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Functional Diversity of Ferredoxin-NADP$^+$ Reductase in Proteobacteria

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The Ferredoxin-NADP$^+$ reductases (FPR or FNR) (EC 1.18.1.2) are ubiquitous, monomeric and reversible flavin enzymes. The FPR displays a strong preference for NADP(H) over NAD(H). They have prosthetic flavin cofactor (FAD) and catalyze the reversible electron exchange between NADPH and either ferredoxin (Fd) or flavodoxin (Fld) [1, 2]. In oxygenic photosynthesis, the Fd is reduced by photosystem, then passes electrons on to NADP$^+$ via the FPR. This reaction provides the cellular NADPH pool needed for CO_2 assimilation and other biosynthetic processes [3]. In heterotrophic organisms, reduced ferredoxin, owing to the reverse enzymatic activity of the FPR, can donate an electron to several Fd-dependent enzymes, such as nitrite reductase, sulfite reductase, glutamate synthase, and Fd-thioredoxin reductase, allowing ferredoxin to function in a variety of systems, including oxidative stress [4-7]. Furthermore, the Fd is known to be involved in the assembly of iron-sulfur clusters [5, 8].

Commonly, bacteria have one *fpr* gene in their chromosome. Interestingly, some proteobacteria including *Pseudomonas putida* genome possess two annotated *fpr* genes (*fprA* and *fprB*) [9, 10]. We have no knowledge of evolution of two *fpr* genes in these bacteria. Because the FprA and FprB have homologues with *A. vinelandii* Fpr (Bacterial subclass I FPR) or *Escherichia coli* Fpr (Bacterial subclass II FPR), respectively, we postulate that FprA and FprB will have a differential catalytic activity and relationship for metabolic commitment. The *fpr* gene in *E. coli* is known to be involved in oxidative stress defense and its expression is regulated by the *soxRS* system [11, 12]. But it has been documented that genetic organization and regulation of the *fpr* genes in *P. putida* differ from those of *E. coli* system. Little is known on the function of FPR in *P. putida*. In the chromosome of *P. putida*, two [2Fe-2S] ferredoxin (FdA, FdB), and two [4Fe-4S] Ferredoxins and one flavodoxin (Fld) are annotated and those functions are unknown. The size of bacterial FPRs is smaller than that of plastid-type FPRs. Bacterial FPRs have less than 20% amino acid similarity with plastid-type FPRs although their 3-D structure are highly conserved. Catalytic activity (*K*cat) of the plastid-type FPRs is much faster (200-500^{-1}) than that of bacterial FPRs (1-2^{-1}) [1, 2]. The bacterial FPR could be further categorized into two subclasses based on their amino acid similarity. The subclass I and

II have a differential C-terminal key residues, which is phenylalanine and tryptophan residues, respectively [1]. The C-terminal key residue of FPR interacts with the adenine moiety of FAD cofactor. To elucidate the function of the FPR and possible redox partners in *P. putida*, all components are cloned and purified and *in vitro* and *in vivo* interactions of each FPR with several Fd and Fld were performed using a variety of biochemical assays, the yeast two-hybrid assay and homology computer modeling.

Both FPRs have higher affinity for NADPH than for NADH in diaphorase assays. Interestingly, the FprA prefers Fld as a redox partner with high catalytic efficiency, compared to its kinetics with two ferredoxins in a NADPH-dependent cytochrome reduction assay. The FprB has the highest specific constant (*K*cat/*K*m) with the FdA. Binding affinity (*K*m) and catalytic activity (*K*cat) of the FprB for NADH are considered to be significant in both assays. Strong *in vitro* interaction of FprB with FdA was also observed in the *in vivo* the yeast two-hybrid system. These data along with the homology computer modeling suggested that the Fld could be the physiological electron partner of the FprA and the FdA is able to interact productively *in vivo* with the FprB. The isothermal calorimeter data have shown that hydrophobic interaction contributes significantly to electron exchange of the FPR with its redox partner. Both FPRs show ferric reductase activities in the presence of free FMN. Interestingly, catalytic ferric reduction of the FprA and FprB prefer NADPH and NADH as an electron donor, respectively. The results of all assays tested here provide evidence that the FprB is able to efficiently accept electrons from NADH. Interestingly, the NAD(P) region of the FprB has homology with that of oxidoreductases in many other protists and plants. Our data has shown that the FprB may be the evolutionary link of the FPR between proteobacteria and plant.

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