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Crystallization and preliminary X-ray diffraction analysis of the C-terminal domain of an archaeal transcription regulator

Eveline Peeters[a], Daniel Charlier[a], Dominique Maes[b]

[a]Erfelijkheidsleer en Micriobiologie. Vrije Universiteit Brussel (VUB). Pleinlaan 2, B-1050 Brussels, Belgium. [b]Laboratorium voor Ultrastructuur, Vrije Universiteit Brussel (VUB) and Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), Pleinlaan 2, B-1050 Brussels, Belgium.

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Little information is available on transcription regulation in Archaea, the third domain of life. Characterized archaeal regulators mostly belong to the Bacterial/Archaeal superfamily of Lrp-like transcription regulators (Lrp= *L*eucine-responsive *R*egulatory *P*rotein) [1]. One such example is Ss-LrpB from *Sulfolobus solfataricus*, which is a hyperthermoacidophilic organism growing optimally at 80°C and a pH between 2 and 3. Crystal structures of two Lrp-like proteins, both archaeal, have been determined: LrpA from *Pyrococcus furiosus* [2] and FL11 from *Pyrococcus* OT3 [3]. Lrp-like proteins are composed of an N-terminal DNA-binding domain with a helix-turn-helix motif and a C-terminal ab-sandwich (babbab-fold), also called RAM domain (RAM=*R*egulation of *A*mino acid *M*etabolism). These two domains are connected by a flexible linker. Ss-LrpB binds its own control region [4], as do most Lrp-like proteins. This binding occurs cooperatively at three regularly spaced 15 bp binding sites with as consensus the palindromic sequence 5'-TTGYAWWWWWTRCAA-3' (Y=pyrimidine, R=purine, W=weak bp). Due to the low solubility of Ss-LrpB, the C-terminal domain (comprising residues 69 to 147 of the full-length protein, omitting the hydrophobic linker) was cloned separately as a His-tag derivative and purified, aiming at a structural analysis. We succeeded in crystallizing this protein using the hanging-drop vapour-diffusion method in two different conditions [5]. Crystals, formed in one condition (100 mM $NaPO_4$, 100 mM KPO_4, 100 mM MES pH 6.5, 2.0 M NaCl), belong to the space group $P2_12_12$ with unit-cell parameters a = 59.35 Å; b = 74.86 Å; c = 38.08 Å and diffracted to 1.8 Å resolution. Preliminary attempts at molecular replacement with the C-terminal part of LrpA as model (27.8 % amino acid identity, 55.7 % similarity), as well the dimer as the monomer, have not been successful. In order to solve the structure a Se-Met derivative is being prepared.

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Structure of UDP-N-Acetylglucosamine Enolpyruvyl Transferase from *Haemophilus influenzae* in Complex with UDP-N-Acetylglucosamine and Fosfomycin: X-ray Crystal Analysis & Docking Study

Hye-Jin Yoon, Se Won Suh

Department of Chemistry, Seoul National University, Korea

Keywords: fosfomycin, UDP-N-acetylglucosamine, UDP-N-acetylglucosamine enolpyruvyl transferase

The bacterial enzyme UDP-N-acetylglucosamine enolpyruvyl transferase (EPT; EC 2.5.1.7) catalyzes the first committed step of peptidoglycan biosynthesis, i.e., transfer of enolpyruvate from phosphoenolpyruvate to UDP-N-acetyl-glucosamine. In order to provide missing structural information on the substrate and inhibitor complexes of EPT and to assist structure-based design of specific inhibitors for antibacterial discovery, we have determined the crystal structure of EPT from *Haemophilus influenzae* in a complex with UDP-N-Acetylglucosamine and fosfomycin, i.e., the substrate and inhibitor-complex form at 2.3 Å resolution. Structural comparisons of the *H. influenzae*, *E. coli* and *E. cloacae* EPT indicate that the overall conformational changes are very small except the loop around residues (Leu113 to Pro123) of active site, adopting the half open conformation at the substrate and inhibitor complex structure of *H. influenzae*, while in the *E. coli* and *E. cloacae* structures, they are in the several conformations from fully open conformation to closed conformation as substrate or inhibitor complex. These flexible loop sites indicate that a conformational change is necessary to bring the inhibitors to substrate closer together for initiating the catalysis. It represents crucial structural information necessary for better understanding of the substrate and inhibitor recognition and the catalytic mechanism. Also, we have employed the docking study to investigate the complex study because we were not able to refine the complex structure due to the existence of sulphate ion instead of inhibitor at the expected inhibitor site. The results of auto docking modeling for new inhibitors will be useful in structure-based inhibitor design.

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