Nucleotides: structure, metabolism, its disturbance, and consequences
Outline of the lecture

- Classification and nomenclature of nucleotides
- Functions of nucleotides
- Ways of purine nucleotide synthesis.
  - De novo synthesis of purino nucleotides
  - “Salvage” pathway of nucleotide synthesis.
- Breakdown of purine nucleotides and excretion of final products
- De novo synthesis of pyrimidine nucleotides
- Breakdowns of pyrimidine nucleotides and excretion of final products.
- Hyperuricemia and podagra
- Orotic aciduria
Nucleotides are complex compounds containing N-base linked to pentose phosphate.
Classification of nucleosides and nucleotides

1. According to the type of pentose
2. According to the type of N-base
3. According to the number of phosphate groups
Ribonucleotides  Deoxiribonucleotides

- These are ribose-containing nucleotides
- These are deoxyribose-containing nucleotides
Classification according to the type of N-base

Purine nucleotides contain purinine bases
adenine arba guanine

Pyrimidine nucleotides contain pyrimididine bases uracil, cytosine or thymine
Purine bases

Adenine

Guanine

Purine nucleotides

Adenosine 5’-monophosphate
Pyrimidine bases

Uracil

Cytosine

Thymine

Uridine 5’-monophosphate
Nomenclature of purine nucleotides: name of nucleotide comes from the name of respective nucleoside + word “phosphate” with a prefix indicating number of those groups, e.g. mono-, di-, tri.

Adenosine (a nucleoside)

Adenosine 5’-monophosphate (AMP)

Adenosine 5’-diphosphate (ADP)

Adenosine 5’-triphosphate (ATP)
Name of a pyrimidine nucleotide comes from the name of respective nucleoside + word “phosphate” with a prefix indicating number of those groups, e.g. mono-, di-, tri.
Functions of nucleotides

- Precursors of nucleic acids.
- Energy carriers (e.g. ATP and GTP)
- Components of coenzymes (NAD\(^+\), FAD, CoA)
- Metabolic regulators (cAMP)
- Activators of substrates (e.g. UDP-glucose; CDP-diacylglycerols)
Formation of purine/pyrimidine nucleotides

- They are produced from N- bases (these come from the diet and from partly degraded nucleotides)
- They are produced by de novo pathways
Origin of atoms in purine rings

- Aspartate amino
- "C_1"–H_4folate
- Glutamine amide
- HCO_3^-
- Glycine
- "C_1"–H_4folate
De novo synthesis of purine nucleotides

- Ribose 5-phosphate from pentose phosphate pathway is used as the first substrate in the synthesis
- Synthesis begins with assembling of purine ring on ribose 5-phosphate, e.i. purine is being formed in the progress of synthesis
- ATP and GTP are required as sources of energy
Scheme of *de novo* pathway of purine synthesis

\[ \text{ATP} + \text{Ribose-5-phosphate} \rightarrow \text{5-Phospho-\(\alpha\)-ribose-1-pyrophosphate (PRPP)} \]

**Phosphoribose pyrophosphate synthetase**

- IMP dehydrogenase
- Adenylosuccinate synthetase
- Adenylosuccinase

\[ \text{GTP} \rightarrow \text{GDP} + \text{P}_i \]

\[ \text{aspartate} \rightarrow \text{adenylosuccinate} \]

\[ \text{IMP} \rightarrow \text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+ \]

\[ \text{AMP} + \text{PP}_i \rightarrow \text{GMP} \]

\[ \text{fumarate} \]

\[ \text{Guanosine 5’-monophosphate (GMP)} \]

\[ \text{Adenosine 5’-monophosphate (AMP)} \]
Control of de novo synthesis of purine nucleotides
Purine nucleotide synthesis from N-bases
Salvage pathway of purine nucleotide production

- Saves purine bases from degradation
- Saves energy
- Prevents over-production of uric acid
- The pathway is important in brain and in RBC
Salvage pathway of purine nucleotide synthesis
Decomposition of purine nucleotides

Guanosine → Guanine
Inosine → Hypoxanthine

GMP → AMP → IMP → Xanthine

Guanine → Hypoxanthine

Allopurinol + Oxygen → Xanthine oxidase

Xanthine → Uric acid

Excretion with urine

$\text{pK}_a = 5.4$
Abnormalities of purine nucleotide metabolism

- Normal concentration of uric acid in blood plasma is: 2.5-7.8 mg/dL for women; 3 – 9 mg/dL for men.
- Hyperuricemia occurs when uric acid concentration is higher than the norm.
- Causes of hyperuricemia: impaired excretion of uric acid in kidney, wrong dietary habits, disturbances in purine synthesis regulation (decreasing in salvage pathway activity and increasing in de novo pathway activity).
- Delayed hyperuricemia results in the gout disease.
- Severe hyperuricemia is characteristic for neurologic Lesh-Nyhan syndrome.
Symptoms of the gout disease

"The Gout" by James Gilray, 1799. Gout depicted as an evil demon attacking a toe.

"By Royal Authority" by George Cruickshank. A gout sufferer helped onto his horse.

Arthritis starting from thumb of foot
Uric acid crystals in sinovial fluid

In polarized light

In non-polarized light

Acute attack of gout at the ankle

Large tophaceous deposits surrounding joints.
Foods rich in purines

- Very High
  Hearts, herring, mussels, yeast, smelt, sardines, sweetbreads

- Moderately High
  Anchovies, grouse, mutton, veal, bacon, liver, salmon, turkey, kidneys, partridge, trout, goose, haddock, pheasant, scallops
Strategy of de novo synthesis of pyrimidine nucleotides

• Synthesis begins with formation of pyrimidine ring.
• Active form of ribose 5-phosphate is being produced as phosphoribose 1-pyrophosphate (PRPP).
• Pyrimide base is added to PRPP.
Origin of atoms in pyrimidine ring

Comes from carbamoylphosphate

Comes from Asp
Synthesis of pyrimidine nucleotides

Gln + CO₂ + 2 ATP → Carbamoylphosphate

CPS II-carbamoylphosphate syntethase II

CPS II

UTP + PRPP → Orotate

PRPP

CO₂

UMP

UDP

UTP

RNA

CTP

Glutamine

NH₄⁺

5,10-Methylene- FH₂

dCTP

dCDP

dCMP

dUMP

dUMP

dTMP

dTDP

dTTP

CPS II-carbamoylphosphate syntethase II
Control of de novo synthesis of pyrimidines

- In mammals dihydroorotate dehydrogenase, orotate phosphoribosyl transferase and orotic acid decarboxylase are organized into multienzyme complex UMP synthase.
- The second place of control is CTP synthase. The product (CTP) inhibits the enzyme. ATP activates CTP synthase.
Cyitosine → Uracil

NADPH + H⁺
NADP⁺

Dihydrouracil

Thymine

NADPH +
NADP⁺

Dihydrothymine

Ureidopropionate

β-ureidoisobutyrate

Beta-alanine

β-aminoisobutyrate

Degradation of pyrimidines
What happens with products of pyrimidine decomposition?

- Beta-alanine is used for synthesis of CoAi
- NH$_3$ is detoxicated in the liver
- CO$_2$ is exhaled or used for biosynthetic purposes as bicarbonate
- **Beta-aminoisobutyrate** is excreted with urine
Synthesis of thymidine phosphates

- Thymidine phosphates are present as deoxyribonucleoside phosphates.
- 1. The priming reaction of deoxythymidine monophosphate synthesis is formation of deoxyuridine diphosphate (dUMP) from UMP.
- 2. Thymidylate synthase is the enzyme producing TMP from dUMP.
Synthesis of dUDP

• The enzyme involed is ribonucleotide reductase (rNDP-reductase).
• rNDP-reductase uses ribonucleoside diphosphates as substrates
• NADPH is a reductant in the synthesis
Sources of e- in NDP reduction
Synthesis of dTMP