

Regulatory mechanism of transcriptional activity

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Abstract: - The biomedical important is de novo synthesis which is permitting purine and pyrimidine analogs with potential as anticancer drugs to be incorporated into DNA. The synthesis rates of purine and pyrimidine oxyribonucleotides and deoxyribonucleotides are subject to precise regulation. The most important regulator of de novo purine biosynthesis is the intracellular concentration of PRPP-phosphoribosylamine. Mammalian 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 synthesizing purines de novo. In this paper sensitivity to product inhibition by reacting nucleotides was involved.

Key-Words: - Biosynthesis, enzyme, catabolism, nucleosides, amidotransferaze, cross-regulation.

1 Introduction

The structure and function of the purines and pyrimidines and their nucleosides and nucleotides were studied in numerous literature. 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 or 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. Mechanisms have evolved to ensure production of these compounds in quantities and at times appropriate to meet varying physiologic demand.

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 regulatory mechanisms of purine and pyrimidine biosynthesis were studied.

2 Synthetic nucleotide

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 oncologist's pharmacopeia is the group of synthetic analogs of purine and pyrimidine nucleobases 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 Regulation of Purine Biosynthesis

The de novo synthesis of IMP (Inosine monophosphate) consumes the equivalent of 6-high-energy phosphodiester bonds (by 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 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).

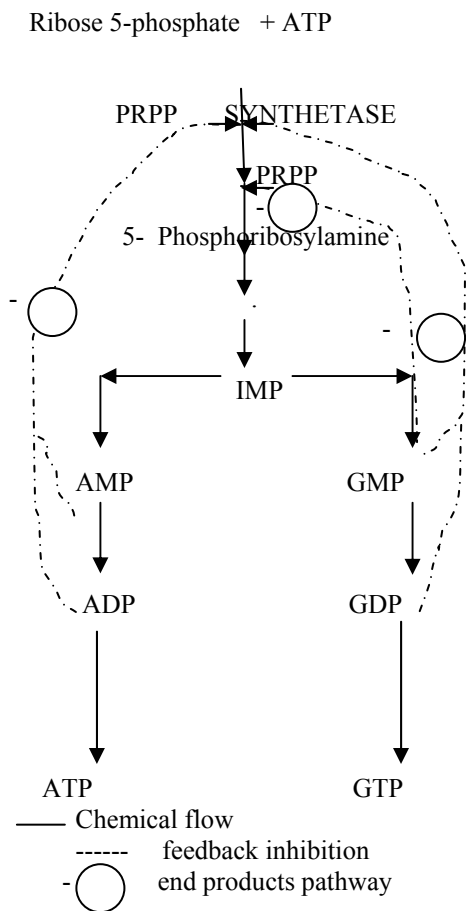
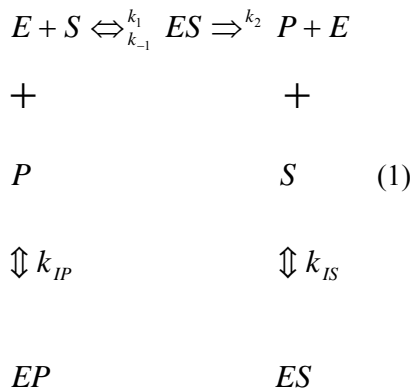


Fig.1 De novo synthesis purine control

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

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

The conversion of IMP to GMP or to AMP 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 dehydrogenase. Furthermore, the conversion of IMP to adenylosuccinate en route to amp requires the presence of 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 phosphoribosyltransferase, which converts hypoxanthine and guanine to IMP and GMP, respectively, is quite sensitive to product inhibition by these same nucleotides. Thus, it can be assumed :



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

4 Catabolism of Purines

In humans the ultimate catabolite (end product) of purines is uric acid. On the basis of observations in humans with inherited enzyme deficiencies, it appears that over 99% of the uric acid is derived from substrates of purine nucleoside phosphorylase

, a component of the purine salvage pathway. The purine products of purine nucleoside phosphorilase, guanine and hypoxanthine, are converted to uric acid by way of xanthine in reaction catalysed by the enzymes guanase and xanthine oxidase, respectively, of liver, small intestine, and kidney.

Xanthine oxidase is an important site for pharmacologic intervention in patients with hyperuricemia and gout. In lower primates and other mammals, but not in humans, uricase hydrolyses uric acid to allantoin, a highly water soluble end product. Amphibians, birds, and reptiles, which lack uricase, excrete uric acid and guanine as the end products of both purine metabolism and nitrogen (protein) metabolism.

5 Regulation of Pyrimidine Biosynthesis

Although the pyrimidine nucleus is simpler and its synthetic pathway briefer than that of the purine structure, they share several common precursors. PRPP, glutamine, CO₂, and aspartate are required for the synthesis of all pyrimidine and purine nucleotides. There is one striking difference between the synthesis 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 thymidine nucleotides and for all purine nucleotides, tetrahydrofolate derivatives 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 added to orotic acid to form orotidylate (orotidine monophosphate, OMP). This reaction is catalyzed by orotate phosphoribosyltransferase, an enzyme analogous to the hypoxanthine-guanine phosphoribosyltransferase, and the adenine phosphoribosyltransferase, involved in the phosphoribosylation of performed purine rings.

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

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 UTP and purine nucleotides but activated by PRPP (Fig.2). Aspartate transcarbamoylase is particularly sensitive to inhibition by CTP. The allosteric properties of the aspartate transcarbamoylase 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 CO₂, 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 described for the further phosphorylation 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).

6 Catabolism of Pyrimidines

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 CO₂ 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. Approximately 25% of tested. 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 converted 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 moiety by hydrolysis of the N-glycosidic 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 phosphorylation 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 overexcretion greater than 600mg/uric acid per 24 hours can be categorized as having secondary hyperuricemia. They have other disease processes such as cancer or psoriasis that lead 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

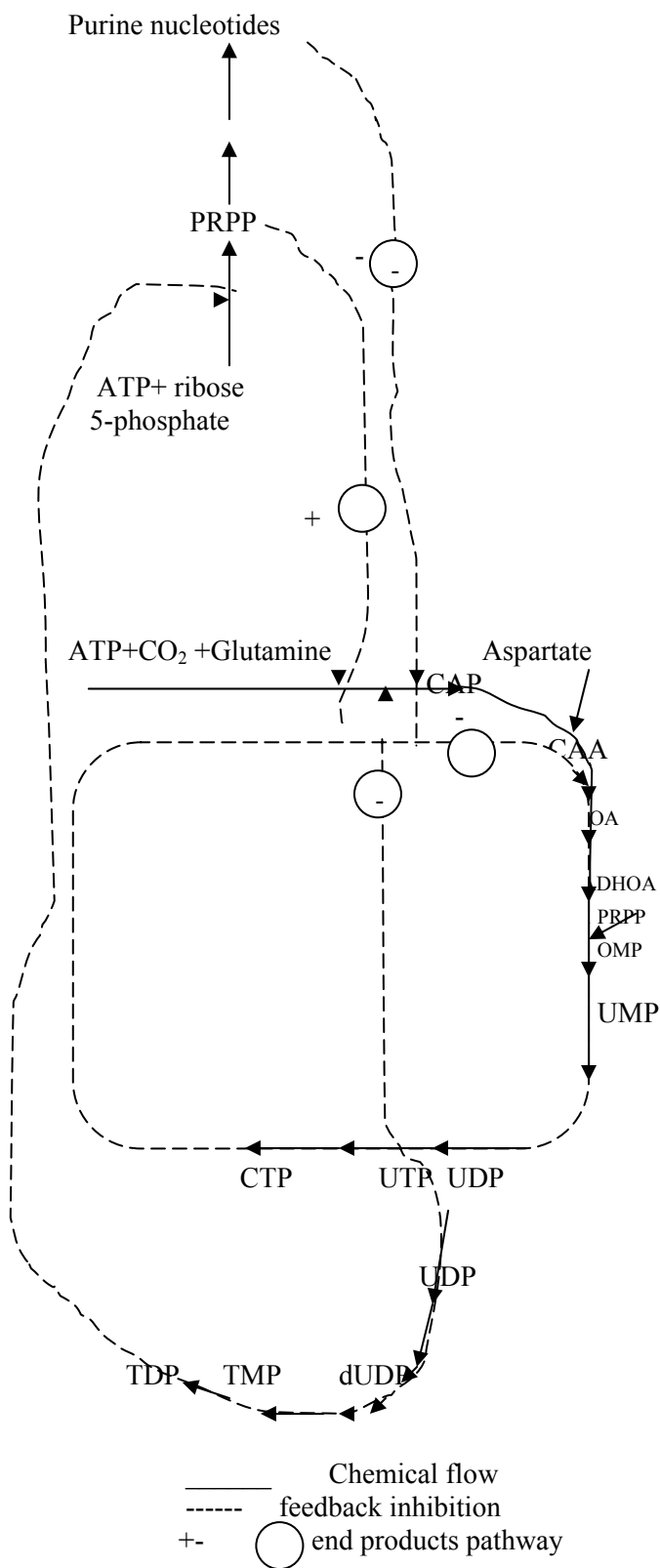


Fig.2 Control of pyrimidine nucleotide synthesis

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 on the molecular bases for their metabolic defects are recognized.

7 Disorders

The predominant form of uric acid is determined by the pH of its milieu (eg, blood, urine, cerebrospinal fluid). Thus, under physiologic conditions ie, at the usual pH of physiologic fluids, only uric acid and its monosodium salt, sodium urate, are found. In a fluid where the pH is less than 5.75, the predominant molecular species will be uric acid. In a fluid at pH 5.75, the concentration of sodium urate will equal that of uric acid. At a pH greater than 5.75, sodium urate will predominate in the solution.

The miscible urate pool in the body is reflected by the sodium urate concentration in the serum. When this level exceeds the solubility of sodium urate in serum (hyperuricemia), crystals of sodium urate may precipitate. The solubility of sodium urate in serum at 37 °C is 7 mg/dl. Crystals of sodium urate can collect and deposit in soft tissues, particularly in or about joints. These urates deposits are referred to as tophi. Accumulation of sodium urate crystals in the tissues including phagocytosis of the crystals by polymorphonuclear leukocytes in joint spaces, can lead to an acute inflammatory reaction called acute gouty arthritis.

The end products of pyrimidine metabolism, unlike those of purine metabolism, are highly water soluble compounds such as CO₂, ammonia, β – alanine, and propionate. Thus, in circumstances where pyrimidine overproduction occurs, clinically detectable abnormalities are rarely evident. In cases of hyperuricemia associated with severe PRPP overproduction, there is concomitant overproduction of pyrimidine nucleotides with increased excretion of compounds such as β –alanine.

In specific liver mitochondrial failure, such as in Reye's syndrome, there is a secondary orotic acid-urea.

Purine deficiency states are rare in humans. These are limited to circumstances attributable primarily to deficiencies of folic acid and perhaps of vitamin B₁₂ when the later results in a secondary deficiency of folate derivatives.

7 Conclusion

Carbamyl phosphate synthase is sensitive to feedback inhibition by both purine and pyrimidine nucleotides and activation by PRPP. The most important regulator of de novo purine biosynthesis is

the intracellular concentration of PRPP. Need to examine, more sites at which there is significant cross-regulation between purine and pyrimidine nucleotide synthesis.

Notation

AMP- adenine monophosphate
 ADP- adenine diphosphate
 ATP- adenine triphosphate
 CTP- cytosine triphosphate
 DP-diphosphate
 GDP- guanine diphosphate
 GMP-guanine monophosphate
 GTP- guanine triphosphate
 HGPRase-hypoxanthine-guanine phosphoribosyl- transferase
 IMP- inosine monophosphate
 MP-monophosphate
 NADPH-cofactor
 OMP- orotidine monophosphate
 PRPP- phosphoribosyl-pyrophosphate
 TMP-thymidine monophosphate
 TP- triphosphate
 UMP- uridine monophosphate
 UTP- uridine triphosphate

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