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Crystallization of the novel flavodoxine-like protein, WrB_A, - on the way to three-dimensional structure.

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Tryptophan (W)-repressor binding protein A, WrB_A, identified as an *E. coli* stationary-phase protein was named for its reported effect on the interaction between tryptophan repressor and DNA [1]. Later work [2] showed that this effect was non-specific, leaving the physiological role of WrB_A unknown. According to sequence analysis and homology modeling [3] WrB_A was identified as the founding member of a new family of flavodoxin-like proteins, which displays low but structurally significant sequence similarity with the flavodoxins. The members of WrB_A family are predicted to share the open, twisted α/β flavodoxin fold, but with a short conserved insertion unique for the new family. This structure motif could account for experimental observations that some family members are dimeric in solution, including also finding that WrB_A creates a dimer-tetramer equilibrium at micromolar concentrations [2]. Unlike typical flavodoxins [4], these proteins bind FMN relatively weakly but still specifically. The computer analysis [3] indicated some structural differences in the flavin-binding pocket, which may explain the lower affinity of WrB_A for FMN. Due to these peculiarities the structural analysis may aid in understanding the physiological roles of WrB_A family members. These factors motivated our research for diffraction-quality crystals. Purified WrB_A apoprotein and holoprotein were used for crystallization trials. Standard and advanced crystallization techniques were applied to crystallize mentioned proteins. WrB_A apoprotein crystals grown in capillaries were measured directly at synchrotron DESY (beamline X13) in Hamburg (Germany). Crystals diffracted to a resolution of 2.2 Å. Attempts with variable growing conditions are performed to improve quality of apo- and holoprotein crystals.

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Structure of superoxide reductase bound to ferrocyanide and active site expansion upon X-ray induced photo-reduction.

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Some sulphate-reducing and microaerophilic bacteria rely on the enzyme superoxide reductase (SOR) to eliminate the toxic superoxide anion radical ($O_2^{\cdot-}$) [1, 2]. SOR catalyses the one-electron reduction of $O_2^{\cdot-}$ to hydrogen peroxide at a non-heme ferrous iron centre [3, 4]. The structures of *Desulfoarculus baarsii* SOR (mutant E47A) alone and in complex with ferrocyanide were solved to 1.15 and 1.7 Å resolution, respectively. The latter structure, the first ever reported of a complex between an organo-metallic compound and a protein, reveals that ferrocyanide entirely plugs the SOR active site, coordinating the active iron through a bent cyano bridge [5]. Surprisingly, biochemical data show only a modest reduction of the SOR activity when ferrocyanide is added, suggesting that the complex is still able to react with $O_2^{\cdot-}$ by adopting an alternate reduction mechanism. The subtle structural differences between the mixed-valence and the fully-reduced SOR-ferrocyanide adducts were investigated by taking advantage of the photo-electrons induced by X-rays. Photo-reduction of the SOR active site was monitored in real-time by online absorption microspectrophotometry, and was found to be a very rapid process under a powerful synchrotron beam. Analysis of composite data sets [6] revealed that photo-reduction from Fe(III) to Fe(II) of the iron centre induces a significant expansion of the SOR active site.

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