

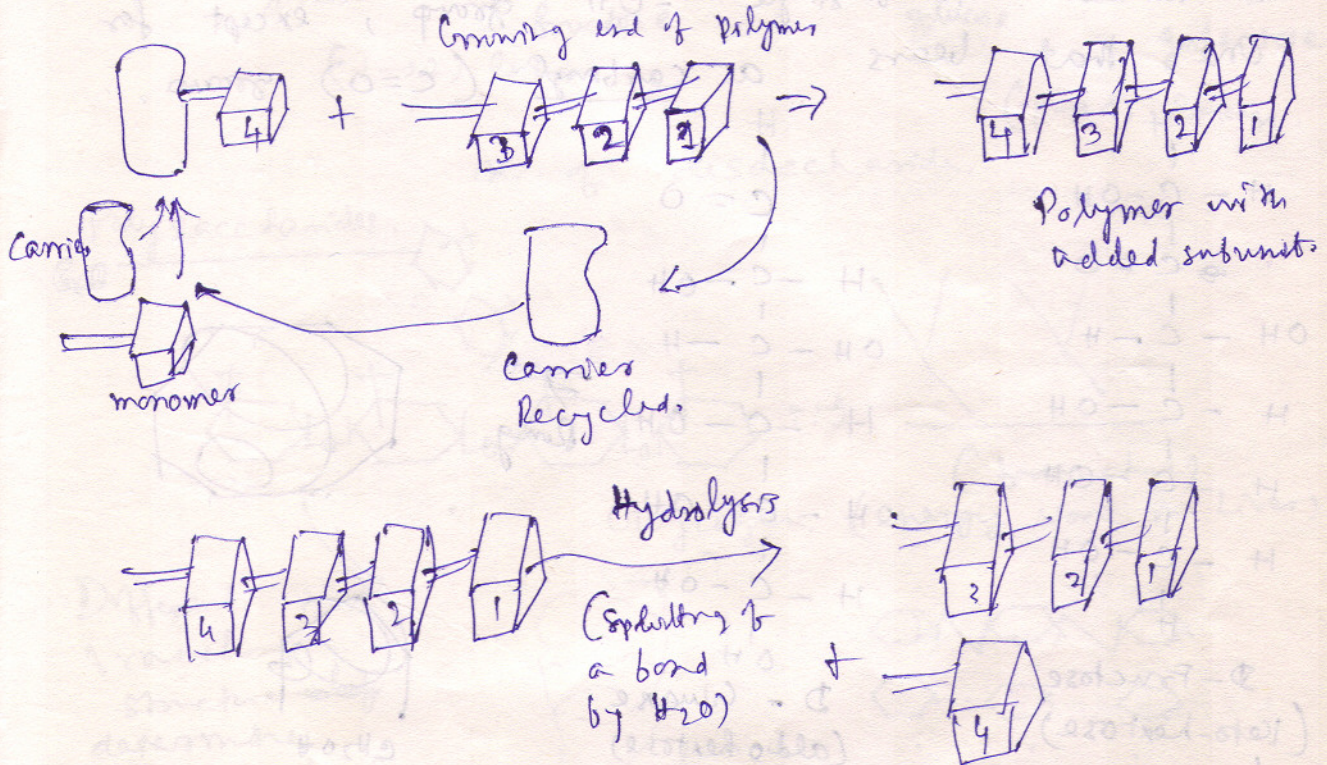
Biological Molecules.

Macromolecules
(dozen to millions
of carbon atoms)

→ Polysaccharides
→ Proteins
→ Nucleic Acids
→ Lipids

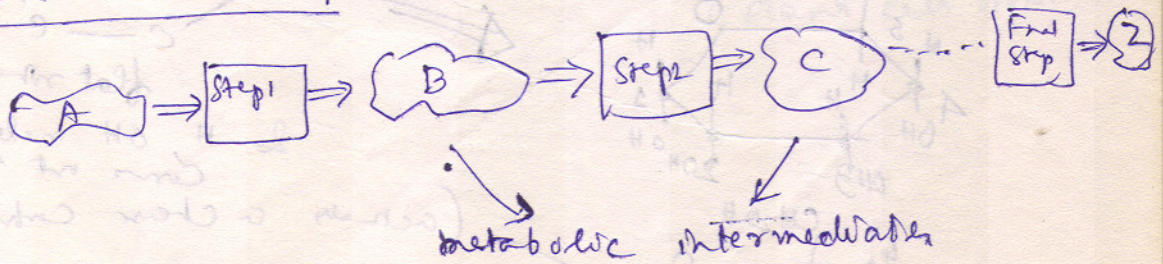
Precursors

→ sugars
→ Amino Acids
→ nucleotides
→ fatty acids



⇒ All these reactions are catalyzed by enzymes.

Metabolic Pathways:

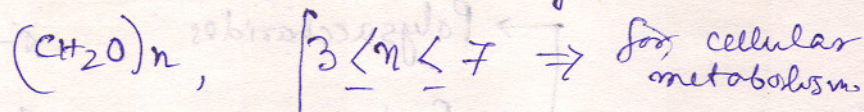


Other molecules:

Vitamins, Steroid, ATP, Urea etc.
Bulk of the cell is composed of macromolecules.

#

Carbohydrates → monosaccharides & polysaccharides.

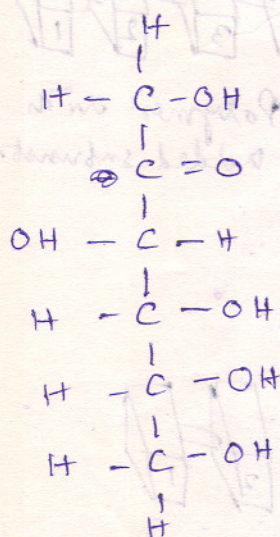


trioses, tetroses, pentoses, hexoses, heptoses

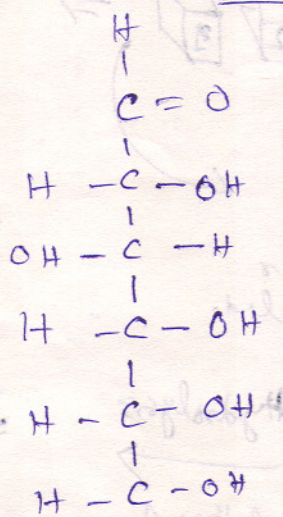
↓
ribose

↓
glucose

Each of the carbon atoms of the backbone is linked to a single -OH group, except for one that bears a carbonyl ($C=O$) group.

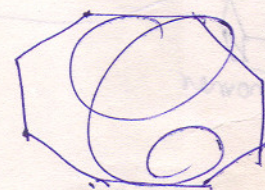


D-Fructose
(keto-hexose)
inside the carbon chain
($C=O$)

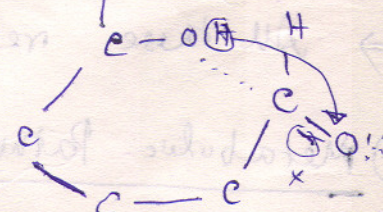


D-glucose
(aldohexose)
↓
 $C=O$ at the terminal location

→
ring



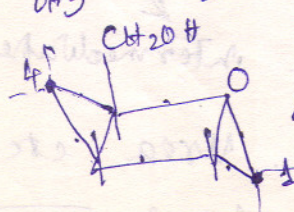
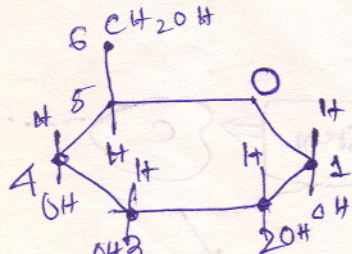
CH_2OH



flat ring

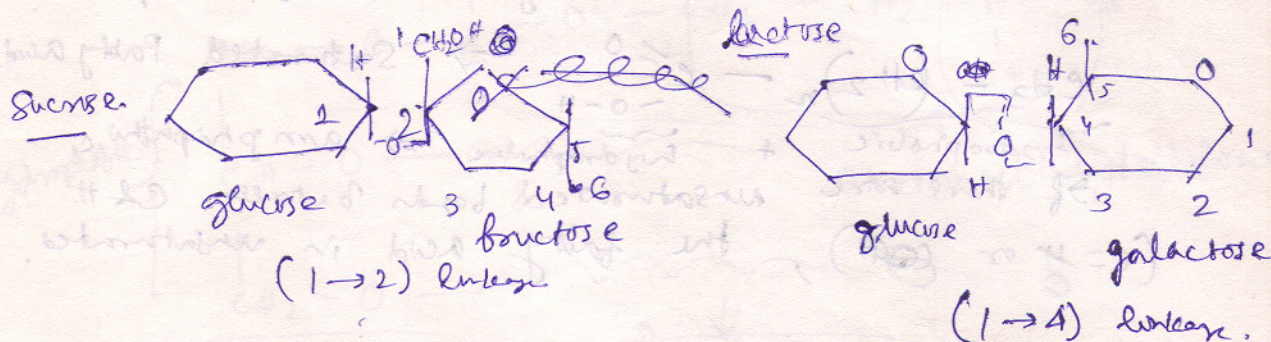
& H OH molecules
comes out of it

(actually a chair conformation)



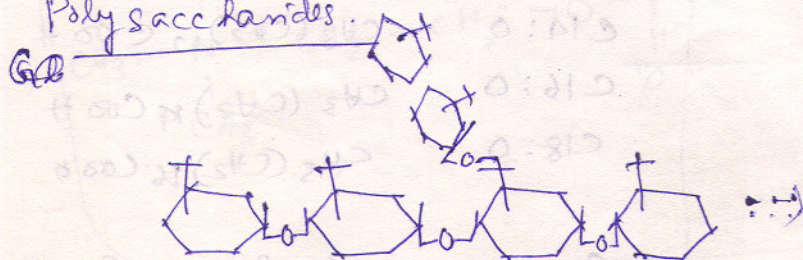
Linking sugars;

glycosidic bonds $\Rightarrow$ between C1 of one sugar & hydroxyl group of another, generating a $-C-O-C-$ linkage.



ex. of disaccharides.

Poly saccharides.

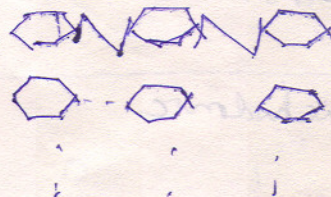


(branched)
Glycogen. (energy store in Liver)

Different branching structure ~~and~~ determine wide variety of chemical prop.



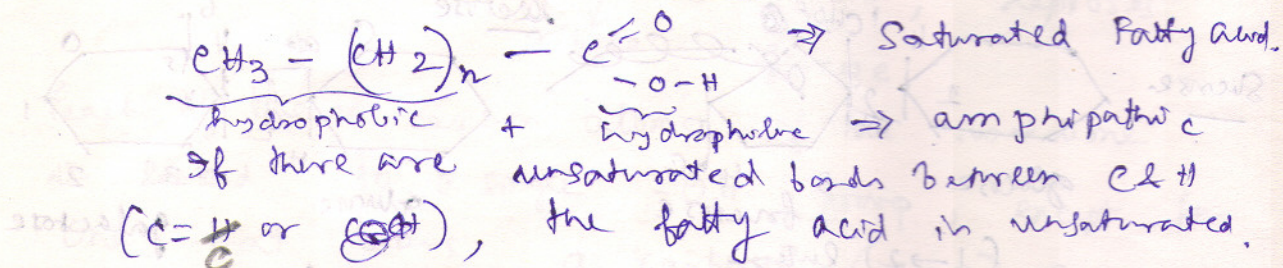
Starch (helical)
(Energy storage in plants)



cellulose
(array)
(plant cell wall)

Lipids: $\begin{cases} \text{Fats} \\ \text{steroids} \\ \text{phospholipids} \end{cases}$

Fats: Fatty acids: long unbranched hydrocarbon chains with a single carboxyl group at one end.



e.g.

Saturated Fatty Acids

Abbreviation

Chemical Formula

Myristic

C14:0

$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$

Palmitic

C16:0

$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$

Stearic

C18:0

$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$

Unsaturated

Oleic

C18:1

$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$

Linoleic

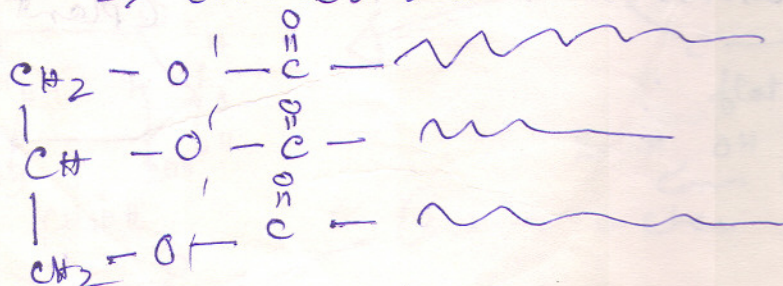
C18:2

$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$

Arachidonic

C20:4

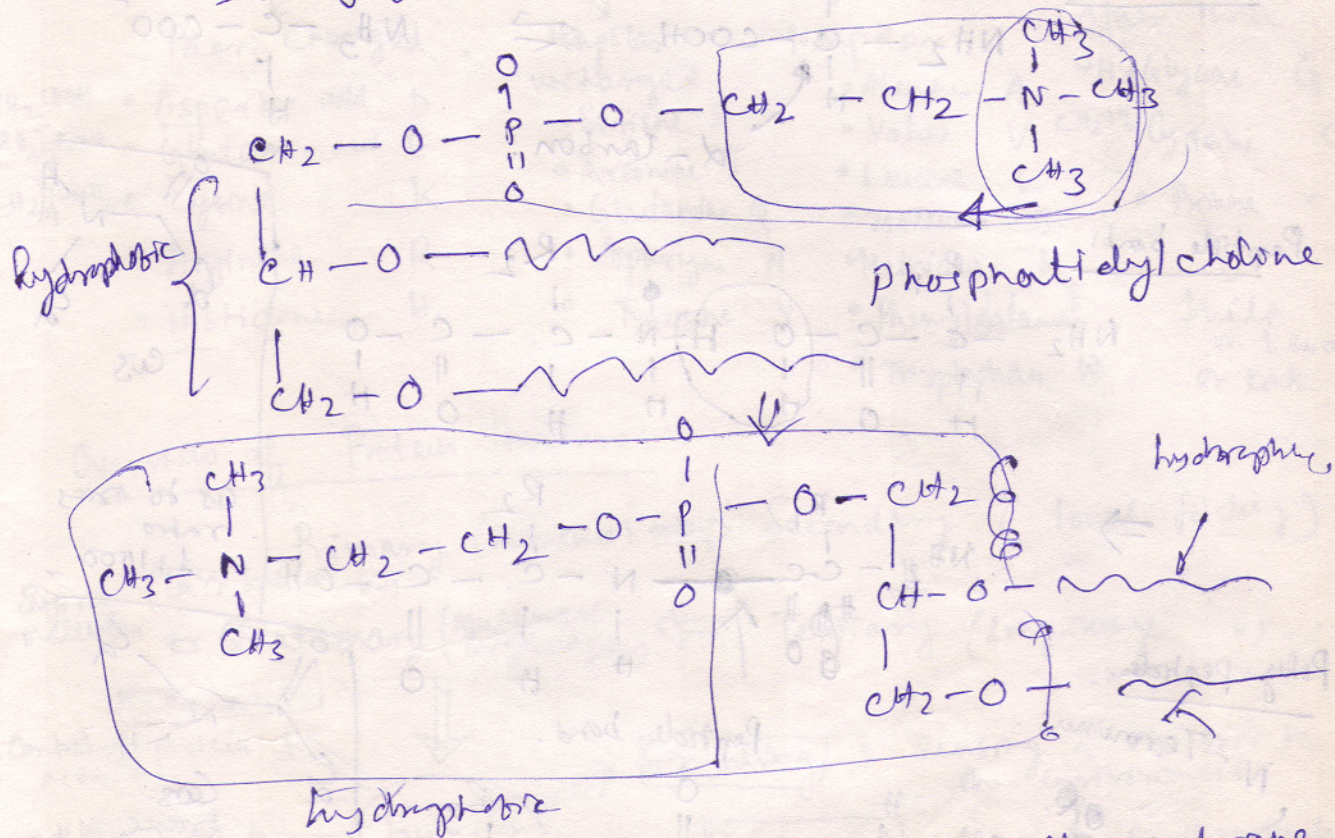
Fats: consists of glycerol molecules linked by ester bonds to three fatty acids.
 $\Rightarrow$ triacylglycerol



Fats that are liquid in room temp are called oils. Fats have polar group & not soluble in water

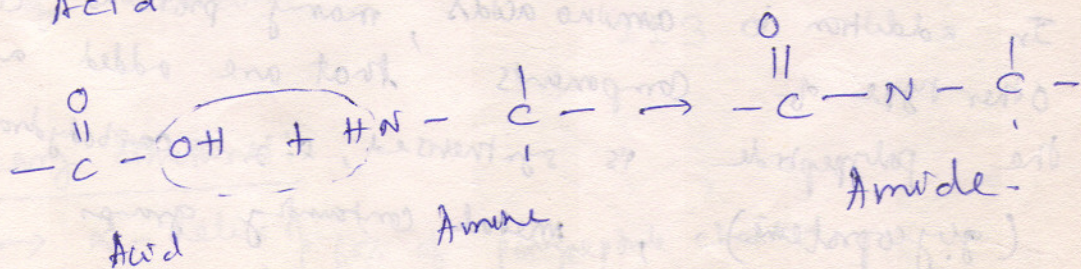
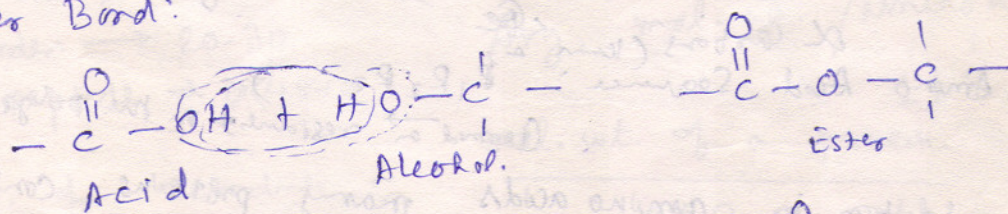
Phospholipids:

Diacylglycerol + phosphate + polar group
(e.g. choline).



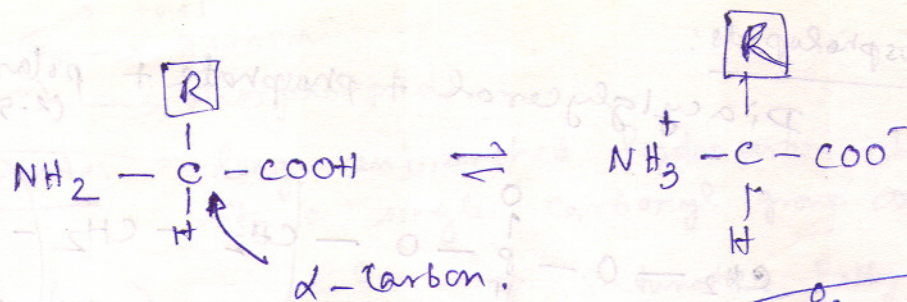
Phospholipids primarily function in cell membrane.

Ester Bond:

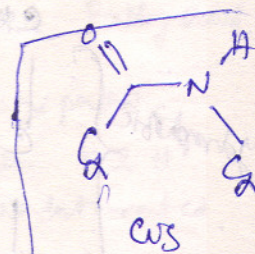
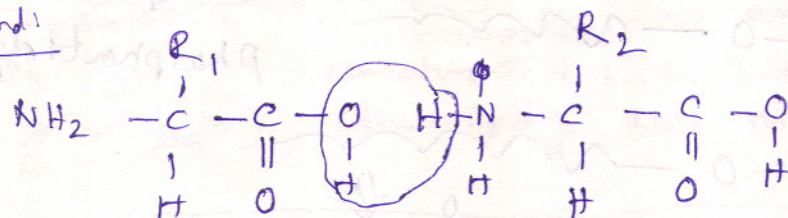


Ⓐ Proteins:

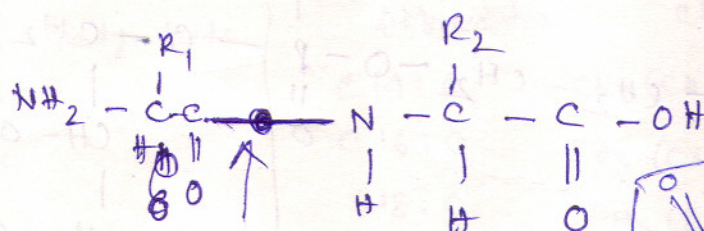
Amino acids:



Peptide bond:



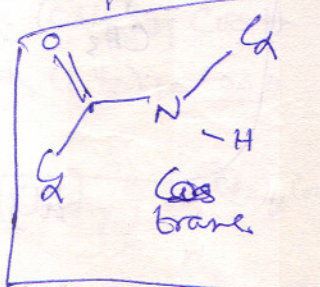
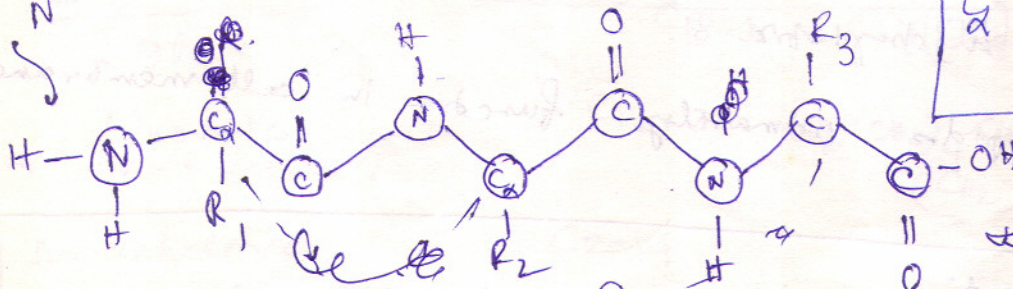
cis to trans ratio 1:1000



Peptide bond.

Poly-peptides.

N-Terminus.



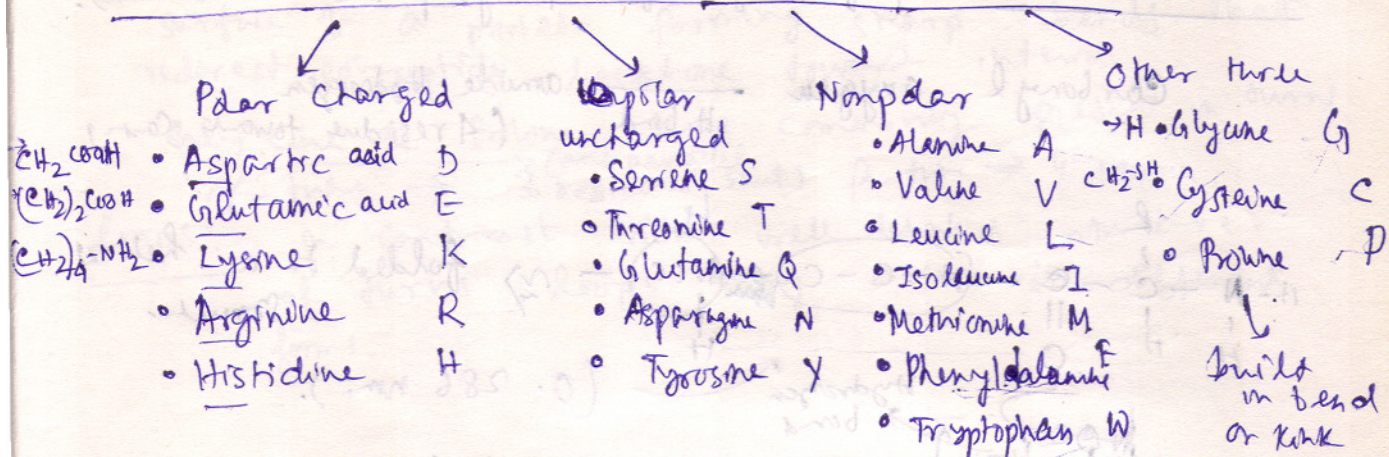
α Carbons (labeled as R₁ R₂ R₃)

< Amino Acid Sequence:

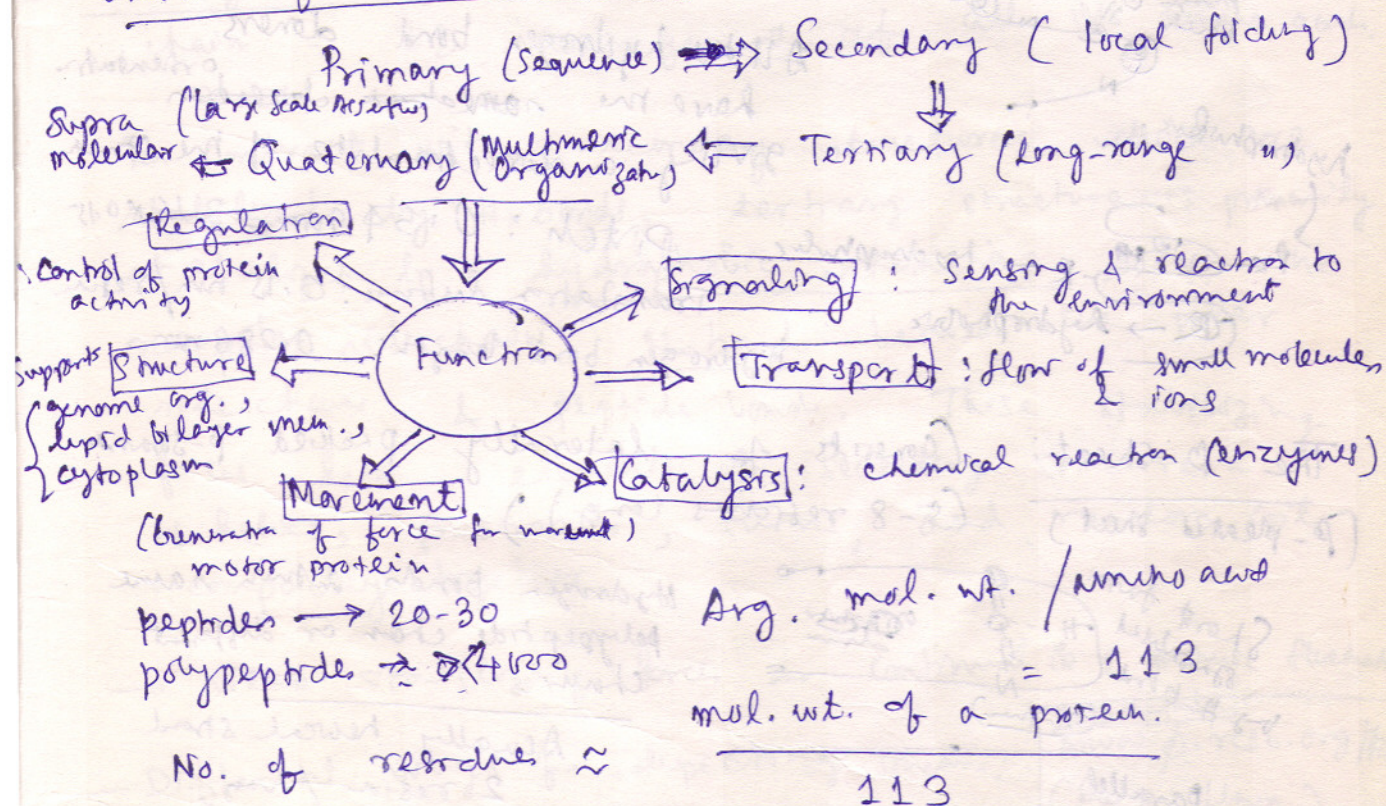
(formed as residues in the polypeptide chain.)

In addition to amino acids, many proteins contain other type of components that are added after the polypeptide is synthesised, e.g. carbohydrates (glycoproteins), metal containing groups (metalloproteins), organic groups (flavoproteins).

Amino acid classifications:



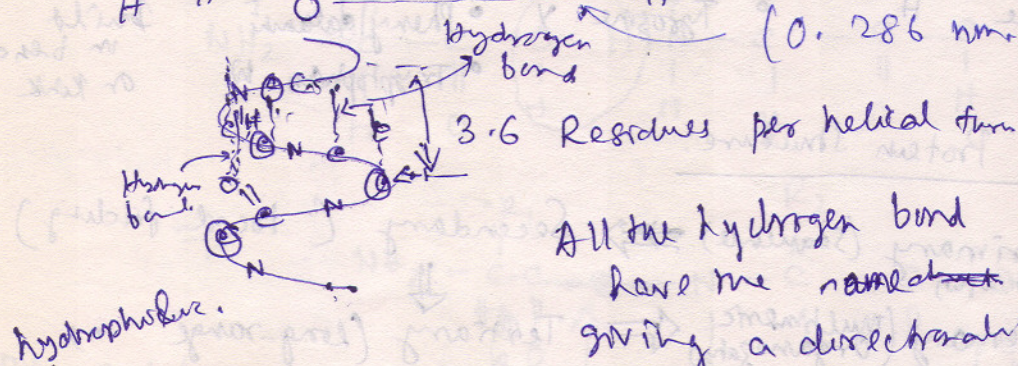
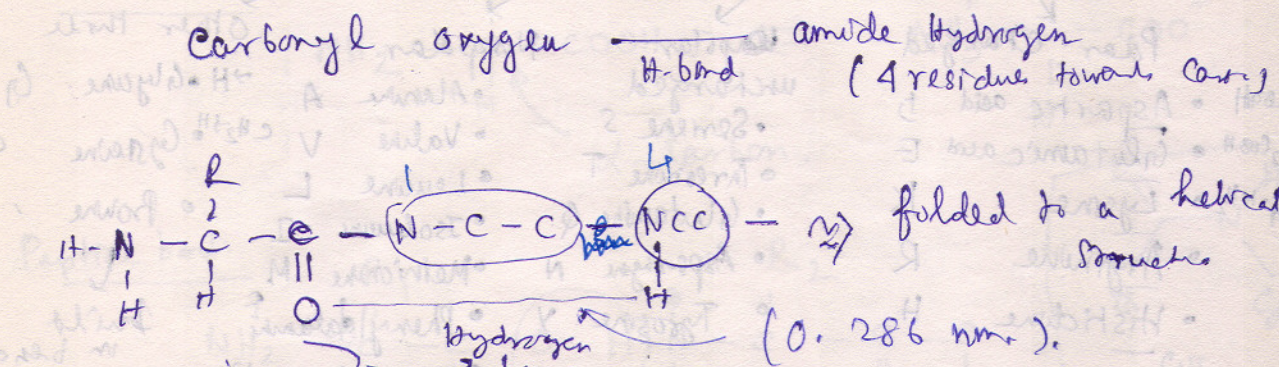
Overview of Protein Structure:



⊛ Secondary Structures!

- α -helix
 - β -sheet
 - U-turns
 - random coils
- 60% of polypeptide chain
- (in the absence of stabilizing non-covalent interact)

The α helix: (folding of polypeptide backbone).



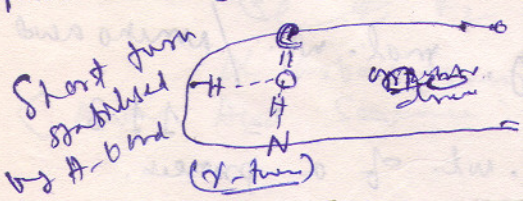
All the hydrogen bond donors have the same orientation giving a directionality of the strand.

Pitch: $0.54 \text{ nm} = 3.6 \times 0.15$

Translation distance: 0.15 nm/residue

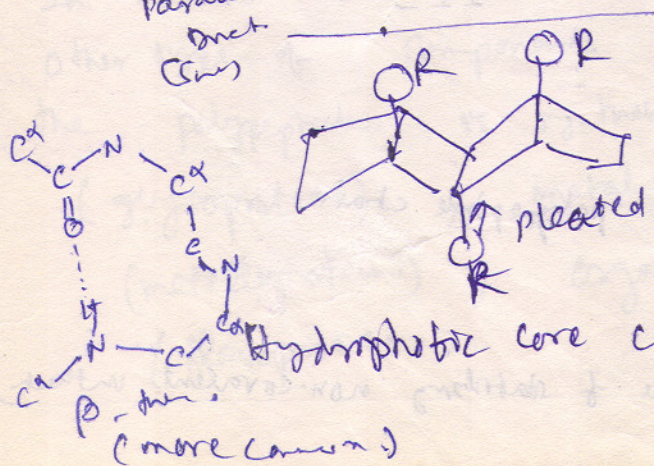
Hydrogen bond length: 0.286 nm .

The β -sheet: (Consists of laterally packed β -strands (5-8 residues long))



Hydrogen bonds within same polypeptide chain or different chains.

Parallel sheet (5nm)



Actually helical strand
2 residues / turn,
translation: 0.34 nm/residue
length $\approx 0.68 \text{ nm}$ (pitch)

pleated sheet structure.

Hydrophobic core contain multiple β -sheets

Turns: (composed of 3-4 residues, located on the surface of a protein forming sharp bends that redirect polypeptide backbone towards interior.

Only α helix & β strand are commonly present in turns.

γ -turn $\Rightarrow$ 3 residues (antiparallel sheet) β -turn $\Rightarrow$ 4 residues.

Loops: In contrast with well defined structure of turns, loops may occur in different forms.

*Tertiary Structure: overall conformation of a polypeptide chain - 3D arrangement of all its amino acids.

In contrast with secondary structures, which are stabilized by H-bonds, tertiary structure is primarily stabilized by hydrophobic interactions between the nonpolar side chains, H-bonds between the polar side chains & peptide bonds. These stabilizing forces hold elements of secondary structure - α -helices, β -strands, turns, and random coils - compactly together.

$\rightarrow$ weak stabilizing forces $\rightarrow$ continual & minute fluctuation.

- Different ways of depicting structure:

$\rightarrow$ α -backbone structure

$\rightarrow$ Ball & stick (all atoms)

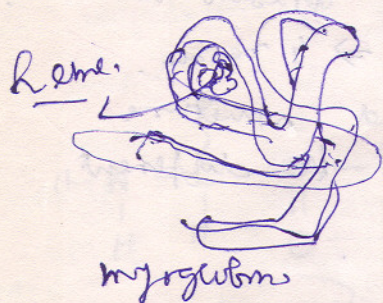
$\rightarrow$ Ribbons

$\rightarrow$ Solvent accessible surface $\rightarrow$ topography of protein surface & the distribution of charge

$\rightarrow$ Determined usually by X-Ray crystallography.

PDB database:
www.pdb.org/pdb
(Rutger Univ.)
PDB format & structures
www.wwpdb.org/docs.html

Tertiary structure fibrous (a highly elongated shape)

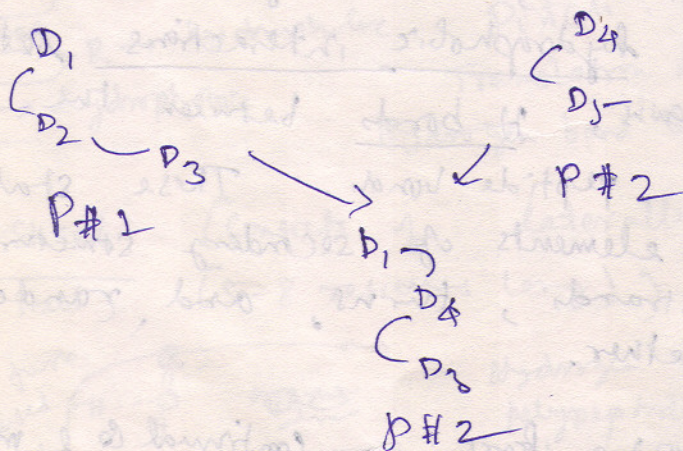


Globular (a compact shape)
 e.g. myoglobin contains eight rod-like stretches of α -helix contain 7-24 amino acids (altogether 153 amino acids, 75% lying in α -helices)
 unlike myoglobin most globular proteins contain α & β structure.

Protein Domains & Motifs:

$\approx 80\%$ of Eucaryotic proteins consist of two or more spatially distinct modules or domains that fold independent of one another.

e.g.



$P \Rightarrow$ Protein

Typical case: (a small fraction)

booster C.

D₁ (α -helices)

D₂ (α -helices)

hair coil

phospholipase C

D₂ (α -helices)

receptor

D₁

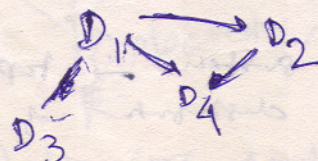
D₂ (α -helices)

synaptic tagma

D₄

(β -sheet)

Evolutionary trace of proteins.



mammalian phospholipase C

(complexly interconnected core structure)

motifs are recurring substructures consisting of a defined arrangements of α -helix & β strands.

Domains that are grouped into the same family have a backbone that is folded into roughly the same configuration; said to share a common fold.

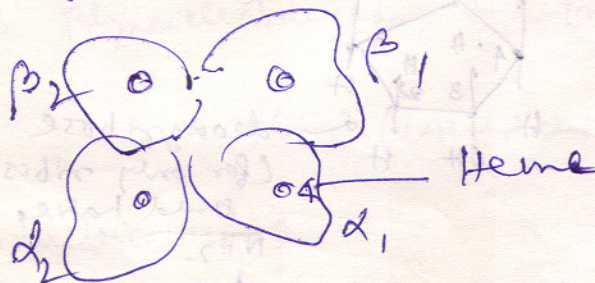
structural biologists want to describe all possible folds in ~~the~~ nature. (estimated at ~ 5000 in no. out of which ~ 1000 reported).

* Quaternary structure:

Many protein $\Leftrightarrow$ single polypeptide chain.

Most of them $\Rightarrow$ multimeric proteins composed of independent subunits (or polypeptide chains) which are held together by noncovalent bonds (some time covalent disulphide bridges).

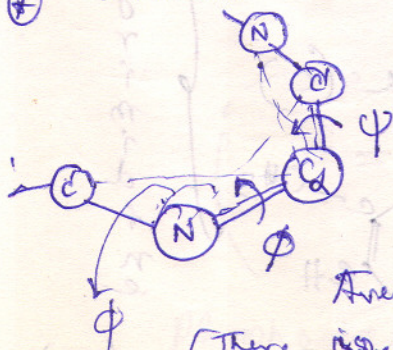
e.g. hemoglobin $\Rightarrow$ 2 α -globin + 2 β -globin.



homodimers $\Rightarrow$ identical subunits. (two)

heterodimers $\Rightarrow$ two different "

* Dihedral angles of alpha helices:



ϕ is defined by the angles between $C'-N-C_{\alpha}-C'$

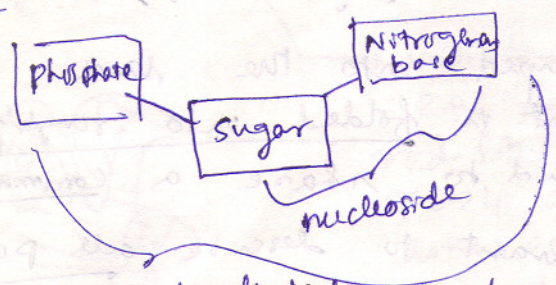
ψ is defined by the angle between $N-C_{\alpha}-C'-N$

Average value of ψ & $\phi \Rightarrow -57^\circ$ & -47°

(There are a range in which these should occur for α -helix, β -strand etc.) $\Rightarrow$ Ramachandran Plot.

Nucleic Acid

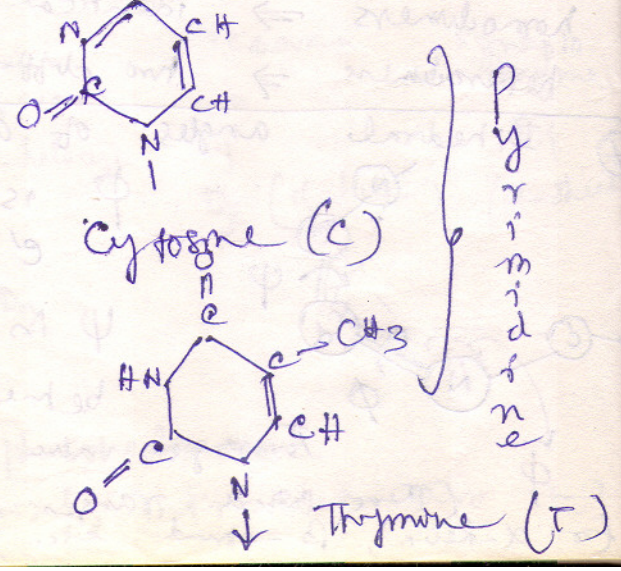
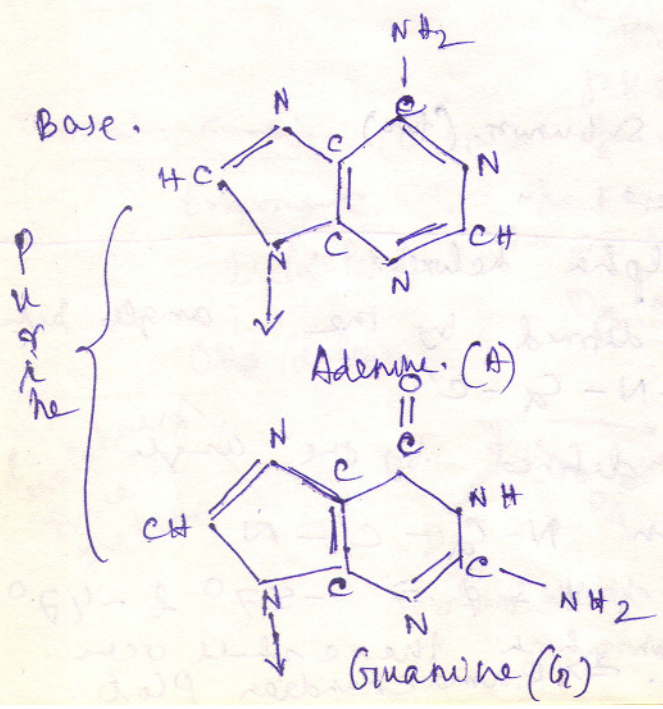
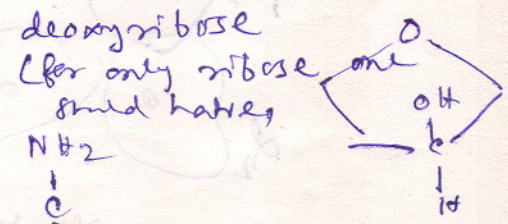
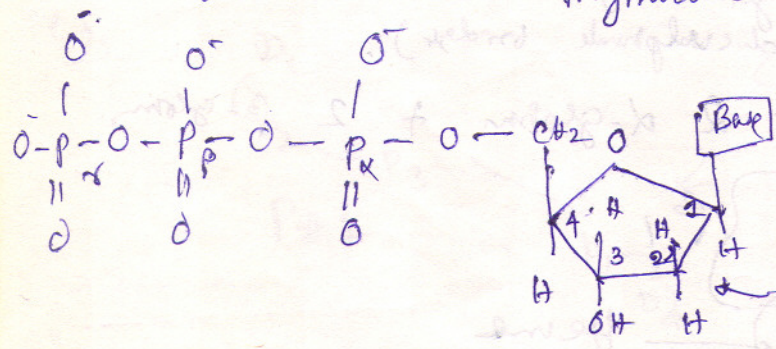
nucleotides:



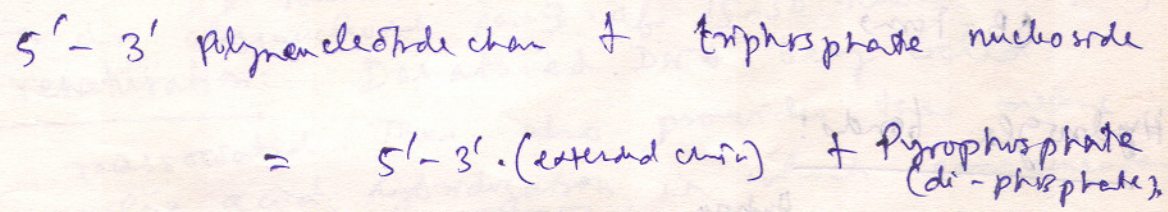
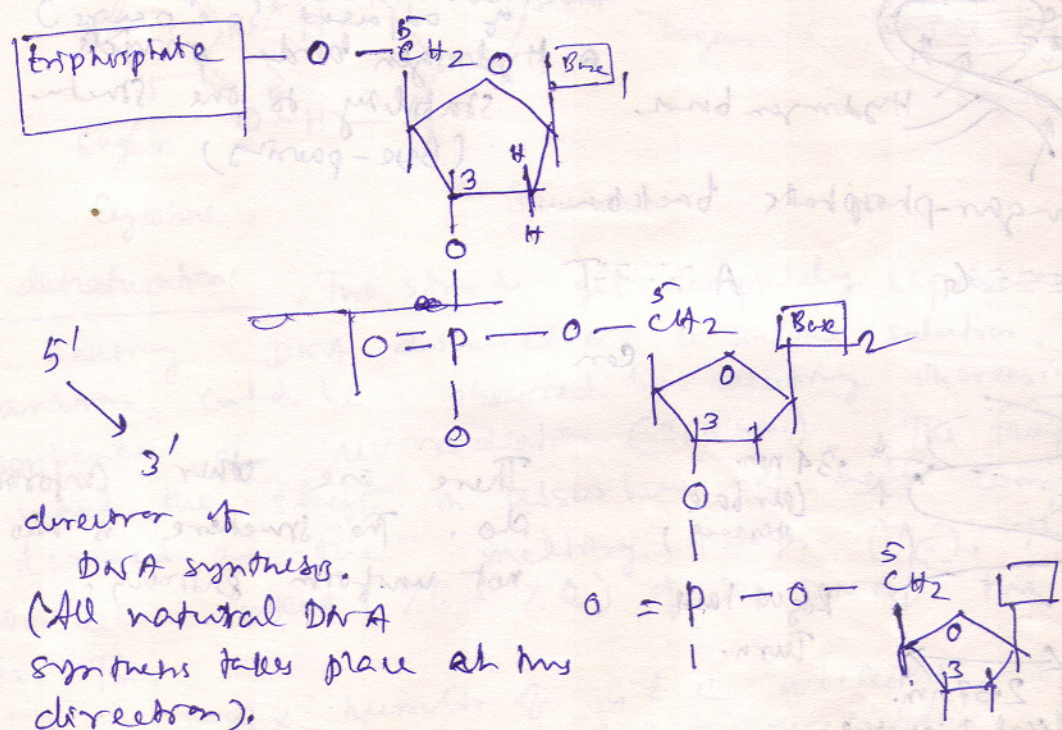
nucleotide = nucleoside + phosphate

Although cell contains 1 or 2 or 3 phosphate groups, only the nucleoside triphosphate act as substrate for DNA synthesis.

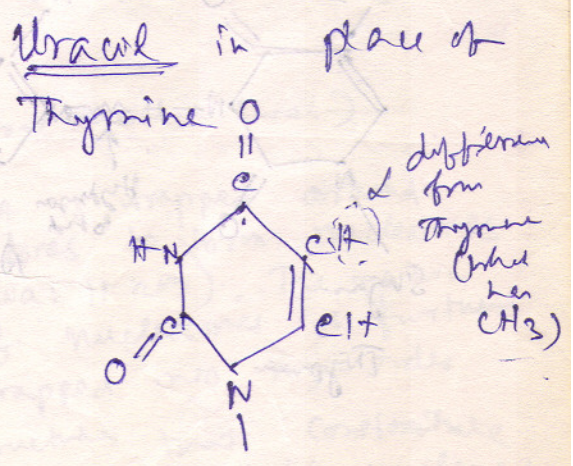
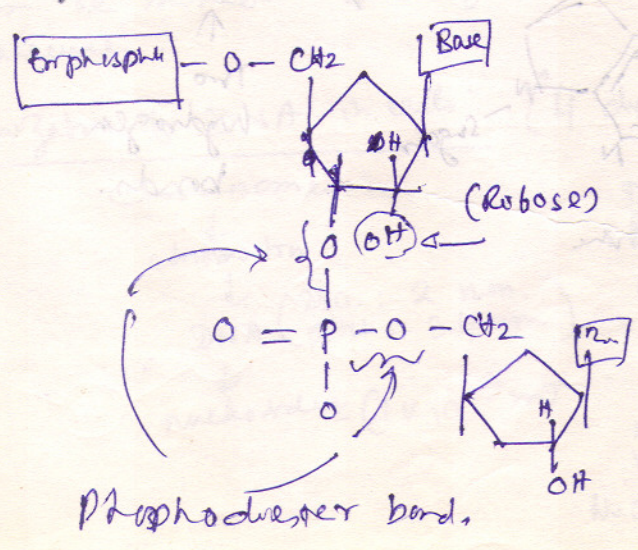
- 2' - deoxyadenosine 5' - triphosphate
- cytidine
- guanosine
- thymidine



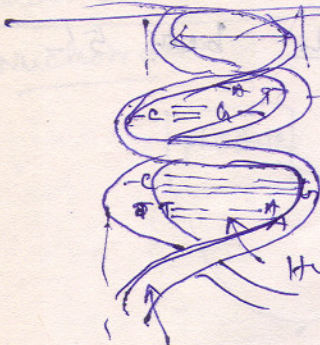
DNA: (Deoxyribonucleic acid)
 In a polynucleotide, individual nucleotides are linked together by phosphodiester bonds betw. 5' & 3' carbons.



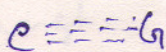
RNA: (Ribonucleic Acid)



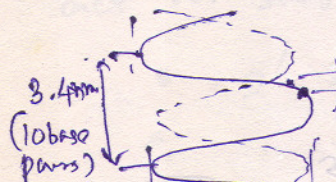
Double helical structure of DNA.



sugar-phosphate backbone.



Con



3.4 nm.
(per base
thereat)

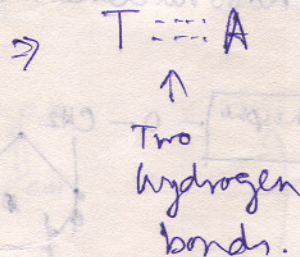
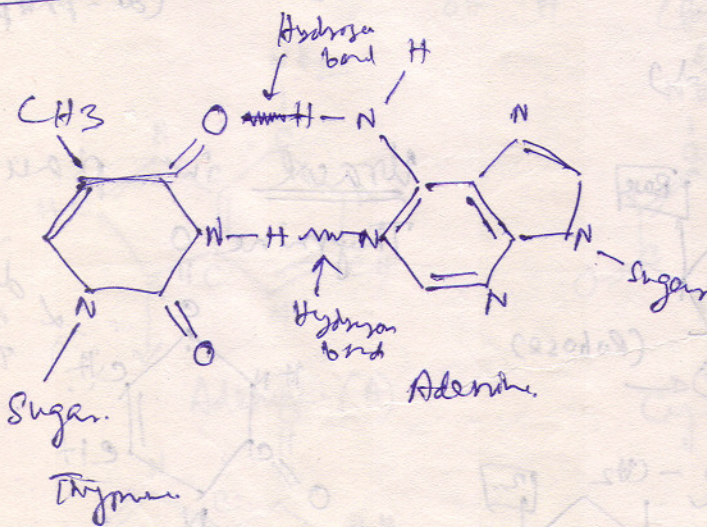
Right hand
Turn.

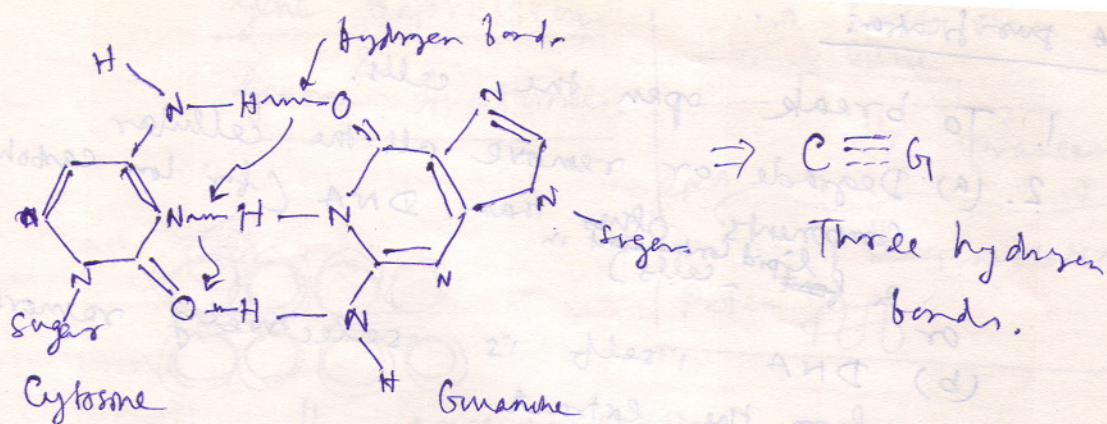
2.37 nm.
(Helical Diameter)

B-Form

There are other conformations
also. The structure is also
not uniform entirely.

Hydrogen bonds:





DNA denaturation: Two strands get completely separated by heating. DNA dissolved in a saline solution. Strand separation could be observed by detecting increasing absorbance of UV radiation (260 nm). The temperature at which the shift in absorbance is half completed is termed as the melting temp. (T_m). The higher the GC content (%G + %C) of the DNA, the higher the T_m .

$$T_m = \frac{4 \times \text{number of G \& C nucleotides} + 2 \times \text{number of A \& T nucleotides}}{\text{For an oligonucleotide seq. of 15-30 nucleotide length}}$$

DNA renaturation: Denatured DNA being cooled gets reassociated. This also provides the means of nucleic acid hybridization, in which complementary strands of nucleic acids from different sources can be mixed & to form double-stranded (hybrid) molecules.

Organization of DNA in cells: (4 distinct structural levels).

Chromosomes
↓
chromatin
↓
DNA (dia.: 2 nm, pitch: 3.4 nm)
↓
nucleosomes (1×10^{-9} m)

DNA is wrapped around histones to form nucleosomes (dia: 11 nm). The chain of nucleosomes is further wrapped into high order structures that constitute the chromosomes (in nucleus). Nucleus (dia: 5 μ m, 5% of cell).

⑧ DNA purification:

1. To break open the cells.
2. (a) Degrade or remove all the cellular components other than DNA (for low carbohydrate or lipid content in cells)
or
(b) DNA itself is selectively removed from the extract.

! Breaking Cells:

Preparation of Cells:

- Cultured animal cell → add detergent such as sodium dodecyl sulfate (SDS)
- plant cells → ~~frozen~~ freezing & grinding.
- Bacteria (e.g. E. coli) → enzyme (lysozyme) & chemical (ethylene diamine tetra-acetate (EDTA)) treatment

2. (A) Degradation of other molecules:

1) Degradation of other molecules:
 Centrifuged at low speed
 ↓
 precipitates cell wall etc
 +
 phenol
 ↓
 precipitates proteins
 +
 aqueous phase
 → test tube
 +
 test tube
 +
 ribonuclease enzymes
 ↓
 RNA molecules broken ethanol added
 ↓ centrifuged
 DNA polynucleotide precipitated & collected.

(b) Add cetyltrimethylammonium bromide (CTAB)

↓
insoluble complex with nucleic acid coprecipitates & collected by centrifugation.

resuspended in a high salt soln. which causes the complex to break down releasing nucleic acids

↓ Ribonuclease treatment + ethanol precipitation
yields pure DNA molecule