Arsenate reductase, a small, almost universal, protein that uses a cysteine thiol cascade for redox chemistry

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Arsenate reductases catalyze the reduction of arsenate to arsenite and participate in the arsenic detoxification systems of prokaryotes and eukaryotes. Arsenate reductases form a very diverse family; they have unrelated structural folds and utilize different sources of reducing equivalents. Arsenate reductase from *Staphylococcus aureus* plasmid pI258 (ArsC) requires thioredoxin, thioredoxin reductase and NADPH for its activity.

ArsC has a PTPase-I fold typical for low molecular weight tyrosine phosphatases (LMW PTPase). It includes a flexible P-loop active site with a characteristic CX_5R sequence motif. Tetrahedral oxyanions structure the dynamic substrate binding-site of ArsC in its active conformation. Remarkably, kinetic experiments show that pI258 ArsC also catalyzes the tyrosine phosphatase reaction in addition to arsenate reduction. These results provide evidence that ArsC from pI258 evolved from LMW PTPase by the grafting of a redox function on a pre-existing catalytic site and that its evolutionary origin is different from those of both arsenate reductases from *Escherichia coli* plasmid R773 and from *Saccharomyces cerevisiae*.

The mechanism of pI258 ArsC catalyzed arsenate reduction involves its flexible P-loop structural motif and three redox active cysteines (Cys10, Cys82 and Cys89). ArsC combines a phosphatase-like nucleophilic displacement reaction with a unique intramolecular disulfide bond cascade. Within this cascade, the formation of a disulfide bond triggers a very significant and reversible 'conformational switch'. A short α-helix bearing two essential cysteines (Cys82 and Cys89) is looped out, allowing a 10 Å translation of Cys89 towards Cys82, necessary to transfer the oxidative equivalents to the surface of the enzyme, while releasing the reduced substrate. To close the catalytic cycle, oxidized ArsC is reduced by thioredoxin. Thioredoxin requires the flexible redox helix of ArsC to be looped out for its interaction. As such, thioredoxin appears to be selective for the oxidized structural fold of ArsC.