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The ubiquitous enzyme purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1.) is the key enzyme of the purine salvage pathway [1]. In mammals homotrimeric PNPs catalyse the reversible phosphorolytic cleavage of the glycosidic bond of 6-oxopurine nucleosides (ribo- and 2'-deoxyribo) and some analogues, as follows: purine nucleoside + orthophosphate ↔ base + pentose-1-phosphate. PNP deficiency in humans leads to inhibition of T-cell response. Potent inhibitors of this enzyme are therefore considered as potential immunosuppressive agents. In the 28 years since the genetic deficiency of PNP has been discovered [2], great efforts have been made to design inhibitors of PNPs with potential medical applications [1]. However, only the transition state analogue of human PNP, immucillin-H (trade name BCX-1777), has reached phase I/II clinical trials against human T-cell leukemia [3]. The important class of potent ground-state analogue inhibitors of trimeric PNPs (K_i in nM range) is composed of so called multisubstrate analogue inhibitors. These are compounds consisting of three structural parts linked together: (1) purine base, (2) acyclic chain or cyclic moiety, and (3) phosphonate or phosphate or other electronegative group [1]. These three parts mimic two substrates of PNP, namely, purine nucleoside (part 1 and 2) and phosphate (part 3) in the phosphorolytic direction or purine base and pentose-1-phosphate in the synthetic direction, hence they are expected to bind to purine, pentose and phosphate binding sites in a binary complex with the enzyme. In the Protein Data Bank there are no reported structures of the complexes of trimeric PNPs with multisubstrate analogue inhibitors. We describe here for the first time the high resolution X-ray structure of trimeric calf spleen PNP, highly homologous to the human PNP, with the potent (K_i^{app} = 16 and 18 nM at pH 7.4 with calf and human enzymes, respectively [4]) multisubstrate analogue inhibitor 9-(5,5-difluoro-5-phosphonopentyl)guanine.

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