Crystal structure of lumazine synthase from *Mycobacterium tuberculosis* complexed with new inhibitors.

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The last two steps in the biosynthesis of riboflavin, an essential metabolite that is involved in electronic transport, are catalyzed by Lumazine Synthase (LS) and Riboflavin Synthase (RS). LS catalyzes the turnover of 3,4-dihydroxy-2-butanone-4-phosphate and 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione to 6,7-dimethyl-8-(D-ribityl)-lumazine and RS catalyzes the formation of one riboflavin molecule from two product molecules of the previous reaction. The compounds developed as inhibitors for LS and RS are considered as a potential basis for the development of the antibacterial drugs for therapeutical purposes. Recently, it was shown that the compounds based on the purinetrione aromatic system demonstrated the highest binding affinity and specificity to the LS from *M.tuberculosis* (MbtLS) in comparison with the LSs from the other bacteria (*1*). We have undertaken the structure determination of the *M.tuberculosis* LS complexed with the difluoro derivative of the purinetrione compound and also with the chloride derivative of pyrimidin compound.

The complexes of MbtLS with 3-(1,3,7-trihydro-9-D-ribityl-2,6,8-purinetrione-7-yl) 1,1 difluoropentane-1-phosphate (TS51) and [4-(6-chloro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)butyl]phosphate (JC33) were obtained by cocrystallization of the native protein in K-phosphate buffer at pH 7.0 with the respective inhibitor solution up to 2.5 mM concentration. The reservoir solution contained 0.1 M of Na-ADA buffer at pH 6.2, 2M of K-acytate, 10 % of MPD and 0.04M of DTT. Small crystals were grown with the vapour diffusion technique. Macroseeding procedure was then applied in order to obtain the crystals suitable for data collection.The data sets for both complexes were collected on a MAR Research 345 Image plate detector system (DESY synchrotron beamline BW7B at the EMBL Outstation, Hamburg, Germany) at 100 K each from a single crystal. Each data set was obtained at a wavelength of 0.85 Å with an oscillation range 1º. Crystals of both complexes belonged to the monoclinic space group C2 with cell dimensions: a=131.4 Å, b=81.2 Å, c=85.8 Å, $\alpha=\gamma=90°$, $\beta=120.2°$ for the MbtLS/TS51 and a=131.6 Å, b=82.3 Å, c=86.4 Å, $\alpha=\gamma=90°$, $\beta=120.3°$ for MbtLS/JC33, respectively. The crystal structures were solved by molecular replacement using the program MOLREP as implemented in CCP4 (*2*). The pentamer of the lumazine synthase from earlier reported structure of the MbtLS/TS44 complex (*3*) (pdb code 1W19) excluding the coordinates for the inhibitor was successfully used as a search model for each data set. The models were refined using REFMAC5 (*2*) at 1.9 Å resolution to an R-factor of 17.5% (R_{free}=21.8%) (MbtLS/TS51) and 14.6% (R_{free}=21.5%) (MbtLS/JC33).

LS from *Mycobacterium tuberculosis* is a homopentameric enzyme. Protein monomer is constructed from a central 4-strand β-sheet flanked from both sides by two and three α-helixes. The active site of LS is located at the interface of two neighbouring subunits and it is built by highly conserved in other LSs hydrophobic and positively charged residues from both subunits (*3*). The density maps for both complexes were well-defined and allowed to build protein molecules and ligands easily. Several well conserved key residues are involved in the characteristic contacts with the both inhibitors. The highly conserved Arg128 and other adjacent residues comprise the phosphate binding site, which is used in the inhibitor complex for the fixation of the phosphate moiety by charge-charge interaction. The ribityl moiety of the TS51 compound is through main and side chain interactions in tight contact with Ser59, Glu61 and Asn114. The aromatic ring system of both inhibitors are effectively packed in the hydrophobic part of the active site formed by Trp27,

Ile60, Val81 and Val82, Ile83, Phe90 and Val93 residues of one subunit. In compound TS51 the substitution of the oxygen atom in phosphate group with the difluoromethyl group is resulted in the slightly shorter 6.8 Å distance between the N4 and P atoms but the PO3 group clearly strives to occupy the same position as in previously known structures. One of the fluoro atoms, F2, forms additional hydrogen bond with nitrogen atom from the main chain NGly85. JC33 is the first compound, among the long list of all known inhibitors of LS, which does not contain the ribityl chain. The positions of the four hydroxyl oxygen atoms of the ribityl chain are occupied with four water molecules in the structure of MbtLS/JC33 complex. The pyrimidin moiety is located in the position which mimics the middle line of the purinetrione ring system of TS51. The stacking interaction between pyrimidin aromatic ring and the indole group of Trp27 seems to be weaker in comparison with the purinetrione inhibitors due to the smaller size of the pyrimidin ring. Chloride atom makes two additional hydrogen bonds with the main chain nitrogen of Ile83 and NE2 of His28.

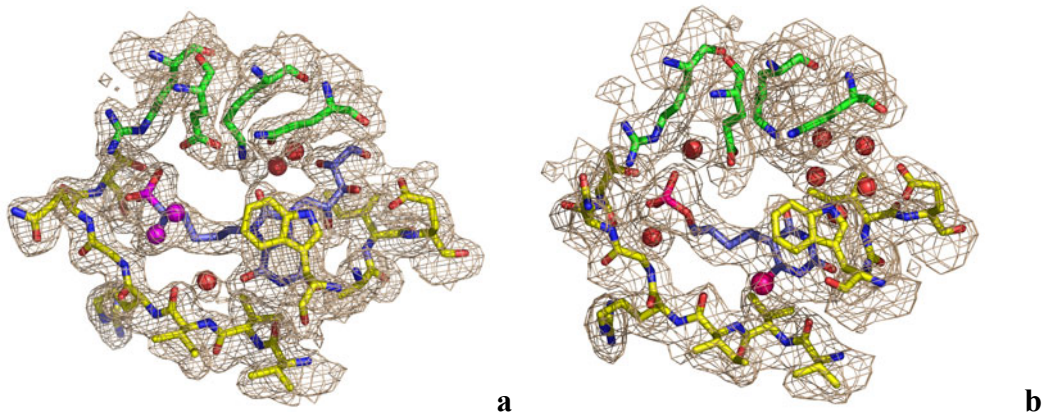

Figure 1. The $2IF_oI\text{-}IF_cI$ electron density map around the active site of MbtLS complexed with TS51 (**a**) and JC33 (**b**).

References

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