Indira Gandhi National
Open University
School of Sciences

BBCCT-101
MOLECULES OF LIFE

Block

4

VITAMINS AND NUCLEIC ACIDS

BLOCK 4 : VITAMINS AND NUCLEIC ACIDS

In block 3 you have studied the structure and functions of lipids and their specialised properties like the immediate energy source, energy storage, signaling molecule and as plant steroids etc. Also, you had an account of membrane lipids and their significance in maintaining the integrity of a cell. The present block consists of 3 units (unit 12 to unit 14).

In unit 12 you will study about structure and functions of water soluble and fat soluble vitamins. The unit also describes significance of vitamins as antioxidants and coenzymes.

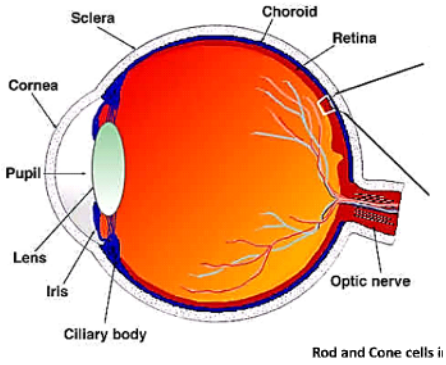
Unit 13 deals with the different aspects of nucleic acids like their constituents, arrangement and properties. In first part of this unit you will study how DNA is acting as a genetic material. The second part of this unit describes about structure, various types and functions of DNA & RNA.

In Unit 14 you will study how various chemical and physical factors influence the structure and functions of DNA. The unit also deals with other significant biological functions of nucleotides such as energy source, coenzymes and secondary messengers.

Objectives

After studying this block you will be able to:

- list various types vitamins soluble in water and fat;
- describe the structure and biological significance of vitamins;
- describe the basic unit of nucleic acids and its structural hierarchy;
- explain the difference between DNA and RNA;
- illustrate how DNA is acting as genetic material;
- explain the biological significance of nucleic acids; and
- recognise and explain the effect of temperature, acid and alkali on DNA structure.



UNIT 12

VITAMINS

Structure

12.1 INTRODUCTION

So far, you have learned about proteins, carbohydrates and lipids which are the major constituents of living cells. In unit 4, you studied about proteins. As you know all enzymes are proteins that function as catalysts in regulating the metabolic pathways and other life processes. Enzymes employ coenzymes or metal ions (cofactor) to assist them in catalysis. Coenzymes are non-protein organic molecules that are mostly precursors of vitamins. Therefore, this unit deals with vitamins.

Vitamins are organic molecules that are essential for proper functioning and growth of the human body. As many of these molecules perform the role of coenzymes and antioxidants in the human body. Vitamins are considered as micronutrients that require in small amounts for maintaining health. Although, human body cannot synthesize vitamins and we must take them from the dietary sources. Generally, whole grains, green vegetables, fruits and animal products are rich sources of vitamins.

However, an inadequate amount of vitamins, may result in vitamin-deficiency (hypovitaminosis) diseases such as night blindness, scurvy and rickets etc. In addition, abnormal accumulation of vitamins in the body leads to toxicity and this condition is known as hypervitaminosis.

Therefore, in this unit, you would learn about classification, structure, active forms and biochemical function of water soluble vitamins and fat soluble vitamins. This unit will also discuss the deficiency diseases, symptoms and recommended daily allowance (RDA) of vitamins. Hypervitaminosis and the role of vitamins as coenzymes and antioxidants will be discussed in this unit.

Objectives

After studying this unit, you should be able to:

- classify vitamins into water soluble vitamins and fat soluble vitamins;
- describe structure, active forms and functions of the water soluble vitamin and fat-soluble vitamins;
- explain deficiency diseases, symptoms, dietary requirements and dietary sources of vitamins;
- illustrate hypervitaminosis; and
- enlist role of vitamins as coenzymes and antioxidants.

12.2 CLASSIFICATION OF VITAMINS

A Polish biochemist Casimir Funk coined the term **'vitamin'** in 1912 who designated it as essential factor for life (Vita means "important" and amine means "possessing amine group"). However, all vitamins are not amines. The structure of all vitamins contain hetrocyclic ring of hydrogen, nitrogen, carbon, oxygen. Vitamins are classified into two broad categories on the basis of their solubility (Fig. 12.1). Vitamins generally are designated by alphabets A, B, C, D, E and K in which alphabet B is referred to the B-complex vitamins by B_1, B_2, B_3, B_4, B_6, B_{12}.

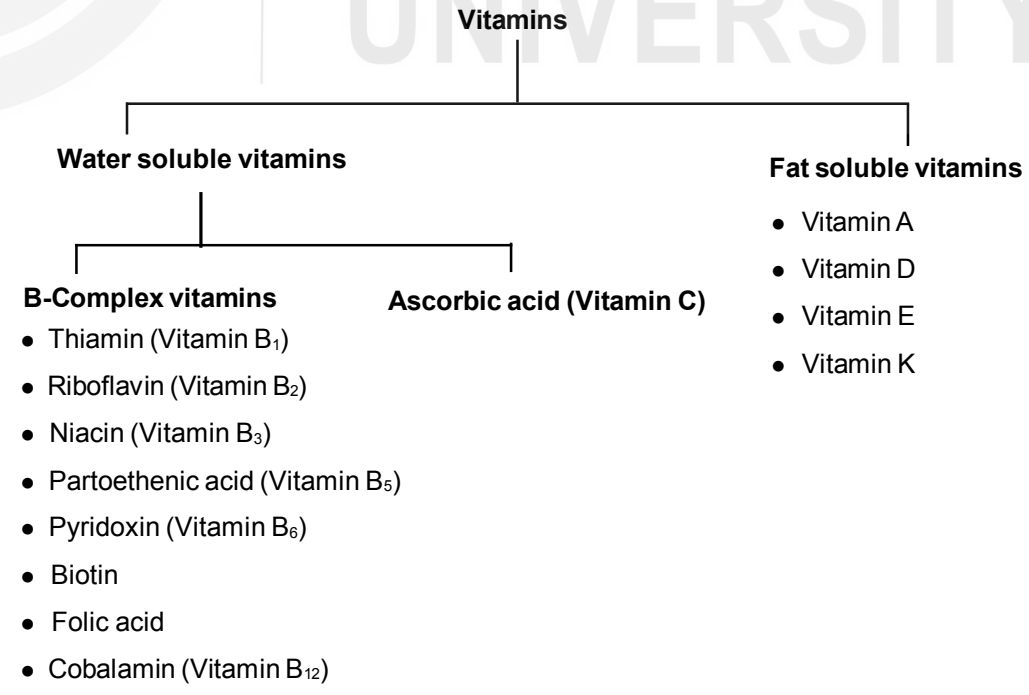


Fig. 12.1 : Classification of Vitamins

12.3 STRUCTURE, ACTIVE FORMS AND FUNCTIONS OF WATER-SOLUBLE VITAMINS

Let us first study about the water soluble vitamins.

As the name implies, water-soluble vitamins are soluble in water and excreted out in urine, thus preventing the toxicity due to overdose. This group composed of B-complex vitamins and vitamin C.

12.3.1 B-complex Vitamins

B-complex vitamins are so called because they are group of eight vitamins like thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), pantothenic acid (vitamin B_5), pyridoxine (B_6), biotin, folic acid and cobalamin (vitamin B_{12}). They serve as a coenzymes in many enzymatic reactions and help to release energy by metabolizing food stuff in the human body.

1. Thiamin (Vitamin B_1)

Thiamin is the first vitamin of B-complex group which was discovered in people affected with beriberi disease. It can be synthesized by bacteria, fungi and plants. It is a colorless compound which is ***found in cereals, groundnuts, pistachios, walnuts, mustard seeds, soybean and capsicum.***

The structure of thiamin consists of pyrimidine and a thiazole ring that are linked by methylene group (CH_2,) as shown in the (Fig. 12.2). Thiamin named as thiol (sulfur group, SH-) containing vitamin. Thiamin is converted into biologically active form, thiamin pyrophosphoate in the human body.

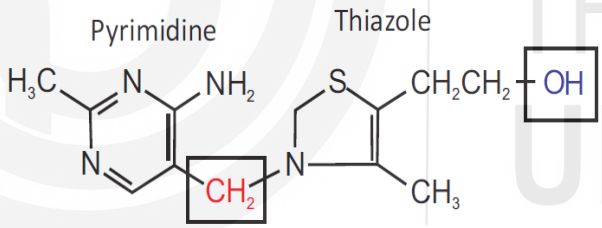


Fig. 12.2: Strucutre of Thiamin

Active form: Thiamin pyrophosphate (TPP) is active form of thiamin. The OH- group of thiazole ring is phosphorylated (two phosphate groups) (Fig. 12.3).

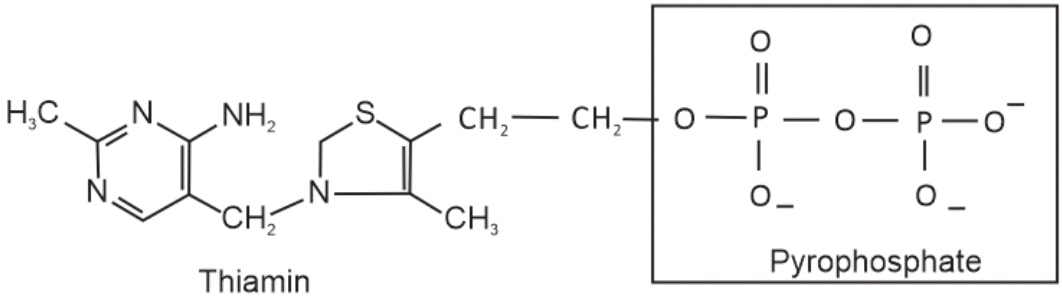


Fig. 12.3: Strucutre of Thiamin pyrophosphate

Biochemical functions: TPP is involved in the dehydrogenation and decarboxylation reactions of carbohydrate and amino acid metabolis.

It participates in the oxidative decarboxylation of α-ketoacids like pyruvate, α- ketogltarate and ketoacids derived from branched chain amino acids, leucine, isoleucine and valine. It serves as a coenzyme in many enzymes like transketolase, pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase and branched-chain amino acid dehydrogenase complex.

Deficiency diseases and symptoms: Thiamin deficiency causes beriberi disease which occurs in three forms:

Recommended Dietary Allowance (RDA): Is the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group (ICMR, 2009)

1) Dry beriberi: It is associated with neurological manifestations.

2) Wet beriberi: It effects on mental confusion, muscle atrophy and edema.

3) Infantile beriberi: Infant's cardiovascular system is generally affected.

The common symptoms are muscular weekness, loss of sensation, vomiting, confusion. It mosly affects neurological and cardiovascular system. Thiamine containing balanced diet or supplements require for the treatment of beriberi disease.

Recommended Dietary Allowance (RDA): 1.4 mg/day for male and 1.1 mg/ day for female.

2) Riboflavin (Vitamin B_2)

Riboflavin is the second vitamin in the the B-complex group known as vitamin B_2. ***Yeast, milk, liver, eggs and leafy vegetables are good sources of this vitamin.***

It consists of ribose (a pentose sugar) and a flavin molecule (an isoalloxazine ring) (Fig. 12.4). It was isolated by Kuhn, Gyorgy and Wagner in 1933. Riboflavin has intense yellow color and is widely used as a food additive.

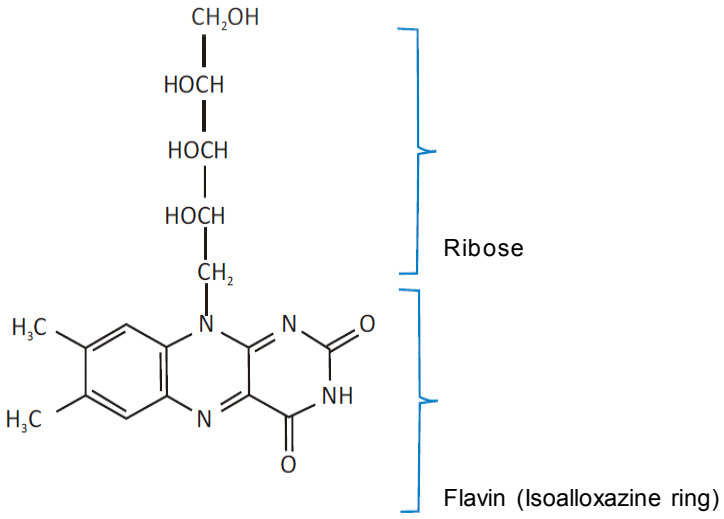


Fig. 12.4: Strucutre of Riboflavin

Active form Flavin adenine dinucleotide (FAD) is a riboflavin derivative (Fig. 12.5).

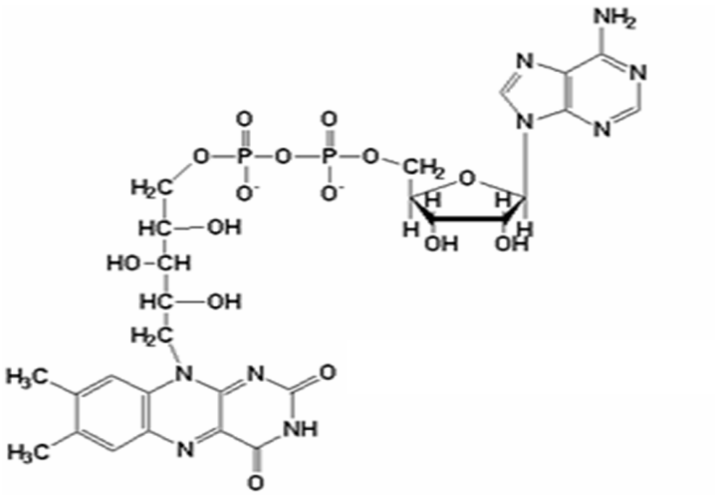


Fig. 12.5: Structure of Flavin Adenine Dinucleotide

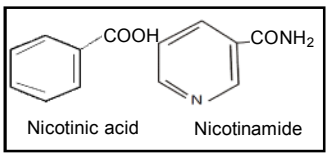
Biochemical functions : Riboflavin involves in the metabolism of carbohydrates, lipids and amino acids. FAD is a coenzyme which play a role in the transferring of electrons in many metabolic reactions. It acts as electron carrier in oxido-reduction reactions of oxidative phosphorylation, fatty acid and amino acid catabolism and citric acid cycle.FAD-dependent enzymes are pyruvate dehydrogenase complex, α- ketoglutarate dehydrogenase complex, succinate dehydrogenase, acyl CoA dehydrogenase, and glycine oxidase.

Deficiency diseases and symptoms: Lack of riboflavin in the body leads to sympotms like fatigue, slow growth; cheilosis, glossitis dry and scaly skin (seborrheic dermatitis), abnormal vision and corneal inflammation.

RDA: 1.7 mg/day for male and 1.3 mg/day for female.

3) Niacin (Vitamin B_3)

The term 'Niacin' is referred collectively to nicotinamide and nicotinic acid. It is known as vitamin B_3. Niacin is the non-toxic derivative of the toxic tobacco alkaloid, nicotine. The amide derivative ($CO\text{-}NH_2$) of nicotinic acid is called nicotinamide. Niacin was discovered as nutrient while studying *pellagra* disease. It is synthesized from tryptophan (an amino acid). ***Peanuts, legumes, meat, eggs and milk are rich sources of niacin.***



Active forms: Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are active forms of niacin. Fig. 12.6 shows the structure of NAD and NADP in which nicotinamide group is attached to adenine dinucleotide. *NADP differs from NAD in the presence of additional phosphate group on 2' position of the Ribose ring that carries adenine moiety*.

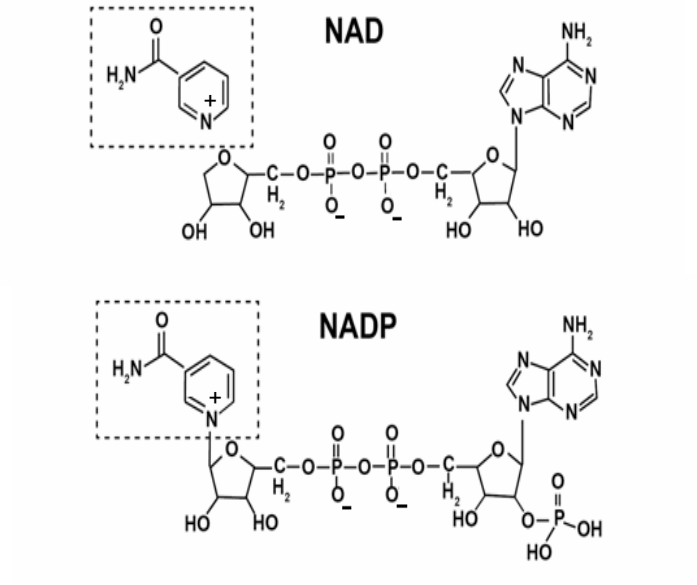


Fig. 12.6: Structures of NAD and NADP

Biochemical functions : NAD and NADP as a coenzyme primarily acts as hydrogen acceptor and electron carrier in wide variety of oxidation and reduction reactions of metabolism. At least 200 reactions of cellular metabolism are known where role of these coenzymes is well defined. Oxidized NAD is an electron acceptor and its reduced form NADPH is electron donor in many biochemical reactions. FAD, NAD-dependent enzymes are dehydrogenase enzymes such as lactate dehydrogenase, pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase etc. Besides, NAD plays an important role in DNA repair and mobilization of calcium ions in the body.

Deficiency disease and symptoms: Pellagra disease results from the deficiency of niacin.

The early symptoms are vague, weakness, anorexia, indigestion and dizziness. Photosensitive dermatitis, skin infections and digestive problems which are associated with niacin or tryptophan deficiency.

RDA: 18 mg/day for male and 14 mg/day for female.

SAQ 1

a) Choose the correct option.

i) Vitamins are essential nutrient compounds. [True/False]

ii) TPP participates in the carboxylation reactions. [True/False]

iii) Niacin vitamin contains Isoalloxazine ring. [True/False]

iv) Niacin deficiency causes pellagra disease [True/False]

v) Riboflavin has a thiazol ring. [True/False]

vi) Thiamin is a precursor of FAD. [True/False]

vii) Thiamine deficiency causes beriberi disease. [True/False]

b) Fill in the blanks with correct answer(s).

i) The first vitamin in the group of Vitamin B complex is …….

ii) Thiamin consists of …….

iii) ……is the coenzyme form of thiamin.

iv) The deficiency disease of thiamin is .....

v) The structure of riboflavin is composed of……. and …….

vi) ……and ……. are active forms of riboflavin.

vii) Niacin synthesized from …….

viii) The coenzyme of niacin are ……. and …….

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4)P antothenic acid (Vitamin B_5)

Pantothenic acid is also known as vitamin B_5 which was identified by Roger J. Williams in 1931. It is the peptide of β-alanine and pentoic acid (Fig.12.7). Pantothenic acid has a central role in energy-yielding metabolism.Human body cannot synthesize pantothenic acid, we must obtain it from ***dietary sources like whole grains, broccoli, avocados, cereals and mushrooms.***

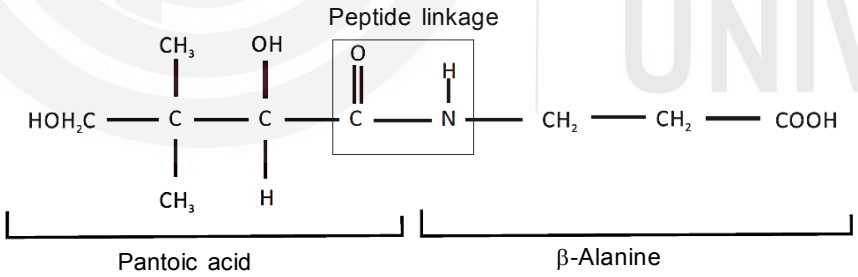


Fig. 12.7: Structure of Pantothenic acid

Biochemical functions : Pantothenic acid involes in the formation of coenzyme A (A for acyl). Coenzyme A (CoA) consists of cysteine, pantothenic acid and phospho-adenosine diphosphate (Fig. 12.8). Pantothenic acid is one of the functional moiety of CoA. It helps to carry acyl groups for many biochemical reactions such as fatty acid synthesis, metabolism of carbohydrate and amino acids. CoA is a component of acyl carrier protein (a component of fatty acid synthase) involves in fatty acid synthesis. CoA requires for the formation of acetyl-CoA and succinyl-CoA in the oxidative decarboxylation of glucose and in the Crebs cycle respectively. It also required for the acetylation of choline to form the neurotransmitter acetylcholine in the brain.

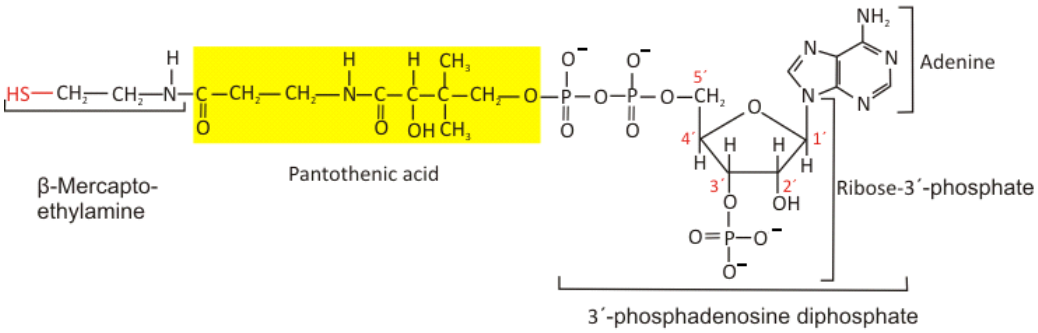


Fig. 12.8: Strucutre of Coenzyme A

Deficiency disease and symptoms: Deficiency of pantothenic acid is often related to low energy-level symptoms including fatigue and weakness.

RDA: The dietary intake of pantothenic acid is 5 mg/day for male and female.

5. Pyridoxine (Vitamin B_6)

This vitamin exists in three different forms -pyridoxine, pyridoxamine and pyridoxal. ***It is found in eggs, liver, yeast, cereals, legumes and milk.***

The structure of vitamin B_6 possess single pyridine ring having hydroxyl goup in pyridoxine, an amino group in the pyridoxamine and aldehyde group in the pyridoxal as shown in Fig. 12.9 .

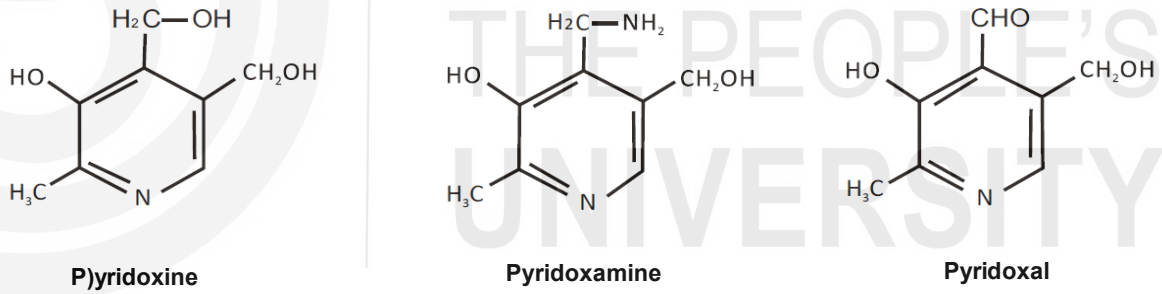


Fig. 12.9: Strcutre of vitamin B_6

Active form: The biologically active form of vitamin B6 is Pyridoxal-5-phosphate (PLP) (Fig.12.10)

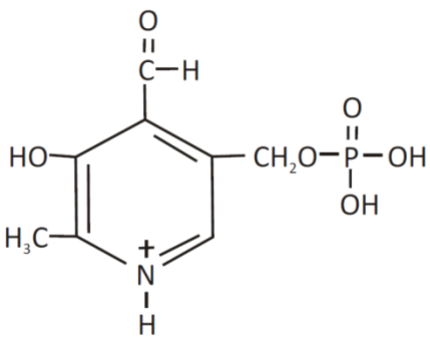


Fig. 12.10: Structure of Pyridoxal-5-phosphate

Biochemical functions: Vitamin B_6 plays an important role in the metabolism of amino acids, fatty acids, glycogen and also as a steroid hormone. Pyridoxal-5-phosphate serves as a coenzyme in many enzymatic reactions of amino acid metabolism e.g. transamination, deamination, decarboxylation, racemization and aldol reactions. Pyridoxal-5-phosphate required for transferring of amino group from one molecule to another in the transamination reaction of amino acid metabolism. Pyridoxal-5-phosphate dependent enzymes are aspartate transaminase, alanine transaminase, glutamic decarboxylase and gamma-aminobutyric acid transaminase involved in many metabolic reactions.

Deficiency diseases and symptoms: The clinical signs and symptoms of pyridoxine deficiency include anemia, dermatitis, peripheral neuropathy, gastrointestinal disorders, nausea and vomiting.

RDA: The diatary intake of vitamin B_6 is about 2.0 mg/day for male and female and 2.5 mg/day for pregnant women.

6) Biotin

Biotin is a water soluble vitamin. It was isolated from the egg by Dutch biochemist Fritz Kogl in 1935 later named it as biotin. It is necessary for cell growth, metabolism of lipids and amino acids. **Yeast, nuts, tomato, animal meat, and egg yolk are some of the well-known sources of biotin.**

The structure of biotin consists of imidazole and thiophene ring along with a fatty acid side chain (Fig. 12.11). It is widely found in many food sources in the form of biocytin. It is linked through a peptide bond with the amino acid lysine.

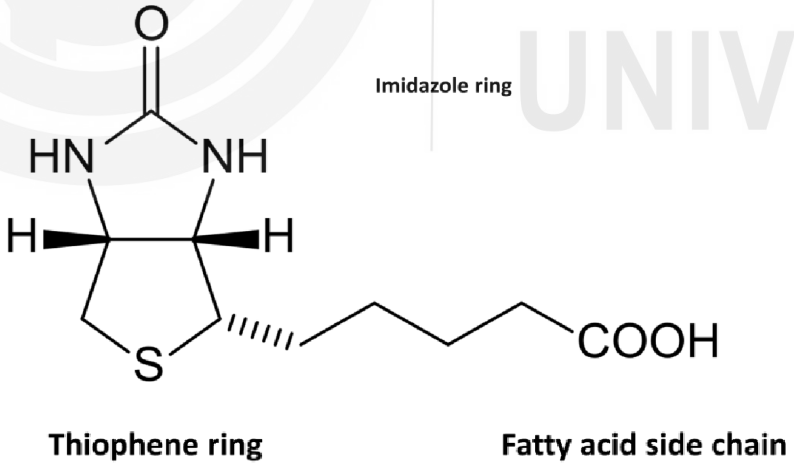


Fig. 12.11: Structure of Biotin

Active form: Biocytin

Biochemical functions: Biotin serves as a coenzyme for many carboxylase enzymes such as pyruvate carboxylase, acetyl-CoA carboxylase and propionyl-CoA carboxylase. It involves in transferring carbondioxide (CO_2) in carboxylation reactions. It is covalently bound to the enzyme via an amide linkage with a lysine residue to form biotincytin (Fig 12.12).

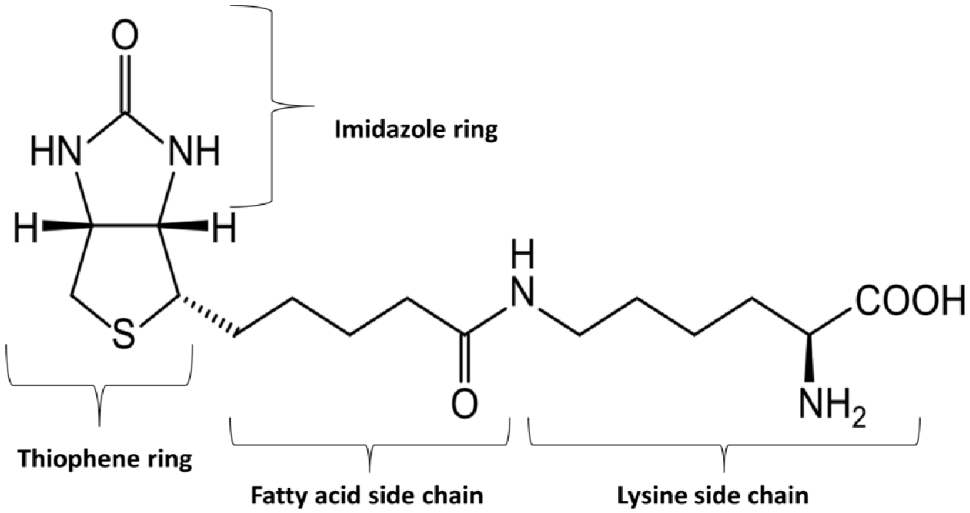


Fig. 12.12: Strcutre of Biocytin

Deficiency diseases and symptoms: The general deficiency symptoms of biotin include loss of appetite and growth; dermatitis, hair loss and thickening of bones.

RDA: About 30 µg/day is recommended for male and female.

7) Folic acid

The term, Folic acid is derived from Latin word 'folium' which stands for 'leaf'. It is also known as pteroylglutamic acid or folacin. Plants are a rich source of folic acid especially, spinach leaves. ***Green vegetables, whole grains, cereals, yeast, soybean and eggs are some of the other important sources of this vitamin.***

Folic acid consists of a ***pteridine*** ring, ***para-amino benzoic acid*** **(PABA)**, and ***glutamic acid*** (Fig. 12.13).

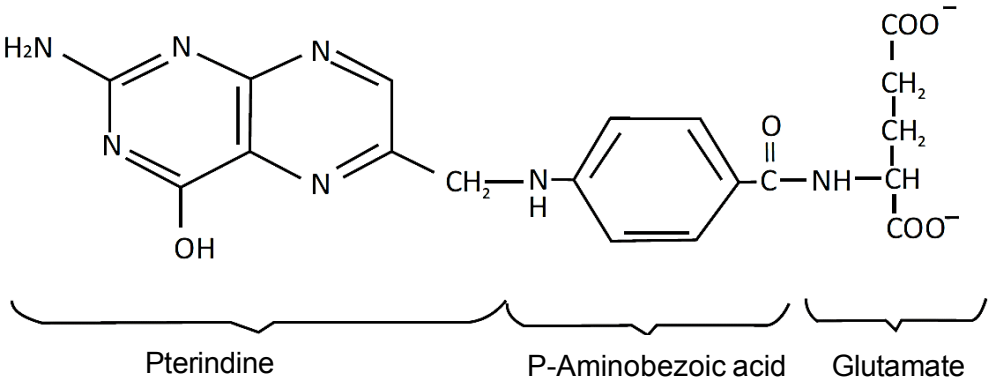


Fig. 12.13: Strucutre of Folic acid

Active forms: Tetrahydrofolate is active form of dietary folic acid (Fig. 12.14). It exits in two forms: 5,10-methylene tetrahydrofolate (THF) and 5-methyltetrahydrofolate in the human body:

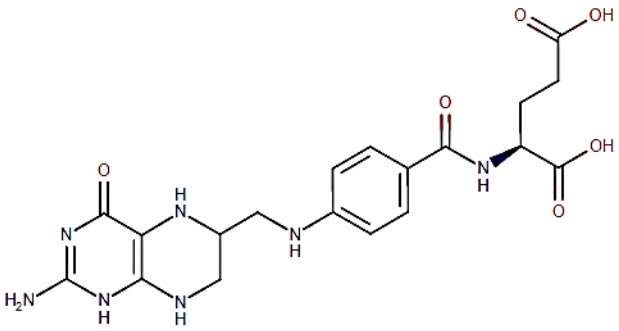
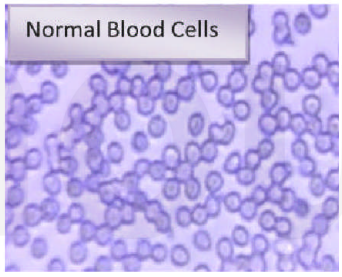


Fig. 12.14: Strucutre of Tetrahydrofolate

Biochemical functions: Tetrahydrofolic acid is a coenzyme in many biochemical reactions, especially in the synthesis of amino acids and nucleic acids (DNA). It works closely with Vitamin B_{12} in the synthesis of blood cells (RBC) and the amino acid homocysteine. 5,10-methylene tetrahydrofolate required for the methylation and repair of nucleic acids while 5-methyltetrahy-drofolate required for the formation of amino acid methionine. In addition, folate involved acid the formation of healthy red RBCs and prevent anemia especially in infants and pregnant women.

Deficiency diseases and symptoms: The deficiency of folic acid affects the synthesis of nucleic acids and amino acids. The deficiency of folic acid is most common in pregnant women. The deficiency in either folic acid or vitamin B_{12} can lead to megaloblastic anemia which cause clinical symptoms like weakness, fatigue, depression, breathing problems and fetal neural tube defect in pregnant womens.

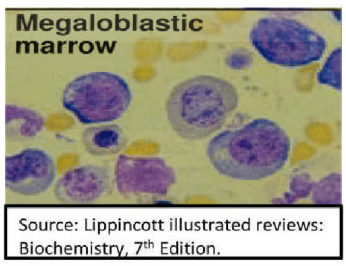
RDA: About 200 μg/day is recommended dose for adult male and female.



Normal blood cells in the blood smear. The size, shape, and color of the red blood cells-show hat they are normal. Mature red blood cells have lost their nuclens.

8) Cobalamin (Vitamin B_{12})

Vitamin B_{12} contains cobalt metal ion in the center of coring ring hence known as cobalamin. It is the heaviest (molecular weight = 1355.4kd) and most complex of all the vitamins. Vitamin B_{12} consists of a class of chemically related compounds known as vitamers (exhibiting vitamin activity). Neither plants nor animals are capable to synthesizing vitamin B_{12}. Bacteria have the enzymes required for the synthesis of vitamin B_{12}. ***Dietary sources of vitamin B_{12} are animal meat (espically liver), milk products, eggs and shellfish.*** Vitamin B_{12} is required for the normal functioning of the brain, nervous system and formation of red blood cells (RBCs). Vitamin B_{12} requires the specific protein, intrinsic factor for its absorption in the human body. Due to lack of this factor causes vitamin B_{12}- deficiency in the human body.



Megaloblastic blood cells in the bone marrow (immature stage of development). They still have their nuclei and are slightly larger than normal red blood cells.

Strucutre: Vitamin B_{12} is found as hydroxocobalamin in bacteria but it is converted into methyl cobalamin and 5'-deoxyadenosylcobalamin in human body. The chemical structure of cyanocobalamin was determined

by *Dorothy Crowfoot Hodgkin* in which 5-deoxyadenosyl group replaces the cyano group attached to cobalt in the sixth coordination position.

Vitamin B_{12} is composed of Cobalt ion (Co^{3+}), corrin ring system, dimethylbenzimidazole *(DMB) and 5 deoxy-adenosine* as shown in **Fig. 12.15**. Corrin stands for "core" component of vitamin B_{12}. A Corrin ring is heterocyclic compound and its structure is similar to the porphyrin ring of hemoglobin.

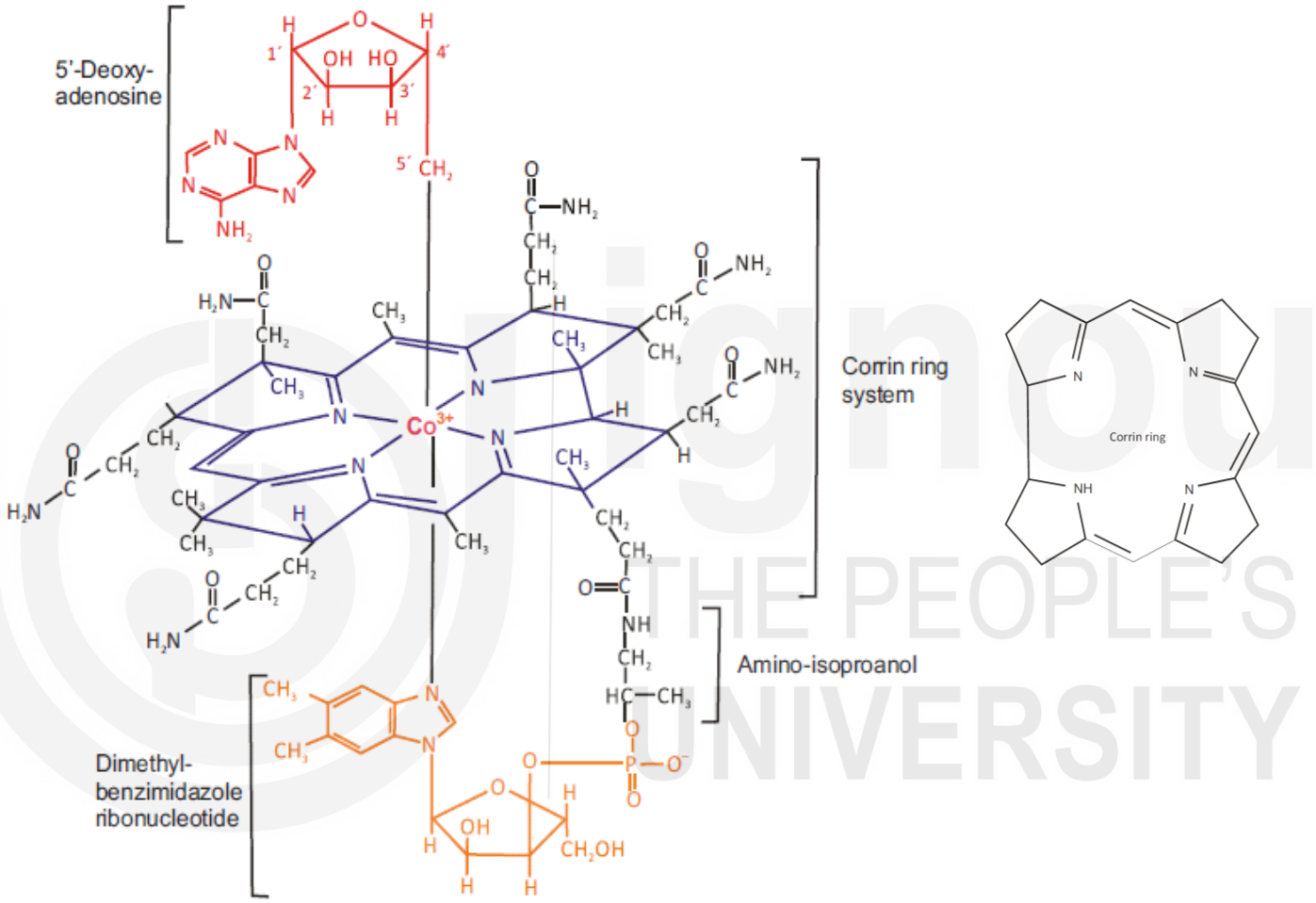


Fig. 12.15: Strcture of Vitamin B_{12}

Active form: Methyl cobalamin and adenosyl cobalamin

Biochemical functions: Methyl cobalamin and adenosyl cobalamin are two coenzymes of Vitamin B_{12} which are involved in metabolism of amino acids and synthesis of hemoglobin. Vitamin B_{12} acts as a coenzyme for *two enzymes: methionine synthase* and *L-methylmalonyl-CoA mutase*. (Fig. 12.16). Vitamin B_{12} participates in the transfer and rearrangement of the methyl group in biochemical reactions.

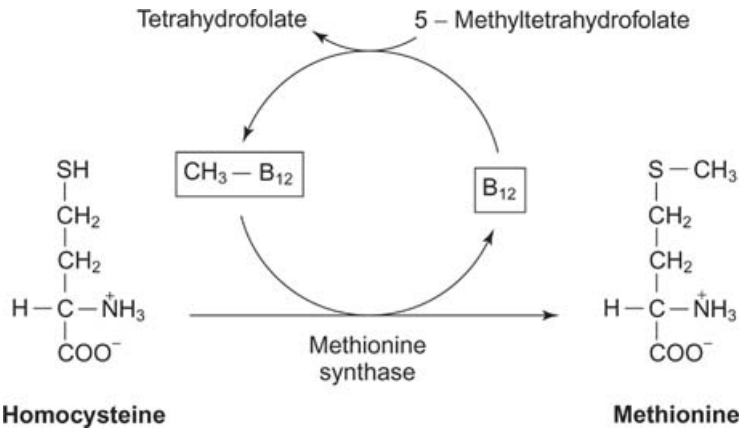
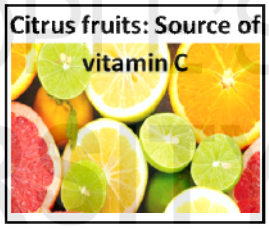


Fig. 12.16: Synthes is to methionine via transfer of methly group form Vit B_{12} to homocysteine

Deficiency diseases and symptoms: The major signs of vitamin B_{12} deficiency are pernicious anemia and neuropathy. The pernicious anemia disease is characterized by low hemoglobin levels, decreased number of erythrocytes and neurologic manifestation.

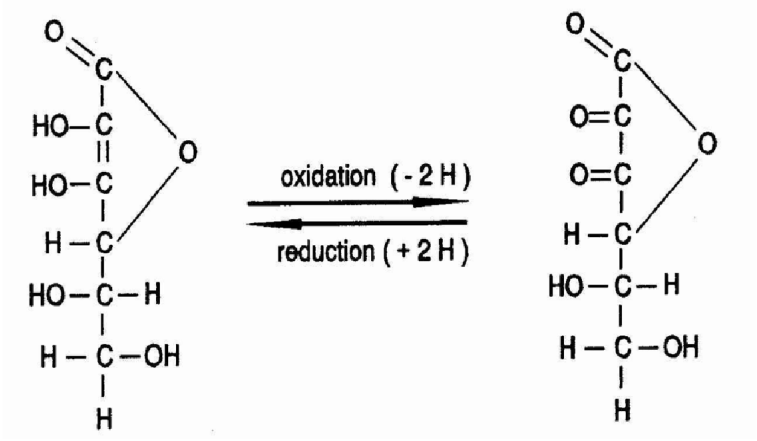
RDA : The recommended doses are 3 μg/day for both male and female and up to 0.5-1.5 μg/day for children.

12.3.2 Vitamin C (Ascorbic Acid)



It is also known as vitamin C. Unlike B-complex vitamins, vitamin C acts as an antioxidant molecule which also falls in the group of water soluble vitamins. ***Citrus fruits, peanuts, tomatoes and green vegetables are rich sources of this vitamin.***

The structure of vitamin C has six corbon atoms with hydroxyl groups that resemble a glucose to some extent. It is synthesized in most of the animals and plants but it cannot be synthesized by human body due to lack of an enzyme *L-gulonolactone oxidase*. Hence it is essential to take this vitamin through diet. This vitamin occurs in two forms; L-ascorbic acid (reduced form) and L-dehydro ascorbic acid (oxidized form) in the cells (Fig. 12.17).

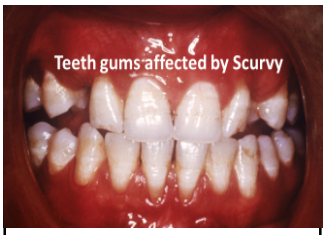


L-Ascorbic acid Dehydroascorbic acid

Fig. 12.17: Structure of Vitamin C

Biochemical functions: Vitamin C serves as a water soluble antioxidant that involve in neutralising free radicals in the cells. It protects cells from oxidative damage. Besides, vitamin C is required for the hydroxylation of proline and lysine for the formation of collagen in the skin. It also involved in the metabolism of folic acid, tyrosine and tryptophan. Vitamin C helps in wound healing, bone and teeth formation, improving immunity and absorption of iron from the diet. It is also widely used as a food additive to prevent oxidation.

Deficiency diseases and symptoms: Vitamin C-deficiency causes scurvy disease in humans. Fragility of blood capillaries, bleeding from gums and tooth loss are common symptoms of Scurvy.



Appearance of teeth and gums in scurvy.

RDA : Human body needs vitamin C about 90 mg/day for male and 75 mg/day for female.

SAQ 2

a) Write the active forms of following vitamins

S.No.	Vitamins	Active forms
1)	Vitamin B_6	
2)	Biotin	
3)	Folic acid	
4)	Vitamin B_{12}	

b) Match the following

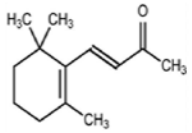
Column A	Column B
a) CoA	i) vitamins B_6
b) Blood cell formation	ii) biotin
c) transfer of amino group	iii) pantothenic acid
d. transfer of CO_2 group	iv) vitamin C
e) collgen formation and antioxidant	v) folic acid and vitamins B_{12}

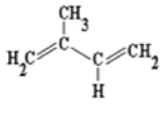
12.4 STRUCTURE, ACTIVE FORMS AND FUNCTIONS OF FAT-SOLUBLE VITAMINS

Unlike water soluble vitamins, fat-soluble vitamins are soluble in fat. Vitamins A, D, E and K are collectively known as fat soluble vitamins. These vitamins stored in liver and adipose tissue and their requirement is something like "demand and supply" *i.e.*, whenever there is a demand. These vitamins accumulate in the body due to over consumption which can cause toxic effects. This group of vitamins play an important role in performing various

physiological functions. Human body needs fat soluble vitamins to maintain healthy skin, hair, eyes, heart and bone. Let us study fat soluble vitamins one by one.

12.4.1 Vitamin A


β-ionone ring


Isoprene

Vitamin A is found in two forms; **retinoids and carotenoids**. All forms of vitamin A consists of a β-ionone cyclic ring and an isoprenoid side chain that is unsaturated and participates in the visual cycle. Carrot, papaya and fish liver oil are the best sources of vitamin A.

i) Retinoids comprise of three isomers of vitamin A: retinol, retinaldehyde, retinoic acid. Vitamin A is a collective term for biologically active isomers as shown in Fig. 12.18.

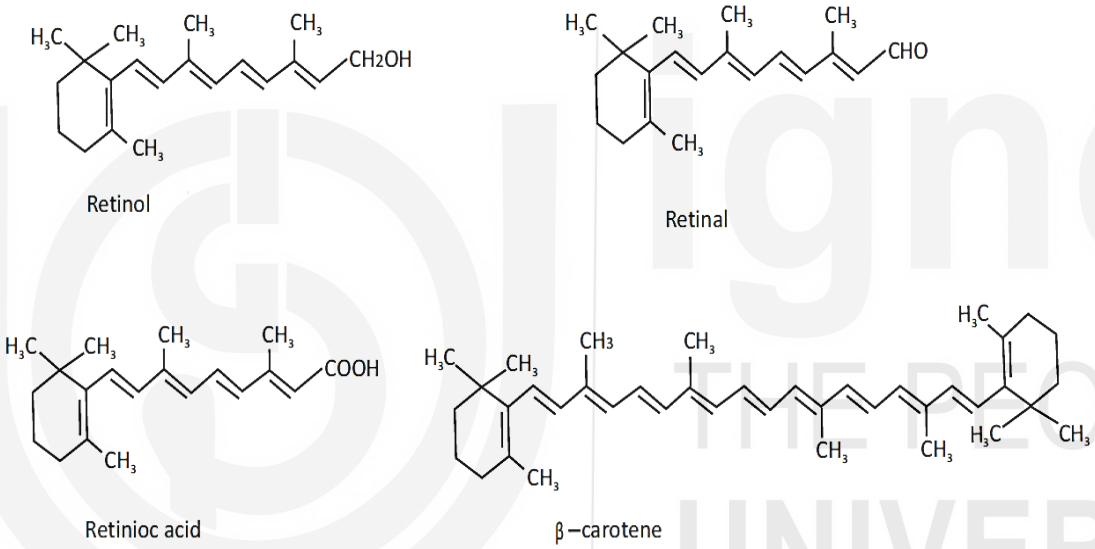


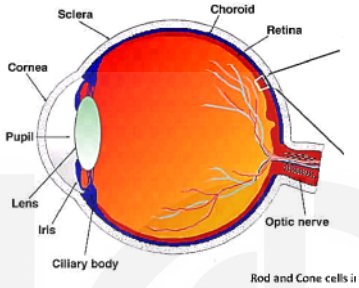
Fig. 12.18: Vitamin A isomers

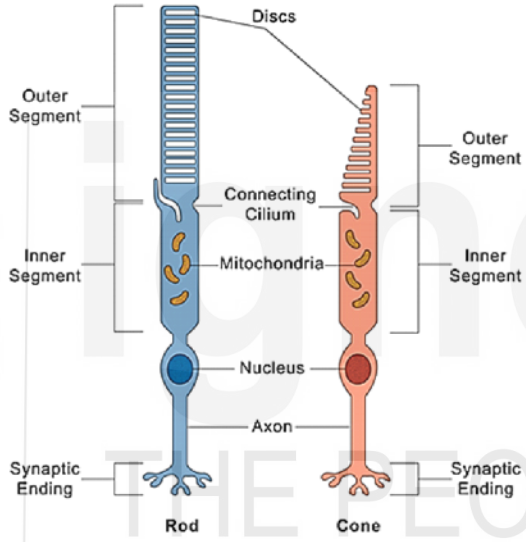
1. **Retinol:** It is primary alcohol (OH) containing β-ionone ring with an unsaturated side chain.
2. **Retinal:** It is an aldehyde (CHO) containing vitamin A which is formed by the oxidation of retinol. Retinal plays a crucial role in the visual system.
3) **Retinoic acid:** It is formed by the oxidation of retinol. It serves as an intracellular messenger that affects gene expression.

ii) Carotenoids: β-Carotene is one of the carotenoids found in the plants. When we consume β-carotene it converts to retinol in the human body. Hence, it is called as provitamin A β-Carotene has two β-ionone rings at both ends of the molecule.

Biochemical functions: Vitamin A plays many vital roles for the proper functioning of our body. It is crucial for vision. Let us discuss in brief about few important functions of vitamin A:

1. **Role of vitamin A in vision:** Role of vitamin A and rhodopsin in visual cycle was elucidated by George Wald. Hence this cycle is also known *Wald's visual cycle or rhodopsin cycle*. In eye, retina has two photosentisitve rod and cone cells. In retina, retinal (Vitamin A) binds to the photon-sensitive opsin protein to form visual pigment rhodopsin (in rod cells) and iodopsin (in cone cells). Rhodopsin is made up of a protein called opsin which firmly bound to 11-cis-retinal. Rhodopsin absorbs light that induces isomerization when 11-cis retinal converts into all-trans retinal and opsin is released (Fig. 12.19a and b). This isomerization leads to the dissociation of all trans-retinaldehyde, that triggers an electrical signal into the brain (nerve impulse in neurons) which processes the signals into an image.





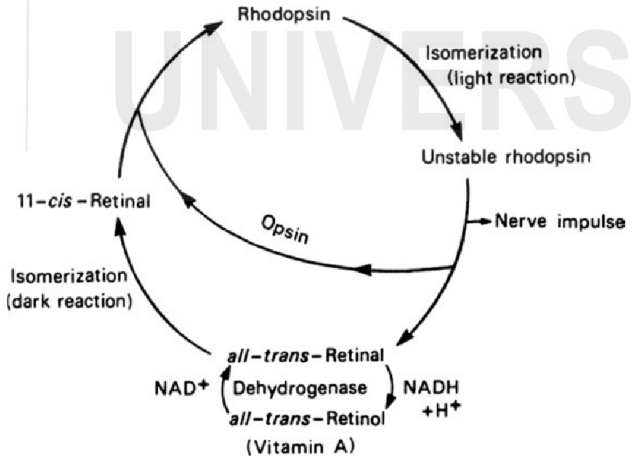


Fig. 12.19(a): Visual cycle

Cis-trans photo isomerization: When light strikes retina of eyes, the 11-*cis*-retinal of rhodopsin absorbs a photon, and it isomerizes into all-trans –retinal in which cis configuration state change into trans state. This configuration occurs at carbon number 11 of retinal in which interconversion of atoms occur.

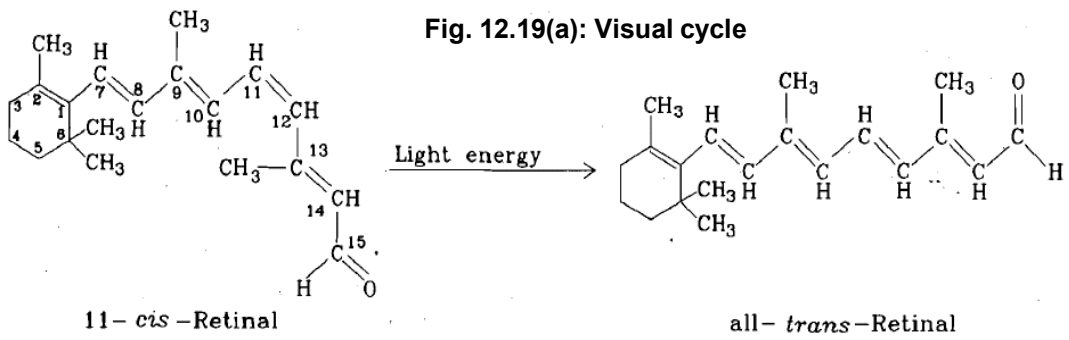


Fig. 12.19(b): Photoisomerization of 11-cis retinal to all-trans retinal in retinal

2. **Regulation of tissue differentiation and gene expression:** Vitamin A (Trans-retinoic acid and cis-retinoic acid) has a major role in regulation of tissue differentiation process and expression of certain genes at the transcriptional level.

3. **Antioxidant**: β-carotene is a pro-vitamin A that shows antioxidant like property to scavange free radicals in the body.

Deficiency diseases and symptoms: Vitamin A-deficiency (VAD) leads to impaired vision which has been recognized as major global problem specially in India and developing countries. The most common cause is inadequate dietary intake of vitamin A, different signs of vitamin A deficiency are:

i) **Night blindness:** It is one of the most common and primary clinical symptom of vitamin A deficiency in which a person cannot see in dim light. It can affect children as well as pregnant and lactating women.

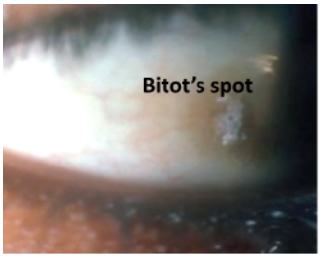
ii) **Bitot spots:** Abnormal squamous cell proliferation and keratinization of the conjunctiva affected in the eye due to VAD.

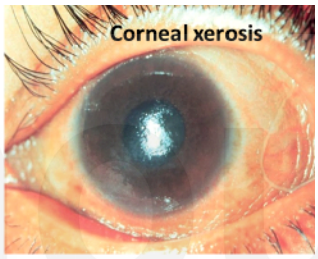
iii) **Xerophthalmia (dryness in eyes):** It is also known as dry-eyes syndrome. It results from keratinization of the cornea and blindness due to sudden acute deficiency of vitamin A. It is generally present in children and can occur in any age group.

iv) **Keratomalacia (dryness in corner):** The most severe form of xerophthalmia is keratomalacia in which one third part of cornia is affected due to chronic deficiency of vitamin A resulting in necrosis.

RDA: 400 μg/day for childern, 600 μg/day for male and female, 800 μg/day for pregnant women.

Vitamin A deficiency diseases in human eye





Source: Clate Gilbert. The eye signs of vitamin A deficiency. Community eye Health, 2013; 26[84]; 66-67. PMCIP : PMC393668

12.4.2 Vitamin D

Vitamin D is also known as calciferol. It is found in two active forms like calciferol (vitamin D_2) and cholecalciferol (vitamin D_3) (Fig. 12.20). Vitamin D is derived from sterol compounds which occur in plants and animals. Natural exposure of skin to sunlight (UV-B radiations) leads to the formation of this vitamin from its exposure to provitamins, ergosterol in plants and 7-dehydrocholesterol in human. When the exposure to sunlight is inadequate, dietary intake of vitamin D is required. ***Cod liver oil is the*** rich source of this vitamin. Besides, sunlight exposure is also required to meet the recommended daily intake of vitamin D in the human body.

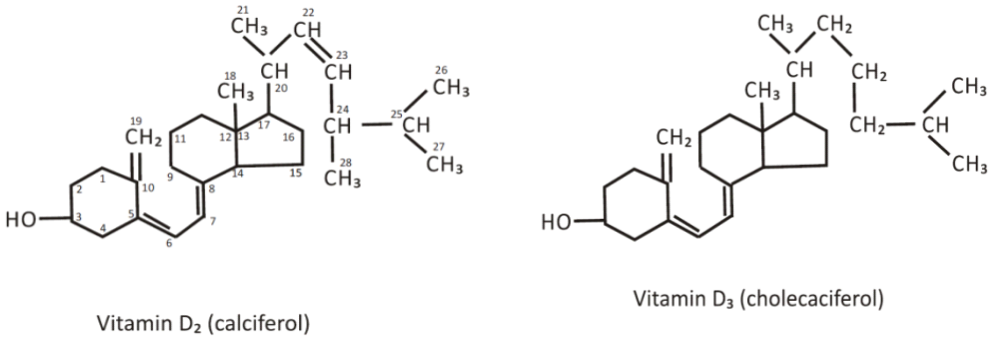


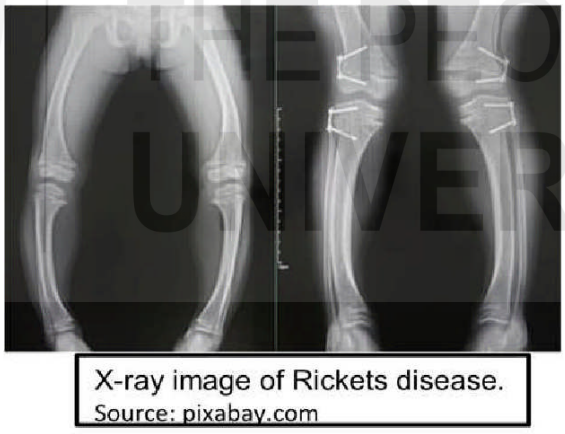
Fig. 12.20: Structure of Vitamin D

Biochemical function

Vitamin D functions like a hormone. It acts as a transcription factor which promotes the expression of gene encoding for calcium binding protein. It regulates the concentration of calcium and phosphate ions in the blood stream, thereby promoting growth and remodeling of bone. It also enhances absorption of calcium, iron and magnesium ions in the intestine.

Deficiency diseases and symptoms

Vitamin D deficiency leads to poor bone mineralization in the body resulting in osteomalacia in old age people and rickets in children. The common symptoms are muscle weakness, softening of tissues and fragility of the bones.



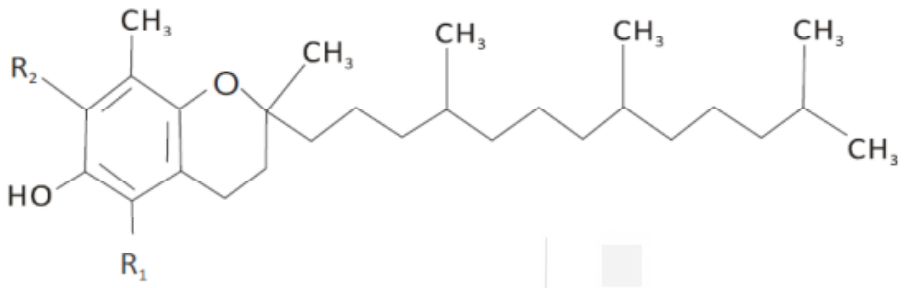
Rickets in Indian child (Vitamin-D Deficiency)

RDA: 15 µg/day for male and female

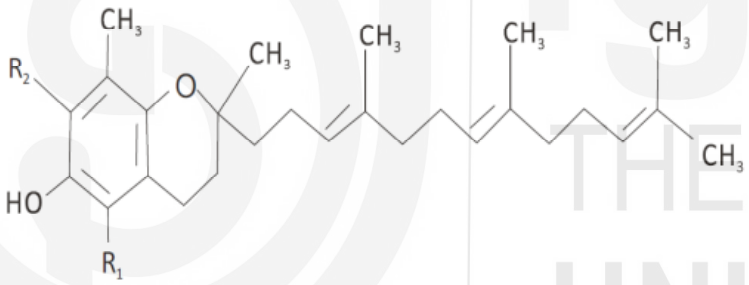
12.4.3 Vitamin E (Tocopherols)

Vitamin E is an essential nutrient because human body cannot make its own. In 1936, Evans and his colleagues isolated vitamin E from wheat-germ oil later named it as tocopherol. Tocopherol also known as vitamin E. It includes eight naturally occurring compounds of two classes: (1) tocopherols and (2) tocotrienols. Tocopherols is synthesized in plants and photosynthetic organisms. ***The rich sources of vitamin E are corn oil, soyabean oil Wheat and germ oil.***

Vitamin E is a derivative of 6-hydroxy chromane ring with phytal side chain The isomers of four-tocopherols and four tocotrienols are homologues which are designated as α (alpha), β (beta), γ (gamma) and δ (delta) based on number and position of methyl groups on the chromane rings (Table 1.1). Tocopherols and tocotrienols have saturated side chain and unsaturated fytal side chain along with chroman ring respectively as shown in Fig. 12.21 a and b. Among these, α-tocopherol is the most active form of tocopherol which is widley available form of vitamin E in foods.



(a) Tocopherol



(b) Tocotrienols

Fig. 12.21: Strucutre of Vitamin E

Table 12.1: Position of methyl group in alpha, beta, gamma and delta tocopherol

	R_1	R_2
α-Tocopherol	CH_3	CH_3
β-Tocopherol	CH_3	H
γ-Tocopherol	H	CH_3
δ-Tocopherol	H	H

Biochemical functions

Most of the biochemical functions of vitamin E relate to its anti-oxidative property. especially in cell membranes where it helps to prevent oxidation of membrane

lipids. It acts as an anti-aging molecule, that reduces oxidative stress in the cells. Vitamin E is essential in maintaining redox homeostasis in the body. Scientifc studies that vitamin E helps to reduce cardiovascular diseases and is essential for normal reproduction.

Deficiency disease and symptoms: Animals with Vitamin E deficiency causes infertility. The other symptoms of vitamin-E deficiency are muscular dystrophy in addition to this liver kidney different times modified.

RDA: About 7.5-10 mg/day (α-tocopherol) recommended for female and male.

12.4.4 Vitamin K

Vitamin K is known as blood clotting vitamin. It was discovered by H. Dam in 1929. The structure of vitamin K consists of a quinine ring that linked to the isoprenoid side chain. ***Spinach, cabbage and green leafy vegetables are rich sources of this vitamin.***

Vitamin K exits in two forms:

1. Vitamin K_1 (phyllo Quinone): It is synthesized by plants. The isoprenoid side chain is saturated as shown in Fig. 12.22.

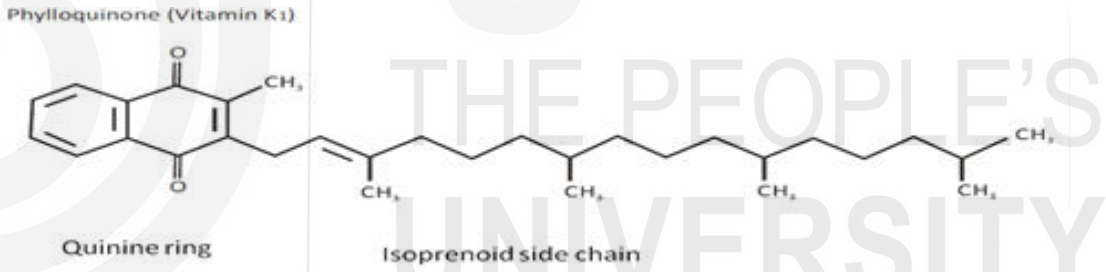


Fig. 12.22: Strucutre of Vitamin K_1

2) Vitamin K_2 (menaquinone): It is synthesized by intestinal bacteria. It is the main storage form in animals. Fig. 12.23 shows the structure of vitamin K_2 in which isoprenoid chain is unstaturated (containing double bonds) as compared to saturated isoprenoids in vitamin K_1.

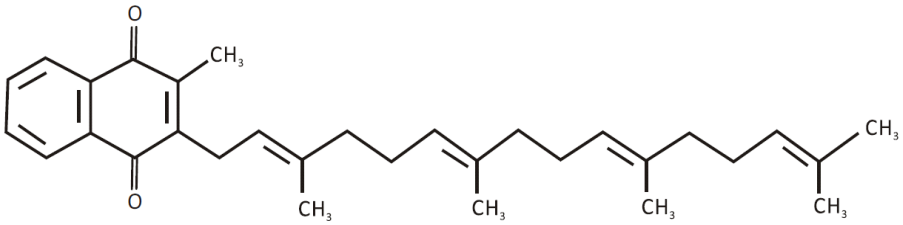


Fig. 12. 23: Structure of Vitamin K_2

Biochemical functions

Vitamin K is essential for the carboxylation of glutamic acid to produce α-carboxy glutamic acid in blood coagulation. Prothrombin and several other proteins of blood clotting system contain γ-carboxy glutamic acid residues. Such modified residues can bind Ca^{2+}, which is required for blood clotting cascade.

Deficiency diseases and symptoms

Deficiency of Vitamin K causes delayed blood cogulation, that results into prolonged clotting time.

RDA: 55 μg/day for males and females.

SAQ 3

a) Write the functions of following vitamins.

S. No.	Vitamins	Functions
1)	Vitamin A	.....
2)	Vitamin D	.....
3)	Vitamin E	.....
4)	Vitamin K	.....

b) Fill in the blanks with correct answer(s).

i) The first sign of vitamin A deficieny .....

ii) In the presence of light, rhodopsin is convereted to ..... from ..... which is essential reaction for human vision.

iii) The structure of tocotrienols differ from tocopherols due to ..... presence of .....

iv) ..... Vitamin acts as hormone.

v) Exposure to ..... stimulates synthesis of Vitamins D

vi) ..... is Anti-aging vitamin.

vii) Vitamin required for the carboxylation of glutamic acid ..... is.....

12.5 HYPERVITAMINOSIS

The term hyper-vitaminosis means that presence of higher amount of vitamins than that of required. This condition leads to toxicity. Hypervitaminosis is primarily associated with fat soluble vitamins. Water soluble vitamins are not

toxic because they do not store in the body except vitamin B_6. When the levels water soluble vitamins exceeds they get excreted through urine. The excess amount of fat soluble vitamins in the body results from over dose of vitamin-supplements.

As you know vitamin supplements are readily available in many different formulations (pills, syrup and tablates) which are recommended for the treatment of vitamin-deficiency diseases. Hence, taking multivitamins for longer periods may lead to metabolic abnotmalities that consequently affect the vital organs and tissues as these organs are storage sites for fat soluble vitamins.

Let us know some the common hypervitaminosis:

Hypervitaminosis A: High intake of vitamin A shows significant toxicity. Storage of vitamin A may cause visual changes, congenital birth defects, dizziness, nausea, headache, discoloration of skin, pain in joints and bones, coma, and even death.

Hypervitaminosis D: Overdose of this vitamin causes adverse health effects such as anorexia, weight loss, polyuria, heart arrhythmias and also vascular and tissue calcification. Higher levels of vitamin D damages heart, blood vessels and kidneys. Prolonged exposure to sunlight does not result in vitamin D toxicity.

Hypervitaminosis E: Higher intake of vitamin E-supplements leads to weakness, headache and gastrointestinal upset. Research studies reported that abnormal storage of vitamins E can affect blood clotting, inhibit platelet aggregation and cause haemorrhage.

Hypervitaminosis K: Higher doses of vitamin K can cause allergic reactions such as skin rashes, itching and redness on the body. Its higher dose can cause hemolysis in newborns and hyperbilirubinemia.

Hyper vitaminosis B_6: Though it is a water soluble vitamin, it shows some adverse effects in the body. High intake of vitamin B_6 or pyridoxine supplements lead to common symptoms such as headache, severe fatigue, nerve damage and mood changes.

12.6 VITAMINS AS COENZYMES AND ANTIOXIDANTS

So for you have studied about the active forms and biochemical functions of vitamins. It is noted that most vitamins serve as coenzymes and antioxidants. Most coenzymes are derivatives of water soluble vitamins (B-complex vitamins) which are required for enzyme-catalyzed reactions. The major functions of coenzymes are to transport chemical groups between substrates and products. Coenzymes are specific to specific enzyme that serve unique function during cellular metabolism (Table 12.2).

NAD^+ and NADH involve in Oxido-reduction reactions (Fig. 12.24) and B_6 in conversion of α-amino acid to α-ketoacid (Fig. 12.25).

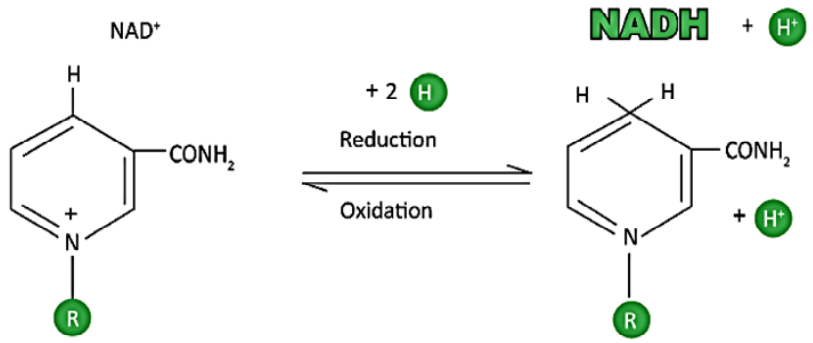
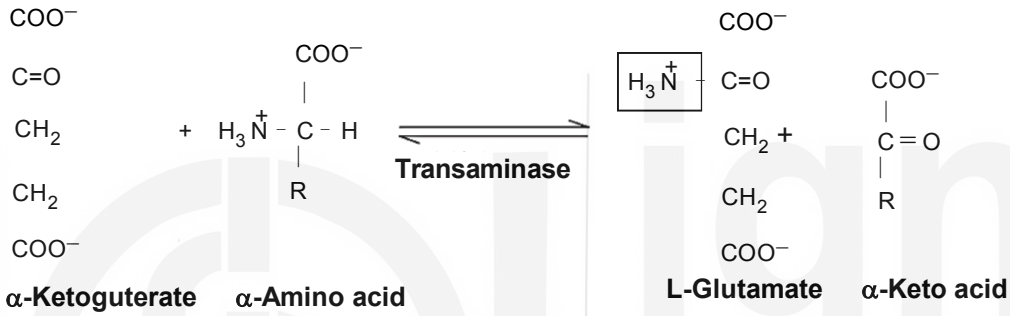


Fig. 12.24: Interconversion of NAD^+ to NADH



α-Ketoguterate **α-Amino acid** **L-Glutamate** **α-Keto acid**

Fig. 12.25: Transamiation reaction

Table 12.2: Water-soluble Vitamins, Coenzymes their and Biochemical Functions

Vitamins	Coenzyme forms	Biochemical function
Thiamin (B_1)	Thiamin-pyrophosphate (TPP)	Participates in the Oxidative decarboxylation reactions
Riboflavin (B_2)	Flavin mono nucleotide (FMN) & Flavin di nucleotide (FAD)	Participates in the Redox reactions
Niacin (B_3)	Nicotinamide adenine dinucleotide (NAD^+) Nicotinamide adenine dinucleotide phosphate (NADP)	Participates in the Redox reactions
Pantothenic acid (B_5)	Coenzyme A	Transfer of acyl group
Pyridoxine (B_6)	Pyridoxal phosphate	Participates in the Transamination reactions
Biotin	Biocytin	Participates in the Carboxylation reactions
Folic acid	Tetrahydrofolate	Transfer of one carbon unit
Vitamin B_{12}	methylcobalamin and 5- deoxyadenosyl cobalamin	Methyl group transfer and its rearrangements

***All water-soluble vitamins (with the exception to vitamin C) are coenzymes or precursors of coenzymes.**

Vitamins as antioxidants: Vitamins that are considered as antioxidants include vitamin A, vitamin C, and vitamin E. You are aware that vitamin C is a water soluble vitamin and vitamins A and E are fat soluble vitamins. Recall the structure of these vitamins having hydroxyl and oxygen groups that play an important role in the scavenging of free radicals. These vitamins serve as a antioxidant molecule to mitigater the oxidative damage in the biological system. Antioxidant molecules neutralize free radicals by accepting or donating electron(s).

α-tocopherol is one of the effective form among other forms of vitamin E that has an antioxidant property. Vitamin E involves in protecting of membrane lipids from oxidation. It terminates the formation of free radicals producing through chain reaction process of lipid peroxidation. **β-carotene** is a precursor of vitamin A has been shown to have antioxidant property. It is considered the most efficient "quencher" of singlet oxygen.

Vitamin C and vitamin E can directly react with or neutralize free redicals such as hydroxyl, alkoxyl and lipid peroxyl (ROO*) and convert them into non-reactive molecules like water molecule , alcohol and lipid hydroperoxides, respectively.

Since, we are discussing about antioxidants let us know in brief about free radicals.Free radicals are highly reactive atoms or group of atoms bearing unpaired electrons. Human body naturally produces free radicals through metabolic reactions involving oxygen.These free radicals are also known as reactive oxygen species(ROS) such as hydroxyl radical ($OH^{\cdot}$), superoxide anion radical, singlet oxygen, hydrogen peroxide (H_2O_2), hypochlorite and nitric oxide. Free radicals react with lipoproteins and unsaturated fatty acids in cell membranes, removing an electron from these molecules and thus generating a new free radical which can start a chain reaction process. Free radicals generated by environmental pollutants, sun light, smoking, stress and exposure to heavy metals and synthetic drugs etc.

SAQ 4

Tick ( ) the correct option given in the brackets

i) (Vitamin A/vitamin E) Protects membrane lipid peroxidation from free radicals

ii) Water soluble antioxidant is (vitamin E/vitamin A/ vitamin C

iii) Reactive oxygen species in cells are also know as antioxidant/ free radicals

iv) Coenzymes are derivatives of water soluble vitamins/fat soluble vitamins

v) Provitamin of vitamin A is (Rotinone /β-carotene.

Table 12.3 : Vitamins at glance:

Vitamin	Functions	Deficiency diseases/symptoms	Dietary sources	*Recommended Dietary Allowances (RDA)
Water soluble vitamins				
Thiamin	Coenzmye in decarboxylation reactions	Beri-beri	**C**ereals, groundnuts, soybean and capsicum.	1.4 mg/day for male and 1.1 mg/day for female.
Riboflavin	Serve as electron donor and acceptor in redox reactions	Fatigue Cheilosis	Yeast, milk, eggs and green vegetables	1.6 mg/day for male and 1.3 mg/day for female.
Niacin	Formation of NAD & NADP	Pellagra	Peanuts, legumes, meat, eggs and milk	18 mg/day for male and 14 mg/day for female.
Pantothenic acid	Functional part of CO-A and transfer acyl group	Peripheral nerve damage	Whole grains and vegetables	5 mg/day
Pyridoxin	Involves in amino group Trnsfer in transamination reactions	Disorders in amino metabolism	eggs, liver, yeast, cereals, legumes and milk	2.0 mg/day for male and female
Biotin	Transfer of CO_2 in biotin dependent enzymes for carboxylation	Impired lipid and carbohydrate metabolism	Liver, kidney, yeast, nuts, tomatoes and egg yolk	30 μg/day for male and female
Folic acid or folate	Transferring of one carbon group in biochemical reactions	Megaloblastic anemia	Green vegetables, whole grains, cereals,	200 μg/day for male and female, 500 ìg/day for pregnant women
Vitamin B_{12}	Formation of Hemoglobin	Pernicious anemia	animal foods, meat, milk, egg and fish.	1.0 μg/day for male or female and 1.2 ìg/day for pregnant women
Vitamin C	Required for collagen biosynthesis and serve as an antioxidant	Scurvy	Citrus fruits, peanuts, tomatoes and green vegetables	40 mg/day for male and female, 60 mg/day for pregnant women
Fat Soluble vitamins				
Vitamin A	Essential for proper vision	Night blindness, xeropthalemia	Carrot, papaya and fish liver oils	400 μg/day for child, 600 ìg/day for male and female, 800 ìg/day for pregnant women
Vitamin D	Help to body to utilize calcium and phosphate from food. Essential for bone formation.	Rickets in children and osteomalacia in adults	Cod liver oil	10 μg/day for male and female
Vitamine E	Is essential for reproduction and serves as an antioxidant	Infertifity	Wheat, seed oil and fish oil	8-10 mg/day
Vitamin K	Participate in Blood clotting mechanism	Prolonged blood coagulation	Spinach, cabbage and green leafy vegetables	55 μg/day for adulsts

12.7 SUMMARY

Vitamins are essential nutrients required for development and maintenance of the healthy body. They perform various functions in the body. Their inadequate levels in body lead to the various deficiency dieseases.

They broadly devided into two groups like water soluble and fat soluble.

B complex vitamins primarily serves as coenzymes to transfer functional groups and assist enzymes to relase energy from food stuffs. Vitamin C serve as an anti-oxidant molecule and essential for collagen formation.

Vitamin A is important for vision. Vitamin D is for absorption of calcium and phosphorus, bone and teeth formation; vitamin E for neutralizing free radicals and improve immune system. Vitamin K for blood clotting.

Over storage of vitamins for longer period in the body results in toxicity. This medical condition is known as hypervitaminosis.

Vitamins serves as coenzymes and antioxidants. Most B-complex vitamins primarily function as coenzymes and vitamins A, C and E serve as antioxidants to protect the cell from oxidative stress.

12.8 TERMINAL QUESTIONS

1) Define vitamins?
2) Name the deficiency diseases or symptoms of the water-soluble and fat-soluble vitamins.
3) Provide the rich sources of the following vitamins?

   Thiamine, Niacin, Folic acid, riboflavin, ascorbic acid and vitamin A
4) Write RDA values the following vitamins.

   A, B_1, B_6, Biotin, D and K.

12.9 ANSWERS

Self Assessment Questions

a) (i) True (ii) True (iii) False (iv) True (v) False (vi) False (vii) True

b) i) Thiamin
   ii) pyrimidine ring and a thiazole ring
   iii) Thiamin pyrophosphate
   iv) beriberi
   v) Flavin and ribose
   vi) FMN and FAD
   vii) tryptophan
   viii) NAD and NADP

2) a) 1. Pyridoxin phosphate

2. Biocytin

3. 5, 10 methylene tetrahydrofolate

5-Methyl tetrahydrofolate

4. Adenosyl cobalamin and methyl cobalamin

b) a) iii b) v c) i d) ii e) iv

3) a) 1. Visual cycle

2. Teeth and bon formation

3. antioxidant and improving immune system

4. Blood clotting formation

b) i) Night blindness

ii) 11-cis retinal to all-trans retinal

iii) Unsaturated fytal side chain

iv) Vitamin D

v) Sunlight

vi) Vitamin E

vii) Vitamin K

4) i) vitamin E ii) vitamin C iii) free radicals

iv) water soluble vitamins v) β–carotene

Terminal questions

1) Refer to section 12.1 for definition of vitamins

2) Refer to section 12.3

3)

Vitamins	**Sources**
Thiamine	Sunflower seeds and brewer's Yeast
Niacin	Rice and wheat
Folic acid	Peanuts, tomato juice and green beans
Riboflavin	Spinach
Vitamin C	Citrus juice and lemon
Vitamin A	Carrots

4) RDA of the following vitamins.

Vitamins	Dietary requirement
Vitamin A	400 μg/day for child, 600 μg/day for male and female, 800 μg/day for pregnant women
Vitamin B_1	1.4 mg/day for male and 1.1 mg/day for female.
Vitamin B_6	2.0 mg/day for male and female
Biotin	30 μg/day for male and female
Vitamin D	15 μg/day for male and female.
Vitamin K	55 μg/day for adults

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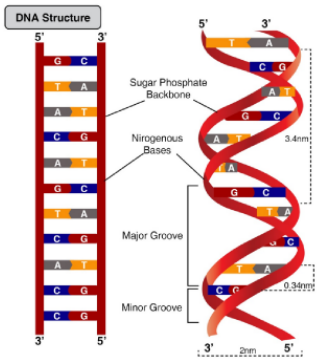


UNIT 13

NUCLEIC ACIDS

Structure

13.1 INTRODUCTION

In the pervious Blocks you have studied about the significance, structure and functional aspects of water and buffers, amino acids, proteins, carbohydrates and lipids. In this unit you will learn about nucleic acids both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

You must have noticed that we resemble either of our parents. Have you ever asked yourself why? Because genetic information is transferred from one generation to another, i.e. from parents to the off springs. This flow of information is a vital condition for life and for the entire cellular processes. The genetic information is stored in the form of nucleotide sequences in the cells nucleus. You will study the structural as well as functional aspects of both DNA and RNA in detail in this unit.

Objectives

After studying this Unit, you should be able to:

- explain how nucleic acids act as genetic material;
- define and draw structures of nitrogenous bases, purines and pyrimidines;
- explain the significence phosphodiester linkage;
- differentiate between DNA and RNA;
- illustrate the structure of DNA double helix; and
- describe various types of RNA and their functions.

13.2 NUCLEIC ACIDS AS GENETIC MATERIAL: EXPERIMENT EVIDENCES

Earlier it was believed that protein is the genetic material because of its vast diversity while nucleic acid was considered to be a static molecule. Friedrich Miescher, a Swiss physician in 1869 isolated and identified a macromolecular substance from the pus cells and from salmon sperm which he termed as **nuclein**.

Meischer's student Richard Altmann in 1899 used the term nucleic acid for phosphorus containing nuclein, which was later proved to be the hereditary material in 1950s.

Friedrich Meischer

In biology course at intermediate level you have studied about nucleic acids briefly. Let us recall and build upon that simultaneously. Nucleic acids constitute about 7 % of the dry weight of a cell. Both the nucleic acids are linear polymers of repeating units called **nucleotides.** Nucleotides are made up of sugar, phosphate and nitrogenous bases which you will study in detail in the next section. Sugar and phosphate form the backbone while sequence of bases linked to sugar moiety characterize the uniqueness of the nucleic acid.

Let's go further. Nucleic acids are very appropriately referred as information storage molecules of a cell. This information is organized in the form of genes, the fundamental unit of genetic information. Information flows from DNA to RNA to protein i.e. DNA directs the synthesis of RNA, which in turn directs the synthesis of proteins. This flow of information is known as **central dogma** of molecular biology (Fig. 13.1). You will study about these processes in detail in later courses.

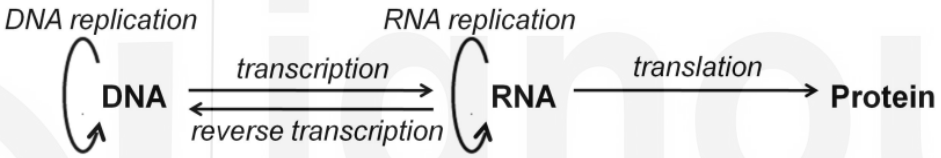


Fig. 13.1: Central dogma of life

Now the question which generally comes to our mind is what qualifies DNA as the genetic material? Why not protein or lipid or some other biomolecule? Today, we know that DNA is the genetic blueprint of life. It functions as the molecule that carries on the genetic information from one generation to another, lets understand how. Nitrogenous bases linked to sugar phosphate backbone in a sequential manner stores and transfer the genetic information. Bases have a property to form pairs with the help of hydrogen bonds. Because of this **base pairing** nucleic acids tend to form a double stranded helical structure, and also base pairing provides a mechanism for copying this genetic information.

If any error occurs in any of the steps involved in expressing the genetic information (replication, transcription and translation) stored in DNA, a genetic disease may occur. Thus, an understanding of how nucleic acids store and deliver genetic information within the cells and from one generation to another becomes necessary for understanding diseases and also to devise strategies for treatment, for example gene therapy.

DNA Carries Genetic Information: Experimental Evidences

DNA is the genetic material in all prokaryotic and eukaryotic organisms. In 1869 Miescher isolated DNA from the pus cells but the first experimental evidence that DNA is the genetic material came much later. Griffith's experiment on transforming principle in the year 1928 suggested that bacteria are capable of transferring genetic information through a process known as **transformation**. However, in the year 1944, Avery, Macleod and McCarty using Griffith's experiment on bacterial transformation provided the evidence that the transforming agent is DNA and it is the genetic material (Fig. 13.2). Griffith found that DNA extracted from a virulent (smooth, disease causing) strain of the bacterium *Streptococcus pneumoniae,* genetically transformed a non-virulent (rough, non-disease causing) strain of this organism into a virulent form.

They observed that:

Stage 1 - Living 'S' strain causes death in mouse.

Stage 2 - Living 'R' strain causes no-death in mouse.

Stage 3 - Heat killed 'S', strain can not cause death in mouse.

Stage 4 - Mixture containing both heat killed 'S', strain and live 'R', strain will cause death in mouths.

Hence, they concluded that in stage 4, some substance causing virulence in 'S', strain is transfered into 'R', strain and is responsible virulence.

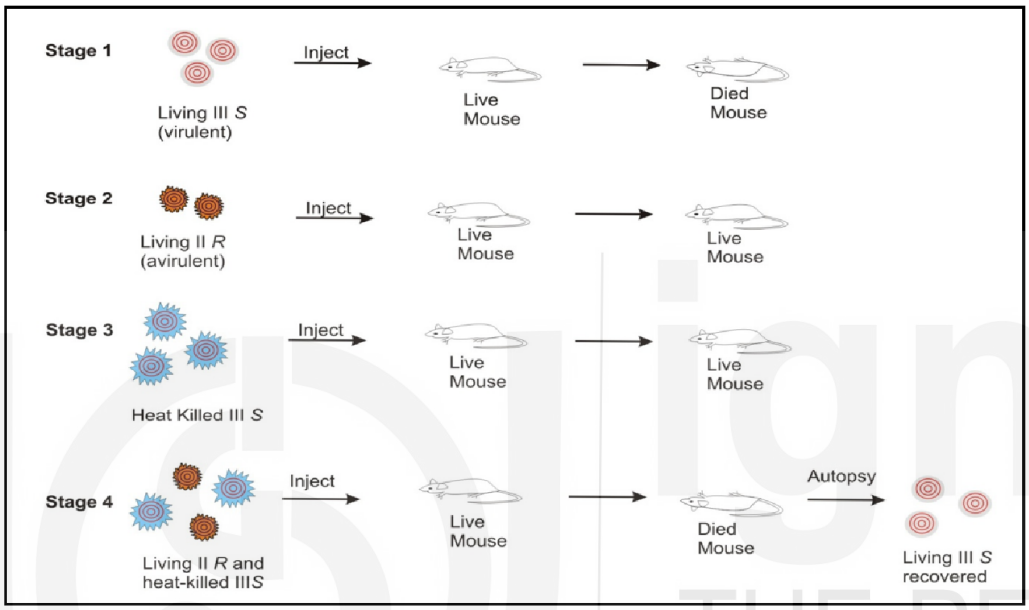


Fig. 13.2: Griffith's experiment on bacterial transformation

Second independent experimental evidence came in the year 1952. Hershey and Chase devised an experiment with T2 bacteriophage (virus) using radio-labeled sulpher and phosphorus (S^{35} and P^{32}). Since T2 phage contains both DNA and proteins, in one experiment the protein part was made radioactive with S^{35} and in the other the DNA was made radioactive with P^{32}.They infected *E. coli* with labeled T2 bacteriophage. After infection, culture was gently agitated in a blender to loosen the phage particles and culture was centrifuged. It was found that when T2 phage containing radioactive protein was used as the infecting agent, the bacterial pellet contained very little radioactivity and most of the radioactivity was in the supernatant. But if T2 phage with radioactive DNA was used, the heavier bacterial pellets were radioactive (Fig. 13.3). This experiment indicated that during infection with the virus, it is the DNA that actually entered the bacteria. This experiment is also known as **Blender** experiment.

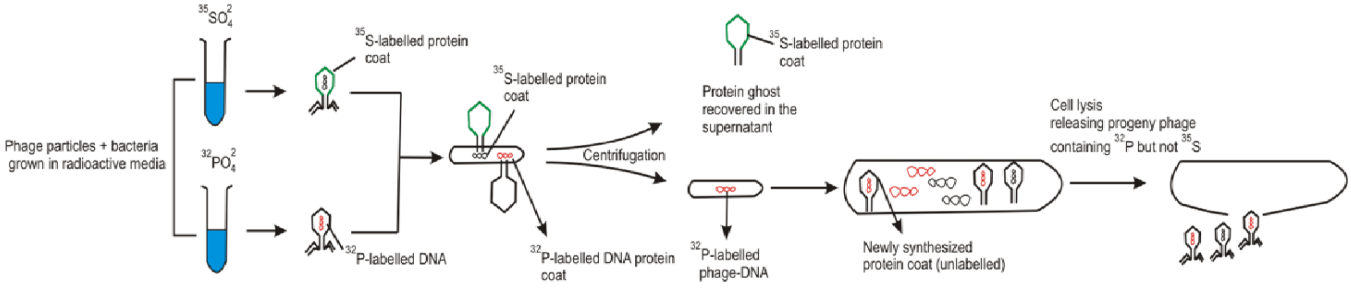


Fig. 13.3: Blenders experiment of Hershey and Chase

Let us now study in detail about the constituents of nucleic acids.

13.3 CONSTITUENTS OF NUCLEIC ACIDS: STRUCTURE AND PROPERTIES

Nucleic acids (both DNA and RNA) are composed of pentose sugar, phosphate and nitrogenous bases. Sugar is deoxyribose in DNA (deoxyribonucleic acid) while ribose in RNA (ribonucleic acid). Deoxyribose means the 2' carbon of sugar lacks the oxygen atom which is present in ribose (Fig. 13.4). Numerals with a prime (2' or 3') distinguish atoms of the sugar from those of the bases.

Have you ever wondered why its ribose and not glucose or any other sugar?

Experiments show that because of steric hindrance other sugars were not suitable to fit and it also led to unstable structure formation with other components of nucleic acid.

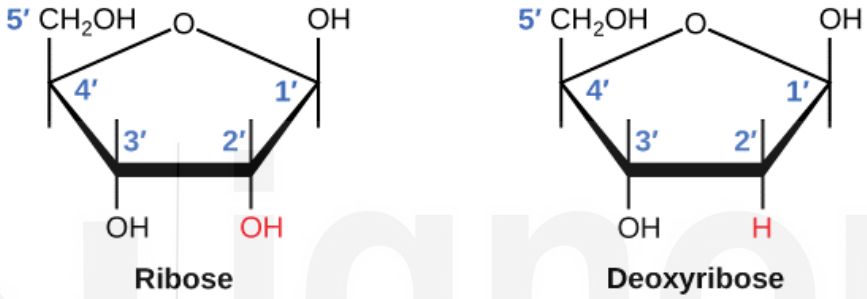


Fig. 13.4: Structure of Ribose and deoxyribose (pentose sugar)

Sugars in the nucleic acid backbone are linked together by phosphodiester bond/linkages/bridges. Phosphate group (PO_4^{3-}) is attached to C-5 of pentose. During the formation of a phosphodiester bond, the C-5 of one sugar moiety is joined to the OH of C-3 with phosphate group (phosphate attached to C-5 of pentose). Look at a dinucleotide in Fig.13.5, the pentose moieties are linked by a 3', 5' phosphodiester bond i.e. two ester bonds are present one at C-3 and other at C-5 of the pentose sugar and thus termed as phospho-di-ester. These phosphodiester bonds are strong covalent bonds and do not break even on heating the DNA molecule at high temperature.

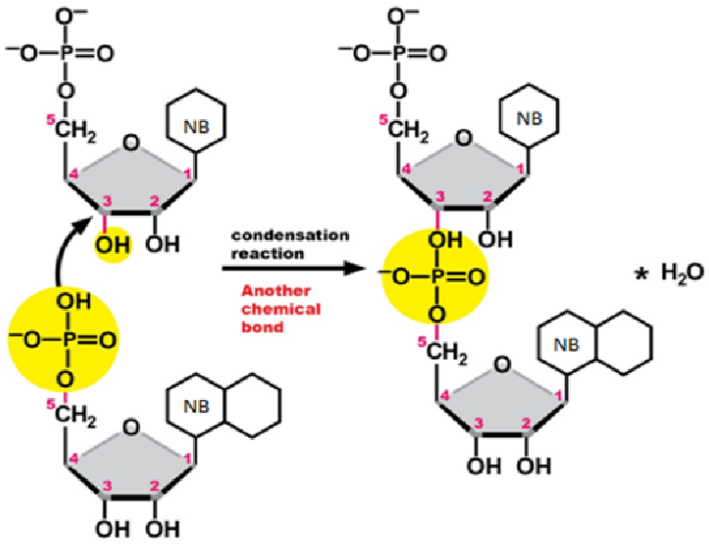


Fig. 13.5: Phosphodiester linkage in a dinucleotide formed between the C-5 phosphate of one sugar moiety and C-3 hydroxyl group of the second to build a polynucleotide.

Till now we discussed how pentose sugar and phosphate are joined together. Now, you will study about nitrogenous bases which are the key component of nucleotide/nucleoside.

The heterocyclic ring of both purine and pyrimidine bases contain oxo (-C?O-) functional group and thus can exist in two tautomeric forms, keto and enol.

Keto forms are more predominant than enol form in both DNA and RNA.

13.3.1 Nitrogenous Bases

Nitrogenous bases were identified in 1880 by Emil Fischer. They belong to heterocyclic group of organic compounds and have planar structure. Nitrogenous bases can be grouped into two: purines and pyrimidines.

Purines are double ring structures, numbered in anti-clockwise direction while pyrimidines contain single ring and are numbered clockwise (Fig. 13.6). The major purines of both DNA and RNA are Adenine (A) and Guanine (G). In DNA major pyrimidines are Cytosine (C) and Thymine (T), while in RNA major pyrimidines are Cytosine (C) and Uracil (U). So, **instead of Thymine (in DNA), Uracil is present in RNA** while other bases are common to both types of nucleic acids.

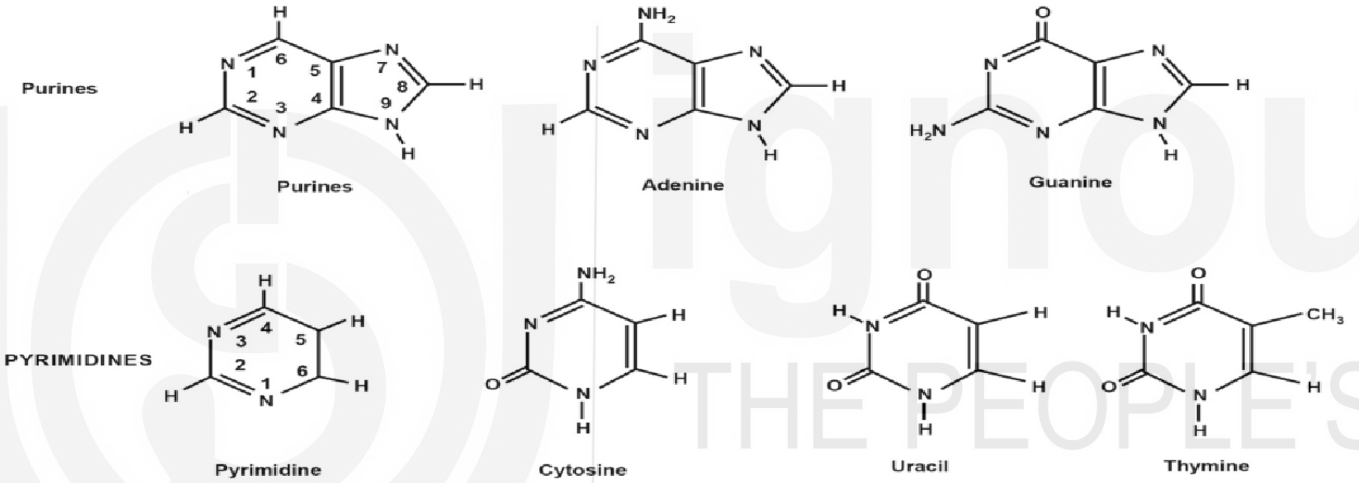


Fig.13.6: Structure of purines and pyrimidines. Atoms within bases are numbered without primes. Purines are double ring structures, numbered in anti-clockwise direction. Pyrimidines contain single ring and are numbered clockwise.

In addition to these five major nitrogenous bases found in nucleic acids, some unusual or minor bases are also found in nature for example hypoxanthine, xanthine, uric acid etc. Hypoxanthine and Xanthine are the metabolites of adenine and guanine and are ultimately converted to uric acid, the end product of purine catabolism. Minor or unusual bases like 5-methycytosine, pseudo uracil, N^6 methyladenine occur mostly in RNA. The IUPAC names of major purines and pyrimidines are given in Table 13.1.

Table 13.1: IUPAC names of major purines and pyrimidines constituting nucleic acids

Common name	IUPAC name	One letter symbol
PURINES		
Adenine	6-amino purine	A
Guanine	2-amino-6-oxy purine	G
PYRIMIDINES		
Cytosine	2-oxy-4-amino pyrimidine	C
Uracil	2,4-dioxy pyrimidine	U
Thymine	2,4-dioxy-5-methyl pyrimidine	T

13.3.2 Nucleosides

A unit consisting of a nitrogenous base bonded to a pentose sugar is termed as nucleoside. Nucleosides are derivatives of purines and pyrimidines. Look at Fig. 13.7 and you will notice that C-1 of sugar is linked to the ring nitrogen, N-9 of a purine or to N-1 of a pyrimidine by a β-N-glycosidic bond (For details refer Carbohydrates Unit 6).

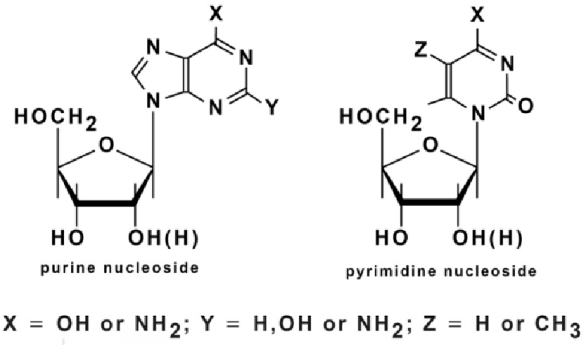


Fig 13.7: Structure of a nucleoside.

13.3.3 Nucleotides

A nucleotide is formed by the joining of nucleoside to one or more phosphate groups by an ester linkage. The most common site of esterification in naturally occurring nucleotides is the hydroxyl group attached to C-5 of the sugar. Thus, nucleotide is the phosphorylated form of nucleoside as you can see in Fig.13.8.

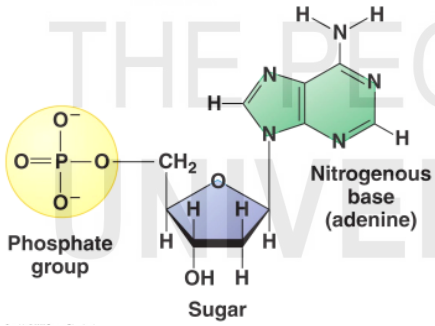


Fig. 13.8: Structure of a nucleotide

Now to put all things together, you know how β-glycosidic linkage joins nitrogenous bases with pentoses to form nucleoside and then this nucleoside is joined to a phosphate molecule with the help of an ester linkage to form nucleotide. These nucleotides are the building blocks of the nucleic acids. One nucleotide is linked to the other nucleotide with the help of a phosphodiester bond to give a polymeric DNA or RNA.

Also, you must notice from Fig. 13. 9, that these phosphodiester linkages give nucleic acid chain a specific polarity because all the phosphodiester linkages have the same orientation along the chain. They join the 3' and 5' carbons of adjacent sugar moieties/nucleotides. Thus, by convention, **the sequence of nucleic acid is written from 5' → 3' direction**, the 5' terminus always being to the left. For example, a tetra-deoxy-ribonucleototide, TAGC means that deoxythymidylate (T) is at the 5' terminal and deoxycytidylate (C) is the 3' terminal residue (Fig. 13.9). Table 13.2 provides you the terminology used to describe nucleic acid components.

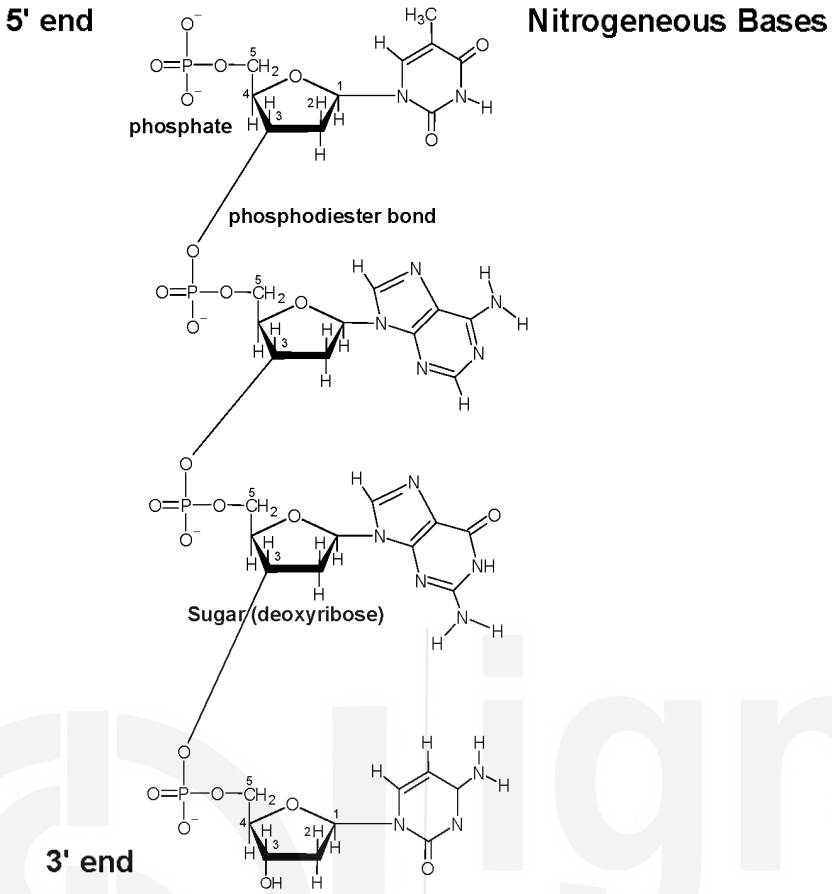


Fig. 13.9: Orientation of nucleotides in a tetra-deoxy-ribonucleototide 5' TAGC 3'

Table 13.2: Nucleic acid nomenclature

Base	Nucleoside	Nucleotide	Abbreviation for nucleotide	Nucleic acid in which it is present
		PURINES		
Adenine (A)	Adenosine	Adenylic acid/ Adenylate/or Adenosine monophosphate	AMP	RNA
	Deoxya-denosine	Deoxyadenylic acid/ Deoxyadenylate/or Deoxyadenosine monophosphate	dAMP	DNA
Guanine (G)	Guanosine/ Guanidine	Guanylic acid/ Guanylic acid/ Guanylate/or Guanosine monophosphate	GMP	RNA
	Deoxyguanosin/ Deoxyguanidine	Deoxyguanylic acid/ Deoxyguanylate/or Deoxyguanosine monophosphate	dGMP	DNA
		PYRIMIDINES		
Cytosine (C)	Cytidine	Cytidylic acid/ Cytidylate/or Cytidine monophosphate	CMP	RNA

	Deoxycytidine	Deoxycytidylic acid/ Deoxycytidylate/or Deoxycytidine monophosphate	dCMP	DNA
Uracil (U)	Uridine	Uridylic acid/ Uridylate/or Uridine monophosphate	UMP	RNA
Thymine (T)	Deoxythymidine	Deoxythymidylic acid/Deoxythy-midylate/or Deoxythymidine monophosphate	TMP	DNA

In the above table you must have noticed that the names of purine nucleosides end in –osine while the names of pyrimidine nucleosides end in –idine. The nucleotides are utilized in multiple ways in the cell, for example as energy source or components of cofactors/coenzymes and so on. You will study about this in detail in the next unit.

SAQ 1

i) Tick [ ] mark the correct option

a) Uracil is a purine. [True/False]

b) Nitrogenous bases are heterocyclic compounds. [True/False]

c) Pyrimidines are double ring structures. [True/False]

d) Thymine is present in RNA. [True/False]

e) Adenine is a 6-aminopruine [True/False]

ii. Fill in the blanks with appropriate words.

a) Each unit of a nucleic acid consisting of a sugar, attached phosphate group, and ..... is a nucleotide.

b) C1 carbon of sugar is linked with N1/N9 of nitrogenous bases by ..... bond.

c) Uridine nucleoside is found only in ..... . A nucleotide containing cytosine is called .....

d) By convention, the sequence of bases in a nucleic acid is usually expressed in the ..... direction.

e) In DNA guanine always pairs with.....

f) In a nucleic acid, the bases are always attached to the ..... carbon of the sugar.

13.4 STRUCTURE OF DNA

Deoxyribonucleic acid (DNA) is the hereditary or genetic component of living beings, like plants and animals. It is primarily the which influence our phenotype (phyiscial characters) i.e. how we will look, color of our eyes, hair, skin etc, whether our hair will be straight or curly, and so on. To understand

these functional aspects of DNA, let us study the structural features first and its various forms in this section.

Till now you have studied about components of nucleic acids (both DNA and RNA) and how they are linked to one another. Let us now understand how this linear polymer (chain of nucleotides) give rise to a two dimensional structure which makes nucleic acids so crucial to our existence.

A DNA is a long, linear, unbranched polymer composed of only four types of deoxy ribonucleotides containing the bases adenine (A), cytosine (C), guanine (G), and thymine (T). The nucleotides are linked together by covalent phospho-diester linkages that join the 5' carbon of one deoxyribose sugar to the 3' carbon of the next. The four kinds of bases are attached to this repetitive sugar-phosphate chain almost like four colors of beads in a necklace. You also know that all the phosphodiester linkages have the same orientation along the chain (look at Fig.13.9), giving each nucleic acid strand a specific polarity and distinct 5' and 3' ends.

Generally, DNA is a double helical structure i.e. it consists of two strands and is known as DNA double helix because the two strands are wound in a regular helical pattern (Fig. 13.10). The two strands in a double helix run in anti-parallel direction (one strand runs in 5' $\rightarrow$ 3' direction while other one runs in 3' $\rightarrow$ 5' direction) and are held together by hydrogen bonds between complementary base pairs (A-T and G-C pairs)and base-stacking interactions.

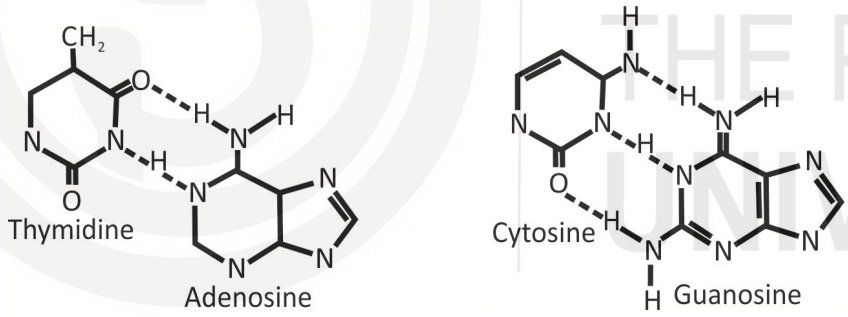


Fig 13.10: In DNA base pairing occur between A and T; and G and C. The dotted lines represent hydrogen bonds. Adenosine forms two hydrogen bonds with thymine. Cytosine forms three hydrogen bonds with guanosine.

The commonly occurring form of DNA helix is right handed i.e. if you look at the double helix from the top; the base residues form a spiral in a clock wise direction. This structure of DNA was proposed by James Watson and Francis Crick in 1953, and is also known as **Watson and Crick model**.

13.4.1 Watson-Crick Model of DNA

An essential feature of the model is that all the bases of the DNA molecule are on the inner side of the double helix, whereas sugar and phosphates are on the outer side. This arrangement brings the bases on one strand extremely close to those on the other strand, and thus facilitates the hydrogen bond formation. *Base pairing* (Fig. 13.10) i.e. hydrogen bond formation between a large purine

(A or G, each of which has a double ring) on one chain and a smaller pyrimidine (T or C, each of which has a single ring) on the other chain occurs effectively without any steric hindrance. Adenine pairs with thymidine and cytosine pairs with guanine.

Here you must also note that the two antiparallel polynucleotide chains of double-helical DNA are not identical in either base sequence or composition. Instead they are **complementary** to each other i.e. wherever adenine occurs in one chain, thymine is found in the other; similarly, wherever guanine occurs in one chain, cytosine is found in the other.

Base ratios of DNAs from several animals and bacterial sources were analyzed by E. Chargaff .This rule named after him "***Chargaff's rule***" of molar equivalence was given by him in the year 1950. This rule of base pairing or molar equivalence explains the phenomenon that whatever the amount of adenine (A) in the DNA of an organism, the amount of thymine (T) is the same similarly, whatever the amount of guanine (G), the amount of cytosine (C) is the same. Also, the specific base pairing suggests that ratio of purines to pyrimidines is always one (A+G=C+T) and could be expressed simply as the %GC.

Now look at Fig. 13.11 and compare the structure of DNA with the structure of a spiral staircase. What do you observe? Covalent backbone of nucleic acid consisting of alternating sugar and phosphate residues can be compared with the railings/backbone of the spiral staircase. Nitrogenous bases present as side groups are joined to the backbone at regular intervals with bases and serve as stacks.

Chargaff's Rule: rules of base pairing (or nucleotide pairing) are:

a) **A with T:** the purine adenine (A) always pairs with the pyrimidine thymine (T) with two hydrogen bonds

b) **C with G:** the pyrimidine cytosine (C) always pairs with the purine guanine (G) with three hydrogen bonds

This is consistent with there not being enough space (20 Å) for two purines to fit within the helix and too much space for two pyrimidines to get close enough to each other to form hydrogen bonds between them.

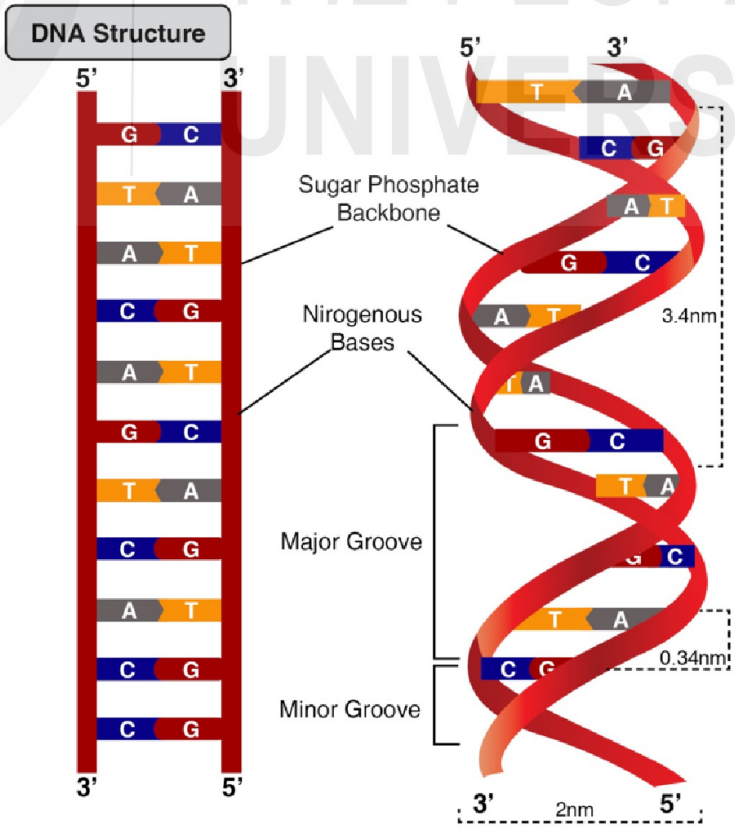


Fig. 13.11: Double helical structure of DNA (B-form)

The important features of the Watson and Crick Model of DNA are:

1) Two linear polynucleotide chains run in opposite directions (i.e. have antiparallel orientation), and are coiled around a common axis to give rise to a right handed helix (Fig. 13.12).

2) The sugar-phosphate backbones are on the outside, the purine and pyrimidine bases lie on the inside of the helix and are nearly perpendicular to the helix axis.

3) Adjacent bases (nucleotides) are separated by the same distance of 3.4 Å. The helical structure repeats every 34 Å, so there are approximately 10 bases per turn of helix. There is a rotation of 36 degrees per base (360 degrees per full turn/10 bases per turn).

4) Strong, straight hydrogen bonds establish specific base pairing between A and T and between G and C. The diameter of the helix is 20 Å.

SAQ 2

a) A sample of DNA contains 20% adenine find the % of GC

13.4.2 Other Forms of DNA

DNA can exist in different three dimensional forms. It is a flexible molecule. The Watson-Crick structure which you studied in the previous section is commonly referred as B form of DNA, or B-DNA. It is *biologically* significant form of DNA since it is commonly and occuring naturally found in most living systems. It is the most stable form under physiological conditions. Other structural forms of DNA also exist due to considerable rotation possible in the number of bonds in sugar-phosphate backbone and temperature changes. Two other structural variants of the DNA have also been characterized. These are A and Z forms of DNA. These structural forms although have significant deviations from Watson–Crick DNA structure but the key properties i.e. base pairing, strand complementarity and anti-parallel orientation of the duplex remains the same.

A-DNA

A-DNA is right handed (Fig. 13.12) but less hydrated than the B-DNA. It is more compact with 11 base pairs per turn of the helix and 23 A^0 in diameter (thicker than B-DNA).

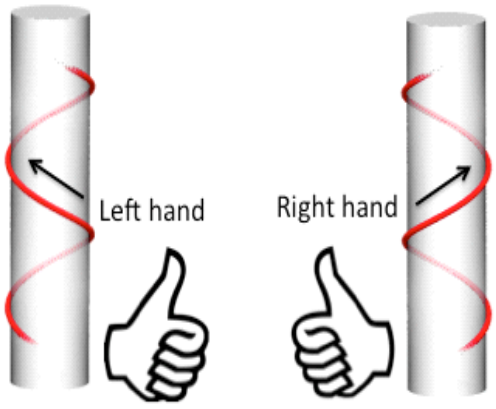


Fig. 13.12: The left and right hand direction of DNA helix.

A-DNA may occur under experimental conditions. Interestingly B-DNA forms A-DNA under high salt concentration. The bases do not lie perpendicular to the halical axis, but instead are titled by 22^{O}.

Z-DNA

Z-DNA is left handed consisting of alternating purines and pyrimidines such as GCGCGCGCGC at very regular intervals. This is called as Z-DNA because of its zig-zag structure. This *zig-zag* structure is due to sugar-phosphate backbone which is not as smooth as in B-DNA. Under high salt concentration, repelling electrostatic forces between negatively charged phosphate groups are reduced with phosphates much closer leading to zig-zag structure. The helix of Z-DNA is 18 A^0 in diameter (thinner than B-DNA) containing 12 base pairs per turn (Fig. 13.13). DNA can flip-flop from B to Z form in those regions of double helix that is rich in sequences having alternating purines and pyrimidines. Z-DNA occurs in eukaryotes, plants and bacteria. Z-form of DNA can coexist with the B-form in the same DNA molecule. The important characteristics of different forms of DNA are given in Table13.3.

In 1979, 25 years after Watson and Crick discovered B-DNA, A. Rich confirmed the findings of Paul and Jovin that left handed forms of DNA also exist.

The double helix has t wo grooves, major and minor. These are binding sites for proteins. The grooves are also acts as sites for initial molecular interactions of certain drugs, antibiotics, carcinogens and poisons.

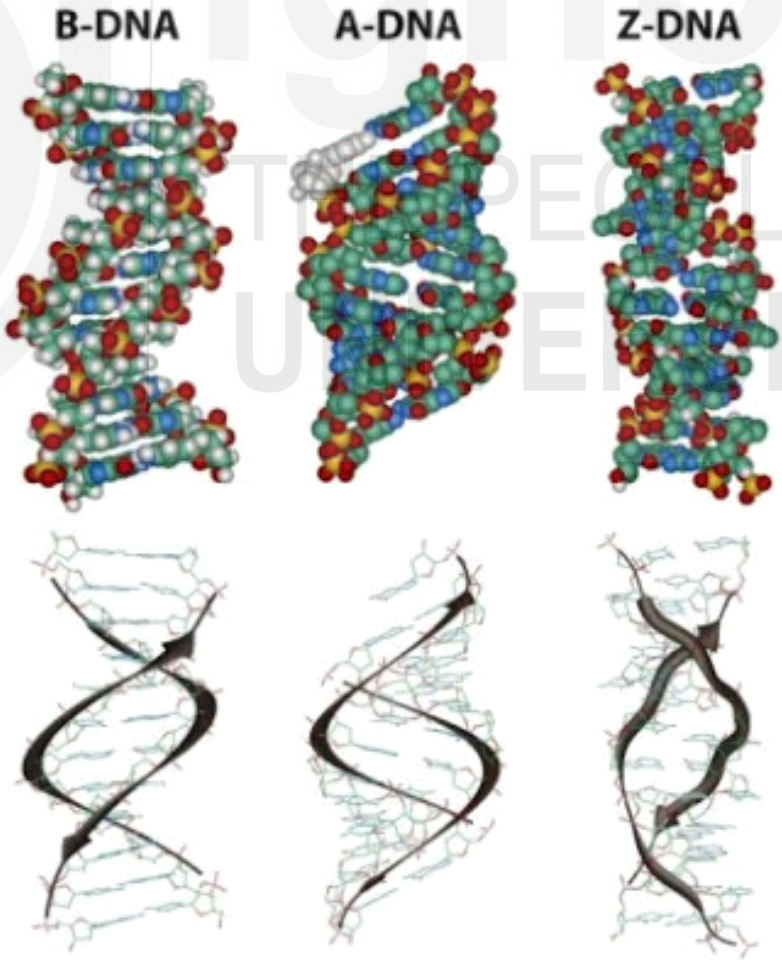


Fig. 13.13: Structure of B, A and Z form of DNA

Table 13.3: Various forms of DNA

Forms of DNA	B-DNA	A-DNA	Z-DNA
Helical turn	Right handed	Right handed	Left handed
Diameter	~20 A^0	~23 A^0	~18 A^0
Base pairs per helical turn	10.4bp	11bp	12bp
Helical rise per base pair	3.4 A^0	2.7A^0	3.7 A^0
Physical Condition	At physiological pH	At 75% relative humidity, high ionic strength	At very high salt concentration

SAQ 3

Tick [ ] mark the correct option

a) B-DNA is also known as Watson and Crick DNA. [True/False]

b) A-DNA is left handed. [True/False]

c) Diameter of B-DNA is 40 A^0. [True/False]

d) B-DNA is converted into A-DNA at high salt concentration. [True/False]

13.5 STRUCTURE OF MAJOR SPECIES OF RNA

RNA, ribonucleic acid, is another major type of nucleic acid. It plays a vital role in expression and regulation of genes. Experimentally it has been observed that a DNA molecule which is located in the nucleus of an organism does not leave the nucleus to participate directly in the process of protein synthesis. Rather it is transcribed into different types of ribonucleic acid molecules for transporting its information from cells nuclei to the site of protein synthesis i.e. ribosomes. Also, RNA serves as the genetic material in some viruses (commonly known as retroviruses). In this section you will study in detail about the structure and various types of RNA's.

All the RNAs are made from DNA. One strand of DNA is used as a template and its complementary strand is synthesized which serves as mRNA, tRNA or rRNA depending upon the information stored in that segment of DNA. For an active gene thousands of RNA transcripts can be made from the same DNA segment in each cell generation.

RNA is structurally similar to DNA with some exceptions. RNA contains ribose as its sugar unit rather than deoxyribose present in DNA (refer to section 13.3). However, the presence of hydroxyl group (2' OH in ribose sugar) makes RNA less stable and prone to hydrolysis in comparison to DNA. Also, instead of thymine which is present in DNA uracil occurs in RNA i.e. the complementary base to adenine in RNA is uracil instead of thymine.

Unlike double stranded DNA, RNA is generally a single stranded molecule with much shorter chain of nucleotides and double stranded in case of t-RNA (transfer-RNA) but non-helical in structure. The self-complementary sequences in biologically active RNAs allow some of its parts to fold and pair to form short double helices looking like hair pin loops.

Major Species of RNA

RNA can be grouped into various types. For example, RNA's involved in protein synthesis, DNA replication, regulatory RNA's, and so on and plays a significant role during biological processes.

Here we will mainly consider three types of RNA which are involved in protein synthesis. A class of RNA molecules called **messenger RNA** (**mRNA**) is the information-carrying intermediate in protein synthesis. Other RNA molecules, such as **transfer RNA (tRNA)** and **ribosomal RNA (rRNA),** are part of the protein-synthesizing machinery. All forms of cellular RNA are synthesized by RNA polymerases that take instructions from DNA templates which you will study later. Let us now learn about different types of RNA and their functions.

Messenger RNA (mRNA)

It carries the message from DNA to the ribosome, the site of protein synthesis (known as translation). The message is encoded in the form of codons (triplet of nucleotides in mRNA coding for an amino acid) for the synthesis of proteins. Each mRNA molecule can be translated into thousands of copies of a polypeptide chain, and thus the information contained in a small region of DNA can direct the synthesis of millions of copies of a specific protein. The key features of mRNA are:

- It comprises only 5% of the total RNA in the cell.
- It is most heterogeneous in terms of size (i.e. number of nucleotides is highly variable) and hence has variable sedimentation coefficient.
- mRNA sequence is complementary to the nucleotide sequence of the template DNA.
- In cytosol the nucleotide sequence is translated into polypeptide chain.

One of the important structural characteristics of eukaryotic mRNA which differentiates it from prokaryotes include the presence of a **long poly A tail** (poly adenosine monophosphate tail) on 3' end and a methylated guanine nucleotide "cap" attached to the 5' end of the RNA chain.

Transfer RNA (tRNA)

Transfer RNA or tRNA is also known as "adapter molecule". It binds to an amino acid and transfer it from the cytosol to a growing polypeptide chain at the site of protein synthesis. It has *sites for amino acid attachment* and anticodon region for codon recognition that binds to a specific sequence on the mRNA chain through hydrogen bonding (Fig. 13.14)

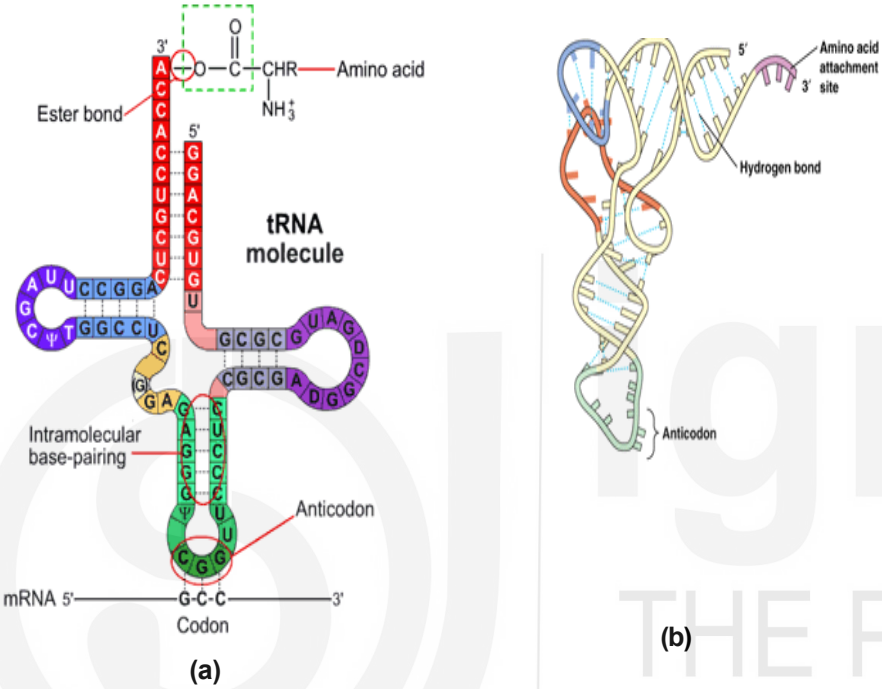
The key features of tRNA are:

- It is the smallest RNA amongst three major categories of RNA i.e. mRNA, rRNA and tRNA having 73 to 93 nucleotide residues.
- Comprises around 15% of the total RNA in the cell.
- Sedimentation coefficient is 4S.

Contain certain unusual bases for example dihydrouridine (D), pseudouridine (Ψ), and extensive intra-chain base pairing resulting into various loops.

There is atleast one tRNA for each amino acid.

- The secondary structure is a characteristic "**clover leaf**" structure (Fig. 13.14 A) while tertiary structure looks like an **inverted L or 7** (Fig. 13.14B).



S is the sedimentation coefficient, i.e. the sedimentation velocity per unit of centrifugal force. It is usually expressed in units of 10^{-13}S, which is known as Svedberg, named after the inventor of the ultracentrifuge.

Fig. 13.14: Structure of tRNA (a) Clover leaf structure (b) Tertiary Structure

Ribosomal RNA (rRNA)

Ribosomal RNA or rRNA plays a very important role. It has a dual role in protein synthesis, *structural and enzymatic.* rRNA is the structural constituent of ribosomes i.e. rRNA along with other ribonucleoproteins constitute ribosomes. The ribosomes bind mRNA and carries out protein synthesis. During protein synthesis several ribosomes (polyribosome) may be attached to a single mRNA. You will study more in detail while studying Gene Expression and Regulation (BBCCT-123). The key features of rRNA are:

It is the structural component of ribosomes.

Most abundant of all the types of RNA. Comprises of 80% of total rRNA in the cell.

Three different rRNA molecules with sedimentation coefficient of 23S, 16S and 5S are present in prokaryotic ribosomes while four different rRNA molecules with sedimentation coeffeicient of 28S, 18S, 5.8S and 5S are found in eukaryotic ribosomes (Fig. 13.15).

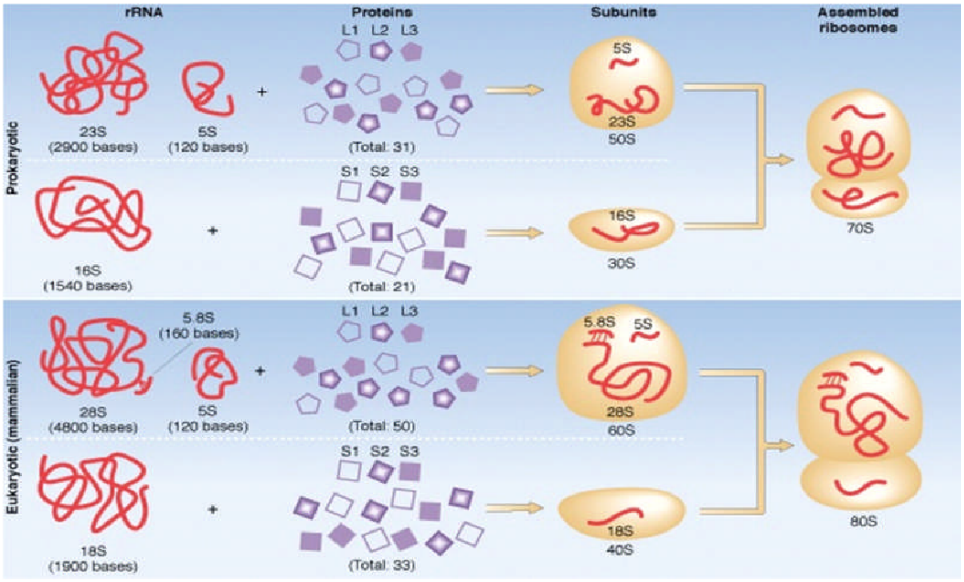


Fig. 13.15: Pictographic depiction of ribosomal assembly comprising of various rRNA and proteins.

SAQ 4

Tick [ ] mark the correct statement

a) mRNA acts as a template during protein synthesis. [True/False]

b) 28S rRNA is present in the eukaryotic mitochondria. [True/False]

c) Function of tRNA is to identify and transport amino acid to the protein synthesizing machinery. [True/False]

d) Secondary structure of tRNA is clover leaf. [True/False]

e) mRNA is the most abundant of all the types of RNA. [True/False]

13.6 SUMMARY

1) Nucleic acids, DNA and RNA are one of the important biomolecules.

2) Either DNA or RNA is the genetic material of all organisms, and carries information from parents to offspring's.

3) Nucleic acids are made up of nucleotides. Nucleotides have three characteristic components: (1) a nitrogenous (nitrogen-containing) base, (2) a pentose, and (3) a phosphate. The molecule without the phosphate group is called a nucleoside. The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine.

4) Nucleotide moieties in a large DNA molecule are linked to one another with phosphodiester bond. The 3', 5' phosphodiester bond not only links the nucleotides but also provide an orientation (directionality) to the molecule.

5) The double helical structure of DNA was proposed by Watson and Crick in 1953. An essential feature of the model is that all of the bases of the DNA molecule are on the inside of the double helix, whereas sugar and

phosphates are on the outside The two strands of polynucleotide run in opposite direction and are joined together by hydrogen bonds, A always pairs with T (A=T) and G pairs with C (G≡C).

6) DNA can exist in many forms. Commonly occurring forms of DNA are B-DNA, A-DNA and Z-DNA.

7) Ribonucleic acid or RNA is another major form of nucleic acid. Structurally it is similar to DNA, except the sugar units in RNA are riboses and instead of thymine, Uracil is present in RNA.

8) There are three different types of RNA. mRNA or messenger RNA carries information for protein synthesis, tRNA or transfer RNA carries amino acid from cytosol to the site of protein synthesis i.e. ribosomes while rRNA or ribosomal RNA is the integral part of ribosomes and provide structural and functional framework to the protein synthesizing machinery.

13.7 TERMINAL QUESTIONS

1) DNA is a genetic material. Explain with the help of an experiment.
2) What is the biological significance of DNA?
3) What are the various nitrogenous bases found in the nucleic acids?
4) Explain phosphodiester linkage.
5) What are the major differences between DNA and RNA?
6) What are the characteristic features of Watson and Crick DNA?
7) Distinguish between left and right handed DNA with suitable example.
8) Describe briefly various types of RNA and their functions.

13.8 ANSWERS

Self Assessment Questions

1) i) a – false; b – true; c – false; d – false; e – true;

   ii) a – nitrogenous base; b – β-glycosidic; c – RNA

   iii) d – $5^1 \rightarrow 3^1$; e – cytosine; f – C-1

2) (3) 60% (Refer chargaff' s rule page 234)

3) a – true; b – false: c – false; d – true

4) a – true; b – false; c – true; d – true, e – false

Terminal Questions

1) Nucleic acids are very appropriately referred as information storage devices of a cell. This information is organized in the form of genes, the fundamental unit of genetic information. Information flows from DNA to RNA to protein. There are two experiments which prove that DNA is the genetic material. For experimental evidence refer section13.2.

2) DNA is the basis of heredity. It is the genetic material with exception to RNA in viruses and contains all the necessary information needed for the function of a cell. It dictates the formation of proteins via RNA intermediate and regulates the activity of a cell.

3) Various nitrogenous bases found in the nucleic acids are mainly of two types: purines (adenine and guanine) and pyrimidines (cytosine, thymine and uracil). For detail refer Section 13.3.

4) Nucleotides are linked together with a phosphodiester bond to give a polymeric DNA or RNA. They join the 3' and 5' carbons of adjacent sugar moieties/nucleotides. Phosphodiester bond/linkage give nucleic acid chain a specific polarity because all the phosphodiester bonds have the same orientation along the chain.

5) DNA contains deoxyribose sugar and Adenine, Guanine, Thymine and Cytosine as nitrogenous bases while RNA consists of ribose sugar and Adenine, Guanine, Cytosine and Uracil.

6) B DNA is also known as Watson and Crick DNA, described in the year 1953. This is the physiologically occurring form of DNA. For more details refer section 13.4.

7) A DNA and B DNA are right handed while Z DNA is left handed. Refer section 13.4.

8) Various types of RNA are mRNA, tRNA and rRNA. Mainly these three types of RNA are involved in protein synthesis. Class of RNA molecules called messenger RNA (mRNA) is the information-carrying intermediate in protein synthesis. Transfer RNA (tRNA) and ribosomal RNA (rRNA), are part of the protein-synthesizing machinery. For detail refer section 13.5.

13.9 FURTHER READING

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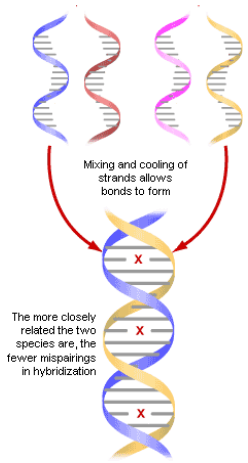

UNIT 14

DYNAMICS OF NUCLEIC ACIDS

Structure

14.1 INTRODUCTION

In the previous Unit you learnt about the structure and functional significance of nucleic acids. Now in this unit you will study about the chemical properties of nucleic acids. How chemical properties are affected by the change in temperature and pH? What are the reasons behind these changes and so on? Nucleotides as you know are the building blocks of nucleic acids. The genetic information is encrypted in the nucleotide sequences. So, apart from storing the genetic information what are the other significant functions in which nucleotides play a role? We will focus on other functions of nucleotides in section 14.3 as the carriers of energy which is stored in their energy rich phosphate bonds, as the component of coenzymes (Coenzyme A, NAD^+ and FAD) and also as second messengers (cAMP and cGMP).

Objectives

After studying this Unit, you should be able to:

- define hyperchromicity;
- explain denaturation of DNA;
- explain the difference between renaturation and hybridization;
- define T_m;
- explain the melting of DNA; and
- discuss the role of nucleotides as energy source.

14.2 NUCLEIC ACID CHEMISTRY

To understand the functional significance of nucleic acids we must understand its chemical properties. We know that DNA is the store house of genetic information and this role can be partly due to its inherent stability. So, what makes this DNA a stable molecule? Actually DNA undergoes chemical transformation very slowly. Although the changes acquired are very slow but get accumulated over a period of time. For example, ageing where slowly accumulated changes over the years result in irreversible modifications in DNA. Here we are not considering mutations. Mutations are sudden, heritable changes in the genetic material. It may be spontaneous or induced.

However, certain transient and non destructive alterations also occur in DNA, like strand separation, which is very much needed during replication and transcription. Moreover, these alterations are reversible and transient in nature.

Now let us study some of the properties of Nucleic acids.

14.2.1 UV Absorption

Nucleic acid absorbs UV light at 260 nm. For example, if we have three solutions (i) double-stranded DNA, (ii) single stranded DNA, and (iii) free bases, each at 50µg/ml, absorption values at 260nm (A_{260}) are like this:

Double stranded DNA	A_{260} = 1.00
Single stranded DNA	A_{260} = 1.37
Free bases	A_{260} = 1.60

The question which comes to our minds is why so much variation in the absorption values when concentration is same? This can be very well understood in terms of denaturation and renaturation properties of nucleic acids.

Denaturation is the separation of the two component strands of a double stranded DNA in a solution by increasing the temperature (above 80°C) or decreasing the salt concentration (alteration of pH). During nucleic acid denaturation, hydrogen bonds between the paired bases are disrupted causing unwinding of the double helix resulting in two single strands (Fig. 14.1). You must note that during denaturation process **no covalent bonds in the nucleic acids are broken**. When a nucleic acid molecule undergoes denaturation, its viscosity decreases sharply, indicating that the DNA has undergone a physical change.

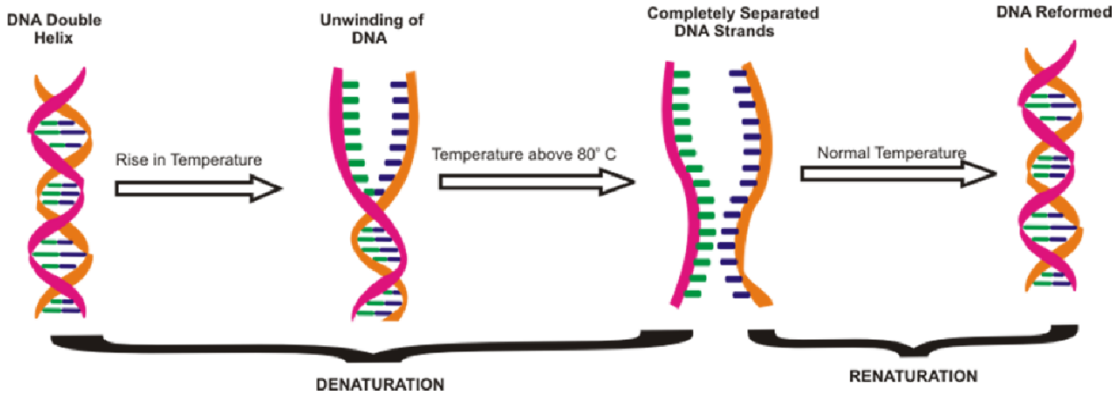

Fig. 14.1: Denaturation and reneturation process of DNA

Denaturation of the DNA molecule results in the **increase of the optical absorbance** of the purine and pyrimidine bases - a phenomenon known as **hyperchromicity**. Under physiological conditions, interaction between stacked bases in a nucleic acid leads to the decrease in the absorption of UV light relative to that of a solution with the same concentration of free nucleotides. The absorption of UV light is decreased further when two complementary nucleic acid strands are paired and this is known as **hypochromic effect**. This is why absorption values of free bases > single stranded DNA > double stranded DNA.

Thus, transition from double strand to single strand i.e. denaturation can be detected by monitoring the absorption of the UV light.

Renaturation or annealing is a process during which the separated strands of DNA join together under appropriate physiological temperature and salt conditions. It is a spontaneous, rapid and one step process. The unwound segments of the two strands spontaneously rewind/anneal, to yield the double helix. But, if the two strands are completely separated, renaturation occurs in two steps. First step is relatively slow; the two strands “find” each other by random collisions and form a short segment of complementary double helix (Fig. 14.1). The second step is much faster where the remaining unpaired bases successively pair themselves together to form the double helix.

Denaturation and renaturation are the basis of **hybridization**. This technique is a powerful tool useful in determining identical or related sequences of DNA or RNA among two different species or within the genome of a single species.

The ability of DNA to anneal has been very beautifully used for investigating genetic similarity between pools of DNA sequences. Closely related sequences form base pairs are said to be complementary. The complementarity of sequences reveals genetic closeness. When genome of several species is compared, genetic distance between two species can be determined. The more closely related the two species are, lesser is the mis-pairing in hybridization (Fig. 14.2).

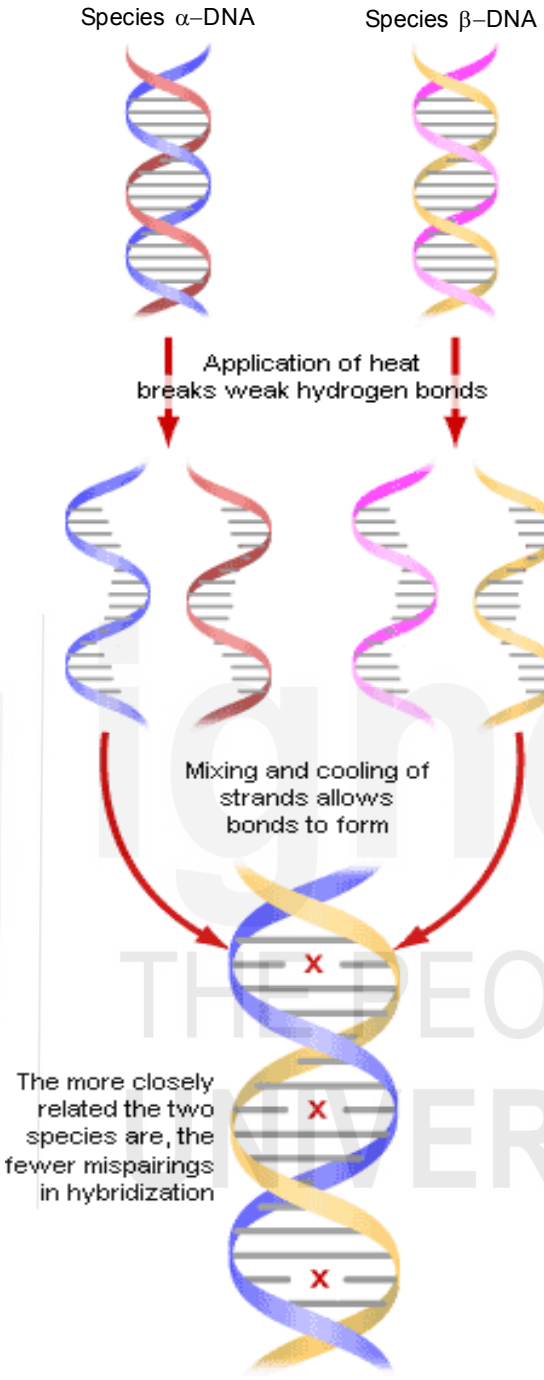

Fig. 14.2: DNA Hybridization

Hybridization technique was developed by biochemist Roy Britten in the 1960s as a way of analyzing the composition of the genome.

With the help of this technique identification of individuals from a single hair found at the crime scene is possible.

Small solutes like formamide and urea form hydrogen bonds with nitrogenous bases and thereby disrupt the interactions between bases in the duplex.

Formamide is commonly used in recombinant DNA experiments to destabilize hydrogen bonding between bases, thereby lowering the T_m.

This allows the strands of DNA or DNA-RNA hybrids to be separated at much lower temperatures and minimizes the phosphodiester bond breakage that occurs at high temperatures.

14.2.2 Effect of Temperature and pH on DNA

Exposure to extremes of temperature (above 80°C) or pH (acids or alkali) leads to disruption of hydrogen bonds in the DNA duplex.

pH below 2.3 or above 10 disrupts the base-pairing potential and thus the DNA duplex is denatured. Alkali is preferred over acid as a denaturating agent because it does not hydrolyze the glycosidic linkage between sugar and purine bases.

The denaturation or unwinding of DNA double helix due to heating is known as **melting of DNA**. Melting temperature or $\mathbf{T_m}$ is the temperature at which half of the helical structure is lost. It is the mid point of a temperature range over

which the strands of a given DNA molecule separate. T_m of a DNA depends on its base composition. DNA rich in G-C pairs melts at a higher temperature than the DNA rich in A-T pairs. Can you explain why? Because, G-C pairs have three hydrogen bonds and are strongly bonded, as compaired to A-T pairs which have two hydrogen bonds.

The melting curve of DNA is sigmoidal (Fig. 14.3). It has been estimated that under normal conditions, increase in 1% G:C in a DNA causes an increase of 0.4 °C in its melting temperature. Tm is also influenced by the salt concentration of the solution. Every ten fold increase in monovalent cations (Na^+, K^+, etc) increases T_m by 16.6 °C.

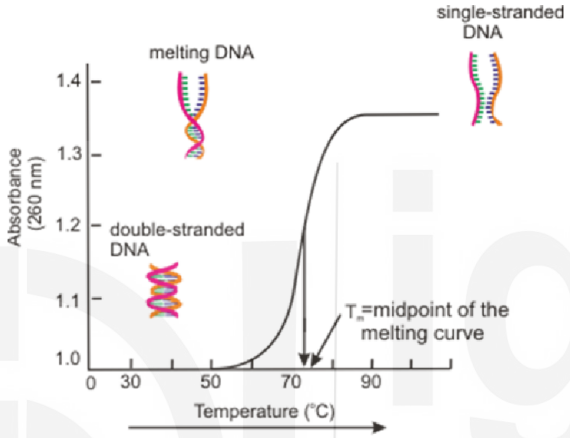

Fig.14.3: Melting curve of DNA. The temperature at the midpoint of the transition (T_m) is the melting point; it depends on pH and ionic strength and on the size and base composition of the DNA.

SAQ 1

i) Tick [ ] mark the correct statement

a) Alteration of temperature and pH can lead to denaturation of DNA. [True/False]

b) T_m of DNA rich in A–T pairs is high. [True/False]

c) Renaturation and hybridization are similar phenomenon. [True/False]

d) Melting curve of DNA is sigmoidal. [True/False]

ii) Fill in the blanks with appropriate words.

a) Every ten fold increase in monovalent cations (Na^+, K^+, etc) increases T_m by ……°C.

b) Denaturation of the DNA or RNA molecule results in the increase of the optical absorbance of the nitrogenous bases- a phenomenon referred to as ……

14.3 OTHER FUNCTIONS OF NUCLEOTIDES

In Unit 13 you learnt about the components of nucleic acids. You also learnt how these various components viz. nitrogenous bases, sugar and phosphate are linked to one another to form a nucleotide. Nucleotides are the building blocks of nucleic acids. Phosphodiester linkages join the two adjacent nucleotide molecules to form a long linear chain of DNA or RNA. In addition to this nucleotides play some other important roles as well. They serve as the energy molecule (ATP) at cellular level, components of coenzyme A and cofactors NAD^+ and FAD, and as second messengers. Let us look at these functions one by one.

14.3.1 Source of Energy

We derive our energy from the food we eat. Carbohydrates are the main source of energy apart from proteins and fats. But these food substances are not stored in our body to give energy whenever required. Rather they are metabolized and the energy is stored in the form of high energy compounds.

Inside a cell source of energy is generally ATP and this is why it is very appropriately referred as energy currency of the cell. Uridine triphosphate (UTP), Guanidine triphosphate (GTP) and Cytidine triphosphate (CTP) are also used in some of the biochemical reactions but ATP is most widely used nucleotide. So, what makes these nucleotides the source of energy? Let's understand this with ATP as an example. In adenosine triphosphate, three phosphate groups are attached with the 5'-OH of ribose (Fig.14.4).

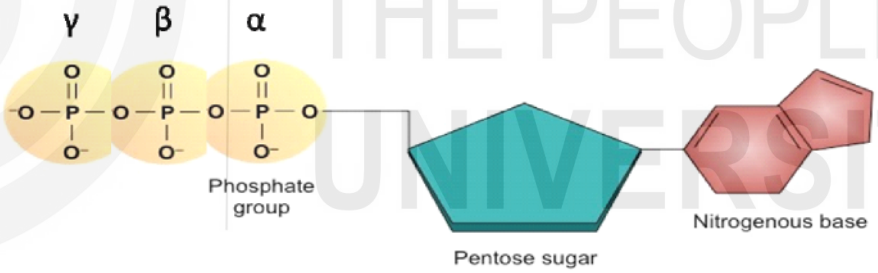

Fig. 14.4: Structure of Adenosine triphosphate

Now look at Fig. 14.4, the three phosphates are generally numbered as α (alpha), β (beta), and γ (gamma). The α-phosphate is linked to ribose sugar with an ester linkage. You may recall this is the same phosphodiester linkage which joins two adjacent nucleotides. However, α–β, and β-γ phosphates are linked by anhydride bonds.

These ester and anhydride bonds release large amount of energy on hydrolysis. Anhydride bond yields about 30kJ/mol, whereas ester bond yields 14kJ/mol energy under standard conditions. Thus, at cellular level for driving various biochemical reactions energy is obtained from these nucleotide molecules.

14.3.2 Component of Co-enzymes/Co-factors

You would have studied about coenzymes and cofactors in class XII biology. Various enzymes require these small molecules for their biological activity.

These helper molecules assist the enzymes in transferring groups from one moiety to another. Coenzyme A, NAD^+ (Nicotinamide adenine dinucleotide) and FAD (Flavin adenine dinucleotide) contain adenosine moiety in their structure (Fig. 14.5). Coenzyme A is involved in acyl group ($-COCH_2$) transfer while NAD^+ and FAD are involved in electron transfer (hydrogen atom). Although adenosine moiety is not directly involved in group transfer reactions but experimental evidences show that removal of adenosine drastically reduces the cofactor activity.

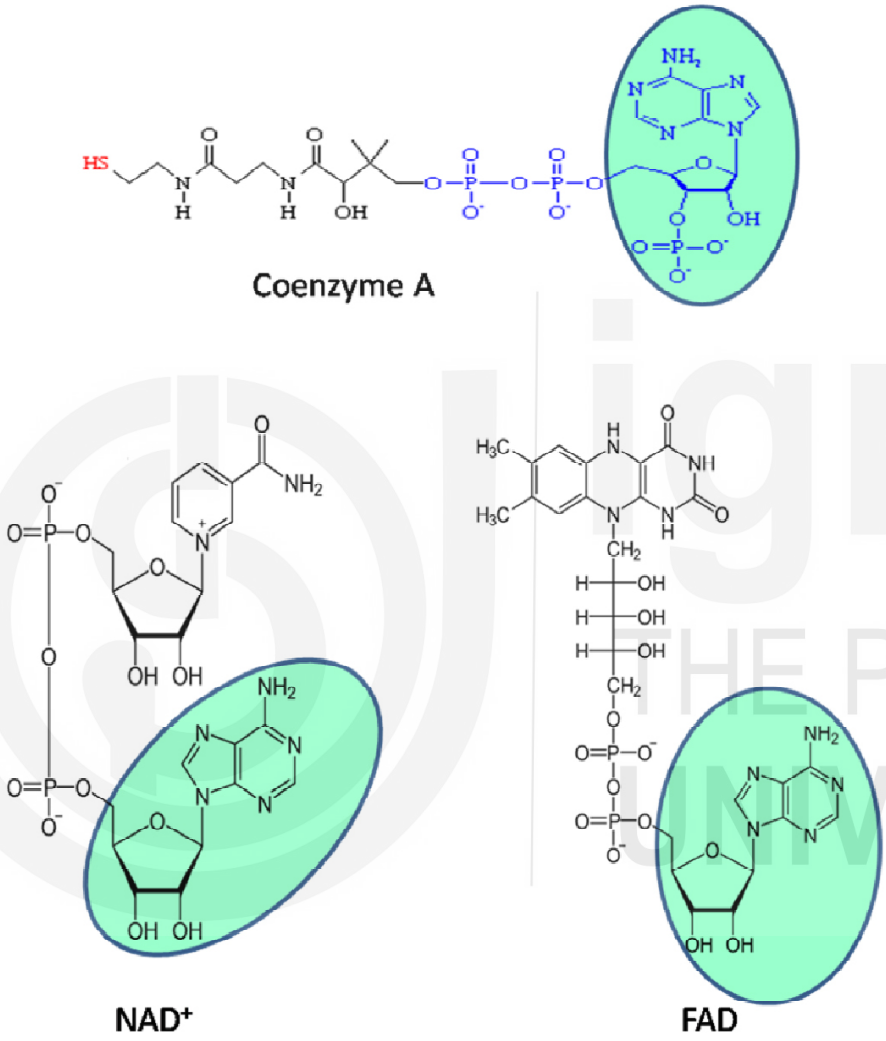

Fig. 14.5: Structure of Coenzyme A, NAD^+ and FAD

14.3.3 Second Messengers

Binding of hormone (first messenger) to receptor is highly specific and initiates a series of events which leads to generation of so-called **second messengers (cAMP, cGMP)** within the cell (you will study later in detail in Core Course BBCCT-119, Hormone: Biochemistry and function). The second messenger then triggers a series of molecular interaction, also termed as signal transduction mechanism leading to a specific response.

Cyclic AMP (cAMP) is one of the most common second messengers. In response to hormone action, adenylate cyclase, a hormone associated with the inner side of cell membrane catalyzes the formation of cyclic AMP from ATP. Cyclic AMP is adenosine 3', 5'-cyclic monophosphate. Guanosine 3',

5'-cyclic monophosphate or cyclic GMP (cGMP) is another nucleotide functioning as secondary messenger (Fig. 14.6).

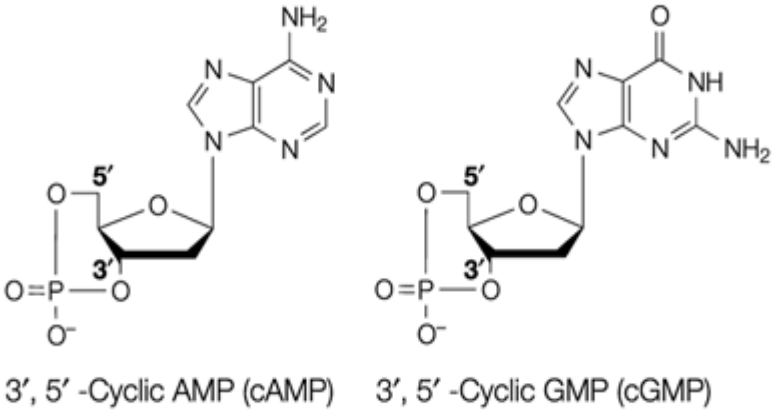

Fig. 14.6: Structure of Cyclic AMP, cyclic GMP

SAQ 2

Fill in the blanks with appropriate words.

a) Anhydride bond yields about ..... kJ/mol energy on hydrolysis.

b) cAMP is a ..... messenger.

c) FAD (Flavin adenine dinucleotide) contains ..... nucleotide moiety in their structure.

d) Adenylate cyclase, a hormone associated with the inner side of cell membrane catalyzes the formation of cyclic AMP from .....

14.4 SUMMARY

1) Nucleic acids exhibit certain chemical properties. They undergo denaturation and renaturation with change in temperature and pH.

2) Denaturation of DNA or RNA molecule results in an increase in the optical absorbance of the nitrogenous bases, this phenomenon is known as hyperchromicity.

3) Renaturation or annealing is a process during which the separated strands of DNA join together under appropriate physiological temperature and salt conditions.

4) Hybridization is a technique based on renaturation and is useful in revealing genetic closeness of two species.

5) The denaturation of DNA double helix due to heating is known as melting of DNA. Melting temperature or T_m is the temperature at which half of the helical structure is lost.

6) In addition to building nucleic acids, nucleotides play some other important roles as well. They serve as the energy molecule (ATP) at cellular level, components of coenzyme A and cofactors NAD^+ and FAD, and as second messengers.

14.5 TERMINAL QUESTIONS

1) Explain denaturation and renaturation.
2) What is melting of DNA? A sample of DNA had a melting temperature of 82 °C while another showed a melting temperature of 86.5 °C. What do you conclude regarding the base composition of the two samples?
3) How hybridization helps in identification of an individual? Explain.
4) Discuss the role of nucleotide as coenzyme.
5) What makes ATP an energy rich molecule?

14.6 ANSWERS

Self Assessment Questions

1) i) a – true; b – false; c – true: d – true
   ii) a – 16.6; b – hyperchromicity
2) a – 30; b – secondary; c – adenosine; d – ATP

Terminal Questions

1) Denaturation is a phenomenon where two strands of a nucleic acid either DNA or RNA unwind and undergo separation while renaturation which is also known as annealing the two separated strands join together under appropriate physiological condition of temperature and pH.
2) Sample with high melting temperature is rich in G-C bases.
3) Denaturation and renaturation are the basis of hybridization. This technique is a powerful tool useful in determining identical or related sequences of DNA or RNA among two different species or within the genome of a single species. Closely related sequences form base paired double helices readily and are said to be complementary. The complementarity of sequences reveals genetic closeness. Based on this genetic closeness between two individuals identification is done using other sophisticated tools as well.
4) Various enzymes require coenzymes/cofactors for their biological activity. These helper molecules assist the enzymes in transferring groups from one moiety to another. Coenzyme A, NAD^+ (Nicotinamide adenine dinucleotide) and FAD (Flavin adenine dinucleotide) contain adenosine moiety in their structure. Coenzyme A is involved in acyl group ($-COCH_2$) transfer while NAD^+ and FAD are involved in electron transfer (hydrogen atom).
5) ATP contains one ester and two anhydride bonds. These bonds are rich in energy. Ester and anhydride bonds release large amount of energy on hydrolysis. Anhydride bond yields about 30kJ/mol, whereas ester bond yields 14kJ/mol energy under standard conditions. Thus, at cellular level for driving various biochemical reactions energy is obtained from these nucleotide molecules.

14.7 FURTHER READING

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