Novel Non-Heme Diiron Peroxidases from Air-Sensitive Bacteria

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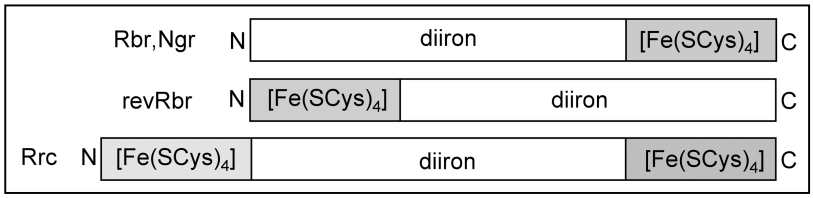
Rubrerythrins (Rbrs) constitute a novel class of hydrogen peroxide reductases (peroxidases) that protect air-sensitive bacteria against oxidative stress. The prototypical Rbr subunit from *Desulfovibrio vulgaris* (Dv) contains two domains: N-terminal four-helix bundle and C-terminal rubredoxin-like; the active site consists of a non-sulfur, His,Glu-ligated diiron site separated by ~12 Å from an [Fe(SCys)$_4$] site (1). Herein we describe three Rbr homologs, namely, Dv nigerythrin (Ngr), Rbo-Rbr-like protein from *Campylobacter jejuni* (Rrc) and reverse Rbr from *Clostridium acetobutylicum* (revRbr), that adopt novel protein domain arrangements to achieve the peroxidase-functional Rbr-type active site. RevRbr and Rrc show significantly different sequential ordering of the protein domains from that of the "classical" Dv Rbr (cf. the schematic diagram). The recombinant purified proteins featured the expected iron sites and all three showed NAD(P)H peroxidase activity in vitro, although revRbr and Rrc differed in their preferences for proximal electron donors. Plasmid-borne expression of either Ngr or Rrc protected a catalase/alkylhydroperoxide reductase-deficient *E. coli* strain against hydrogen peroxide exposure. Crystal structures of Ngr were solved to 1.4-Å resolution in three different oxidation states. The oligomeric structure of Ngr implies that the active site is contained within each subunit, which differs from the functional "head-to-tail" homodimer seen for Dv Rbr. Nevertheless, the redox-dependent His↔Glu ligand "toggling" of one iron of the Ngr diiron site is reminiscent of that seen in Dv Rbr and can, therefore, be considered a characteristic feature of Rbrs. Dv Rbr, Ngr, revRbr and Rrc, thus, represent four different protein domain arrangements that achieve the peroxidase-functional Rbr-type active site.



1. Jin, S., Kurtz, D. M., Jr., Liu, Z.-J., Rose, J., Wang, B.-C. *J. Am. Chem. Soc.* **2002**, *124*, 9845-9855.