FrnE: A CADMIUM INDUCIBLE PROTEIN IS CHARACTERIZED AS A DISULFIDE ISOMERASE HAVING ROLE IN OXIDATIVE STRESS TOLERANCE IN DEINOCOCCUS rADIODURANS

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Abstract

Deinococcus radiodurans R1 is characterized for its extraordinary resistance to DNA damaging agents including gamma radiation, UV rays, desiccation and genotoxic chemicals like H$_2$O$_2$ and MMC. These stressors produce oxidative stress and cause severe damage of DNA and proteins, which eventually lead to cell death. It can tolerate extensive DNA damage and oxidative stress without a measurable loss of cell viability. The antioxidant enzymes like catalase, superoxide dismutase, antioxidant metabolites like thioredoxins, pyrroloquinoline quinine, deinoxanthin and Mn$^{2+}$ involved processes have been contributing to the extraordinary oxidative stress tolerance in this bacterium. Its genome also encodes homologues of putative Disulfide bond (Dsb) forming proteins. D. radiodurans exposed to a lethal dose of cadmium shows differential expression of a large number of genes including frnE and others involved in DNA repair and oxidative stress tolerance. Levels of frnE expression increased by nearly 7 fold in response to cadmium treatment. FrnE mutant of D. radiodurans showed $\sim$15 and $\sim$3 fold less tolerance to 10 mM cadmium and diamide respectively, as compared to wild type. Recombinant FrnE showed disulfide isomerase activity on scrambled Rnase A substrate and could maintain insulin in its reduced form in presence of dithiothreitol (DTT). In trans expression of FrnE could complement the loss of DTT resistance in dsbC and dscbCD mutants of E. coli. Equimolar amount of purified recombinant FrnE protected malate dehydrogenase completely from thermal denaturation at 420C, indicating the chaperon role of FrnE in vitro. The results suggesting FrnE role in oxidative stress tolerance of D. radiodurans most likely by protecting the proteins from oxidative damage would be presented.

**Introduction**

Deinococcus radiodurans R1 a member of Dienococceae family is characterised for its extraordinary resistance to DNA damaging agents including γ radiation, UV rays, desiccation and genotoxic chemicals like H₂O₂ and MMC (Misra et al., 2013). It can tolerate extensive DNA damage and oxidative stress without a measurable loss of cell viability. The antioxidant enzymes like catalase and superoxide dismutase, thioredoxins, antioxidant metabolites like pyrroloquinoline quinone, deinoxanthin and Mn²⁺ complexes of small molecules involved processes are attributed to extraordinary oxidative stress tolerance in D. radiodurans. Its genome also encodes putative Dsb homologues (Heras et al., 2009), which have not been characterized in greater details. Recently, it has been observed that D. radiodurans exposed to lethal doses of cadmium and gamma radiation upregulates the transcription of DR_0659 by nearly 7 fold (Joe et al., 2011). DR_0659 encodes a putative FrnE type protein in this bacterium and the levels of expression of proteins belonging to this family are induced in response to oxidative damage in other bacteria. Proteins belonging to thioredoxin family are involved in disulfide bond formation (Dsb) in many organisms including in eukaryotes. In prokaryotes, the Dsb proteins are located in periplasm and six members of this class have been identified, which are named as DsbA-E and G redox proteins (Singh et al., 2008). Roles of Dsb redox proteins have been demonstrated in virulence of many pathogenic bacteria (Peek and Paylor, 1992). These proteins have redox-active dithiol Cys-X-X-Cys motif in the active site. DsbA and DsbB are involved in disulfide bond formation while DsbC and DsbD are in the disulfide bond isomerisation function. Disulfide bond plays an important role in proper folding, stability and secretion of such proteins (Tomb, 1992, Missiakas and Raina, 1997) and therefore, mutations in dsb genes lead to incorrect folding of cellular proteins involved in various processes including oxidative stress tolerance (Kamitani et al., 1992). During severe oxidative stress, these systems get inactivated or overburdened, thus the cytosolic cysteine residues become susceptible to oxidation. Most organisms encode machineries that protect proteins from oxidative damage and thioredoxin superfamily proteins play important roles in this process. Therefore, the characterization of Dsb proteins and their roles in oxidative stress tolerance in bacteria belonging to the Deinococceae family would be worth undertaking. Here, we characterized DR0659 protein (hereafter referred as drFrnE) a member of Dsb family as a disulfide isomerase and demonstrated its roles in bacterial resistance to oxidative stress producing agent in vivo and in protection of malate dehydrogenase from thermal inactivation in vitro.

**Results and conclusion**

Genomic copy of drfrnE gene was inactivated and its effects of normal growth and oxidative stress response of D. radiodurans was evaluated. Inactivation of this gene led to the sensitivity to Cd by ~15 fold, ~3 fold for diamide, ~6 fold to γ rays and ~ 2 fold losses to hydrogen peroxide at 40mM, suggesting the role of drFrnE in oxidative stress tolerance of D. radiodurans (Khairnar et al., 2013). Recombinant drFrnE was produced in our laboratory and various activities related to Dsb proteins were checked. The recombinant protein could reduce insulin and reactivate scrambled RNase in vitro (Khairnar et al., 2013). These results suggested that drFrnE is a member of protein disulfide isomerase (PDI) family protein having roles in oxidative stress tolerance in D. radiodurans. The PDIs function in the reactivation of scrambled RNaseA by correcting the wrong disulfide interaction to correct disulfide bond formation. Such proteins are known to act as molecular chaperon in vitro. Since, drFrnE does reactivate scrambled RNaseA in vitro, the possibility of drFrnE having chaperon like activity was tested using malate dehydrogenase (MDH) in vitro. Both wild type as well as active site mutant as generated through site directed mutagenesis, derivatives were made and drFrnE role in protection of MDH from higher temperature was evaluated. Results showed that wild type drFrnE protected MDH from heat denaturation at
42°C in a concentration dependent manner (Khairnar et al., 2013). This activity was not observed with active site mutant of FrnE. This indicated that the possible mechanisms underlying in vitro protection of MDH from inactivation could be through both holdase as well as stabilization of disulfide bonds by PDI function of drFrnE. Nevertheless, these results suggested that wild type drFrnE could protect MDH from thermal denaturation and seems to have chaperon functions at least in vitro.

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References