Archael Purine nucleoside phosphorylase and anticancer gene therapy

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Purine nucleoside phosphorylases (PNP) are ubiquitous enzymes involved in the purine salvage pathway where they catalyze the reversible phosphorolytic cleavage of the glycosidic bond of purine nucleosides to the corresponding free nucleobase and ribose-1-phosphate. Unlike the enzymes of the *de novo* synthesis, PNP are able to use nucleosides and nucleobases as substrates. This prerogative is extremely advantageous for the biotechnological utilization of this class of enzymes in the synthesis and activation of cytotoxic nucleosides.
In contrast with human PNP,*E. coli* PNP recognizes purine nucleosides with 6-oxo, 6-amino and some nonstandard 6-substituents and is able to activate some adenosine analogues, such as 6-methyl-purine-2'-deoxyribonucleoside and 9-(b-D-arabinofuranosyl)-2-fluoroadenine (fludarabine) that are not substrates for the human enzyme. The different substrate specificity between *E. coli* and human PNP has been exploited in gene therapy for cancer to develop a suicide gene/prodrug system (1). Expression of *E. coli* PNP in cancer cells converts adenosine analogs to highly cytotoxic membrane-permeable compounds that interfere with the synthesis of DNA, RNA, and proteins thus being active against both dividing and quiescent tumor cells. The combination of the gene of the *E. coli* PNP and fludarabine represents one of the most promising systems in the gene therapy of solid tumors. The activation of fludarabine by *E. coli* PNP generates intratumorally the highly toxic agent 2-fluoroadenine that is characterized by a strong bystander activity. The association of *E. coli* PNP with fludarabine has demonstrated efficient cytotoxicity in hepatocellular carcinoma and in prostate cancer. Unfortunately, the use of fludarabine is limited by the poor capacity of *E. coli* PNP to efficiently activate this prodrug which, consequently, has to be administered in high doses that cause serious side effects. Many attempts are currently being made to overcome this obstacle including the use of a mutated *E. coli* PNP gene that is able to express an enzyme with higher catalytic efficiency for fludarabine with respect to the *wild-type*. In an attempt to identify enzymes with a better catalytic efficiency than *E. coli* PNP towards fludarabine we have selected 5'-deoxy-5'-methylthioadenosine phosphorylase (SsMTAP) (2,3) and 5'-deoxy-5'-methylthioadenosine phosphorylase II (SsMTAPII) (4,5), two PNPs isolated from the hyperthermophilic archaeon *Sulfolobus solfataricus* characterized by unusual features of stability and substrate specificity.
The detailed kinetic analysis carried out with SsMTAP and SsMTAPII with fludarabine evidenced the high catalytic power of the archaeal PNPs toward this prodrug. SsMTAP and SsMTAPII, share with *E. coli* PNP a comparable low affinity for fludarabine but are better catalysts of fludarabine cleavage with kcat/K_m values that are 12.8-fold and 6-fold higher, respectively, than those reported for the bacterial enzyme. Moreover, a computational analysis of the interactions of fludarabine in the active sites of *E. coli* PNP, SsMTAP, and SsMTAPII allowed to identify the crucial residues involved in the binding with this substrate, and provided structural information to improve the catalytic efficiency of *E. coli* PNP by enzyme redesign.

1. Parker, W. B., King, S. A., Allan, P. W., Bennett Jr, L. L., Secrist III, J. A., Montgomery, J. A., Gilbert, K. S., Waud, W. R., Wells, A. H., Gillespie, G. A. & Sorscher, E. J. Hum. Gene Ther. 8, 1637-1644, 1997.
2. Cacciapuoti, G., Porcelli, M., Bertoldo, C., De Rosa, M. & Zappia, V. J. Biol. Chem. 269, 24762–24769, 1994.
3. Appleby, T. C., Mathews, I. I., Porcelli, M., Cacciapuoti, G. & Ealick, S. E. J. Biol. Chem. 276, 39232-39242, 2001.
4. Cacciapuoti, G., Forte, S., Moretti, M.A., Brio, A., Zappia, V. & Porcelli M. FEBS J. 272, 1886-1899, 2005.
5. Zhang, Y., Porcelli, M., Cacciapuoti, G. & Ealick, S. E. J. Mol. Biol. 357, 252-262, 2006.