Biological Science

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Crystal structures of 1-Deoxy-D-xylulose 5-phosphate reductoidomerase complexed with bisphosphonate inhibitors

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Introduction

In all living organisms, isoprenoids such as steroid hormones, carotenoids, and ubiquinone or menaquinone play important roles. There are several routes to the production of isoprenoids, including the mevalonate pathway, the mevalonate-independent or methylerythritol phosphate (MEP) pathway, as well as less common pathways involving leucine catabolism and the pentose phosphate pathway. In recent work, it has been found that the MEP pathway is of particular importance in many pathogenic bacteria and in addition, it is the pathway used in the protozoan parasite *Plasmodium falciparum*. It was also shown that an old antibiotic, fosmidomycin was a potent inhibitor of the enzyme deoxyxylulose-5-phosphate reductoisomerase (DXR) from bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, plants and *P. falciparum* and in combination with clindamycin, provided parasitological cures of uncomplicated falciparum malaria. These results show that DXR is a valid drug target and prompted us to search for additional inhibitors.

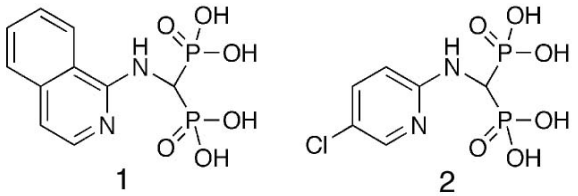
In other work, we recently found that a variety of bisphosphonates inhibited the growth of *P. falciparum* both *in vitro* and *in vivo*, and those compounds inhibit DXR activity. We thus initiated crystallographic structure study to investigate the binding mode of the bisphosphonate inhibitors to assist the new drug design against the enzyme.

Materials and Methods

E. coli DXR tagged with 6xHis at N-terminus was crystallized with the mother liquor containing 1.65 M ammonium sulfate, 0.06 M potassium sodium (+)-tartrate in 0.1 M sodium citrate buffer, pH 5.6. The space group was $P2_12_12$ and the unit cell dimension was a = 182.4, b = 59.0, c = 87.0 Å. The crystals were soaked with the bisphosphonate compound of either 0.2 M (3-isoquinolylamine) methylene-1,1-bisphosphonate (**1**) or 0.2 M [{(5-chloro-2-pyridinyl)amino} methylene]-1,1-bisphosphonate (**2**) for 15 min. The structures were solved by the molecular replacement method with the coordinate of 1JVS [1] as a target model.

Results and Discussion

The bisphosphonates dock into the same site as does the hydroxamate moiety of fosmidomycin, there is close register of two bisphosphonate oxygens with the two hydroxamate oxygens, which are bound to Mn^{2+}[2]. The fosmidomycin phosphonate-binding site is occupied in both structures by a sulfate ion and there is no bound NADPH. In each structure, the aromatic (isoquinoline or pyridine) side-chains of the bisphosphonate are located in a hydrophobic cleft containing Trp-211, Met-213, Pro-273 and Met-275, while the bisphosphonate groups appear to interact with Lys-124 and the conserved Asp-149, Glu-151 and Glu-230 cluster. While it might be expected that the bisphosphonate would bind to a divalent metal in this active site region, as does fosmidomycin, there is little evidence for electron density in this region and indeed, these three residues have a generally similar structural arrangement in the apo-enzyme structure, where it was proposed that they could form a hydrogen bonding network, so it seems possible that the bisphosphonates may interact with this network. When taken together, these results are of general interest since they represent a new class of inhibitors of the alternate-mevalonate or MEP pathway, of importance because of to its presence in many pathogenic microorganisms and where, at present, there is only one published structure of an enzyme-inhibitor complex. These results are also of interest since they suggest the possibility of synergistic interactions of bisphosphonates with multiple enzymes involved with isoprene biosynthesis, which could help explain the potent activity of species such as the n-alkyl bisphosphonates versus *P. falciparum*e.



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