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Unit - I: Bioenergetics

Important terms in Bioenergetics:

Bioenergetics: Bioenergetics or biochemical thermodynamics is the quantitative study of energy transductions in living cells and the physical-chemical nature underlying these processes.

Thermodynamics: It is a branch of science which deals with study of different forms of energy and their interconversions. It deals with energy changes in physical and chemical processes.

Significance of Thermodynamics

- To predict whether any chemical reaction can occur under specified conditions
- To determine the extent of chemical reaction before the equilibrium is reached
- To derive important laws like law of equilibrium

System: specified part of universe in which energy changes are taking place.

Surroundings: the remaining portion of universe excluding the system.

The reacting system and its surroundings together constitute the universe.

Universe = System + Surroundings

Types of System:

- **Open system:** Mass and energy are exchanged with surroundings.
- **Closed system:** No exchange of mass but exchange of energy with surroundings takes place.
- **Isolated system:** Neither exchange of mass nor energy with surroundings.

Based on the nature of constituents, systems are classified into:

- **Homogenous system:** All the constituents are present in the same phase and the composition of system is uniform throughout.
- **Heterogenous system:** It contains two or more phases and the composition of system is uniform throughout.

State of the System:

The state of a thermodynamic system is its measurable properties.

On the basis of the dependency of a particular property on the mass of the system, thermodynamic properties can be classified into two groups:

1. Intensive property: Properties that is independent of the mass of the concerned system. For ex- Pressure, Temperature, Density, Concentration, Melting point, Boiling point, Surface tension, Viscosity, etc.

2. Extensive property: Properties that depend of the mass of the concerned system. For ex- Mass, Volume, Internal energy, Heat capacity, Enthalpy, Entropy, Gibbs energy, Helmholtz energy, etc.

State variables:

These are the properties of the system such as temperature, pressure, volume. When these are changed, the system also changes.

State functions:

Properties whose value only depend upon the state of the system and are independent of the path by which state has been reached.

Internal Energy:

Internal energy represents the total energy (potential and kinetic energy) of a system. Potential energy is the stored energy and kinetic energy is the energy generated due to the motion of molecules. It includes the energy of chemical bonds and intermolecular forces in addition to kinetic energy. The internal energy is given by the symbol U and the change in the internal energy is given as ΔU.
Change in internal energy (ΔU) takes place when:

- Heat is passed into or out of the system
- Work is done on or by the system
- Mass enters or leaves the system

When change of state is brought about both by doing work and by transfer of heat, internal energy is given by:

$$\Delta U = Q+W$$

Where, ΔU is the change in internal energy, Q is the heat transferred, W is the work done on or by the system.

In an isolated system, the internal energy is constant i.e. the energy transfer is zero and no work is done. Then $\Delta U = 0$.

When the ΔU is a negative value, then the system release heat and work is done by the system.

If heat flows into a system or the surroundings do work on it, the internal energy increases and the signs of Q and W are positive. Conversely, if heat flow out of the system or work done by the system on the surroundings, it will take place at the expense of the internal energy, and Q and W will therefore be negative or "The change in internal energy of a system is equal to the heat added to the system minus the work done by the system".

- **Adiabatic process**: An adiabatic process transfers no heat, therefore $Q = 0$;
  When a system expands adiabatically, W is positive (the system does work) so ΔU is negative;
  When a system compresses adiabatically, W is negative (work is done on the system) so ΔU is positive.
- **Isothermal process**: An isothermal process is a constant temperature process. Any heat flow into or out of the system must be slow enough to maintain thermal equilibrium.
  For ideal gases, if ΔT is zero, $\Delta U = 0$; Therefore, $Q = W$; any energy entering the system (Q) must leave as work (W).
- **Isobaric process**: An isobaric process is a constant pressure process.
  ΔU, W, and Q are generally nonzero, but calculating the work done by an ideal gas is straight forward;
  $W=P\cdot\Delta V$
- **Isochoric process**: An isochoric process is a constant volume process.

When the volume of a system doesn't change, it will do no work on its surroundings. $W = 0$; $\Delta U=0$

In all the above processes, the heat capacity Q is expressed as the amount of heat required to raise a certain mass of a material by a certain temperature and is defined as:

$$Q=mc_x\Delta T$$

The constant c_x is called the specific heat of substance X, and in SI units is defined as J/kg-K.

The heat capacity of an ideal gas at constant volume (C_V): C_V =3/2R; The heat capacity of an ideal gas at constant pressure (C_P): C_P =5/2R. (universal gas constant R = 8.314 J/mol-k).

Thus, for constant volume, Q can be written as:

$$Q=nC_V\Delta T=\Delta U$$

Entropy:

It is defined as a measure of the degree of randomness or disorder in a system. For example, the release of water from nonpolar surfaces responsible for the hydrophobic effect is favorable because water molecules free in solution are more disordered than they are when they are associated with nonpolar surfaces. The entropy of a perfect crystal approaches zero as the temperature approaches absolute zero. The units of entropy are joules/mole.Kelvin (J/mol.K).

Enthalpy:

Enthalpy reflects number and kinds of chemical bonds in the reactants and products and can be defined as the amount of heat energy that is being absorbed or evolved during a chemical reaction. The enthalpy is represented by the symbol H. The change of enthalpy is given as ΔH where the symbol Δ indicates the change of enthalpy. The units of ΔH are joules/mole or

calories/mole. When a chemical reaction releases heat, i.e. exothermic; the heat content of the products is less than that of the reactants and ΔH has a negative value. When a reaction is endothermic, ΔH has a positive value.

Enthalpy also depicts the relationship between the system and the surrounding. In other words, enthalpy is the sum of the internal energy of a system, because the internal energy is changed during a chemical reaction and the change is measured as the enthalpy. The enthalpy of a process that occurs at a constant pressure can be given by:

$$H = U + PV$$

Where, H is the enthalpy, U is the sum of the internal energy, P is the pressure of the system, V is the volume of the system.

Actually, enthalpy is the sum of internal energy and the energy required to maintain the volume of a system at a given pressure. The term "PV" indicates the work that has to be done on the surroundings in order to make space for the system.

Significance of enthalpy: The change in enthalpy indicates whether a particular reaction is endothermic or exothermic. Reaction is endothermic, if the value of ΔH is a positive; conversely, reaction is exothermic, if the value of ΔH is a negative. Furthermore, change in enthalpy occurs when there is change of phase or state of substances. For example, if a solid is converted to its liquid form, enthalpy is changed known as the heat of fusion**.** When a liquid is converted to the gaseous form, the enthalpy change is called the heat of vaporization.

Gibbs free energy:

Gibbs free energy, G, expresses the amount of an energy capable of doing work during a reaction at constant temperature and pressure. When a reaction proceeds with the release of free energy, the free-energy change, ΔG, has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and ΔG is positive. The units of ΔG are joules/mole or calories/mole.

All chemical reactions are influenced by two forces: the tendency to achieve the most stable bonding state and to achieve the highest degree of randomness. The net driving force in a reaction is ΔG, the free-energy change, which represents the net effect of these two factors. Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation

$$\Delta G = \Delta H - T\Delta S$$

Where, ΔG is the change in Gibbs free energy of the reacting system, ΔH is the change in enthalpy of the system, T is the absolute temperature, and ΔS is the change in entropy of the system.

1.1 Laws of thermodynamics, internal energy, enthalpy, entropy

Laws of Thermodynamics

Various forms of energy may be transformed from one form to another, under certain conditions. Thermodynamics deal with initial and final states of the system undergoing the change, instead of the rate of transformations. Laws of thermodynamics apply only when a system is in equilibrium or moves from one equilibrium state to another equilibrium state. Macroscopic properties like pressure and temperature do not change with time for a system in equilibrium state. Biological energy transformations obey the laws of thermodynamics.

First law of thermodynamics

The first law of thermodynamics (also known as the law of conservation of energy) states that energy can neither be created nor destroyed; it can only be transferred or changed from one form to another. For ex- turning on a light would seem to produce energy; however, it is electrical energy, which is converted. In other words, the first law of thermodynamics states that the total energy of a system and its surroundings is constant.

The first law of thermodynamics can be stated as "any change in the internal energy (ΔU) of a system is given by the sum of the heat (Q) that flows across its boundaries and the work (W) done on the system by the surroundings". Mathematically, it can be expressed as

$\Delta U=Q+W$, which states that the energy of an isolated system is constant.

Second law of thermodynamics

It states that the total entropy of a system plus that of its surroundings always increases. In other words, for a process to take place, the entropy of the universe must increase. Total entropy is given by

$$\Delta S_{universe} = \Delta S_{system} + \Delta S_{surroundings}$$

The entropy (S) of the system may change in the course of a chemical reaction by an amount ΔS_{system}. If heat flows from the system to its surroundings, then the heat content, often referred to as the enthalpy (H), of the system will be reduced by an amount ΔH_{system}.

If heat flows from the system to the surroundings, then the entropy of the surroundings will increase. The temperature of the system has a great influence on the change in the entropy of the surroundings; i.e. the change in the entropy of the surroundings will be proportional to the amount of heat transferred from the system and inversely proportional to the temperature (T) of the surroundings.

In biological systems, T [in kelvins (K), absolute temperature] is usually assumed to be constant. Thus, a change in the entropy of the surroundings is given by:

$$\Delta S_{surroundings} = -\Delta H_{system}/T \qquad \text{Equation 1}$$

The total entropy change is given by:

$\Delta S_{total} = \Delta S_{system} + \Delta S_{surroundings}$ Equation 2

Substituting Equation 1 into Equation 2, we obtain

$\Delta S_{total} = \Delta S_{system} - \Delta H_{system}/T$ Equation 3

Multiplying both sides by –T gives,

$-T\Delta S_{total} = \Delta H_{system} - T\Delta S_{system}$ Equation 4

The function - TΔS has units of energy and is referred to as free energy or **Gibbs free energy**, after Josiah Willard Gibbs, who developed this function in 1878:

$\Delta G = \Delta H_{system} - T\Delta S_{system}$ Equation 5

The free-energy change (ΔG) is used to describe the energetics of biochemical reactions. It takes into account both the entropy of the system (directly) and the entropy of the surroundings (in the form of heat released from the system).

As per second law of thermodynamics, total entropy will increase if

$\Delta S_{system} > \Delta H_{system}/T$ Or, $T\Delta S_{system} > \Delta H_{system}$ Equation 6

Therefore, entropy will increase if and only if

$\Delta G = \Delta H_{system} - T\Delta S_{system} < 0$ Equation 7

Thus, the free-energy change must be negative for a process to take place spontaneously. There is negative free-energy change when and only when the overall entropy of the universe is increased.

1.2 Concept of free energy, redox potential, energy rich compounds

1.2.1 Free energy of a Reaction

The composition of a reacting system (a mixture of chemical reactants and products) tends to continue changing until equilibrium is reached. At the equilibrium concentration of reactants and products, the rates of the forward and reverse reactions are exactly equal and no further net change occurs in the system. The concentrations of reactants and products at equilibrium define the equilibrium constant, K_{eq}

For a reaction,

$$aA + bB \rightleftharpoons cC + dD$$

where, a, b, c, and d are the number of molecules of A, B, C, and D participating, and [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium, the equilibrium constant (K_{eq}) is given by

$$K_{eq} = \left(\frac{[C]^c [D]^d}{[A]^a [B]^b} \right) \qquad \text{Equation 8}$$

When a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force, the magnitude of which can be expressed as the free-energy change for the reaction, ΔG. Under standard conditions (298 K = 25°C), when reactants and products are initially present at 1 M concentrations or, for gases, at partial pressures of 101.3 kilopascals (kPa), or 1 atm, the force driving the system toward equilibrium is defined as the standard free-energy change, $\Delta G'^0$.

The standard transformed free-energy change ($\Delta G'^0$), is a physical constant that is characteristic of a given reaction and can be calculated from the equilibrium constant for the reaction:

$$\Delta G'^0 = -RT \ln K'_{eq} \qquad \text{Equation 9}$$

Each chemical reaction has a characteristic standard free-energy change, which may be positive, negative, or zero, depending on the equilibrium constant of the reaction.

If $K'_{eq} > 1.0$, its $\Delta G'^0$ is negative, the reaction will proceed in a forward direction under standard conditions (when the initial concentration of each component is 1.0 M, the pH is 7.0, the temperature is 25°C, and the pressure is 101.3 kPa (1 atm)). Similarly, if $K'_{eq} < 1.0$, $\Delta G'^0$ is positive, the reaction will proceed in a reverse direction under standard conditions. At K'_{eq} = zero, $\Delta G'^0$ is zero, the reaction will be at equilibrium.

The actual free-energy change, ΔG, is a variable that depends on $\Delta G'^0$ and on the concentrations of reactants and products.

The ΔG and $\Delta G'^0$ for any reaction $aA + bB \rightleftharpoons cC + dD$ are related by the equation

$$\Delta G = \Delta G'^0 + RT \ln \left(\frac{[C]^c [D]^d}{[A]^a [B]^b} \right) \qquad \text{Equation 10}$$

Here,

$[C]^c[D]^d/[A]^a[B]^b$ is called the mass-action ratio, Q.

Thus, Equation 10 can be expressed as:

$$\Delta G = \Delta G'^0 + RT \ln Q$$

When ΔG is large and negative, the reaction tends to go in the forward direction; when ΔG is large and positive, the reaction tends to go in the reverse direction; and when ΔG = 0, the system is at equilibrium.

The free-energy change for a reaction is independent of the pathway by which the reaction occurs; it depends only on the nature and concentration of the initial reactants and the final products. Free-energy changes are additive; the net chemical reaction that results from successive reactions sharing a common intermediate has an overall free-energy change that is the sum of the ΔG values for the individual reactions.

Standard free energy changes are additive but equilibrium constants are multiplicative

In the case of two sequential chemical reactions, A⇌B and B⇌C, each reaction has its own equilibrium constant and each has its characteristic standard free-energy change, $\Delta G'^{0}_{1}$ and $\Delta G'^{0}_{2}$.

For the overall reaction A⇌C, $\Delta G'^{0}_{total}$ is equal to the sum of the individual standard free-energy changes, $\Delta G'^{0}_{1}$ and $\Delta G'^{0}_{2}$, of the two reactions and can be written as:

$$\Delta G'^{0}_{total} = \Delta G'^{0}_{1} + \Delta G'^{0}_{2} \qquad \text{Equation 11}$$

Although, the $\Delta G'^{0}$ values for two reactions that sum to a third, overall reaction are additive, the K'_{eq} for the overall reaction is the product of the individual K'_{eq} values for the two reactions. In other words, equilibrium constants are multiplicative and is given by

$$K'_{eq3} = (K'_{eq1}).(K'_{eq2}) \qquad \text{Equation 12}$$

1.2.2 Redox potential

The affinity of an oxidation reduction system for electrons is referred to as oxidation-reduction potential or redox potential. It provides a quantitative measure of the oxidizing or reducing power of a molecule. The effective redox potential depends on the proportion of the oxidized and reduced forms. Redox potential is measured in Volts (V). The standard reduction potential, E_0, is a measure (in volts) of this affinity. High redox potential signifies high electron affinity or greater tendency to accept electrons and low redox potential signifies low electron affinity or greater tendency to lose electrons.

Oxidation: it is defined as the removal of electrons.
Reduction: it is defined as the addition of electrons.

For ex- NADPH/NADP couple is much better biological reducing agent than NADH/NAD despite the fact that the standard redox potentials are identical.

The oxidation-reduction reactions are catalyzed by a set of enzymes:

Oxidoreductases: Enzymes involved in oxidation and reduction reactions are called oxidoreductases. There are four groups of Oxidoreductases:

1. Oxidases: Catalyze the removal of hydrogen from a substrate with the involvement of oxygen as a H-acceptor, for ex- Cytochrome oxidase (copper containing)-the terminal component of Electron transport chain which transfer the electron finally to O_2. Others are D-amino acid oxidase (FAD linked), L-amino acid oxidase (FMN linked), Xanthine oxidase (molybdenum containing), Aldehyde dehydrogenase (FAD-linked)

2. Dehydrogenases: They are involved in transfer of hydrogen from one substrate to other in a coupled oxidation reduction reactions. They are components of Electron transport chain. Dehydrogenases use coenzymes – nicotinamides & riboflavin - as hydrogen carriers.

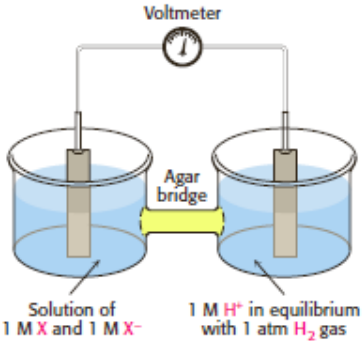
3. Hydroperoxidases: They use hydrogen peroxide as substrate. For ex- catalase and peroxidases.

4. Oxygenases: They catalyze the direct transfer and incorporation of oxygen into a substrate molecule. Monooxygenases or mixed function oxidases incorporate only one atom of molecular oxygen into the substrate $A - H + O_2 + ZH_2 \rightarrow A - OH + H_2O + Z$, while Dioxygenases incorporate both atoms of oxygen molecule into the substrate; $A + O_2 \rightarrow AO_2$.

5. Superoxide dismutase (SOD): SOD protects aerobic organisms against oxygen toxicity due to superoxide anion free radical ($O^{-2\cdot}$)

Redox couple

Consider a substance that can exist in an oxidized form X and a reduced form X^-. Such a pair is called a redox couple and is designated $X : X^-$. The sample half-cell consists of an electrode immersed in a solution of 1 M oxidant (X) and 1 M reductant (X^-). The standard reference half-cell consists of an electrode immersed in a 1 M H^+ solution that is in equilibrium with H_2 gas at 1 atmosphere (1 atm) of pressure. The electrodes are connected to a voltmeter, and an agar bridge allows ions to move from one half-cell to the other, establishing electrical continuity between the half-cells. Electrons then flow from one half-cell to the other through the wire connecting the two half-cells to the voltmeter.



If the reaction proceeds in the direction

$$X^- + H^+ \rightarrow X + 1/2\ H_2$$

the reactions in the half-cells (referred to as half-reactions or couples) must be

$$X^- \rightarrow X + e^-$$

$$H^+ + e^- \rightarrow 1/2\ H_2$$

Thus, electrons flow from the sample half-cell to the standard reference half-cell, and the sample-cell electrode is taken to be negative with respect to the standard-cell electrode. The reduction potential of the $X : X^-$ couple is the observed voltage when X, X^-, and H^+ are 1 M with 1 atm of H_2. The reduction potential of the H^+: H_2 couple is defined to be 0 volts. In oxidation–reduction reactions, the donor of electrons, in this case X, is called the reductant (reducing agent), whereas the acceptor of electrons, H^+ here, is called the oxidant.

A negative reduction potential means that the oxidized form of a substance has lower affinity for electrons than does H_2. A positive reduction potential means that the oxidized form of a substance has higher affinity for electrons than does H_2.

For ex- a strong reducing agent (such as NADH) is poised to donate electrons and has a negative reduction potential, whereas a strong oxidizing agent (such as O_2) is ready to accept electrons and has a positive reduction potential.

Standard reduction potentials can be used to calculate free energy change

Biological oxidation-reduction reactions can be described in terms of two half-reactions, each with a characteristic standard reduction potential, E'^0. When two electrochemical half-cells, each containing the components of a half-reaction, are connected, electrons tend to flow to the half-cell with the higher reduction potential. The strength of this tendency is proportional to the difference between the two reduction potentials (ΔE) and is a function of the concentrations of oxidized and reduced species.

The standard free-energy change ($\Delta G'^0$) for an oxidation-reduction reaction is directly related to the change in reduction potential ($\Delta E'^0$) of the two half-cells:

$$\Delta G'^0 = - nF\Delta E'^0$$

Where,

n = Number of electrons transferred in a redox reaction

F = Faraday's constant = 96.48 kJ mol^{-1} V^{-1} (23.06 kcal mol^{-1} V^{-1},

$\Delta E'^0$ = in volts; difference in standard reduction potential of oxidizing and reducing agents i.e. (E'^0 of half reaction containing oxidizing agent) – (E'^0 of half reaction containing reducing agent)

$\Delta G'^0$ = in kilojoules or kilocalories per mole.

We consider the reduction of pyruvate by NADH, catalyzed by lactate dehydrogenase, during lactic acid fermentation.

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+ \qquad \text{Equation A}$$

The reduction potential of the NAD^+ : NADH couple, or half-reaction, is -0.32 V, whereas that of the pyruvate : lactate couple is -0.19 V.

As per convention, partial reactions can be written as

$\text{Pyruvate} + 2\ H^+ + 2\ e^- \rightarrow \text{lactate}$ $E'^0 = -0.19$ V Equation B

$NAD^+ + H^+ + 2\ e^- \rightarrow \text{NADH}$ $E'^0 = -0.32$ V Equation C

Or

$NADH \rightarrow NAD^+ + H^+ + 2\ e^-$ $E'^0 = +0.32$ V Equation D

For reaction B, the free energy can be calculated with n = 2.

$$\Delta G'^0 = -2 \times 96.48 \text{ kJ mol}^{-1} \text{ V}^{-1} \times -0.19 \text{ V}$$
$$= +36.7 \text{ kJ mol}^{-1} (+8.8 \text{ kcal mol}^{-1})$$

Similarly, for reaction D,

$$\Delta G'^0 = -2 \times 96.48 \text{ kJ mol}^{-1} \text{ V}^{-1} \times +0.32 \text{ V}$$
$$= -61.8 \text{ kJ mol}^{-1} (-14.8 \text{ kcal mol}^{-1})$$

Therefore, the free energy for reaction A can be given by

$$\Delta G'^0 = \Delta G'^0 \text{ (for reaction B)} + \Delta G'^0 \text{ (for reaction D)}$$
$$= +36.7 \text{ kJ mol}^{-1} + (-61.8 \text{ kJ mol}^{-1})$$
$$= -25.1 \text{ kJ mol}^{-1} (-6.0 \text{ kcal mol}^{-1})$$

1.2.3 Energy rich compounds

It may be of two types:

1. High Energy Compounds: Compounds in biological system which on hydrolysis yield free energy equal to or greater than that of ATP, i.e. $\Delta G = -7.3$ kcal / mol. Most of the high energy compounds contain phosphate group [except acetyl CoA] hence they are also called **high energy phosphates.** The bonds in the high energy compounds which yield energy upon hydrolysis are called high energy bonds. These bonds are notated by the symbol "squiggle" (~P), a notation invented by Fritz Albert Lipmann.

2. Low Energy Compounds: Compounds that yield energy less than -7.3 kcal / mol. For ex- ADP, Glucose-6-P, Fructose-6-P, etc.

High energy compounds are mainly classified into 5 groups:

1. **Pyrophosphates:** The energy bonds in pyrophosphates are acid anhydride bonds. These bonds are formed by the condensation of acid groups [mainly phosphoric acid] or its derivatives. An example for pyrophosphates is ATP. It has two high energy diphosphate bonds – phosphoanhydride bonds.

2. **Enol phosphates:** The bond present here is enolphosphate bond. It is formed when phosphate group attaches to a hydroxyl group which is bounded to a carbon atom having double bond. Example : phosphoenolpyruvate.

3. **Acyl phosphates:** The high energy bond in this compound is formed by the reaction between carboxylic acid group and phosphate group. An example for acyl phosphate is 1,3-bisphosphoglycerate.

4. **Thiol phosphates:** Here high energy phosphate bond is absent. Instead high energy thioester bond is present. Thioester bond results from the reaction between thiol and carboxylic acid group. Example : Acetyl CoA

5. **Guanido phosphates or phophagens:** Also known as phophagens. The bond is known as guanidine phosphates bonds. It is formed by the attachment of phosphate group to guanidine group. Most important compound with this bond is phosphocreatine.

ADENOSINE TRIPHOSPHATE (ATP)

- Adenosine-5'-triphosphate (ATP), also known as **energy currency of the cell,** is a nucleotide consisting of adenine, a ribose, and a triphosphate unit.
- The active form of ATP is usually a complex of ATP with Mg^{2+} or Mn^{2+}.
- ATP is an energy-rich molecule because its triphosphate unit contains two phosphoanhydride bonds. ATP is often called a high-energy phosphate compound, and its phosphoanhydride bonds are referred to as high-energy bonds.
- ATP hydrolysis is exergonic. A large amount of free energy is liberated when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (P_i) or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PP_i).

$$ATP + H_2O \rightleftharpoons ADP + Pi$$
$$\Delta G'^0 = -30.5 \text{ kJ mol}^{-1} (-7.3 \text{ kcal mol}^{-1})$$
$$ATP + H_2O \rightleftharpoons AMP + PPi$$
$$\Delta G'^0 = -45.6 \text{ kJ mol}^{-1} (-10.9 \text{ kcal mol}^{-1})$$

$\Delta G'^0$ for these reactions depends on the ionic strength of the medium and on the concentrations of Mg^{2+} and other metal ions. Under typical cellular concentrations, the actual ΔG for these hydrolyses is approximately -50 kJ mol^{-1} (-12 kcal mol^{-1}).

ATP hydrolysis drives metabolism by shifting the equilibrium of coupled reactions

We consider a chemical reaction that is thermodynamically unfavorable without an input of free energy.

Suppose that the standard free energy of the conversion of compound A into compound B is +16.7 kJ mol^{-1} (+4.0 kcal mol^{-1}):

$$A \rightleftharpoons B \qquad \Delta G'^0 = +16.7 \text{ kJ mol}^{-1} (+4.0 \text{ kcal mol}^{-1})$$

The equilibrium constant K'_{eq} of this reaction at 25°C is related to $\Delta G'^0$ (in units of kilojoules per mole) by

$$K'_{eq} = [B]_{eq}/[A]_{eq} = 10^{-\Delta G'^0/5.69} = 1.15 \times 10^{-3}$$

Thus, net conversion of A into B cannot take place when the molar ratio of B to A is equal to or greater than 1.15×10^{-3}.

However, A can be converted into B under these conditions if the reaction is coupled to the hydrolysis of ATP. Under standard conditions, the $\Delta G'^0$ of hydrolysis is approximately -30.5 kJ mol^{-1} (-7.3 kcal mol^{-1}). The new overall reaction is

$$A + ATP + H_2O \rightleftharpoons B + ADP + P_i$$
$$\Delta G'^0 = -13.8 \text{ kJ mol}^{-1} (-3.3 \text{ kcal mol}^{-1})$$

Its free-energy change of -13.8 kJ mol^{-1} (-3.3 kcal mol^{-1}) is the sum of the value of $\Delta G'^0$ for the conversion of A into B [+16.7 kJ mol^{-1} (+4.0 kcal mol^{-1})] and the value of $\Delta G'^0$ for the hydrolysis of ATP [-30.5 kJ mol^{-1} (-7.3 kcal mol^{-1})].

At pH 7, the equilibrium constant of this coupled reaction is

$$K'_{eq} = \frac{[B]_{eq}}{[A]_{eq}} \times \frac{[ADP]_{eq}\,[P_i]_{eq}}{[ATP]_{eq}}$$

At equilibrium, the ratio of [B] to [A] is given by

$$\frac{[B]_{eq}}{[A]_{eq}} = \frac{K'_{eq}[ATP]_{eq}}{[ADP]_{eq}[P_i]_{eq}}$$

which means that the hydrolysis of ATP enables A to be converted into B until the [B]/[A] ratio reaches a value of 2.67 x 10^2. In other words, coupling the hydrolysis of ATP with the conversion of A into B under standard conditions has changed the equilibrium ratio of B to A by a factor of about 10^5. Thus, a thermodynamically unfavorable reaction sequence can be converted into a favorable one by coupling it to the hydrolysis of a sufficient number of ATP molecules in a new reaction.

Main functions of ATP are: Synthesis of compounds, such as polysaccharides, amino acids, DNA, RNA etc.; Supplying energy for mechanical work, such as muscle contraction; Help in active transport for transporting organic substances through the cell membrane.

Other energy rich compounds include:
Nucleoside triphosphates (GTP: Guanosine triphosphate; CTP: Cytosine triphosphate; UTP: Uridine triphosphate); Deoxynucleoside triphosphates (dATP, dGTP, dTTP, and dCTP)

Energy shuttles:
NADH: Nicotinamide Adenine Dinucleotide. It yields 2.5 (~3) ATP during oxidative phosphorylation.
$FADH_2$: Flavin adenine dinucleotide. It yields 1.5 (~2) ATP during oxidative phosphorylation.

1.3 Mitochondrial electron transport chain and oxidative phosphorylation

Electron transport chain and oxidative phosphorylation:

The electron transport chain reaction is coupled with transport of protons (H^+) across the inner mitochondrial membrane to inter membrane space, resulting in an electrochemical proton gradient. The electron transport chain or mitochondrial respiratory chain, consisting of four large protein complexes (complexes I–IV), are embedded in the inner mitochondrial membrane and allow a series of oxidation–reduction reactions that transfer electrons from NADH and $FADH_2$ to oxygen, thereby generating water (H_2O).Further re-entry of this proton gradient from inter-membrane space to mitochondrial matrix through ATP-synthase (complex V), is used for the phosphorylation of adenosine diphosphate (ADP) into ATP (Figure 1).

Oxidative phosphorylation is the cellular metabolic process in which cells use enzymes to oxidize different biomolecules such as carbohydrates, fats, and proteins to release energy in the form of ATP. In oxidative phosphorylation, the electron-transfer potential of NADH or $FADH_2$ is converted into the phosphoryl-transfer potential of ATP. These reactions are summarized as below:

$$NADH + 1/2O_2 + H^+ \rightarrow H_2O + NAD^+$$
$$\Delta G'^0 = -220.1 \text{ kJ mol}^{-1} (-52.6 \text{ kcal mol}^{-1}) \qquad \text{Equation 1}$$

$$ADP + P_i + H^+ \rightarrow ATP + H_2O$$
$$\Delta G'^0 = +30.5 \text{ kJ mol}^{-1} (+7.3 \text{ kcal mol}^{-1}) \qquad \text{Equation 2}$$

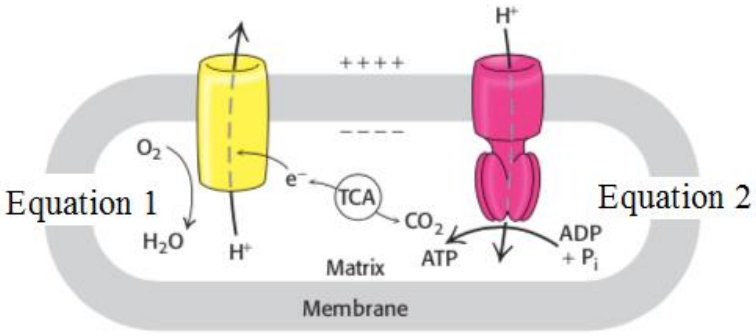


Figure 1: Electron transport is tightly coupled to phosphorylation.

Complex I (*NADH:ubiquinone oxidoreductase or NADH dehydrogenase*): It is largest and the first enzyme of the respiratory chain, oxidizes NADH, generated through the Krebs cycle in the mitochondrial matrix, and uses the two electrons to reduce Coenzyme Q (CoQ) to ubiquinol (the reduced form of ubiquinone) ($CoQH_2$). The generated ubiquinol in complex-I acts as the entry point for electrons from $FADH_2$ (Complex-II) of flavoproteins (Figure 2).

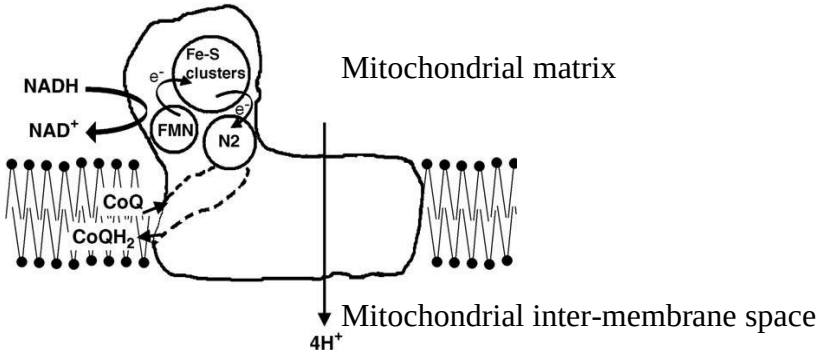


Figure 2: Schematic picture of Complex I in the inner mitochondrial membrane. (Taken from, Brandt et al., 2003).

NADH dehydrogenase catalyzes reaction showing:

$$NADH + Q + 5H^+_{matrix} \rightarrow NAD^+ QH_2 + 4H^+_{cytoplasm}$$

The binding of NADH leads to the transfer of two high-potential electrons to the *flavin mononucleotide* (FMN), a prosthetic group of complex-I, providing the reduced form of $FMNH_2$. The flow of these electrons takes place through Fe-S clusters to coenzyme Q. The overall reaction leads to the pumping of four hydrogen ions out of the matrix to the mitochondrion inter-membrane space.

Complex II (*Succinate dehydrogenase*): The succinate dehydrogenase *or succinate-Q reductase complex* is an integral membrane protein of the inner mitochondrial membrane (Figure 3), and catalyzes the conversion of succinate to fumarate. The oxidation of succinate to fumarate occurs by succinate dehydrogenase and accepts the hydrogen from $FADH_2$. $FADH_2$ does not leave the

complex. Rather, its electrons are transferred to Fe-S centers and finally to Q to form QH_2, which then is ready to transfer electrons further down the electron transport chain.

$$FADH_2 \rightarrow FAD + 2\ H^+ + 2\ e^-$$

The succinate-Q reductase complex does not pump protons from one side of the membrane to the other. Consequently, less ATP is formed from the oxidation of $FADH_2$ than NADH.

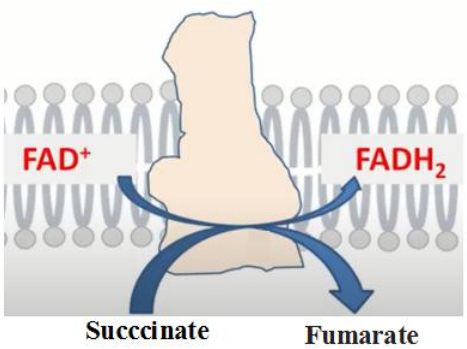


Figure 3: Showing Complex II as an integral membrane protein of the inner mitochondrial membrane.

Complex III (*ubiquinone:cytochrome c oxidoreductase or cytochrome bc1 complex)*: In Complex III, electrons flow from ubiquinol (QH_2) to cytochrome *c*. The function of *ubiquinone-cytochrome c oxidoreductase* is to catalyze the transfer of electrons from QH2 to oxidized *cytochrome* c (Cyt *c*), and pump protons out of the mitochondrial matrix to inter-membrane space. The pair of electrons flow leads to the effective net transport of 2 H+. The net reaction is shown below:

$$QH_2 + 2\ \text{Cyt}\ c_{(\text{oxidized})} + 2\ H^+_{\text{matrix}} \rightarrow Q + 2\ \text{Cyt}\ c_{(\text{reduced})} + 4H^+_{\text{cytoplasm}}$$

The enzyme *ubiquinone-cytochrome c oxidoreductase* contains an iron–sulfur protein with a 2Fe-2S center, also known as *Rieske center,* in this complex one of the iron ions is coordinated by two histidine residues instead of two cysteine residues, that coordinates the stabilization of the *Rieske* center in its reduced form, and raise its reduction potential so that it can readily accept electrons from QH_2.

Q cycle: The mechanism for the coupling of electron transfer from Q to cytochrome *c* (**Q Cycle funnels electrons from a two-electron carrier to a one-electron carrier and pumps protons)** shown in **Figure 4**. Two QH_2 molecules bind to the complex consecutively, each giving up two electrons and 2 H^+. *These protons are released to the cytoplasmic side of the membrane.* The first QH_2 to exit the Q pool binds to the first Q binding site (Qo), and its two electrons travel through the complex to different destinations. One electron flows, first, to the Rieske 2Fe-2S cluster; then, to cytochrome *c*1; and, finally, to a molecule of oxidized cytochrome *c*, converting it into its reduced form. The reduced cytochrome *c* molecule is free to diffuse away from the enzyme to continue down the respiratory chain.

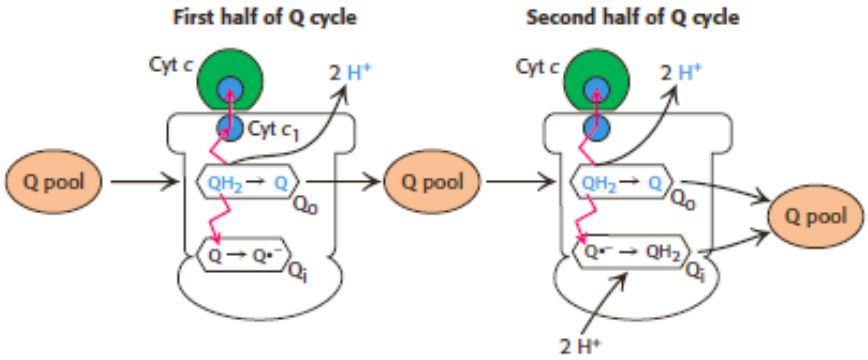


Figure 4: The Q cycle takes place in Complex III, which is represented in outline form. In the first half of the cycle, two electrons of a bound QH_2 are transferred, one to cytochrome c and the other to a bound Q in a second binding site to form the semiquinone radical anion $Q^{\cdot-}$. The newly formed Q dissociates and enters the Q pool. In the second half of the cycle, a second QH_2 also gives up its electrons to complex II, one to a second molecule of cytochrome c and the other to reduce $Q^{\cdot-}$ to QH_2. This second electron transfer results in the uptake of two protons from the matrix. The path of electron transfer is shown in red (**Taken from Stryer Biochemistry, 7th edition**).

A second molecule of QH2 binds to the Qo site of Q-cytochrome *c* oxidoreductase and reacts in the same way as the first. One of the electrons is transferred to cytochrome *c*. The second electron passes through the two heme groups of cytochrome *b* to partly reduced ubiquinone bound in the QI binding site. On the addition of the electron from the second QH2 molecule, this quinone radical anion takes up two protons from the matrix side to form QH2. *The removal of these two protons from the matrix contributes to the formation of the proton gradient.*

$$2QH_2 + Q + 2\ \text{Cyt}\ c_{(\text{oxidized})} + 2\ H^+_{\text{matrix}} \rightarrow 2Q + QH2 + 2\ \text{Cyt}\ c_{(\text{reduced})} + 4H^+_{\text{cytoplasm}}$$

The overall four protons are released on the cytoplasmic side, and two protons are removed from the mitochondrial matrix in Q cycle.

Complex IV (*cytochrome* c *oxidase)*: Cytochrome *c* oxidase catalyzes the transfer of electrons from the reduced form of cytochrome *c* to molecular oxygen as final acceptor. Four electrons are channeled to O_2 to completely reduce it to H_2O, and protons are pumped from the matrix to the cytoplasmic side of the inner mitochondrial membrane.
Four molecules of cytochrome *c* bind consecutively to the enzyme and transfer an electron to reduce one molecule of O_2 to H_2O. Thus, the overall process catalyzed by cytochrome *c* oxidase is summarized as:

$$4\ \text{Cyt}\ c_{(\text{reduced})} + 8\ H^+_{\text{matrix}} + O_2 \rightarrow 4\ \text{Cyt}\ c_{(\text{oxidized})} + 2\ H_2O + 4H^+_{\text{cytoplasm}}$$

Complex V (*ATP synthase*): The proton gradient powers generated during electron transfer chain are used for the synthesis of ATP. *The flow of protons from high concentration (inter-membrane space) to the low concentration of the matrix through F0-F1 complex in the presence of* ATP synthase leads to the assembly of ADP+Pi to from ATP. The F_0 portion is embedded in the inner membrane and provides a channel that allows protons flow back from the inter-

membrane space to the matrix. The energetically favorable return of protons to the matrix is coupled to ATP synthesis by the F_1 subunit, which catalyzes the synthesis of ATP from ADP and phosphate ions (P_i) (Figure 5). The oxidation of one NADH leads to the synthesis of three molecules of ATP, whereas the oxidation of one $FADH_2$, yields only two ATP molecules.

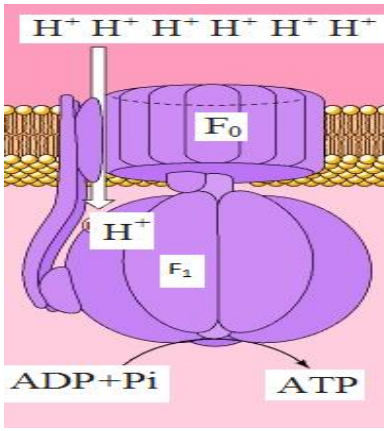


Figure 5: A multi protein Complex V with F0-F1 subunit.

Overall, 4 protons are required to generate one ATP, thus 1 NADH transport 10 H^+ and generate 2.5 ATP, similarly 1 $FADH_2$ transport 6 H^+ and generate 1.5 ATP.

Uncoupling agents: The molecules associated with the uncoupling of oxidative phosphorylation from ATP synthesis to generate heat. In certain conditions, electrons are transferred from succinate or NADH to O_2, but no ATP synthesis is coupled to this respiration. It may be through several means such as increased mitochondrial membrane permeability, reduced proton gradient, increased oxygen consumption, and inhibition of ATP synthase. In the presence of uncoupling agents, proton gradient generates heat instead of synthesis of ATP. The most Common uncoupling agents are Aspirin (in high concentrations), 2,4-Dinitrophenol, Thermogenin etc.

Respiratory chain inhibitors: Reduces the proton gradient generation during inhibition of electron chain complexes.

Complex I (NADH:ubiquinone oxidoreductase) inhibitor: Rotenone, Amytal, Piericidin
Complex III (ubiquinone:cytochrome c oxidoreductase) inhibitor: Antimycin A
Complex IV (cytochrome c oxidase) inhibitor: Cyanide, carbon monoxide, azides
Complex V (ATP synthase) inhibitor: Oligomycin

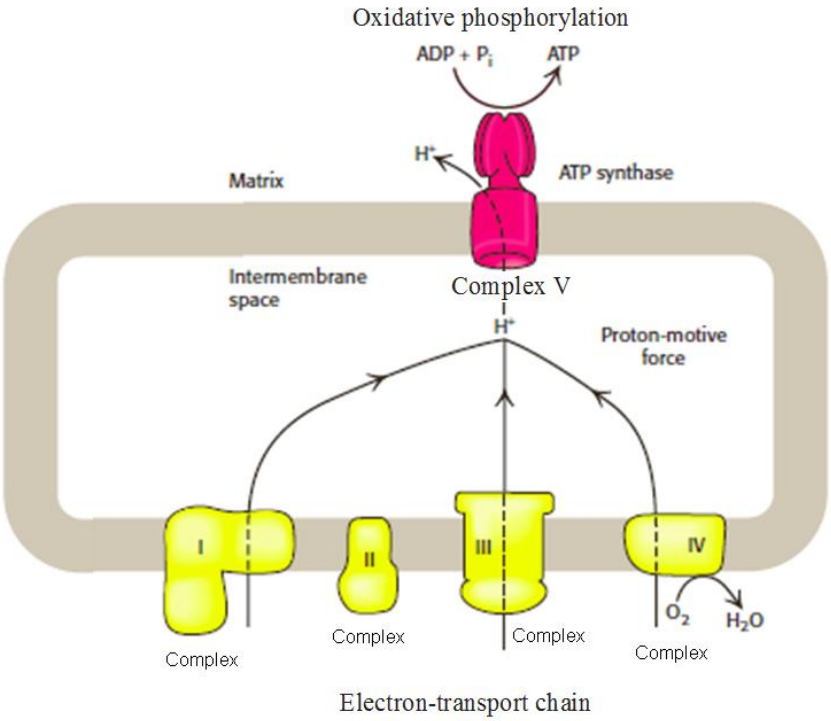


Figure 6: Overview of mitochondrial Electron-transport chain and Oxidative phosphorylation. In oxidative phosphorylation, the synthesis of ATP is coupled to the flow of electrons from NADH or FADH2 to O2 by a proton gradient across the inner mitochondrial membrane. Electron flow through three asymmetrically oriented transmembrane complexes results in the pumping of protons out of the mitochondrial matrix and the generation of a membrane potential. ATP is synthesized when protons flow back to the matrix through a channel in an ATP-synthesizing complex, called ATP synthase (also known as F_0F_1-ATPase).

References:

Lehninger Principles of Biochemistry, 6th edition.
Stryer Biochemistry, 7th edition.

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