

Bisubstrate Analog Inhibitors of 6-Hydroxymethyl-7,8-dihydropterin Pyrophosphokinase: Synthesis, Biochemical and Crystallographic Studies

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Introduction: 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) catalyzes the transfer of pyrophosphate from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP), leading to the biosynthesis of folate cofactors. Like other enzymes in the folate pathway, HPPK is an ideal target for the development of antimicrobial agents because the enzyme is essential for microorganisms but is absent from human and animals.

Methods and Materials: Single crystal X-ray diffraction.

Results: Three bisubstrate analogs have been synthesized for HPPK and characterized by biochemical and X-ray crystallographic analyses. All three bisubstrate analogs consist of a pterin, an adenosine moiety and a link composed of 2-4 phosphoryl groups. P¹-(6-hydroxymethylpterin)-P²-(5'-adenosyl)diphosphate (HP₂A) shows little affinity and inhibitory activity for *E. coli* HPPK. P¹-(6-hydroxymethylpterin)-P³-(5'-adenosyl)triphosphate (HP₃A) shows moderate affinity and inhibitory activity with a K_d of 4.25 μM in the presence of Mg²⁺ and an IC₅₀ of 1.27 μM. P¹-(6-hydroxymethylpterin)-P⁴-(5'-adenosyl)tetrakisphosphate (HP₄A) shows the highest affinity and inhibitory activity with a K_d of 0.47 μM in the presence of Mg²⁺ and an IC₅₀ of 0.44 μM. The affinity of MgHP₄A for HPPK is ~116 and 76 times higher than MgADP and HP respectively. The crystal structure of HPPK in complex with MgHP₄A (HPPK•MgHP₄A) has been determined at 1.85 Å resolution with a crystallographic *R* factor of 0.185.

Conclusions: The crystal structure shows that HP₄A occupies both HP- and ATP-binding sites and induces significant conformational changes in HPPK. The biochemical and structural studies of the bisubstrate analogs indicate that the bisubstrate analog approach can produce more potent inhibitors for HPPK and the minimum length of the link for a bisubstrate analog is ~7 Å.

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