European Journal of Medical Practitioners

Volume 01| Issue 01 | July - Sep | 2024

Journal homepage: https://ejmp.org.uk/

Research Article

Regulation of Purine and Pyrimidine Analogs in DNA Incorporation: Insights into Phosphoribosyl-Pyrophosphate Synthetase Activity

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Submission Date: 28 May 2024 | Published Date: 08 July 2024

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Abstract

Synthesis which is permiting purine and pyrimidine analogs with potential as anticancer drugs to be incorporated into DNA. The most important regulator of de novo purine biosynthesis is the intracellular concentration of phosphoribosyl-pyrophosphate. The synthesis rates of purine and pyrimidine oxyribonucleotides and deoxyribonucleotides for parameters determination in cross regulation. Liver is a major site of purine nucleotide synthesis and provides purines in the form of bases or nucleosides to be salvaged and utilized by those tissues' incapable of synthetizing purines. In this paper the catalytic activity of phosphoribosyl-pyrophosphate synthetase was studied.

Keywords: Enzymes, biosynthesis rate, nucleotides, phosphoribosyl-pyrophosphate.

1. Introduction

The structure and function of the purines and pyrimidines and their nucleosides and nucleotides were studied in numerous literature [1]-[7]. Synthetic analogs of naturally occurring nucleotides find application in cancer chemotherapy as enzyme inhibitors and can replace the naturally occurring nucleotides in nucleic acids. Therapeutic attempts to inhibit the growth of cancer cells or certain viruses have often employed administration of analogs of bases, nucleosides, or nucleotides that inhibit the synthesis of either DNA deoxiribonucleic acid (DNA) or ribonucleic acid (RNA). Allopurinol, a purine analog, is widely used in the treatment of gout.

Biomedical important it neither nucleotides nor their parent purine and pyrimidine bases in the diet are incorporated into human tissue nucleic acids or into purine or pyrimidine coenzymes. Even when a diet rich in nucleoproteins is ingested, human subjects form the constituents of tissue nucleic acids from amphibolic intermediates. This de novo synthesis permits purine and pyrimidine analogs with potential as anticancer drugs to be incorporated into DNA. The rates of synthesis of purine and pyrimidine oxy- and deoxyribonucleotides are subject to precise regulation [8]. Mechanisms have evolved to ensure production of these compounds in quantities and at times appropriate to meet varying physiologic demand [9]-[12].

In addition to de novo synthesis, these include "salvage" pathways for reutilization of purine or pyrimidine bases released by degradation of nucleic acids in vivo. Human diseases that involve abnormalities in purine or pyrimidine metabolism include gout, Lesch-Nyhan syndrome, Reye's syndrome, adenosine deaminase deficiency, and purine nucleoside phosphorylase deficiency.

In this paper rate of purine and pyrimidine biosynthesis were studied.

2. Nucleotides transformation

Purine and pyrimidine bases that occur in the nucleotides are derived by substitution on the ring structures of the parent

substances, purine or pyrimidine. The positions on the rings are numbered according to the international system. Note that the direction of the numbering of the purine ring is different from that of the pyrimidine ring but that the number 5

carbon is the same in both heterocycle compounds. Because of their π electron clouds, both the purine and pyrimidine bases are planar molecules.

The tree major pyrimidine bases present in the nucleotides of both prokaryotes and eukariotes are cytosine, thymine, and uracil. The purine bases adenine and guanine are the two major purines found in living organisms. Two other purine bases, hypoxanthine and xanthine, occur as intermediates in the transformation of adenine and guanine (Fig. 1). In humans, a completely oxidizes purine base uric acid, is formed as the end product of purine catabolism.

In natural materials, numerous minor bases occurring addition to the 5 major bases. Some of these unusual substituted bases are present only in the nucleic acids of bacteria and viruses, but many are also found in the DNA and transfer RNAs of both prokaryotes and eukaryotes. For example, both bacterial and human DNA contain significant quantities of 5-methylcytosine, bacteriophages contain 5-hydroxymethilcytosine. Unusual bases present in the messenger RNA molecules of mammalian cells include N^6-methyladenine, N^{6-}, N^6-dimethyladenine, and N^7-methylguanine. An uracil modified at the N^3-position by the attachment of an (α- amino, α-carboxyl)-propyl group has also been detected in bacteria.

In plant, a series of purine bases containing methyl substituents occurs. Many have pharmacologic properties. Examples are coffee, which contains caffeine (1,3,7-trimethylxanthine), tea which contains theophylline (1,3-dimethylxanthine), and cocoa, which contains the obromine (3,7-dimethylxanthine).

Synthetic analogs of nucleobases, nucleosides, and nucleotides are widely used in the medical sciences and clinical medicine. In the past, most of these uses have depended upon the role of nucleotides as components of nucleic acids for cellular growth and division. For a cell to divide, its nucleic acids must be replicated. This requires that the precursors of nucleic acids -the normal purine and pyrimidine deoxy- be readily available. One of the most important components of the oncologict's pharmacopeia is the group of synthetic analogs of purine and pyrimidine nucleotides and nucleosides.

The pharmacologic approach has been to use an analog in which either the heterocyclic ring structure of the sugar moiety has been altered in such a way as to induce toxic effects when the analog becomes incorporated into various cellular constituents. Many of these effects results from inhibition by the drug of specific enzyme activities necessary for nucleic acids synthesis or from the incorporation of metabolites of the drug into the nucleic acids where they alter the base pairing essential to accurate transfer of information.

3. Biosynthesis regulation

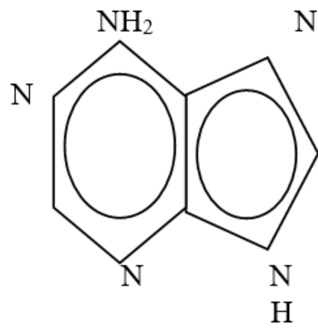
The de novo synthesis of IMP (inosine monophosphate) consumes the equivalent of 6-high-energy phosphodiester bonds (by adenosine triphosphate (ATP) hydrolysis) along with the other required precursors, glycine, glutamine, methenyltetrahydrofolate, and aspartate. It is important for the conservation of energy and nutrients that the cell economically regulate its rate of de novo purine biosynthesis. The most important regulator of de novo purine biosynthesis is the intracellular concentration of phosphoribosyl-pyrophosphate (PRPP). As with so many other intracellular compounds, PRPP concentration depends upon its rates of synthesis, utilization, and degradation.

The rate of synthesis of PRPP is dependent upon availability of its substrates, particularly ribose 5-phosphate, and the catalytic activity of PRPP synthetase, which is dependent upon the intracellular phosphate concentration as well as the concentrations of purine and pyrimidine ribonucleotides acting as allosteric regulators (Fig.1 and Fig. 2).

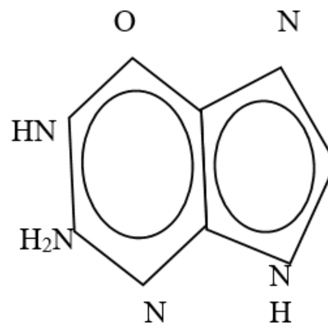
The rate of utilization of PRPP is dependent to a large extent on its consumption by the salvage pathway that phosphoribosylate hypoxantine and guanine to their respective nucleotides.

The first enzymes uniquely committed to de novo purine synthesis, PRPP amidotransferaze, demonstrates in vitro a sensitivity to feedback inhibition by purine nucleotides, particularly adenosine monophosphate and guanosine monophosphate (Fig.1).

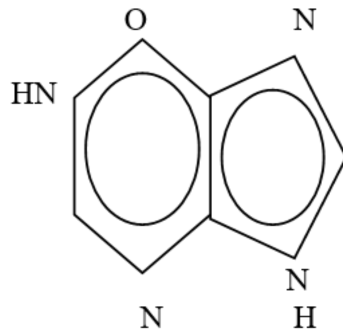
The conversion of IMP to guanozine monophosphate (GMP) or to adenosine monophosphate (AMP) to is regulated by reduction of purine and pyrimidine ribonucleotides. AMP feedback regulates its own synthesis as the level of adenylosuccinate synthetase, GMP regulates its own synthesis by feedback inhibition of IMP dehidrogenase. Furthermore, the conversion of IMP to adenylosuccinate end route to amp requires the presence of guanozine triphosphate (GTP). The conversion of xanthinylate to GMP requires. There is significant cross-regulation between the divergent pathways in the metabolism of IMP. This regulation prevents the synthesis of one purine nucleotide when there is a deficiency of the other. Hypoxanthine - guanine phosphoribosyl transferase, which converts hypoxanthine and guanine to IMP and GMP, respectively, is quite sensitive to product inhibition by these same nucleotides.



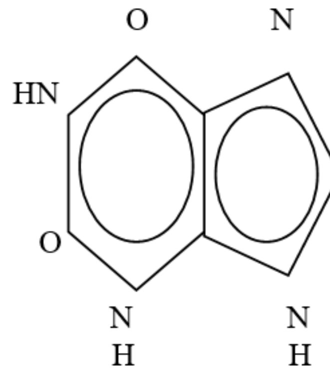
Adenine
(6-aminopurine)



Guanine
(2-amino-6-oxypurine)



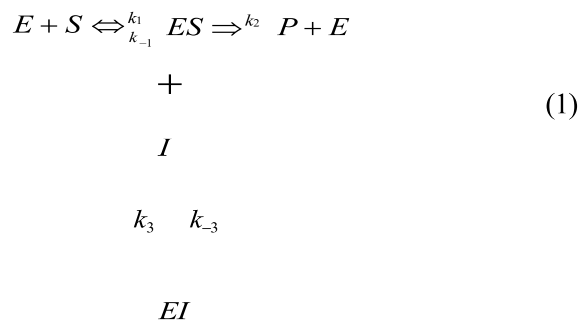
Hypoxanhine
(6-oxypurine)



Xanthine
(2,6-dioxypurine)

Fig.1 The major purine bases present in nucleotide

Thus, it can be assumed:



Enzyme complex rate can be defined.

$$\frac{dc_{ES}}{dt} = k_1 \cdot c_E \cdot c_S - k_{-1} \cdot c_{ES} - k_2 \cdot c_{ES} \qquad (2)$$

Product formation rate with inhibition:

$$\frac{dc_P}{dt} = k_2 c_{ES} \; - k_3 c_E c_I \qquad (3)$$

where S is substrate, E is enzyme, ES and EI are complexes, and k_1, k_{-1}, k_2, k_3, k_{-3} are specific biochemical constants.

The reduction of ribonucleoside diphosphates to deoxyribonucleoide diphosphates is subject to complex regulation.

Though the pyramidine nucleus is simpler and its synthetic pathway briefer than that of the purine structure, the share several common precursors. PRPP, glutamine, CO_2, and aspartate are required for the synthesis of all pyramidine and purine nucleotides. There is one striking difference between the syntesis of pyrimidine nucleotides and that of purine nucleotides, namely, that the synthesis of the purine nucleotides commences with ribose phosphate as an integral part of the earliest precursor molecule, whereas the pyrimidine base is formed and attachment of the ribose phosphate moiety delayed until the later steps of the pathway. For the thymidne nucleotides and for all purine nucleotides, tetrahydrofolate derivates are also necessary. The first step uniquely committed to the biosynthesis of pyrimidines is the formation of carbamoyl aspartate by the condensation of carbamoyl phosphate and aspartate, a reaction catalyzed by the enzyme aspartate transcarbamoylase.

In a subsequent dehydrogenation step catalyzed by dihydroorotate dehydrogenase and utilizing NAD as a cofactor, orotic acid is formed. Then a ribose phosphate moiety is aidded to orotic acid to form orotidylate (orotidine monophosphate, OMP). This reaction is catalyzed by orotate phosphoribosyl - transferase, an enzyme analogous to the hypoxanthine - guanine phosphoribosyl - transferase, and the adenine phosphoribosyl - transferase, involved in the phosphoribosylation of performed purine rings.

The first true pyrimidine ribonucleotide is formed by the decarboxylation of orotidylate to form uridilate (UMP-uridine monophosphate) (Fig.2).

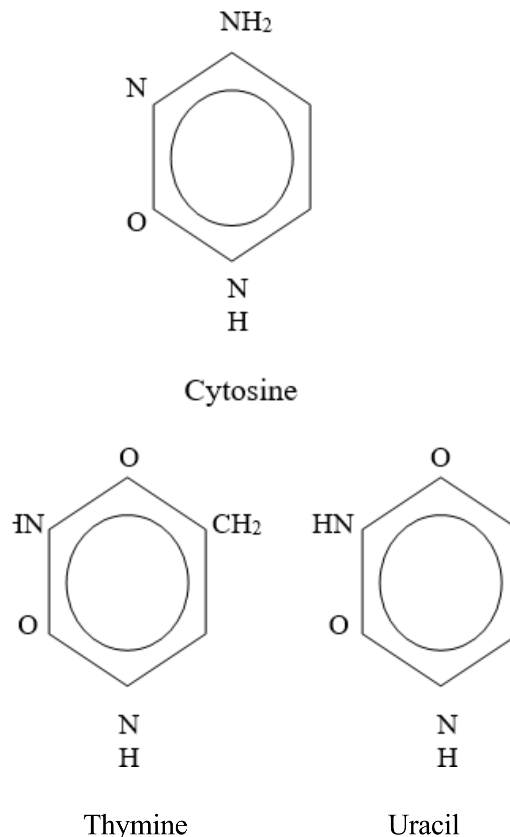


Fig.2 The major pyrimidine bases present in nucleotides

The pathway of nucleotide biosynthesis is regulated by the general mechanisms. The first two enzymes in the pathway are sensitive to allosteric regulation, while the first 3 enzymes are regulated by an apparently coordinate repression and depression, as are the last 2 enzymes of the pathway. Carbamoyl phosphate synthase is inhibited by uridine triphosphate (UTP) and purine nucleotides but activated by PRPP. Aspartate transcarbamoilase is particularly sensitive to inhibition by cytozine triphosphate (CTP). The allosteric properties of the aspartate transcarbamylase in microorganisms have been the subject of extensive and now classic studies of allostery.

On a molar basis, the rate of pyrimidine biosynthesis parallels that of purine biosynthesis, demonstrating a coordinate control of purine and pyrimidine nucleotide synthesis. PRPP synthetase, an enzyme that forms a necessary precursor for both purine and pyrimidine nucleotide biosynthesis, is subject to feedback inhibition by both purine and pyrimidine nucleotides.

Synthesis of the pyrimidine ring commences with the formation of carbamoyl phosphate from glutamine, ATP, and CO_2, in a reaction catalyzed by the carbamoyl phosphate synthase in the cytosol. The carbamoyl phosphate synthase enzyme responsible for the early steps in urea synthesis resides in the mitochondria.

Furthermore, carbamoyl phosphate synthase is sensitive to feedback inhibition by both purine and pyrimidine nucleotides and activation by PRPP. Thus, there are several sites at which there is significant cross-regulation between purine and pyrimidine nucleotide synthesis.

By mechanisms analogous to those desribed for the further phosphorylaton of the purine nucleoside monophosphates, the pyrimidine nucleoside monophosphates are converted to their diphosphate and triphosphate derivatives. UTP is aminated to CTP by glutamine and ATP. The reduction of the pyrimidine nucleoside diphosphates occurs by a mechanism also analogous to that described for the purine nucleotides.

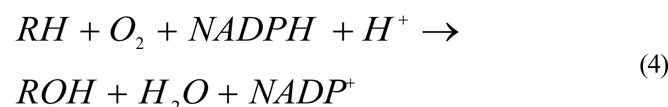
The formation of thymidylate (TMP - thymidine monophosphate) is the one reaction in pyrimidine nucleotide biosynthesis that requires a tetrahydrofolate donor of a single carbon compound.

In order to continue to use the folate carrier, the cell must reduce dihydrofolate to tetrahydrofolate, a reaction carried out by the enzyme dihydrofolate reductase. Thus, dividing cells that by necessity are generating TMP and dihydrofolate are especially sensitive to inhibitors of dihydrofolate reductase. An example of such an inhibitor is methotrexate (amethopterin, a widely used anticancer drug).

4. Cytochrome P450 enzyme treatment

Cytochrome P450 represent a coupled enzyme system composed of the heme-containing cytochrome P450 and the nicotin amide, adenine dinucleotide phosphate(H) (NADPH)[5]-[7] containing cytochrome 450 reductase. This flovoprotein has a preference for NADPH as its cofactor and transfers either one or two electrons from NADPH to cytochrome 450. The phospholipid matrix is crucial for enzymatic activity since is fascilitates the interaction between both enzymes. Individual enzymes are regulated in their expression by a variety of factors such as treatment with xenobiotics, species, organ, sex, diet. In mammals, two general classes of cytochrome P450 exist: six families involved steroid metabolism and bile acid biosynthesis, and four families containing numerous individual cytochromes P450 mainly responsible for toxic materials biotransformation.

Reaction catalyzed by cytochrome P450:



$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+ \quad (4)$$

where RH is substrate.
Cytochrome 450 enzymes are monooxigenases. These enzymes utilize one of the atoms of molecular oxygen and incorporate it into the destructed cell in the noted stoichiometry (eq.4).

The second oxygen atom is reduced to water with consumption of NADPH as reducing cofactor. There are the likely mechanisms of electron transfer and xenobiotic oxidation.

In the first step of catalytic cycle, the xenobiotic combines with the oxidized form of cytochrome P450 (Fe^{3+}) followed by one electron reduction by NADPH cytochrome P450 reductase to form a reduced (Fe^{2+}) cytochrome P450 substrate complex. This complex then combines with molecular oxygen, and another electron from NADPH is accepted. In the last step of the catalytic cycle, the oxidized substrate dissociates and regenerates to oxidized form of cytochrome P450. Examples of oxidation reactions catalyzed by cytochromes P450 are aliphatic hydroxylation, N-dealkylation, O-dealkylation and epoxidatuion.

Cytochromes P450 may catalyze the hydroxylation of carbon hydrogen bonds to transform hydrocarbons to the corresponding alcohols. In larger aliphatic chains, oxidative N-, O- or S- dealkylations and oxidative dehalogenations are similar in mechanism to aliphatic hydroxylation, but give different end products due to secondary reactions of the intermediate products formed. Olefins are also oxidized by cytochrome P450, and with some substrates, epoxides are formed as products. The reaction, however, does not proceed in a concerted fashion, but involves discrete ionic intermediates. These may also rearrange to products other than epoxides as shown for chloroolefins.

Sulfur or nitrogen is oxidized by the addition of oxygen at the one electron pair on the sulfur or nitrogen atom. The products formed may be stable like many sulfoxides, may be further oxidized by other enzymes in the organism (hydoxylamines), or may decompose to sulfur and the corresponding oxo compound.

5. Catabolism

The catabolism of pyrimidines, which occurs mainly in the liver, produces highly soluble end products. This contrasts with the production of the sparingly soluble uric acid and sodium urate by purine catabolism. The release of respiratory CO_2 from the ureido carbon of the pyrimidine nucleus represents a major pathway for the catabolism of uracil, cytosine, and thymine. β - alanine and β - aminoisobutyrate are the major end products of cytosine, uracil, and thymine catabolism, respectively.

Thymine is the precursor β - aminoisobutyrate in laboratory animals and in humans. The excretion of β - aminoisobutyrate is increased in leukemia as well as after the body has been subjected to X-irradiation. This is undoubtedly a reflection of increased destruction of cells and their DNA. A familial occurrence of an abnormally high excretion of β - aminoisobutyrate has also been observed in otherwise normal individuals. This genetic trait is traceable to recessively expressed gene. High excretors results only when the trait is homozygous. Although little is known about the mechanism whereby β - aminoisobutyrate is degraded in humans, an enzyme that catalyzes the reversible transamination reaction has been identified in pig kidney. The β - aminoisobutyrate is convereted to methylmalonic semialdehyde and thence to propionate, which in turn proceeds to succinate.

The initial steps in degradation of pyrimidine nucleotides, including the removal of the sugar phosphate moitely by hydrolysis of the N-glucosidic bond are similar to reversing the later part of the synthetic pathway. For pseudouridine, which is formed in t - RNA by a rearrangement reaction, there is no mechanism to catalyze the hydrolysis or phosphorylsis of this unusual nucleoside to its respective pyrimidine base, uracil. Consequently, pseudouridine is excreted unchanged in the urine of normal persons. Some individuals with urate overexctretion greater than 600 mg/uric acid per 24 hours can be categorized as having secondary hyperuricemia. They have other disease processes such as cancer or psoriasis that led enhanced tissue turnover.

Finally, there are persons with identifiable enzyme defects, including abnormalities of PRPP synthetase, the HGPRT- ase (hypoxanthine - guanine phosphoribosyl - transferase) deficiencies, both the complete Lesch - Nyhan syndrome and incomplete deficiencies and glucose – 6 - phosphatase deficiency von Gierke’s disease. There exists also a group of patients exhibiting idiopathic overproduction hyperuricemia, which will certainly be regarded as a heterogeneous group of disease ones the molecular bases for their metabolic defects are recognized.

6. Conclusion

The most important regulator of the nucleotides biosynthesis is the intracellular concentration of PRPP. The kinetic equations for enzymes rate definition were derived and the rate of PRPP can be control. Carbamyl phosphate synthase is sensitive to feedback inhibition by both purine and pyramidine nucleotides and activation by PRPP.

A coupled enzyme cytochrome P 450 decreased destruction cells and their DNA.

Examination, in the framework of this paper, shows enzymes effects in significant cross - regulation between purine and pyramidine nucleotide synthesis and can be put in function of cancer treatment.

Notation

E - enzyme

ES – complex

c - concentration, mol/cm^3

I - inhibitor

k - specific biochemical constant, s^{-1}

S - substrate

P - product

t - time, s

Subscript
E - enzyme
P - product
S - substrate

Abbervation

AMP - adenine monophosphate
ADP - adenine diphosphate
ATP - adenine triphosphate
CTP - cytosine triphosphate
DNA - deoxynucleic acid
DP - diphosphate
GDP - guanine diphosphate
GMP - guanine monophosphate
GTP - guanine triphosphate
HGPRT – ase - hypoxanthine - guanine
Phosphoribosyl - transferase
IMP - inosine monophosphate
MP - monnophosphate
NADPH - cofactor
OMP - orotidine monophosphate
PRPP - phosphoribosyl-pyrophosphate
RNA – ribonucleic acid
TMP - thymidine monophosphate
TP- triphosphate
UMP - uridine monophosphate
UTP - uridine triphosphate

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