Nucleic Acids

The discovery of DNA

Between 1868-1869 Friedrich Miescher separated white blood cells from dressing and concluded that weak basic solutions cause swelling and degradation of cell nuclei. In result new chemical compound appeared. He found it also in yeast and nuclei of cells. It contained C, H, O, N, P and got the name nuclein.

In 1884 Oscar Hertwig concluded that nuclein is responsible for the transfer of inheritance.

In 1909 scientists from Rockefeller Institute concluded that rybose is the sugar in nucleic acids.

The discovery of DNA

In 20-ies of XX century it was concluded that in some forms of nucleic acids ribose with missing oxygen is present and it was named deoxyribose.

At the same time Oswald Avery and his team working on pneumococci concluded that the substance responsible for the conversion of avirulent colonies (R) into virulent (S) is DNA.

In the middle of XX century James Watson and Francis Crick described the structure of DNA what allows for understanding genetic information and the way of its transferring.

Teaching aims

- Gaining new knowledge about the structure and function of nucleic acids
- Gaining new knowledge about processes lying upon the biosynthesis of proteins

Learning effects

- Understanding the meaning of multiplication, transcription and translation of genetic material for appropriate function of cells and bodies
- Understanding the meaning of genetic engineering for diagnostics

Dalmatian

- Owners noticed the pain of one joint of their dog and after certain time other joints as well. Most often pain occured at night.
- Dog liked to eat offal.

Nucleic acids

Polynucleotide chains where single nucleotides are bound by phosphodiester bonds between C`-5 of one and C`-3 of another.

Nucleotides

Phosphate ester of nucleoside, where phosphoric acid is ester linked to one of hydroxyl groups of pentose. In deoxyribonucleotides - 3` i 5`,

in ribonucleotides – 2`, 3`, i 5`.

The meaning of nucleotides:

- Building blocks of nucleic acids
- Participate in indirect metabolism and in energy conversion reactions
- Energy carriers
- Coenzymes in reactions of transportation of acetate residues, sugars and amines
- Coenzymes of oxidoreductases

Nitrogen bases:

Pyrimidines – uracil, thymine, cytosine (rare – 5-methyl-cytosine, 5-hydroxy-methyl-cytosine)

Purines – pyrimidine + imidazol ring – adenine, guanine (rare – 2-methyladenine, 1-methylguanine)

- They exist in tautomeric forms
  (lactim – OH --- lactam = O)
- They absorb ultraviolet light at 260-280nm
- They are weakly soluble in water

Plant nitrogen bases

Methylated purines in plants:

Teophiline (1,3-dimethylxantine)
Teobromine (3,7-dimethylxantine)
Coffeine (1,3,7-trimethylxantine)

Conseqences of the presence of tautomeric forms - pyrimidines

Keto-enol tautomery resulting from the the movement of H protons causes the presence of different tautomeric forms of nitrogen bases:

Lactam (ketone form =O), or

Lactim (enol form –OH)

In physiological conditions lactam of thymine and uracil dominate while cytosine is present in form of lactim.

Mutagenic effect of pyrimidine bases tautomery results from the fact that lactim of thymine formates complementary pair with guanine instead of adenine.

Consequences of the presence of tautomeric forms - purines

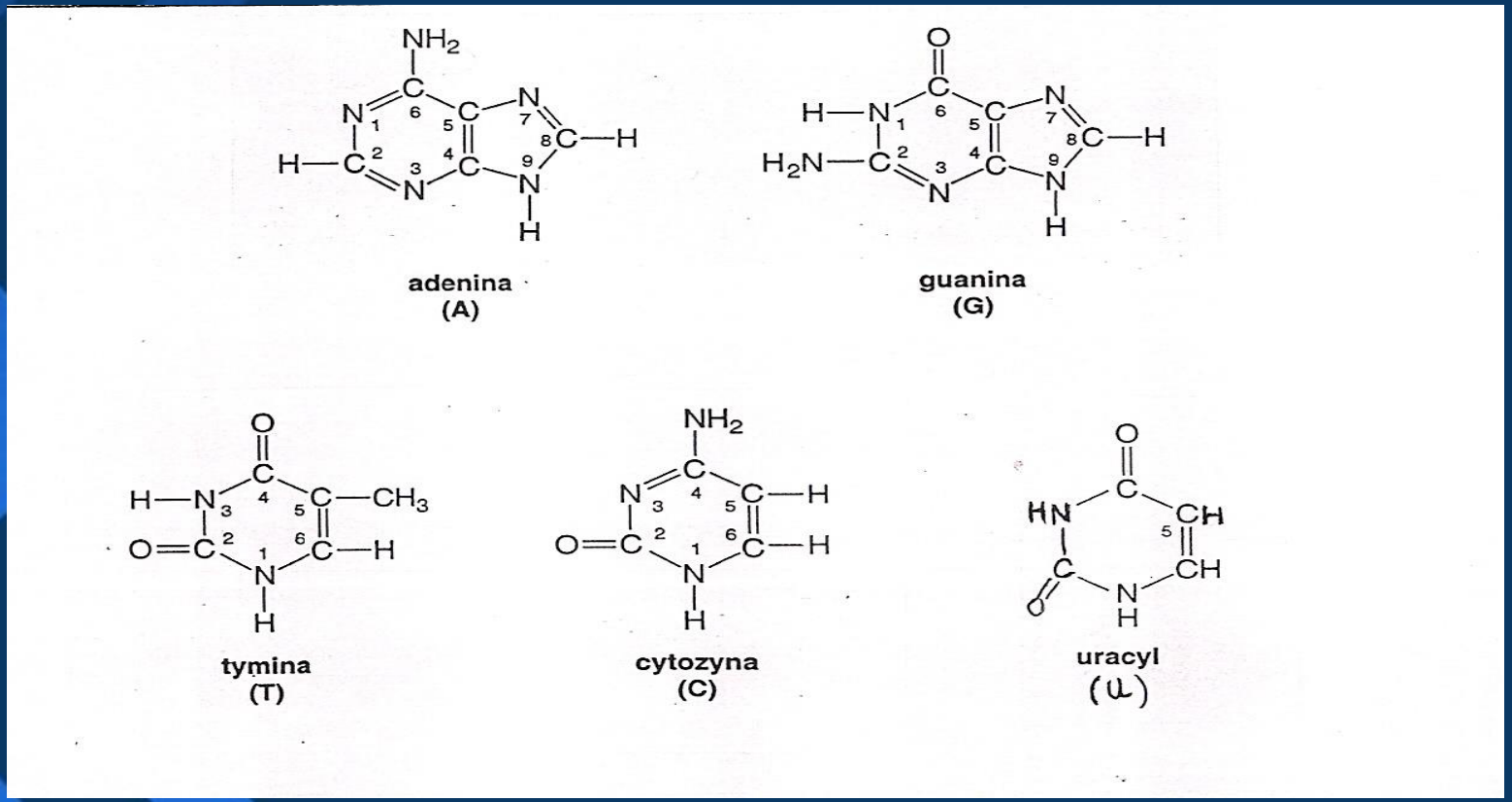
In physiological conditions main tautomeric forms of guanine and hypoxantine are lactams while dominating form of adenine is lactim.

Lactam tautomeric form of adenine formates pair with cytosine what may be related to mutagenesis.

2. 5-Carbon sugar

Ribose or deoxyribose

3. Phosphate residues



Nucleoside

N-glycoside of purine or pyrimidine bases with 5-C sugar bound via C1 atom of pentose and N-1 atom of pyrimidine or N-9 atom of purine.

Physico-chemical properties

- Molecules of acid are long in comparison to diameter that is why their solutions have a high viscosity
- DNA is resistant to strong bases. In similar conditions RNA can hydrolyse due to the presence of OH group at C2 of ribose.
- Acidic hydrolysis provides with degradation products depending on the duration of the process and kind of used acid

Denaturation and renaturation of DNA

- Denaturation is related to the damage to second and third order structure – high temp, high pH, alcohols, fenols, radiation, ultrasound
- Hydrogen bonds of double helix are broken
- After removal of denaturating agent renaturation is possible

Deoxyribonucleic acid (DNA)

It is a polymer consisting of monomers – deoxy-ribonucleotides**.** Carriers of genetic information are nitrogen bases which are present in DNA molecules while sugar and phosphate residues are of structural meaning. Purine bases in DNA are adenine and guanine, while pyrimidine bases – thymine and cytosine.

DNA chain shows polarity – one of its ends has 5`OH group (on left side) and the second 3`OH (on right side).

Model of DNA alpha helix

1. Two helical chains running in opposite directions entwine the common axis.
2. Purine and pyrimidine bases are inside while phosphates and deoxyribose residues are outside the helix, the planes of the sugar rings are arranged perpendicular to the bases.
3. Diameter of helix is 2.0 nm, the distances between bases are 0.34^{o}, there are10 nucleotide pairs per helix stroke.

Model of DNA alpha helix

4. Two chains are bound by hydrogen bonds between bases which formate **COMPLEMENTARY PAIRS** :

   Adenine – Thymine (two hydrogen bonds)

   Guanine – Cytosine (three hydrogen bonds)

5. Order of bases in chain is not limited. Precisely defined sequence of bases carries genetic information.

The synthesis of DNA

DNA POLYMERASES (DNA nucleotidyl transferases) – catalyse the formation of phosphodiester bonds. They also exert the activity of endodeoxyribo-nuclease, which is used for the removal of incorrectly introduced nucleotides

DNA+5`triphosphate of deoxyribonucleotide → DNA+1+PPi

Indispensable are; DNA starter, all four triphosphates of deoxyribonucleotides and DNA strand

The synthesis of DNA

DNA LIGASES – enzymes that bind the ends of two DNA chains or one chain creating spherical molecule. They require the presence of NAD which acts as the source of adenyl group.

- They catalyse the repair in location where one of two DNA strands was interrupted
- They cooperate with DNA polymerases during the replication of both opposite polar DNA strands.

Mitochondrial DNA

Human mitochondrial DNA is spherical double stranded molecule containing 16 569 bp. It codes 13 proteins, 22 tRNA and 2 rRNA. It uses 22 tRNA (while in cytosol there are 61 tRNA).

AGA i AGG are STOP codons (in cytosol they code arginin), AUA codes methionin instead of isoleucine, UGA codes tryptophan (in cytosol it is STOP codon).

Ribonucleic acid (RNA)

It is long, not-branched polymer consisting of rybonucleotides bound by 3`--5` phosphodiester bonds.

Differences in comparison to DNA:

- Ribose is sugar residue
- One of main pyrimidine bases is uracyl instead of thymine
- Does not formate double helix
- Proportions of concentrations of particular bases do not follow the rule of complementarity

Ribonucleic acid (RNA)

MESSENGER (mRNA) – contains genetic information about protein synthesis which was transcribed from DNA. Separate nRNAs are synthesised for each gene or group of genes.

TRANSFER (tRNA) – carries activated aminoacids to ribosomes where they are bound to each other by peptide bond in sequence determined by information from mRNA. There is at least one tRNA for each of 20 aminoacids.

RIBOSOMAL (rRNA) – main component of ribosomes, it has catalytic and structural functions during protein synthesis.

mRNA

Cap – it protects pre-mRNA and mRNA from the action of 5` exonucleases, it participates in mRNA maturation and the transportation from nucleus to cytoplasm and in translation

Coding Sequence (translation region) – contains pre-mRNA exons after excising introns (splicing) during the maturation of mRNA

poli(A) Tail – it is the sequence of 200-250 adenin nucleotides located at 3` mRNA end. It stabilizes mRNA and makes possible the binding of proteins that protect from the action of 3` exonucleases. It helps in translation.

tRNA

rRNA

The synthesis of RNA

RNA POLYMERASES (RNA nucleotydyltransferases) – catalyse the formation of phosphodiester bonds. Do not exert endonuclease activity. Do not require starter.

RNA REPLICASES – produced when the cell is infected by virus containing RNA. They exert polymerase activity but on the strand of viral RNA.

Enzymes that hydrolyse nucleotides

Exonucleases:

- **Group a** – hydrolyse selectively ester bonds between 3` OH group and phosphate residues

Phosphodiesterase from venom of rattle snake – it acts on DNA and RNA by liberating nucleotides as nucleosido 5`phosphates

- **Group b** – hydrolyse selectively ester bonds between phosphate residues and 5` OH end of phosphodiester bridge

Phosphodiesterase from bovine spleen – it acts on DNA and RNA by liberating nucleotides as nucleosido 3` phosphates

Enzymes that hydrolyze nucleotides

Endonucleases – act inside the chain, do not require the presence of free OH groups

- **Group a**

Deoxyribonuclease I from bovine pancreas – it acts on DNA and hydrolyses the bonds between pairs pyrimidine – purine in chain

- **Group b**

Deoxyribonuclease II from spleen and thymus - it acts on DNA

Ribonuclease from bovine pancreas – it acts on RNA and hydrolyses the bond b at which bond a binds pyrimidine nucleotide. Nucleosido 3` phosphates containing pyrimidine and oligonucleotides ending with pyrimidine nucleotide with phosphate group in 3` position are liberated

Enzymes that hydrolyze nucleotides

NUCLEOTIDASES decompose hydrolytically nucleotides from nucleosides

NUCLEOSIDE PHOSPHORYLASES catalyse phosphorolytic breakdown of nucleosides to free bases and riboso-1-phosphate (or deoxyribozo -1-phosphate)

PHOSPHORIBOMUTASE izomerises ribose-1-phosphate to ribose-5-phosphate (substrate in the synthesis of PRPP)

Restriction enzymes (restrictases)

It is a special group of endonucleases. They are synthesised by bacteria and their aim is to degrade foreign DNA (for example viral) in this cell. They are characterised by almost absolute specificity, they recognise usually 4 – 8 nucleotide sequence where they breakdown phosphodiester bonds.

Restrictases recognise palindrom sequences in DNA (identical when read at both strands in direction 5`→3`). They are used in molecular biology for the recombination of DNA molecules.

Synthesis of purine nucleotides

AMP is formated by introducing of amino group at C-6 instead of carbonyl oxygen – aspartate is a donor, GTP is used

GMP is formated by the oxidation of inosinate and the introduction of amino group at C-2, which comes from glutamine, two high energy bonds are used (ATP$\rightarrow$AMP)

Synthesis of pyrimidine nucleotides

Di and tri phosphates are converted by nucleosidodiphosphate kinases

CTP is formated from UTP – carbonyl oxygen at C-4 is replaced by amino group (from glutamine), ATP is required

Deoxyribonucleotides are formated by the reduction of ribonucleotides by use of rybonucleotide reductase complex. Group 2` OH of sugar residue is replaced by hydrogen atom, NADPH is the reductor.

dUMP is methylated dTMP – derivative of tetrahydrofolate is the donor of methyl group.

Synthetic analogs of nucleosides

Derivatives of nitrogen bases and nucleosides where the structure of base ring or sugar was changed in order to obtain molecule with altered biologic alactivity what can be used therapeutically.

- The inhibition of enzymes that use physiological substitutes of analogs as substrates
- The consequences of incorporating nucleoside analogs in DNA or RNA resulting from incorrect base pairing nad inhibition of replication or transcription

Synthetic analogs of nucleosides

- Allopurynol inhibits xanthine oxidase and enzymes of purine biosynthesis – used for treatment in hyperurykemia and gout
- 9-(2-hydroxyetoxymethyl)guanine – acyklowir – used for treatment in viral diseases
- 3`-azydo-2`-deoxythymidine – zidowudyna – used for treatment in viral diseases

Mechanism of action of antibiotics

From the point of view of mechanism of action 3 groups of antibiotics stand out which can act via:

1. The inhibition of biosynthesis of bacterial cell wall (penicilin, cephalosporins, bacytracin, cycloserin, novobiocin, wankomycin, ristocetin)
2. The damage to cytoplasmatic membrane leading to alterations in permeability for electrolytes, aminoacids and nucleotides (streptomycin, neomycin, polymyxins B and E)
3. The inhibition of protein and nucleic acids synthesis (streptomycin, tetracyclins, chloramphenicol, erytromycin, kanamycin, peptolide antibiotics)

Mechanism of action of antibiotics

Daktinomycin formates hydrogen bonds with DNA guanine and takes place where normally polymerase, which catalyse the synthesis of mRNA on DNA, is bound.

Fleomycin inhibits the action of DNA polymerase and blocks the synthesis of nucleic acids.

Mitomycin formates cross-bonds between DNA chains and blocks their unwinding and the same replication.

Streptomycin blocks the binding of mRNA to ribosomes and inhibits translation.

Puromycin, as structural analog of aminoacylo-tRNA inhibits protein synthesis on the stage of translation. Peptidyltransferase that catalyse the formation of peptide bonds catalyses also the synthesis of peptidylpuromycin with unfinished polypeptide chain.

Diagnostic meaning

Recombinant DNA technology is based on nucleic acid enzymology and complementarity of bases.

RESTRICTION ENZYMES are used for cutting up DNA on specific fragments which are subjects to analysis and different manipulations more easily as primary molecule.

PCR – polymerase chain reaction – multiple multiplication of selected DNA sequences

Synthesis of cDNA

Reverse transcriptase from selected viruses possesses the ability to catalyse the transcription in reverse direction. In this way one stranded DNA complementary to mRNA is obtained. Then single stranded cDNA is a pattern for the synthesis of complementary strand. Double stranded DNA containing only coding sequences of selected genes without intron sequences is formated.

Recombination of DNA

- Selected restriction enzymes cut double stranded DNA in a way that 4-6 nucleotide fragments are left so called „sticky ends”. These ends express the tendency to the formation of complementary junctions with fitting molecules, also synthetic.
- Recombination is related to the insertion of certain fragment of foreign DNA into double stranded circular molecule of DNA in plasmids.

Plasmids

Plasmids are small molecules of DNA which are outside chromosomes but are able to autonomous replication and are responsible for some selected properties of bacteria such as drug resistance.

In recombination with selected DNA fragment they may transfer genetic information to bacteria.

Diagnostic meaning

SOUTHERN BLOTTING – method for the identification of seeking DNA fragment via the hybridisation with isotope labelled probe.

After electrophoresis fractions are transfered to nitrocellulose, are hybridized with the probe and via autoradiography the location of seeking fragments which are compatible to used probe is shown.

NORTHERN BLOTTING – concerns RNA

WESTERN BLOTTING – the detection of proteins with the reaction with specific antibodies

Mechanism of action of steroid hormones

Steroid hormones (estrogens, androgens, progestagens, mineralo- and glycocorticoids) as well as **hormones of thyroid glands** act via the connection with the receptor localised intracellularly and influence on gene expression.

Influencing in a selective way on transcription of particular gene and the production of adequate mRNA they may change the concentrations of chosen proteins and metabolic processes.

Dalmatian

Uric acid is the product of purine degradation in hepatocytes. It is eliminated via digestive tract in 30% while in 70% via kidneys.

The concentration of uric acid depends on glomerular filtration rate and can be helpful in the monitoring of renal function. The increase in the concentration of uric acid is the result of disturbances in purine metabolism.

Acidification of urine decreases the reabsorption of urates what favors the precipitation of crystals and the formation of urinary stones.

Dalmatian

The accumulation of sodium urate comes as the result:

- decreased excretion through the kidneys
- increased formation of uric acid
- excessive supply of purines with diet

Due to the fact that urates can precipitate in temp around 30^0 C even at low concentrations (4 mg/dl), the deposition of urate crystals in poorly vascularized tissues is observed (eg. tendons, ligaments) as well as in non-vascularized tissues eg. cartilages of the earlobes and around peripheral joints.

Dalmatian

Physico-chemical properties of uric acid which is not soluble in water are the base of gout attacks. In non-physiological, high concentrations uric acid starts to crystalize mainly in bradytrophic tissues such as metatarsophalangeal joints.

Precipitating crystals of uric acid strongly stimulate inflammatory cells – neutrophils and macrophages. Neutrophils surround and phagocytize urate crystals and liberate proinflammatory cytokines. During this process neutrophils are damaged and liberate lysosomal enzymes which also act as proinflammatory agents to tissues. In concequence severe gout attack is clinically observed.

Dalmatian

Diagnostics:

- The analysis of synovial fluid – the presence of monosodium urate in synovial fluid
- The concentration of uric acid in plasma not always correlate with gout attack
- High resolution USG and computer tomography – crystal deposits in tissues