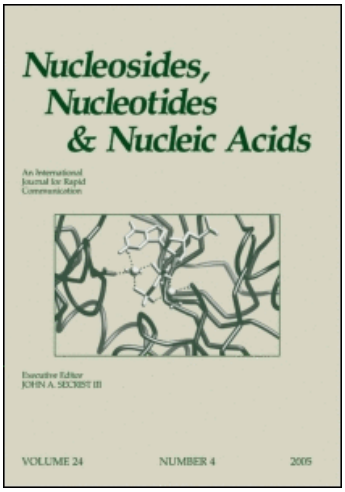

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Crystal Structure of Calf Spleen Purine Nucleoside Phosphorylase in a Complex with Multisubstrate Analogue Inhibitor with 2,6-Diaminopurine Aglycone

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ABSTRACT

The crystal structure at 2.05 Å resolution of calf spleen PNP complexed with stoichiometric concentration of acyclic nucleoside phosphonate inhibitor, 2,6-diamino-(S)-9-[2-(phosphonomethoxy)propyl]purine, in a new space group $P2_12_12_1$ which contains two full trimers in the asymmetric crystal unit is described.

Key Words: Purine nucleoside phosphorylase; X-ray crystallography; Multisubstrate analogue inhibitor; New space group; Conformational change; Mechanism.

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INTRODUCTION

Trimeric purine nucleoside phosphorylases (PNP, E.C. 2.4.2.1.) are important targets for drug design since their potent selective inhibitors are considered promising immunosuppressive and antiparasitic agents.[1] Trimeric PNPs are found in mammals and some other organisms and catalyze the reversible phosphorolysis of inosine, guanosine and their 6-oxo analogues, as follows: β-purine nucleoside + orthophosphate ⇔ purine base + α-D-pentose-1-phosphate. The mechanism of action of phosphorylases is still not elucidated. Open questions regard electronic state of the purine ring in a transition state as well as possible interactions of enzyme subunits.[1] For trimeric PNPs negative cooperativity in binding of immucillins, transition state analogue inhibitors, and hypoxanthine in the absence of phosphate, was observed,[2,3] while possible cooperative binding of ground state analogues, including phosphate, was not sufficiently documented. Recent detailed studies in solution indicate that for trimeric calf spleen enzyme cooperativity is not responsible for the complex kinetic characteristic observed.[4]

RESULTS AND DISCUSSION

Here we describe the crystal structure at 2.05 Å resolution of calf spleen PNP complexed with stoichiometric concentration of acyclic nucleoside phosphonate inhibitor, 2,6-diamino-(S)-9-[2-(phosphonomethoxy)propyl]purine (S)-PMPDAP.[5] The structure was obtained in a new space group $P2_12_12_1$ that in contrast to the previous cubic $P2_13$ space group[6–9] contains two full trimers in the asymmetric crystal unit. Hence differences between monomers forming the biologically active trimer if present would be detected.

However, no such differences were noticed. In the crystal structure all active sites of both trimers present in the asymmetric unit were found occupied by (S)-PMPDAP, and hydrogen bond pattern for ligand binding was found to be similar in all monomers forming the two trimers. Therefore we conclude that (S)-PMPDAP binds uniformly to all three sites of the calf spleen PNP. This finding is in agreement with the parallel studies in solution conducted for this and other acyclic nucleoside phosphonate inhibitors binding to trimeric PNPs from calf spleen and *Cellulomonas* using fluorimetric and calorimetric titrations (see two related papers in this volume). It is also in line with recent results indicating that non-Michaelis kinetics of calf PNP is not caused by the cooperativity between enzyme subunits but rather by complex kinetic mechanism.[4]

Moreover, in the present crystal structure of the calf spleen enzyme the loop between Thr60 and Ala65 was found in a different conformation than observed up to now in crystal structures of trimeric PNPs.[1] The active site of the calf spleen enzyme is buried in the protein structure and the walls of the binding pocket appear to be fairly compact. However, in the crystal structure obtained in a new space group $P2_12_12_1$, due to the above mentioned change of the conformation of the loop involving residues 60–65, an entrance into the active site pocket is wide open. This possible entrance is closed in all previously reported structures of the calf enzyme.[6–9] Hence our present crystal structure provides no obvious indication for obligatory

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binding of one of the substrates before the second one; it is rather consistent with random binding of substrates. This result than also supports the hypothesis that non-Michaelis kinetics of calf PNP is caused by complex kinetic mechanism, involving random binding of substrates, unusually slow and hence strongly rate limiting dissociation of some products (purine bases guanine and hypoxanthine), and dual function of phosphate acting as a substrate and as a modifier.[4]

(S)-PMPDAP is the first known and co-crystallized potent inhibitor of the calf spleen PNP that does not have oxygen at purine position 6, which was up to now considered as defining the specificity of trimeric PNPs. In the present crystal structure the distance between Glu201 $O^{\varepsilon 1}$ and nitrogen N(1) of the base is 2.8 Å, suggesting relatively strong hydrogen bond interaction. Hence putative hydrogen bonds in the active site indicate that inhibitor binds in the 6-imino form. This points to the crucial role of N(1)-H·····Glu201 contact in defining specificity of trimeric PNPs and is in line with one of the proposed mechanisms of catalysis for trimeric PNPs in which this interaction helps to stabilize the negative charge that accumulates on the O(6) of purine base in the transition state.[10]

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