

The **transfer of phosphoryl groups** is a central feature of metabolism. Equally important is another kind of **transfer, electron transfer in oxidation-reduction(OxRed)** reactions. These reactions involve the loss of electrons $-e^-$ by one chemical species, which is thereby **oxidized**, and the gain of electrons $+e^-$ by another, which is **reduced**. The flow of electrons $\Rightarrow e^-$ in **oxidation-reduction (OxRed)** reactions is responsible, directly or indirectly, for all work (W) done by living organisms. In **non-photo synthetic** organisms, the sources of electrons e^- are **reduced compounds (foods)**; in **photo synthetic** organisms, the initial electron $-e^-$ donor is a chemical species excited by the absorption **A** of **light $\sim h\nu = E$ energy**. The path of electron $-e^-$ flow \Rightarrow in metabolism is complex. Electrons e^- move from \rightarrow various metabolic intermediates to specialized electron e^- carriers in **enzyme** - catalyzed reactions. The **carriers e^-** in turn **donate** electrons e^- to **acceptors** with higher electron e^- **affinities**, with the release of **energy**. Cells contain a variety of molecular **energy transducers**, which convert the **energy** of electron e^- flow into useful work: $W = E \cdot F \cdot n$; where, **E** potential between species (in volts **V**); **F** = **96485 C** (coulomb) **1 mol** of electrons e^- electric charge in **C**; **n** number of electrons e^- involved in species **OxRed** reactions - between **oxidized** state (**Ox**) and **reduced** state (**Red**) of compound. Established so called **OxRed** system with own **RedOx** potential **E** :

$$Ox + ne^- \rightleftharpoons Red ; E = E^\circ + \frac{\ln(10) \cdot R \cdot T}{F \cdot n} \cdot \lg\left(\frac{[Ox]}{[Red]}\right) \quad (1)$$

where **E°** - standard potential of given **OxRed** system measured at conditions when **E = E°** (as **[Ox] = [Red]**); natural logarithm of number **10** - **ln(10) = 2.302585093** ; universal gas constant - **R = 8.3144 J/mol/K** ; absolute thermodynamics temperature **T = 273.16° + 25° C = 298.16° K** at standard temperature conditions measured : as Kelvin scale value **273.16 K** at zero **0°C** point plus on Celsius scale measured **25°C** but human body temperature **37° C** that will be higher **T = 273.16° + 37° (C) = 310.16° K** non-standard conditions; Faraday's constant - **F = 96 485 C** (coulomb) **1 mol** of electrons e^- electric charge in **C**; number of electrons e^- involved in **OxRed** system - **n** ; decimal logarithmic function - **lg()** of argument as ratio (**[Ox]/[Red]**) between **oxidized form** concentration - **[Ox]** as multiple over **reduced form** concentration **[Red]**.

We begin our discussion with a description of the general types of metabolic reactions in which electrons e^- are **transferred**. After considering the theoretical and experimental basis for measuring the **energy** changes ΔG in **oxidation** reactions in terms of **electromotive force (EMF)**, we discuss the relationship between this **force**, expressed in volts **V**, and the **free-energy** change ΔG , expressed in joules **J**. We conclude by describing the structures and **oxidation-reduction chemistry** of the most common of the specialized electron e^- carriers, which you will encounter repeatedly in later discussions.

The Flow of Electrons can do Biological Work

Every time we use a motor, an electric light or heater, or a spark to ignite gasoline in a car engine, we use the flow of electrons e^- to accomplish work $W = E \cdot F \cdot n$, where **n** (in units **mol**) is the **electron number of moles** moving from **reduced** form **Red** to **oxidized** form **Ox** . In the circuit that powers a motor, the source of electrons e^- can be a **battery** containing two **2** chemical species that differ in **affinity** for electrons e^- . Electrical wires provide a **pathway** for electron e^- flow from the chemical species **reduced form Red₁** at one pole(-) of the **battery**, through the motor, to the chemical species **oxidized form Ox₂** at the other second pole(+) of the **battery**. $(-)Red_1 \rightleftharpoons Ox_1 + ne^-$ Electrical transfer **n** number of electrons ne^- flow to $Ox_2 + ne^- \rightleftharpoons Red_2(+)$ as **electron-carriers** in **biological Ox-Red systems** transfer **n** number of electrons ne^- flow from **E₁** to **E₂** for closed circuit **electric-motion force** is calculated as difference **EMF = E₁ - E₂** in volts **V** . Because the two **2** chemical species differ in their **affinity** difference for electrons e^- , electrons e^- flow spontaneously through the circuit, driven by a **force** proportional to the difference in electron e^- **affinity**, the **electro-motive force (EMF)** . The **electromotive force** (typically a few volts $\pm 1 \div 3.5$ V) can accomplish work $W = EMF \cdot F \cdot n$ if an appropriate **energy transducer** in this case a motor is placed in the circuit. The motor can be coupled to a variety of mechanical devices to accomplish useful work $W = EMF \cdot F \cdot n$.

Living cells have an analogous biological "circuit," with a relatively **reduced** compound such as **glucose** as the source of electrons e^- . As **glucose** is **enzymatic oxidized**, the electrons e^- released flow spontaneously through a series of **electron-carrier** intermediates to another chemical species, such as **O₂**.

This electron e^- flow is **exoergonic** because **O₂** has a higher affinity for electrons e^- than do the **electron-carrier** intermediates. The resulting **electro-motive force** provides **energy** to a variety of molecular **energy transducers** (**enzymes** and other proteins) that do biological work **W**. In the mitochondrion, for example, **membrane-bound enzymes** couple electron e^- flow to the production of a **trans-membrane pH** difference, accomplishing **osmotic** and **electrical work**. The proton **H⁺** gradient thus formed has **potential energy**, sometimes called the **proton-motive force** by analogy with **electro-motive force**. Another **enzyme**, **ATP synthase** in the inner mitochondrial membrane, uses the **proton-motive force** E_{membrane} to do chemical work **W**: synthesis of **ATP** from **ADP** and **Pi** as protons **H⁺** flow spontaneously across the membrane. Similarly, membrane-localized **enzymes** in *E. coli* convert **electro-motive force** to **proton-motive force**, which is then used to power flagella motion.

The principles of electrochemistry that govern energy changes in the macroscopic circuit with a motor and **battery** apply with equal validity to the molecular processes accompanying electron e^- flow in living cells. We turn now to a discussion of those principles.

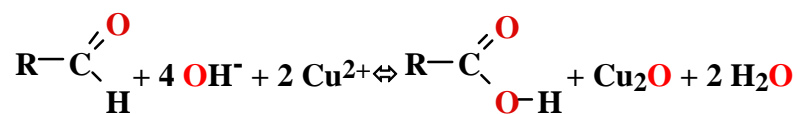
Oxidation-Reductions Can Be Described as Half-Reactions of two Ox \leftrightarrow Red Systems

Although **oxidation** and **reduction** must occur together, it is convenient when describing electron e^- transfers to consider the two halves (**2OxRed** systems) of an **oxidation-reduction** reaction separately. For example, the **oxidation** of ferrous ion **Fe²⁺** by cupric ion **Cu²⁺**,

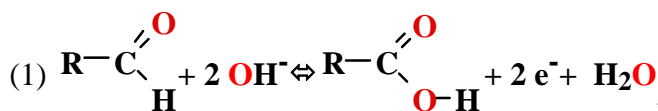
$\text{Fe}^{2+} + \text{Cu}^{2+} \Rightarrow \text{Fe}^{3+} + \text{Cu}^+$ can be described in terms of two **2** half-reactions (**Ox \leftrightarrow Red** systems):

- (1) $\text{Fe}^{2+} \leftrightarrow \text{Fe}^{3+} + e^-$ The electron-donating e^- molecule in an **oxidation-reduction** reaction is called
- (2) $\text{Cu}^{2+} + e^- \leftrightarrow \text{Cu}^+$ the **reducing agent** or **reductant**; the electron-accepting $+ e^-$ molecule is the **oxidizing agent** or **oxidant**. A given agent, such as an iron cation existing in the ferrous (**Fe²⁺**) or ferric (**Fe³⁺**) state, functions as a conjugate **reductant-oxidant** pair (**RedOx** pair), just as an **acid** and corresponding **base** function as a conjugate **acid-base** pair. Recall from **Acid-Base Equilibrium** that in **acid-base** reactions we can write a general equation: proton donor $\leftrightarrow \text{H}^+$ + proton acceptor. In **RedOx** reactions we can write a similar general equation: electron **donor** $\leftrightarrow ne^-$ + electron **acceptor**. In the reversible half reaction (1) above, **Fe²⁺** is the electron **donor** and (2) **Fe³⁺** is the electron **acceptor**; together, **Fe²⁺** and **Fe³⁺** constitute a **conjugate RedOx pair**.

The electron e^- transfers in the **oxidation-reduction** reactions of organic compounds are not fundamentally different from those of inorganic species. In **Reducing Sugars** we considered the **oxidation** of a **reducing sugar** (an free aldehyde or ketone) by cupric ion **Cu²⁺** (see **reducing sugars**):



This overall reaction can be expressed as two **2** half-reactions using **OxRed** systems:



- Because two **2** electrons $2 e^-$ are removed from the aldehyde carbon $-(\text{C}=\text{O})-\text{H}$, the second half-reaction (the one-electron reduction of cupric **Cu²⁺** to cuprous ion **Cu⁺**) must be doubled **2** to balance the overall equation two **2** electrons $2 e^-$ are gained on two cupric **Cu²⁺** cations converting to two cuprous ions **Cu⁺**.

Biological Oxidations Often Involve Dehydrogenation

The carbon in living cells exists in a range of **oxidation** states (Fig. 1). When a carbon atom shares an electron pair : with another atom (typically **H**, **C**, **S**, **N**, or **O**), the sharing is unequal in favor of the more **electronegative** atom. The order of increasing **electro negativity**: $\text{H} < \text{C} < \text{S} < \text{N} < \text{O}$ respectively

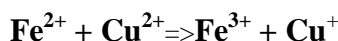
$$2.2 < 2.55 < 2.58 < 3.04 < 3.44.$$

In over simplified but useful terms, the more **electronegative** atom "owns" the bonding electrons e^- it shares with another atom. For example, in methane (CH_4) carbon **C** is more **electronegative** than the four **4** hydrogens **H** bonded to it, and the **C** atom therefore "owns" all eight **8** bonding electrons 8 e^- (Fig. 1). In **ethane**, the electrons e^- in the C-C bond are shared equally, so each :C:C: atom owns only seven **7** of its eight **8** bonding electrons e^- . In **ethanol**, **C-1** is less electronegative than the **oxygen O** to which it is bonded, and the **O** atom therefore "owns" both electrons 2 e^- of the C-O bond, leaving :C-1 with only five **5** bonding electrons e^- . With each formal loss of electrons e^- , the carbon **C** atom has undergone **oxidation** even when no **oxygen O** is involved, as in the conversion of an **alkene** ($\text{CH}_2=\text{CH}_2$) to an **alkyne** ($\text{CH}\equiv\text{CH}$). In this case, **oxidation** (loss of electrons e^-) is coincident with the loss of hydrogen - **H**. In biological systems, **oxidation** is often synonymous, with **dehydrogenation**, and many **enzymes** that catalyze **oxidation** reactions are **dehydrogenases**. Notice that the more **reduced** compounds in Figure 1 (top) are richer in hydrogen **H** than in **oxygen O**, whereas the more **oxidized** compounds (bottom) have more **oxygen O** and less **hydrogen H**.

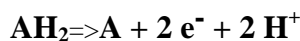
Not all biological **oxidation-reduction** reactions involve carbon **C**. For example, in the conversion of molecular **nitrogen N₂** to ammonia NH_3 : $6\text{H}^+ + 6\text{e}^- + \text{N}_2 \Rightarrow 2\text{NH}_3$, the **nitrogen N** atoms are **reduced**.

Electrons e^- are transferred from one molecule (electron e^- **donor**) to another (electron e^- **acceptor**) in one of four different ways:

1. Directly as electrons e^- . For example, the $\text{Fe}^{2+} / \text{Fe}^{3+}$ RedOx pair can transfer an electron e^- to the $\text{Cu}^+ / \text{Cu}^{2+}$ RedOx pair:

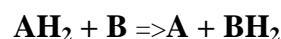


2. As **hydrogen H** atoms. Recall that a hydrogen **H** atom consists of a proton H^+ and a single electron e^- . In this case we can write the general equation:



where AH_2 is the **hydrogen/electron donor**.

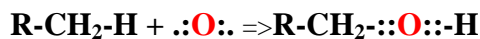
(Do not mistake the above reaction for an acid dissociation; the H^+ arises from the removal of a hydrogen atom ($\text{H}^+ + \text{e}^-$).) AH_2 and **A** together constitute a conjugate **RedOx** pair (A / AH_2), which can **reduce** another compound **B** (or **RedOx** pair, B / BH_2) by transfer of hydrogen **H** atoms:



3. As a **hydride ion (:H^-)**, which has two **2** electrons e^- .

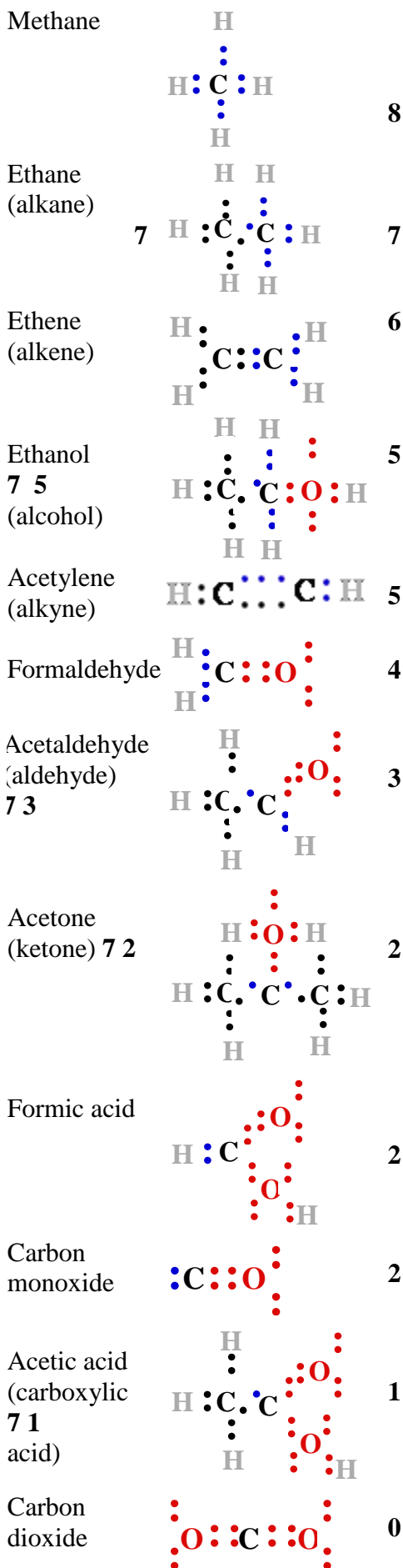
This occurs in the case of **NAD-linked dehydrogenases**, described below.

4. Through direct combination with **oxygen O₂**. In this case, **oxygen O₂** combines with an **organic reductant** and is **covalently incorporated** in the product, as in the **oxidation** of a hydrocarbon to an alcohol by $\frac{1}{2}\text{O}_2$ presented as **O**:



The **hydrocarbon** is the electron e^- **donor** and the **oxygen O** atom is the electron e^- **acceptor**.

All four **4** types of electron e^- transfer occur in cells. The neutral term **reducing equivalent** is commonly used to designate a single **1** electron e^- **equivalent** participating in an **oxidation-reduction** reaction, no matter whether this **equivalent** is an electron e^- per se, a hydrogen **H** ($\text{H}^+ + \text{e}^-$) atom, or a hydride ion :H^- , or whether the electron e^- transfer takes place in a reaction with **oxygen O** to yield an **oxygenated** product. Because biological **fuel** molecules are usually **enzymatic dehydrogenated** to lose two **2** **reducing equivalents** at a time, and because each **oxygen O** atom can **accept** two **2** **reducing equivalents**, biochemists by convention regard the unit of biological **oxidations** as two **2** **reducing equivalents** passing from **substrate** \Rightarrow **oxygen O**.



7 Figure 1. Oxidation states of carbon **C** occurring in the biosphere.

The **oxidation** states are illustrated with some representative **compounds**. Focus on the black carbon **C** atom and its bonding electrons e^- .

When this carbon **C** is bonded to the less \downarrow **electro negative H** atom, both bonding electrons (blue - : •) are assigned to the carbon **C**.

When carbon **C** is bonded to another carbon **C**, bonding electrons e^- are shared equally, so one blue • of the two 2 electrons e^-e^- is assigned to the black carbon **C**.

When the black carbon **C** of our interest is bonded to the more **electronegative O** atom, the bonding electrons e^- are assigned to the **oxygen O**.

The number to the right of each compound is the number **n** of electrons e^- "owned" by the black carbon **C** of our interest, a **rough expression** of the **oxidation** state of that carbon **C**.

When the black carbon **C** undergoes **oxidation** (loses electrons e^-), the number **n** \downarrow gets smaller.

Thus the order of increasing \uparrow **oxidation** state is

methane <
 < **alkane** <
 < **alkene** <
 < **alcohol** <
 < **alkyne** <
 < **aldehyde** <
 < **ketone** <

< **formic acid** <

< **carbon monoxide**.

< **carboxylic acid** <

< **carbon dioxide**.

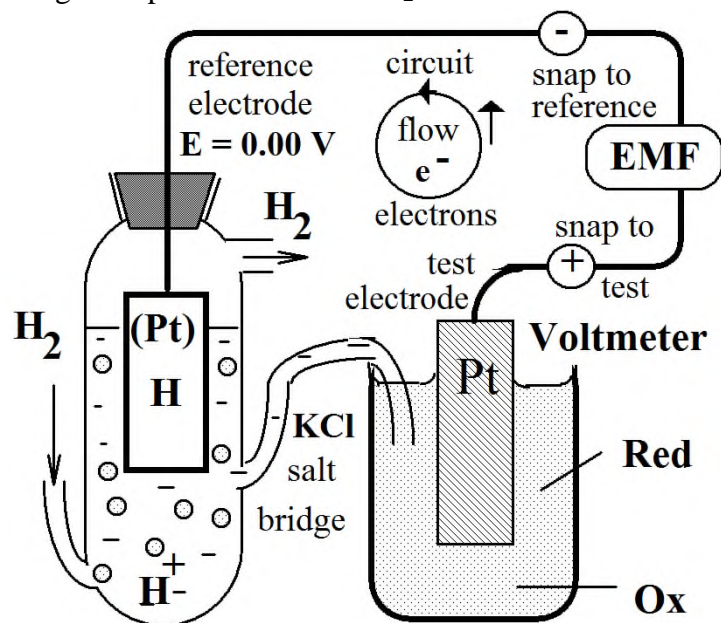
Reduction Potentials Measure Affinity for Electrons

When two 2 conjugate RedOx pairs are together in solution, electron e^- transfer from the electron e^- donor of one RedOx pair to the electron e^- acceptor of the other may occur spontaneously. The tendency for such a reaction depends on the relative **affinity** of the electron e^- acceptor of each RedOx pair for electrons e^- . The **standard reduction potential**, E° a measure (in volts V) of this **affinity**, can be determined in an experiment such as that described in Figure 2. Electrochemists have chosen as a **standard of reference** the half-reaction $H^+ + e^- \rightleftharpoons H_2$ (Pt), ($1/2 H_2$) Hydrogen saturated platinum (Pt) H_2 is a solid metal- first I type electrode.

$$E = E^\circ + \frac{\ln(10) \cdot R \cdot T}{F \cdot 1} \cdot \lg([H_3O^+]); \text{ because of Brønsted water protonation } H^+ + H_2O \rightleftharpoons H_3O^+ \quad (2)$$

$$E = -0.05916 \cdot \text{pH}; \text{ because } -\lg([H_3O^+]) = \text{pH}, \text{ and } \frac{\ln(10) \cdot R \cdot T}{F} = 0.05916 \text{ V at standard conditions} \quad (3)$$

The electrode at which this half-reaction occurs (called a half-cell) is arbitrarily assigned a **standard reduction potential** E° of **0.00 V**. When this **hydrogen electrode** is connected through an external circuit to another half-cell in which an **oxidized** species and its corresponding **reduced** species are present as pure H_2 compound at standard concentrations C (each solute at **1 M**, each gas at **101.3 kPa** or **1 atm**), electrons e^- tend to flow through the external circuit from the half-cell of lower **standard reduction potential** E°_1 to the half-cell of higher **standard reduction potential** E°_2 . By convention, the half-cell with the stronger tendency to acquire electrons e^- is assigned a positive value of $E^\circ_2 > 0.00 \text{ V}$.



hydrogen electrode
pure H_2 gas at standard pressure saturates platinum Pt metal with H hydrogen forming reference cell with known $E = 0.00 \text{ V}$

Test cell containing $\text{pH}=7$ concentration of the **oxidized** [H^+] and **reduced** $H(\text{Pt})$ (like solid as left) species of the RedOx pair to be examined

From the observed **EMF** and the known $E = 0.00 \text{ V}$ for **reference** cell, the E_o of the **test** cell containing the RedOx pair is obtained as calculation $\text{EMF} + E = E_o = -0.41412 \text{ V}$. The cell that gains electrons has, by convention, the more positive $E > E_o$ **reduction potential** and so as well as $E = 0.00 \text{ V} > -0.41412 \text{ V} = E_o$.

The **reduction potential** E of a half-cell depends not only on the chemical species present but also on their activities, approximated by their concentrations C . About a century ago, Walther Nernst derived an equation that relates **standard reduction potential** (E') to the **reduction potential** (E) at any concentration of **oxidized** [Ox] and **reduced** [Red] species in the cell:

Voltmeter the device for measuring **EMF** connected to reference snap and test snap **Figure 2**.

Measurement of the **standard reduction potential** (E_o) of a RedOx pair at $\text{pH}=7$ condition.

Electrons e^- flow from the **test** electrode to the **reference** electrode if **standard reduction potential** is negative $E_o < 0.00 \text{ V}$ or vice versa if positive $E_o > 0.00 \text{ V}$. The ultimate **reference** half-cell is the **hydrogen electrode**, as shown here.

Electro-chemists have chosen as a **standard of reference** the half-reaction which this half-reaction occurs is arbitrarily assigned a **standard reduction potential** $E = E^\circ$ of **0.00 V** $\text{pH}=0$.

The **electro-motive force** ($\text{EMF} = E_o - E$) at $\text{pH}=7$, E_o for the **hydrogen electrode** RedOx system

$H^+ + e^- \rightleftharpoons H_2(\text{Pt})$ is $-0.41412 \text{ V} = E_o = -0.05916 \cdot \text{pH}$ and

$\text{EMF} = -0.41412 - 0.00 \text{ V}$. The direction of electron e^- flow \rightarrow depends on the relative electron "pressure" or **potential** of the two 2 cells. A salt bridge containing a saturated **KCl** solution provides a path for counter-ion movement between the **test** cell and the **reference** cell.

| Half-reaction - OxRed systems | Data source | E ₀ (V) | E _M (V) | E° (V) | E°H ₂ O(V) | E°37(V) |
|---|--------------------|--------------------|--------------------|---------|-----------------------|---------|
| $\text{H}_2\text{O}_2 + 2\text{H}_3\text{O}^+ + 2\text{e}^- = 4\text{H}_2\text{O}$ | Suchotina | 1.7356 | 1.6734 | 1.776 | 1.9821 | 1.9742 |
| $\text{O}_2^- + 2\text{H}_3\text{O}^+ + \text{e}^- = \text{H}_2\text{O}_2 + 2\text{H}_2\text{O}$ | David Harris | 0.305 | 0.1806 | 1.2764 | 1.48246 | 1.4251 |
| $\text{O}_{2g} + 4\text{H}_3\text{O}^+ + 4\text{e}^- = 6\text{H}_2\text{O}$ | Suchotina | 0.813 | 0.751 | 1.2288 | 1.38334 | 1.3732 |
| $\text{NO}_3^- + 3\text{H}_3\text{O}^+ + 2\text{e}^- = \text{HNO}_2 + 4\text{H}_2\text{O}$ | University Alberta | 0.2889 | 0.1957 | 0.9275 | 1.13355 | 1.1291 |
| $\text{NO}_3^- + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{NO}_2^- + 3\text{H}_2\text{O}$ | David Harris | 0.3913 | 0.3291 | 0.8351 | 0.98967 | 0.95138 |
| $\text{p-quinone} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{Hydroquinone} + 2\text{H}_2\text{O}$ | | 0.2336 | 0.1714 | 0.6994 | 0.80243 | 0.79365 |
| $\text{O}_{2aq} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{H}_2\text{O}_{2aq} + 2\text{H}_2\text{O}$ | University Alberta | 0.2336 | 0.1715 | 0.6945 | 0.7975 | 0.7937 |
| $\text{Fe}^{3+} + \text{e}^- = \text{Fe}^{2+}$ | University Alberta | 0.783 | 0.783 | 0.7690 | 0.7690 | 0.7830 |
| $\text{Ubiquinone} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{Ubiquinol} + 2\text{H}_2\text{O}$ | | 0.0197 | 0.0819 | 0.4591 | 0.56215 | 0.5404 |
| $\text{Fumarate}^{2-} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{Succinate}^{2-} + 2\text{H}_2\text{O}$ | | 0.0332 | 0.0953 | 0.4451 | 0.54815 | 0.52695 |
| $\text{CrotonylCoA} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{ButyrylCoA} + 2\text{H}_2\text{O}$ | | -0.0774 | -0.1395 | 0.3991 | 0.50215 | 0.48273 |
| $\text{C}_6\text{H}_6\text{O}_6 + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{AscorbicAcid} + 2\text{H}_2\text{O}$ | DC.Harris | -0.0862 | -1.483 | 0.3900 | 0.4930 | 0.47395 |
| $\text{Glyoxylate} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{glycolate} + 2\text{H}_2\text{O}$ | D.C.Harris 25°C | -0.111 | -0.171 | 0.324 | 0.42715 | 0.42715 |
| $\text{Cytochrome F Fe}^{3+} + \text{e}^- = \text{Fe}^{2+}$ | David Harris | 0.3509 | 0.3509 | 0.3650 | 0.3650 | 0.3509 |
| $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-} + \text{e}^- = [\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ | University Alberta | 0.3258 | 0.3258 | 0.3557 | 0.3557 | 0.3258 |
| $\text{Oxalo-acetate}^{2-} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{Malate}^{2-} + 2\text{H}_2\text{O}$ | | -0.2225 | -0.2847 | 0.2481 | 0.35115 | 0.33757 |
| $\text{Cytochrome a3 Fe}^{3+} + \text{e}^- = \text{Fe}^{2+}$ | | 0.3365 | 0.3365 | 0.3500 | 0.3500 | 0.3365 |
| $\text{Pyruvate}^- + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{lactate}^- + 2\text{H}_2\text{O}$ | | -0.2408 | -0.3030 | 0.2291 | 0.33215 | 0.3193 |
| $\text{FADfree} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{FADH}_2 + 2\text{H}_2\text{O}$ | * | -0.2735 | -0.3356 | 0.1951 | 0.29815 | 0.28662 |
| $\text{CH}_3\text{CHO} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{CH}_3\text{CH}_2\text{OH} + 2\text{H}_2\text{O}$ | KortlyShucha | -0.2784 | -0.3406 | 0.1900 | 0.2930 | 0.28169 |
| $\text{Cytochrome a Fe}^{3+} + \text{e}^- = \text{Fe}^{2+}$ | | 0.2788 | 0.2788 | 0.290 | 0.290 | 0.2788 |
| $\text{GlutaS-Sthione} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = 2\text{GlutathSH} + 2\text{H}_2\text{O}$ | | -0.2841 | -0.3462 | 0.1841 | 0.28715 | 0.27604 |
| $\text{S}_{rhb} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{HSH} + 2\text{H}_2\text{O}$ | University Alberta | -0.2859 | -0.3480 | 0.1739 | 0.27693 | 0.27424 |
| $\text{Cytochrome c Fe}^{3+} + \text{e}^- = \text{Fe}^{2+}$ | | 0.2442 | 0.2442 | 0.254 | 0.254 | 0.2442 |
| $\text{LipoicAcidS-S} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{LipSHSH} + 2\text{H}_2\text{O}$ | | -0.3417 | -0.4039 | 0.1241 | 0.22715 | 0.21837 |
| $\text{Cytochrome c1 Fe}^{3+} + \text{e}^- = \text{Fe}^{2+}$ | | 0.2115 | 0.2115 | 0.220 | 0.220 | 0.2115 |
| $\text{AcetoAcetate}^- + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \beta\text{-OHB} + \text{Butyrate}^- + 2\text{H}_2\text{O}$ | | -0.3956 | -0.4577 | 0.0681 | 0.17115 | 0.16453 |
| $\alpha\text{-Ketoglutarate}^{2-} + \text{CO}_2 + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{isocitrate}^{2-} + 2\text{H}_2\text{O}$ | | -0.4283 | -0.4904 | 0.0341 | 0.13715 | 0.13185 |
| $\text{H}_3\text{O}^+ + \text{e}^- = \text{H(Pt)} + \text{H}_2\text{O}$ | | -0.4611 | -0.5232 | 0.000 | 0.10303 | 0.09904 |
| $\text{Cytochrome b Fe}^{3+} + \text{e}^- = \text{Fe}^{2+}$ | | 0.074 | 0.074 | 0.077 | 0.077 | 0.074 |
| $\text{CH}_3\text{COOH} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{CH}_3\text{CHO} + 3\text{H}_2\text{O}$ | Suchotina | -0.5784 | -0.6407 | -0.118 | 0.03654 | 0.03513 |
| $13\text{PGlycerate}^{4-} + 2\text{H}_3\text{O}^+ + 2\text{e}^- = \text{Glycaldeh3-P}^{2-} + 2\text{H}_2\text{O} + \text{P}^{2-}$ | | -0.5873 | -0.6496 | -0.1314 | -0.0284 | -0.0273 |
| $\text{NADP}^+ + \text{H}_3\text{O}^+ + 2\text{e}^- = \text{NADPH} + \text{H}_2\text{O}$ | | -0.3429 | -0.3740 | -0.117 | -0.0654 | -0.0629 |
| $\text{NAD}^+ + \text{H}_3\text{O}^+ + 2\text{e}^- = \text{NADH} + \text{H}_2\text{O}$ | David Harris | -0.3391 | -0.3702 | -0.113 | -0.0614 | -0.0590 |
| $\text{O}_{2g} + \text{e}^- = \text{O-2aq}$ | Suchotina | -0.2355 | -0.2355 | -0.245 | -0.245 | -0.2355 |
| $\text{Ferredoxin Fe}^{3+} + \text{e}^- = \text{ferredoxin Fe}^{2+}$ | | -0.415 | -0.415 | -0.432 | -0.432 | -0.415 |
| $2\text{C}_3\text{H}_4\text{O}_3 + 4\text{H}_3\text{O}^+ + 4\text{e}^- = \text{C}_6\text{H}_{12}\text{O}_6 + 4\text{H}_2\text{O}$ | Stryer | -0.9975 | -1.060 | -0.5427 | -0.4397 | -0.4373 |
| $\text{H}_2\text{O} + \text{e}^- = \text{H(Pt)} + \text{OH}^-$ | Suchotina | -0.5938 | -0.6559 | -0.828 | -0.9311 | -0.8951 |

Table 1. Standard Reduction Potentials E₀ and E_M of Some Biologically Important Half-Reactions, at 37 °C for pH=7.36 and 8.37 (in mitochondria), E° at standard conditions 298.16 K, pH=0 for H⁺/H reference electrode E°=0.00 V, E°_{H₂O} corrected to water concentration [H₂O] = 997.07/18.0153 = 55.3457 M from equations where involved, and E°₃₇. at body temperature conditions 310.16 K(37 °C) calculated from E°_{H₂O}

Data mostly from: 1. Loach, P.A. (1976) In Handbook of Biochemistry and Molecular Biology,

2. 3rd edn (Fasman, G.D. ed.), Physical and Chemical Data, Vol. 1, pp. 122-130 e, CRC Press,

3. A.M. Suchotina, Handbook of Electro-Chemistry, Petersburg, 1981. "Chimia"©

4. S.Kortly and L.Shucha. Handbook of chemical equilibria in analytical chemistry. 1985.EllisHorwood Ltd.©

5. University Alberta Data Tables Molar Thermodynamic Properties of Pure Substances 1997.,

<http://www.vhem.ualberta.ca/courses/plambeck/p101/p00403.htm>

6. Boca Raton, FL. "This is the value for free FAD;

FAD bound to a specific flavo-protein (for example succinate dehydrogenase) has a different E°

7. David A. Harris, "Bio-energetic at a Glance". b Blackwell Science Ltd ©, 1995, p.116.

8. Daniel C. Harris, "Quantitative chemical analysis". W.H.Freeman and Company ©, 5th ed.1999, p545

$$E = E^\circ + \frac{\ln(10) \cdot R \cdot T}{F \cdot n} \cdot \lg \left(\frac{[\text{Ox}]}{[\text{Red}]} \right)$$

where E° - **standard potential** of given **OxRed** system measured at conditions when $E = E^\circ$ (as $[\text{Ox}] = [\text{Red}]$); natural logarithm of number 10 - $\ln(10) = 2.302585093$; universal gas constant - $R = 8.3144 \text{ J/mol/K}$; absolute thermodynamics temperature $T = 273.16^\circ + 25^\circ(\text{C}) = 298.16^\circ \text{ K}$ at standard temperature **conditions** measured: as Kelvin scale value **273.16 K** at zero 0° C point plus on Celsius scale measured **25°C** but human body temperature **37°C** that will be higher $T = 273.16^\circ + 37^\circ(\text{C}) = 310.16^\circ \text{ K}$ non-standard **conditions**; **Faraday's** constant - $F = 96\,485 \text{ C}$ (coulomb) **1 mol** of electrons e^- electric charge in **C**; At **298 K (25 °C)** and at **310.16 K (37 °C)**, this expression reduces to respectively following expressions:

$$E = E^\circ + \frac{0.05916\text{V}}{n} \cdot \lg \left(\frac{[\text{Ox}]}{[\text{Red}]} \right) ; E = E^\circ + \frac{0.0615\text{V}}{n} \cdot \lg \left(\frac{[\text{Ox}]}{[\text{Red}]} \right)$$

Many half-reactions of interest to biochemists involve protons H^+ or thermodynamically corrected reality **hydronium ion** H_3O^+ . As in the definition of ΔG° , biochemists define the **standard** state for **oxidation-reduction** reactions as **pH 7.36** and express the **standard reduction potential** as E_0 , the standard reduction potential at **pH 7.36**. The **standard reduction potentials** given in Table 1 and used throughout this book are values for E_0 and are therefore only valid for systems at neutral **pH**. Each value represents the potential difference when the conjugate **RedOx** pair, at **equal** concentrations $[\text{Ox}] = [\text{Red}]$ and **pH = 7.36**, is connected with the **standard (pH=0) hydrogen electrode**. Notice in Table 1 that when the conjugate pair H^+/H at **pH 7** is connected with the **standard hydrogen electrode (pH 0)**, electrons e^- tend to flow from the **pH 7** cell \Rightarrow to \Rightarrow the **standard (pH 0)** cell; the measured E_0 for the H^+/H pair according (3) is $-0.05916 \cdot 7 = -0.41412 \text{ V}$

Standard Reduction Potentials Can Be Used to Calculate the Free-Energy Change

The usefulness of **reduction potentials** stems from the fact that when **reduction potentials** E_1 and E_2 values have been determined for any two half-cells, relative to the **standard hydrogen electrode potential** $E^\circ = 0.00 \text{ V}$ as reference, their **EMF** = $E_{\text{Ox}2} - E_{\text{Red}1}$ values relative to each other are also known. We can then predict the direction **Red₁ \Rightarrow Ox₂** in which electrons e^- will tend to flow when the two half-cells are connected through an external circuit or when components **Red₁ \Rightarrow Ox₂** of both half-cells are present in the same solution. Electrons e^- tend to flow to the half-cell with the more positive E° and the strength of that tendency is proportional to the difference in **reduction potentials**, ΔE° .

The energy ΔG made available by this spontaneous electron e^- flow by **work** action $\Delta G = W$ (the **free-energy** change ΔG for the **oxidation-reduction** reaction) is proportional \sim to ΔE . According **RedOx** system positive charged **oxidized** form Ox^{n+} - formed with lost electrons ne^- - flows from metal site (mostly used platinum **Pt** and bearing free electron ne^- gas) to solution. In this movement process **RedOx** system are accomplished the chemical work $W = E \cdot F \cdot n$ by spending given **RedOx** system **free energy** $-\Delta G = -(G_{\text{Ox}} - G_{\text{Red}})$ in conversion of **reduced** form **Red** to **oxidized** form Ox^{n+} :



Here n represents the number of electrons ne^- transferred in the reaction.

Chemical Potential of Species μ

Professor Ilya Prigogine and co has shown that **chemical potential** μ of compound **A** show, how much change of **free energy** ΔG_A brings into system of our interest when adding of **1 mol** amount of compound **A**.

In a fact: how great amount of free energy belongs to one **1 mol** of compound.

It means how much free energy ΔG_A has itself per **1 mol** compound **A**,

- **chemical potential** μ of compound **A** if amount of compound in molar numbers is $\Delta n_A = 1 \text{ mol}$

$$\mu_A = \frac{\Delta G_A}{\Delta n_A} ; \mu_A = \Delta G^\circ + R \cdot T \cdot \ln(X_A) , \text{ where } X_A \text{ is concentration of A unit less mol fraction } X_A = \frac{n_A}{n_{\text{total}}} \quad (5)$$

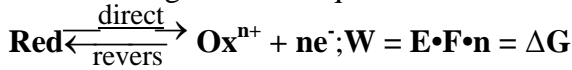
For pure compound **A** when $n_A = n_{\text{total}}$ **mol fraction** is $X_A = 1$ so $\ln(1) = 0$ and $\mu = \Delta G^\circ$ that present **standard free energy** of formation the **1 mol** compound **A** from elements.

Reaction proceeds completely until end only when products of reaction have hardly little disposition to reverse change back. In other words these products of reaction have trifling or zero value of **chemical potential**.

Data from: I. Prigogine, R. Defey. "Chemical Thermodynamics".1954, Longmans Green and co ©.

Conditions of chemical equilibrium.

Provided **chemical potential** of reaction products is taking into consideration (it has anything remarkable level of value), then reaction proceeds not completely until end, go not on but one can observe the setting in **equilibria**. In state of **equilibria** sum of **chemical potentials** for initial compounds is equal to sum of **chemical potentials** for products – according chemical equation



initial compounds products work accomplished by movement of positively ($n+$) charged Ox^{n+} form from metal surface, comprising electron $n\text{e}^-$ gas, and touching **Red** form, to solution of **RedOx** system.

For **RedOx** system due to electric work of charged Ox^{n+} movement between metal and **RedOx** system solution sides are not equal $\mu_{\text{Red}} \neq \mu_{\text{Ox}^{n+}} + n \mu_{\text{e}^-}$ but coefficient $n\text{e}^-$ means $\text{e}^- + \text{e}^- + \text{e}^- \dots n$ times electron e^- takes a part in reaction as is seen in expression of **equilibrium** (4). Free energy change ΔG for any chemical reaction is to calculate as **chemical potential** sum subtraction: the product $\Sigma \mu_{\text{product}}$ minus initial $\Sigma \mu_{\text{initial}}$:

$\Delta G = (\Sigma \mu_{\text{Ox}^{n+}} + n \mu_{\text{e}^-}) - \Sigma \mu_{\text{Red}} = E \cdot F \cdot n$, and **equilibrium** establishes when electric work is compensated by free energy change $\Delta G = W = E \cdot F \cdot n$ and formation on metal (**Pt**) **electrode reduction potential E**.

$$\Sigma \mu_{\text{Red}} + E \cdot F \cdot n = \Sigma \mu_{\text{Ox}^{n+}} + n \mu_{\text{e}^-} \quad (6)$$

At **equilibrium** the **chemical potential** sum of initial compounds and products are equal and **reduced** form includes the compensating free energy change $\Delta G = W = E \cdot F \cdot n$ forming on metal (**Pt**) **electrode reduction potential E**. Becomes obvious that **chemical potential** sum of **oxidized** form has the number n additional **chemical potential** of free electrons $n \mu_{\text{e}^-}$ those values for all known **RedOx** systems are different and mostly laying in side interval between $-90 \div +90$ kJ/mol. Electrons $n\text{e}^-$ are occupied metal (**Pt**) free electron gas solid phase and as pure solid compound has mol fraction concentration $X_{\text{e}^-} = 1$. Expressing above mentioned meaning of **chemical potentials** (5) we can calculate the free energy change ΔG in given **RedOx** system for **Red** form:

$$\Delta G^\circ_{\text{Red}} + R \cdot T \cdot \ln(X_{\text{Red}}) + E \cdot F \cdot n = \Delta G^\circ_{\text{Ox}^{n+}} + R \cdot T \cdot \ln(X_{\text{Ox}^{n+}}) + n \Delta G^\circ_{\text{e}^-} + n \cdot R \cdot T \cdot \ln(X_{\text{e}^-})$$

$$\Delta G^\circ_{\text{Red}} + R \cdot T \cdot \ln(X_{\text{Red}}) + E \cdot F \cdot n = \Delta G^\circ_{\text{Ox}^{n+}} + R \cdot T \cdot \ln(X_{\text{Ox}^{n+}}) + n \Delta G^\circ_{\text{e}^-} \quad (7)$$

$$\Delta G = E \cdot F \cdot n = \Delta G^\circ_{\text{Ox}^{n+}} + n \Delta G^\circ_{\text{e}^-} - \Delta G^\circ_{\text{Red}} + R \cdot T \cdot \ln\left(\frac{X_{\text{Ox}^{n+}}}{X_{\text{Red}}}\right)$$

$$\Delta G^\circ = E^\circ \cdot F \cdot n = \Delta G^\circ_{\text{Ox}^{n+}} + n \Delta G^\circ_{\text{e}^-} - \Delta G^\circ_{\text{Red}} \quad (8)$$

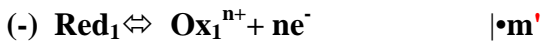
Standard free energy change **RedOx** system for **reduced** form **Red** is expressed as $\Delta G^\circ_{\text{Red}} = E^\circ \cdot F \cdot n$ and for **oxidized** form Ox^{n+} as

$$\Delta G^\circ_{\text{Ox}^{n+}} = -E^\circ \cdot F \cdot n$$

$$\Delta G_{\text{Red}} = E \cdot F \cdot n = E^\circ \cdot F \cdot n + R \cdot T \cdot \ln\left(\frac{X_{\text{Ox}^{n+}}}{X_{\text{Red}}}\right); \Delta G_{\text{Ox}^{n+}} = -E \cdot F \cdot n = -E^\circ \cdot F \cdot n - R \cdot T \cdot \ln\left(\frac{X_{\text{Ox}^{n+}}}{X_{\text{Red}}}\right) \quad (9)$$

With this equation we can calculate the **standard free-energy** change ΔG° for any **RedOx** system from the values of E° in a table of **reduction potentials** (Table 1) and the **free-energy** change ΔG according (9) at known concentrations X_{Red} and $X_{\text{Ox}^{n+}}$ of the each species form (ΔG_{Red} and $\Delta G_{\text{Ox}^{n+}}$) participating in the **RedOx** system.

Considerable **oxidation-reduction** reaction is composed from two **2RedOx** systems (half-reactions) using compounds reaction equivalence law $|+m' \cdot \text{ne}^-| = |-n' \cdot \text{me}^-|$ we have balanced **oxidation-reduction** reaction and can get the summary reaction of both half-reactions:



$m' \cdot \text{Red}_1 + n' \cdot \text{Ox}_2^{m+} \Rightarrow m' \cdot \text{Ox}_1^{n+} + n' \cdot \text{Red}_2$; **initial compounds** forming **products** direction of reaction.

With this equation we can calculate the **standard free-energy** change ΔG° for equi-molar amount of any **oxidation-reduction** reaction from the values of E° in a table of **reduction potentials** (Table 1)

$\Delta G^\circ = m' \cdot \Delta G^\circ_{\text{Red}_1} - n' \cdot \Delta G^\circ_{\text{Ox}_2^{m+}} = m' \cdot E^\circ_{\text{Red}_1} \cdot F \cdot n - n' \cdot E^\circ_{\text{Ox}_2^{m+}} \cdot F \cdot m = (E^\circ_{\text{Red}_1} - E^\circ_{\text{Ox}_2^{m+}}) \cdot F \cdot (m' \cdot n = n' \cdot m)$, where $n' \cdot m' = nm/N$ is equivalent - common number of electrons e^- involved in **RedOx** reaction can be less $n' \cdot m' \leq n \cdot m$. by number N of common divider Red_1 or Ox_2^{m+} and the **free-energy** change ΔG according (9) at known concentrations X_{Red} and $X_{\text{Ox}^{n+}}$ of the each species (ΔG_{Red} and $\Delta G_{\text{Ox}^{n+}}$) participating in the reaction.

$$\Delta G = m \cdot \Delta G_{\text{Red1}} - n \cdot \Delta G_{\text{2Ox}^{n+}} = m \cdot E_{\text{Red1}} \cdot F \cdot n - n \cdot E_{\text{2Ox}^{n+}} \cdot F \cdot m = (E_{\text{Red1}} - E_{\text{2Ox}^{n+}}) \cdot F \cdot (m \cdot n = n \cdot m) =$$

$$= (E^{\circ}_{\text{Red1}} - E^{\circ}_{\text{2Ox}^{n+}}) \cdot F \cdot (m \cdot n = n \cdot m) + R \cdot T \cdot \ln \left(\frac{X_{\text{1Ox}^{n+}}^m \cdot X_{\text{2Red}}^n}{X_{\text{1Red}}^m \cdot X_{\text{2Ox}^{n+}}^n} \right), \text{ where (10)}$$

$$K_{\text{eq}} = \frac{X_{\text{1Ox}^{n+}}^m \cdot X_{\text{2Red}}^n}{X_{\text{1Red}}^m \cdot X_{\text{2Ox}^{n+}}^n}$$

is homeostasis ratio as a multiple **products** over **initial** compounds concentrations.

Consider the reaction in which **acet-aldehyde** is **reduced** by the biological electron carrier **NADH**:



The relevant half-reactions and their E_{37} values are:



By convention (10) balanced $n = 2 = m$ number of electrons $2e^- \Delta E_{37}$ is expressed as E^1_{37} of the electron **donor** minus E^2_{37} of the electron **acceptor**. Because **acet-aldehyde** is **accepting** electrons from **NADH** in our example $\Delta E_{37} = E^1_{37} - E^2_{37} = -0.0590 \text{ V} - 0.28169 \text{ V} = -0.34069 \text{ V}$, and n is 2. Therefore $-0.0590 - 0.28169 = -0.34069$

$$0.34069 \cdot 2 \cdot 96485 = -65742.9495/1000 = -65.7429495 \text{ kJ/mol}$$

$$\Delta G^{\circ} = \Delta E_{37} \cdot F \cdot n = -0.34069 \text{ V} \cdot 2 \text{ mol} \cdot 96485 \text{ C/mol} = -65.7429495 \text{ kJ/mol}$$

This is the free-energy change for the **oxidation-reduction** reaction at equilibrium state when $\Delta G = 0$ and so :

$$\Delta G^{\circ} = -R \cdot T \cdot \ln(K_{\text{eq}}); \frac{X_{\text{NAD}^+} \cdot X_{\text{ethanol}} \cdot X_{\text{H}_2\text{O}}}{X_{\text{NADH}} \cdot X_{\text{Acetaldehyde}} \cdot X_{\text{H}_3\text{O}^+}} = K_{\text{eq}} = e^{-\frac{\Delta G^{\circ}}{R \cdot T}} = e^{-\frac{-65743}{8.314 \cdot 310.15}} = 1.18065 \cdot 10^{11}$$

equilibrium is shifted far to **products** as shows Equilibrium constant $K_{\text{eq}} = 1.181 \cdot 10^{11}$. Anaerobic fermentation conditions NAD^+ concentration exceeds **NADH ratio 70** times at $\text{pH} = 7.36$. At presence of air **oxygen O_2 ratio** $[\text{NAD}^+]/[\text{NADH}]$ is ten times higher **700** times over concentration **NADH**, what cause reaction condition to **oxidize ethanol** and **acet-aldehyde** as well known aerobic fermentation forms acetic acid. If **ethanol ratio** of concentrations is one as produced **ethanol** equal the same **acet-aldehyde** amount in aerobic fermentation: than calculated free energy change is positive $\Delta G = 5195 \text{ J/mol} = (-65.7429495 + 70.938) 27.51 \cdot 8.3144 \cdot 310.15 \cdot \ln(8.85352 \cdot 10^{11} \cdot 1) \Delta G = -742 \text{ J/mol} = (-65.7429495 + 65.00071) 25.21 \cdot 8.3144 \cdot 310.15 \cdot \ln(8.85352 \cdot 10^{11} \cdot 0.1)$ produces 10% ethanol over acetaldehyde and reaction shifted toward acetic acid:

$$\text{Anaerobic } \Delta G \text{ shifted to ethanol negative: } \Delta G = (E_{\text{Red1}} - E_{\text{2Ox}^{n+}}) \cdot F \cdot (m \cdot n = n \cdot m) + R \cdot T \cdot \ln \left(\frac{X_{\text{1Ox}^{n+}}^m \cdot X_{\text{2Red}}^n}{X_{\text{1Red}}^m \cdot X_{\text{2Ox}^{n+}}^n} \right) =$$

| |
|--|
| $= (E^{\circ}_{\text{NADH}} - E^{\circ}_{\text{Acetaldehyde}}) \cdot F \cdot 2 + R \cdot T \cdot \ln \left(\frac{X_{\text{NAD}^+} \cdot X_{\text{ethanol}} \cdot X_{\text{H}_2\text{O}}}{X_{\text{NADH}} \cdot X_{\text{Acetaldehyde}} \cdot X_{\text{H}_3\text{O}^+}} \right)$ |
| $= \Delta G^{\circ} + R \cdot T \cdot \ln \left(\frac{X_{\text{NAD}^+} \cdot X_{\text{ethanol}} \cdot X_{\text{H}_2\text{O}}}{X_{\text{NADH}} \cdot X_{\text{Acetaldehyde}} \cdot X_{\text{H}_3\text{O}^+}} \right)$ |
| $= -65.7429495 + R \cdot T \cdot \ln \left(\frac{X_{\text{NAD}^+} \cdot X_{\text{ethanol}} \cdot X_{\text{H}_2\text{O}}}{X_{\text{NADH}} \cdot X_{\text{Acetaldehyde}} \cdot X_{\text{H}_3\text{O}^+}} \right)$ |
| $= -65.7429495 + 8.3144 \cdot 310.15 \cdot \ln(8.85352 \cdot 10^{10} \cdot 0.1)$ |

$$= -65.7429495 + R \cdot T \cdot \ln \left(\frac{X_{\text{NAD}^+} \cdot X_{\text{H}_2\text{O}} \cdot X_{\text{ethanol}}}{X_{\text{NADH}} \cdot X_{\text{H}_3\text{O}^+} \cdot X_{\text{Acetaldehyde}}} \right) = \left(\frac{70}{1} \cdot \frac{55.21}{10^{-7.36}} \cdot \frac{1}{10} \right) = -6680 \text{ J/mol}$$

$$= -65.7429495 \text{ kJ/mol} + 8.3144 \cdot 310.16 \cdot \ln(8.85352 \cdot 10^{10} \cdot 0.1) = -65.7429495 + 59.063 = -6.680$$

Calculation the free-energy changes ΔG show possible modulation biological **RedOx reaction** at any concentrations X for the **reaction** driving forces and as regulation direction for favorable products formation.

<http://aris.gusc.lv/BioThermodynamics/BioThermodynamics.pdf> page 13 erythrocyte -53.47 kJ/mol $T=310,16 \text{ K}$

Cellular Oxidation of Glucose to Carbon Dioxide Requires Specialized Electron Carriers

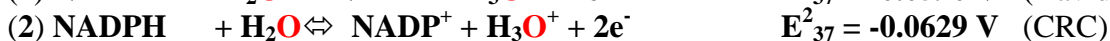
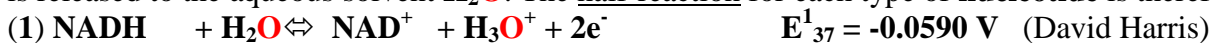
The principles of **oxidation-reduction energetic** described above apply to the many metabolic reactions that involve electron e^- transfers. For example, in many organisms, the **oxidation** of **glucose** supplies **energy** for the production of ATP. The complete **oxidation** of **glucose**: $C_6H_{12}O_6 + 6O_{2(aqua)} + 6H_2O \Rightarrow 6HCO_3^- + 6H_3O^+ + \Delta G^\circ + Q$ has a ΔG° of **-3049,55 kJ/mol**. This is a much larger release of free energy than is required for ATP synthesis erythrocyte and mitochondrial **pH = 7.36** use **-53.47kJ/mol** 55,4% of 100% **120 kJ/mol**. Cells do not convert **glucose** to $CO_{2(aqua)}$ in a single, high-energy-releasing reaction, but rather in a series of controlled reactions, some of which are **oxidations**. The **free energy** released in these **oxidation** steps is of the same order of magnitude as that required for ATP synthesis from ADP, with some **energy** to spare. Electrons e^- removed in these **oxidation** steps are transferred to **coenzymes** specialized for carrying two electrons $2e^-$, such as **NADH** and **FADH₂**(described below). **A Few Types of Coenzymes and Proteins Serve as Universal Electron Carriers**

The multitude of **enzymes** that catalyze cellular **oxidation channel** electrons e^- from their hundreds **100** of different **substrates** into just a few types of universal **electron carriers**. The **reduction** of these **carriers** in catabolic processes results in the conservation of **free energy** released by **substrate oxidation**. **NAD⁺**, **NADP⁺**, **FMN**, and **FAD** are water-soluble **coenzymes** that undergo reversible \Leftrightarrow **oxidation** and **reduction** in many of the electron-transfer e^- reactions of metabolism. The **nucleotides** **NAD⁺** and **NADP⁺** move readily from one **enzyme** to \rightarrow another; the **flavin nucleotides** **FMN** and **FAD** are usually very tightly bound to the **enzymes**, called flavo-proteins, for which they serve as **prosthetic** groups. Lipid-soluble **quinones** such as **ubiquinone** and **plastoquinone** act as **electron carriers** and **proton donors** in the non-aqueous environment of membranes. **Iron-sulfur proteins** and **cytochromes**, which have tightly bound **prosthetic** groups that undergo reversible \Leftrightarrow **oxidation** and **reduction**, also serve as electron e^- carriers in many **oxidation-reduction** reactions. Some of these proteins are water-soluble, but others are **peripheral** or **integral membrane proteins**.

We conclude this chapter by describing some chemical features of **nucleotide coenzymes** and some of the **enzymes** (**dehydrogenases** and **flavo-proteins**) that use them. The **oxidation- reduction** chemistry of **quinones**, **iron-sulfur proteins**, and **cytochromes** is discussed in Oxidative Phosphorylation and Photo-Phosphorylation. **NADH and NADPH Act with Dehydrogenases as Soluble Electron Carriers**

Nicotin-amide adenine dinucleotide (**NAD⁺** in its **oxidized** form) and its close analog **nicotin-amide adenine dinucleotide phosphate** (**NADP⁺**) are composed of two **2nucleotides** joined through their **phosphate** groups by a **phospho-anhydride** bond (Fig. 3). Because the **nicotinamide** ring resembles **pyridine**, these compounds are sometimes called **pyridine nucleotides**. The vitamin **niacin** is the source of the **nicotin-amide** moiety In **nicotin-amide nucleotides**.

Both **coenzymes** undergo reversible \Leftrightarrow **reduction** of the **nicotinamide ring** (Fig. 3). As a **substrate** molecule undergoes **oxidation** (**dehydrogenation**), giving up two **2** hydrogen **H** atoms, the **oxidized** form of the **nucleotide** (**NAD⁺** or **NADP⁺**) **accepts** a **hydride ion** ($:H^-$ the equivalent of a proton **H⁺** and two **2** electrons e^-) and is transformed into the **reduced** form (**NADH** or **NADPH**). The second **2nd** proton **H⁺** removed from the **substrate** is released to the aqueous solvent **H₂O**. The half-reaction for each type of **nucleotide** is therefore



Reduction of **NAD⁺** or **NADP⁺** converts the **benzenoid ring** of the **nicotin-amide** moiety (with a fixed positive (+) charge on the ring **nitrogen N**) to the **quinonoid** form (with no charge $^\circ$ on the **nitrogen N**). Note that the **reduced nucleotides** absorb light at **340 nm**: the **oxidized** forms do not (Fig. 13). The plus sign in the abbreviations **NAD⁺** and **NADP⁺** does not indicate the no charge on these molecules (they are both negatively (-) charged), but rather that the **nicotin-amide ring** is in its **oxidized** form, with a positive (+) charge on the **nitrogen N⁺** atom. In the abbreviations **NADH** and **NADPH**, the "**H**" denotes the added **hydride ion**. To refer to these **nucleotides** without specifying their **oxidation** state, we use **NAD** and **NADP**.

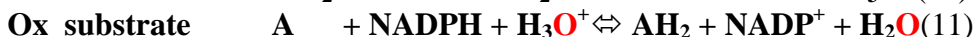
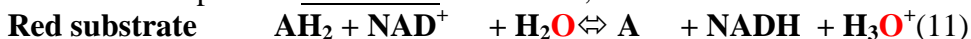
The total concentration of **NAD⁺** + **NADH** in most tissues is about **10⁻⁵M**; that of **NADP⁺** + **NADPH** is about **10** times lower \downarrow . In many cells and tissues, the ratio of **NAD⁺(oxidized)** to **NADH (reduced)** is high, favoring **hydride H⁻** transfer from a **substrate** to **NAD⁺** to form **NADH**. By contrast, **NADPH (reduced)** is generally present in greater \uparrow amounts than its **oxidized** form, **NADP⁺**, favoring **hydride H⁻** transfer from **NADPH** to a **substrate**. This reflects the specialized metabolic roles of the two **2coenzymes**: **NAD⁺** generally functions in **oxidations** - usually as part of a catabolic reaction; and **NADPH** is the usual coenzyme in **reductions** nearly always as part of anabolic reaction. A few **enzymes** can use either **coenzyme**. but most show a strong preference for one over the other. This functional specialization allows a cell to maintain two **2** distinct pools of **electron carrier**, swith two **2** distinct functions, in the same cellular compartment.

More than **200 enzymes** are known to catalyze reactions in which **NAD⁺** (or **NADP⁺**) **accepts** a **hydride :H⁻** ion

from a **reduced substrate** AH_2 , or **NADPH** (or **NADH**) **donates** a hydride $:H^-$ ion to an **oxidized substrate** **A**. The general reactions are $H_3C-CH_2-OH + NAD^+ + H_2O \rightleftharpoons H_3C-CH=O + NADH + H_3O^+$ (11)

Ethanol Acet-aldehyde

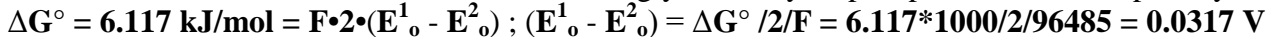
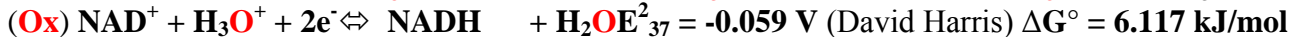
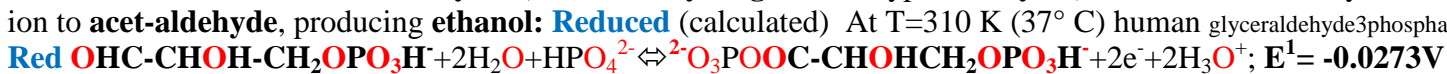
where AH_2 is the **reduced substrate** and **A** the **oxidized substrate**. The general name for an **enzyme** of this type is **oxidoreductase**; they are also commonly called **dehydrogenases**. For example, **alcohol dehydrogenase** catalyzes the first **1st** step in the **catabolism** of **ethanol**, in which **ethanol** is **oxidized** to **acet-aldehyde**:



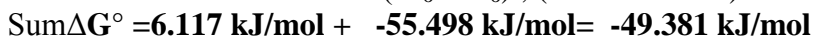
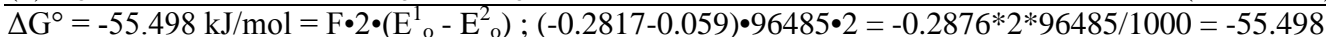
Notice that one of the carbon atoms $-CH_2-OH$ in **ethanol** has lost a hydrogen H^- atom as hydride and dissociates $-OH \Rightarrow H^+$ proton ;the compound has been **oxidized** from an **alcohol** to an **aldehyde** (Fig. 3a).

When NAD^+ or $NADP^+$ is **reduced** the hydride $:H^-$ ion could in principle be transferred to either side of the **nicotin-amide ring**: the front (**A** side) or the back (**B** side) as represented in Figure 3. Studies with isotopically labeled *** substrates** have shown that a given **enzyme** catalyzes either an **A-type** or **B-type** transfer, but not both. For example, **yeast alcohol dehydrogenase** and **lactate dehydrogenase** of vertebrate **heart** transfer a hydride $:H^-$ ion to (or remove a hydride $:H^-$ ion from) the **A** side of the **nicotin-amide ring**; they are classed as type **A dehydrogenases** to distinguish them from another group of **enzymes** that transfer a hydride $:H^-$ ion to (or remove a hydride $:H^-$ ion from) the **B** side of the **nicotin-amide ring** (Table 2).

The association between a **dehydrogenase** and **NAD** or **NADP** is relatively loose; the **coenzyme** readily diffuses from one **enzyme** to another, acting as a **water-soluble carrier** of electrons e^- from one **1metabolite** to another. For example, in the production of **alcohol** during **fermentation** of **glucose** by, **yeast** cells, a hydride $:H^-$ ion is removed from **glycer-aldehyde 3-phosphate** by, one **1 enzyme** (**glycer-aldehyde 3-phosphate dehydrogenase**, a type **B enzyme**) and transferred to NAD^+ . The **NADH** produced then leaves the **enzyme surface** and diffuses to another **enzyme** (**alcohol dehydrogenase**, a type **A enzyme**), which transfers a hydride $:H^-$ ion to **acet-aldehyde**, producing **ethanol**: **Reduced** (calculated) At $T=310\text{ K}$ (37° C) human glycer-aldehyde 3-phosphate

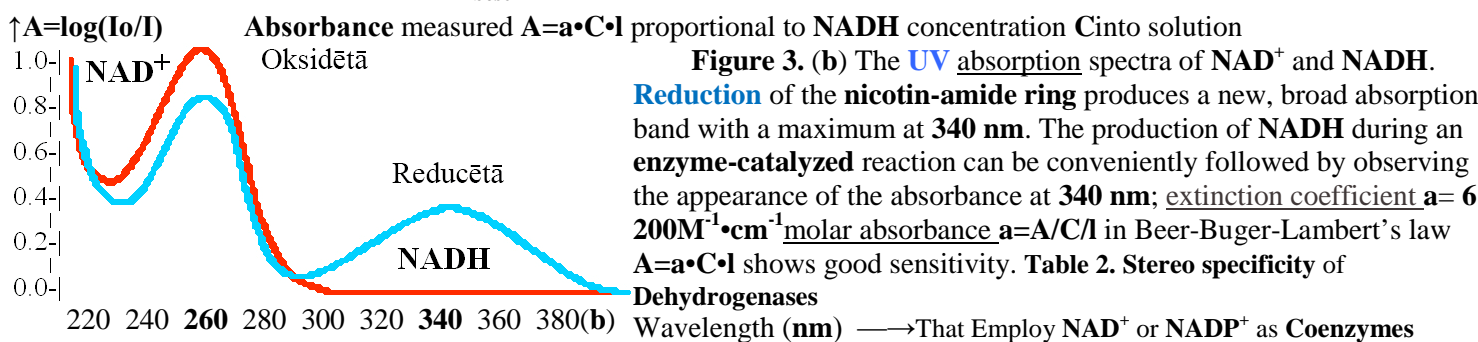
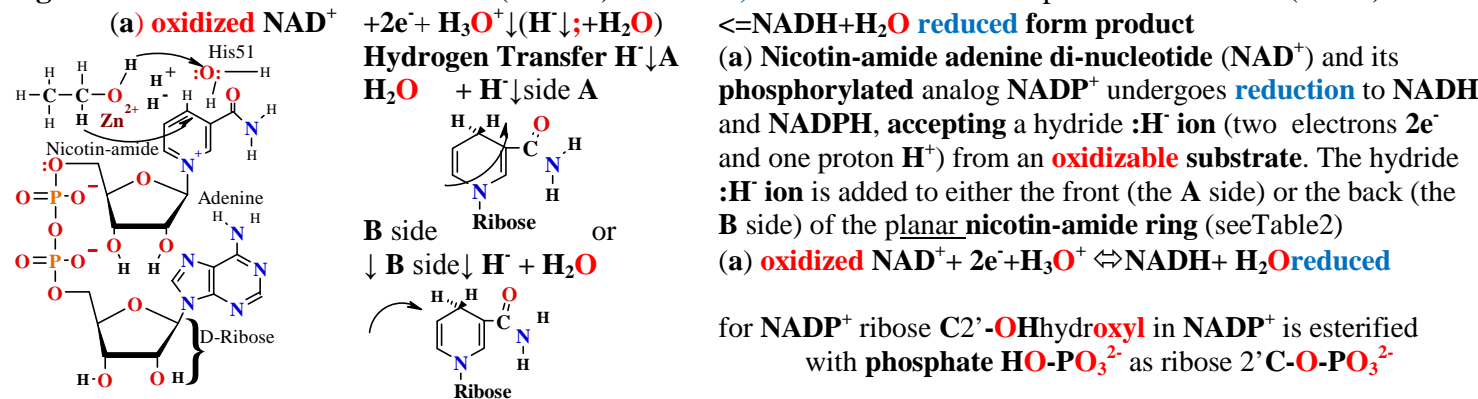


Glycer-aldehyde 3-phosphate \Rightarrow 1,3-phospho-glycerate (Carnegie Mellon Univ.)



Notice that in the overall reaction there is no net production or consumption of NAD^+ or $NADH$; the **coenzymes** function catalytically and are recycled repeatedly without a net change in the concentration **C** of $[NAD^+] + [NADH]$.

Figure 3. NAD and $NADP$ $NAD^+ + H^- (2e^- + H^+) \rightleftharpoons NADH$; $E_o = -0.059\text{ V}$ standard potential $T=310\text{ K}$ (37° C)



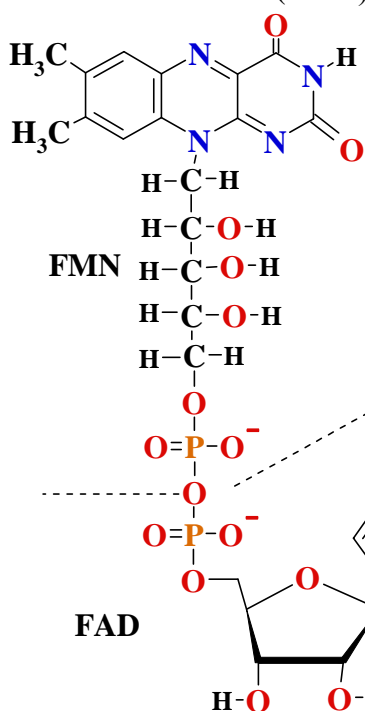
Enzyme Coenzyme Stereo chemical specificity nicotin-amide ring (A or B)

| | | | |
|--|---------------------------------------|--|---|
| Iso-citrate dehydrogenase | NAD ⁺ | | A |
| α-Keto-glutarate dehydrogenase | NAD ⁺ | | B |
| Glucose 6-phosphate dehydrogenase | NADP ⁺ | | B |
| Malate dehydrogenase | NAD ⁺ | | A |
| Glutamate dehydrogenase | NAD ⁺ or NADP ⁺ | | B |
| Glyceraldehyde 3-phosphate dehydrogenase | NAD ⁺ | | B |
| Lactate dehydrogenase | NAD ⁺ | | A |
| Alcohol dehydrogenase | NAD ⁻ | | A |

Table 3. Some Enzymes (Flavo-proteins) That Employ Flavin Nucleotide Coenzymes

| Enzyme | Flavin | Nucleotide | Enzyme |
|-------------------------------|--------|------------------------------------|--------|
| Fatty acyl-CoA dehydrogenase | FAD | | |
| Di-hydro-lipoyl dehydrogenase | FAD | Glycerol 3-phosphate dehydrogenase | |
| Succinate dehydrogenase | FAD | Thio-redoxin reductase | |
| NADH dehydrogenase Complex I | FMN | Glycolate dehydrogenase | |

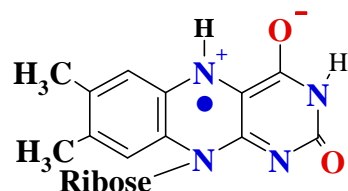
↓flavin mono-nucleotide (FMN)→



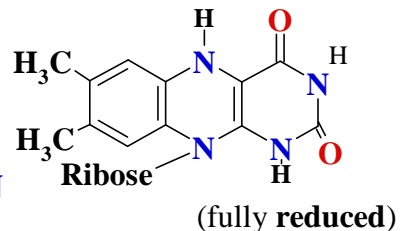
isoalloxazine ring

+e⁻+H⁺→↓ FADH* (FMNH*)

↓ (semi-quinone)



+e⁻+H⁺→↓ FADH₂ (FMNH₂)



(fully reduced)

← Flavin adenine dinucleotide FAD

Flavin Nucleotides Are Tightly Bound in Flavo-proteins

Figure 4. Structures of oxidized and reduced FAD and FMN. FMN consists of the structure above the dashed line shown on the **oxidized (FAD) structure**. The **flavin nucleotides accept two hydrogen 2H atoms (two electrons 2e⁻ and two protons 2H⁺), both of which appear in the flavin ring system**. When **FAD or FMN accepts only one 1 hydrogen H atom**, the **semi-quinone**, a stable free radical, forms.

Flavo-proteins (Table 3) are **enzymes** that catalyze **oxidation-reduction** reactions using either **flavin mono-nucleotide (FMN)** or **flavin adenine dinucleotide (FAD)** as **coenzyme** (Fig. 4). These **coenzymes** are derived from the **vitamin riboflavin**. The **fused ring structure of flavin nucleotides (the isoalloxazine ring)** undergoes reversible **reduction**, accepting either one **1** or two **2** electrons e⁻ in the form of one **1** or two **hydrogen 2H atoms** (each atom an electron e⁻ plus a proton H⁺) from a **reduced substrate**. The fully **reduced** forms are abbreviated **FADH₂** and **FMNH₂**. When a fully **oxidized flavin nucleotide accepts only one 1 electron e⁻** (one hydrogen **H** atom), the **semi-quinone** form of the **isoalloxazine ring** is produced, abbreviated **FADH*** and **FMNH***. Because **flavo-proteins** can participate in either one-**1** or two electron **2e⁻** transfers, this class of **proteins** is involved in a greater diversity of reactions than the **pyridine nucleotide-linked dehydrogenases**.

Like the **nicotin-amide coenzymes** (Fig. 14-15), the **flavin nucleotides** undergo a shift in a major absorption band on reduction. **Oxidized flavo-proteins** generally have an absorption maximum near **570 nm**; when **reduced**, the absorption maximum shifts → to about **450 nm**. This change can be used to **assay** reactions involving a **flavo-protein**.

The **flavin nucleotide** in most **flavo-proteins** is bound rather tightly to the **protein**, and in some **enzymes**, such as **succinate dehydrogenase**, it is bound **covalently**. Such tightly bound **coenzymes** are properly called **prosthetic groups**. They do not transfer electrons e⁻ by diffusing from one **1enzyme** to another second **2nd**; rather, they provide a means by which the **flavo-protein** can temporarily **hold** electrons e⁻ while it **catalyzes** electron e⁻ transfer from a **reduced substrate** to an electron e⁻ **acceptor**. One important feature of the **flavo-proteins** is the variability in the **standard reduction potential (E°)** of the bound **flavin nucleotide**. Tight association between the **enzyme** and **prosthetic group** confers on the **flavin ring** a **reduction potential E** typical of that particular **flavo-protein**, sometimes quite different from that of the **free flavin nucleotide**. **FAD** bound to

succinate dehydrogenase, for example, has an $E_M = 0.0953 \text{ V}$ compared with -0.3356 V for **free FAD**.

Flavo-proteins are often very complex: some have, in addition to a **flavin nucleotide**, tightly bound inorganic ions (iron Fe^{n+} or molybdenum Mo^{n+} , for example) capable of participating in electron e^- transfer.

Summary

Living cells constantly perform work **W** and thus require energy **E** for the maintenance of highly organized **structures**, for the **synthesis** or cellular components, for **movement**, for the generation of **electric currents**, for the production of **light**, and for many other processes. **Bio-energetic** is the quantitative study of energy **E** relationships and energy **E** conversions in biological systems. **Biological energy E** transformations obey the laws of **thermodynamics**. All chemical reactions are influenced by two **2** forces: the tendency to achieve the most stable bonding state (for which enthalpy, **H**, is a useful expression to show minimum reach at the most stable bonding state) and the tendency to achieve the highest \uparrow degree of dispersed energy, **T•S** (called **bound energy**), which measure is the entropy, **S**. The production of entropy in a reaction as positive difference

$\Delta S = S_{\text{products}} - S_{\text{reactants}} > 0$ is entropy increase \uparrow from $S_{\text{reactants}}$ to S_{products} due to dispersion of energy among the members of reaction. The net driving force in a reaction is the free-energy **G** decrease \downarrow from $G_{\text{reactants}}$ to G_{products} and negative difference $\Delta G = G_{\text{products}} - G_{\text{reactants}} < 0$, which represents the net effect of these two **2** factors:

$T \cdot \Delta S + \Delta G = \Delta H$. Cells require sources of free energy **G** to perform work **W**.

The standard transformed free-energy change, ΔG° , is a physical **constant** characteristic for a given **reaction** and can be calculated from the **equilibrium constant** K_{eq} for the reaction: $\Delta G^\circ = -R \cdot T \cdot \ln(K_{\text{eq}})$. The actual free-energy change, ΔG , is a variable, which depends on, ΔG° and on the concentrations **C** of **reactants** and **products**: $\Delta G = \Delta G^\circ + R \cdot T \cdot \ln([\text{products}]/[\text{reactants}])$. When ΔG is large and negative $\Delta G < 0$, the reaction tends to go in the forward \rightarrow direction; when it is large and positive $\Delta G > 0$, the reaction tends to go in the reverse \leftarrow direction; and when $\Delta G = 0$, the system is at **equilibrium**. The free-energy change ΔG for a reaction is independent of the **pathway** by which the reaction occurs only on **initial** ($G_{\text{reactants}}$) and **final** (G_{products}) states. Free-energy changes ΔG are additive; the net chemical reaction that results from the successive occurrence of reactions sharing a common **intermediate** has an overall free-energy change $\Delta G_{\text{reaction}}$ that is the sum of the $\Delta G_{\text{reaction}} = \Delta G_{\text{reaction1}} + \Delta G_{\text{reaction2}}$ values for the individual reactions **reaction1** and **reaction2**.

ATP is the chemical link between catabolism and anabolism. It constitutes the energy currency of the living cell. Its **exoergonic** conversion to **ADP** and **P_i**, or to **AMP** and **PP_i**, is coupled to a large number of **endoergonic** reactions and processes. In general, it is not **ATP hydrolysis**, but the transfer of a **phosphoryl**, **pyro-phosphoryl**, or **adenylyl** group from **ATP** to a **substrate** or **enzyme** molecule that couples the energy of **ATP breakdown** to **endoergonic** transformations of **substrates**. By these group transfer reactions, **ATP** provides the energy for anabolic reactions, including the **synthesis** of informational molecules, and for the **transport** of molecules and ions across membranes against \leftrightarrow concentration **C** gradients and electrical potential **E** gradients. Muscle contraction is one of several exceptions to this generalization; the conformational changes that produce muscle contraction are driven by **ATP hydrolysis** directly.

Cells contain other **metabolites** with large, negative $\Delta G < 0$, free energies of **hydrolysis**, including **phospho-enol-pyruvate**, **1,3-bis-phospho-glycerate**, and **phospho-creatine**. These high-energy compounds, like **ATP**, have a high **phosphoryl group transfer potential**; they are good **donors** of the **phosphoryl** group. **Thio-esters** also have high \uparrow free energies **G** of **hydrolysis**.

Biological **oxidation-reduction** reactions can be described in terms of two **2half-reactions** (called **RedOx** systems), each with a characteristic **standard reduction potential**, E° . When two **2electro-chemicalhalf-cells**, each containing the components (**oxidized** and **reduced** forms) of a half-reaction, are connected, electrons e^- tend to flow \rightarrow to the half-cell with the higher \uparrow **reduction potential E**. The strength of this tendency is proportional to the difference between the two **2reduction potentials** (ΔE) and is a function of the concentrations **C** of **oxidized** [**Ox**] and **reduced** [**Red**] species. The standard free-energy change ΔG° for an **oxidation-reduction** reaction is directly proportional to the difference in **standard reduction potentials** ΔE° of the two **2half-cells**:

$$\Delta G^\circ = F \cdot n \cdot \Delta E^\circ.$$

Many biological **oxidation** reactions are **dehydrogenation** in which one **1** or two **2** hydrogen **H** atoms (electron e^- and proton H^+) are transferred from a **substrate** to a hydrogen **H acceptor**. **Oxidation-reduction** reactions in cells involve specialized electron e^- **carriers**. **NAD** and **NADP** are the freely diffusible **coenzymes** of many **dehydrogenases**. Both NAD^+ and NADP^+ **accept** two **2** electrons e^- and one **1** proton H^+ . **FAD** and **FMN**, the **flavin nucleotides**, serve as tightly bound **prosthetic** groups of flavo-proteins. They can **accept** either one **1** or two **2** electrons e^- . In many organisms, a central energy-conserving process is the **stepwise oxidation** of **glucose** to **CO₂**, in which some of the energy of **oxidation** is conserved in **ATP** as electrons e^- are passed to **O₂**.

Further Reading

Bio-energetic and Thermodynamics

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Phosphoryl Group Transfers and ATP

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Biological Oxidation- Reduction Reactions

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An excellent two-volume collection of authoritative reviews. Among the most useful are the problems by Kaplan, Westheimer, Veech, and Ohno and Ushio.

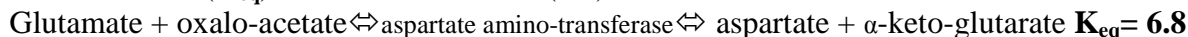
Problems 1. Entropy Changes during Egg Development

Consider a system consisting of an egg in an incubator. The white and yolk of the egg contain proteins, carbohydrates, and lipids. If fertilized, the egg is transformed from a single meiotic cell to a complex mitotic cells in organism. Discuss this irreversible process in terms of the entropy changes ΔS in the system, surroundings, and universe. Be sure that you first clearly define the system and surroundings-environment.

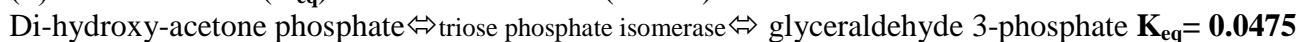
2. Calculation of ΔG° from Equilibrium Constants

Calculate the standard free-energy changes ΔG° the following metabolically important enzyme-catalyzed reactions at **25°C** and **pH 7.0** from the equilibrium constants K_{eq} given.

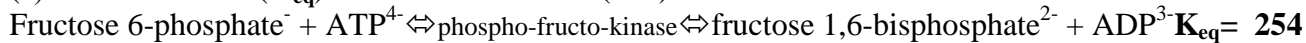
(a) $\Delta G^\circ = -R \cdot T \cdot \ln(K_{eq}) = -8.3144 \cdot 298.16 \cdot \ln(6.8) = -2479.0215 \cdot 1.916923 = -4752.093331 = -4.752 \text{ kJ/mol}$



(b) $\Delta G^\circ = -R \cdot T \cdot \ln(K_{eq}) = -8.3144 \cdot 298.16 \cdot \ln(0.0475) = -2479.0215 \cdot -3.04703 = 7553.65288 = 7.553 \text{ kJ/mol}$



(c) $\Delta G^\circ = -R \cdot T \cdot \ln(K_{eq}) = -8.3144 \cdot 298.16 \cdot \ln(254) = -2479.0215 \cdot 5.537334 = -13727.170039 = -13.727 \text{ kJ/mol}$



$\Delta G^\circ = -R \cdot T \cdot \ln(K_{eq})$; $\Delta G = \Delta G^\circ + R \cdot T \cdot \ln(K_{eq})$

3. Calculation of Equilibrium Constants K_{eq} from ΔG°

Calculate the equilibrium constants K_{eq} for each of the following reactions at **pH 7.0** and **25°C**, using the ΔG°

values of Table 1-1 $K_{eq} = \text{EXP}\left(\frac{\Delta G^\circ}{R \cdot T}\right) = -13.8 \cdot 1000 / 8.3144 / 298.16 = \text{EXP}(-5.5667) = 261.573$ corrected by

$\Delta G^\circ = -R \cdot T \cdot \ln(K_{eq}) = -8.3144 \cdot 298.16 \cdot \ln(299.9981 \cdot 10^{-7}) = -2479.0215 \cdot -15.0195 = 37233.6634$

$\Delta G^\circ = \Delta G^\circ_o + G^\circ_{\text{HPO}_4} + G^\circ_{\text{H}_3\text{O}^+} - G^\circ_{\text{H}_2\text{PO}_4} - G^\circ_{\text{H}_2\text{O}} =$

$= -13.8 + (-1282) + (-284.7) - (-1323) - (-306.7) = 49.306 \text{ kJ/mol}$

$\Delta G^\circ = -R \cdot T \cdot \ln(299.9981 \cdot 10^{-9}) = 37.2336 \text{ kJ/mol}$; $K_{eq} = 1.1469 \cdot 261.573 \cdot 10^{-9} = 299.9981 \cdot 10^{-9}$;

$K_{7eq} = 3$; $\frac{[\text{Glc}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{Glc} - 6\text{P}^-] \cdot [\text{H}_2\text{O}]^2} = 3 \cdot 10^{-7} = K_{\text{H}_2\text{PO}_4} \cdot K_{eq} = 1.15 \cdot 10^{-9} \cdot 261.573 = K_{eq}$; $\Delta G^\circ_{eq} = 37.2 \text{ kJ/mol}$



(a) Glucose 6-phosphate⁻ + H₂O \leftrightarrow glucose 6-phosphatase \leftrightarrow glucose + H₂PO₄⁻; $K_{eq} = 261.573$; $\Delta G^\circ_o = -13.8 \text{ kJ/mol}$

Glucose 6-phosphate⁻ + 2H₂O \leftrightarrow glucose 6-phosphatase \leftrightarrow glucose + HPO₄²⁻ + H₃O⁺; $K_{eq} = 3 \cdot 10^{-7}$; $\Delta G^\circ = 49.3 \text{ kJ/mol}$

Equilibrium is shifted toward formation of Glucose 6-phosphate⁻ and is affected by H₃O⁺ concentration.

$-15.9 \cdot 1000 / 8.3144 / 298.16 = \text{EXP}(-6.4138) = 610.221 = [\text{Glc}] \cdot [\text{Gal}] / ([\text{Lactose}] \cdot [\text{H}_2\text{O}]) = K_{eq} \cdot [\text{H}_2\text{O}] =$

$= 610.221 \cdot 55.3457 = 33773.1084 = K_{eq}$

(b) $K_{eq} = \frac{[\text{Glc}] \cdot [\text{Gal}]}{[\text{Lactose}] \cdot [\text{H}_2\text{O}]} = 610.221$; $\Delta G^\circ = -15.9 \text{ kJ/mol}$

(b) Lactose + H₂O \leftrightarrow β -galactosidase \leftrightarrow glucose + galactose $K_{eq\text{H}_2\text{O}} = 33773$

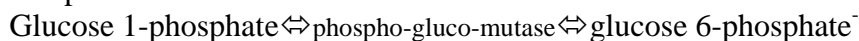
(c) Malate \leftrightarrow fumarase \leftrightarrow fumarate + H₂O $K_{eq\text{H}_2\text{O}} = 0.004615$

$3.1 \cdot 1000 / 8.314400 / 298.16 = \text{EXP}(-1.2505) = 0.28636 = [\text{fumarate}] \cdot [\text{H}_2\text{O}] / ([\text{Malate}]) = K_{eq} / [\text{H}_2\text{O}] =$

$= 0.255400 / 55.3457 = 0.004615 = K_{eq}$; $K_{eq} = \frac{[\text{Fumarate}] \cdot [\text{H}_2\text{O}]}{[\text{Malate}]} = 0.28636$; $\Delta G^\circ = 3.1 \text{ kJ/mol}$

4. Experimental Determination of K_{eq} and ΔG°

If a **0.1 M** solution of **glucose 1-phosphate** is incubated with a catalytic amount of **phospho-gluco-mutase**, the **glucose 1-phosphate** is transformed to **glucose 6-phosphate**. At equilibrium, the concentrations of the reaction components are:



$[\text{Glc1P}] = 4.5 \cdot 10^{-3} \text{ M}$ $9.6 \cdot 10^{-2} \text{ M} = [\text{Glc6P}^-]$

$= 0.096 / 0.0045 = 21.3333 = K_{eq}$ $\Delta G^\circ = -R \cdot T \cdot \ln(21.3333) = -8.3144 \cdot 298.16 \cdot 3.06027 / 1000 = -7.58648$

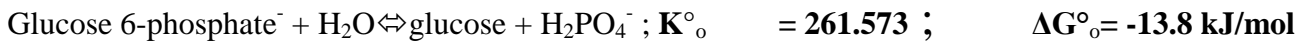
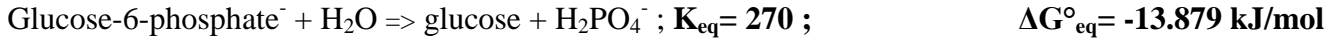
Calculate $K_{eq} = [\text{Glc6P}^-] / [\text{Glc1P}] = 21.3$ and $\Delta G^\circ = -R \cdot T \cdot \ln(21.33) = -7.586 \text{ kJ/mol}$ for this reaction at **25°C**.

5. Experimental Determination of ΔG° for ATP Hydrolysis

A direct measurement of the standard free-energy change ΔG° associated with the **hydrolysis** of **ATP** is technically demanding because the minute amount of **ATP** remaining at equilibrium is difficult to measure accurately. The value of ΔG° can be calculated indirectly, however, from the equilibrium constants of two **2** other **enzymatic** reactions having less favorable equilibrium constants:

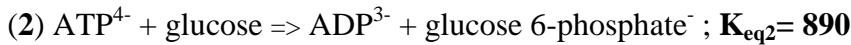
$$\Delta G^\circ_1 = \Delta G^\circ_o + G^\circ_{\text{HPO}_4} + G^\circ_{\text{H}_3\text{O}^+} - G^\circ_{\text{H}_2\text{PO}_4} - G^\circ_{\text{H}_2\text{O}} = -13.8 + (-1282) + (-284.7) - (-1323) - (-306.7) = 49.306 \text{ kJ/mol}$$

$$270 * 1.1469 * 10^{-9} = 3.096630 * 10^{-7} = K^\circ_{\text{eq}} = K_{\text{H}_2\text{PO}_4} * K_{\text{eq}}; \Delta G^\circ = -R \cdot T \cdot \ln(270) = -13879 \text{ kJ/mol}$$



$$K^\circ_{\text{eq}} * K^\circ_{\text{H}_2\text{PO}_4} = K^\circ_{\text{eq}1} = \frac{[\text{Glc}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{Glc-6P}^-] \cdot [\text{H}_2\text{O}]^2} = 3.1 * 10^{-7};$$

$$= 4.7262 * 10^{-7} = 1.1469 * 261.573 * 10^{-9} = \Delta G^\circ_{\text{eq}1} = 37.16 \text{ kJ/mol}$$



$$K^\circ_{\text{eq}2} = \frac{[\text{ADP}^{3-}] \cdot [\text{Glc-6P}^-]}{[\text{Glc}] \cdot [\text{ATP}^{4-}]} = 890; \Delta G^\circ_{\text{eq}2} = -16.836 \text{ kJ/mol}$$

Using this information, calculate the standard free energy ΔG° of hydrolysis of **ATP** at **25°C**.

$$K^\circ_{\text{eq}3} = \frac{[\text{ADP}^{3-}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{ATP}^{4-}] \cdot [\text{H}_2\text{O}]^2} = K^\circ_{\text{eq}1} * K^\circ_{\text{eq}2} = \frac{[\text{Glc}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{Glc6P}^-] \cdot [\text{H}_2\text{O}]^2} \cdot \frac{[\text{ADP}^{3-}] \cdot [\text{Glc6P}^-]}{[\text{Glc}] \cdot [\text{ATP}^{4-}]}$$

$$3.09663 * 890 * 10^{-7} = 2.7560 * 10^{-4} = K^\circ_{\text{eq}3}; \Delta G^\circ_1 + \Delta G^\circ_{\text{eq}2} = -16.836 + 49.3 = 32.464 = \Delta G^\circ_3$$

$$-R \cdot T \cdot \ln(K^\circ_{\text{eq}3}) = -8.1344 * 298.16 * \ln(0.0002756) = 20.3194 \text{ kJ/mol} = \Delta G^\circ_{\text{eq}3}$$

$$\Delta G^\circ_3 = \Delta G^\circ_o + G^\circ_{\text{HPO}_4} + G^\circ_{\text{H}_3\text{O}^+} - G^\circ_{\text{H}_2\text{PO}_4} - G^\circ_{\text{H}_2\text{O}} = -30.5 + (-1282) + (-284.7) - (-1323) - (-306.7) = 32.606 \text{ kJ/mol}$$



$$K_{\text{eq}^\circ_o} = 0.0002756 / 1.1469 * 10^{-9} = 240300; -28981 = \Delta G_{\text{eq}^\circ_o} = -R \cdot T \cdot \ln(K^\circ_o) = -8.3144 * 298.16 * \ln(240300) = -30714; K^\circ_o = \text{EXP}(-\Delta G^\circ / R / T) = \text{EXP}(-30500 / 8.3144 / 298.16) =$$

$$K_{\text{eq}^\circ_o} = \frac{[\text{ADP}^{3-}] \cdot [\text{H}_2\text{PO}_4^-]}{[\text{ATP}^{4-}] \cdot [\text{H}_2\text{O}]^2} = K^\circ_{\text{eq}3} / K^\circ_{\text{H}_2\text{PO}_4} = 240300; \Delta G_{\text{eq}^\circ_o} = -30.714 \text{ kJ/mol}$$



6. Difference between ΔG° and ΔG Consider the following inter conversion, which occurs in **glycolysis** :
Fructose 6-phosphate $^- \rightleftharpoons$ **glucose 6-phosphate** $^-$; $K^\circ_{\text{eq}} = 1.97$

$$K^\circ_{\text{eq}} = \frac{[\text{Glc6P}^-]}{[\text{Fruc6P}^-]} = 1.97 = 531 \text{ 331}; \Delta G^\circ = -R \cdot T \cdot \ln(K^\circ_{\text{eq}}) = -1.5399 \text{ kJ/mol}$$

$$= 0.5 / 1.5 = 0.3 = K^\circ_{\text{eq}} \Delta G^\circ = -R \cdot T \cdot \ln(1.97) = -8.3144 * 298.16 * 3.06027 / 1000 = -1539.9$$

(a) What is ΔG° for the reaction (assuming that the temperature is **25°C**)?

(b) If the concentration of **[Fruc6P $^-$]** is adjusted to **1.5 M** and that of

$$[\text{Glc6P}^-] \text{ is adjusted to } 0.5 \text{ M, what is } \Delta G? -1539.9 + R \cdot T \cdot \ln(0.3) =$$

(c) Why are ΔG° and ΔG different? $\Delta G = \Delta G^\circ + R \cdot T \cdot \ln\left(\frac{[\text{Glc6P}^-]}{[\text{Fruc6P}^-]}\right) = -1539.9 + -2723.54 = -4.263.4 \text{ kJ/mol}$

7. Dependence of ΔG on pH. The free energy ΔG released by the **hydrolysis** of **ATP** under standard conditions at **pH=7** is $\Delta G^\circ = -30.5 \text{ kJ/mol}$. If **ATP** is **hydrolyzed** under standard conditions but at **pH=5.0**, is more or less free energy released? Why?

$$K_{eq3}^\circ = \frac{[\text{ADP}^{3-}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{ATP}^{4-}] \cdot [\text{H}_2\text{O}]^2} = 1.94 \cdot 10^{-6}; K_{eq3}^\circ / K_{H_2PO_4}^\circ = K_{eq3}^\circ = \frac{[\text{ADP}^{3-}] \cdot [\text{H}_2\text{PO}_4^-]}{[\text{ATP}^{4-}] \cdot [\text{H}_2\text{O}]^2} = 220409$$

$$\text{EXP}(-32606/8.3144/298.16) = \text{EXP}(-13.1528) = 1.94 \cdot 10^{-6} = K_{eq3}^\circ; K_{eq3}^\circ = 1.94 \cdot 10^{-6} =$$

$$= \Delta G^\circ; K_{eq3}^\circ = \text{EXP}(-30.5 \cdot 1000/8.3144/298.16) = \text{EXP}(12.3032) = 220409;$$

$$\Delta G_{eq3}^\circ = \Delta G^\circ + G^\circ_{HPO_4} + G^\circ_{H_3O^+} - G^\circ_{H_2PO_4} - G^\circ_{H_2O} = -30.5 + (-1282) + (-284.7) - (-1323) - (-306.7) = 32.606 \text{ kJ/mol}$$

$$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \frac{[\text{ADP}^{3-}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{ATP}^{4-}] \cdot [\text{H}_2\text{O}]^2} = 32606 + R \cdot T \cdot \ln(0.0001/0.01/\text{aoHOH}/\text{aoHOH} \cdot 0.01 \cdot 10^{\text{pH}})$$

$$\text{pH}) = 19397.7 - 7981.3 = -10.11 \text{ kJ/mol (pH=0); at T=298 K (25 }^\circ\text{C)}$$

$$-38.65 \text{ kJ/mol (pH=5); } -50.06 \text{ kJ/mol (pH=7); } -52.12 \text{ kJ/mol (pH=7.36); } -57.88 \text{ kJ/mol (pH=8.37)}$$

$$-11.83 \text{ kJ/mol (pH=0); at T = 310 K (37 }^\circ\text{C) in mitochondria}$$

$$-41.52 \text{ kJ/mol (pH=5); } -53.39 \text{ kJ/mol (pH=7); } -55.53 \text{ kJ/mol (pH=7.36); } -61.53 \text{ kJ/mol (pH=8.37)}$$

8. The ΔG° for Coupled Reactions

Glucose 1-phosphate⁻ is converted into fructose 6-phosphate⁻ in two **2** successive reactions:

Glucose 1-phosphate⁻ \Rightarrow glucose 6-phosphate⁻; $\Delta G^\circ_1 = -7.3 \text{ kJ/mol}$

Glucose 6-phosphate⁻ \Rightarrow fructose 6-phosphate⁻; $\Delta G^\circ_2 = +1.7 \text{ kJ/mol}$

Using the ΔG° values in Table 1.1, calculate the equilibrium constant,

$$\Delta G^\circ = \Delta G^\circ_1 + \Delta G^\circ_2 = -7.3 + 1.7 = -5.6 \text{ kJ/mol for the sum of the two 2 reactions at } 25^\circ\text{C:}$$

$$\text{Glucose 1-phosphate}^- \Rightarrow \text{fructose 6-phosphate}^-; K_{eq}^\circ = K_{eq1} \cdot K_{eq2} = \text{EXP}(5600/8.314400/298.16) =$$

$$\text{EXP}(2.258956) = 9.57309$$

9. Strategy for Overcoming an Unfavorable Reaction: ATP-Dependent Chemical Coupling

The **phosphorylation** of **glucose** to glucose 6-phosphate⁻ is the initial step in the catabolism of **glucose**. The direct **phosphorylation** of **glucose** by **H₂PO₄⁻** and **HPO₄²⁻** is described by the equation at **T = 310.16 K**:

(a) Glucose + **H₂PO₄⁻** \Rightarrow glucose 6-phosphate⁻ + H₂O, $\Delta G^\circ = 13.8 \text{ kJ/mol}$

$$K_{a1}^\circ = \frac{[\text{Glc6P}^-] \cdot [\text{H}_2\text{O}]}{[\text{Glc}] \cdot [\text{H}_2\text{PO}_4^-]}; K_{a2}^\circ \cdot K_{H_2PO_4}^\circ = \frac{[\text{Glc6P}^-] \cdot [\text{H}_2\text{O}]^2}{[\text{Glc}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]} \cdot \frac{[\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{H}_2\text{PO}_4^-] \cdot [\text{H}_2\text{O}]}$$

$$\text{EXP}(49306/RF/T) = 2.01195544 \cdot 10^{+8} = K_{eq3}^\circ; \Delta G^\circ_1 + \Delta G^\circ_{eq2} = -16.836 + 49.3 = 32.464 = \Delta G^\circ_3$$

$$-R \cdot T \cdot \ln(K_{eq3}^\circ) = -8.1344 \cdot 298.16 \cdot \ln(0.0002756) = 20.3194 \text{ kJ/mol} = \Delta G^\circ_{eq3} \cdot 0.0000105738$$

(a2) Glucose + **HPO₄²⁻** + H₃O⁺ \Rightarrow glucose 6-phosphate⁻ + 2 H₂O, $\Delta G^\circ = -49.306 \text{ kJ/mol}$

$$K_{a2}^\circ = \frac{[\text{Glc6P}^-] \cdot [\text{H}_2\text{O}]^2}{[\text{Glc}] \cdot [\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]} = \frac{[\text{Glc6P}^-] \cdot \left(1 + \frac{[\text{H}_3\text{O}^+]}{K_{H_2PO_4} \cdot [\text{H}_2\text{O}]}\right) \cdot [\text{H}_2\text{O}]^2}{[\text{Glc}] \cdot 4.8 \cdot [\text{H}_3\text{O}^+]} = 2.0119 \cdot 10^{+8}$$

$$\Delta G^\circ = \Delta G^\circ_0 + G^\circ_{H_2O} - G^\circ_{H_3O^+} = 13.8 + (-284.7) - (-306.7) = -8.231 = 30.83876;$$

$$K_{H_2PO_4}^\circ = \frac{[\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{[\text{H}_2\text{PO}_4^-] \cdot [\text{H}_2\text{O}]}; P_i = 4.8 \text{ mM} = [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}]; [\text{HPO}_4^{2-}] = 4.8 \cdot \frac{[\text{HPO}_4^{2-}] \cdot [\text{H}_3\text{O}^+]}{K_{H_2PO_4} \cdot [\text{H}_2\text{O}]}$$

$$[\text{HPO}_4^{2-}] + [\text{HPO}_4^{2-}] \cdot \frac{[\text{H}_3\text{O}^+]}{K_{H_2PO_4} \cdot [\text{H}_2\text{O}]} = 4.8 = [\text{HPO}_4^{2-}] \cdot \left(1 + \frac{[\text{H}_3\text{O}^+]}{K_{H_2PO_4} \cdot [\text{H}_2\text{O}]}\right);$$

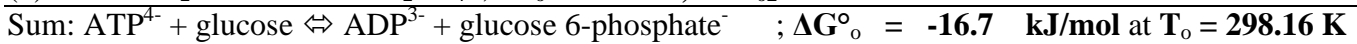
$$[\text{HPO}_4^{2-}] = \frac{4.8}{\left(1 + \frac{[\text{H}_3\text{O}^+]}{K_{H_2PO_4} \cdot [\text{H}_2\text{O}]}\right)}; [\text{Glc6P}^-] = \frac{2.012 \cdot [\text{Glc}] \cdot 4.8 \cdot [\text{H}_3\text{O}^+]}{\left(1 + \frac{[\text{H}_3\text{O}^+]}{K_{H_2PO_4} \cdot [\text{H}_2\text{O}]}\right) \cdot [\text{H}_2\text{O}]^2} = 1.0574 \cdot 10^{-5} \text{ M}$$

(a) Calculate the equilibrium constant K_a for the above reaction. In the rat **hepatocyte** $\text{pH}=7.36$ and at $\text{pH}=7$ the physiological concentrations of **glucose** and $[\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}]$ are maintained at approximately **4.8 mM**. What is the equilibrium concentration of glucose 6-phosphate⁻ obtained by the direct **phosphorylation** of **glucose** by $\text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-}$? Respectively $[\text{Glc6P}^-] = 8.5 \cdot 10^{-8} \text{M}$ and $1.275 \cdot 10^{-7} \text{M}$ ($\text{pH } 7.36$ and 7) Does this reaction represent a reasonable metabolic step for the catabolism of **glucose**? Explain.

(b) In principle, at least, one way to increase the concentration of glucose 6-phosphate⁻ is to drive the equilibrium reaction to the right by increasing the intracellular concentrations of **glucose** and $\text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-}$. Assuming a fixed concentration of $\text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-}$ at **4.8 mM**, how high would the intracellular concentration of **glucose** have to be to give an equilibrium concentration of glucose 6-phosphate⁻ of $[\text{Glc6P}^-] = 250 \mu\text{M}$ (normal physiological concentration)? Would this route be physiologically reasonable, given that the maximum solubility of **glucose** is less than **1 M**?

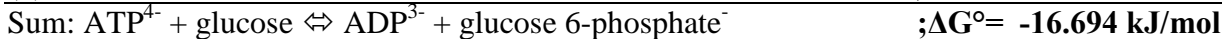
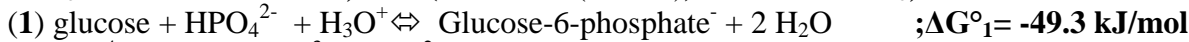
$$[\text{Glc}] = \frac{[\text{Glc6P}^-] \cdot \left(1 + \frac{[\text{H}_3\text{O}^+]}{K_{\text{H}_2\text{PO}_4} \cdot [\text{H}_2\text{O}]} \right) \cdot [\text{H}_2\text{O}]}{K_{a2} \cdot 4.8/1000 \cdot [\text{H}_3\text{O}^+]} = 23.64 \text{M at pH} = 7.36 \quad 23.64259868$$

(c) The **phosphorylation** of **glucose** in the cell is coupled to the **hydrolysis** of **ATP**; that is, part of the free energy of **ATP hydrolysis** is utilized to effect the **endoergonic phosphorylation** of **glucose** at $T = 310.16 \text{ K}$:



$$K^\circ_o = 842.63 \leftarrow \text{EXP}(-\Delta G^\circ/R/T) = 649.3 = K^\circ; K^\circ_{\text{eqo}} = \frac{[\text{ADP}^{3-}] \cdot [\text{Glc6P}^-]}{[\text{ATP}^{4-}] \cdot [\text{Glc}]} = 890 ; \Delta G^\circ_{\text{eqo}} = -16.836 \text{ kJ/mol}$$

$$\Delta G^\circ_o = 13.8 + (-30.5) = -16.7 ; \text{EXP}(-16.7/RF/(T_o+25)) = 842.631 = K^\circ_o ; K^\circ = 649.2998 = \text{EXP}(-16700/RF/T)$$



$$\Delta G^\circ_2 = \Delta G^\circ_{o2} + G^\circ_{\text{HPO}_4} + G^\circ_{\text{H}_3\text{O}^+} - G^\circ_{\text{H}_2\text{PO}_4} - G^\circ_{\text{H}_2\text{O}} = -30.5 + (-1282) + (-284.7) - (-1323) - (-306.7) = 32.606 \text{ kJ/mol}$$

$$[\text{Glc}] = \frac{[\text{ADP}^{3-}] \cdot [\text{Glc6P}^-]}{K^\circ \cdot [\text{ATP}^{4-}]} = \frac{1.32 \cdot 0.25/1000}{649.2998 \cdot 3.38} = 1.504 \cdot 10^{-7} \text{M not depend on concentration } [\text{H}_3\text{O}^+]$$

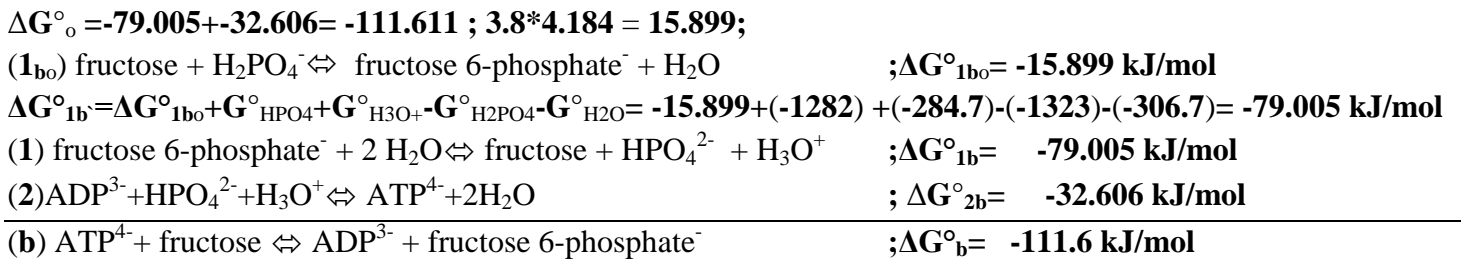
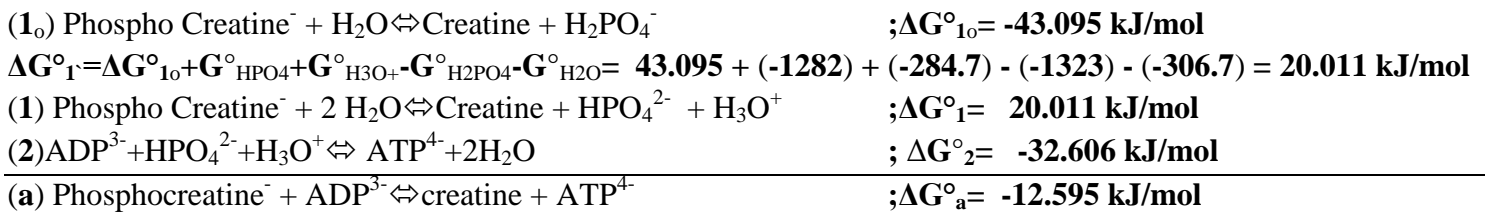
$$[\text{Glc}] = 1.32 \cdot 0.25/1000/649.2998/3.38 = 1.50366804509526 \text{E-}07$$

Calculate **K** for the overall reaction. For the **ATP-dependent phosphorylation** of **glucose**, what concentration of **glucose** is needed to achieve a **250 μM** intracellular concentration of glucose 6-phosphate when the concentrations of **ATP** and **ADP** are **3.38** and **1.32 mM**, respectively? Does this coupling process provide a feasible route, at least in principle, for the **phosphorylation** of **glucose** in the cell? Explain.

(d) Although coupling **ATP hydrolysis** to **glucose phosphorylation** makes thermodynamic sense, how this coupling is to take place has not been specified. Given that coupling requires a common intermediate, one conceivable route is to use **ATP hydrolysis** to raise the intracellular concentration of $\text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-}$ and thus drive the unfavorable phosphorylation of **glucose** by $\text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-}$. Is this ~i reasonable route? (Think about the solubility products of metabolic intermediates.)

(e) The **ATP-coupled phosphorylation** of **glucose** is catalyzed in **hepatocytes** by the enzyme **gluco kinase**. This enzyme binds **ATP** and **glucose** to form a **glucose-ATP-enzyme** complex, and the **phosphoryl** group is transferred directly from **ATP** to **glucose**. Explain the advantages of this route.

10. Calculations of ΔG° for ATP-Coupled Reactions From data in Table 1-2 calculate the ΔG° value for the reactions: $\Delta G^\circ_o = 20.011 + (-32.606) = -12.595$; $-10.3 \cdot 4.184 = -43.095$;



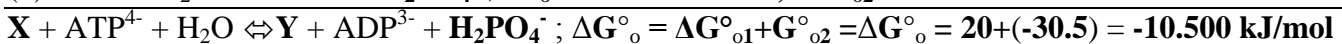
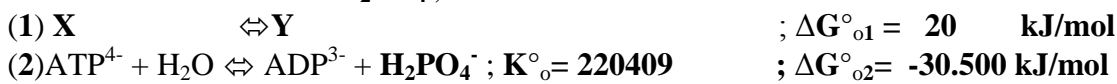
11. Coupling ATP Cleavage to an Unfavorable Reaction.

To explore the consequences of coupling ATP hydrolysis under physiological conditions to a thermodynamically unfavorable biochemical reaction, consider the hypothetical transformation X \rightarrow Y, for which $\Delta G^\circ = 20$ kJ/mol.

(a) What is the ratio [Y]/[X] at equilibrium? $K^\circ_o = 3.135 \cdot 10^{-4}$

$$K_{\text{eq}} = \frac{[Y]}{[X]} = \text{EXP}(-\Delta G^\circ_{o1}/R/T) = \text{EXP}(-20000/R/T) = 0.0003135;$$

(b) Suppose X and Y participate in a sequence of reactions during which ATP⁴⁻ is hydrolyzed to ADP³⁻ and H₂PO₄⁻, The overall reaction is :



$$K^\circ_{\text{eqo}} = \frac{[\text{ADP}^{3-}] \cdot [\text{H}_2\text{PO}_4^-] \cdot [\text{Y}]}{[\text{ATP}^{4-}] \cdot [\text{H}_2\text{O}] \cdot [\text{X}]} = 69.1 ; K^\circ_{\text{eqo}} \cdot [\text{H}_2\text{O}] = \frac{[\text{Y}]}{[\text{X}]} = 3810$$

$$\frac{[\text{Y}]}{[\text{X}]} = \frac{[\text{ATP}^{4-}] \cdot [\text{H}_2\text{O}] \cdot K^\circ_{\text{eqo}} \cdot \left(1 + \frac{K_{\text{H}_2\text{PO}_4} \cdot [\text{H}_2\text{O}]}{[\text{H}_3\text{O}^+]} \right)}{8.05/1000 \cdot [\text{ADP}^{3-}]} = 1.994 \cdot 10^6 ; [\text{H}_2\text{PO}_4^-] = \frac{8.05/1000}{1 + \frac{K_{\text{H}_2\text{PO}_4} \cdot [\text{H}_2\text{O}]}{[\text{H}_3\text{O}^+]}}$$

$$P_i = 8.05 \text{ mM} = [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] ; [\text{H}_2\text{PO}_4^-] = 8.05/1000 \cdot \frac{[\text{H}_2\text{PO}_4^-] K_{\text{H}_2\text{PO}_4} \cdot [\text{H}_2\text{O}]}{[\text{H}_3\text{O}^+]}$$

$$K^\circ_o = \text{EXP}(-\Delta G^\circ_{o1}/R/T) = \text{EXP}(-10500/R/T) = 69.0991 ; a_{\text{HOH}} \cdot 69.1 / 8.05 \cdot 1000 \cdot (1 + 2.543489 \cdot 10^{-9} \cdot a_{\text{HOH}} / 10^{-7.36})$$

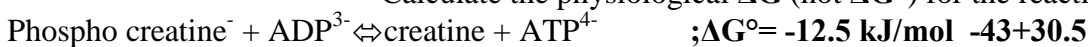
$$[\text{Y}]/[\text{X}] = a_{\text{HOH}} \cdot 8.05 \cdot 69.0991 / 0.93 / 8.05 \cdot 1000 = 1994007.686579$$

Calculate [Y]/[X] for this reaction at equilibrium. Assume that the concentrations of [ATP⁴⁻], [ADP³⁻], and ([H₂PO₄⁻] + [HPO₄²⁻]) are all 1 M when the reaction is at equilibrium T = 310.16 K.

(c) We know that [ATP⁴⁻], [ADP³⁻], and [H₂PO₄⁻] are not 1 M under physiological conditions. Calculate [Y]/[X] for the ATP-coupled reaction when the values of [ATP⁴⁻], [ADP³⁻], and [H₂PO₄⁻] are those found in rat myocytes (Table 1-3).

12. Calculations of ΔG at Physiological Concentrations.

Calculate the physiological ΔG (not ΔG°) for the reaction : at T=310.16 K



$$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \frac{[\text{ATP}^{4-}] \cdot [\text{Cr}]}{[\text{ADP}^{3-}] \cdot [\text{PCr}^-]} = -12500 + R \cdot T \cdot \ln(2.6 \cdot 1 / 0.73 / 4.7) = -13215.2 = -13.215$$
 kJ/mol

at 37 °C as it occurs in the cytosol of neurons, in which phospho creatine⁻ is present at [PCr⁻] = 4.7 mM, creatine at [Cr] = 1.0 mM, ADP³⁻ at 0.73 mM, and ATP⁴⁻ at 2.6 mM.

13. Free Energy Required for ATP Synthesis under Physiological Conditions.

In the **cytosol** of rat **hepatocytes**, the **mass-action ratio** is :

$$R_o = \frac{[ATP^{4-}]}{[ADP^{3-}] \cdot ([HPO_4^{2-}] + [H_2PO_4^-])} = 5.33 \cdot 10^{-2} M^{-1} \quad ; \text{ at } 37^\circ C \text{ T} = 310.16 \text{ K}$$

$$P_i = [H_2PO_4^-] + [HPO_4^{2-}] ; [H_2PO_4^-] = \frac{[ATP^{4-}]}{[ADP^{3-}] \cdot R_o} - \frac{[H_2PO_4^-] \cdot K_{H_2PO_4} \cdot [H_2O]}{[H_3O^+]}$$

$$[H_2PO_4^-] = \frac{\frac{[ATP^{4-}]}{[ADP^{3-}] \cdot R_o}}{\left(1 + \frac{K_{H_2PO_4} \cdot [H_2O]}{[H_3O^+]}\right)} ; \Delta G = \Delta G^\circ + R \cdot T \cdot \ln(R_o \cdot [H_2O] \left(1 + \frac{K_{H_2PO_4} \cdot [H_2O]}{[H_3O^+]}\right)) = 36.99 \text{ kJ/mol}$$

$$ATP^{4-} + H_2O \Leftrightarrow ADP^{3-} + H_2PO_4^- ; K^\circ = 220409 \quad ; \Delta G^\circ = -30.500 \text{ kJ/mol}$$

$$+30500 + RF \cdot T \cdot \ln(0.0533 \cdot a_{HOH} \cdot (1 + 2.543489E-9 \cdot a_{HOH} / 10^{(-7.36)})) = 36988.76$$

Calculate the free energy ΔG required to synthesize ATP^{4-} in a rat hepatocyte.

14. Daily ATP Utilization by Human Adults.

(a) A total of **30.5 kJ/mol** of free energy ΔG is needed to synthesize ATP^{4-} from ADP^{3-} and $H_2PO_4^-$ when the **reactants** and **products** are at **1 M** concentration (standard state). Because the actual **physiological** concentrations of ATP^{4-} , ADP^{3-} , and $H_2PO_4^-$ are not **1 M**, the free energy ΔG required to synthesize ATP^{4-} under physiological conditions is different from ΔG° . Calculate the free energy ΔG required to synthesize ATP^{4-} in the human hepatocyte when the physiological concentrations of

ATP^{4-} , ADP^{3-} , ($H_2PO_4^- + HPO_4^{2-}$) are **3.5, 1.50, 5.0 mM** and **pH=7.36**, respectively, at **37°C**.

$$K_{H_2PO_4}^\circ = \frac{[HPO_4^{2-}] \cdot [H_3O^+]}{[H_2PO_4^-] \cdot [H_2O]} ; P_i = 5 \text{ mM} = [H_2PO_4^-] + [HPO_4^{2-}] ; [H_2PO_4^-] = 5 - \frac{[H_2PO_4^-] \cdot K_{H_2PO_4} \cdot [H_2O]}{[H_3O^+]}$$

$$K_{H_2PO_4}^\circ = 1.1469 \cdot 10^{-9} ; \Delta G^\circ = -R \cdot T \cdot \ln(K_{H_2PO_4}^\circ) = 51.034 \text{ kJ/mol at T} = 298.16 \text{ K}$$

$$\Delta G^\circ = -RF \cdot (T_0 + 25) \cdot \ln(1.1469E-9) = 51033.6 ;$$

$$K_{H_2PO_4}^\circ = \text{EXP}(-51033.6 / T / RF) = 2.543489 \cdot 10^{-9} \text{ at T} = 310.16 \text{ K}$$

$$[H_2PO_4^-] = \frac{5/1000}{\left(1 + \frac{K_{H_2PO_4} \cdot [H_2O]}{[H_3O^+]}\right)} ; \Delta G^\circ = -R \cdot T \cdot \ln \frac{[ATP^{4-}] \cdot [H_2O]}{[ADP^{3-}] \cdot [H_2PO_4^-]} \text{ at T} = 310.16 \text{ K}$$

$$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \left(\frac{[ATP^{4-}] \cdot [H_2O] \cdot \left(1 + \frac{K_{H_2PO_4} \cdot [H_2O]}{[H_3O^+]}\right)}{[ADP^{3-}] \cdot 5/1000} \right) = 60.3976 \text{ kJ/mol at T} = 310.16 \text{ K}$$

$$ATP^{4-} + H_2O \Leftrightarrow ADP^{3-} + H_2PO_4^- ; K^\circ = 220409 \quad ; \Delta G^\circ = -30.500 \text{ kJ/mol}$$

$$+30500 + RF \cdot T \cdot \ln(3.5/1.5 \cdot a_{HOH} / 5 \cdot 1000 \cdot (1 + 2.543489E-9 \cdot a_{HOH} / 10^{(-7.36)})) = 60397.598 = 58998.4$$

(b) A **68 kg (150 lb)** adult requires a caloric intake of **2 000 kcal (8 360 kJ)** of food per day (**24 h**). The food is metabolized and the free energy ΔG is used to synthesize ATP^{4-} , which then provides energy ΔG for the body's daily chemical and mechanical work $W = -\Delta G$. Assuming that the efficiency of converting food energy E into ATP^{4-} is **50%**, calculate the weight m_{ATP} of ATP^{4-} used by a human adult in **24 h**. What percentage of the body weight does this represent?

$$n_{ATP} = 8360 / 60.397598 / 2 = 69.208 \text{ mol} ; m_{ATP} = n_{ATP} \cdot M_{ATP} = 69.208050 \cdot 506.91 = 35082 \text{ g}$$

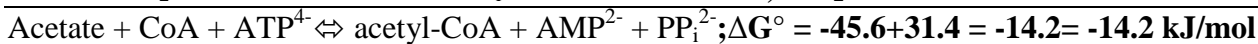
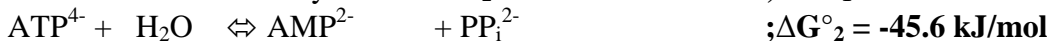
(c) Although adults synthesize large amounts of ATP^{4-} daily, their body weight, structure, and composition do not change significantly during this period. Explain this apparent contradiction.

15. Rates of Turnover of α and β Phosphates of ATP⁴⁻ A-O-OPO⁻-O-OPO⁻-O-OPO⁻-O⁻ (A- α - β - γ -O⁻).

If a small amount of ATP⁴⁻ labeled with radioactive phosphorus in the terminal position, [γ -³²P] ATP⁴⁻, is added to a yeast extract, about half $1/2$ of the ³²P activity is found in H₂PO₄⁻ within a few minutes, but the concentration of [ATP⁴⁻] = const remains unchanged. Explain. If the same experiment is carried out using ATP⁴⁻ labeled with ³²P in the central position, [β -³²P] ATP⁴⁻, the ³²P does not appear in H₂PO₄⁻ within such a short time. Why?

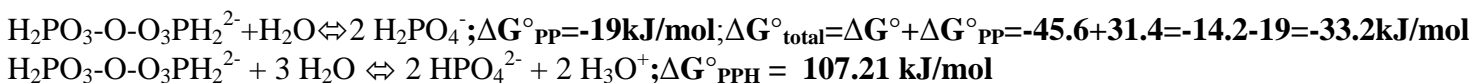
16. Cleavage of ATP to AMP and PP_i during Metabolism

The synthesis of the activated form of acetate (**acetyl-CoA**) is carried out in an ATP-dependent process:



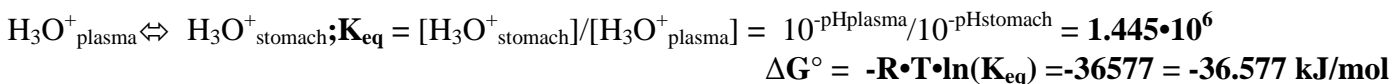
(a) The ΔG° for the hydrolysis of **acetyl-CoA** to **acetate** and **CoA** is **-31.4 kJ/mol** and that for hydrolysis of ATP⁴⁻ to AMP²⁻ and PP_i²⁻ is **-45.6 kJ/mol**. Calculate ΔG° for the **ATP-dependent** synthesis of **acetyl-CoA**.

(b) Almost all cells contain the enzyme inorganic **pyro-phosphates**, which catalyzes the **hydrolysis** of PP_i²⁻ to H₂PO₄⁻. What effect does the presence of this enzyme have on the synthesis of **acetyl-CoA**? Explain!



17. Energy for H₃O⁺ Pumping The parietal cells of the stomach lining contain membrane "pumps" that transport hydrogen ions **H₃O⁺** from the **cytosol** of these cells (**pH_{plasma} 7.36**) into the **stomach**, contributing to the acidity of **gastric juice** (**pH_{stomach} 1.2**). Calculate the free energy required to transport **1 mol** of hydrogen **H₃O⁺** ions through these pumps. (Hint: See Oxidative Phosphorylation.)

Assume a temperature of **37 °C** or **T = 310.16 K**. **1445440**



18. Standard Reduction Potentials The **standard reduction potential, E^o**, of any **RedOx** pair is defined for the half-cell reaction in equilibrium of each **RedOx** system:

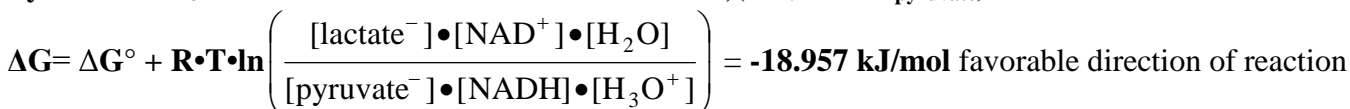


The **E^o** values for the **NAD⁺/NADH** and **pyruvate/lactate** conjugate **RedOx** pairs are **-0.113** and **0.2291 V**, respectively but **E^o₃₇: -0.059** and **0.3193 V**.

(a) Which conjugate pair has the greater tendency to lose electrons? Explain.

(b) Which is the stronger oxidizing agent? Explain.

(c) Beginning with **1 M** concentrations of each **reactant** and **product** at **pH 7.36**, in which direction will the following reaction proceed?



$$\Delta G^\circ_o = 96485 \cdot 2 \cdot (-0.059 - 0.3193) = -73000.5510 ;$$

$$\Delta G = -73000.5510 + R \cdot T \cdot \ln(1/1 \cdot 1/1 \cdot a_{\text{HOH}}/10^{(-7.36)}) = -18957.02$$

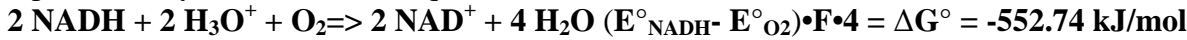
(d) What is the standard free-energy change (ΔG°) at **37 °C** for the conversion of **pyruvate** to **lactate**

(e) What is the equilibrium constant (**K_{eq}**) for this reaction?

$$K_{\text{eq}} = \frac{[\text{lactate}^-] \cdot [\text{NAD}^+] \cdot [\text{H}_2\text{O}]}{[\text{pyruvate}^-] \cdot [\text{NADH}] \cdot [\text{H}_3\text{O}^+]} = 6.149 \cdot 10^{12}; [\text{H}_3\text{O}^+] \cdot K_{\text{eq}} = \frac{[\text{lactate}^-] \cdot [\text{NAD}^+] \cdot [\text{H}_2\text{O}]}{[\text{pyruvate}^-] \cdot [\text{NADH}]} = 268417$$

$$\text{EXP}(-73000.5510/R/T) = 6149086393492.1 \cdot 10^{(-7.36)} = 268417.356457188 = K^\circ_o;$$

19. Energy Span of the Respiratory Chain Electron e^- transfer in the mitochondrial respiratory chain may be represented by the net reaction equation



$$\Delta G^\circ = 96485 \cdot 4 \cdot (-0.059 - 1.3732) = -552743.2680/52000 = 10.6297$$

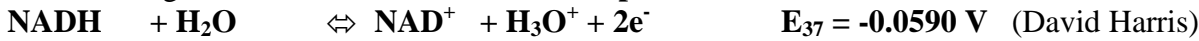
(a) Calculate the value of ΔE° for the net reaction of mitochondrial electron e^- transfer at 37°C .

(b) Calculate ΔG° for this reaction. $\Delta E^\circ = E^\circ_{\text{NADH}} - E^\circ_{\text{O}_2} = -0.059 - 1.3732 = -1.4322 \text{ V}$

(c) How many **nATP** molecules can theoretically be generated by this reaction if the free energy of **ATP** synthesis under cellular conditions is **52 kJ/mol**? **n = 10.63**

20. Dependence of Electromotive Force on Concentrations

Calculate the electromotive force **EMF** (in volts **V**) registered by an electrode immersed in a solution containing the following mixtures of **NAD⁺** and **NADH** at **pH 7.36** and **37 °C**, with reference to a half-cell of **E° 0.00 V**.



$$E = -0.059 + R \cdot T / F \cdot 2 \cdot \ln(1 \cdot 10^{(-7.36)} / 10 / a_{\text{HOH}}) = -0.36983 \text{ V}$$

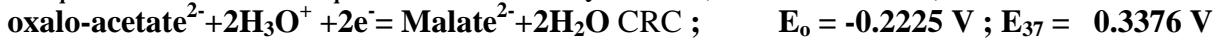
$$\text{EMF} = E = E_{37} + R \cdot T / F \cdot 2 \cdot \ln \left(\frac{[\text{NAD}^+] \cdot [\text{H}_2\text{O}]}{[\text{NADH}] \cdot [\text{H}_3\text{O}^+]} \right) = -0.36983 \text{ V}$$

(a) **1.00 mM NAD⁺** and **10.0 mM NADH**; **E = -0.36983 V**

(b) **1.00 mM NAD⁺** and **1.00 mM NADH**; **E = -0.33906 V**

(c) **10.0 mM NAD⁺** and **1.00 mM NADH**; **E = -0.30829 V**

21. Electron Affinity of Compounds List the following substances in order of increasing \square tendency to accept electrons e^- at **pH = 7.36** by **RedOx potential E_o** values:



(a) α-keto-glutarate + CO₂ (yielding iso-citrate);

(b) oxalo-acetate;

(c) O₂;

(d) NADP⁺.

22. Direction of Oxidation-Reduction Reactions

Which of the following reactions would you expect to proceed in the direction shown under **standard conditions pH = 7.36** and **37°C**, assuming that the appropriate **enzymes** are present to catalyze them?

$$(E^\circ_{\text{Red}} - E^\circ_{\text{Ox}}) \cdot F \cdot n = \Delta G^\circ \text{ kJ/mol};$$

$$\Delta G^\circ = 96485 \cdot 2 \cdot (0.33757 - 0.059) = 53755.65290 = 10.6297$$

$$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \left(\frac{[\text{oxaloacetate}^{2-}] \cdot [\text{NADH}] \cdot [\text{H}_3\text{O}^+]}{[\text{malate}^{2-}] \cdot [\text{NAD}^+] \cdot [\text{H}_2\text{O}]} \right) = 10.053 \text{ kJ/mol unfavorable direction} \Rightarrow \text{for (a)}$$

$$\Delta G = 53755.65290 + R \cdot T \cdot \ln(10^{(-7.36)}) = 10052.76$$

(a) **Malate²⁻ + NAD⁺ + H₂O => oxalo-acetate²⁻ + NADH + H₃O⁺**; $(E^\circ_{\text{malate}} - E^\circ_{\text{NAD}^+}) \cdot F \cdot 2 = \Delta G^\circ = 53.756 \text{ kJ/mol}$

$$\Delta G^\circ = 96485 \cdot 2 \cdot (-0.059 - 0.16453) = -43134.58410$$

$$\Delta G = -43134.58410 - R \cdot T \cdot \ln(10^{(-7.36)}) = 568.3108$$

$$\Delta G = \Delta G^\circ - R \cdot T \cdot \ln(10^{-7.36}) = 0.568 \text{ kJ/mol} \leq \text{direction favorable to left for (b)}$$

(b) **aceto-acetate⁻ + NADH + H₃O⁺ => β-hydroxy-butyrate⁻ + NAD⁺ + H₂O**; $\Delta G^\circ = -43.135 \text{ kJ/mol}$

$$\Delta G^\circ = 96485 \cdot 2 \cdot (-0.059 - 0.3193) = -73000.5510$$

$$\Delta G = -73000.5510 - R \cdot T \cdot \ln(10^{(-7.36)}) = -29297.7$$

$$\Delta G = \Delta G^\circ - R \cdot T \cdot \ln(10^{-7.36}) = -29.298 \text{ kJ/mol} \Rightarrow \text{direction favorable to right}$$

(c) **Pyruvate⁻ + NADH + H₃O⁺ => lactate⁻ + NAD⁺ + H₂O**; $\Delta G^\circ = -73.001 \text{ kJ/mol} \Rightarrow \text{direction}$

$$\Delta G^\circ = 96485 \cdot 2 \cdot (0.16453 - 0.3193) = -29865.96690$$

(d) **Pyruvate⁻ + β-hydroxy-butyrate⁻ => lactate⁻ + aceto-acetate⁻**; $\Delta G^\circ = -29.866 \text{ kJ/mol} \Rightarrow \text{direction}$

$$\Delta G^\circ = 96485 \cdot 2 \cdot (0.33757 - 0.3193) = 3525.56190$$

(e) **Malate⁻ + pyruvate⁻ => oxalo-acetate⁻ + lactate⁻**; $\Delta G^\circ = 3.526 \text{ kJ/mol} \leq \text{direction}$

$$\Delta G^\circ = 96485 \cdot 2 \cdot (0.52695 - 0.286255) = 46446.914150$$

(f) **Acetaldehyde + succinate²⁻ => ethanol + fumarate²⁻**; $\Delta G^\circ = 46.447 \text{ kJ/mol} \leq \text{direction}$