

## Desulfovibrio gigas flavodiiron protein affords protection against nitrosative stress in vivo

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**Abstract:** Desulfovibrio gigas flavodiiron protein (FDP), rubredoxin: oxygen oxidoreductase (ROO), was proposed to be the terminal oxidase of a soluble electron transfer chain coupling NADH oxidation to oxygen reduction. However, several members from the FDP family, to which ROO belongs, revealed nitric oxide (NO) reductase activity. Therefore, the protection afforded by ROO against the cytotoxic effects of NO was here investigated. The NO and oxygen reductase activities of recombinant ROO in vitro were tested by amperometric methods, and the enzyme was shown to effectively reduce NO and O<sub>2</sub>. Functional complementation studies of an Escherichia coli mutant strain lacking the ROO homologue flavorubredoxin, an NO reductase, showed that ROO restores the anaerobic growth phenotype of cultures exposed to otherwise-toxic levels of exogenous NO. Additional studies in vivo using a D. gigas roo-deleted strain confirmed an increased sensitivity to NO of the mutant strain in comparison to the wild type. This effect is more pronounced when using the nitrosating agent S-nitrosoglutathione (GSNO), which effectively impairs the growth of the D. gigas Delta roo strain. roo is constitutively expressed in D. gigas under all conditions tested. However, real-time reverse transcription-PCR analysis revealed a twofold induction of mRNA levels upon exposure to GSNO, suggesting regulation at the transcription level by NO. The newly proposed role of D. gigas ROO as an NO reductase combined with the O<sub>2</sub> reductase activity reveals a versatility which appears to afford protection to D. gigas at the onset of both oxidative and nitrosative stresses.