Purine Nucleoside Phosphorylase from Hyperthermophilic *Archaea* as Biotechnological Tools for Drug Synthesis and Prodrug Activation

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Cytotoxic nucleoside analogues and nucleobases were among the first chemiotherapeutic agents to be introduced for the medical treatment of cancer. These agents behave as antimetabolites, compete with physiologic nucleosides, and interact with a large number of intracellular targets to induce cytotoxicity. Purine and pyrimidine analogues are widely used not only as antileukemic agents, but also as cytotoxic agents to treat solid tumours. However, the clinical use of these compounds is limited by important side-effects and primary or acquired drug resistance. Thus, there is an unmet medical need for the development of new analogs characterized by higher selectivity and minor toxicity. Actually, the biocatalyst technology typically replaces multistep chemical processes and considerable progress in the preparation of nucleoside analogues was achieved by advisable combination of chemical methods and biochemical transformation. Among the biocatalysts, great interest has been paid to enzymes isolated from hyperthermophilic *Archaea*, for their capacity of survival and reproduction at temperatures near or above the boiling temperature of water. The dramatic increase of newly isolated extremophilic microorganisms, the analysis of their genomes and investigations of their enzymes by academic and industrial laboratories demonstrate the great potential of extremophiles in industrial biotechnology. The unique stability of archaeal enzymes at high temperatures, extremes of pH and pressure, in combination with their tolerance to salt concentrations, organic solvents, and metals, make them a powerful tool for the industrial processes of biotrasformations which very often occur in drastic conditions.

Purine nucleoside phosphorylases (PNP) play a key role in the nucleoside salvage pathway catalyzing, with a phosphorolytic mechanism the release of the free bases from the corresponding nucleosides. PNP, being able to utilize purines and their analogs as substrates can be employed both in the synthesis and in the activation of cytotoxic nucleosides. In particular, PNP from hyperthermophilic *Archaea* because of their unusual stability features and for their peculiar and wider substrate specificity than those of bacterial and eukaryotic counterparts, can be considered as potential biotechnological tools for the development of new drugs and for the activation of prodrugs with reduced systemic toxicity compared to those currently in use.

The research project aims the structural and functional characterization and engineering of new PNP from *Sulfolobus solfataricus and Pyrococcus furiosus*, two microorganisms belonging to *Archaea*. Such enzymes will be produced in high amounts by means of optimized expression systems. Substrate specificity will be analyzed and conformational stability will be studied through spectroscopic and calorimetric methods. Moreover, kinetic characterization of the reverse synthetic reaction catalyzed by PNP in both standard experimental conditions and after modifications of some parameters of the physicochemical environment of the reaction will be carried out. Finally, the elucidation of the three-dimensional structure of hyperthermophilic PNP will be performed. The structural information obtained on the enzyme/ligand complex will give a valuable contribution either to the identification of new molecules to be utilized as substrates in the synthetic reaction and to the definition of protein/ligand interaction useful for the redesign of *E. coli* PNP active site and for the design of new prodrugs.