10. NUCLEIC ACIDS

10.1 DNA Structure and Function

Nucleotides are the constituents of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the molecular repositories of genetic information. The structure of every protein, every biomolecule and cellular component is a product of information stored in nucleic acids. The ability to store and transmit genetic information from one generation to the next is fundamental for continuity of life. Nucleotides have various roles in cellular metabolism. They are the energy currency in metabolic pathways and the structural components of metabolic intermediates. Moreover, they are cofactors of some enzymes and mediator molecules that respond hormones and other extracellular stimuli.

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

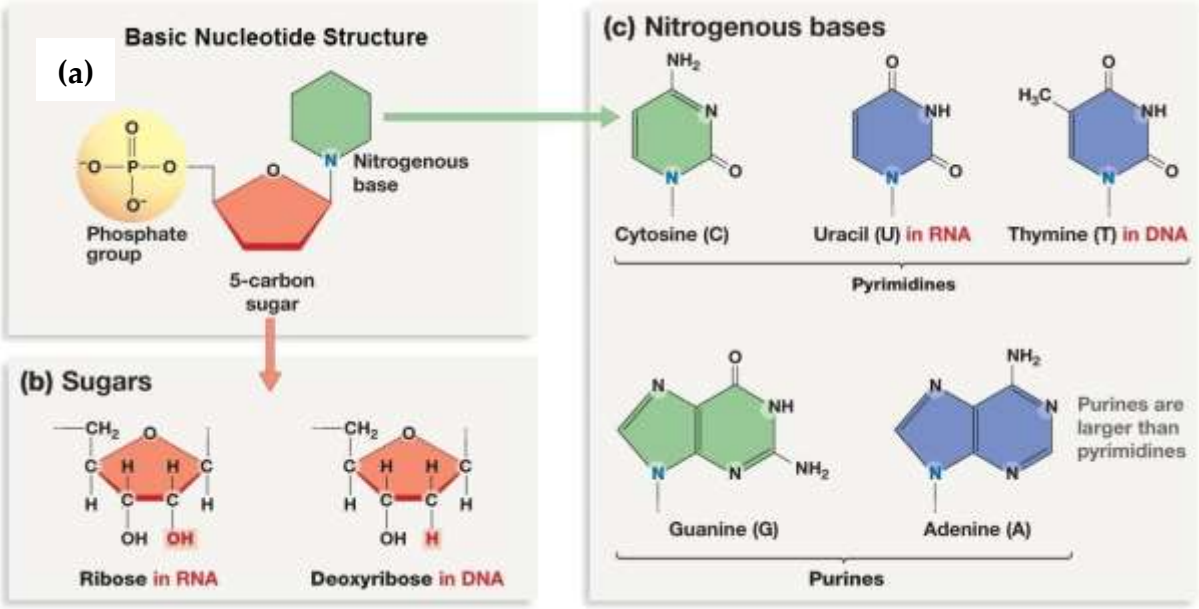

Figure 10.1 (a) Nucleotide and (b) Sugar structure (c) Nitrogenous bases

Both DNA and RNA contain two purine bases, adenine (A) and guanine (G), and pyrimidines. In both DNA and RNA one of the pyrimidines is cytosine (C), but the second major pyrimidine is not the same in both: it is thymine (T) in DNA and uracil (U) in RNA.

Nucleic acids have two kinds of pentoses. The deoxyribonucleotide units of DNA contain **2deoxy-D-ribose**, and the ribonucleotide units of RNA contain **D-ribose**.

Figure 10.2 Phosphodiester linkages in the covalent backbone of DNA and RNA (primer structure).

The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group **"bridges**," in which the 5-phosphate group of one nucleotide unit is joined to the 3-hydroxyl group of the next nucleotide, creating a **phosphodiester bond.** Thus the covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular interval.

Figure 10.3 Hydrogen-bonding patterns in the base pairs defined by Watson and Crick (seconder structure).

The backbones of both DNA and RNA are hydrophilic. The most important functional groups of pyrimidines and purines are ring nitrogens, carbonyl groups, and amino groups. Hydrogen bonds involving the amino and carbonyl groups are the second important mode of interaction between bases in nucleic acid molecules. Hydrogen bonds between bases permit a complementary association of two (and occasionally three or four) strands of nucleic acid. The most important hydrogen-bonding patterns are those defined by James D. Watson and Francis Crick in 1953, in which A bonds specifically to T (or U) and G bonds to C.

In 1953 Watson and Crick postulated a three dimensional model of DNA structure that accounted for all the available data. It consists of two helical DNA chains wound around the same axis to form a right handed double helix. The hydrophilic backbones of alternating deoxyribose and phosphate groups located at the outside of the double helix are surrounded by water molecules. The purine and pyrimidine bases of both strands are stacked inside the double helix, with their hydrophobic and nearly planar ring structures very close together. Each nucleotide base of one strand is paired in the same plane with a base of the other strand. Watson and Crick found that structure created by the hydrogen-bonded base pairs, G with C and A with T, suitable with Chargaff's rule. Chargaff's rule states that three hydrogen bonds can form between G and C, but only two can form between A and T.

As a result of the base pairing between two DNA strands, 10 base pairs build up one complete DNA coiling of two strands around each other. 3D conformation is formed based on this

coiling in space. Hydrogen bonds and hydrophobic interactions provide thermodynamic stability to the double stranded DNA molecule (see Fig. 10.4)

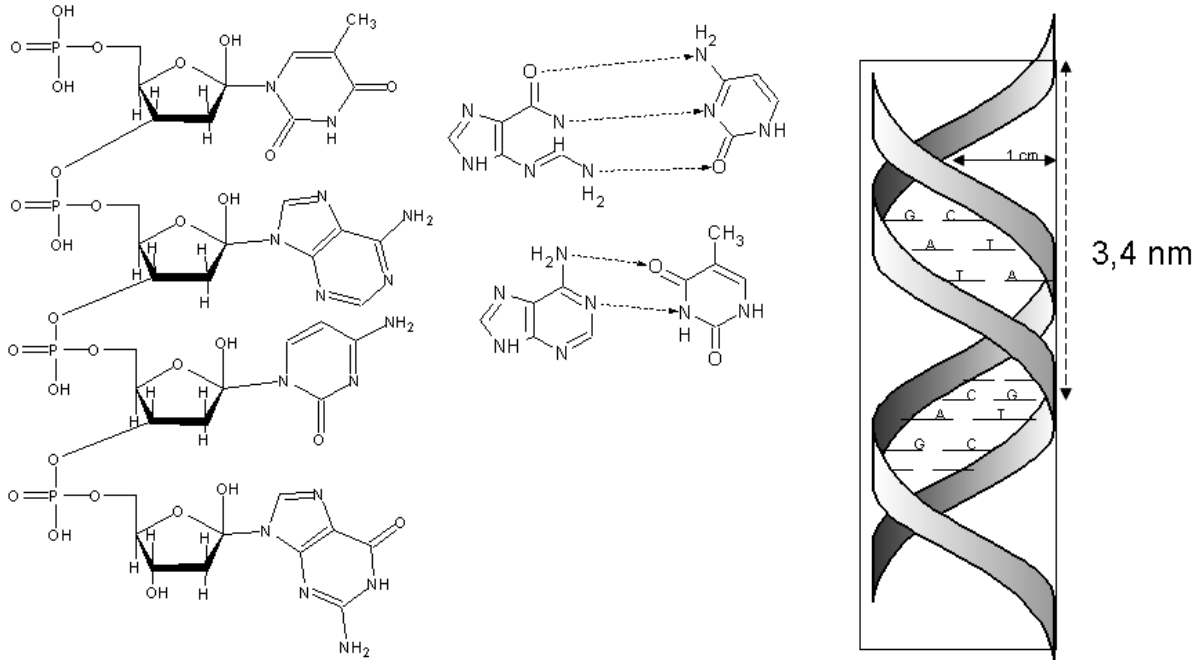

Figure 10.4 Primary, secondary and tertiary structure of DNA

Denaturation and Renaturation

Solutions of carefully isolated, native DNA are highly viscous at pH 7.0 and room temperature (25 ^{o}C). When such a solution is subjected to extremes of pH or to temperatures above 80 ^{o}C, its viscosity decreases sharply, indicating that the DNA has undergone a physical change. Just as heat and extremes of pH denature globular proteins, they also cause denaturation, or melting, of double-helical DNA. Disruption of the hydrogen bonds between paired bases and of base stacking causes unwinding of the double helix to form two single strands, separate from each other along the entire length or part of the length (partial denaturation) of the molecule. No covalent bonds in the DNA are broken.

The close interaction between stacked bases in a nucleic acid has the effect of decreasing its absorption of UV light relative to that of a solution with the same concentration of free nucleotides, and the absorption is decreased further when two complementary nucleic acids strands are paired. This is called **the hypochromic effect**. Denaturation of a double-stranded nucleic acid produces the opposite result: an increase in absorption called the **hyperchromic effect**. The transition from

double-stranded DNA to the single-stranded, denatured form can thus be detected by monitoring the absorption of UV light at 260 nm.

DNA has a characteristic denaturation temperature, or melting point (Tm) which increases as the GC base pair content in the DNA increases. This is because GC base pairs, with three hydrogen bonds, require more heat energy to dissociate than the heat energy required to dissociate A=T base pairs which has two hydrogen bonds. The melting point of a given DNA, under fixed conditions of pH and ionic strength, can yield an estimate of its base composition. If denaturation conditions are carefully controlled, regions that are rich in A=T base pairs will specifically denature while most of the DNA remains double-stranded.

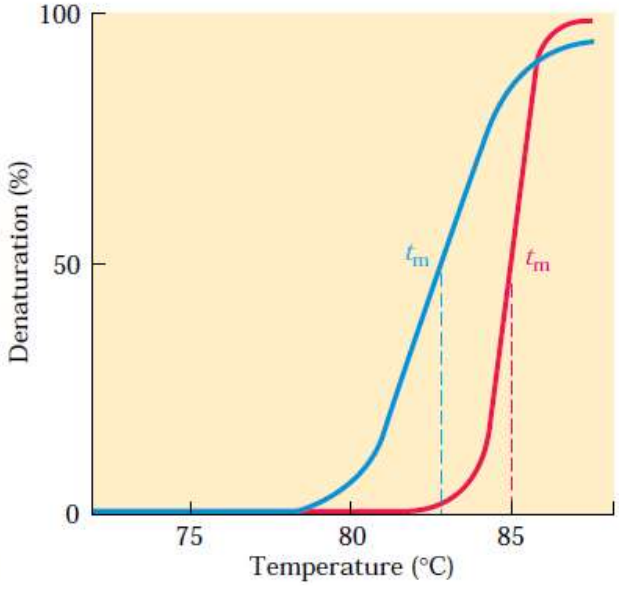

Figure 10.5 Heat denaturation of DNA and melting point (Tm)

Experiments

10.2 Qualitative Tests for Nucleic Acids

10.2.1 Qualitative Tests for Purines

Principle:

Hydrolysis of N_beta glucosidic bonds between purine bases and ribose or deoxyribose results in a release of purine bases (A and G) caused by NH_3. Purine bases form insoluble white salts with Ag^+ in tissue hydrolysate.

Procedure:

1. Take 1 mL tissue hydrolysate, add litmus paper which is a small acid indicator paper (red) into tube and several drop NH_3 until the red color of litmus paper turns into blue.

2. After, add 1-2 mL 2.6% $AgNO_3$. The while salt precipitation indicates purine presence in the medium.

10.2.2 Bial's Test for Quantitative Analysis of Pentoses

Principle

Bial's Test is performed to determine the presence of ribose (pentoses 5C sugars) in muscle DNA. The components of this reagent are resorcinol, HCl, and ferric chloride. In this test, the pentose is dehydrated to form furfural and the solution turns bluish and a precipitate may form.

Procedure:

1. To 1 ml of tissue hydrolysate solution, add 3 mL of Bial's reagent and boil.
2. Upon boiling, the green-blue color formed.

10.2.3 Qualitative Test for Phosphates

Principle

Yellow precipitate is obtained if phosphates of DNA present due to reaction of ammonium molybdate with phosphoammonium molybdate which is a positive result.

Procedure:

1. Take 1 mL tissue hydrolysate solution.
2. Add 1 mL 5% ammonium molybdate solution.
3. Yellow crystal precipitation is an indication for presence of phosphate ions in the medium.

10.3 Quantitative Test for Nucleic Acids

10.3.1 DNA Analysis via Diphenylammine Method

Principle

The principle underlying estimation of DNA using diphenylamine is the reaction of diphenylamine with deoxyribose sugar producing blue-coloured complex. The DNA sample is boiled under extremely acidic conditions; this causes depurination of the DNA followed by dehydration of deoxyribose sugar into a highly reactive **ω-hydroxylevulinylaldehyde.** The ω-hydroxylevulinylaldehyde, under acidic conditions, reacts with diphenylamine to produce a blue-coloured complex that absorbs at 595 nm. The mechanism of reaction of deoxyribose sugar with diphenylamine is shown in attached figure.

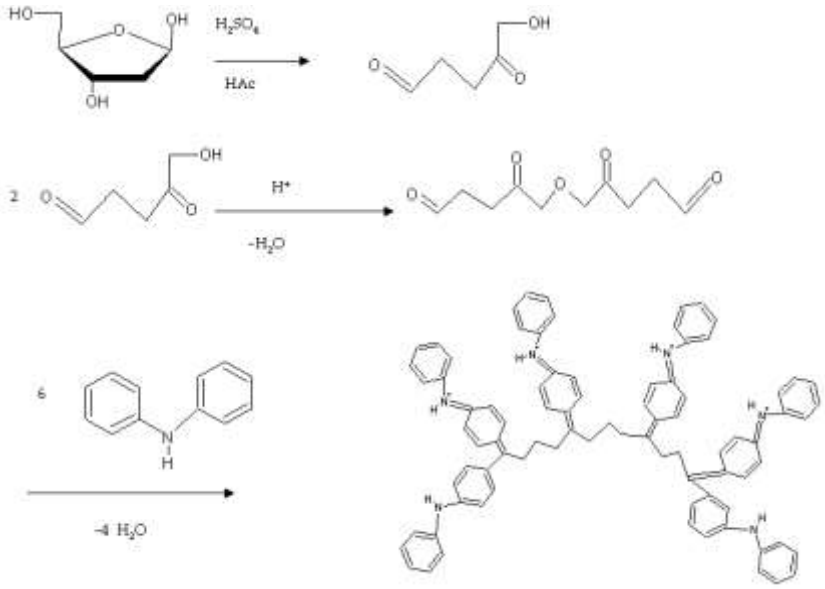

Figure 10.6 Determination of DNA concentration using diphenylammine

Reagents and Solution:

Standard DNA: Dissolve 50 mg DNA in 100 ml PCA while boiling it.

Muscle hydrolysate: Boil 1 g muscle in 20 ml 1 N PCA and filtrate. The filtrate is muscle hydrolysate that is going to be used in the experiments.

B Reactive: Dissolve 1.5 g dipenylammine in 100 ml concentrated acetic acid, 1.5 ml conc. H_2SO_4 and 0.5 ml hydrous acetaldehyde (16 mg/ml).

Procedure:

Take 5 tubes and add solutions according to the table

Solutions (mL)	**Blank**	**Sample**	**Std.1 DNA**	**Std.2 DNA**	**Std.3 DNA**
Standard DNA	-	-	0.2	0.4	0.8
Tissue hydrolysate	-	1.6	-	-	-
1N PCA	2	0.4	1.8	1.6	1.2
B Reactive	4	4	4	4	4

Put tubes into a boiling water bath and keep boiling for 10 min. Absorbance of the known standards and unknown sample are determined by spectrophotometer at 600 nm. The concentration of the unknown sample can be directly calculated from the standard graph.

10.3.2 Extraction of DNA from Pea

Procedure:

1. Take 30 mL peas. They are must be cold.
2. Mash the peas and add 30 mL cold distilled water.
3. Filter through several layers of gauze into a beaker.
4. Add 10 mL of detergent and mix well.
5. Take 3 tubes and add 3 mL of this solution to each.
6. Add a few (2 or 3) drop lens solution to each tube and slowly add 3 mL 70% cold EtOH.
7. Leave the tubes, undisturbed, for 30 min in room temperature.
8. DNA will be released to the solution white forms white cloudy precipitate into the upper (ethanol) layer.

Figure 10.7 Appearance of isolated DNA molecules from pea