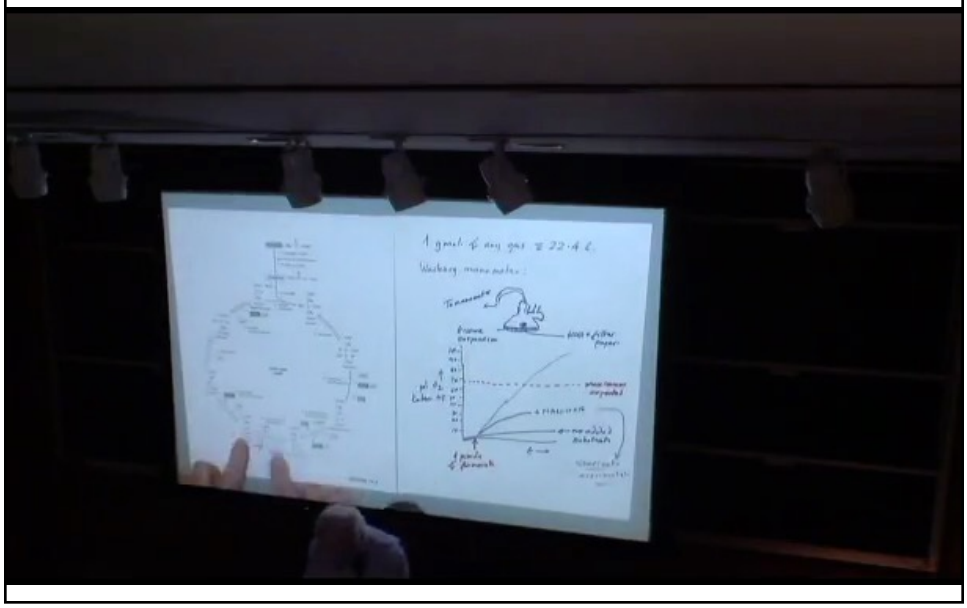
$3O_2 + C_4H_4O_4 \rightarrow 4CO_2 + 2H_2O$

∴1 µmole fumarate would consume 3 µmole O_2

33

Time B.C. (Before the Cycle)

O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

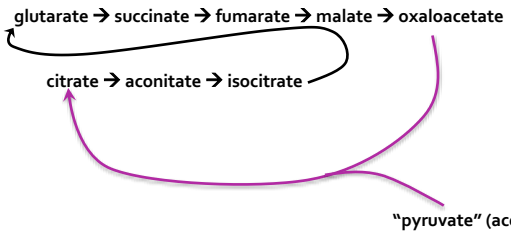


34

Time B.C. (Before the Cycle)

O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

Hans Krebs
1900-1981



He observed much more than 3:1!!

Krebs confirmed that the pathway was consistent with succinate, fumarate, and malate proved to be useful because all these molecules increased oxygen consumption in the pigeon breast muscle.

The first clue came from an experiment with fumarate. Krebs did careful measurements using the Warburg manometer. Fumarate gave greater than expected oxygen consumption in the pigeon breast muscle.

$3O_2 + C_4H_4O_4 \rightarrow 4CO_2 + 2H_2O$

∴1 µmole fumarate would consume 3 µmole O_2

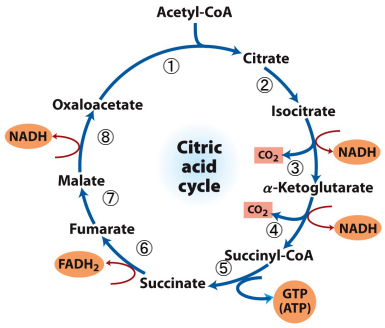
1) Malonic acid inhibition of the **succinate → fumarate** step prevented this increase…But succinate **accumulated**
2) How can fumarate give rise to succinate? There must be a cycle
3) Tested by showing that using succinate or fumarate you could detect the formation of citrate.

Later in 1937, he proposed that pyruvate would combine with oxaloacetate to make citrate in a cycle he called the Citric Acid Cycle. Later, Fritz Lipmann showed that it was acetyl-CoA and not pyruvate.

35

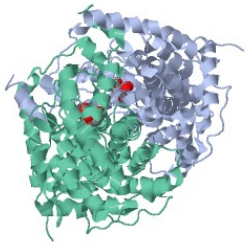
The Citric Acid Cycle

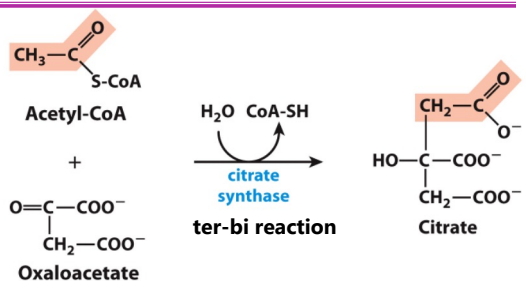
- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give $FADH_2$
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH



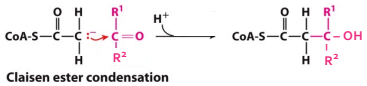
37

The Citric Acid Cycle: Citrate Synthase





- Joining of acetyl-CoA and oxaloacetate with C-C bond formation
- Highly thermodynamically favorable/irreversible ($\Delta G^{\circ\prime} = -7.7$ kcal/mol)
  - regulated by substrate availability and product inhibition
- Activity largely depends on [oxaloacetate].
- Rate-limiting step of CAC
- Uses acid/base catalysis
  - Carbonyl of oxaloacetate is a good electrophile.
  - Methyl of acetyl-CoA is not a good nucleophile…
  - …unless activated by deprotonation to form a carbanion.

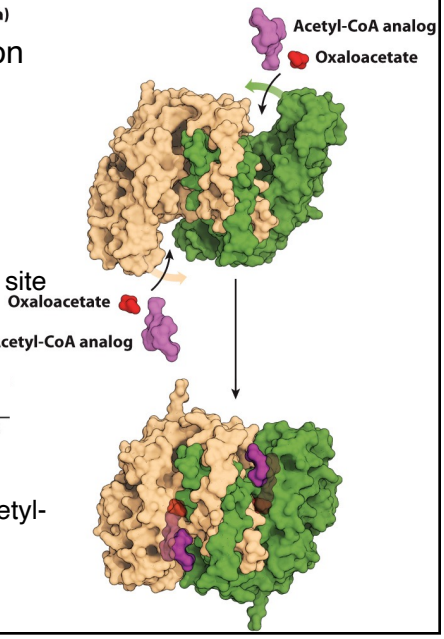


38

The Citric Acid Cycle: Citrate Synthase

Mechanism

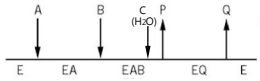
- Conformational change occurs upon binding oxaloacetate.
- Avoids unnecessary hydrolysis of thioester in acetyl-CoA



a) Open conformation:

Free enzyme does not have a binding site for acetyl-CoA. Ordered binding.

Sequential ordered ter bi



b) Closed conformation:

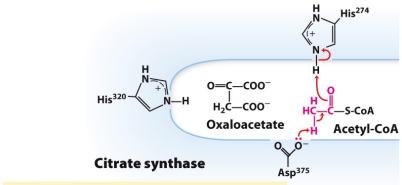
Binding of OAA creates binding for acetyl-CoA.

Reactive carbanion is protected.

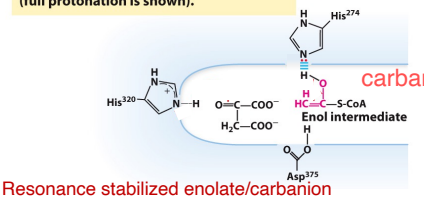
39

The Citric Acid Cycle: Citrate Synthase

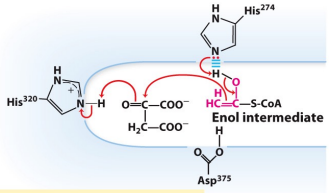
Mechanism



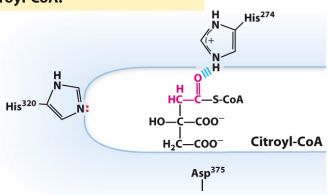
The thioester linkage in acetyl-CoA activates the methyl hydrogens. Asp375 abstracts a proton from the methyl group, forming an enolate intermediate. The intermediate is stabilized by hydrogen bonding to and/or protonation by His274 (full protonation is shown).



- Resonance stabilized enolate/carbanion
- Low barrier H-bond with His 274



The enol(ate) rearranges to attack the carbonyl carbon of oxaloacetate, with His274 positioned to abstract the proton it had previously donated. His320 acts as a general acid. The resulti generates citroyl-CoA.



41