NUCLEOTIDE METABOLISM

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Nucleotides serve various metabolic functions. For example, they are:
- Substrates (building blocks) for nucleic acid biosynthesis and repair,
- The main storage form of “high energy phosphate”,
- Components of many “so-called” co-enzymes (NAD, NADP, FAD, CoA),
- Components of many activated metabolic intermediates (such as UDPG, SAM),
- Major allosteric effectors (such as AMP, ADP, ATP, GTP),
- Major second messengers (such as 3’,5’ cAMP), and
- Precursors for the biosynthesis of a variety of important compounds (such as biopterin and histidine).

Nucleotide biochemistry can be treated both as an aspect of nitrogen metabolism, along with such compounds as amino acids and porphyrins, and as an aspect of nucleic acid metabolism. When the focus is on the biosynthesis and degradation of nucleotides, in other words on their turnover, the treatment is similar to that of other nitrogenous compounds. When the focus is on the role of nucleotides in overall nucleic acid metabolism, the treatment is included in molecular biology. Both aspects will be considered here with the major emphasis directed toward relating defects in nucleotide turnover to either metabolic diseases or chemotherapy.

I. Nomenclature (pages 11 and 12).

II. Overall metabolic pathways (page 4).

1) PRPP synthetase.

\[
\text{Ribose 5 Phosphate} \quad \xrightarrow{\text{ATP, AMP}} \quad \text{PRPP (5 PhosphoRibosyl-1 Pyrophosphate)}
\]

2) Nucleoside phosphorylases.

\[
\text{Ribo- or Deoxyribonucleoside} \quad \xleftrightarrow{\text{B, P_i}} \quad \text{Ribose or Deoxyribose 1 phosphate}
\]

3) Phosphoribosyl transferases.

\[
\text{PRPP} \quad \xrightarrow{\text{B, PP_i}} \quad \text{Ribonucleotide}
\]
III. Biosynthesis of purines and pyrimidines (pages 5, 6, 8). A minimum amount of time will be spent discussing these pathways in lecture. Please examine them carefully in the text and note that:

1) Purine synthesis begins at the nucleotide level, while pyrimidine synthesis does not.

2) Both syntheses are regulated at their committed steps.

3) Glutamine and tetrahydrofolic acid derivatives are important donors of amino groups and one carbon residues, respectively.

4) The conversion of IMP to AMP requires GTP and is inhibited by AMP, while the conversion of IMP to GMP requires ATP and is inhibited by GMP.

IV. Degradation of purines and pyrimidines (pages 4 and 7).

V. Disease states associated with defects in purine metabolism.

1) Lesch-Nyhan syndrome (pages 6-7) The Lesch-Nyhan syndrome is a sex linked defect of the Hypoxanthine, guanine, phosphoribosyl transferase (HGPRT) gene characterized by little or no HGPRT activity. Patients accumulate very high levels of uric acid (actually uric acid plus urate) in urine and blood, and also exhibit severe neurological abnormalities including self mutilation. The high uric acid levels are associated with an increase in de novo purine biosynthesis. Since Phosphoribosyl amine synthase (the enzyme catalyzing the committed step in purine biosynthesis) and HGPRT both use PRPP as a substrate, elimination of the HGPRT reaction results in an increased PRPP concentration and an increased rate of phosphoribosylamine synthesis. The Lesch-Nyhan syndrome is a rare and extreme form of a more common condition known as Gout.

2) Gout (pages 6-7). Gout is a condition characterized by above normal levels of uric acid plus urate in the urine and/or blood. Primary gout is associated with increased de novo purine synthesis, while secondary gout is associated with problems in uric acid (urate) excretion. Two genetic defects have been identified as the probable cause of primary gout in some patients. One is a partial HGPRT deficiency and the other is an overactive form of the enzyme that catalyzes the conversion of ribose 5-phosphate to PRPP (PRPP synthetase). (Allopurinol, a suicide substrate inhibitor of xanthine oxidase, is used to treat severe cases of gout.)
3) Severe combined immunodeficiency due to adenosine deaminase deficiency (page 7).
Adenosine deaminase (ADA) deficiency is due to autosomal recessive defects of the ADA gene and is characterized by increased levels of adenosine and deoxyadenosine in blood and urine. Total or nearly total loss of enzyme activity results in a severe combined immunodeficiency affecting both B and T lymphocyte functions, and it has been found that deoxyadenosine at the high levels accumulating under these conditions is toxic to these cells. A variety of explanations have been proposed to explain this toxicity, but one that is particularly attractive involves the efficient conversion of deoxyadenosine to dATP in lymphocytes, and the fact that dATP is a potent inhibitor of ribonucleotide reductase (page 9). (Overall lymphocyte function requires cell proliferation.)

4) Impaired cellular immunity due to purine nucleoside phosphorylase deficiency (page 7).
Purine nucleoside phosphorylase (PNP) deficiency is due to a defective PNP gene (autosomal recessive) and is characterized by increased levels of the purine nucleosides (inosine, deoxyinosine, guanosine, deoxyguanosine) in the blood and urine. The increase in one of them, deoxyguanosine, is toxic to T cells and results in a selective defect in cell mediated immunity. As in the case of ADA deficiency, a variety of explanations have been proposed to explain this toxicity, one of which involves the efficient conversion of deoxyguanosine to dGTP in T cells, and the fact that dGTP is an inhibitor of ribonucleotide reductase.

VI. Synthesis of deoxyribonucleotides (page 9).

VII. Cancer chemotherapy (page 10).
OUTLINE OF NUCLEOTIDE METABOLISM

Nucleoside triphosphates → Nucleic Acids

Nucleoside diphosphates

Nucleosides

5'-Nucleotides

Polynucleotides (some free nucleotides)

x H₂O (ribonucleases and deoxyribonucleases)

y H₂O (phosphodiesterases)

5'-phosphoribosyl pyrophosphate (PRPP)

(biosynthetic enzymes)

P₂O₅

PP₃

ATP

H₂O

Pᵢ

Ribose-1-phosphate, or Deoxyribose 1-phosphate

(nucleoside phosphorylases)

Pᵢ

(degradative enzymes)

Uric acid

β-alanine

β-aminoisobutyric acid

Pᵢ

PRPP

(phosphoribosyltransferases)
ADDITIONAL POINTS OF INTEREST

1) Ribonucleoside diphosphates can be converted to deoxyribonucleoside diphosphates by ribonucleotide reductase.

2) Not all nucleosides are phosphorylytically cleaved with the same efficiency; in general, inosine, guanosine, uridine, and thymidine are preferred. AMP and adenosine are converted by deaminases to IMP and inosine, while CMP and cytidine are converted by deaminases to UMP and uridine. The relative rates of these deaminations and various nucleoside phosphorylytic cleavages differ from species to species, and from tissue to tissue within a species. In humans, it appears that the conversions of AMP and adenosine to IMP and inosine are important for the further metabolism of adenine.

3) An improper concentration of 5'-nucleotides due to impairment of de novo synthesis, breakdown, or formation by phosphoribosyl transferases results in a variety of diseases.

PURINE NUCLEOTIDE BIOSYNTHESIS

1) COMMITTED STEP OF DE NOVO PURINE NUCLEOTIDE BIOSYNTHESIS

The enzyme catalyzing this reaction, PRPP-Glutamine Amidotransferase, is also referred to as “Phosphoribosylamine Synthase”. The enzyme exhibits extreme cooperativity (sigmoidal kinetics) with respect to PRPP concentration, and is inhibited by each of IMP or AMP or GMP, as well as by combinations of these nucleotides.
2) OUTLINE OF *DE NOVO* AND SALVAGE PURINE NUCLEOTIDE BIOSYNTHESIS

The conversion of phosphoribosylamine to IMP involves the addition of building blocks derived from glycine, glutamine, aspartate, CO₂, and one-carbon (“1C”) FH₄ derivatives at the formate oxidation level.

AMP, IMP, and GMP are negative allosteric effectors of the committed step in *de novo* purine biosynthesis. The conversion of some hypoxanthine and guanine to ribonucleotides through the Hypoxanthine Guanine PhosphoRibosyl Transferase (HGPRT) reaction is essential for maintaining normal nucleotide balance. Partial or nearly total loss of HGPRT results in increased *de novo* purine biosynthesis due to both an increased PRPP level and decreased IMP and GMP.
levels. A partial loss is a form of gout, and nearly total loss represents the Lesch-Nyhan Syndrome. Interestingly, in glucose-6-phosphatase deficiency, increased shunting of glucose through the pentose phosphate pathway also increases liver PRPP levels and results in mild gout.

PURINE NUCLEOTIDE BREAKDOWN

The letter d in parentheses, (d), before a nucleotide or nucleoside indicates that it may be either a ribo- or a deoxyribo- derivative. Under normal circumstances both ribo- and deoxyribo-nucleoside levels are barely detectable. Not shown in this figure are Adenosine, Deoxyadenosine, and Deoxyguanosine kinase activities that convert (d)Adenosine to (d)AMP, and Deoxyguanosine to dGMP. Mammalian cells do not contain Inosine, Deoxyinosine or Guanosine kinase activities. The normal flow of nucleosides is in the direction of deamination and phosphorolysis.
OUTLINE OF *DE NOVO* PYRIMIDINE NUCLEOTIDE BIOSYNTHESIS

\[
\text{Gln} + \text{HCO}_3^- + 2 \text{ATP} + \text{H}_2\text{O} \rightarrow \text{Glu} + 2 \text{ADP} + \text{Pi} + \text{NH}_2\text{C-O-} \hat{\text{P}} \]

*CPSII*

- a) Cytosolic (not mitochondrial, as CPSI)
- b) Gln substrate (not NH\(_3\), as CPSI)
- c) Inhibited by UTP (not activated by NAc-Glu, as CPSI)

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*Orotic Acid*

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nucleotides - 8
SOME IMPORTANT NUCLEOTIDE INTERCONVERSIONS -
GENERATION OF DEOXYRIBONUCLEOTIDES

Enzymes
I - Ribonucleotide Reductase
II - Nucleoside Diphosphate Kinase
III - dCMP Kinase
IV - dUTP Diphosphohydrolase
V - dCMP Deaminase
VI - Thymidylate Synthase
VII - FH₂ Reductase

As might be expected, ribonucleotide reductase is a highly regulated enzyme. It is a hetero-
tetramer containing two substrate binding sites, and four allosteric regulatory sites.

Two identical regulatory sites, known as “low affinity, general activity” sites, bind either ATP or
dATP. ATP stimulates the reduction of all four ribonucleoside diphosphates (UDP, CDP, GDP
and ADP), while dATP inhibits their reduction. High intracellular [dATP] can inhibit the enzyme
completely.

Two other identical regulatory sites, known as “high affinity, substrate specificity” sites, bind

nucleotides - 9
either ATP, dATP, dTTP or dGTP. ATP and dATP stimulate the reduction of UDP and CDP, dTTP stimulates the reduction of GDP, but inhibits the reduction of UDP and CDP, and dGTP stimulates the reduction of ADP, but inhibits the reduction of UDP and CDP.

The regulation here is complex but presumably allows for the generation of a balanced mixture of deoxyribonucleoside triphosphates. For our purposes, the key point is that high concentrations of either dATP or dGTP can have a major effect on the concentrations of deoxyribonucleotides available for DNA synthesis.

**THYMIDYLATE SYNTHASE AND DIHYDROFOLATE REDUCTASE AS TARGET ENZYMES IN CANCER CHEMOTHERAPY**

![Thymidylate Synthase and Dihydrofolate Reductase Diagram](image-url)
HETEROCYCLIC RING SYSTEMS

pyrrole  pyrrolidine  imidazole  thiazole

pyridine  pyrimidine  uracil  thymine  cytosine

purine  adenine  hypoxanthine  xanthine  guanine

pteridine  isoalloxazine  corrin
MAJOR TAUTOMERS OF SOME COMMON NUCLEOSIDES