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Structural Investigation of the GAF Domain Protein BPSL2418 from Burkholderia pseudomallei

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Abstract : A new family of methionine-sulfoxide reductase (Msr) was recently discovered and was named free methionine sulfoxide reductase (fRMsr). This family includes enzymes with a reductase activity toward the free R isomer of a methionine sulfoxide substrate. The fRMsrs have a GAF domain topology, a domain, which was previously identified as having in some cases a cyclic nucleotide phosphodiesterase activity. The classification of fRMsrs as GAF domains revealed a new function can be added to the GAF domain family. Interestingly the four members identified in the fRMsr family share the GAF domain structure and the presence of three conserved cysteines in the active site with free R methionine sulfoxide substrate specificity. This thesis presents the crystal structures of reduced, free Met-SO substrate-bound and MES-bound forms of a new fRMsr from Burkholderia pseudomallei (BPSL2418). BPSL2418 was cloned, overexpressed and purified to enable protein crystallization. The crystallization trials for reduced, Met-SO-bound and MES-bound forms of BPSL2418 were prepared and reasonable crystals of each form were produced. The crystal structures of BPSL2418MES, BPSL2418Met-SO and BPSL2418Reduced were solved at 1.18, 1.4 and 2.0Å, respectively by molecular replacement. The BPSL2418MES crystal belongs to space group P 21 21 21 while BPSL2418Met-SO and BPSL2418Reduced crystals belong to space group P 1 21 1. All three forms share the GAF domain structure of six antiparallel β-strands and four α-helices with connecting loops. The antiparallel β-strands (β1, β2, β5 and β6) are located in the center of the BPSL2418 structure flanked on one side by a three α-helices (α1, α2 and α4) and on the other side by a (loop1, β3, loop2, α3, β4 loop4) unit where loop4 forms a capping flap and covers the active site. The structural comparison of the three forms of BPSL2418 indicates that the catalytically important cysteine is CYS109, where the resolving cysteine is CYS75, which forms a disulfide bond with CYS109. They also suggest that the third conserved cysteine in the active site, CYS85, which is located in α3, is a non-essential cysteine for the catalytic function but it may play a role in the binding of the substrate. The structural comparison of the three forms reveals that conformational changes appear in the active site particularly involving loop4 and CYS109 during catalysis. The 3D structure of BPSL2418 shows strong structure similarity to fRMsrs enzymes, which further suggests that BPSL2418 acts as a free Met-R-SO reductase and shares the catalytic mechanism of fRMsr family.

Keywords : Burkholderia pseudomallei, GAF domain protein, methionine sulfoxide reductase, protein crystallization
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