Biochemistry II (BI/CH 422 & BI/CH 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

Chemical Reactivity

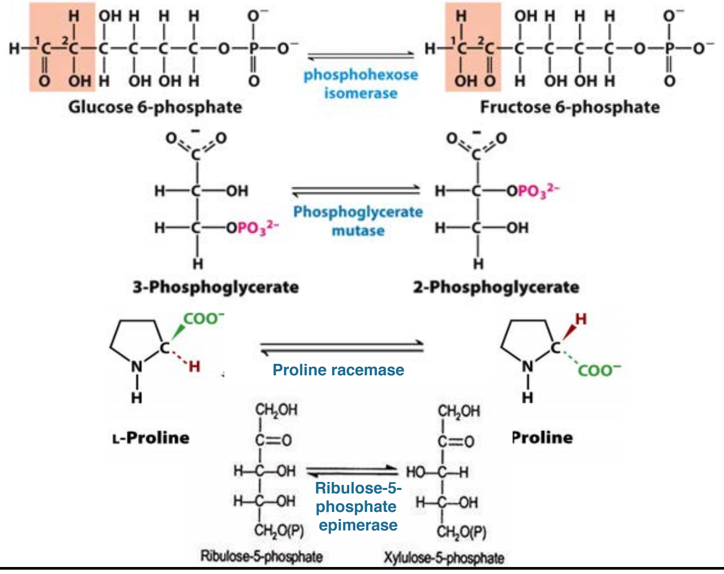
Most biochemical reactions fall within only a few categories:

- •Cleavage and formation of C–C bonds
- •Cleavage and formation of polar covalent bonds (Addition-Elimination Reactions)
  - –nucleophilic substitution mechanism
  - –addition–elimination mechanism
    - o hydrolysis and condensation reactions
    - o Eliminations (without cleavage)
- •Internal rearrangements
- •Group transfers (H^+, CH_3^+, PO_3^{2-})
- •Oxidations-reductions (e^- transfers)

Chemical Reactivity

Rearrangements

Isomerizations and Eliminations:
(No Change in Oxidation State)



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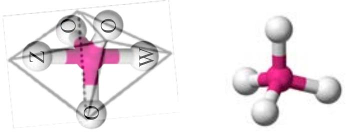
Chemical Reactivity

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

Z = R—OH (nucleophile)
W = ADP (leaving group)



Substitution from sp^3 phosphorous proceeds via the nucleophilic substitution (usually associative, S$_N$2-like) mechanism.

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

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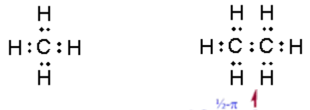
Re-dox

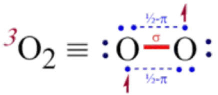
Chemical Reactivity

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.



- 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.

Re-dox

Chemical Reactivity

Oxidation-Reduction Reactions

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

Relative ΔG°' of oxidation per carbon (kcal/mol)

Most energy

$—CH_2—CH_3$ **Alkane** −196

−168

−125

−68

Least energy 0

Re-dox

Chemical Reactivity

Oxidation-Reduction Reactions

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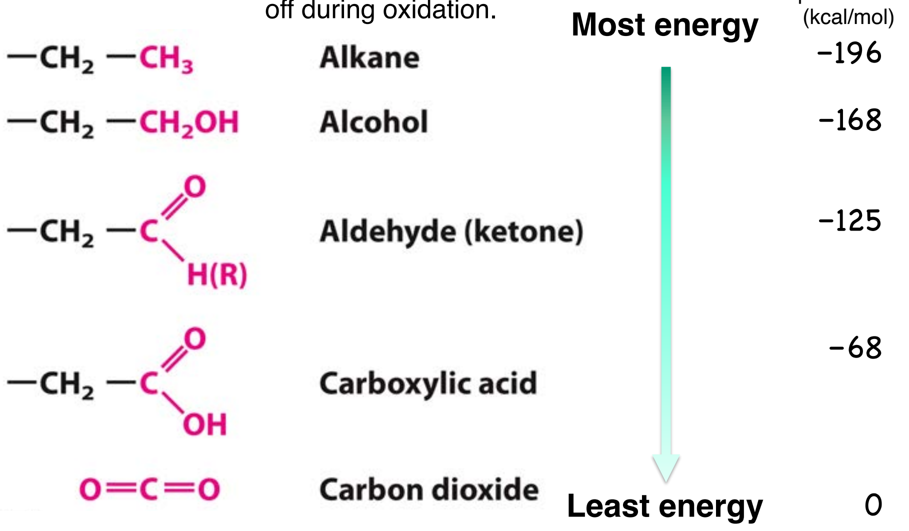


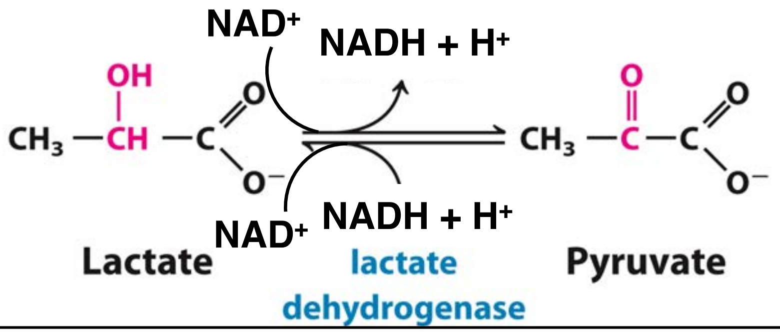
		Relative ΔG°' of oxidation per carbon (kcal/mol)
$-CH_2-CH_3$	**Alkane**	−196
$-CH_2-CH_2OH$	**Alcohol**	−168
	Aldehyde (ketone)	−125
	Carboxylic acid	−68
$O=C=O$	**Carbon dioxide**	0

Re-dox

Chemical Reactivity

EXAMPLE: Reversible Oxidation of a Secondary Alcohol to a Ketone

- Many biochemical oxidation-reduction reactions 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 ($:H^-$).



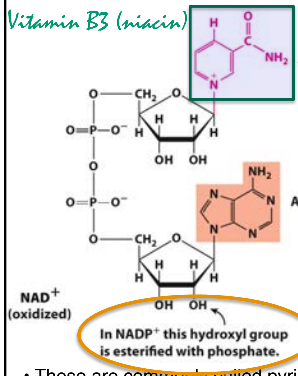
Re-dox

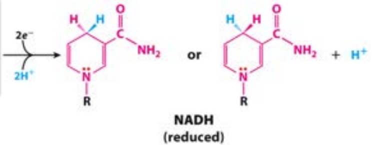
Chemical Reactivity

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

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

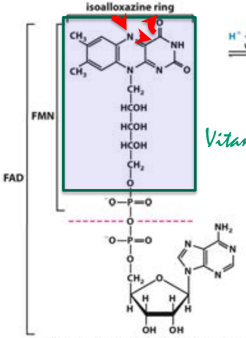
Vitamin B3 (niacin)



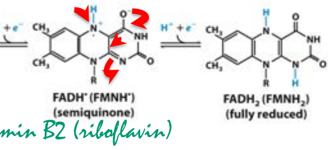


- Measure the change of absorbance at 340 nm
- Very useful signal when studying the kinetics of NAD-dependent dehydrogenases

- 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.



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)



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:

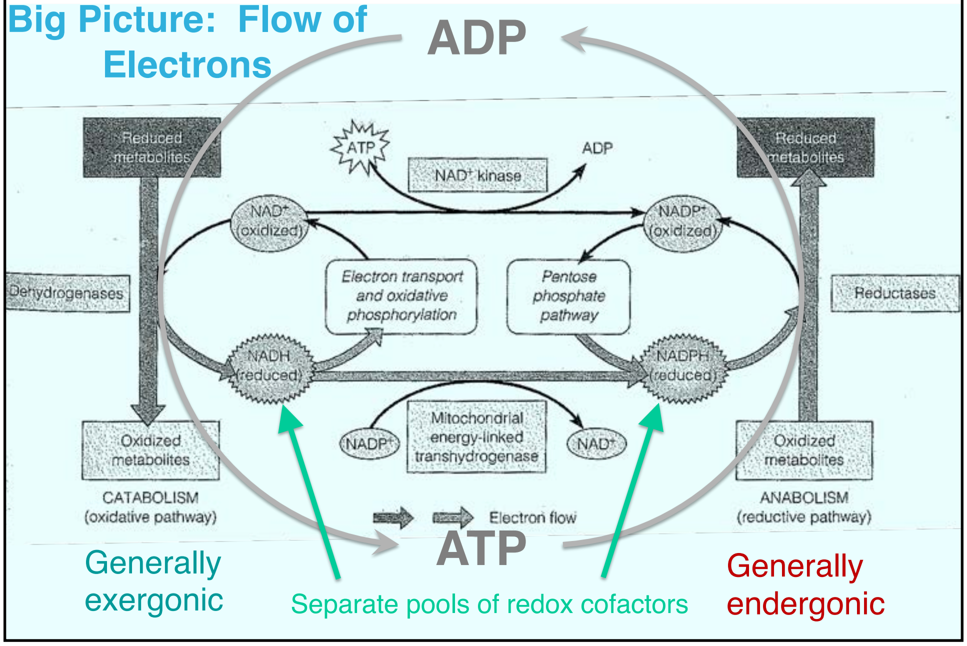
Bioenergetics

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: $\Delta G^\circ = 0$
- 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

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

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

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

- For negative ΔG, need positive ΔE

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

Bioenergetics

Reduction Potential

determines the Flow of Electrons

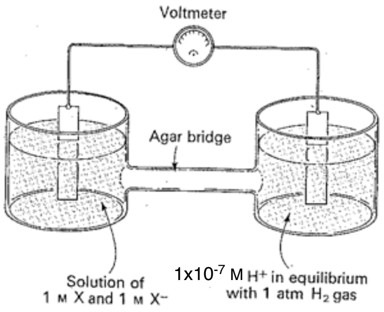
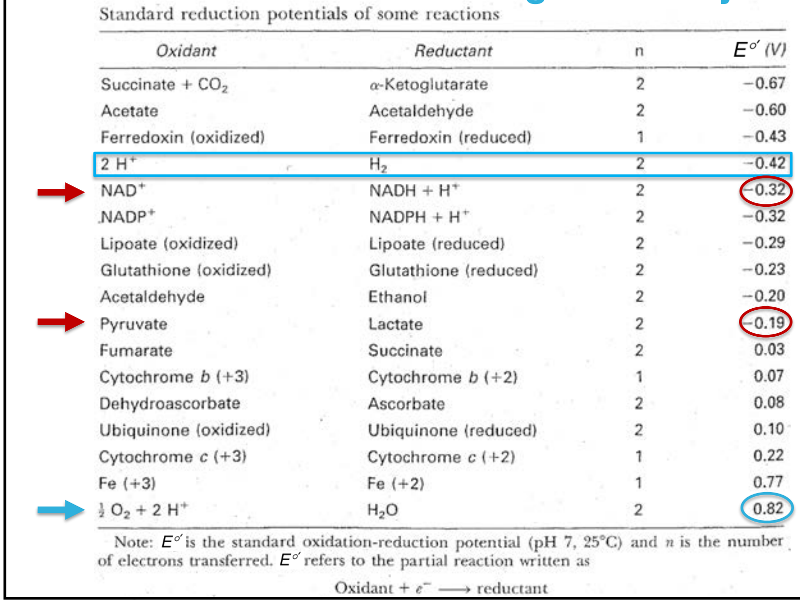


TABLE 13-7a Standard Reduction Potentials of Some Biologically Important Half-Reactions

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
$NAD^+ + H^+ + 2e^-$ → NADH	–0.320
$NADP^+ + H^+ + 2e^-$ → 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

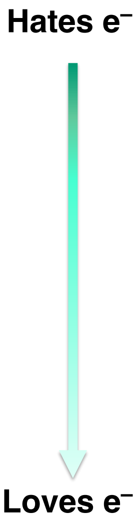


Standard reduction potentials of some reactions

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

$$\text{Oxidant} + e^- \longrightarrow \text{reductant}$$

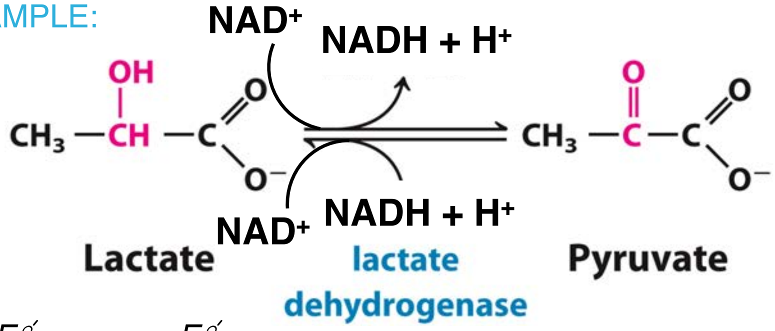


Hates e⁻

Loves e⁻

Bioenergetics

EXAMPLE:



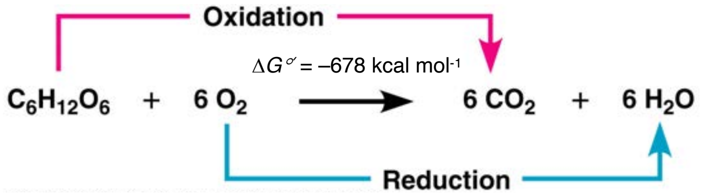
$$\Delta E^{\circ\prime} = E^{\circ\prime}_{(reduction)} - E^{\circ\prime}_{(oxidation)}$$
$$= -0.32\ \text{V} - (-0.19\ \text{V})$$
$$= -0.13\ \text{V}$$

$$\Delta G^{\circ\prime} = -n\mathscr{F}\Delta E^{\circ\prime}$$
$$= -(2)(23.06\ \text{kcal}\,\text{V}^{-1}\text{mol}^{-1})(-0.13\ \text{V})$$
$$= +6\ \text{kcal}\,\text{mol}^{-1}$$

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

Bioenergetics

EXAMPLE:



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

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

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

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

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

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

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

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

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
  - Reduced cofactors can be used in biosynthesis, or may serve as a source of energy for ATP synthesis.