NUCLEOTIDE METABOLISM

General Overview

- Structure of Nucleotides
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  - Purines and Pyrimidines
  - Nucleosides
  - Nucleotides
- De Novo Purine Nucleotide Synthesis
  - PRPP synthesis
  - 5-Phosphoribosylamine synthesis
  - IMP synthesis
  - Inhibitors of purine synthesis
  - Synthesis of AMP and GMP from IMP
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- Salvage pathways for purines
- Degradation of purine nucleotides
- Pyrimidine synthesis
  - Carbamoyl phosphate synthesisOrotik asit sentezi
- Pirimidin nükleotitlerinin yıkımı
- Ribonükleotitlerin deoksiribonükleotitlere dönüşümü

Basic functions of nucleotides

- They are precursors of DNA and RNA.
- They are the sources of activated intermediates in lipid and protein synthesis (UDP-glucose→glycogen, S-adenosylmathionine as methyl donor)
- They are structural components of coenzymes (NAD(P)+, FAD, and CoA).
- They act as second messengers (cAMP, cGMP).
- They play important role in carrying energy (ATP, etc).
- They play regulatory roles in various pathways by activating or inhibiting key enzymes.

Structures of Nucleotides

- Nucleotides are composed of

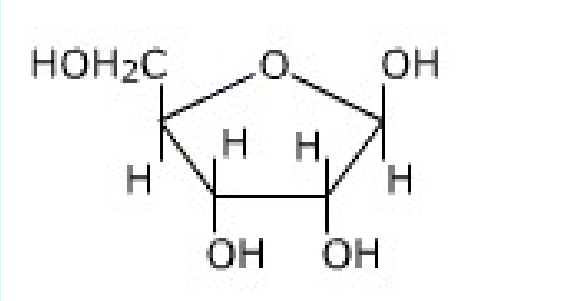
  1) A pentose monosaccharide (ribose or deoxyribose)

  2) A nitrogenous base (purine or pyrimidine)

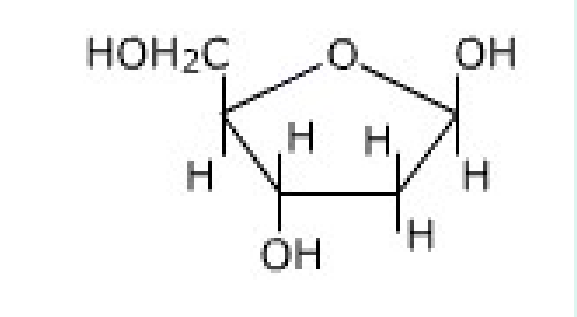
  3) One, two or three phosphate groups.

Pentoses

1.Ribose



2.Deoxyribose

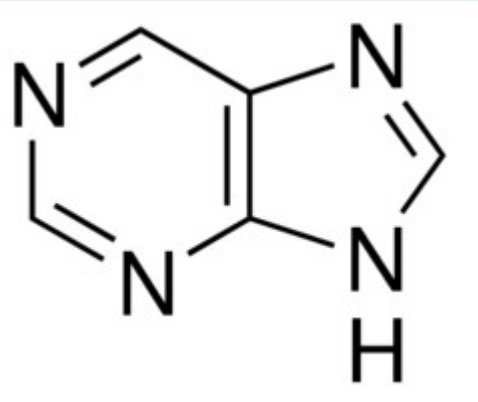


•Deoxyribonucleotides contain deoxyribose, while ribonucleotides contain ribose.

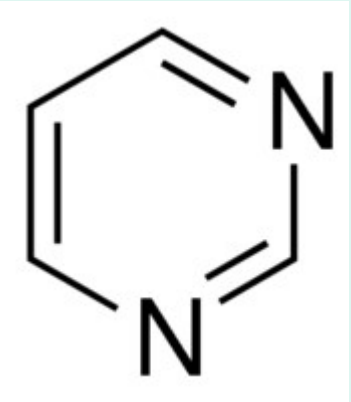
•Ribose is produced in the pentose phosphate pathway. Ribonucleotide reductase converts ribonucleoside diphosphate deoxyribonucleotide.

Nucleotide structure-Base

1.Purine



2.Pyrimidine



•Adenine and guanine, which take part in the structure of DNA and RNA are purine nucleotide bases.

•Cytosine is the pyrimidine base. Among the pyrimidine bases, Uracil is usually only found in RNA, whereas thymine is only found in DNA.

Nucleoside-Nucleotide

Base	Ribonucleoside	Ribonucleotide
Adenine (A)	Adenosine	Adenosine 5’-monophosphate (AMP)
Guanine (G)	Guanine	Guanosine 5’- monophosphate (GMP)
Cytosine (C)	Cytidine	Cytidine 5’- monophosphate (CMP)
Uracil (U)	Uridine	Uridine 5’- monophosphate (UMP)

Nucleoside-Nucleotide

Base	Deoxyribonucleoside	Deoxyribonucleotide
Adenine(A)	Deoxyadenosine	Deoxyadenosine 5’-monophosphate (dAMP)
Guanine(G)	Deoxyguanosine	Deoxyguanosine 5’-monophosphate (dGMP)
Cytosine(C)	Deoksisitidin	Deoxycytidine 5’-monophosphate (dCMP)
Thymine (T)	Thymidine or deoxythmidine	Deoxythymidine 5’-monophosphate (dTMP)

Nucleotide synthesis

•Nucleotides can be synthesised *de novo* or in salvage pathways from existing forms.

•Nucleotides are first formed as ribonucleotides and then converted to deoxyribonucleotides which are required for DNA.

De novo synthesis of pyrimidine nucleotides

Carbamoyl phosphate synthesis

- In mammalian cells, the first step in pyrimidine biosynthesis is carbamoyl phosphate synthesis from glutamine and CO2. This reaction, catalyzed by the carbamoyl phosphate synthetase (CPS II) enzyme and involves numerous steps. CPS II enzyme contains more than one active site for catalysis.

Orotic acid synthesis

- The second step in the synthesis of the pyrimidine is carbamoyl aspartate formation catalyzed by aspartate transcarbamoylase. Then, the pyrimidine ring is hydrolytically closed with dihydroorotase and the resulting dihydroorotate is oxidised to orotic acid.

De novo synthesis of pyrimidine nucleotides

Formation of pyrimidine nucleotide

- The first formed pyrimidine ring (orotate) then combines with a phosphorylated form of the ribose, called PRPP, and forms the nucleotide primid, orotidylate (OMP).

In the following steps,

- UMP, is phosphorylated to UDP and UTP'ye fosforile olur. nucleoside monophosphate kinases and nucleoside diphosphokinases catalyse these phosphorylation reactions.

- UTP is then converted to CTP. Glutamine and ATP energy are used for this reaction.

- **In addition,**

dUMP can also be converted to dTMP in a reaction which uses tymidilate synthase enzyme and N5, N10 methylene tetrahydrofolate as a coenzyme. This step is used as a target in the development of trimetoprim (antibacterial) and 5-fluorouracil (anticancer) agents.

Disorders of pyrimidine nucleotide synthesis

Orotic aciduri,

Low activities of orotate phosphoribosyl transferase and OMP decarboxylase enzymes that catalyze the conversion of OMP to UMP and orotate to OMP result with growth retardation, megaloblastic anemia and excretion of urinary orotic acid.

Degradation of pyrimidine nucleotides

The pyrimidine ring is opened in human cells and is degraded by formation of highly soluble beta alanine, beta aminoisobutyric acid, ammonia and carbon dioxide.

Pyrimidine *de novo* biosynthesis Overview

- PRPP is the source of sugar in pyrimidine nucleotides.
- Amnoacids contribute to nitrogen atoms.
- Pyrimidines are first synthesized as free rings called orotates and then they are combined with PRPP to form the pyrimidine nucleotide called OMP.
- Cytidine nucleotides are synthesized by the conversion of UTP to CTP.
- CTP is the feedback inhibitor of ATCase (prokaryotes), which catalyzes the first step of pyrimidine biosynthesis, whereas ATP is the positive regulator of the same enzyme. This regulatory mechanism provides a balance of purine and pyrimidine nucleotides. In mammals, the control point is CPSII, while PRPP and ATP activate this enzyme while UDP and UTP inhibit it.

Purine biosynthesis- *De Novo*

- In de novo purine biosynthesis, the base is built on ribose sugar. This is different from pyrimidine biosynthesis, in which bases are first built and then joined to the ring.

- During the synthesis of the purine ring, various groups enter the reaction steps to form the source of the different components of the ring. Glycine amino acid, folate derivatives, aspartic acid, and glutamine, all act as donors of carbons, nitrogens, or other components of the ring. In addition, fumarate, the product of the citric acid cycle, is formed as an intermediate in this pathway.

- **PRPP synthesis**

  PRPP is synthesised from ATP and ribose phosphate by the catalysis of PRPP synthatase.

- **Phosphoribosylamine sentezi**

  Phosphoribosylamine is synthesised from PRPP and glutamine in a reaction catalysed by glutamine phosphoribosyl pyrophosphate amidotransferase This step is the regulatory point in purine synthesis pathway. It is inhibited by AMP and GMP and is activated by PRPP.

Purine biosynthesis-*De Novo*

AMP and GMP synthesis from IMP

- IMP is the branch point for the AMP and GMP synthesis. While GTP energy is used for AMP synthesis, ATP energy is used for GMP synthesis. The main reason for this is that the levels of AMP and GMP can be maintained at appropriate rates.

- AMP is an allosteric inhibitor of GMP synthesis from IMP, whereas GMP is an allosteric inhibitor of AMP synthesis from IMP.

- The conversion of monophosphate forms to diphosphate forms in purine nucleotides is carried out by specific kinases.

  AMP + ATP ↔ 2ADP (adenylate kinase)
  GMP + ATP ↔ GDP + ADP (guanylate kinase)

Purine biosynthesis-*De Novo*

- The purine diphosphates thus formed are then converted to triphosphate forms by reactions catalyzed by nucleoside diphosphokinase enzymes (NDPK). Thus, NDPK is the enzyme that converts the nucleoside diphosphate forms of both purines and pyrimidines to nucleoside triphosphates.

- The purines require carbon groups from the tetrahydrofolate derivatives in the two steps in de novo biosynthesis. Tetrahydrofolate and its derivatives have to be regenerated for continuing purine synthesis. This step is an important target for the development of anticancer therapeutics. Synthetic inhibitors of purine synthesis (such as sulfonamides) are used therapeutically to inhibit the growth of rapidly growing organisms without affecting human cell functions.

De novo Purine biosynthesis Overview

- PRPP, which is synthesized from ribose-5 fosfato in pentose pathway, is the source for the synthesis of purines.
- The nitrogen of the purines comes from glutamine, aspartate and glycine.
- Formyl-tetrahydrofolate is needed in two steps in purine biosynthesis.
- The branch point in purine biosynthesis is IMP. The IMP may then be converted to AMP or GMP. This step is regulated by feedback mechanisms by AMP and GMP. In addition, ATP energy is used for GMP synthesis and GTP energy is used for AMP synthesis.

Purine Biosynthesis- Salvage Pathways

- Purines are synthesized de novo as well as salvage pathways (synthesis of nucleotides from existing species).
- These pathways are hypoxanthine guanine phosphoribosyl transferase and adenine phosphoribosyl transferase enzymes catalyse the reactions in salvage pathways

- Guanine + PRPP ↔ GMP + PPi (catalysed by HGPRT)
  Hypoxanthine + PRPP ↔ IMP + PPi (catalysed by HGPRT)

- Adenine + PRPP ↔ AMP + PPi (catalysed by APRT)

- Lesch-Nyhan syndrome (HGPRT deficiency).

Synthesis of deoxyribonucleotides

- Deoxyribonucleotides are synthesized from ribonucleoside diphosphates in reactions catalyzed by ribonucleotide reductases. Reactions catalyzed by ribonucleotide reductases are:

  ADP ↔ dADP

  GDP ↔ dGDP

  CDP ↔ dCDP

  UDP ↔ dUDP

Synthesis of deoxyribonucleotides

- Ribonucleotide reductase contains two subunits that are not identical. These subunits are specific for the reduction of purine nucleoside diphosphates and primidyne nucleoside diphosphates to deoxy forms. The two sulfhydryl groups required for the reduction of the 2 'hydroxyl groups are present in the enzyme.

- During the synthesis of deoxyribonucleotides, in the reduction of the 2'-deoxy carbon, thioredoxin acts as a source of reducing equivalent and gives its Hydrogen atoms to ribonucleotide reductase.

- The resulting oxidised thioredoxin is regenerated to its reduced form by the reducing equivalents provided by NADPH + H +.

- The ribonucleotide reductase enzyme is important for balanced synthesis of deoxyribonucleotides and undergoes allosteric regulation.

- ATP inhibits the enzyme It is inhibited by dATP and activated by ATP.

Degradation of Purine nucleotides

- Nucleic acids are cleaved to nucleotides by nucleases released from pancreas. In intestinal intestinal mucosa cells, purine nucleotides are cleaved into nucleosides and free bases as a series of reactions. The end product of this pathway is uric acid.

- De novo synthesized purine nucleotides are mainly cleaved in the liver.

- Diseases associated with purine cleavage pathways include gut disease and adenosine deaminase (ADA) deficiency.