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1. Review of electrophiles and nucleophiles in biology

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<th>Nucleophiles</th>
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<tr>
<td>$H^+$</td>
<td>$ROH \rightleftharpoons RO^{-}$ + $H^+$</td>
</tr>
<tr>
<td>$M^{n+}$</td>
<td>$RSH \rightleftharpoons RS^{-}$ + $H^+$</td>
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<td>Protons</td>
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2. Carbonyl Chemistry: Mechanisms of C-C Bond Formation

- There are three general ways to form C-C bonds:
  1. Aldol reactions where an aldehyde is condensed in a reversible reaction with another aldehyde or a ketone;
  2. Claisen reaction in which a Coenzyme A thioester is condensed with an aldehyde or a ketone;
  3. Prenyl transfer reactions where two 5 carbon units are condensed to form isoprenoids.

- The aldol and claisen reactions involve carbonyl chemistry. These reactions are prevalent in glycolysis, fatty acid biosynthesis and degradation and the pentose phosphate pathway.

1. Aldol reaction generalizations:
   a. All aldol reactions occur with retention of configuration.
   b. All aldol reactions are reversible.
   c. All aldol reactions involve carbanion intermediates.

- Generic mechanism:
The increase in acidity at the a hydrogens involves two mechanisms:

a. Activation by imine formation with a lysine in the active site (class I aldolases found in eucaryotes)

b. Use of Zn\(^{2+}\) (class II aldolases found in procaryotes)

In addition, the “C” of \(\text{C} \equiv \text{Y}\) is activated by both mechanism (a.) and (b.) for nucleophilic attack.
2. Claisen reaction generalizations:
   a. All Claisen reactions occur with inversion of configuration.
   b. All Claisen involve carbanion intermediates.
   c. Most Claisen reactions involve thioesters of Coenzyme A (RS⁻).
   - The phosphopantetheine arm (shown in red) can also be covalently attached to a serine of an Acyl Carrier Protein (ACP).
   
   - Generic mechanism:
• Why has nature chosen thioesters and not oxygen esters?
  - Thioesters have two essential properties:
    i. They are activated for nucleophilic attack relative to oxygen esters
    ii. Their $\alpha$ hydrogens are more acidic than oxygen esters (pKa of 18 vs 22).
• The thioester is more ketone-like than the oxygen ester because in the oxygen ester, the non-bonded electrons from the oxygen of the ester can be resonance delocalized with the $\pi$ electrons of the carbonyl.
  a. With the thioester, this delocalization is limited because of the poor electron overlap due to energetic differences.
  b. With the $\alpha$ hydrogen of the thioester, the delocalization is more favorable with the thioester than oxygen ester, for similar reasons.

![Diagram of thioester and oxygen ester comparisons](image)

a. $\text{H}_2\text{C} - \text{O} - \text{OR}$ vs $\text{H}_2\text{C} - \text{O}^+ - \text{R}$

![pKa values](image)

b. $\text{H}_3\text{C} - \text{H} - \text{C} - \text{O} - \text{OE}$ vs $\text{H}_3\text{C} - \text{H} - \text{C} - \text{O} - \text{SE}$

pKa = 10.5 vs pKa = 8.5
3. Prenyl transfer reactions: A third way to form C-C bonds
(not covered in 5.07)

3. Prenyl transfer reaction generalizations:
   a. All prenyl transfer reactions involve dimethylallyl pyrophosphate (DMAPP) and isopentenylpyrophosphate (IPP)
   b. Most reactions involve carbox cation intermediates

• Generic mechanism:
4. Redox Chemistry: Flavins (FAD, FMN, riboflavin) and nicotinamide adenine dinucleotides (NAD\(^+\) or NADP\(^+\))

- Three forms of flavin:

- Generalizations:
  a. FAD (FMN) are major mediators between 2 e\(^-\) donors and 1 e\(^-\) acceptors.
  b. Redox potentials can be modulated over 600 mv (E\(^o\) = -450 to +150 mv) by the protein environment.
  c. Flavins are tightly or covalently bound to most proteins, therefore they need to be reoxidized on the protein to complete the catalytic cycle.
  d. Reduced flavins (FADH\(_2\) or FMNH\(_2\)) are very O\(_2\) labile, that is, rapidly oxidized with O\(_2\).
  e. Chemistry of flavins occurs at the C4a position or the N5 position. (Ex hydride from NADH is transferred to the N5 of the flavin, see example on next page.)
Redox states accessible to flavins under physiological conditions

**Oxidized (yellow)**
- Neutral

**Semiquinoine (blue and red)**
- Reduced (no visible absorption at 400nm)

**Anionic**
- pKa = 10
- pKa = 8.3
- pKa = 6.7

Chemical structures and pKa values are shown with arrows indicating oxidation and reduction processes.
N5 and C4a chemistry of flavins

- **N5 addition of hydride**

  \[
  \text{FAD or FMN (Fl}_{\text{ox}}) \quad \rightarrow \quad \text{FADH}_2 \text{ or FMNH}_2 \text{ (FlH}_2) 
  \]

- **C4a addition of RS\(^{-}\)**

  \[
  \text{FAD or FMN (Fl}_{\text{ox}}) \quad \rightarrow \quad \text{Fl-4a-thiol} 
  \]

- **C4a addition of O\(_2\)\(^{-}\) to FlH\(^{\bullet}\) (not covered in 5.07)**

  \[
  \text{FAD or FMN (Fl}_{\text{ox}}) \quad \rightarrow \quad \text{Fl-4a-OO}^{-}\text{ or Fl-4a-OOH} 
  \]
Difference in absorption is basis for spectrophotometric assay.

Generalizations:

a. NAD\(^+\) mediates only 2 e\(^-\) chemistry.
b. Redox potential does not vary with protein environment (E\(^o\) = -320 mv).
c. NAD functions as a substrate, comes off and on the enzyme on every turnover.
d. NADH is not O\(_2\) sensitive.
e. The chemistry of NAD involves hydride (H\(^-\)) and proton (H\(^+\)) transfer. Don’t forget the proton as it can dramatically effect equilibrium measurements.
f. NAD in limited cases can be tightly (non-covalently) bound and be involved in masked redox chemistry, that is, it is reduced and reoxidized during conversion of substrate to product.
Example of how NADH reduces flavins through N5 chemistry

- Hydride transfer mechanism:
5. **Mg\(^{2+}\)**-ATP is the energy currency of the cell

- ATP is kinetically stable and thermodynamically labile.

- Generalizations:
  a. Major mechanism for dehydration of carboxylates requires either:
     i. adenylation (attachment of AMP to make a better leaving group) or
     ii. phosphorylation (attachment of phosphate to make a better leaving group).

  i. **Adenylation mechanism:**

  

  ![Adenylation mechanism diagram](attachment://adenylation.png)

  charges neutralized Mg\(^{2+}\) (K, R)

  

  ![Adenylation mechanism diagram](attachment://adenylation.png)

  ii. **Phosphorylation mechanism:**

  

  ![Phosphorylation mechanism diagram](attachment://phosphorylation.png)

  charges neutralized Mg\(^{2+}\) (K, R)

  

  ![Phosphorylation mechanism diagram](attachment://phosphorylation.png)
ATP is almost universally coordinated to Mg\(^{2+}\). Without neutralizing the charges on the phosphates, a nucleophile cannot attack the P. In general, nucleophiles attack either the \(\gamma\) or \(\alpha\) phosphate, almost never the \(\beta\) phosphate.

\[
\begin{align*}
\text{Mg}^{2+} \text{ can coordinate } & \alpha, \beta; \text{ and } \alpha, \beta, \gamma; \text{ as well as } \beta, \gamma \text{ (shown)} \\
\end{align*}
\]

- Thermodynamically labile

- The free energy of hydrolysis of ATP is large and negative and therefore ATP is frequently used in coupled reactions to drive unfavorable reactions.

- What is unique about P chemistry relative to C chemistry that you have learned about?
  1. Chemistry at phosphate can occur by associative and dissociative mechanisms, analogous to Sn1 and Sn2 reactions in carbon chemistry.
  2. In the dissociative case (Sn1 like), a metaphosphate transition state (ts) is observed (see next page). This ts is electron deficient (similar to a carbocation) and is activated toward nucleophilic attack.
  3. In the associative mechanism, a trigonal bipyramidal (pentacoordinate) ts or intermediate ts is generated. This reaction is analogous to an Sn2 reaction. P because of its low lying 3d orbitals can form stable pentacoordinate structures (although none have been detected in biology).
Proposed mechanisms for phosphoryl transfer

- Role in metabolism:
  - phosphoryl transfer
    - mostly in nucleic acid metabolism

6. Thiamine pyrophosphate (TPP) Vit B1

- Role in metabolism for TPP enzymes:
  1. Oxidative decarboxylations
  2. Non-oxidative decarboxylations
  3. Aldol reactions

1. Mechanistic generalizations:
   a. Formation of a ylid on the thiazolium ring by abstraction of the C2 proton.
   b. Nucleophilic attack of the resulting carbanion on the carbonyl of the substrate to generate a tetrahedral intermediate equivalent to a \( \beta \)-keto acid.
   c. These types of species rapidly decarboxylate to generate hydroxyethyl-TPP.
   d. This intermediate undergoes different reactions depending on the enzyme.
      i. For non-oxidative decarboxylation, protonation occurs.
      ii. For oxidative decarboxylation, reaction with oxidized lipoic acid occurs.
      iii. For aldol reaction, reaction with an aldehyde occurs.
Example of ending via protonation

Protonation

Ending with aldehyde

Ending with oxidized lipoic acid
Structures of TPP enzymes reveal an unanticipated role of the pyrimidine amino group in ylid formation.
7. Lipoic acid

- **Role in metabolism:**
  - A redox cofactor covalently attached to proteins through a lysine
  - Examples are pyruvate dehydrogenases (PDH) and α-ketoglutarate dehydrogenases (KGDH) of the TCA cycle

![Chemical structures of lipoic acid, lipoamide, dihydrolipoamide, and reaction equations]
8. S-adenosylmethionine (SAM or Adomet), methylcobalamin and N-5 methyltetrahydrofolate are all methylating agents

**Role in metabolism:**
- Universal methylating agent

**Mechanism generalizations:**

a. Nucleophilic attack of the activated methyl group via an Sn2 reaction. The reaction occurs with an inversion of configuration.

b. Newly discovered role in metabolism – radical SAM superfamily (SF). In this SF, SAM binds to a unique Fe in the [4Fe4S]^{1+} cluster and is reductively cleaved to generate 5'-deoxyadenosyl radical (AdoChl, 5'-dA\*). This radical is involved in radical chemistry (not covered in 5.07).

- Bioinformatics studies suggest that > 40,000 reactions will use this chemistry.
9. Pyridoxal phosphate (PLP)

- The first step in metabolism of any amino acid involves PLP.

- **Generalizations:**
  a. PLP is able to mediate chemistry at the $\alpha$, $\beta$ and $\gamma$ positions of the amino acid.
     i. At $\alpha$ position: transamination, epimerization, decarboxylation and aldol reactions
     ii. At $\beta$ position: eliminations and replacement
     iii. At $\gamma$ position: eliminations and replacements
  b. PLP is always covalently bound to the enzyme as a Schiffs base (imine) with an active site lysine (lys).
  c. The first step is a transimination reaction with the amino acid, liberating the lys which then functions as a general acid catalyst (gac) or general base catalyst (gbc) in further steps in substrate transformation.
  d. The second step in all PLP reactions is generation of a carbanion at the $\alpha$ carbon of the amino acid. This can be done by the removal of the proton from the $\alpha$ position or by decarboxylation of the amino acid. This intermediate is the precursor to both $\beta$ and $\gamma$ elimination reactions and $\beta$ and $\gamma$ replacement reactions.
  e. The final step in all PLP reactions is transimination to reform the Schiffs base with the lysine at the active site.
• Dunathan’s hypothesis provides the chemical rational for the first chemistry that occurs subsequent to transimination. The bond to be cleaved (C-H or C-C bond in decarboxylation) is positioned by the enzyme, perpendicular to the plane of the π aldimine system to maximize electron overlap of the resulting anion with the pyridine ring.

Amino acid-PLP Schiff base

Delocalized α carboanion
Example of the first half reaction of a transimination reaction

1. Transimination

\[ \alpha\text{-Amino acid} + \text{Enzyme-PLP (Schiff base)} \rightarrow \text{Geminal diamine intermediate} \]

2. Removal of the \( \alpha \) hydrogen

\[ \text{Amino acid-PLP (Schiff base, aldimine)} \rightarrow \text{Resonance-stabilized intermediate} \]

3. Hydrolysis

\[ \text{Ketimine} \rightarrow \text{Carbinolamine} \rightarrow \text{Pyridoxamine phosphate (PMP)-Enzyme} + \text{\( \alpha \)-Keto acid} \]
Example of a $\beta$ elimination reaction: Serine dehydratase

- The first half reaction was shown on the previous page; a second $\alpha$-keto acid ($R_1\text{--}\text{COCO}_2^-$) then converts PMP back to Enzyme-PLP and liberates the corresponding amino acid ($R_1\text{--CH(NH}_3^+\text{)--CO}_2^-$).

- As shown in both examples, the reactions often make use of the Lys in the active site of all PLP-requiring enzymes, which functions as both a gbc and gac.
10. Biotin: carboxylation reactions involved in FA biosynthesis

The carboxylated biotin then transfers the CO$_2$ to the $\alpha$ carbon of acetylCoA (or propionylCoA in a claisen type reaction).
11. Hemes

- As you have seen with Mb and Hb, heme is the cofactor involved in reversible O$_2$ binding in many organisms. Hemes are very versatile cofactors and are involved in hydroxylation of unactivated C-H bonds, epoxidation of olefins, oxidation of sulfides to sulfoxides. These types of reactions are found in the liver, which is the organ largely responsible for removal of xenobiotics (environmental toxins).

- Heme dependent enzymes also catalyze disproportionation of hydrogen peroxide to O$_2$ and water, removing toxic hydrogen peroxide.

- The active site of the enzyme and the residues on the distil and proximal face of the heme govern which types of reactions are observed. The distil face of the heme is the one that binds O$_2$ to Fe, while the proximal face in the case of Mb and Hb has a His that coordinates Fe. Cysteine and tyrosine can also coordinate Fe on the proximal face in other enzymatic systems.
Different Hemes in ET (electron transfer) respiration

- Function: O₂ carriers, 1 e⁻ transfers. With Hb (Mb), the iron of the heme is in the +2 (Fe²⁺) oxidation state. If the iron gets oxidized to ferric heme (Fe³⁺) and superoxide (O₂⁻), then the iron must be re-reduced before binding O₂. In the case of ET in respiration, the iron toggles between the +2 and +3 oxidation states.
12. Metal cofactors (The players) in ET pathways

Iron sulfur clusters are involved in ET in the respiratory pathway. The irons change between the +2 and +3 oxidation states.

Other cofactors in the respiratory pathway: complex IV (cytochrome oxidase), which reduces $O_2$ to $H_2O$. 
13. Coenzyme Q (ubiquinone)

Functions in 1 e⁻ or 2 e⁻ transfers and is associated with membranes due to the hydrophobic tail that contains a long isoprenoid unit (see section of C-C bond formation). One electron chemistry (proton coupled electron transfer) is shown above.
14. Electron transfer in biology over long distances

- The theory of electron transfer (ET) makes predictions about the dependence of ET rates on the structures of the electron donor (D) and electron acceptor (A) (oxidized and reduced states), the distance between D and A, and the driving force for the ET process. The e\(^{-}\) can be treated as a particle and a wave and the theories for ET are based on quantum-mechanical expressions of the wave functions of the D and A involved in electron donation and acceptance. The wave functions are coupled weakly by the media (protein) in between the A and D.

- Dealing with electron movement is distinct from dealing with proton movement because of the differences in mass of the e\(^{-}\) vs the H\(^{+}\) (H\(^{+}\) is 2000 x). Thus, while electrons can “tunnel” over 10 to 15 Å at very fast rates (10\(^{8}\) s\(^{-1}\)), protons can only tunnel over 0.7 Å. Most metal clusters that you will encounter in biology (ex, respiratory chain) are spaced 10-15 Å apart and the rates are limited by physical steps, conformational gating, and not \(k_{ET}\).
Semiclassical theory describing ET (Gray and Winkler):

\[ k_{ET} = \sqrt{\frac{4\pi^3}{h^2 \lambda k_B T}} H_{AB}^2 \exp \left\{ -\frac{(\Delta G^\circ + \lambda)^2}{4 \lambda k_B T} \right\} \]

\( k_{ET} \) is the rate constant for ET between donor (D) and acceptor (A), 
\( \Delta G^\circ \) is the driving force for the reaction, 
\( \lambda \) is the nuclear reorganization energy
The electronic coupling element (HAB) reflects the strength of the interaction between D and A at the nuclear configuration of the transition state. Hopfield’s model predicts that coupling will depend exponentially on the distance, \( r \), between redox centers.

\[
H_{AB}(r) = H_{AB}(r_0) \exp \left\{ -\frac{1}{2} \beta (r - r_0) \right\}
\]

\( \beta \) depends on the media separating D and A. For a vacuum \( \beta = 3-5 \text{ Å}^{-1} \); through \( \text{H}_2\text{O} \) it = 1.65 Å\(^{-1} \) and for inhomogeneous proteins it has been measured by Gray/Winkler to be 1.1 Å\(^{-1} \).

Figure 5 from Tezcan, F. Akif, Brian R. Crane, Jay R. Winkler, and Harry B. Gray. "Electron tunneling in protein crystals." Proceedings of the National Academy of Sciences 98, no. 9 (2001): 5002-5006.
15. Folates: One carbon transfers at all oxidation states

All three oxidation states of C can be transferred

Methyl equivalent

Methylene or formaldehyde equivalent

Formic acid equivalent

N⁵-Methyl-THF

N⁵,10-Methylene-THF

N⁵,10-Methyldine-THF
Folate is the major player in one carbon transfers. The carbon is obtained from serine. The carbon can be transferred to its acceptor in all three oxidation states. This type of reaction is prevalent in purine and pyrimidine biosynthetic pathways that we will not discuss in 5.07.

The single carbon can be transferred as a CH$_3$ group from N$^5$-MeTHF; at the aldehyde level with N$^{5,10}$-methylene-THF; and at the acid oxidation state from N$^{10}$-formyl THF.
16. Methylcobalamin/Adenosylcobalamin

In mammalian systems, these cofactors are not widely used, but are essential.

- **CH₃-Cbl** is essential for methionine biosynthesis.
- **AdoCbl** is essential for odd chain fatty acid biosynthesis.
• Mechanism generalizations:
  a. Three oxidation states are available to cobalt in the cobalamin cofactors: Co(I), Co(II), Co(III).

  ![Cob(III)alamin](image1)
  ![Cob(II)alamin](image2)
  ![Cob(I)alamin](image3)

  b. All cobalamin cofactors have distinct visible spectra.

  a. For AdoCbl, homolytic cleavage of the C-Co bond to form Co$^{II}$ shown below and on the next page with methylmalonyl-CoA mutase.

  b. For CH$_3$Cbl, heterolytic cleavage of the C-Co bond to form Co$^I$ shown on the next page with methionine synthase.

  a. Generic mechanism for AdoCbl:
• Example for AdoCbl reaction: Methylmalonyl-CoA mutase (MMCoA mutase) 2 possible mechanisms.

a.

\[
\begin{align*}
\text{H}_2\text{C} & \text{CH} \quad \text{COO}^- \\
\text{Co(III)} & \text{CH}_2\text{Ad} \\
\text{Co(II)} & \text{H} \rightarrow \text{CH}_2\text{Ad} \\
\end{align*}
\]

b.

\[
\begin{align*}
\text{H}_2\text{C} & \text{CH} \quad \text{COO}^- \\
\text{Co(II)} & \text{H} \rightarrow \text{CH}_2\text{Ad} \\
\end{align*}
\]

• Example for CH\textsubscript{3}Cbl reaction: Methionine synthase (MS)

\[
\begin{align*}
\text{H}_3\text{C} & \text{S} \quad \text{NH}_3 \quad \text{O}^- \\
\text{Homocysteine} & \text{Methionine} \\
\end{align*}
\]

\[
\begin{align*}
\text{MS} \quad \text{Co(I)} & \text{5N-CH}_3\text{-THF} \\
\text{THF (tetrahydrofolate)} & \\
\end{align*}
\]