

The crystal structure of lumazine synthase from *Mycobacterium tuberculosis* as a target for rational drug design: binding mode of a new class of purinetrione inhibitors.

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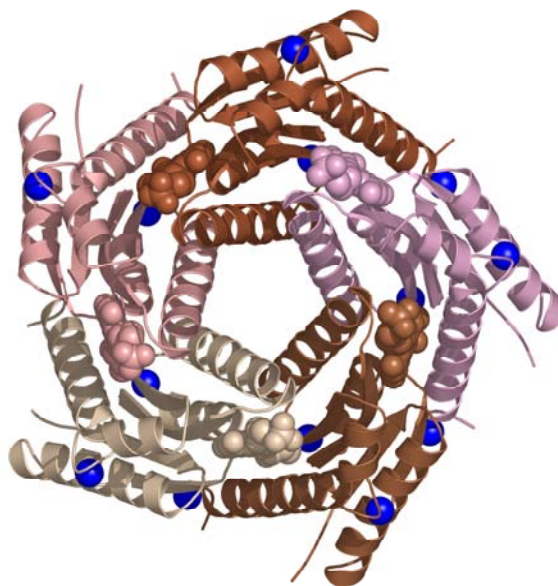
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The enzymes involved in the biosynthesis of riboflavin represent attractive targets for the development of drugs against bacterial pathogens, because the inhibition of these enzymes is not likely to interfere with enzymes of the mammalian metabolism. Lumazine synthase catalyses the penultimate step of riboflavin biosynthesis pathway. A number of substituted purinetrione compounds represent a new class of highly specific inhibitors of lumazine synthase from *Mycobacterium tuberculosis*. In order to develop potent antibiotics for the treatment of tuberculosis we have determined the structure of lumazine synthase from *Mycobacterium tuberculosis* in complex with two purinetrione inhibitors and have studied binding via isothermal titration calorimetry.

MbtLS was crystallized in presence of two inhibitor compounds 3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)propane-1-phosphate (TS-44) and 3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl)butane-1-phosphate (TS-70). The crystals were obtained by the vapor diffusion technique with the following macroseeding procedure. The space group was determined as monoclinic C2 for both complexes with slightly different cell dimensions $a=131.3$ Å, $b=80.7$ Å, $c=86.2$ Å, $\alpha=\gamma=90^\circ$, $\beta=120.3^\circ$ for MbtLS/TS44 and $a=131.4$ Å, $b=80.8$ Å, $c=86.0$ Å, $\alpha=\gamma=90^\circ$, $\beta=120.2^\circ$ for the MbtLS/TS70 complex. The asymmetric unit contained one pentamer.

The structures were solved by molecular replacement using lumazine synthase from *Saccharomyces cerevisiae* as search model and refined at 2 and 2.3 Å resolution. The respective R-factors were 14.7% and 17.4% and the respective free R-factors were 19.3% and 26.3%. The enzyme was found to be a pentamer constituted by five subunits related by five-fold local symmetry.



The comparison of the active site architecture with the active site of previously determined lumazine synthase structures reveals a largely conserved topology with the exception of the residues Gln141 and Glu136, which participate in different charge-charge interactions in the core space of the active site. Glu136, is introduced in the active site as a replacement for the uncharged Gly133. Glu136 can probably form a salt bridge to Lys138 and can therefore act as negatively charged counterpart for Lys138. The variation of its charge environment might hamper Lys138 in its conformational variability and thus decrease its ability to mediate a proper reorientation of the phosphate bearing aliphatic chain that is needed to make the subsequent ring closure possible [1].

References

- [1] Morgunova, E., Meining, W., Cushman, M., Illarionov, B., Haase, I., Jin, G., Bacher, A. Cushman, M., Fischer, M. & Ladenstein, R. (2004). The crystal structure of lumazine synthase from *Mycobacterium tuberculosis* as a target for rational drug design: binding mode of a new class of purinetrione inhibitors. *Biochemistry*, in press