2

CHAPTER

CELL ENVIRONMENT

• Water as universal solvent • pH and buffer solution • Measurement of pH • Biochemical buffer

All living cells contain inorganic ions, small organic molecules and other macromolecules. The intracellular and extracellular environment is different. Water is present in all environments and is a major chemical component of living organisms. It has unique physical properties like ability to solvate organic and inorganic molecules, dipolar structure and forming hydrogen bonds. Water is an excellent nucleophile and therefore is a product or reactant in many metabolic reactions. It can dissociate into hydroxide ions and protons.

Various buffers maintain the pH of a cell. pH is the negative log of $[H^+]$.

WATER AS UNIVERSAL SOLVENT

The structure of water molecule is bended tetrahedral. The bond angle between H—O—H is 104.5° and the average hydrogen-oxygen interatomic distance is 0.0965 nm (Fig. 1). This arrangement of electrons in the water molecule gives it electrical asymmetry. The highly negative oxygen atom tends to withdraw the single electrons from the hydrogen atoms, leaving the hydrogen nuclei bare. As a result, each of the two hydrogen atoms has a local partial positive charge (δ+) (Fig. 2). The oxygen atom, in turn, has a local partial negative charge (δ–) located in the zone of the unshared orbitals. Thus, although the water molecule has no net charge, it is an electric dipole.

Water molecules interact with each other because positively charged hydrogen atoms on one molecule are

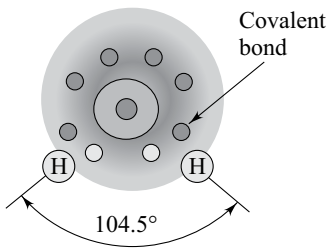

Fig. 1 The two non-bonding electron pairs remain closer to the oxygen atom. These exert a stronger repulsion against the two covalent bonding pairs, effectively pushing the two hydrogen atoms closer together. The result is a distorted tetrahedral arrangement in which the H—O—H angle is 104.5°.

glutamate, serine, arginine, are present on the surface in contact with water whereas amino acids with hydrophobic side chains are in the interior.

Hydrophobic interactions: When non-polar compounds self-associate in an aqueous environment, then it refers to hydrophobic interaction. Water molecules near to a hydrophobic group are restricted in the number of orientation that permit them to participate in the maximum number of energetically favourable hydrogen bonds. Non-polar molecules tend to form droplets with minimal exposed surface area, reducing to number of water molecules affected.

Van der Waals forces: This attraction arises between transient dipoles generated by the rapid movement of electrons on all neutral atoms. It is a weaker bond but there large number plays a big role.

Electrostatic Interactions: This is the interaction between charged groups. It is also termed salt bridges. They cover long distances and equal to the strength of hydrogen bonds.

Electrolytes and Non-electrolytes

Substances that dissociate in water into a cation (positively charged ion) and an anion (negatively charged ion) are classified as **electrolytes** because these ions make easy conductance of an electrical current through water. Sugars or alcohols are classified as **non-electrolytes** because they dissolve without difficulty in water but do not carry a charge or dissociate into charged species. Compounds and salts of organic acids dissociate completely in biological systems at low concentration like sodium lactate and sodium chloride.

Weak Electrolytes

Many acids, when dissolved in water, do not dissociate totally but rather establish equilibrium between undissociated and dissociated components. For example, is lactic acid dissociates partially into lactate anion and a proton. Such compounds on a molar basis have a lower capacity to carry an electrical charge in comparison to those that dissociate totally. Therefore a they are called **weak electrolytes**.

Partial dissociation of a weak electrolyte is represented by HA, the concentration of various species can be determined from the equilibrium equation

$$K'_{eq} = \frac{[H^+][A^-]}{[HA]}$$

K'_{eq} is a physical constant, A represents the dissociated anion and square brackets indicate concentration of each component in units such as moles per liter (mol L^{-1} or M) or millimoles per liter (mmol L^{-1} or mM). The extent of dissociation of an acid increases with increasing temperatures.

pH AND BUFFER SOLUTIONS

Most biological processes in the cell take place in the water-based environment. Water has many important chemical and physical properties. When a water molecule binds to a proton to form H_3O^+, it is acting as a base, whereas when it forms OH^-, it is acting as an acid

$$K'_{eq} = [H^+][OH^-]/[H_2O] = 1.8 \times 10^{-16}\ M$$

where concentrations are expressed in molarity.

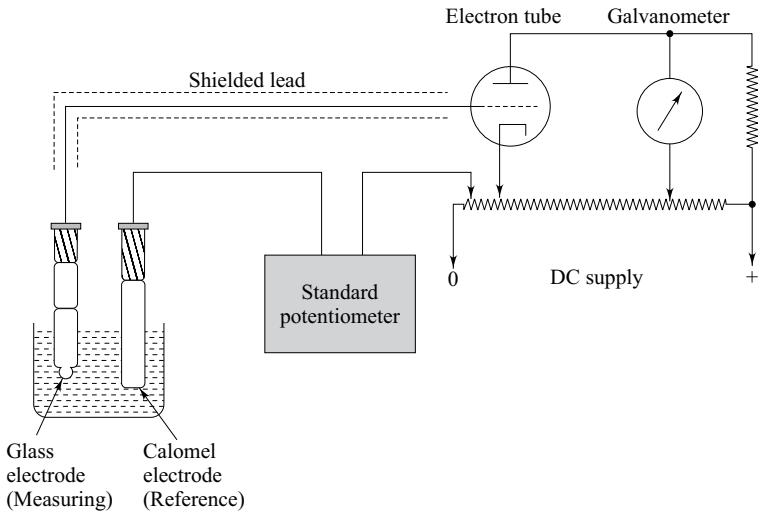

Fig. 3 A general scheme of pH-meters using a simple null-detector method.

$$HA \underset{K_2}{\overset{K_1}{\longleftrightarrow}} H^+ + A^-$$

K_1 is the rate constant for dissociation of the acid and K_2 is the rate constant for association of the conjugate base and H^+.

According to the law of mass action, K_a the acid dissociation constant or equilibrium constant. It is defined as

$$K_a = \frac{K_1}{K_2} = \frac{[H^+][A^-]}{[HA]}$$

and

$$[H^+] = \frac{K_a\,[HA]}{[A^-]}$$

Taking negative logarithms

$$-\log_{10}[H^+] = -\log_{10} K_a + -\log_{10}\frac{[HA]}{[A^-]}$$

or

$$pH = pK_a + \log_{10}[A^-]/[HA]$$

In general terms,

$$pH = pK_a + \log_{10}[\text{Conjugate base}]/[\text{Acid}]$$

This relationship is known as the **Henderson-Hasselbalch equation**. pK_a is the negative logarithm of the acid dissociation constant of a weak acid.

At a pH where in a solution equal amounts of positive and negative charges are present that value is referred to as the **isoelectric point (pI) or the isoelectric pH**. Like at pH 6.0 alanine has equal amounts of positive and negative charges.

Buffer Solutions

A buffer solution is one that resists pH change on the addition of the acid or the alkali. From the Henderson-Hasselbalch equation, the pH of a buffer solution depends on two factors: one is the pK_a value and the other is the ratio of salt to acid. This ratio is regarded to be the same as the amount of salt and acid mixed together over the pH range 4 – 10, where the concentration of hydrogen and hydroxyl ions is very low and can be ignored. Let us consider buffers consisting of a mixture of acetic acid and sodium acetate.

$$CH_3COOH \longleftrightarrow CH_3COO^- + H^+$$

$$CH_3COONa \longrightarrow CH_3COO^- + Na^+$$

Since acetic acid is only weakly dissociated, the concentration of acetic acid is almost the same as the amount put in the mixture; likewise the concentration of acetate ion can be taken to be the same as the concentration of sodium acetate placed in the mixture, since the salt is completely dissociated.

Buffer Value

Buffer solutions vary in the extent to which they resist pH changes. In order to compare different buffer solutions, Van Slyke introduced the term buffer value. When acid or alkali is added to a buffer solution, a titration curve is obtained. The slope of this curve is given by dB/d (pH), where dB is the increment of strong acid or strong base added in mol/litre and d(pH), the change in the pH increment. This slope is the buffer value B, which is always positive, since dB is negative when acid is added causing a negative change in pH.

Common Laboratory Buffers

Commonly used laboratory buffers are phosphoric acid, citric acid, carbonic acid, acetic acid, barbituric acid, Tris, HEPES etc. The actual buffer chosen for a particular experiment needs to be selected with care. The pH in many biological experiments often needs to be kept constant in the range of pH 6 – 8.

Phosphate: It is the most popular buffer but it readily forms complexes with heavy metals. Phosphate is inconvenient as it plays an active part in a number of biochemical reactions where it can act as an activator, an inhibitor or a metabolite. Its buffering capacity above pH 7.5 is poor.

Bicarbonate: It spontaneously liberates carbon dioxide and therefore, must be maintained in an atmosphere of carbon dioxide. Bicarbonate has a pK_a of 6.1, the buffering capacity around pH 7.4 is poor.

Tris: It can be used with heavy metals but it also acts as an inhibitor in some biochemical systems. Tris penetrates membranes, which can be a disadvantage. Tris has a poor buffering capacity below pH 7.5.

HEPES: It is a zwitterionic buffer. It contains both negative and positive groups, so do not readily penetrate membranes.

Buffer for life Processes

Most cells can only function within very narrow range of pH and require buffer systems to resist the changes in pH. The three main buffer systems in living material are protein bicarbonate and phosphate. The most important buffering system in mammalian plasma is bicarbonate:

$$H_2CO_3 \longleftrightarrow HCO_3^- + H^+$$

From the Henderson-Hasselbalch equation,

$$pH = pK_a + \log_{10} \frac{[HCO_3^-]}{[H_2CO_3]}$$

The plasma pH depends on the ratio of bicarbonate to carbonic acid. Any tendency for the pH to change is buffered and can be corrected by adjusting this ratio. Large quantities of acids formed during normal metabolism react with bicarbonate to form the weakly dissociated carbonic acid, so that free hydrogen ions are effectively cleaned up. At the same time, carbonic acid is removed at the lungs as carbon dioxide, thus maintaining the pH of the plasma. The kidneys also play an important role in the maintenance of acid-base balance by adjusting the excretion of acid or base in the urine, so that the pH of urine can normally vary from 4.8 to 7.5 in man.

Proteins and amino acids are good buffers. The buffering capacity of hemoglobin depends on its oxygenation and deoxygenation. The average cytoplasmic pH is similar to that of animals, but the cell sap of land plants is acidic and lies within pH 5.2 – 6.5. Bacteria grow well at pH 6 or 9 but their internal pH is around 7 i.e. neutral.

Summary

1. The structure of water molecule is bended tetrahedral. The bond angle between H—O—H is 104.5° and the average hydrogen oxygen interatomic distance is 0.0965 nm. The arrangement of electrons in the water molecule gives it electrical asymmetry.
2. Water molecules interact with each other because positively charged hydrogen atoms on one molecule are attracted to a negatively charged oxygen atom on another, with formation of a weak bond between two molecules. This bond is called hydrogen bond.
3. Due to the polar nature and ability to form hydrogen bonds, water has unique solvent properties. Polar molecules easily disperse in water. Salts dissolve in water because electrostatic forces in the crystal can be overcomed by attraction of individual of charges to the dipole of water.
4. Substances that dissociate in water into a cation (positively charged ion) and an anion (negatively charged ion) are classified as electrolytes because these ions make easy conductance of an electrical current through water.
5. Many acids, when dissolved in water do not dissociate totally but rather establish equilibrium between undissociated and dissociated components. Such compounds on a molar basis have a lower capacity to carry an electrical charge in comparison to those that dissociate totally. They are called **weak electrolytes**.
6. When a water molecule binds to a proton to form H_3O^+, it is acting as a base, whereas when it forms OH^-, it is acting as an acid.
7. The majority of intracellular processes occur at a pH maintained near neutrality. To maintain pH in biological systems, efficient buffering systems are present. The major buffering systems found in cellular fluids involve phosphate, bicarbonate, amino acids and proteins.

References

Dick, D.A.T. Cell Water. Washington, DC: Butterworths, 1966.

Eisenberg, D. and Kauzmann, W. The Structures and Properties of Water. Fairlawn, NJ: Oxford University Press, 1969.

Morris, J.G. A Biologist's Physical Chemistry. Reading, MA: Addison-Wesley, 1968.

Wiggins, P.M. Role of water in some biological processes. *Microbiol. Rev*, 54: 432, 1990.

exergonic processes to the endergonic processes. **ATP** is a **nucleoside triphosphate** containing adenine, ribose and three phosphate groups. Three phosphoryl groups are esterified to the 5′ position of the ribose moiety in phosphoanhydride bonds. The two terminal phosphoryl groups are designated as energy-rich or **high-energy bonds**. ATP was regarded a means of transferring phosphate radicals in the process of phosphorylation (Fig. 1). Examples are AMP, glucose-1-phosphate, glycerol-3-phosphate. There are two other energy groups. One group is low energy phosphates whose $\Delta G^{0\prime}$ values are smaller than that of ATP, while the other group is high-energy phosphates where $\Delta G^{\circ\prime}$ value is higher than that of ATP. Example are carbamoyl phosphate, creatine phosphate, etc. Other biologically important high energy compound is thiol esters involving coenzyme-A, acyl carrier protein, uridine diphosphate, glucose, etc. ATP contains two high energy phosphate groups; ADP contains one whereas AMP contains low energy phosphate, which is a normal ester link. ADP can accept high energy phosphate to form ATP. ATP is generated from ADP by coupled or linked phosphorylation reactions at the expense of energy yielded by degradation of fuel molecules. The ATP so generated donates its terminal phosphate group to specific acceptor molecules, to energize them for carrying out various energy-requiring functions in the cell e.g., the biosynthesis of cell macromolecules, the active transport of inorganic ions and cell nutrients across membranes against concentration gradients and the contraction of muscles. As the energy is provided to these energy requiring processes, the ATP undergoes cleavage to ADP and inorganic phosphate (Fig. 1). The ADP is then rephosphorylated at the expense of energy yielding oxidation of fuels to yield ATP, thus completing the cellular energy cycle. The terminal phosphate group of ATP was thus visualized as undergoing constant turnover, being constantly transferred to acceptor molecules and continuously replaced by phosphate groups that become energized during the catabolic degradation of cell fuels.

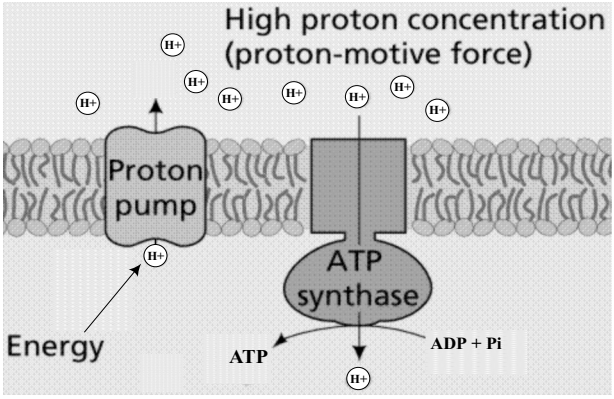

Fig. 1 Generalized view of an electron transport system.

Besides ATP, there are other high energy phosphates like **creatine phosphate** and **arginine phosphate** (Fig. 2).

Consider the following reaction: L-arginine + ATP ⟶ N-phospho-L-arginine + ADP

In muscles, creatine phosphate shuttle transports high-energy phosphate from mitochondria to the sarcolemma.Creatine is charged with energy by the enzyme creatine kinase (CK), which transfers the high-energy (~) phosphate bond of ATP to make creatine~phosphate.

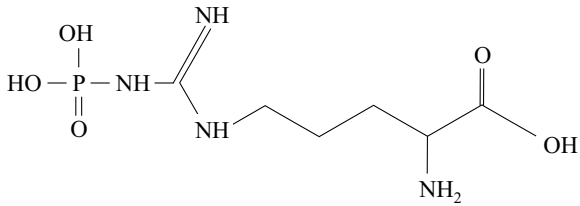

Fig. 2 Structure of arginine phosphate.

$$\text{ATP} + \text{Creatine} \rightleftharpoons \text{Creatine} \sim \text{phosphate} + \text{ADP} + H^+$$

Creatine and creatine~phosphate exist in a reversible equilibrium in skeletal muscle. Creatine~phosphate, by reversal of the above reaction, plays an important role in recharging ADP to ATP during maximal anaerobic burst-type exercise. The reaction contributes significantly to ATP resynthesis for about 10 to 20 seconds of maximal exercise.

This is followed by a proportionate increase in other pathways of ATP resynthesis, such as anaerobic glycolysis or aerobic oxidation of fat and carbohydrate. So creatine~phosphate functions as a 'battery' that stores the excess ATP energy. In skeletal muscle, approximately one-fourth exists as free creatine and three-fourths as creatine phosphate.

Creatine is synthesized primarily by the liver, kidneys and pancreas at a rate of 1 to 2 g/day. An additional 1 to 2 g/day is obtained in the diet, mainly from fish and meat. Excretion of creatine by the kidneys at a rate of 1 to 2 g/day is via irreversible conversion to creatinine in skeletal muscle.

Creatinine is just a waste product formed by the slow, spontaneous degradation of creatine phosphate (Fig. 3). However, the 'level' (concentration) of creatinine in the serum is diagnostically useful for assessing kidney function. In most types of kidney malfunction, even if only transient, the serum creatinine level rises.

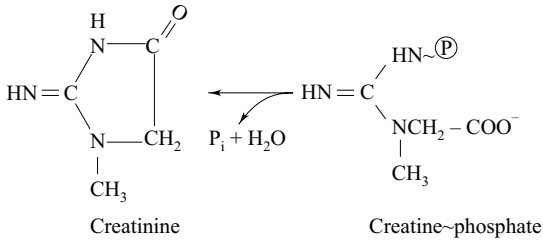

Fig. 3 Conversion of creatine~phosphate to creatine.

There are various reasons why certain compounds or bonding arrangements are energy rich. First, products of hydrolysis of an energy-rich bond may exist in more resonance forms than the precursor molecule. The more possible resonance forms in which a molecule can exist stabilize that molecule. Fewer resonance forms can be written for ATP or pyrophosphate (PPi) than for phosphate (Pi). Second, many high-energy bonding arrangements have groups of similar electrostatic charges located in close

proximity to each other in such compounds. Because like charges repel one another, hydrolysis of energy-rich bonds alleviates this situation and again lends stability to products of hydrolysis. Third, hydrolysis of certain energy-rich bonds results in formation of an unstable compound, which may isomerize spontaneously to form stable compounds.

ATP, ADP and AMP

ATP, ADP and AMP have been found in all forms of life examined, in both animal and plants. The sum of their concentrations in the aqueous phase of living cells remains nearly constant. In actively metabolizing cells, the concentration of ATP is greater. These three nucleotides are present only in soluble cytoplasm but also such organells as mitochondria and nuclei. At pH 7.0, both ATP and ADP are highly charged anions. ATP has four ionizable protons in its condensed phosphate groups; ADP has three. In intact cells, ATP and ADP are largely present as the 1:1 $MgATP^{2-}$ and $MgADP^{-}$ complexes, because of the high affinity of the pyrophosphate groups for divalent cations and the relatively high concentrations of Mg^{2+} in intracellular fluid. The affinity of ATP for Mg^{2+} is about 10 times that of ADP.

Nearly all metabolic reactions in the cell proceed in consecutive sequences. In the consecutive reactions responsible for energy transfers via ATP, chemical energy is transferred from a high energy phosphate donor group to ADP and is conserved in the form of ATP as a reaction product. In the succeeding reaction, in which ATP is a substrate, its terminal phosphate group is transferred to an acceptor molecule, causing the latter to be changed to a compound with higher energy content. ATP is thus a common intermediate in enzymatic reactions involving the transfer of phosphate groups and is thus a vehicle for the transfer of chemical energy. Many chemical groups and acetyl groups are enzymatically transferred by means of consecutive reactions having common intermediates.

ATP does not function primarily as a reservoir of chemical energy. It acts as a transmitter or carrier of energy (Fig. 4). The amount of ATP in the cell at any given time is sufficient for only a short period. However, some cells do have phosphate compounds that function as reservoirs of energy e.g. phosphocreatine, which is formed by direct enzymatic transfer of a phosphate group from ATP to creatine whenever ATP is at a high concentration. The only known pathway for the dephosphorylation of phosphocreatine is the reversal of the reaction by which it is formed. The phosphocreatine reservoir

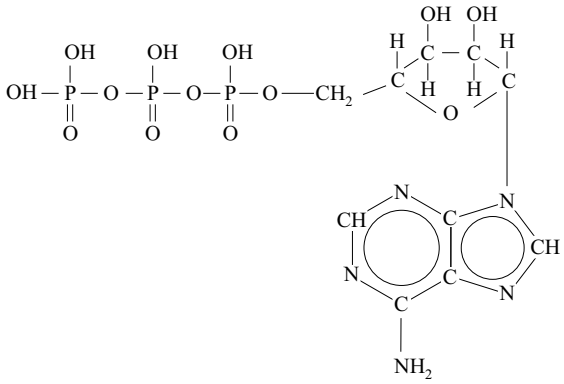

Fig. 4 Structure of ATP.

is, therefore, filled with phosphate groups whenever ATP is present in high concentrations. Whenever the ATP concentration falls, thus raising the concentration of ADP, phosphate groups are transferred back to ADP from phosphocreatine. The phosphocreatine system is especially important in skeletal muscle, where it can provide the chemical energy required for several minutes of contractions. It is also found in smooth muscle and nerve cells, but only in very small amount in liver, kidney and other mammalian tissues and not at all in bacteria. Phosphoarginine functions in a similar way in muscles of some invertebrates e.g. the crab and lobster. Some microorganisms store high-energy phosphate groups in the form of insoluble granules containing polymetaphosphate, a linear polymer of indefinite size. Phosphate groups can be released from polymetaphosphate by specific enzymes.

Other nucleosides and 2-deoxyribonucleosides also participate in cellular energy transfers. The 5′-di- and triphosphates of various ribonucleosides not only serve as energized precursors in RNA synthesis but also channel high-energy phosphate groups to other specific biosynthetic reactions. They function through the action of the enzyme nucleoside diphosphate kinase, which is found in mitochondria; and in the soluble cytoplasm of cells, it catalyzes reversible reactions. This enzyme not only transfers phosphate between ATP and any NDP but also between any NTP and any NDP.

$$\begin{aligned}
&\text{ATP} + \text{UDP} \longleftrightarrow \text{ADP} + \text{UTP}\\
&\text{ATP} + \text{CDP} \longleftrightarrow \text{ADP} + \text{CTP}\\
&\text{ATP} + \text{GDP} \longleftrightarrow \text{ADP} + \text{GTP}\\
&\text{GTP} + \text{UDP} \longleftrightarrow \text{GDP} + \text{UTP}\\
&\text{ATP} + \text{dCDP} \longleftrightarrow \text{ADP} + \text{dCTP}\\
&\text{GTP} + \text{dADP} \longleftrightarrow \text{GDP} + \text{dATP}
\end{aligned}$$

In many ATP-utilizing reactions in the cell, the two terminal phosphate groups of ATP are enzymatically removed in one piece as pyrophosphate (PPi), leaving AMP as the other product. The two important enzymes that allow AMP and pyrophosphate to return to the mainstream of phosphate-group transfer via the ATP-ADP cycle are **inorganic pyrophosphatase** and **adenylate kinase**. The first one catalyzes the hydrolysis of inorganic pyrophosphate (PPi) to form two molecules of inorganic orthophosphate (Pi)

$$\text{PPi} + \text{H}_2\text{O} \longleftrightarrow 2\text{Pi}$$

The orthophosphate so formed can then be utilized in the regeneration of ATP from ADP. Adenylate kinase catalyzes the rephosphorylation of AMP to ADP in the following reaction:

$$\text{ATP} + \text{AMP} \longleftrightarrow \text{ADP} + \text{ADP}$$

Control of ATP production

The rate of ATP production in cells is adjusted to the rate of ATP utilization in a dynamic state. If a sudden work loss is placed on a cell and a higher amount of ATP is needed, then first the concentration of ATP in the cell decreases and ADP will rise. This change is a signal that causes acceleration of the ATP-generating reactions of glycolysis and respiration, which then occurs at higher rates to fulfill ATP requirement. When workload is suddenly reduced, the ATP concentration instantly increases and ADP falls, thus signaling the ATP-yielding reactions to slow down. It indicates that terminal phosphate group of ATP must undergo very fast turnover in the cell.

ELECTROCHEMICAL POTENTIAL

Electrochemical potential is the chemical and electrical potential for an electrolyte (A).

$$\mu = \mu^\circ + RTIn\,[A] + nF\Delta E$$

In electrochemical cell, solutions are held at different concentrations in adjacent compartments by creating different electrical potential. This arrangement maintains osmotic balance between cells to facilitate water absorption.

REDUCTION-OXIDATION (REDOX) REACTIONS

Many biological molecules are known to exist in either a reduced form or an oxidized form as a result of either gaining or losing electrons to molecules. Such substances are called as redox couples.

$$BH + A \longleftrightarrow AH + B$$

Many catabolic processes are oxidative in nature because the carbons in the substrates, carbohydrates, fats and proteins are in a partially or highly reduced state. Reducing equivalents are released from substrates in the form of hydride ions (a proton containing two electrons $H:^-$), which are transferred from the substrates to nicotinamide adenine dinucleotide (NAD^+) (Fig. 5) by enzyme called **dehydrogenase** with the formation of NADH.

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

The NADH is then transported to mitochondria where reducing equivalents are transferred in a series of reactions by the electron transport chain to O_2 as the ultimate electron acceptor. The oxidative reactions in mitochondria are exergonic, producing energy used for synthesis of ATP in a process called

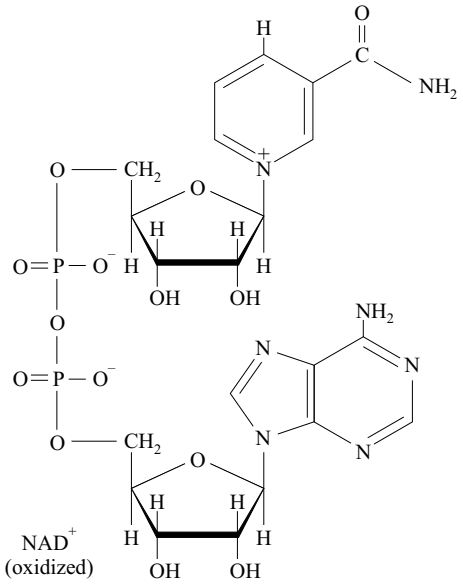

Fig. 5 Structure of NAD^+.