Biological Redox Reactions

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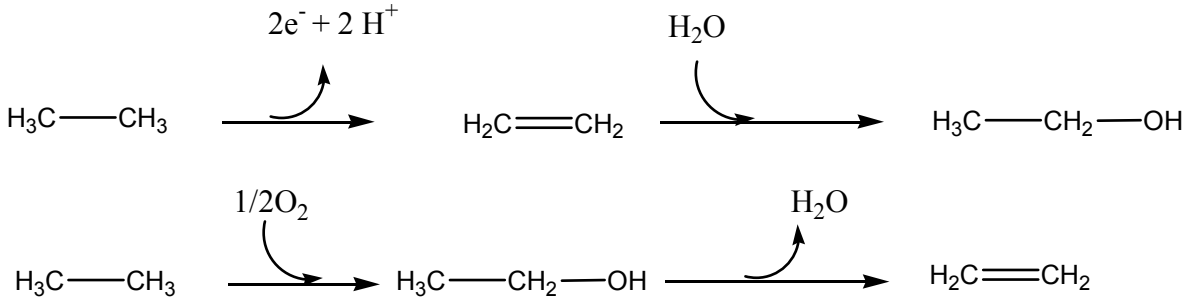
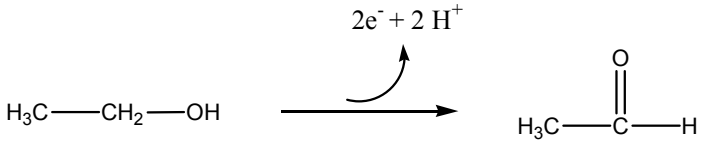
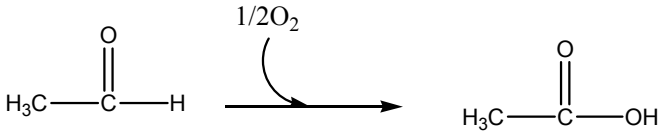
The transfer of electrons is equally as important as the transfer of phosphoryl groups. Oxidation is the loss of electrons, reduction is the gain of electrons.

It is easy to tell of an organic compound has been oxidized or reduced. If an organic molecule gains oxygen or loses hydrogen is has been oxidized. I.e.

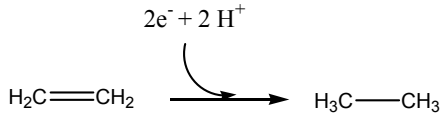


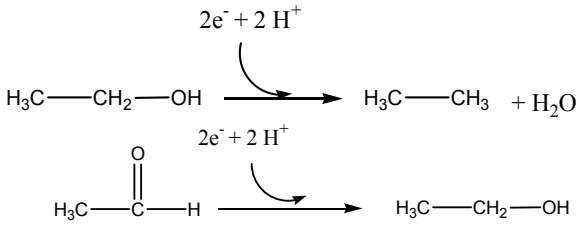
Note that $CH_2{=}{=}CH_2$ and CH_3CH_2OH are at the same oxidation state. No oxidation-reduction occurs during the interconversion of ethylene into ethanol.

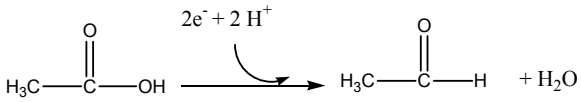




If an organic molecule loses oxygen or gains hydrogen, then is has been reduced.







Electrons are transferred from one molecule to another in four different ways.

1. **Directly as electrons**. I. e. $Fe^{2+} + Cu^{2+} \rightarrow Fe^{3+} + Cu^{+}$
2. **As Hydrogen atoms**. The hydrogen atom contains a proton, H^{+} and an electron, e^{-}.
   $AH_2 \rightleftharpoons A + 2e^{-} + 2H^{+}$
3. **As a Hydride ion :H^{-}.** The hydride ion has two electrons and is highly reactive. In biological systems it is directly transferred to NAD-linked dehydrogenases.
4. **Through the direct combination with oxygen.** Molecular oxygen combines with organic reactants to oxidize hydrocarbons to alcohols, aldehydes to acids ect. I.e.
   $R—CH_3 + 1/2O_2 \rightarrow R—CH_2–OH$

In biological systems, oxidation is often coincident with the loss of hydrogen, **dehydrogenation**. The enzymes that catalyze these oxidations are called **dehydrogenases**.

Oxidation-reduction reactions (Redox reactions) must occur together. Electrons are transferred from the reducing agent to the oxidizing agent such that the reducing agent is oxidized and the oxidizing agent is reduced. It is convenient however to describe the electron transfer reaction as two half reactions, one for the oxidation of the reduced species and one for the reduction of the oxygen species. I.e. The oxidation of the ferrous ion by the cupric ion,

$Fe^{2+} + Cu^{2+} \rightarrow Fe^{3+} + Cu^{+}$

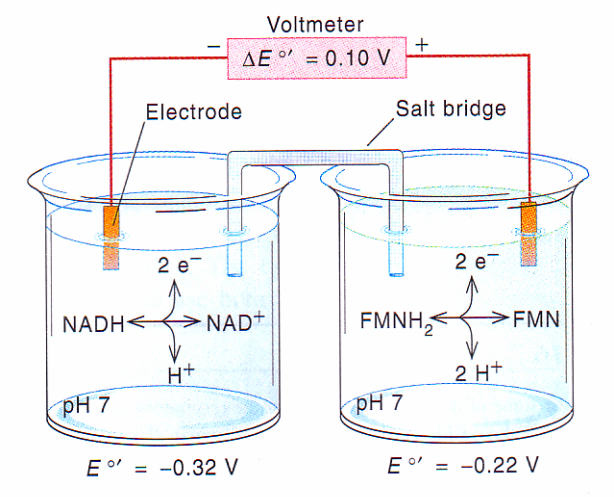
Can be described by the two half reactions:

(1) $Fe^{2+} \rightarrow Fe^{3+} + e^{-}$

(2) $Cu^{2+} + e^{-} \rightarrow Cu^{+}$

A half reaction consists of an electron donor and its conjugate electron acceptor. In the first half-reaction shown above, Fe^{2+} is the electron donor and Fe^{3+} is the conjugate electron acceptor. Together these constitute a **conjugate redox pair**.

The two half-reactions of a redox reaction can be physically separated to form an electrochemical cell. In such a device, each half reaction takes place in a separate half-cell and the electrons are passed between the two cells by a wire connecting two electrodes. A salt bridge is necessary to complete the electrical circuit by providing a conduit for ions to migrate in the maintenance of cell neutrality.



The free energy of an oxidation-reduction reaction can be easily determined by measuring the voltage difference between the two half cells.

Consider the general redox reaction:

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

In which n moles of electrons are transferred from B_{red} to A_{ox}^{n+}. The free energy of this reaction is given by the equation:

$$\Delta G = \Delta G^{o\prime} + RT \ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right)$$

Now at constant temperature and pressure under reversible conditions, $\Delta G = -w$, where w is non-pressure volume work, in this case electrical work is being done. $\Delta G = -w_{el}$. According to the laws of electrostatics, the work required to transfer n moles of electrons through an electric potential of $\Delta\xi$ is: $w_{el} = nF\Delta\xi$ where F = Faradays constant which is the electrical charge of 1 mole of electron = 96,494 J/Vmol.

$\Delta G = -w_{el} = -nF\Delta\xi$

$$\Delta G = \Delta G^{o\prime} + RT\ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right); \Delta G = -nF\Delta\xi = -nF\Delta\xi^{o\prime} + RT\ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right)$$

$$-nF\Delta\xi = -nF\Delta\xi^{o\prime} + RT\ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right); \Delta\xi = \Delta\xi^{o\prime} - \frac{RT}{nF}\ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right);$$

$$\Delta\xi = \Delta\xi^{o\prime} - \frac{RT}{nF}\ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right)$$

The last expression is the Nerst equation. $\Delta\xi$ is called the electromotive force or redox potential. The quantity $\Delta\xi^o$ is the redox potential when all of the components are in their standard states and is called the standard redox potential. In this class we are going to use the biochemists standard state $\Delta\xi^{o\prime}$.

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

The component half reactions can be written as reductions and assigned reduction potentials, ξ_A and ξ_B.

$A_{ox}^{n+} + ne^- \rightleftharpoons A_{red}$ $\xi_A = \xi_A^{o\prime} - (RT/nF)\ln([A_{red}]/[A_{ox}^{n+}])$

$B_{ox}^{n+} + ne^- \rightleftharpoons B_{red}$ $\xi_B = \xi_B^{o\prime} - (RT/nF)\ln([B_{red}]/[B_{ox}^{n+}])$

For the redox reaction of any two half reactions:

$\Delta\xi^{o\prime} = \xi_{acceptor} - \xi_{donor}$

For our example reaction

$\Delta\xi^{o\prime} = \xi_A^{o\prime} - \xi_B^{o\prime}$

Standard reduction potentials are defined with respect to the standard hydrogen half-reaction.

$2H^+ + 2e^- \rightleftharpoons H_2(g)$

In which $[H^+]$ is 1M, T= 298°K, P=1 atm, Pt electrodes. This half cell is assigned a standard reduction potential, $\xi^o = 0$ V.

In the biochemical convention, the standard state is $[H^+] = 10^{-7}$M (pH 7), T= 298°K, P=1 atm, Pt electrodes, $\xi^{o\prime} = -0.421$ V.

Note than when $\Delta\xi$ is positive, ΔG is negative. Spontaneous, exergonic

When $\Delta\xi$ is negative, ΔG is positive. Nonspontaneous, endergonic.

The more positive the standard reduction potential, the greater the tendency for the redox couple's oxidized form to accept electrons and thus become reduced.

The usefulness of reduction potentials is that many reduction potentials have been determined and tabulated. We can predict the direction that electrons will flow if two half-cells are connected. Electrons will flow towards the half-cell with the more positive E. The free energy available from spontaneous electron flow is proportional to $\Delta\xi$,

$\Delta G = -nF\Delta\xi$ or $\Delta G^{o'} = -nF\Delta\xi^{o'}$

TABLE 15-4. STANDARD REDUCTION POTENTIALS OF SOME BIOCHEMICALLY IMPORTANT HALF-REACTIONS

Half Reaction	$\mathscr{E}^{\circ\prime}$ (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$	0.815
$SO_4^{2-} + 2H^+ + 2e^- \rightleftharpoons SO_3^{2-} + H_2O$	0.48
$NO_3^- + 2H^+ + 2e^- \rightleftharpoons NO_2^- + H_2O$	0.42
Cytochrome a_3 (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome a_3 (Fe^{2+})	0.385
$O_2(g) + 2H^+ + 2e^- \rightleftharpoons H_2O_2$	0.295
Cytochrome a (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome c (Fe^{2+})	0.235
Cytochrome c_1 (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) + $e^- \rightleftharpoons$ cytochrome b (Fe^{2+}) (*mitochondrial*)	0.077
Ubiquinone + $2H^+ + 2e^- \rightleftharpoons$ ubiquinol	0.045
Fumarate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ succinate$^-$	0.031
FAD + $2H^+ + 2e^- \rightleftharpoons FADH_2$ (*in flavoproteins*)	~0.
Oxaloacetate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ malate$^-$	−0.166
Pyruvate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ lactate$^-$	−0.185
Acetaldehyde + $2H^+ + 2e^- \rightleftharpoons$ ethanol	−0.197
FAD + $2H^+ + 2e^- \rightleftharpoons FADH_2$ (*free coenzyme*)	−0.219
$S + 2H^+ + 2e^- \rightleftharpoons H_2S$	−0.23
Lipoic acid + $2H^+ + 2e^- \rightleftharpoons$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \rightleftharpoons$ NADH	−0.315
$NADP^+ + H^+ + 2e^- \rightleftharpoons$ NADPH	−0.320
Cystine + $2H^+ + 2e^- \rightleftharpoons$ 2 cysteine	−0.340
Acetoacetate$^-$ + $2H^+ + 2e^- \rightleftharpoons$ β-hydroxybutyrate$^-$	−0.346
$H^+ + e^- \rightleftharpoons \frac{1}{2}H_2$	−0.421
Acetate$^-$ + $3H^+ + 2e^- \rightleftharpoons$ acetaldehyde + H_2O	−0.581

Source: Mostly from Loach, P.A., *In* Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), *Physical and Chemical Data*, Vol. I, *pp.* 123–130, CRC Press (1976).

For example consider the follow redox reaction.
Acetaldehyde + NADH + H^+ → ethanol + NAD^+
The relevant half reactions are:

(1) Acetaldehyde + $2H^+ + 2e^-$ → ethanol $\quad \xi^{o'} = -0.197$ V

(2) $NAD^+ + 2H^+ + 2e^-$ →NADH + H+ $\quad \xi^{o'} = -0.320$ V

Remember,

$\Delta\xi^{o'} = \xi_{acceptor} - \xi_{donor}$

$\Delta\xi^{o'} = -0.197 - (-0.320) = 0.123$ V

n = 2

F = 96.5 kJ/V•mol

$\Delta G^{o'} = -nF\Delta\xi^{o'} = -2(96.5 \text{ kJ/ V•mol})(0.123\text{V}) = -23.7$ kJ/mol

This is the free energy change when acetaldehyde, ethanol, NADH and NAD^+ are 1 molar and the pH is 7.0.

What would the free energy change be if [Acetaldehyde]=1M, [NADH] = 1M, [ethanol] = 0.1 M and $[NAD^+]$ = 0.1 M?

$$\Delta\xi = \Delta\xi^{o'} - \frac{RT}{nF}\ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right)$$

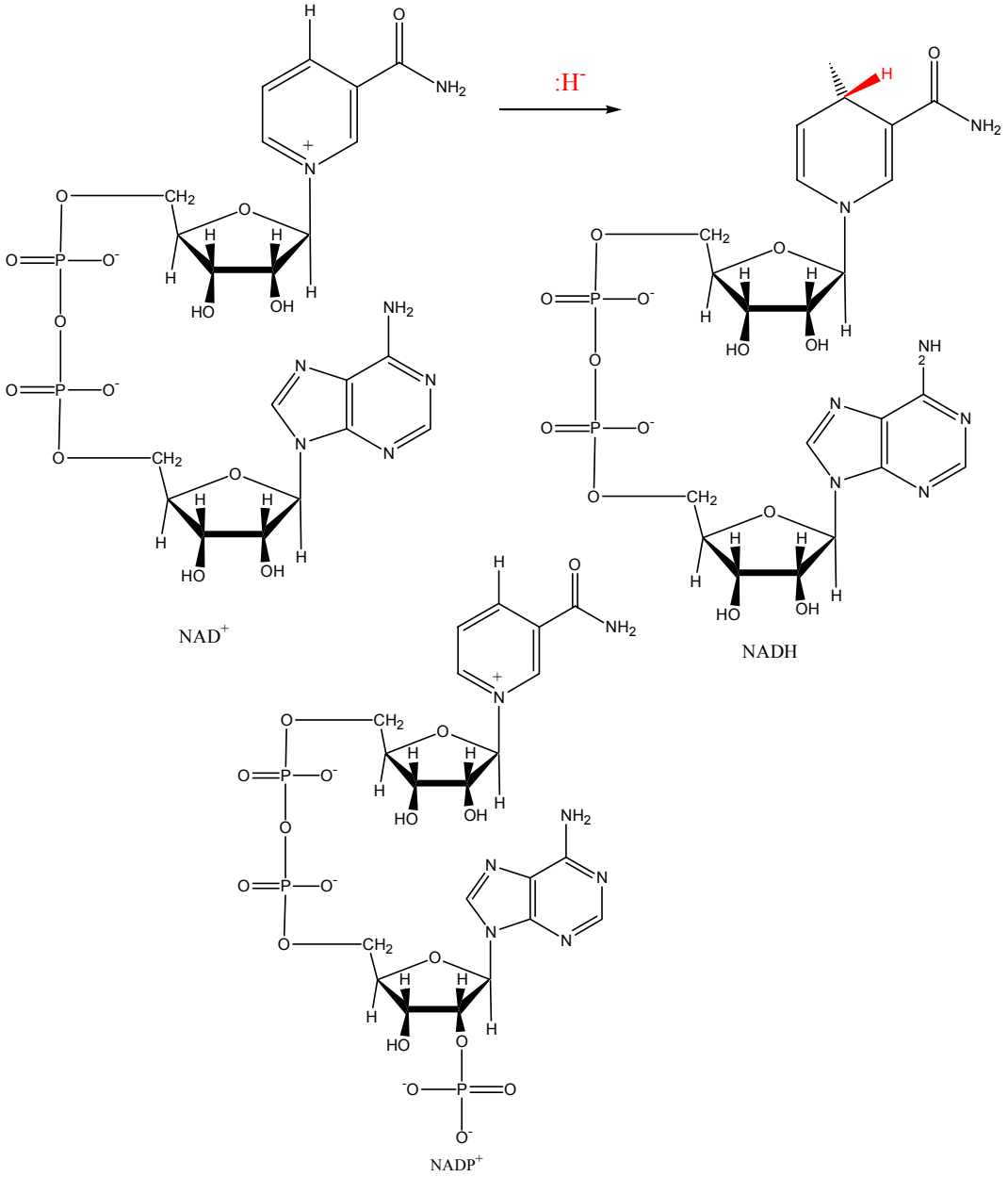
$\Delta\xi = 0.123$ V – ([8.3145X10^{-3} Kj/mol•^{o}K]*298^{o}K])/[(2)(96.5 kJ/V•mol)]ln([0.1M][0.1M])/([1M][1M])

$\Delta\xi = 0.123$ V – (0.0128V) -4.6 = 0.182 V

$\Delta G = -nF\Delta\xi = -2(96.5 \text{ kJ/ V•mol})(0.182\text{V}) = -35.1$ kJ/mol

Coenzymes that serve as universal electron carriers.

I. NADH and NADPH



Nicotinamide adenine dinucleotide (NAD^+)and its close analog nicotinamide adenine dinucleotide phosphate ($NADP^+$) undergo reversible reduction of the nicotinamide ring. The substrate undergoes oxidation (dehydrogenation), giving up two hydrogen atoms. The oxidized nicotinamide of either NAD^+ or $NADP^+$ accepts a hydride ion and is transformed into the reduced nicotinamide (NADH of NADPH).

The vitamin niacin is the source of the nicotinamide moiety.
The half reactions for the reduction potentials are

$$NAD^+ + H^+ + 2e^- \rightarrow NADH \quad \Delta\xi^{o\prime} = -0.315\ V$$

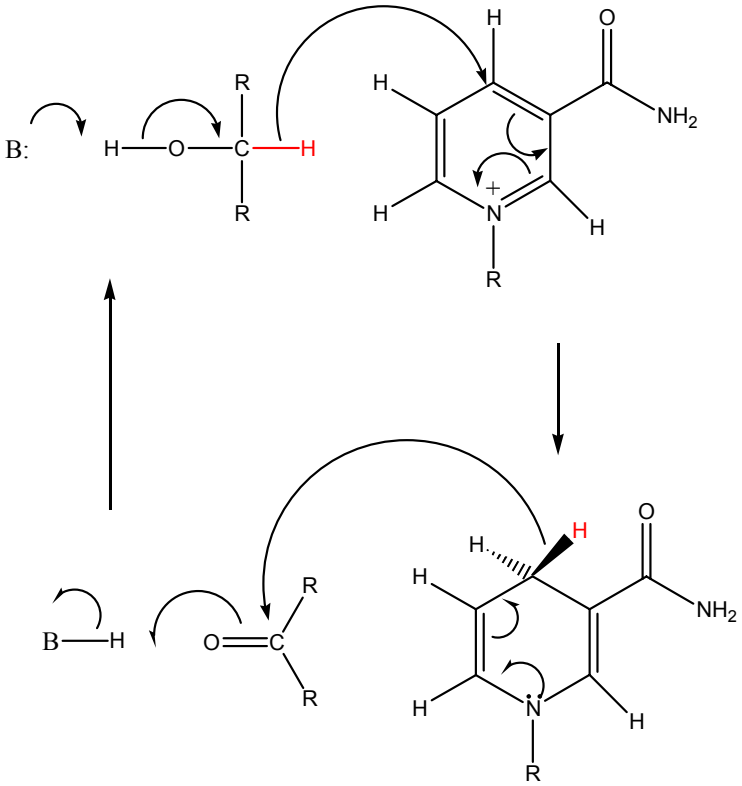
$$NADP^+ + H^+ + 2e^- \rightarrow NADPH \quad \Delta\xi^{o\prime} = -0.320V$$

Both NAD and NADP are water soluble cofactors that move readily from enzyme to another.
NAD generally functions in oxidations, usually in a catabolic pathway.
NADP generally functions in reductions, typically in an anabolic pathway.
A few enzymes can use either NAD or NADP, but most are specific for one or the other. This functional specialization allows cells to maintain two pools of electron carriers with each pool having its own specific function. The general reactions of these cofactors are:

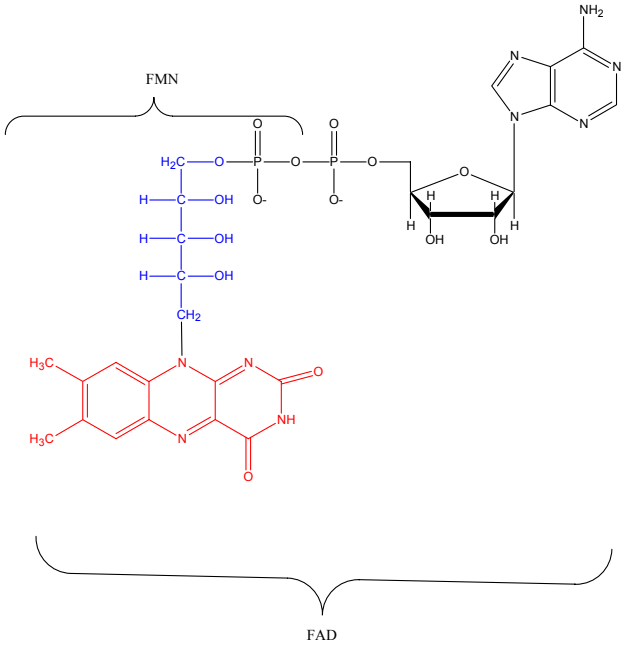
$AH_2 + NAD^+ \rightarrow A + NADH + H^+$

$A + NADPH + H^+ \rightarrow AH_2 + NADP^+$

The enzymes that catalyze these reactions are **oxidoreductases**, commonly called dehydrogenases. The example shown below is yeast alcohol dehydrogenase.



II. Flavin Nucleotides



Flavoproteins are enzymes that catalyze redox reactions using either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzymes.

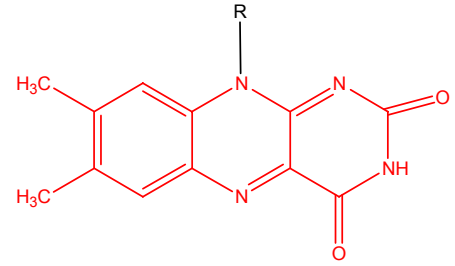
These coenzymes are derived from the vitamin riboflavin.

Although the flavin coenzymes are water soluble, they are bound tightly to the enzyme. Tightly bound coenzymes are called prosthetic groups. As a result the flavin coenzymes to do not transfer electrons from one enzyme to another, but allow the flavoprotein to temporarily hold the electrons to catalyze an electron transfer from a substrate to the electron acceptor.

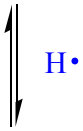
The fused ring shown in red is an isoalloxazine ring which undergoes reversible reduction.

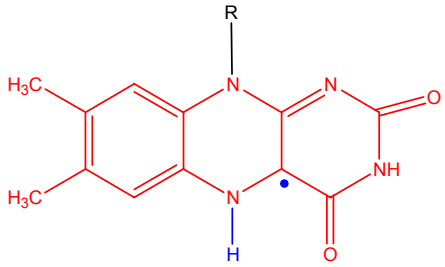
The isoallozazine ring can accept either one electron or two.

The fully reduced flavins are abbreviated $FADH_2$ or $FMNH_2$.

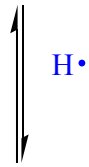


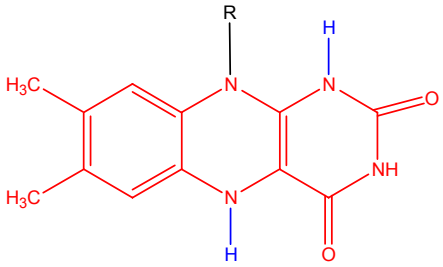
FAD the oxidized or quinone form.





FADH• the radical or semiquinone form.





$FADH_2$ the reduced or hydroquinone form

For the flavin coenzymes in solution, the standard redox potentials are:

$FAD + 2H+ + 2e- \rightarrow FADH_2 \quad \Delta\xi^{o'} = -0.219$ V

$FMN + 2H+ + 2e- \rightarrow FMNH_2 \quad \Delta\xi^{o'} = -0.219$ V

Flavoproteins have a high variability in the standard reduction potential of the bound flavin nucleotide.

$FAD + 2H+ + 2e- \rightarrow FADH_2$ or $FMN + 2H+ + 2e- \rightarrow FMNH_2$ $\Delta\xi^{o'}$ ranges from 0.003 to 0.091 V