Redox Reactions and Cofactors

Lecture 27

Key Concepts

• Reduction potentials are a measurement of electron affinity

• Coenzymes provide reactive groups that function in enzyme catalysis

• The pyruvate dehydrogenase complex is a metabolic machine

What does the $\Delta E$ value of a coupled redox reaction tell you about electron transfer potential?

What are coenzymes and how do they function in the pyruvate dehydrogenase reaction?
Reduction potentials are a measurement of electron affinity.

Redox reactions (oxidation-reduction) in the citrate cycle are a form of energy conversion involving the transfer of electron pairs from organic substrates to the carrier molecules NAD$^+$ and FAD. The energy available from redox reactions is due to differences in the electron affinity of two compounds and is an inherent property of each molecule based on molecular structure.

Coupled redox reactions consist of two half reactions,

1) an oxidation reaction (loss of electrons)
2) a reduction reaction (gain of electrons).

Compounds that accept electrons are called oxidants and are reduced in the reaction, whereas compounds that donate electrons are called reductants and are said to be oxidized by loss of electrons.

Each half reaction consists of a conjugate redox pair represented by a molecule with and without an electron (e$^-$). For example, Fe$^{2+}$/Fe$^{3+}$ is a conjugate redox pair in which the ferrous ion (Fe$^{2+}$) is the reductant that loses an e$^-$ during oxidation to generate a ferric ion (Fe$^{3+}$) the oxidant:

$$\text{Fe}^{2+} \leftrightarrow \text{Fe}^{3+} + \text{e}^-$$

reductant oxidant

Similarly, the reductant cuprous ion (Cu$^+$) can be oxidized to form the oxidant cupric ion (Cu$^{2+}$) plus an e$^-$ in the reaction:

$$\text{Cu}^+ \leftrightarrow \text{Cu}^{2+} + \text{e}^-$$

reductant oxidant

The two conjugate redox pairs in these reactions are Fe$^{2+}$/Fe$^{3+}$ and Cu$^+$/Cu$^{2+}$. The e$^-$ functions as a shared intermediate in the coupled reaction.

$$\text{Fe}^{2+} \leftrightarrow \text{Fe}^{3+} + \text{e}^- \quad (\text{oxidation of Fe}^{2+})$$

$$\text{Cu}^{2+} + \text{e}^- \leftrightarrow \text{Cu}^+ \quad (\text{reduction of Cu}^{2+})$$

$$\text{Fe}^{2+} + \text{Cu}^{2+} \leftrightarrow \text{Fe}^{3+} + \text{Cu}^+ \quad (\text{coupled redox reaction})$$
Glucose is fully metabolized by aerobic metabolism to CO₂ and H₂O. The e⁻ donor is glucose which functions as the reductant, and O₂ is the e⁻ acceptor (oxidant) that is reduced in the last step of the electron transport chain to form H₂O. The two conjugate redox pairs NAD⁺/NADH and FAD/FADH₂ serve as the e⁻ carriers linking glycolysis to the citrate cycle and electron transport chain.

It is useful to think of glucose as a biochemical "battery" containing stored energy in the form of electrons that can be used to synthesize ATP in the mitochondria as a result of proton motive force and oxidative phosphorylation.

Methane (CH₄), the most highly reduced form of carbon, has 8 e⁻ than can be donated. The carbon in carbon dioxide (CO₂) has all of its e⁻ shared with O and tightly associated with the more electronegative O. The more electrons a carbon atom has available to donate in a redox reaction, the more reduced (less oxidized) it is.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Number of e⁻ that can be donated by C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>H:\ H: C: H</td>
<td>8</td>
</tr>
<tr>
<td>Acetaldehyde (aldehyde)</td>
<td>H: C: H: O: H: H</td>
<td>3</td>
</tr>
<tr>
<td>Acetic acid (carboxylic acid)</td>
<td>H: C: O: H: H: H: O: H</td>
<td>1</td>
</tr>
</tbody>
</table>

Hydrogen is less electronegative than carbon, and therefore electrons in C-H bonds are considered "owned" by the carbon. Similarly, since oxygen is more electronegative than carbon, the electrons in C-O and C=O bonds are all "owned" by the oxygen atom.
Redox reactions in the citrate cycle (and indeed most all enzyme-catalyzed redox reactions) involve the transfer of electron pairs (2 e⁻) to the electron carrier molecules NAD⁺ and FAD. The reduction of NAD⁺ to NADH involves the transfer of a hydride ion (\(:\text{H}^-\) ), which contains 2 e⁻ and 1 H⁺, and the release of a proton (H⁺) into solution.

\[
\text{NAD}^+ + 2 \text{e}^- + 2 \text{H}^+ \leftrightarrow \text{NADH} + \text{H}^+ 
\]

In contrast, FAD is reduced by sequential addition of one hydrogen (1 e⁻ and 1 H⁺) at a time to give the fully reduced FADH₂ product.

\[
\text{FAD} + 1 \text{e}^- + 1 \text{H}^+ \leftrightarrow \text{FADH} + 1 \text{e}^- + \text{H}^+ \leftrightarrow \text{FADH}_2
\]

Free energy changes can be calculated from reduction potential differences.

The change in standard free energy of a reaction under biochemical conditions (\(\Delta G^{°'}\)) is a measure of the spontaneity of the reaction in kJ/mol and reflects the tendency of compound A to be converted to compound B (\(\text{A} \rightarrow \text{B}\)). A negative \(\Delta G^{°'}\) means the reaction is favored in the direction written from left to right (product B will accumulate), whereas, a positive \(\Delta G^{°'}\) means the reverse reaction is favorable (A will accumulate).

In redox reactions, we use the term reduction potential (\(E\)), measured in volts (V), to represents the electron affinity of a given conjugate redox pair. Analogous to biochemical standard conditions that define Gibbs Free Energy, \(G^{°'}\), the term \(E^{°'}\) refers to the biochemical standard reduction potential under the same conditions. \(E^{°'}\) values are determined in the laboratory using an apparatus called an electrochemical cell that measures the relative e-affinity of a test redox pair, compared to that of the hydrogen half-reaction (\(2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2\)), which has been chosen as the standard.
Depending on the relative electron affinity of the test oxidant compared to \( H^+ \), the electrons will either move from the hydrogen half-cell toward the test half-cell or from the test half-cell toward the hydrogen half-cell.

The two half-cells are connected by an agar bridge containing potassium chloride that permits counter-ion movement to balance the charge.

The \( E^{\circ} \) of oxidants with a higher affinity for electrons than \( H^+ \) are recorded as positive \( E^{\circ} \) values (\( E^{\circ} > 0 \)), and oxidants with a lower affinity for electrons than \( H^+ \) are recorded as negative \( E^{\circ} \) values (\( E^{\circ} < 0 \)).

You should understand general trends here

Know approximate values for important half reactions
The amount of energy available from a coupled redox reaction is directly related to the difference between two reduction potentials and is defined by the term $\Delta E^{\circ \circ}$. By convention, the $\Delta E^{\circ \circ}$ of a coupled redox reaction is determined by subtracting the $E^{\circ}$ of the oxidant (e- acceptor) from the $E^{\circ}$ of the reductant (e- donor) using the following equation:

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

Moreover, the $\Delta E^{\circ \circ}$ for a coupled redox reaction is proportional to the change in free energy $\Delta G^{\circ \circ}$ as described by the equation:

$$\Delta G^{\circ \circ} = -nF\Delta E^{\circ \circ}$$

in which n is the number of electrons transferred in the reaction (usually 2 in biochemical redox reactions), and F is the Faraday constant (96.48 kJ/V•mol). As can be seen by this equation, when the difference in reduction potentials for a coupled redox reaction is positive then the reaction is favorable since $\Delta G^{\circ}$ will be negative. This means that for a coupled redox reaction to be favorable, the reduction potential of the e- acceptor needs to be more positive than that of the e- donor.

To see how $\Delta G^{\circ}$ and $\Delta E^{\circ}$ are related, we can use the biochemical standard reduction potentials ($E^{\circ}$) to calculate the change in biochemical standard free energy ($\Delta G^{\circ}$) for the citrate cycle isocitrate dehydrogenase reaction.

**Isocitrate + NAD$^+$ → α-ketoglutarate + CO$_2$ + NADH**

Note that in a spontaneous coupled redox reaction the e- flow is from the reductant in the conjugate redox pair with the lower $E^{\circ}$ value (more negative) toward the oxidant in the conjugate redox pair with the higher $E^{\circ}$ value (less negative).

The two standard half reactions are written below as reduction reactions. Note that NAD$^+$ is the e- acceptor (less negative) and α-ketoglutarate is the e- donor (more negative):

- $\alpha$-ketoglutarate + CO$_2$ + 2 e$^-$ + 2 H$^+$ → Isocitrate ($E^{\circ} = -0.38$ V)
- NAD$^+$ + 2 e$^-$ + 2 H$^+$ → NADH + H$^+$ ($E^{\circ} = -0.32$ V)

isocitrate is the reductant (e- donor) and NAD$^+$ is the oxidant (e- acceptor)
\( \alpha\)-ketoglutarate + CO\(_2\) + 2 e\(^-\) + 2 H\(^+\) → Isocitrate (\(E^\circ\) = -0.38 V)
NAD\(^+\) 2 e\(^-\) + 2 H\(^+\) → NADH + H\(^+\) (\(E^\circ\) = -0.32 V)

For this coupled redox reaction in which isocitrate is the reductant (e\(^-\) donor) and NAD\(^+\) is the oxidant (e\(^-\) acceptor), we can calculate \(\Delta E^\circ\) using the equation:

\[
\Delta E^\circ = (E^\circ e^- \text{ acceptor}) - (E^\circ e^- \text{ donor})
\]
\[
\Delta E^\circ = (E^\circ \text{ NAD}^+) - (E^\circ \text{ isocitrate})
\]
\[
\Delta E^\circ = (-0.32 \text{ V}) - (-0.38 \text{ V}) = +0.06 \text{ V}
\]

and then convert this \(\Delta E^\circ\) value to \(\Delta G^\circ\) using the relationship:

\[
\Delta G^\circ = -nF\Delta E^\circ
\]
\[
\Delta G^\circ = -2 \cdot (96.48 \text{ kJ/mol\cdotV}) \cdot +0.06 \text{ V}
\]
\[
\Delta G^\circ = -11.6 \text{ kJ/mol}
\]

showing that the conversion of isocitrate to \(\alpha\)-ketoglutarate is a favorable reaction (\(\Delta G^\circ < 0\)) under standard biochemical conditions.

You should be able to do such calculations if given standard half reactions!

**Coenzymes provide reactive groups that function in enzyme catalysis**

**Pyruvate** must be transported from the cytosol into the mitochondrial matrix before it can serve as a source of reducing power for the cell or as a precursor for glucose synthesis. Pyruvate that is destined for the citrate cycle (or fatty acid synthesis) is converted to acetyl-CoA by the enzyme **pyruvate dehydrogenase**.

Pyruvate dehydrogenase contains several coenzymes, and works with other coenzymes to do its job. Because it makes acetyl-CoA, it is a very important enzyme complex in cell metabolism.

The pyruvate dehydrogenase complex catalyzes the oxidative decarboxylation of pyruvate to form CO\(_2\) and acetyl-CoA using a **five step reaction mechanism** that requires **three distinct enzymes** and five different coenzymes.
Acetyl-CoA is an important and versatile molecule.

It can be metabolized by the citrate cycle to **convert redox energy to ATP** by oxidative phosphorylation.

It can be used as a form of **stored energy by conversion to fatty** acids that are transported to adipocytes (fat cells) as triglycerides.

It has other fates in plants and microbes, where it is used to acetylate many compounds.

It is used by all cells to acetylate histones, which affects chromatin structure and gene expression.

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**Why are there coenzymes?**

Amino acids can only provide a **finite number of chemical groups** for enzyme reactions. One way evolution has solved this problem is by selecting for enzyme complexes that bind (through covalent or non-covalent interactions) reactive biomolecules that provide additional reactive groups for catalytic mechanisms.

Amino acid side chains cannot catalyze oxidation/reduction reactions, carboxylations, decarboxylations, to name a few. These are essential for life, so something else must be used: coenzymes.

Five examples of coenzymes (NAD\(^+\), FAD, CoA, thiamine pyrophosphate (TPP) and lipoic acid) are utilized in the pyruvate dehydrogenase reaction.
<table>
<thead>
<tr>
<th>Coenzyme</th>
<th>Vitamin</th>
<th>Types of reactions</th>
<th>Nutrient source</th>
<th>Symptoms of dietary deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinamide adenine dinucleotide (NAD⁺)</td>
<td>Niacin (B₃)</td>
<td>Redox reactions (transfer of hydride ion)</td>
<td>Poultry, fish, vegetables</td>
<td>Causes the disease pellagra</td>
</tr>
<tr>
<td>Flavin adenine dinucleotide (FAD)</td>
<td>Riboflavin (B₂)</td>
<td>Redox reactions (transfer of electrons)</td>
<td>Dairy, almonds, asparagus</td>
<td>Causes cheliosis (swelling, cracked lips)</td>
</tr>
<tr>
<td>Coenzyme A (CoA)</td>
<td>Pantotheic acid (B₅)</td>
<td>Acyl group transfer</td>
<td>Chicken, yogurt, avocados</td>
<td>Rarely observed</td>
</tr>
<tr>
<td>Thiamin pyrophosphate (TPP)</td>
<td>Thiamin (B₁)</td>
<td>Aldehyde transfer</td>
<td>Lentils, brown rice, fortified cereals</td>
<td>Causes beriberi</td>
</tr>
<tr>
<td>alpha-lipoic acid (lipoamide)</td>
<td>Not a vitamin</td>
<td>Acyl group transfer</td>
<td>Tomatoes, broccoli, spinach</td>
<td>None reported</td>
</tr>
<tr>
<td>Biocytin (biotin-lysine)</td>
<td>Biotin</td>
<td>Carboxyl group transfer</td>
<td>Breads, cooked eggs, vegetables</td>
<td>skin rash, hair loss,</td>
</tr>
</tbody>
</table>

**Nicotinamide adenine dinucleotide (NAD⁺)**

Derived from the water-soluble vitamin niacin which is also called vitamin B3.

NAD⁺, and its phosphorylated form NADP⁺, are involved in over 200 redox reactions in the cell which are characterized by the transfer of 2 e⁻ in the form of hydride ions (:H⁻).

Severe niacin deficiency causes the disease pellagra which was first described in Europe in the early 1700s amongst peasants who relied on cultivated corn as their primary source of nutrition.
Flavin adenine dinucleotide (FAD)

Derived from the water-soluble vitamin riboflavin which is also called vitamin B2. Riboflavin is the precursor to FAD and to the related molecule flavin mononucleotide (FMN), both of which are often tightly associated with enzymes that catalyze redox reactions. FAD is a coenzyme in the pyruvate dehydrogenase complex and is also covalently bound to a histidine residue in the citrate cycle enzyme succinate dehydrogenase. FAD can accept one electron at a time and form a partially reduced intermediate called a semiquinone (FADH•).

Coenzyme A (CoA)

Derived from the water-soluble vitamin pantothenic acid which is also called vitamin B5. CoA is absolutely essential for life as it is required for energy conversion by the citrate cycle, it is also a cofactor in fatty acid, acetylcholine, heme and cholesterol biosynthetic pathways, among others. A major role of CoA is to function as a carrier molecule for acetate units in the form of acetyl-CoA.
**Thiamin pyrophosphosphate (TPP)**

Derived from the water-soluble vitamin thiamin (or thiamine) which is also called vitamin B1. Thiamin was one of the first organic compounds to be identified as a vitamin because thiamin deficiency causes the human disease beriberi. Thiamin is absorbed in the gut and transported to tissues where it is phosphorylated by the enzyme thiamin kinase in the presence of ATP to form thiamin pyrophosphate (TPP) and AMP.

Thiamin deficiency was first described in Chinese literature over four thousand years ago and is the cause of beriberi, a disease characterized by anorexia, cardiovascular problems and neurological symptoms. Beriberi has been found in populations that rely on white polished rice as a primary source of nutrition (milling rice removes the bran which contains thiamin). Beriberi has also been observed in chronic alcoholics who have poor nutrition and obtain most of their calories for energy conversion from alcohol.

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**α-Lipoic acid (lipoamide)**

A coenzyme synthesized in plants and animals as a 6,8-dithiooctanoic acid. The role of α-lipoic acid in metabolic reactions is to provide a reactive disulfide that can participate in redox reactions within the enzyme active site. Lipoamide, the naturally occurring form of α-lipoic acid, is a covalent linkage of α-lipoic acid to a lysine epsilon-amino group on proteins.

The pyruvate dehydrogenase complex contains the lipoamide at the end of a polypeptide tether which functions as a "ball and chain" that moves the lipoamide back and forth across a 50 Å span in the interior of the complex. α-Lipoic acid is not considered a vitamin because it is synthesized at measurable levels in humans, however, because of its potential to function as an antioxidant in the reduced form, α-lipoic acid is promoted as a nutritional supplement.
The pyruvate dehydrogenase complex is a metabolic machine

The conversion of pyruvate to acetyl-CoA by the pyruvate dehydrogenase complex is an oxidative decarboxylation reaction that represents another amazing example of protein structure and function. The eukaryotic pyruvate dehydrogenase complex contains multiple subunits of three different catalytic enzymes that work together as a metabolic machine to carry out the following net reaction:

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH}$$

$$\Delta G^\circ = -33.4 \text{ kJ/mol}$$

Coenzymes provide a chemical platform for the catalytic reactions of pyruvate dehydrogenase

Five Distinct Catalytic Steps

Steps 1, 2 and 3 lead to the formation of acetyl-CoA

Steps 4 and 5 serve to regenerate the oxidized form of lipoamide and in the process, transfer 2 e- to NAD⁺
The E1, E2 and E3 subunits of the mammalian pyruvate dehydrogenase are packed together in a huge ~400 Å diameter sphere with a combined molecular weight of ~7800 kDa. The pyruvate dehydrogenase complex consists of an inner core made up of tightly packed E2 subunits that are in close contact with the E3 subunits with the E1 subunits forming an outer shell that is ~50 Å away from the E2 core. The colored E1 (yellow), E2 (green) and E3 (red) pyruvate dehydrogenase complex subunits are labeled, and the linker region connecting E2 to E1 is shaded blue.

The lipoamide moiety of the E2 subunit is attached near the end of a ~200 amino acid long segment of the protein that functions as both a structural linker connecting the E2 and E1 subunits (gray region in the figure above), and as a type of lipoamide "ball and chain." An important component of the linker region is the E1-binding domain that serves as a "pivot" for the ball and chain. The hydrocarbon extension on the lipoamide moiety itself is sometimes referred to as a "swinging arm."
A naturally occurring inhibitor of lipoamide coenzyme function is the element arsenic (As) which in the form of arsenite \( (\text{AsO}_3^{3-}) \) creates bidentate adducts on dihydrolipoamide. Inadvertent ingestion arsenite can lead to an untimely death by irreversibly blocking the catalytic activity of lipoamide-containing enzymes such as the pyruvate dehydrogenase and \( \alpha \)-ketoglutarate dehydrogenase complexes.

A tragic example of this is the accidental arsenic poisoning of thousands of people in Bangladesh and India which has occurred over the last 20 years. During the 1970s and 1980s, UNICEF and other relief organizations helped drill thousands of wells in small villages as an humanitarian effort to circumvent public water supplies that had become biologically contaminated. About ten years later when large numbers of villagers in the Ganges delta region developed skin lesions and cancers, it was realized that these wells contained water with toxic levels of arsenic.