Glucose potential energy

The Tricarboxylic Acid Cycle

Chapter 19

Summary of aerobic energy production

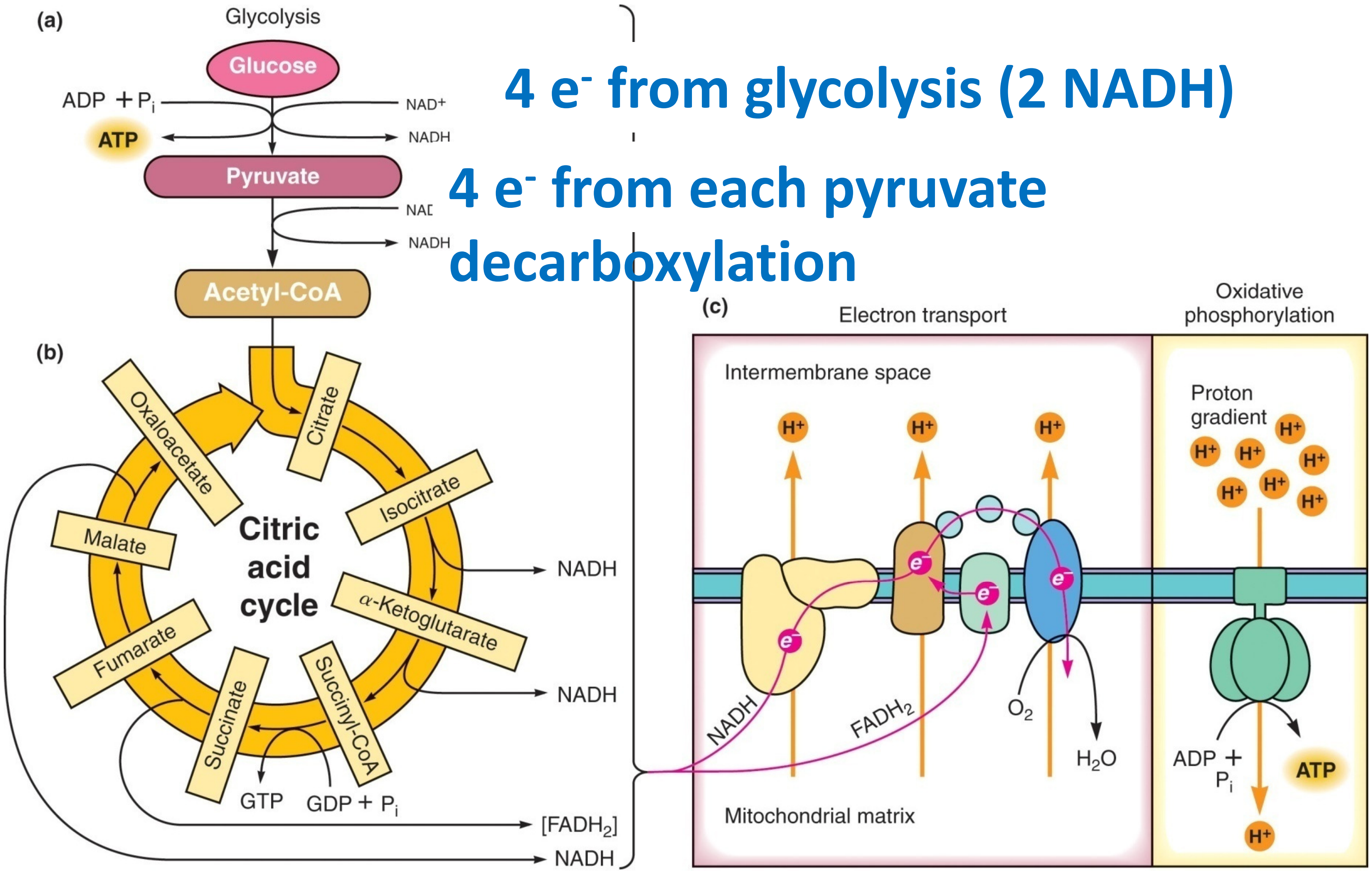
- Pyruvate → acetyl-CoA → CO_2
- Electrons released → NADH and $FADH_2$ → O_2

- Electron transfer → proton gradient across membrane
- ATP synthesis in ETC = oxidative phosphorylation

Summary of aerobic energy production

4 e⁻ from glycolysis (2 NADH)

4 e⁻ from each pyruvate decarboxylation



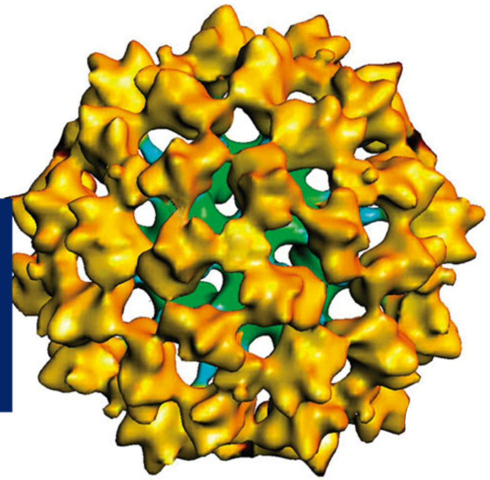
Pyruvate as a source of acetyl-CoA

- Glycolysis → TCA cycle

- Oxidative decarboxylation of pyruvate:

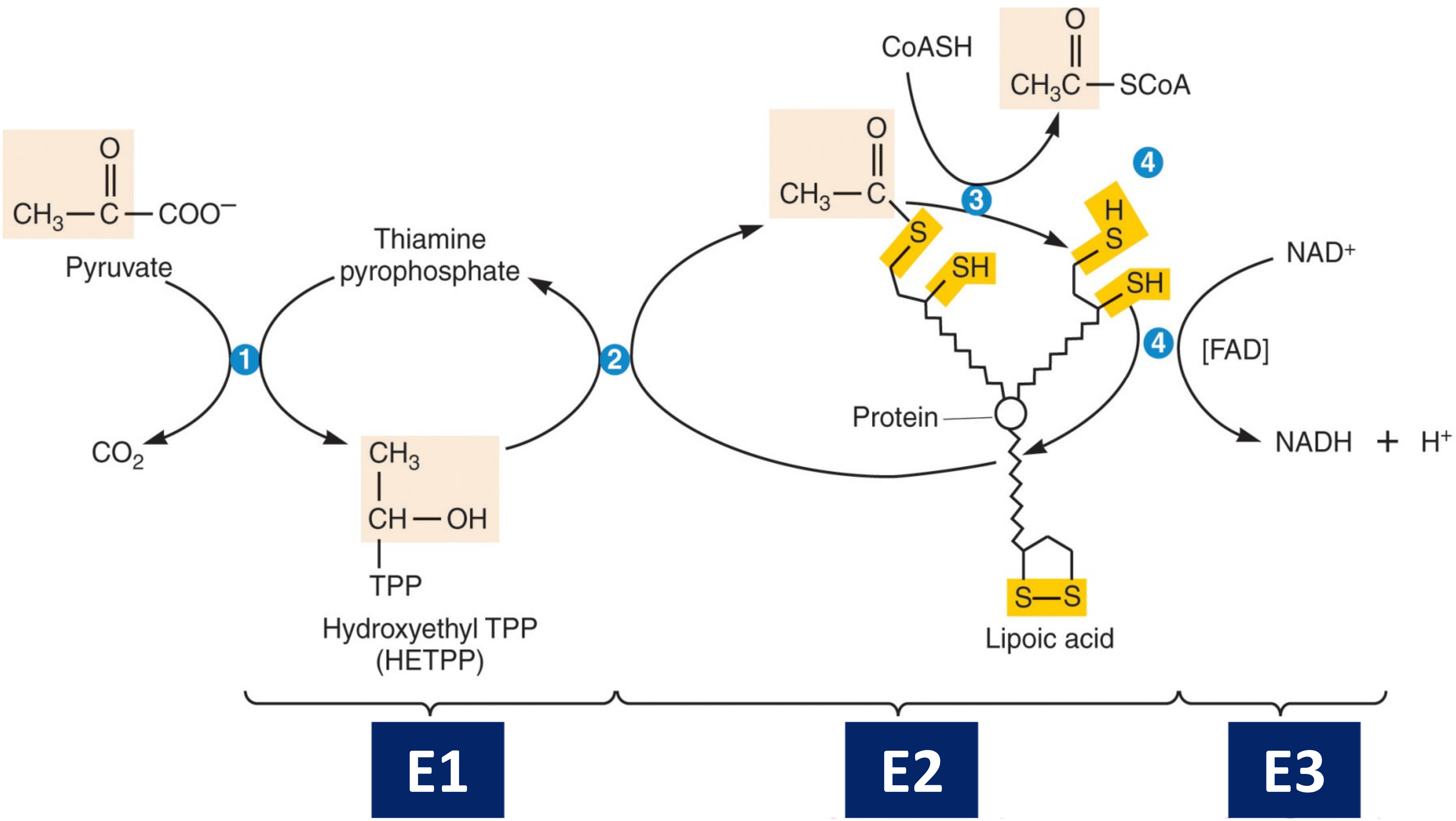
Pyruvate dehydrogenase complex (PDC)

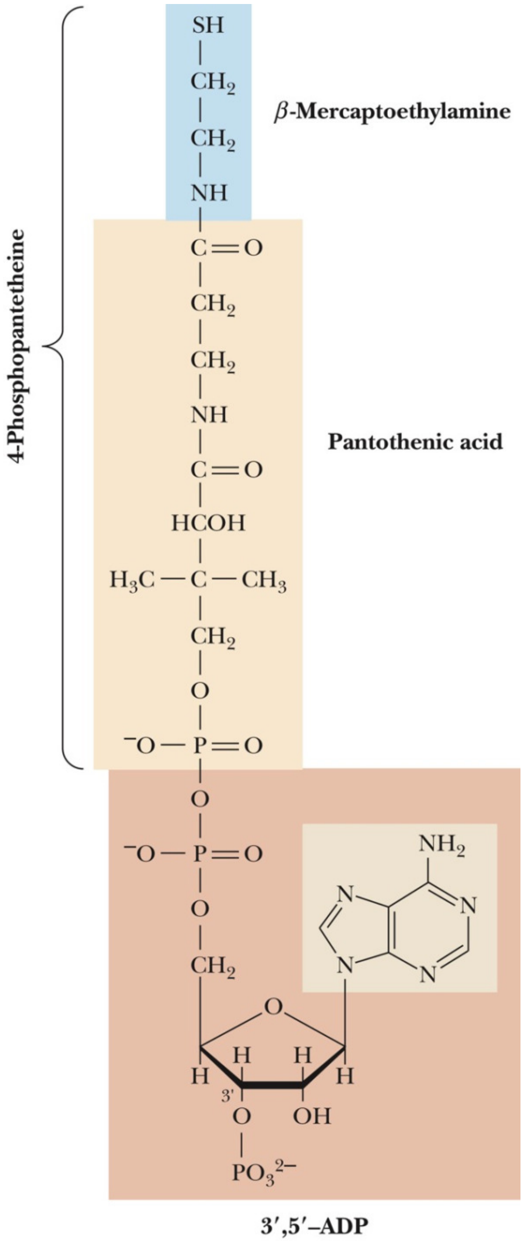
- Pyruvate dehydrogenase (E1)
- Dihydrolipoamide acetyltransferase (E2)
- Dihydrolipoamide dehydrogenase (E3)



Reaction mechanism of PDC

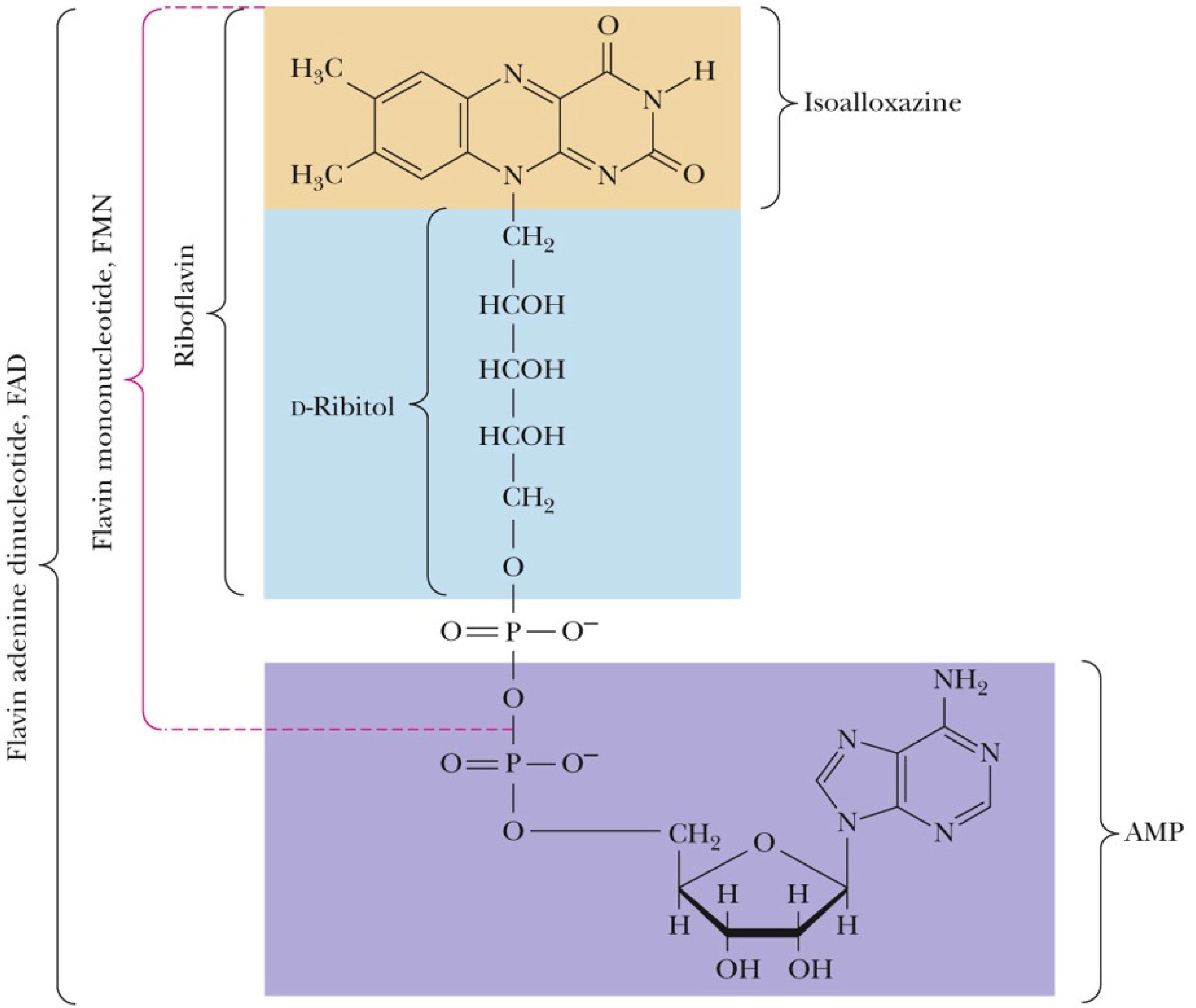
1. Decarboxylation of pyruvate
2. Transfer of 2C unit to lipoic acid
3. Formation of acetyl Co-A
4. Reoxidation of lipoic acid



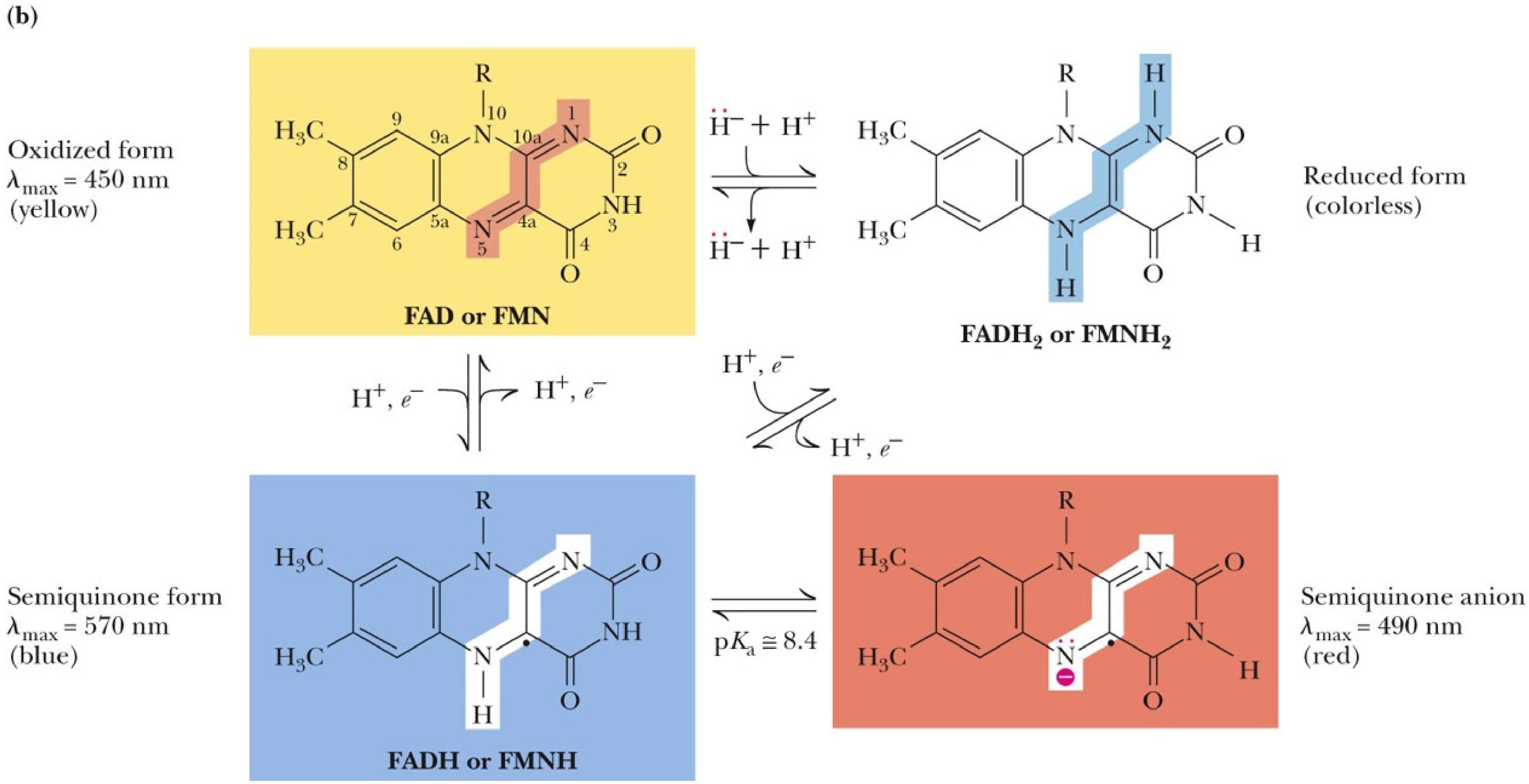


β-Mercaptoethylamine
Pantothenic acid
4-Phosphopantetheine
3′,5′–ADP

(a)


Flavin adenine dinucleotide, FAD
Flavin mononucleotide, FMN
Riboflavin
Isoalloxazine
D-Ribitol
AMP

Isoalloxazine moiety of the Flavin Co-enzymes



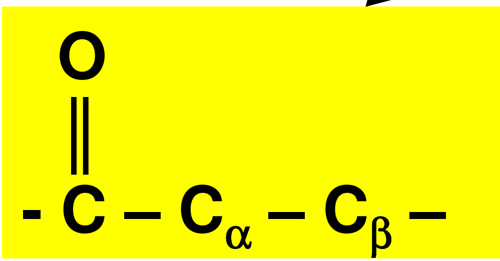
A snapshot of the TCA cycle reactions

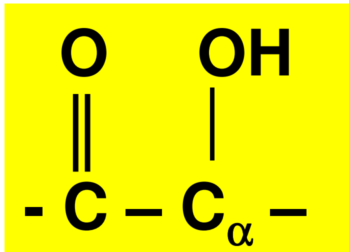
- **Reaction 1:** 2C acetyl group from acetyl-CoA transferred to oxaloacetate → 6C citrate
- **Reaction 2:** isomerisation of citrate to isocitrate
- **Reaction 3:** first oxidative decarboxylation → αketoglutarate
- **Reaction 4:** second oxidative decarboxylation → Succinyl-CoA
- **Reaction 5:** substrate level phosphorylation, succinyl-CoA → Succinate
- **Reaction 6:** Succinate oxidised to fumerate in an FAD-dependant reaction
- **Reaction 7:** trans hydration of fumerate → L-malate
- **Reaction 8:** malate oxidised back to oxeloacetate

A chemically feasible way for cleaving C – C

- $CH_3COO^- \rightarrow CO_2 + CO_2$

C – C cleavage in biological systems





Condense acetate with oxaloacetate for β cleavage

All the TCA cycle reactions and their thermodynamics

TABLE 19.1 The Enzymes and Reactions of the TCA Cycle

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1. Acetyl-CoA + oxaloacetate + $H_2O \rightleftharpoons$ CoASH + citrate	Citrate synthase	−31.4	−53.9
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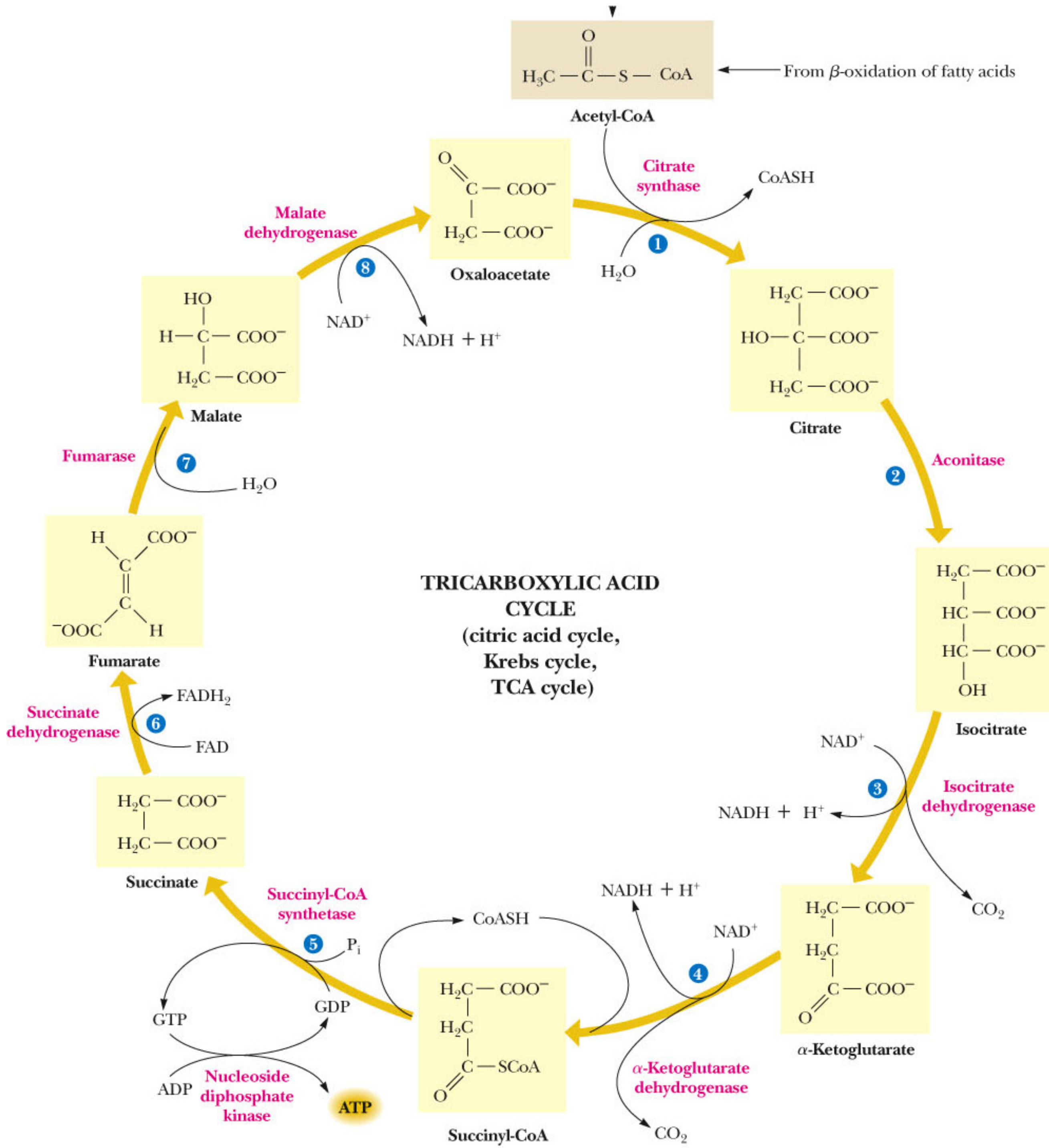
4. α-Ketoglutarate + CoASH + $NAD^+ \rightleftharpoons$ succinyl-CoA + NADH + CO_2	α-Ketoglutarate dehydrogenase complex	−30	−43.9
5. Succinyl-CoA + GDP + $P_i \rightleftharpoons$ succinate + GTP + CoASH	Succinyl-CoA synthetase	−3.3	≈0
6. Succinate + [FAD] $\rightleftharpoons$ fumarate + [$FADH_2$]	Succinate dehydrogenase	+0.4	≠0
7. Fumarate + $H_2O \rightleftharpoons$ L-malate	Fumarase	−3.8	≈0
8. L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase	+29.7	≈0

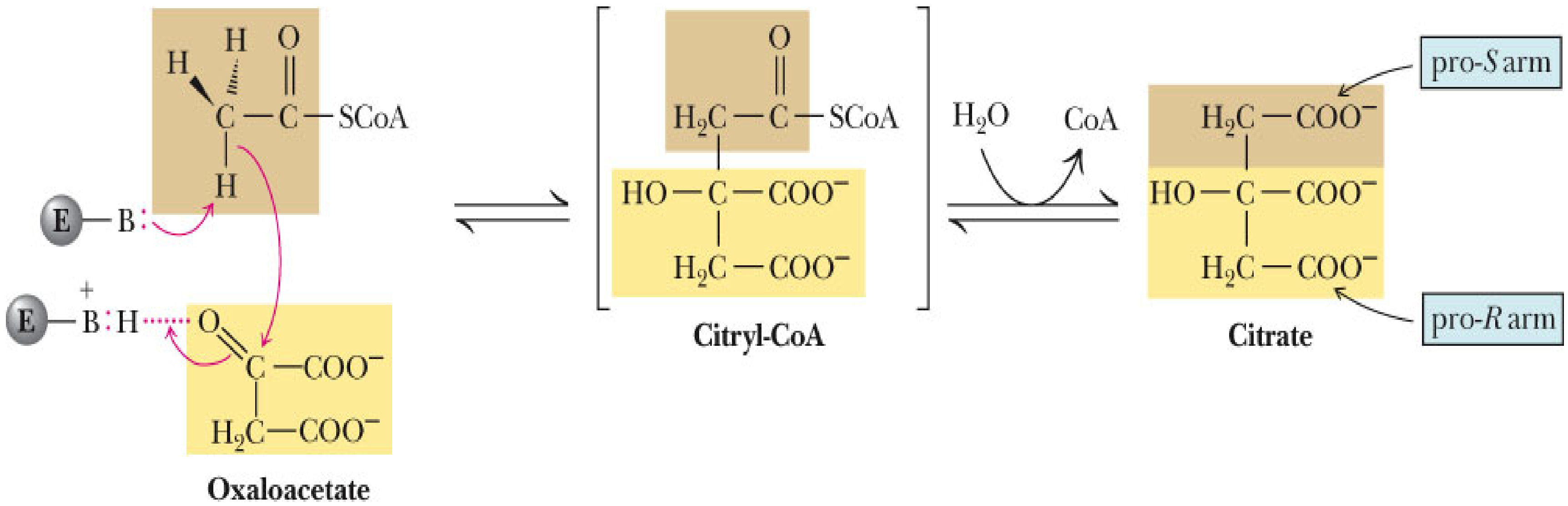


From β-oxidation of fatty acids
Acetyl-CoA
Citrate synthase
CoASH
H_2O
Oxaloacetate
Citrate
Aconitase
Isocitrate
NAD^+
$NADH + H^+$
Isocitrate dehydrogenase
CO_2
α-Ketoglutarate
α-Ketoglutarate dehydrogenase
NAD^+
$NADH + H^+$
CO_2
CoASH
Succinyl-CoA
Succinyl-CoA synthetase
P_i
GDP
GTP
ADP
Nucleoside diphosphate kinase
ATP
Succinate
Succinate dehydrogenase
FAD
$FADH_2$
Fumarate
Fumarase
H_2O
Malate
Malate dehydrogenase
NAD^+
$NADH + H^+$
TRICARBOXYLIC ACID CYCLE
(citric acid cycle,
Krebs cycle,
TCA cycle)

The citrate synthase reaction

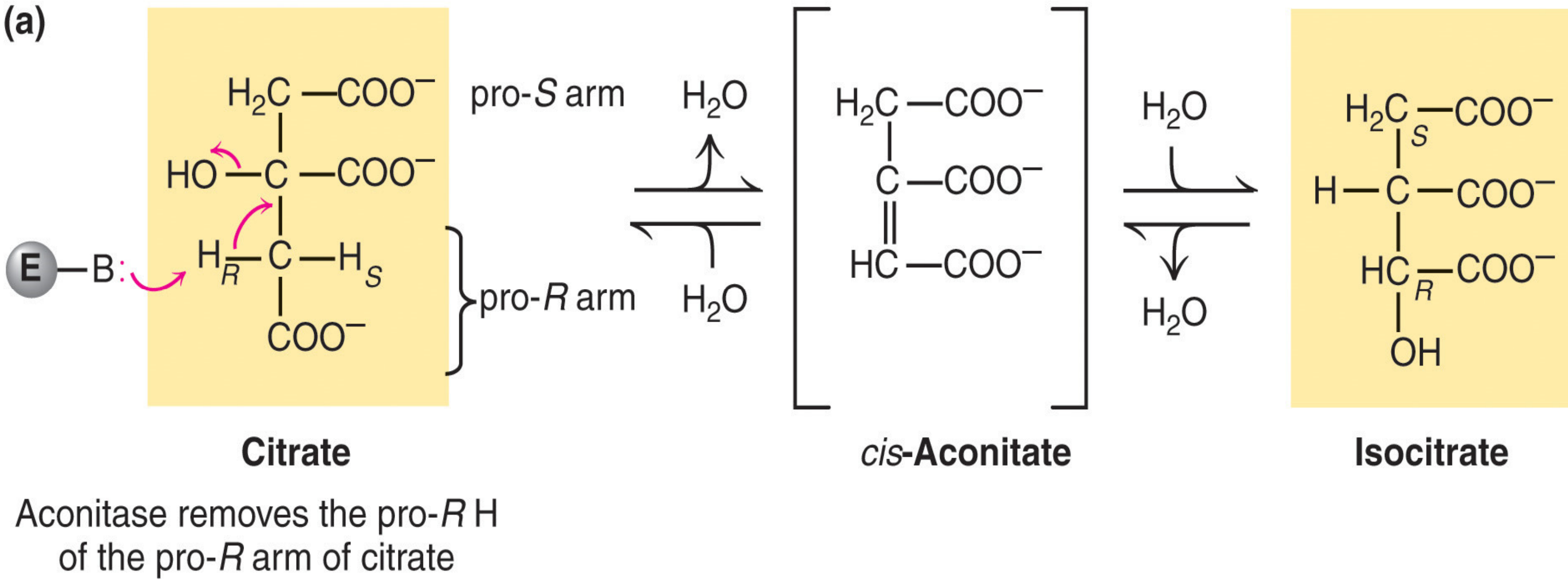
- Carbon atoms introduced to the cycle
- Condensation reaction

Acetyl-CoA + oxaloacetate + H_2O → CoASH + citrate
$\Delta G^{o\prime}$ = -31 kJ/mol **Highly regulated enzyme**



The aconitase reaction

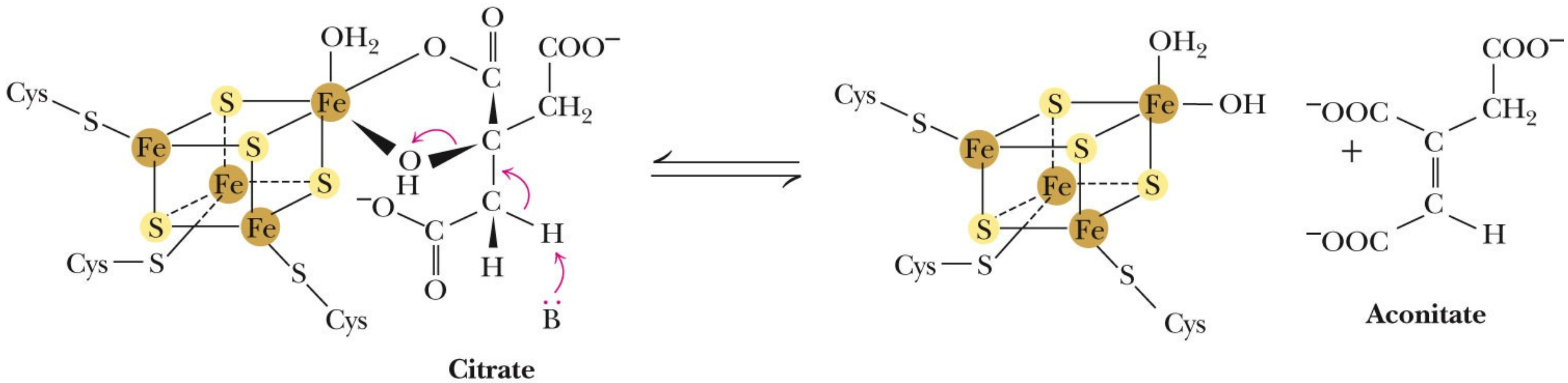
- 2-step process
- H_2O removed to form aconitate and then re-added to form isocitrate



The iron-sulfur cluster of aconitase

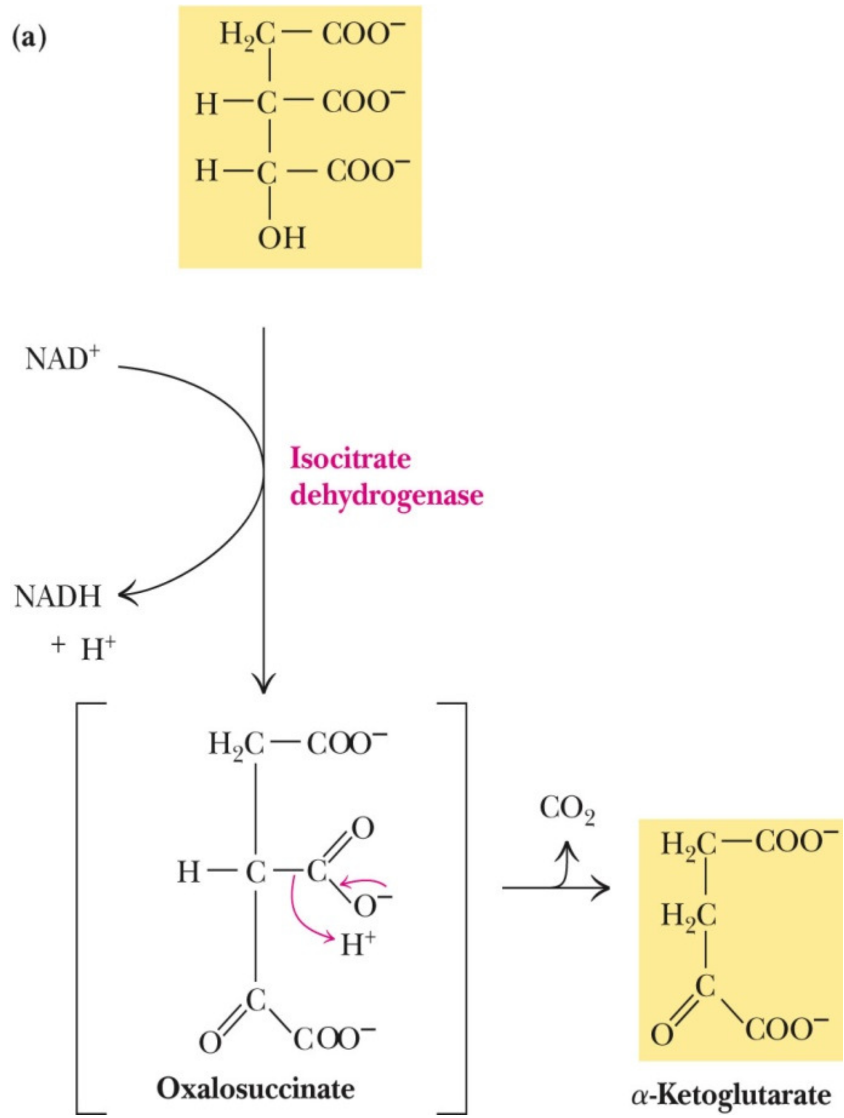
- 3 irons and 4 sulfurs
- 4^{th} iron activates the enzyme

Fe^{3+} in vacant position coordinates carboxyl and OH



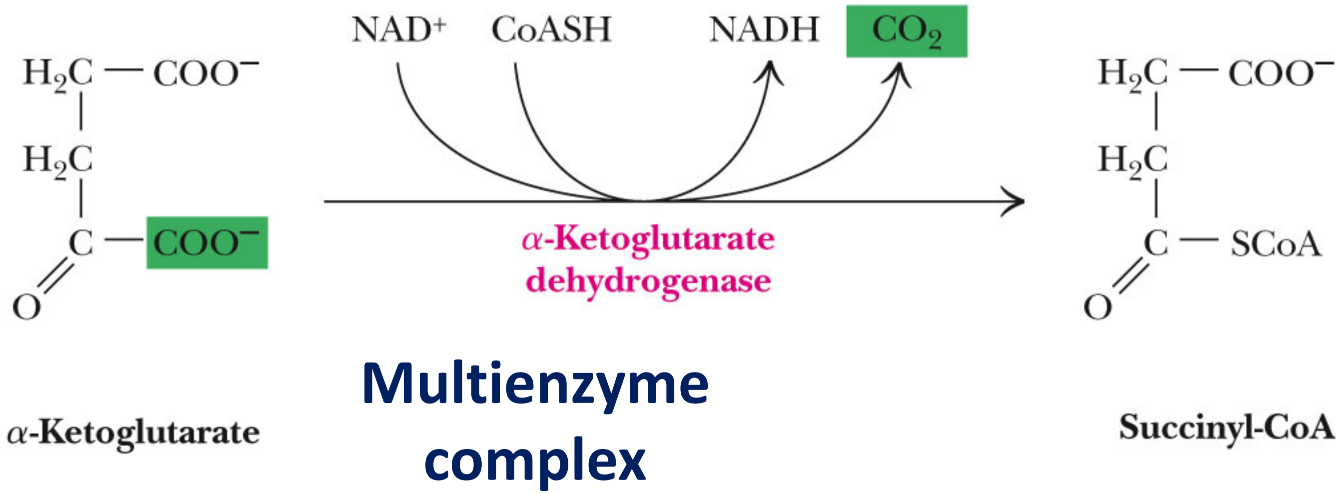
The isocitrate dehydrogenase reaction

- Oxidative decarboxylation
- Links the TCA cycle with the electron transport chain and oxidative phosphorylation



NADH and ATP = allosteric inhibitors
ADP = allosteric activator

The α-ketoglutarate dehydrogenase reaction



- Second oxidative decarboxylation reaction
- NADH, CO_2 and succinyl-Co-A are the products

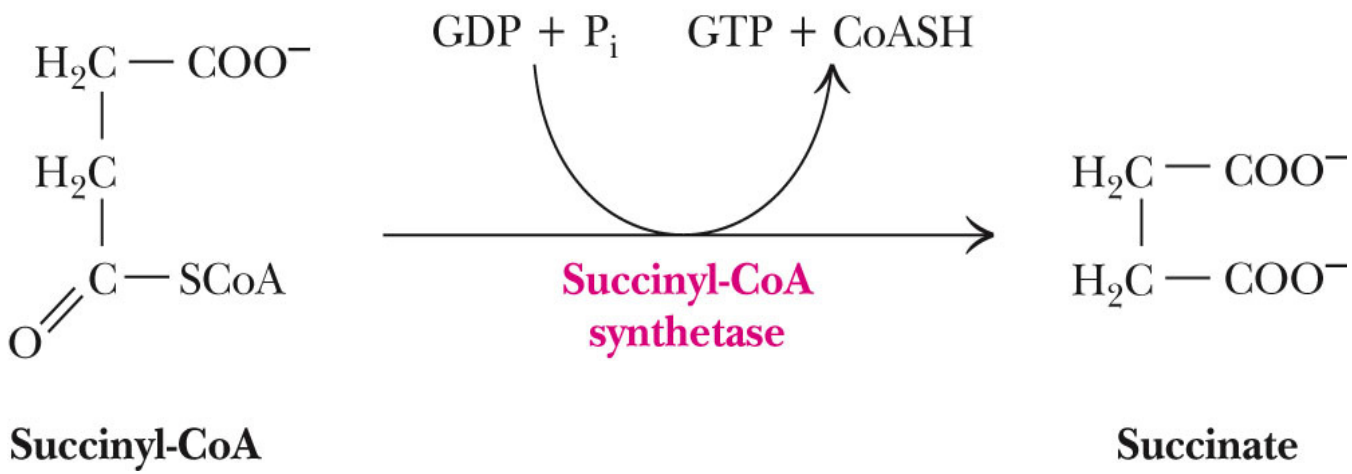
2 energy rich products for use in subsequent reactions

The succinyl Co-A synthetase reaction

2 high energy molecules from previous reaction

Succinyl- CoA

NADH



GTP + ADP → ATP + GDP

The succinate dehydrogenase reaction

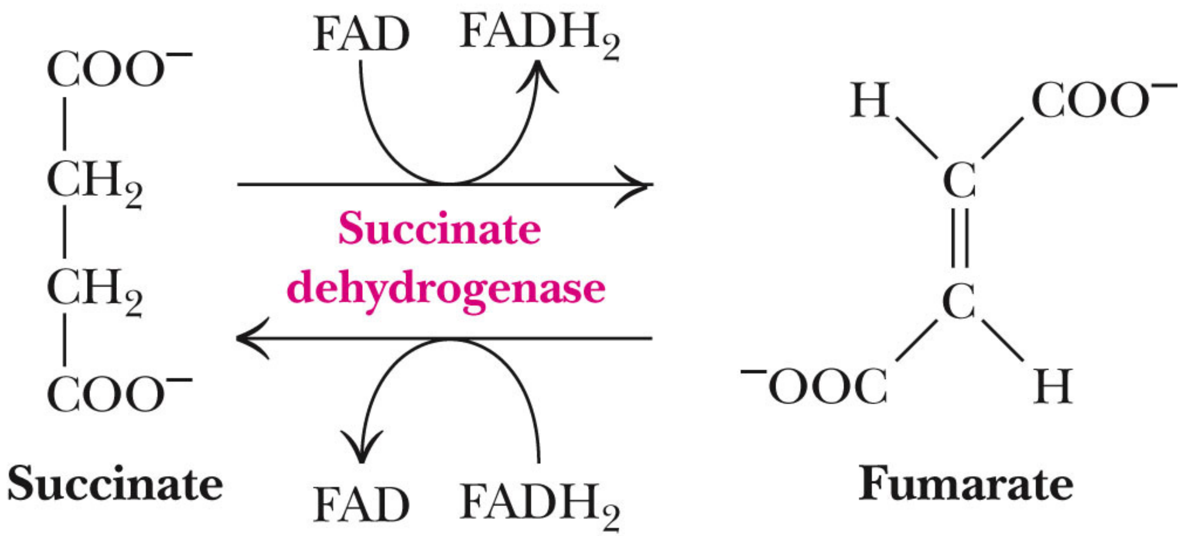
- Membrane-bound enzyme
- Part of the ETC
- Heterodimer

Binds FAD covalently via His residue

- 3 different Fe-S clusters
- Carries out oxidation of succinate to fumerate

- Removal of H across a C-C bond
- Oxidation of an alkane to an alkene
- Not sufficient energy to reduce NAD^+
- Stereospecific reaction

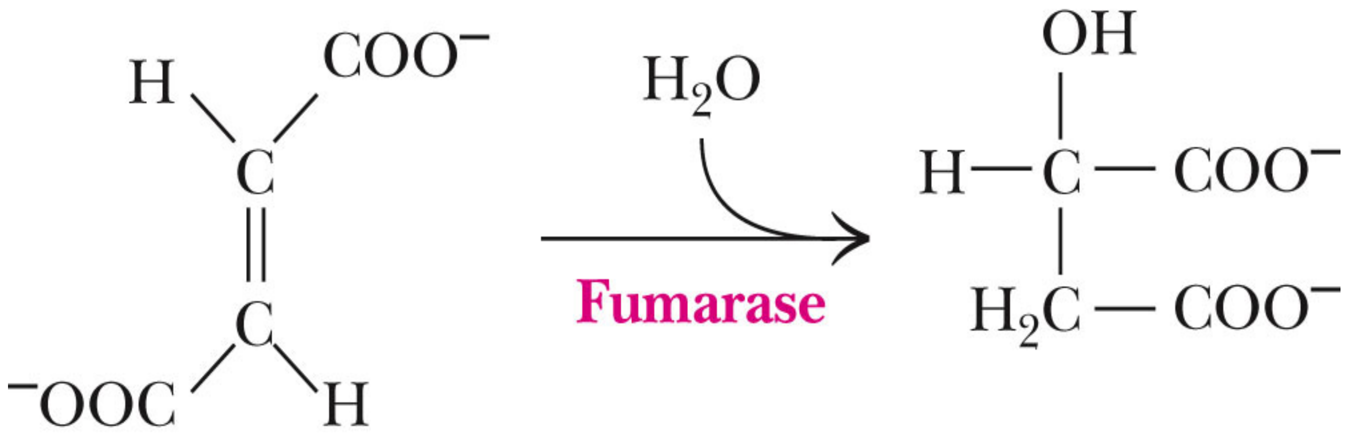
- Electrons → [FAD] →Fe-S clusters → co-Q → ETC



Trans **fumerate formed**

The fumarase reaction

- Trans addition of the elements of water across the double bond

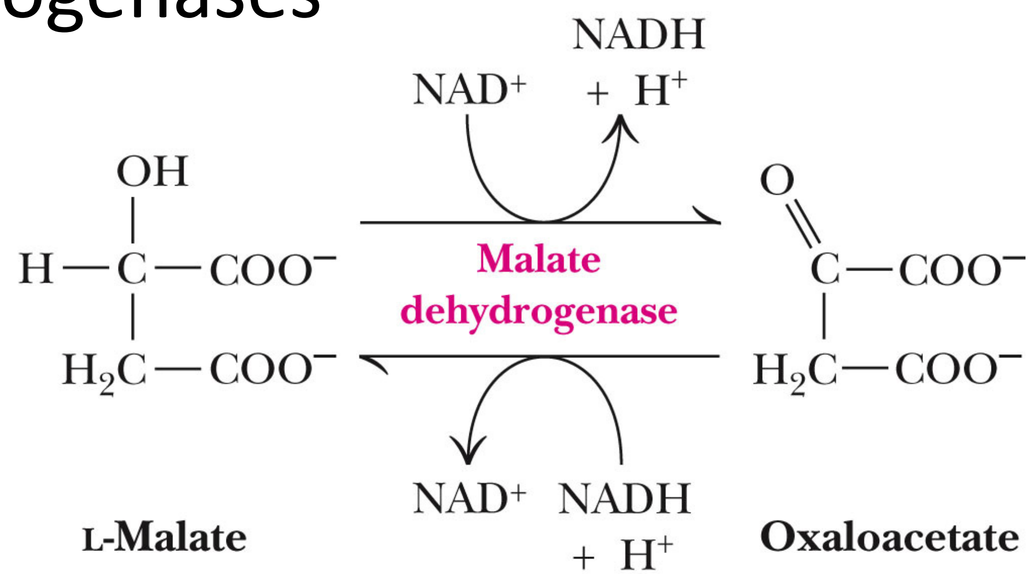


Fumarate

L-Malate

The malate dehydrogenase reaction

- Oxidation reaction coupled to reduction of NAD^+ **4th coenzyme reduced via oxidation of single acetate unit**
- $\Delta G^{o\prime}$ = + 30 kJ/mol
- Structurally and functionally similar to other dehydrogenases



Energetic consequences of TCA cycle

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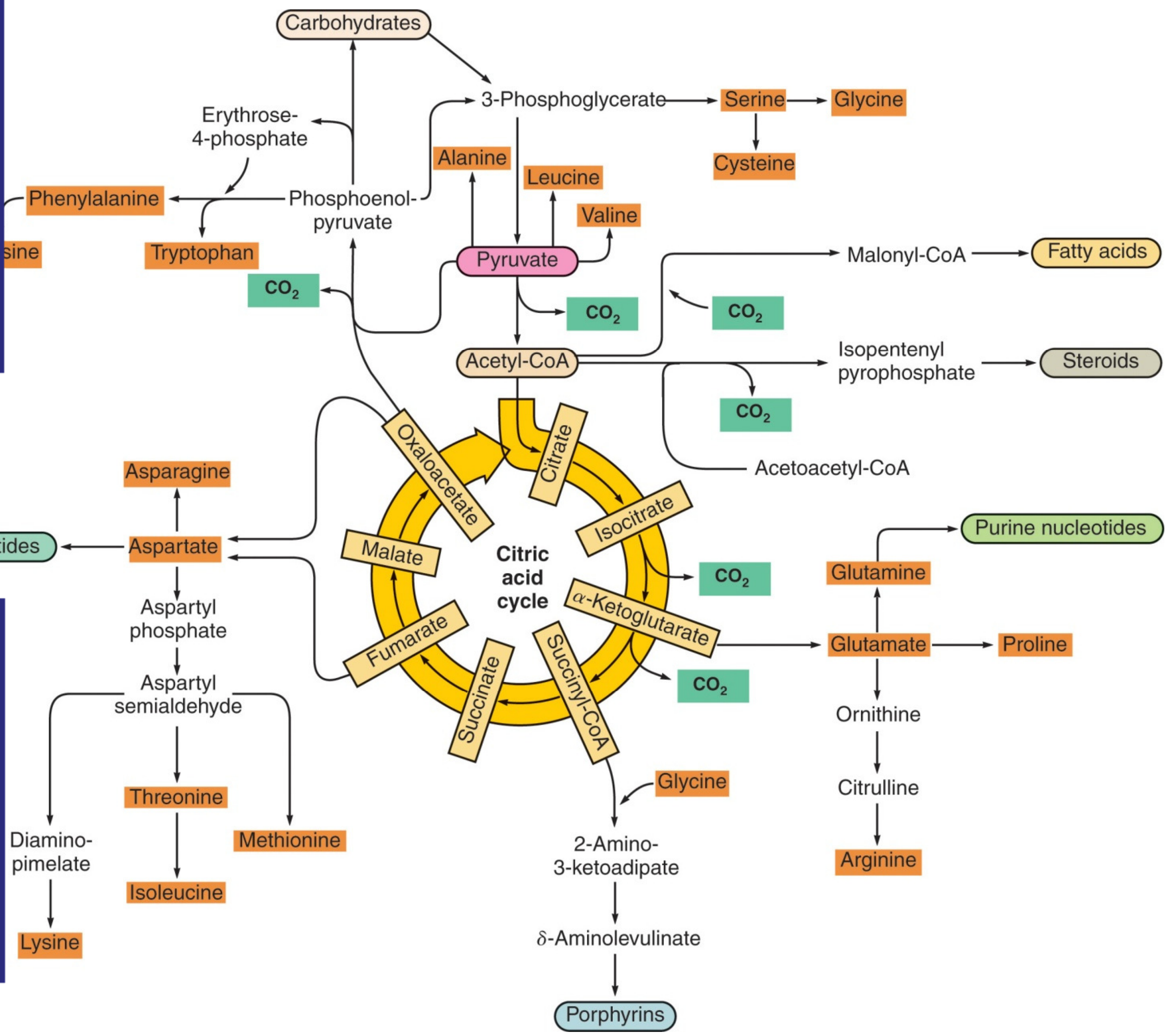
8. L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase	+29.7	≈0

Intermediates serve as starting point for many biosynthetic processes



Anaplerotic reactions feed intermediates back into the cycle

Regulation of the TCA cycle

- Link between glycolysis and ETC
- Must be carefully controlled
- Sites of regulation?

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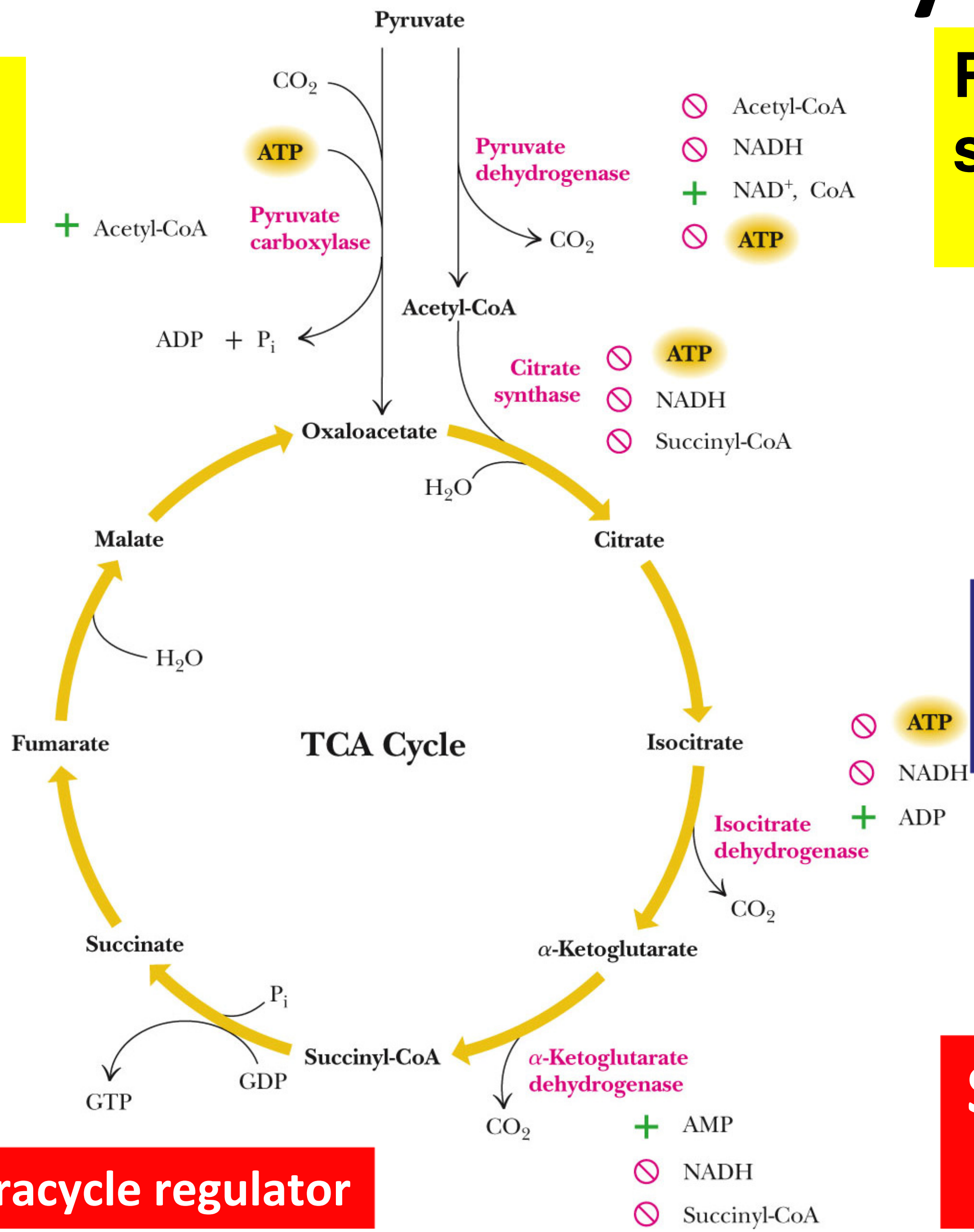
Regulation of the TCA cycle

Sites of regulation:

Regulatory signals:



Inhibits all 4 enzymes

Shuts down cycle

Intracycle regulator

Reduction potentials – chapter 3

- **Standard reduction potential ($\mathcal{E}_o'$)** quantifies the tendency of chemical species to be reduced or oxidised

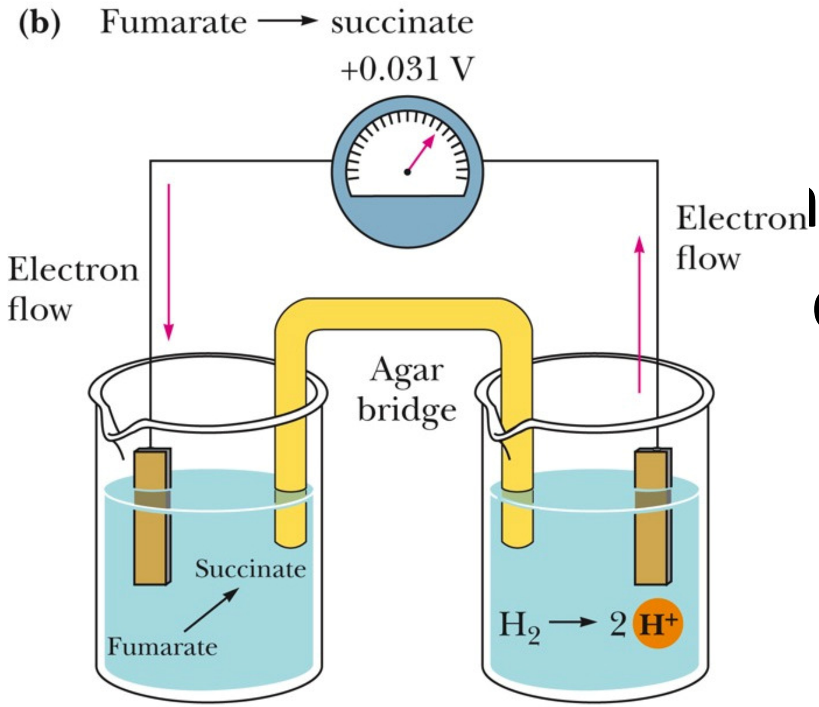
Reduced donor **Oxidised acceptor**

ne^-

Oxidised donor **Reduced acceptor**

- $\mathcal{E}_o'$ is related to the free energy of a process
- ($\mathcal{F}$= Faraday's constant: 96.485 kJ/mol.V)
- **Δ $\mathcal{E}_o'$** =difference in reduction potentials between donor and acceptor. **n** = number of e^- transferred

Measuring standard reduction potential



half cells containing 1 M
e redox couple

Sample Half cell

Reference Half cell

e^- flow towards sample half cell → **+ve** potential

e^- flow away from sample half cell → **-ve** potential

Predicting direction of redox reactions

- Tabulated as **reduction potentials**
- **Positive sign**: substance has tendency to accept electrons
- **Negative sign**: tendency to gain electrons

TABLE 3.5 Standard Reduction Potentials for Several Biological Reduction Half-Reactions

Reduction Half-Reaction	$\mathscr{E}_o'$ (V)
$\frac{1}{2}O_2 + 2\,H^+ + 2\,e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
Photosystem P700	0.430
$NO_3^- + 2\,H^+ + 2\,e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome f (Fe^{3+}) + e^- $\longrightarrow$ cytochrome f (Fe^{2+})	0.365
Cytochrome a_3(Fe^{3+}) + e^- $\longrightarrow$ cytochrome a_3(Fe^{2+})	0.350
Cytochrome a(Fe^{3+}) + e^- $\longrightarrow$ cytochrome a(Fe^{2+})	0.290
Rieske Fe-S(Fe^{3+}) + e^- $\longrightarrow$ Rieske Fe-S(Fe^{2+})	0.280
Cytochrome c (Fe^{3+}) + e^- $\longrightarrow$ cytochrome c (Fe^{2+})	0.254
Cytochrome c_1(Fe^{3+}) + e^- $\longrightarrow$ cytochrome c_1(Fe^{2+})	0.220
UQH· + H^+ + e^- $\longrightarrow$ UQH_2 (UQ = coenzyme Q)	0.190
UQ + 2 H^+ + 2 e^- $\longrightarrow$ UQH_2	0.060
Cytochrome b_H(Fe^{3+}) + e^- $\longrightarrow$ cytochrome b_H(Fe^{2+})	0.050
Fumarate + 2 H^+ + 2 e^- $\longrightarrow$ succinate	0.031
UQ + H^+ + e^- $\longrightarrow$ UQH·	0.030
Cytochrome b_5(Fe^{3+}) + e^- $\longrightarrow$ cytochrome b_5 (Fe^{2+})	0.020
[FAD] + 2 H^+ + 2 e^- $\longrightarrow$ [$FADH_2$]	0.003–0.091*
Cytochrome b_L(Fe^{3+}) + e^- $\longrightarrow$ cytochrome b_L(Fe^{2+})	−0.100
Oxaloacetate + 2 H^+ + 2 e^- $\longrightarrow$ malate	−0.166
Pyruvate + 2 H^+ + 2 e^- $\longrightarrow$ lactate	−0.185
Acetaldehyde + 2 H^+ + 2 e^- $\longrightarrow$ ethanol	−0.197
FMN + 2 H^+ + 2 e^- $\longrightarrow$ $FMNH_2$	−0.219
FAD + 2 H^+ + 2 e^- $\longrightarrow$ $FADH_2$	−0.219
Glutathione (oxidized) + 2 H^+ + 2 e^- $\longrightarrow$ 2 glutathione (reduced)	−0.230
Lipoic acid + 2 H^+ + 2 e^- $\longrightarrow$ dihydrolipoic acid	−0.290
1,3-Bisphosphoglycerate + 2 H^+ + 2 e^- $\longrightarrow$ glyceraldehyde-3-phosphate + P_i	−0.290
NAD^+ + 2 H^+ + 2 e^- $\longrightarrow$ NADH + H^+	−0.320
$NADP^+$ + 2 H^+ + 2 e^- $\longrightarrow$ NADPH + H^+	−0.320
Lipoyl dehydrogenase [FAD] + 2 H^+ + 2 e^- $\longrightarrow$ lipoyl dehydrogenase [$FADH_2$]	−0.340
α-Ketoglutarate + CO_2 + 2 H^+ + 2 e^- $\longrightarrow$ isocitrate	−0.380
2 H^+ + 2 e^- $\longrightarrow$ H_2	−0.421
Ferredoxin (spinach) (Fe^{3+}) + e^- $\longrightarrow$ ferredoxin (spinach) (Fe^{2+})	−0.430
Succinate + CO_2 + 2 H^+ + 2 e^- $\longrightarrow$ α-ketoglutarate + H_2O	−0.670

Analysis of energy changes in redox reactions

$NAD^+ + \text{isocitrate} \rightarrow NADH + H^+ + \alpha kg + CO_2$

$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H$ $\mathcal{E}_o{}' = -0.32V$

$\alpha kg + CO_2 + 2H^+ + 2e^- \rightarrow \text{isocitrate}$ $\mathcal{E}_o{}' = -0.38V$

$$\Delta\mathcal{E}_o{}' = \mathcal{E}_o{}'(\text{acceptor}) - \mathcal{E}_o{}'(\text{donor})$$

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