Biochemistry II (BB 422/622)

OUTLINE

Review of 421
Goals of 422
Review of chemical principles
  Thermodynamics & ENERGY
  C/O cycles
  Overview of Metabolism
  ATP cycle
Energy Coupling
Chemical Reactivity
Bioenergetics
Membranes and Transport

Chemical Reactivity

First, we mentioned the typical type of bond cleavage and kinds of nucleophiles and electrophiles commonly used by the cell in biochemical reactions

Chemical Reactivity

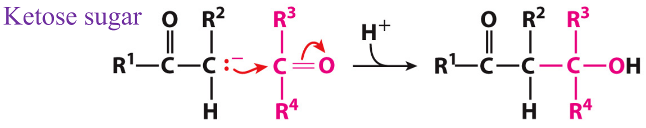
Most biochemical reactions fall within only a few categories:

- Cleavage and formation of C–C bonds
- Cleavage and formation of polar covalent bonds
  - –Nucleophilic substitution mechanism
  - –Addition–elimination mechanism
    - ○ Hydrolysis and Condensation reactions
    - ○ Eliminations (without cleavage)
- Internal rearrangements
- Group transfers (H^+, CH_3^+, PO_3^{2-})
  - –Nucleophilic substitution mechanism
- Oxidations-reductions (e^- transfers)

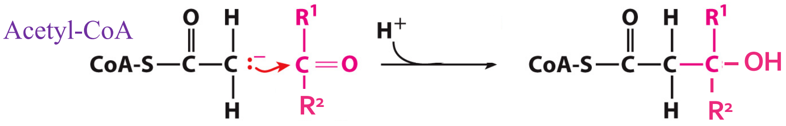
C–C bonds

Chemical Reactivity

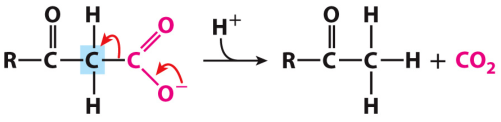
Examples of Nucleophilic Carbon-Carbon Bond Formation and Cleavage Reactions



Aldol condensation



Claisen ester condensation

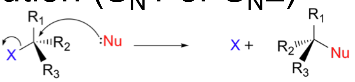


Decarboxylation of a β-keto acid

Chemical Reactivity

Nucleophilic Substitution & Addition-Elimination

- Substitution from sp^3 carbon proceeds normally via the nucleophilic substitution (S_N1 or S_N2) mechanism.

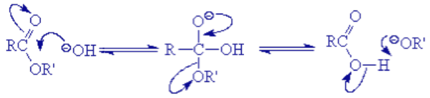


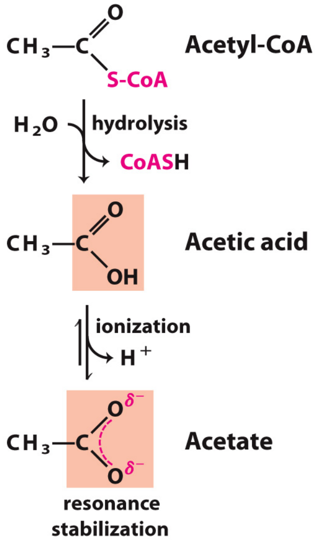
- Substitution from the sp^2 carbon proceeds normally via the nucleophilic addition–elimination mechanism.
  - – nucleophile adds to the sp^2 center, giving a tetrahedral intermediate
  - – leaving group eliminates from the tetrahedral intermediate
  - – leaving group may pick up a proton

Chemical Reactivity

Addition-Elimination

Hydrolysis of Esters, Amides & Thioesters



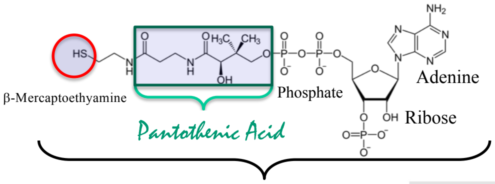


•Hydrolysis of thioesters is strongly favorable.

–EXAMPLE: Acetyl-CoA is an important donor of acyl group

–feeding two-carbon units into metabolic pathways

–synthesis of fatty acids



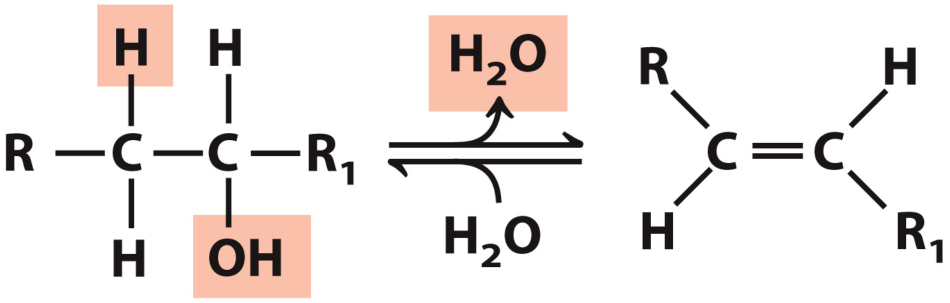
Coenzyme A

$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$
$$\Delta G'^\circ = -31.4 \text{ kJ/mol}$$

Chemical Reactivity

Addition-Elimination

Elimination without Cleavage

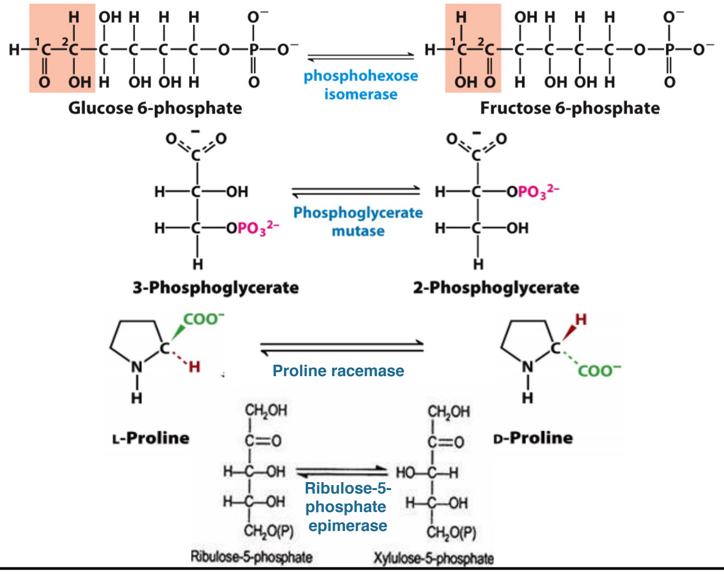


Enzymes will do this stereo-specifically

Chemical Reactivity

Rearrangements

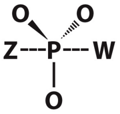
Isomerizations:
(No Change in Oxidation State)



Chemical Reactivity

Group Transfers

- Proton transfer, very common
- Methyl transfer, various biosyntheses
- Acyl transfer, biosynthesis of fatty acids
- Glycosyl transfer, attachment of sugars
- Phosphoryl transfer, to activate metabolites
  - also important in signal transduction
  - proceeds through a nucleophilic substitution mechanism

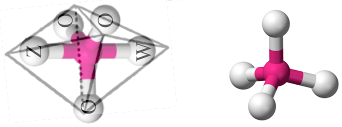


Example of ATP hydrolysis

Z = R—OH (nucleophile)

W = ADP (leaving group)

Sometimes called "bi-pyramidal"



Substitution from sp^3 phosphorous proceeds via the nucleophilic substitution (usually associative, S_N2-like) mechanism.

The nucleophile forms a partial bond to the phosphorous center, giving a pentacovalent intermediate or a penta-coordinated transition state.

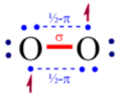
Chemical Reactivity

Re-dox

Lessons from Quantum Chemistry

- Most organic molecules, including the reduced fuels, are in the singlet spin state.
  - All electrons are paired.

- Molecular oxygen is in the triplet spin state.
  - Two electrons are unpaired.

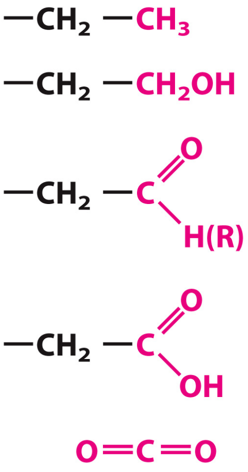
$^3O_2 \equiv$


- Direct electron transfer from a singlet reduced species to a triplet-oxidizing species is quantum-mechanically unfavorable.
- This is why spontaneous oxidation of biomolecules (carbon oxidation states differ by $2e^-$) does not occur readily, despite the exergonic reaction thermodynamics.
- Cofactors, such as transition-metal ions and flavin adenine dinucleotide (FAD), are able to catalyze consecutive single-electron transfers needed for utilization of O_2.

Chemical Reactivity

Oxidation-Reduction Reactions

Reduced organic compounds serve as fuels from which electrons can be stripped off during oxidation.



Most energy

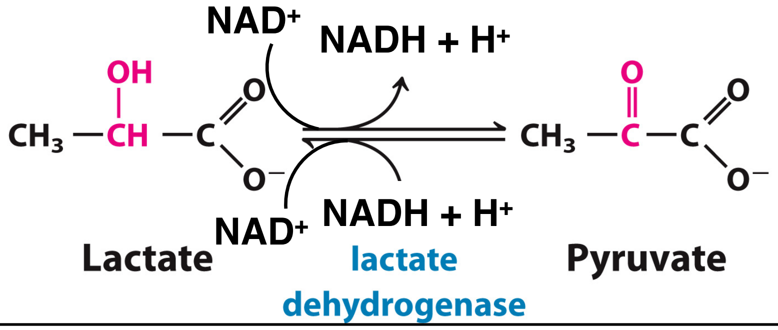
	Relative ΔG°' of oxidation per carbon (kcal/mol)
Alkane	−196
Alcohol	−168
Aldehyde (ketone)	−125
Carboxylic acid	−68
Carbon dioxide	0

Least energy

Chemical Reactivity

EXAMPLE: Reversible Oxidation of a Secondary Alcohol to a Ketone

- Many biochemical oxidation-reduction reactions, especially those of carbon, involve transfer of two electrons.
- In order to keep charges in balance, proton transfer often accompanies electron transfer.
- In many dehydrogenases, the reaction proceeds by a stepwise transfers of proton (H^+) and hydride ($:H^-$).



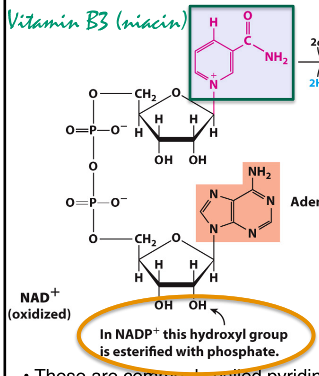
Chemical Reactivity

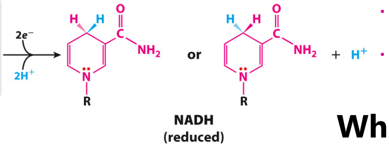
Re-dox

Common Redox Cofactors: NADH, NADPH, and $FADH_2$

- Niacin Cofactors allow Hydride (2 e⁻) Electron Transfers
- Flavin Cofactors allow single Electron Transfers

Vitamin B3 (niacin)

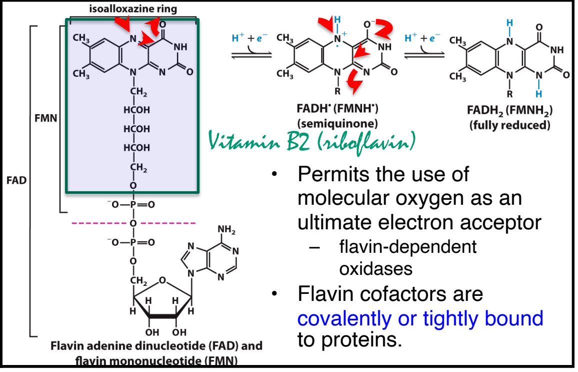




- Measure the change of absorbance at 340 nm
- Very useful signal when studying the kinetics of NAD⁺-dependent dehydrogenases

- These are commonly called pyridine nucleotides.
- They can dissociate from the enzyme after the reaction.
- In a typical biological oxidation reaction, hydride from an alcohol is transferred to NAD⁺, giving NADH.

What about FAD?



Vitamin B2 (riboflavin)

- Permits the use of molecular oxygen as an ultimate electron acceptor
  - flavin-dependent oxidases
- Flavin cofactors are covalently or tightly bound to proteins.

Bioenergetics

Energetics of Biochemical Reactions:

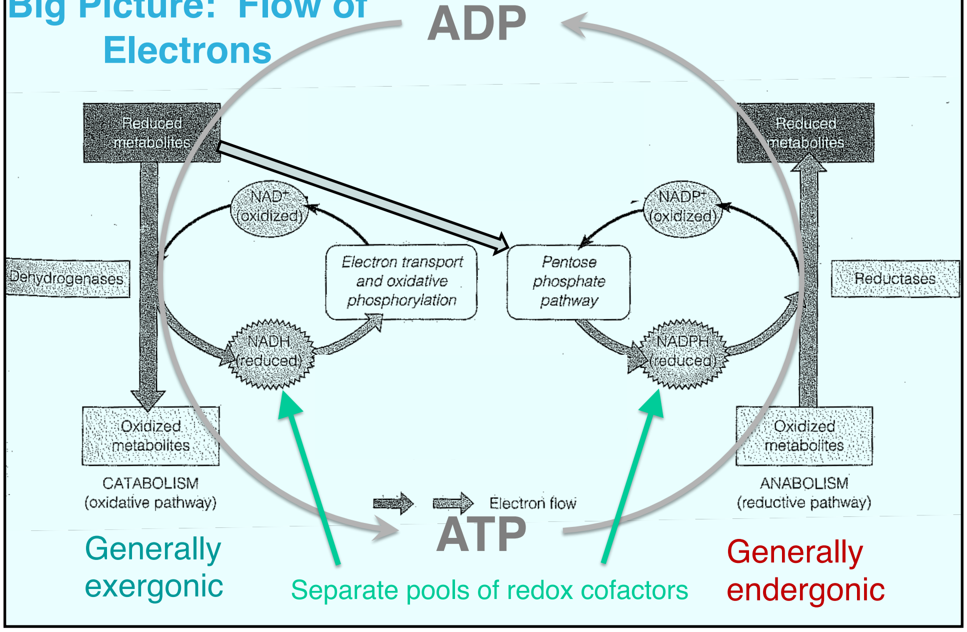
Bioenergetics

Energetics of Some Chemical Reactions:

- Hydrolysis reactions tend to be strongly favorable ~~(spontaneous).~~
  - Recall that being thermodynamically favorable is not the same as being kinetically rapid.
- Isomerization reactions have smaller free-energy changes.
  - isomerization between enantiomers: $\Delta G^{\circ\prime} = 0$
- Complete oxidation of reduced compounds (redox reactions) is strongly favorable.
  - This is how heterotrophs (sometimes called chemotrophs) obtain most of their energy.
  - In biochemistry, the oxidation of reduced fuels with O_2 is stepwise and controlled.

Bioenergetics

Big Picture: Flow of Electrons



Generally exergonic

Separate pools of redox cofactors

Generally endergonic

Bioenergetics

Reduction Potential determines the Flow of Electrons

$A_{ox} + B_{red} \rightleftharpoons A_{red} + B_{ox}$

- Reduction potential (E)
  - affinity for electrons; higher E, higher affinity
  - electrons transferred from lower E to higher E

$$\Delta E^{\circ\prime} = E^{\circ\prime}_{(e-\ \text{acceptor})} - E^{\circ\prime}_{(e-\ \text{donor})}$$

(that which is reduced) (that which is oxidized)

$$\Delta E^{\circ\prime} = (RT / n\mathscr{F})\ln (K_{eq}) = -\Delta G^{\circ\prime}/n\mathscr{F}$$

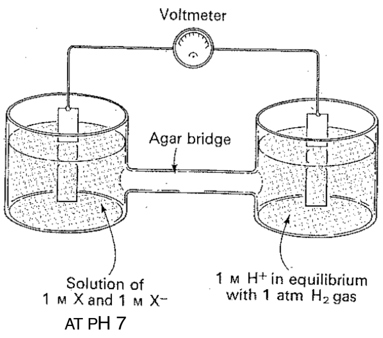
$$\boldsymbol{\Delta G^{\circ\prime} = -n\mathscr{F}\Delta E^{\circ\prime}}$$

- For negative ΔG, need positive ΔE

$$E^{\circ\prime}_{(\text{acceptor})} > E^{\circ\prime}_{(\text{donor})}$$

Bioenergetics

Standard Reduction Potential ($E^{o\prime}$) determines the Flow of Electrons



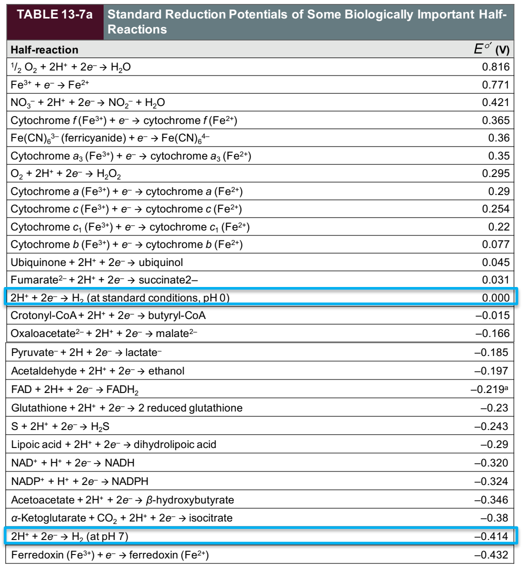
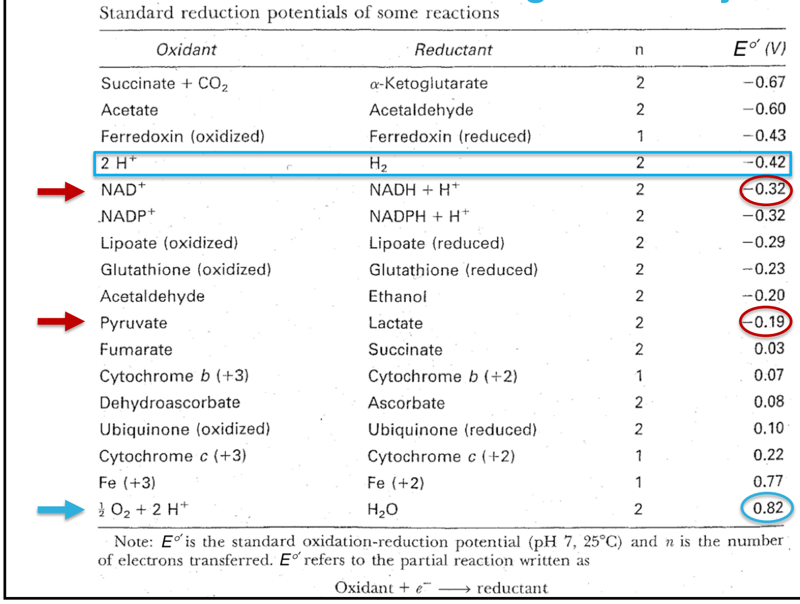


TABLE 13-7a Standard Reduction Potentials of Some Biologically Important Half-Reactions

Half-reaction	$E^{\circ\prime}$ (V)
$^1/_2\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	0.421
Cytochrome *f* (Fe^{3+}) + e^- → cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + e^- → $Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + e^- → cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + e^- → cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* (Fe^{3+}) + e^- → cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + e^- → cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* (Fe^{3+}) + e^- → cytochrome *b* (Fe^{2+})	0.077
Ubiquinone + $2H^+$ + $2e^-$ → ubiquinol	0.045
Fumarate^{2-} + $2H^+$ + $2e^-$ → succinate2–	0.031
$2H^+ + 2e^- \rightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+$ + $2e^-$ → butyryl-CoA	–0.015
Oxaloacetate^{2-} + $2H^+$ + $2e^-$ → malate^{2-}	–0.166
Pyruvate$^-$ + 2H + $2e^-$ → lactate$^-$	–0.185
Acetaldehyde + $2H^+$ + $2e^-$ → ethanol	–0.197
FAD + 2H+ + $2e^-$ → $FADH_2$	–0.219[a]
Glutathione + $2H^+$ + $2e^-$ → 2 reduced glutathione	–0.23
$S + 2H^+ + 2e^- \rightarrow H_2S$	–0.243
Lipoic acid + $2H^+$ + $2e^-$ → dihydrolipoic acid	–0.29
$NAD^+ + H^+ + 2e^- \rightarrow NADH$	–0.320
$NADP^+ + H^+ + 2e^- \rightarrow NADPH$	–0.324
Acetoacetate + $2H^+$ + $2e^-$ → *β*-hydroxybutyrate	–0.346
α-Ketoglutarate + CO_2 + $2H^+$ + $2e^-$ → isocitrate	–0.38
$2H^+ + 2e^- \rightarrow H_2$ (at pH 7)	–0.414
Ferredoxin (Fe^{3+}) + e^- → ferredoxin (Fe^{2+})	–0.432

Bioenergetics

Reduction Potentials with higher affinity at bottom



Standard reduction potentials of some reactions

Oxidant	Reductant	n	$E^{\circ\prime}$ (V)
Succinate + CO_2	α-Ketoglutarate	2	−0.67
Acetate	Acetaldehyde	2	−0.60
Ferredoxin (oxidized)	Ferredoxin (reduced)	1	−0.43
2 H^+	H_2	2	−0.42
NAD^+	NADH + H^+	2	−0.32
$NADP^+$	NADPH + H^+	2	−0.32
Lipoate (oxidized)	Lipoate (reduced)	2	−0.29
Glutathione (oxidized)	Glutathione (reduced)	2	−0.23
Acetaldehyde	Ethanol	2	−0.20
Pyruvate	Lactate	2	−0.19
Fumarate	Succinate	2	0.03
Cytochrome *b* (+3)	Cytochrome *b* (+2)	1	0.07
Dehydroascorbate	Ascorbate	2	0.08
Ubiquinone (oxidized)	Ubiquinone (reduced)	2	0.10
Cytochrome *c* (+3)	Cytochrome *c* (+2)	1	0.22
Fe (+3)	Fe (+2)	1	0.77
$\frac{1}{2}O_2 + 2H^+$	H_2O	2	0.82

Note: $E^{\circ\prime}$ is the standard oxidation-reduction potential (pH 7, 25°C) and *n* is the number of electrons transferred. $E^{\circ\prime}$ refers to the partial reaction written as

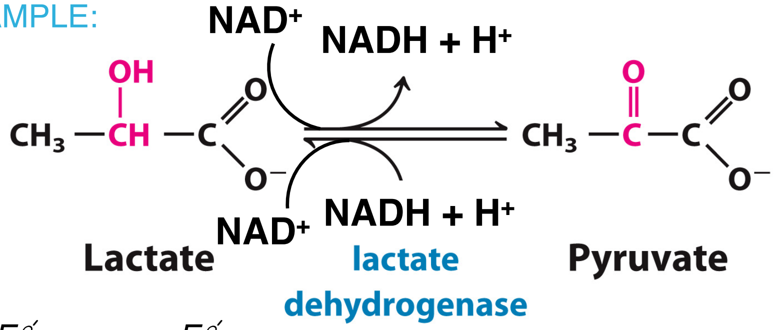
Oxidant + e^- ⟶ reductant

Hates e^-

Loves e^-

Bioenergetics

EXAMPLE:



$$\Delta E^{\circ\prime} = E^{\circ\prime}_{(reduction)} - E^{\circ\prime}_{(oxidation)}$$

$$= -0.32\ \text{V} - (-0.19\ \text{V})$$

$$= -0.13\ \text{V}$$

This is why we always ran the reaction the other way!

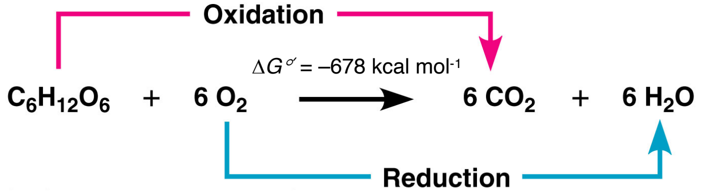
$$\Delta G^{\circ\prime} = -n\mathscr{F}\Delta E^{\circ\prime}$$

$$= -(2)(23.06\ \text{kcal mol}^{-1}\ \text{V}^{-1})(-0.13\ \text{V})$$

$$= +6\ \text{kcal mol}^{-1}$$

Bioenergetics

EXAMPLE:



Oxidation

$\Delta G^{o\prime} = -678$ kcal mol^{-1}

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O$$

Reduction

What is the $\Delta E^{o\prime}$ for the complete oxidation of glucose?

$$\Delta G^{o\prime} = -n\,\mathscr{F}\,\Delta E^{o\prime}$$

$$-678 \text{ kcal mol}^{-1} = -(24)(23.06 \text{ kcal V}^{-1}\text{mol}^{-1})(x \text{ V})$$

$$\Delta E^{o\prime} = +1.25 \text{ V}$$

What is the reduction potential ($E^{o\prime}$) for Glc → CO_2?

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

$$+1.25 \text{ V} = +0.82 \text{ V} - (E^{o\prime}_{(\text{Glc } oxidation)})$$

$$E^{o\prime}_{(\text{Glc } oxidation)} = -0.43 \text{ V}$$

Summary

We learned that:

- The rules of thermodynamics and organic chemistry still apply to living systems
- Reactions are favorable when the free energy of products is much lower than the free energy of reactants
- Biochemical phosphoryl transfer reactions are favorable when:
  - the phosphate donors are destabilized by electrostatic repulsion
  - the reaction products are often stabilized by resonance
- Unfavorable reactions can be made possible by chemically coupling a highly favorable reaction to the unfavorable reaction
- Oxidation-reduction reactions commonly involve transfer of electrons from reduced organic compounds to specialized redox cofactors in generally favored reactions
  - Reduced cofactors can be used in biosynthesis, or may serve as a source of energy for ATP synthesis.

Biological Membrane Transport

"Crossing the Berlin Wall of the cell - Membrane Transport"
– Dr. Kornberg

Physical Properties of Membranes

- Not permeable to large polar solutes and ions
- Permeable to nonpolar compounds and some small polar compounds (e.g., water)
- Permeability can be artificially increased by chemical treatment.
  - when we want to get DNA into the cell
- Very stable yet dynamic and flexible structures
- Can exist in various phases and undergo phase transitions
- Can fuse; and can have shapes, e.g., endocytosis
- Additional functionality provided by embedded proteins
  - recall peripheral, integral, and amphitropic proteins
  - recall that membrane proteins have hydrophobic regions