Nucleotide Metabolism – Purines and Pyrimidines

Nucleotide metabolism results in the synthesis of the four nucleotides that form DNA. These are further divided in two categories: purines and pyrimidines. Their construction and degradation takes place in a progression of single steps, while different enzymes and cofactors are involved in the process. Beside degradation, there is also a form of reprocessing purine and pyrimidine nucleotides, defined as Salvage-pathways.

Nucleotide Construction

The four nucleotides serve as DNA (Deoxyribonucleic Acid) components. A nucleotide is, in turn, composed of a deoxyribose (sugar), a phosphate group and a nitrogen basis.

There are four different nitrogen bases, and, therefore, four sorts of nucleotides. The four bases are:

- Adenine
- Guanine
- Cytosine
- Thymine

The resulting nucleotide is named accordingly. If the base is e.g. adenosine, it is named deoxyadenosine-5'-monophosphate. The respective nucleotides are therefore
indicated, in short form, with the initial of their base, i.e. A, G, C and T.

Nitrogen bases are alike two by two since their construction, and are grouped into two categories. Thus, adenine and guanine constitute the **purine** category, while cytosine and thymine form the **pyrimidine** class.

![Image: "Nucleotides" by philschatz. License: CC BY 4.0](image)

**Nucleoside Construction**

A nucleoside is composed of **deoxyribose** and a **nitrogen basis**, i.e. contrary to a nucleotide; it doesn’t have a phosphate group anymore. This means that, on the other hand, a nucleotide can come from a nucleoside through the removal of a phosphate group.

**Purine Nucleotide de Novo Synthesis Process**

**Purine nucleotides** are synthesized directly at the nucleotide’s **ribose** within their synthesis process. This represents an important difference from **pyrimidine synthesis** (see below), in which the ribose is only added subsequently to the complete formation of the pyrimidine ring.

The purine nucleotide synthesis takes place in multiple individual steps. During the first step, **Ribose-5-Phosphate** becomes Phosphoribosylpyrophosphate (PRPP) – consuming ATP to AMP. The enzyme involved is called PRPP-synthetase, in accordance with its final product.

Meanwhile, the rate-limiting reaction, the second step of the purine nucleotide synthesis, takes place. During this reaction, the combination of **PRPP** and **Glutamine** with the
separation of Pyrophosphate (PP) results in 5-Phosphoribosylamine. The configuration on the C1 atom of the ribose is converted from an α structure to a β structure.

The enzyme involved in this rate-limiting reaction is glutamine-phosphoribosylpyrophosphate-amidotransferase. Substances like IMP, GMP and AMP hinder the enzyme and the reaction with it. The reaction can be re-driven through pyrophosphate hydrolysis.

During the third step of the purine nucleotide synthesis, which is represented by an ATP-dependent reaction, 5-phosphoribosylamine becomes a glycinamide-ribonucleotide. The glycinamide-kinosynthase enzyme mediates this reaction.

Through a formylation, the glycinamide-ribonucleotide becomes a formyl-glycinamide-ribonucleotide. The necessary residual formyl comes from a \(N^{10}\)-Formyltetrahydrofolate.

In the following step, an N-Atom with Glutamine serves as a donor and a closed five-membered ring is formed through a H\(_2\)O separation. Glutamine thus becomes glutamate.

A CO\(_2\)-molecule is formed in the closed five-membered ring. Another N-atom is subsequently added through an ATP-dependent reaction, which, in this case, is provided by aspartate (similarly to the urea cycle).

Aspartate becomes fumarate through the loss of the N-atom. Alongside this process, another C1-fragment is incorporated in the five-member ring, which becomes a six-member ring through the separation of H\(_2\)O. The C1-fragment is obtained from the \(N^{10}\)-Formyltetrahydrofolate.

The resulting product is called inosine monophosphate (IMP). It serves as a pre-amplifier to the adenosine monophosphate (AMP) and guanosine monophosphate (GMP) synthesis.

The synthesis to AMP is also GTP-dependent and aspartate-dependent. Within this reaction, the keto-group is replaced with a NH\(_2\)-group in position C\(_6\).

The synthesis of GTP from IMP is, on the contrary, composed of two steps. In the first step, IMP is oxidized to xanthine monophosphate in a NAD\(^+\)-dependent reaction. Xanthine monophosphate, however, is later aminated, and the amine group (-NH\(_2\)) is obtained from the glutamine. The second step is both glutamine- and ATP-dependent.

**Note:** Glutamine and aspartate belong to the amino acids involved in the purine nucleotide synthesis, where they serve as donors of N atoms and thus become glutamate or fumarate.
Role of Folic Acids

Folic acid is a very important component of an efficient purine nucleotide synthesis. A lack of folic acids leads to strongly reduced purine nucleotide synthesis. This reduction becomes particularly clear in processes with high cell turnover, like e.g. erythropoiesis.

A consequence of a folic acid deficiency in this context is a **megaloblastic anemia**, where a malfunction is present, both in DNA synthesis, as well as in the nuclear maturation of the myelopoiesis, which leads to the appearance of megaloblasts.

Besides a folic acid deficiency, a megaloblastic anemia can also occur due to a lack of B12 vitamin, which, overall, is a more frequent cause of megaloblastic anemia than a folic acid deficiency.

Further symptoms of a folic acid deficiency are, among others, **gastritis** or **dermatitis**. The lack of folic acids during a pregnancy can somewhat increase the risk of the baby being born with **spina bifida**.

Folic Acid Metabolism Process

Folic acids are composed of a **p-aminobenzoic acid** rest, a **glutamine** rest and a **pteridine** rest. It is available in its biologically active form as **tetrahydrofolic acid** (TH4), in which it is involved, among other things, in the construction of the purine nucleotide (see above).

The active form of folic acid serves all in all as a coenzyme in the C1 transmission, in which the rest of the C1 position is bound to the N atoms of position five and ten of the pteridine or 4-aminobenzoic acid rest. Possible rests that could be transferred in this context are methyl, hydroxyl, formic and formyl rests.

The conversion to the active form takes place, among others, in a reaction which is dependent on **NADP**⁺ and **vitamin C**. In its first step **7, 8-dihydrofolic acid** is created from folic acid – with the investment of **NADPH + H⁺** and mediated by **folate reductase**.

In a **NADPH + H⁺**-dependent reaction, **5, 6, 7, 8-tetrahydrofolate** is also created by this process. **Tetrahydrofolate** synthesis, that is, in its active form, takes place thanks to the dihydrofolate reductase enzyme. This enzyme can be inhibited through a series of substances like e.g. **trimethoprim**.

Pyrimidine Nucleotide De Novo Synthesis Process

The temporary product of the pyrimidine synthesis is initially a **ribonucleotide**. During the process, the ribose is reduced to a **2’-deoxyribose**, so it can be incorporated in the DNA as such.
The entire pyrimidine nucleotide biosynthesis takes place in multiple individual steps which involve different enzymes. Here, the **pyrimidine ring** is synthesized first, and **ribose** is subsequently added to it.

Within the first step of the pyrimidine synthesis, **carbamoyl phosphate** and **aspartate** react and produce **carbamoyl aspartate** through a phosphate release. The involved enzyme is called aspartate **transcarbamoylase**.

![Diagram of the first step of pyrimidine synthesis](image)

**Note**: The pyrimidine synthesis key reaction represents the reaction between carbamoyl phosphate and aspartate to carbamoyl aspartate.

**Dihydroorotic** acid develops from carbamoyl aspartate through a water (H₂O) separation. This second step of the pyrimidine synthesis includes a carbamoyl aspartate cyclisation and involves the **dihydroorotase** enzyme.

![Diagram of the second step of pyrimidine synthesis](image)

The following step is an oxidation of dihydroorotic acid to **orotic acid** through the **orotic-acid-dehydrogenase** enzyme. This enzyme has NAD⁺ as coenzyme, which comes out as NADH + H⁺ at the end of this reaction.

![Diagram of the third step of pyrimidine synthesis](image)

Orotic acids form **orotidine-5-phosphate** with phosphoribosyl pyrophosphate (PRPP) through the separation of pyrophosphate (PP). This reaction is mediated by the **orotate-phosphoribosyltransferase**.
From orotidine-5-phosphate, uridine-5-phosphate (UMP) develops within a decarboxylation process (that is, through a separation of CO₂) through the orotidine-5-phosphate-decarboxylase enzyme.

![Chemical diagram]

The uridine-5-phosphate constitutes the primary product of a series of further reactions. Firstly, UDP results from UMP via phosphorylation, which can be converted to UTP through further phosphorylation. The phosphate group required for this is obtained through an ATP to ADP-reaction.

In turn, through an ATP and glutamine-dependent reaction, CTP (cytidine triphosphate) can be obtained from UTP. This reaction is mediated by the CTP-synthetase enzyme.

On the other hand, uridine-5-phosphate can be reduced to d-UMP through d-TMP-synthetase (thymidylate-synthase). This reduction is NADPH + H⁺-mediated. The following step of the d-TMP-synthetase consists of a methylation of d-UMP to d-TMP. The necessary methyl group is obtained through the \( \text{N}^5-\text{N}^{10}\)-Methylene-H₂-Folate, which subsequently emerges as H₂-Folate.

### Deoxy-forms Synthesis of Purine and Pyrimidine Nucleotides

The final product of both the purine and the pyrimidine nucleotide synthesis (see above) is the ribonucleotide, which must be reduced further into the 2'-deoxy-form in order to be incorporated in the DNA.

The enzyme involved in this is ribonucleotide-reductase, which has thioredoxin as a cofactor. Thioredoxin, in turn, contains two SH-groups, which are converted to a disulfide form in the reduction process. Thioredoxin in its disulfide form is reconverted to its original form through NADP⁺-dependent thioredoxin reductase.

### Degradation of Purine Nucleotide or Purine Bases

Like the purine nucleotide construction, their degradation also takes place in multiple individual steps. In this process, each step’s differentiation from the others is partly based on their dependence from their respective purine base (adenosine or guanosine).

However, the first step of the degradation is a conversion from nucleotide to nucleoside. This takes place through a hydrolytic separation through the nucleotidase enzyme. Additionally, a phosphorylic separation in a free base (purine or pyrimidine) and in ribose-1-phosphate takes place. The nucleoside phosphorylase enzyme mediates this step. The degradation of the purine bases adenosine and guanosine takes place afterwards.
In a first step, adenosine is converted into inosine through NH$_3$ separation (deamination), involving the adenosine deaminase enzyme. The second step is identical for both inosine and guanosine. Both are converted to hypoxanthine (inosine) or guanine (guanosine) through an ATP-dependent ribose separation. The enzyme involved in this is nucleoside phosphorylase.

During the next step, xanthine is obtained from inosine and hypoxanthine. However, this step is different for both cases. Guanine is deaminated to xanthine, while hypoxanthine is oxidized to xanthine through xanthine oxidase.

**Note:** Xanthine oxidase is an iron-bearing flavoprotein, which contains a molybdate atom in its active centrum.

A further step which is mediated by xanthine oxidase is the conversion of xanthine into uric acid. This step also represents an oxidation, where molecular oxygen serves as a means for oxidation. Uric acid will be pruned over urine.

### Enzyme Defects That Can Lead to an Altered Uric Acid Level

The uric acid level plays a clinical role since, during a solubility products exceedance (ca. 7 mg / 100 ml), a urate crystal deficit in the tissue can occur. Moreover, this can cause local inflammations - the corresponding clinically manifest disease is called **gout**.

Urate crystals fail particularly in badly capillarized tissues, probably because a low temperature promotes a urate crystal deficit. That is why e.g. the metatarsophalangeal joint (podagra), but also the cornea or the lens, are particularly affected by this.

The gout-affected joint appears flushed, overheated and swollen, besides causing a very strong pain. These clinical signs are the cardinal ones of an inflammation (rubor, calor, tumor and dolor).

Along with purine metabolism, enzyme defects can lead to either an increased or a diminished uric acid level. Among others, enzyme defects that can cause an increased uric acid level are a partial and a complete lack of hypoxanthine-guanine-phosphoribosyltransferase. A partial lack prevents the recycling of IMP to GMP, thus increasing the de novo purine synthesis. The respective disease is called **Kelley Seegmiller syndrome**.

A complete hypoxanthine-guanine-phosphoribosyltransferase lack is called **Lesch Nyhan syndrome**. Clinically, affected children present a trio of hyperuricemia, progressive kidney insufficiency and neurologic symptoms, for example the tendency to self-mutilation.

One enzyme defect, which, on the other hand, can lead to a diminished uric acid level, is a reduced xanthine oxidase activity (**xanthinurie**).

**Note:** Xanthine oxidase activity can be intentionally diminished through the allopurinol agent within a gout therapy.

Besides enzyme defects, other factors, like the renal function (uric acid secretion), an increased cell turnover (e.g. in diseases like leukemia) or a strongly purine-based diet (e.g. flesh), can also influence the uric acid level.
Pyrimidine Nucleotide and Bases Degradation

The first step of pyrimidine nucleotide degradation is, similarly to the purine nucleotide degradation process (see above), the conversion of nucleotides to nucleosides. Here, the respective steps are identical.

The degradation of cytosine and thymine, which has been obtained during the first step of the pyrimidine bases degradation, takes place in the liver. Here, the pyrimidine ring is broken down through multiple steps and pyrimidine bases are consequently degraded.

**Note:** The ring is degraded during the pyrimidine nucleotide degradation, while the purine ring is preserved during the pyrimidine nucleotide degradation.
The two bases go through two independent degradation ways in which the reaction steps are identical, except for the first step regarding cytosine degradation.

In the first step, cytosine is degraded to uracil through a separation of the amino group. Uracil and thymine are, in turn, reduced to dihydrouracil and dihydrothymine through a NADPH + H⁺-dependent reaction.

Through a separation of CO₂ and NH₃, β-alanine develops from dihydrouracil, while β-aminobutyrate results from dihydrothymine.

β-alanine and β-aminobutyrate are partially further degraded into respectively acetate and propionate, NH₃ and CO₂ via multiple intermediate steps. The nitrogen atoms that were obtained by this become part of the urea cycle.

Recycle of Purine and Pyrimidine Nucleotides
(Salvage-Pathways)

Since purine nucleotide degradation doesn’t result in any energy gain and pyrimidine nucleotide degradation only provides a small one, while the synthesis of both needs a great amount of energy, recycling is energetically more convenient.

However, the exact steps of recycling are only known for purine bases, which is why only those are covered here.

First, during the purine bases recycling, they are phosphoribosylized to nucleotides through PRPP. The transmission of each purine base to PRPP takes place for adenine through adenine-phosphoribosyltransferase and for hypoxanthine and guanine through hypoxanthine-guanine-phosphoribosyltransferase.

Through the synthesized final product, both enzymes are inhibited. The final product is AMP for adenine, while hypoxanthine results in IMP. The end product of guanine is GMP.

Review Questions

The answers can be found below the references.

1. **Which of the following substances does not belong to nucleotides?**
   A. Adenine
   B. Guanine
C. Thymine
D. Cytosine
E. Glutamine

2. Which statement about purine metabolism is not correct?

A. The final product of purine degradation in humans is uric acid.
B. Adenine-Phosphoribosyltransferase is responsible for adenine recycling.
C. The rate-limiting reaction in purine nucleotide synthesis is mediated by glutamine-
phosphoribosylpyrophosphate-amidotransferase.
D. The AMP synthesis is ATP-dependent.
E. The GMP synthesis is NAD^+-dependent and ATP-dependent.

3. Which statement about pyrimidine metabolism is not correct?

A. The key reaction in pyrimidine nucleotide synthesis includes the reaction of
   carbamoyl phosphate and aspartate to carbamoyl aspartate.
B. Uridine Monophosphate (UMP) is produced through a decarboxylation from
   orotidine-5-phosphate.
C. The first step of pyrimidine nucleotide degradation is the conversion from
   nucleosides to nucleotides.
D. The nitrogen atoms that were obtained during degradation are introduced in the
   urea cycle.
E. Cytosine is degraded through the separation of NH3 to uracil.

4. Which sentence about folic acids is not correct?

A. Folic acids consist of a pteridine rest, a p-aminobenzoic acid rest and a cytosine
   rest.
B. The biologically active form is tetrahydrofolic acid (TH4).
C. Folic acid activation is ATP-dependent.
D. The product of dihydrofolate reductase is 7, 8-dihydrofolic acid.
E. The function of the active form is to transmit NH3-groups.

References

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Correct answers: 1E, 2D, 3C, 4B

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