Metabolism of nucleotides nucleosides and nucleobases in microorganisms

Edited by

AGNETE MUNCH-PETERSEN

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University Institute of Biological Chemistry B, Copenhagen, Denmark

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The last decade has witnessed an immense expansion in the field of nucleic acid research. In parallel to this there has been an accumulation of data concerning the metabolism of compounds involved in, or related to, the cellular synthesis of nucleic acids. Procaryotic as well as eucaryotic cells have been shown to command numerous enzymatic reactions, through which they can scavenge nucleic acid precursors from the surroundings and metabolize them according to their immediate needs, whether they be for nucleic acid synthesis or as carbon or nitrogen sources.

Besides being involved in nucleic acid synthesis, the role of certain nucleotides as important regulatory compounds in cell metabolism has been well documented. In addition there has been an increasing pharmacological use of nucleoside and nucleotide analogs as antimetabolites in the treatment of disease. This in turn emphasizes the practical importance of knowledge about the enzymatic reactions which may be the targets for the therapeutic treatment, since the analogs are metabolized mainly by the same enzymes as are the natural intermediates in nucleic acid metabolism.

The main objective of the present book has been to present a detailed description of the anabolic and catabolic pathways which may function when nucleic acid precursors (or their analogs) are made available to growing cells. The book has developed as a joint project at our Institute. The different contributors are all past or present members of the staff and each author has been a researcher within the particular field which is treated in the chapter.

The first chapter deals with the metabolism of 5-phosphoribosyl 1-pyrophosphate, a key compound in nucleotide metabolism. While the enzyme responsible for the synthesis of this compound has been characterized in detail, its regulation on the genetic level is still quite obscure. Mutants with defects in the synthesis of 5-phosphoribosyl 1-pyrophosphate have now been isolated as mentioned in Chapter 1, but at present they have only been partially characterized. Chapters 2, 3, and 4 describe in detail the multitude of enzymatic reactions and pathways which are brought into operation when exogenous purine or pyrimidine compounds are utilized by the cells for nucleic acid synthesis. Each compound is individually treated and a practical

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aspect of this information is that it should enable the reader, who wishes to do experiments with labelled nucleic acids, to work out labelling procedures, optimally tailored to the enzymatic patterns found in the individual bacterial strains. The two last chapters deal specifically with the transport of the nucleosides into the cells and the subsequent intracellular catabolism. While the enzymatic background for the latter has been known in detail for some years, the transport systems are only beginning to be unravelled. It is known, however, that transport and catabolism are regulated by the same cellular control systems and thus closely linked in the metabolism of the cells. The control systems have proved to be of considerable complexity and they are described in some detail in Chapter 5.

Throughout the chapters the importance of well characterized mutants with defects in the different enzymes has been emphasized, and selection procedures have been described or referred to whenever possible.

An appendix concludes the book. It enlists the genes from Salmonella typhimurium and Escherichia coli, which are known to be involved in the metabolism of nucleic acid precursors and thus are relevant to the subjects treated in the book. In addition the appendix contains a linkage map, indicating the location of these genes on the circular chromosome of S. typhimurium and E. coli.

The dramatic developments within molecular genetics during the last few years perhaps tend to overshadow the enzymological and metabolic aspects of nucleic acid precursors in the cells, although they form the basis for nucleic acid synthesis and for many cellular control systems. It has been our aim to produce a useful book on these subjects, which are too specialized for textbooks and yet of importance for those who carry on experimental research are he natural assermediates in nucleic held metaloping in the field.

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May 1983 Agnete Munch-Petersen

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Metabolism of 5-phosphoribosyl 1-pyrophosphate (PRPP) in Escherichia coli and Salmonella typhimurium

KAJ FRANK JENSEN

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I. INTRODUCTION

The discovery of 5-phosphoribosyl-α-1-pyrophosphate (PRPP) was intimately connected to the metabolic function of the compound in nucleotide biosynthesis (33, 55). In searching for the origin of the phosphoribosyl moiety which is being added to preformed purine and pyrimidine bases during their conversion to nucleotides, Kornberg et al. (33) discovered and partially purified an enzymatic activity from pigeon liver extracts which synthetized PRPP from ribose 5-phosphate and ATP:

ribose 5-phosphate + ATP-PRPP + AMP

The following two reactions were used to monitor PRPP formation:

orotate + PRPP
$$\rightleftharpoons$$
OMP + PP_i
OMP \rightleftharpoons UMP + CO₂

From the stoichiometry of reaction [I] and from studies of the decomposition of the product in acid solution the structure of PRPP was deduced (Fig. 1.1) and it was suggested that the pyrophosphoryl group of ATP was transferred intact to ribose 5-phosphate in the reaction. Evidence supporting this was provided shortly after by Khorana et al. (31) who showed that the phosphate group which is being esterified to the anomeric hydroxyl group of ribose 5-phosphate in the reaction derives from the β -position of ATP. The α -configuration at carbon number one of PRPP was deduced from the observation that PRPP is broken down to 5-phosphoribosyl-1.2-cyclic phosphate and orthophosphate in alkaline solution (31, 55, 67).

FIG. 1.1. Structure of 5-phosphoribosyl- α -1-pyrophosphate (PRPP); Haworth projection.

After the discovery of PRPP synthetase in pigeon liver Kornberg et al. (33) showed the enzyme to be present in various other tissues and organisms including Escherichia coli.

PRPP was shown to function in the *de novo* synthesis of pyrimidine and purine nucleotides (18, 19, 33), in the biosynthesis of histidine, tryptophan, and pyridine nucleotide coenzymes (45, 52, 53, 70), and was also found to participate in the utilization of preformed purine and pyrimidine bases for nucleotide synthesis (9, 34, 38, 55). Thus the PRPP synthetase reaction may be regarded as the first step in a highly branched biosynthetic pathway leading to the formation of these compounds (Fig. 1.2) and may therefore be expected to be subject to rigorous metabolic control.

The literature concerning PRPP synthesis in mammalian cells has been reviewed by Becker and Seegmiller (6). In this chapter we shall deal only with the synthesis and utilization of PRPP in the two enteric bacteria Escherichia coli and Salmonella typhimurium. For other microorganisms the knowledge of PRPP metabolism, notably PRPP synthesis, is scarce and sporadic.

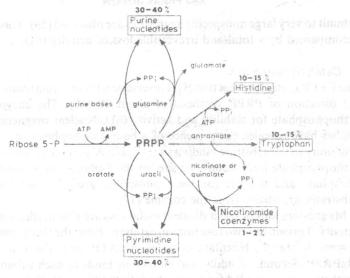


FIG. 1.2. Consumption of PRPP. The endproducts of the various biosynthetic pathways that utilize PRPP are shown in boxes. The numbers next to the boxes indicate the percentage of total PRPP which is consumed by the different pathways when E. coli or S. typhimurium is grown in a glucose salt minimal medium.

II. REGULATION OF PRPP SYNTHESIS

A. Properties of PRPP synthetase (EC 2.7.6.1)

1. Enzyme structure

PRPP synthetase has been purified to homogeneity and in high yield from an extract of *Salmonella typhimurium* (61). A molecular weight of the subunit of 31 000 was determined by a variety of methods (59). Proline was found to be the only aminoterminal residue indicating that the enzyme contains only one kind of polypeptide chains. This is also consistent with sulfhydryl titration data (56, 59).

The aggregated state of the native enzyme is not well defined. In the presence of inorganic phosphate the majority of the enzyme is found in two species with molecular weights of about 160 000 and 320 000 suggesting that the predominant forms are pentamers and decamers or possibly hexamers and dodecamers. The relative distribution of enzyme in the two major forms depends on the experimental conditions. In the electron microscope an asymmetric pentameric structure has been observed (59).

When inorganic phosphate is removed PRPP synthetase completely loses a defined quaternary structure and all aggregational states from the size of the

subunit to very large nonspecific aggregates are observed (59). This situation is accompanied by a total and irreversible loss of activity (61).

2. Catalytic properties

The PRPP synthetase reaction [I] is reversible with an equilibrium constant in the direction of PRPP synthesis of about 29 (61). The enzyme requires orthophosphate for stability and activity (61). Nuclear magnetic resonance studies have revealed the presence of a bound orthophosphate ion in close proximity to the substrate binding site (17, 37). Also these studies showed that orthophosphate ions occupy the site for the 5-phosphate group of ribose 5-phosphate and the site for the γ -phosphate group of ATP when these substrates are absent from the enzyme (17).

Magnesium ions or other divalent metal ions are also needed for enzymatic activity. This ion has two functions in catalysis. First, the true substrate for the enzyme is the β , γ -bidentate complex MgATP, and the true product is MgPRPP. Second, an additional metal ions binds to each subunit of PRPP synthetase and is needed for enzymatic activity (Fig. 1.3). This was concluded from the observation that the exchange stable β , γ -bidentate complex

FIG. 1.3. Arrangement and conformations of substrates and activators at the active site of PRPP synthetase of Salmonella typhimurium consistent with the determined distances from enzyme-bound Mn^2 + (17) and from the Cr^3 + atom of exchange-stable, substitution inert α, β, γ -tridentate Cr-ATP (37). Ribose 5-phosphate is appropriately positioned for nucleophilic attack with inversion on the β -phosphorous atom of ATP. The broken line from the 1- α -D-ribose oxygen to the β -phosphorous atom of ATP shows the reaction coordinate. Reproduced (with permission) from Granot et al. (17).

Co(III)(NH₃)₄-ATP is a substrate for PRPP synthetase only if a second cation such as Mg²⁺ or Mn²⁺ is present (36). The conclusion was confirmed by nuclear magnetic resonance spectroscopy using paramagnetic ions (17, 37). These studies also revealed that the enzyme-bound metal ion does not directly interact with the bound substrates (17).

Kinetic studies indicate a sequential reaction mechanism for PRPP synthetase (62, 64). This implies that the reaction takes place as a direct substitution while both substrates are bound to the enzyme. The combined data agree best with an obligatory reaction order where binding of MgATP precedes binding of ribose 5-phosphate (14, 17, 61, 62).

When the reaction is carried out in $H_2^{18}O$, oxygen-18 is recovered in PRPP, but not in AMP. This indicates that a β P-O bond is broken and hence that the reaction proceeds via a direct nucleophilic attack (S_N2) by the α -1-OH group of ribose 5-phosphate on the β -phosphorous atom of the polyphosphate chain of ATP, displacing AMP and forming PRPP (43). Nuclear magnetic resonance studies using paramagnetic metal ions (17, 37) showed that the spatial arrangement of the substrates in the active centre of the enzyme is favourable for such a nucleophilic attack to occur; the distance between the α -1-OH group of ribose 5-phosphate and the β -phosphorous atom of ATP being about 3.8 Å (37). Figure 1.3 shows a model of the arrangement of substrates and activators in the active site of PRPP synthetase.

The enzyme is inactivated by oxidation of a single specific cystein residue (56) and by chemical modification of a single specific lysine residue (57). Since the reactivity of either of the two residues to chemical modification is reduced by the presence of substrates they are probably located near the active site, but their roles in catalysis are unknown (56, 57).

3. Regulatory properties

The effect of purine and pyrimidine nucleotides, tryptophan, histidine, and nicotinamide coenzymes on the reaction kinetics of purified PRPP synthetase has been investigated since these compounds may be regarded as endproducts of a branched biosynthetic pathway with the PRPP synthetase reaction being the first step (Fig. 1.2).

All pyrimidine and purine nucleotides except ADP and dADP inhibit the enzyme competitively with ATP. In general, purine nucleotides are stronger inhibitors ($K_i \sim 2$ mM) than pyrimidine nucleotides ($K_i \sim 5$ mM). Very weak competitive inhibition is observed with NAD⁺, NADP⁺, and ppGpp, while histidine and tryptophan have no effect on PRPP synthetase activity (66).

ADP is by far the most powerful inhibitor of PRPP synthetase activity. The K_i is 10 to 100 times smaller than for other purine nucleotides, dependent on the concentration of ribose 5-phosphate. The mode of action of this inhibitor is complex. At low ribose 5-phosphate concentrations ADP produces a linear

competitive inhibition towards ATP, but at high concentrations of ribose 5-phosphate this inhibition becomes a parabolic function of the concentration of ADP, indicating that this compound interacts with more than one enzyme form in the reaction. The presence of ADP also induces a pronounced substrate inhibition exerted by ribose 5-phosphate. The higher the concentration of ADP is the lower is the concentration of ribose 5-phosphate needed to produce an evident substrate inhibition (Fig. 1.4).

Equilibrium dialysis experiments have shown that ADP can bind to the active site of PRPP synthetase in the absence of ribose 5-phosphate. This binding is sensitive to competition by ATP and by the reaction inert analog, α, β -methylene ATP. However, in the presence of ribose 5-phosphate an additional site becomes available for ADP, and the binding to this site is not subject to competition with α, β -methylene ATP (14). Thus ADP seems to be a

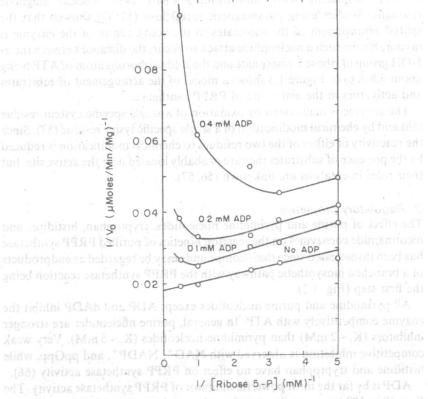


FIG. 1.4. Effect of ADP on the saturation kinetics of PRPP synthetase with ribose 5-phosphate. Reproduced with permission from Switzer and Sogin (66).

true allosteric inhibitor of PRPP synthetase (14, 66). DeoxyADP produces an inhibition pattern which is qualitatively similar to that of ADP, but it is a somewhat weaker inhibitor (66).

Since ATP is a substrate for the enzyme, AMP is a product, and ADP is a strong inhibitor, it has been suggested that PRPP synthetase activity is regulated by "energy charge" (2, 35). As the inhibition by ADP depends on the concentration of ribose 5-phosphate, the effect of "energy charge" on PRPP synthetase activity varies with the concentration of ribose 5-phosphate (66).

In summary, the in vitro studies indicate that PRPP synthetase activity is regulated by the entire pool of purine and, to a lesser extent, pyrimidine nucleotides. In particular ADP is a potent inhibitor. The supply of ribose 5-phosphate in combination with "energy charge" may also be of physiological significance.

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B. Physiological evidence for regulatory properties of PRPP synthetase

Measurements of the intracellular concentration of PRPP in growing cells have given some information concerning the physiological significance of the regulatory properties found *in vitro* for PRPP synthetase.

An inverse relationship has been observed between the size of the PRPP pool and the pools of ribonucleoside triphosphates in *E. coli* K-12 under conditions where RNA synthesis is suddenly stimulated (3). The stimulation was accomplished by addition of leucine or chloramphenicol to a leucine-starved culture or by addition of methionine to an exponentially growing culture. At the onset of the enhanced RNA synthesis the nucleoside triphosphate pools drop transiently to about one third of their initial size, whereas the ADP pool remains unchanged. Concomitantly with this the PRPP pool increases substantially and returns to normal simultaneously with the purine nucleoside triphosphate pools. This rise in PRPP concentration may be interpreted as the result of a release of feed-back inhibition of PRPP synthetase activity caused by the fall in the purine ribonucleoside triphosphate pools (3, 66).

When a purine requiring mutant of *S. typhinurium (purF)* is starved for purine both the purine and the pyrimidine nucleoside triphosphate pools fall to low levels. Under these conditions PRPP accumulates rapidly and the concentration increases more than one hundred fold. This accumulation of PRPP continues for hours indicating that no factor which inhibits PRPP synthesis is operative in this condition (58).

When an adenine requiring mutant of S, typhimurium is starved for adenine, the adenine nucleotide pools fall to low levels while GTP and the pyrimidine

nucleotide pools remain relatively normal. Also in this case PRPP accumulates (58).

During guanine starvation of S. typhimurium (guaB) mutants the guanine nucleotide pools are depleted while the pools of adenine nucleotides increase and the pyrimidine nucleotide pools remain relatively constant. This condition of starvation is accompanied by a reduction of the PRPP pool. Such a reduction of the PRPP pools is also seen during starvation of pyrimidine auxotrophic mutants, where the pyrimidine nucleotide pools decrease and the purine nucleotide pools increase (58).

Guanine requiring mutants of S. typhimurium that harbour the mutation in guaA respond differently to guanine starvation with regard to the behaviour of ATP and PRPP than the corresponding guaB mutants (Fig. 1.5). During starvation of a guaA strain the ATP pool initially falls and then increases again. In contrast, the PRPP pool first increases, then reaches a maximum and decreases again. The maximal PRPP pool is observed simultaneously with the minimal ATP pool (Fig. 1.5).

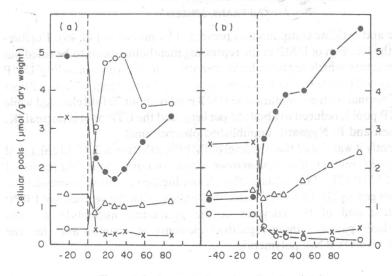
Thus it appears that starvation conditions which lead to high adenine nucleotide pools result in a reduction of the PRPP pool, while conditions which lead to reduced pools of adenine nucleotides result in an accumulation of PRPP. Hence the in vivo experiments demonstrate the presence of a physiologically significant inhibitor amongst the adenine nucleotides; this could well be ADP (58).

At the onset of a nitrogen starvation of *S. typhimurium* the PRPP pool is rapidly and totally depleted even though the specific activity of PRPP synthetase remains unchanged, the ribonucleoside triphosphate pools decrease, and the energy charge ratio remains constant. This indicates that the enzyme activity becomes immediately inhibited. This phenomenon is of interest as all compounds that require PRPP for their synthesis contain nitrogen. Since there is no known *in vitro* property of purified PRPP synthetase which might explain this phenomenon it was suggested that the synthesis of PRPP terminates due to decreased availability of ribose 5-phosphate (58).

At present there is no strong evidence which supports the hypothesis that energy charge *per se* might be a relevant physiological regulator of PRPP synthetase activity. However, experiments relating to this problem has been discussed by others (3, 58).

C. Control of enzyme synthesis

The specific activity of PRPP synthetase in extracts of *S. typhimurium* depends on the carbon source used for growth but appears not to be correlated to the growth rate (69).



Time relative to onset of guanine-starvation (min)

FIG. 1.5. Reciprocal relation between the pool of ATP and the pool of PRPP during starvation of guanine requiring mutants of Salmonella typhimurium. The bacteria were grown in a glucose-salt minimal medium supplemented with adenine (15 μ g/ml) and guanine (6 μ g/ml), labelled with [32 P]-orthophosphate, and allowed to exhaust their guanine supply (time equal zero min). At various times samples were withdrawn for determination of pools of nucleotides and PRPP (24). (a) Starvation of KP-1489 (guaA); ATP (\bullet - \bullet), GTP (\times - \times), UTP (\triangle - \triangle), and 0.5 \times PRPP (\bigcirc - \bigcirc). (b) Starvation of KP-1476 (guaB); 0.25 \times ATP (\bullet - \bullet), GTP (\times - \times), UTP (\triangle - \triangle), and PRPP (\bigcirc - \bigcirc). The pool of CTP responded essentially as the UTP pool and has not been shown for clarity reasons. (K. F. Jensen, unpublished results.)

The possibility that enzyme synthesis might be controlled by repression by some of the biosynthetic end products has been investigated in *S. typhimurium* (69). The addition of purines, pyrimidines, histidine, or tryptophan to the medium has only marginal repressive effects on the specific activity of PRPP synthetase (69).

Pyrimidine starvation was found to be the only starvation condition which leads to derepression of the synthesis of PRPP synthetase (69). By total starvation the level of PRPP synthetase increases two to three fold. Slowfeeding of pyrimidine auxotrophic mutants by the use of orotate as pyrimidine source may lead to a ten fold elevated enzyme level. Thus it appears that the synthesis of the enzyme is under repressive control by pyrimidine nucleotides (69). The repressing pyrimidine compound seems to be a uridine nucleotide rather than a cytidine nucleotide (49) since PRPP synthetase activity only increases by starvation for uridine nucleotides and not by starvation for cytidine nucleotides in mutants where the interconversion of