علوم الحياة / كورس الأول

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المادة: جزيئي عملي / 2+1

المرحلة: الرابعة

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السعر (500)

Molecular structure of nucleic acid

- DNA and RNA are molecular structures compose of regular repeating polymers formed from nucleotides called polynucleotide.

- The basic building blocks of nucleic acids are nucleotides composed of:
  1. Nitrogenous base of purines (A,G) and pyrimidines [C, T (DNA) & U (RNA)].
  2. Pentose carbon sugar (2-deoxyribose in DNA & ribose in RNA).
  3. Phosphate group.

- Nitrogenous base bind to pentose carbone sugar by glycosidic linkage forming nucleoside.

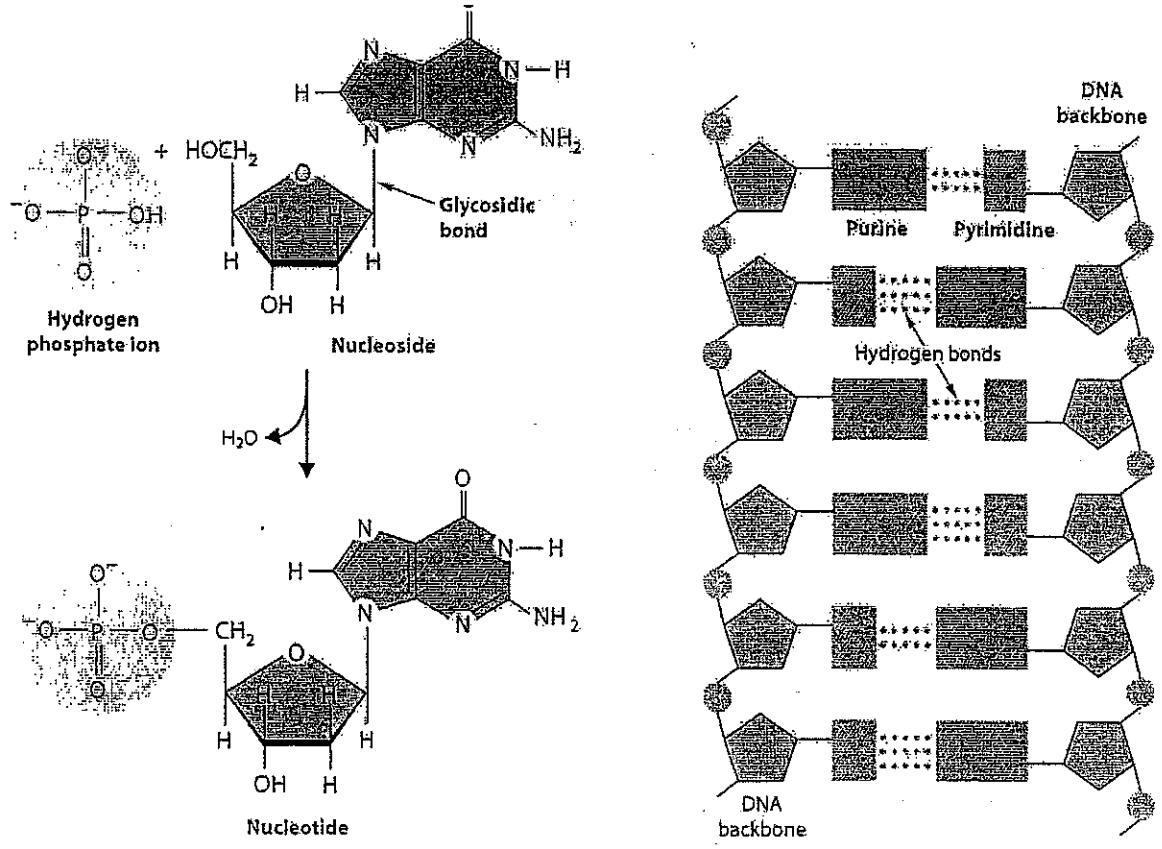
- Nucleoside binds to phosphate forming nucleotide by attachment of phosphate to the 5′ position of nucleoside by an ester bond.

- Nucleotides joined together by formation second ester bond between 5′ phosphate group of one nucleotide and the 3′ hydroxyl group of another nucleotide generating 5′ to 3′ phosphodiester bond which represent backbone of DNA and RNA.

- DNA molecule described as antiparallel structure has two polynucleotide strands, each have free 5′ at one end and 3′ to another end in opposite directions. RNA molecule has one strand.

- The antiparallel strands of DNA are held together by:
  1. hydrogen bonds between Nitrogenous bases.
  2. Partly hydrophobic interactions between staked base pairs.

Diameter of double helix DNA = 20° A
Double of nucleotides = base pair (bp)
Kilo base pair (Kbp) = 10^3 bp
1bp = 618 dalton ⟩ 1000 bp (1 Kbp)= 618000 dalton
Mega dalton (mega dal.) = 10^6 Dalton
Three nucleotides = codon (coded for one amino acid)
The average of M.wt to bp = 618 dalton
The average of M.wt to ribonuclotide = 320 dalton
The average of M.wt to amino acid =120 dalton



Calculation of Molecular structure of DNA

Ex. 1 Calculate the length, volume and number of turns to double helix DNA molecule if the M.wt of this DNA molecule is equal to 3×10^7 dalton.

Solution: No. of nucleotide pairs = 3×10^7 dalton / 618 dalton= 48544 bp

Length of DNA strand = 48544× 3.4A= 165049 A= 16.5×10^{-4} cm

The shape of DNA molecule is cylindrical. The length of cylinder is 16.5×10^{-4} cm and the diameter is 20A (20×10^{-8} cm)

Volume = $3.14\times r^2 \times L$

vol. = $3.14\times (10\times10^{-8}\ cm)^2 \times (16.5\times10^{-4}) = 5.18\times10^{-17}\ cm^3$

No. of turns = 48544 bp / 10 bp $turn^{-1}$ = 4854 turn

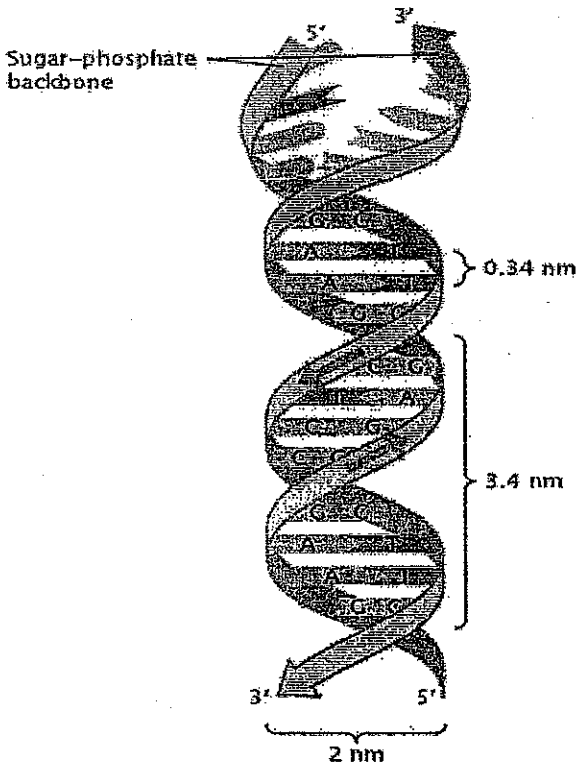
Ex. 2 The M.wt of T4 DNA is equal to 1.3×10^8 dalton, calculate:

a. No. of amino acid that coded from this DNA.
b. No. of proteins which have 55000 dalton that coded by T4 DNA.

Solution: No. of bp of T4 DNA 1.3×10^8 dalton/ 618 dalton= 2.1×10^5 bp
No. of codons of T4 DNA= 2.1×10^5 bp / 3= 7×10^4 codons
No. of amino acids = 55000/ 120= 458 amino acids
No. of different proteins = 7×10^4 / 458 = 153 proteins

Ex. 3 Calculate the M.wt of mRNA that coded to protein if the M.wt of this protein is equal to 75000 dalton.

Solution: No. of amino acids = 75000/ 120= 625 amino acids
No. of ribonuclotide of mRNA = 3 × 625 =1875 ribonuclotides
M.wt of mRNA = $1875\times320= 6\times10^5$ dalton



Buffers

Buffer is a solution containing either a weak acid and its salt or a weak base and its salt, which is resistant to changes in pH. **Buffer** is a solution which resists change in pH value on dilution or on addition on an acid or alkali solution such as phosphate buffer, citrate buffer, Tris buffer and blood.

Buffer capacity represents the ability of a buffer to resist changes in pH.

Buffering agent, the weak acid or weak base in a buffer solution.

Properties of buffer solution any buffer solution acidic or basic possesses of following properties:

1. It has defined pH.
2. It has pH value does not change with long time or on dilution.
3. It has pH value remains practically constant when small quantities of strong acid or strong base are added to the buffer.

The purpose function of biological systems require control of pH, since most metabolic processes are inactivated outside a certain narrow range of concentration of hydrogen ions.

Kinds of buffers used in molecular biology

TBE buffer: Tris-borate -EDTA buffer.
TSE buffer: Tris- sucrose -EDTA buffer.
STET buffer: sucrose- Tris- EDTA-TritonX-100

These buffers are often used in procedures of nucleic acid such as in electrophoresis.

Tris-acid solutions are effective buffers for slightly basic conditions, which keep DNA deprotenated and soluble in water.

EDTA is a chelator agent of divalent cations, particularly of Mg^{+2} that act as co-factor for many enzymes including contaminant nucleases therefore the role of EDTA is protected the nucleic acid from enzymatic degradation.

Mg^{+2} is also act as co-factor for many useful DNA-modifying enzymes such as restriction enzymes and DNA polymerase, the concentration of EDTA in TBE or TSE buffers is generally kept low (typically around at 1 mM).

Methodology

1. Preparation of TE buffer

TE buffer contains of (10 mM of Tris + 1 mM of EDTA)

- Prepare stock solution of 500 mM of Tris (M.wt=121) and 500 mM of EDTA (M.wt=372).
- Prepare working solution with (V=10 ml) from stock solution by dilution of stock solution to get 10 mM of Tris and 1 mM of EDTA.
- Measure pH by pH meter and adjust pH=8.

2. Preparation of STET buffer

STET buffer contains equal volume of [8% sucrose (w /v%) +50 mM Tris + 10 mM EDTA + 3% TritonX-100 (v/v%)].

- Weight 8g sucrose, 0.605g Tris, 0.372g EDTA.
- Add 3ml of TritonX-100 to the Mixture above.
- Complete it to 100 ml with D.W.
- Measure pH by pH meter and adjust pH=8.