s1.m7.p31 **Crystallization of the novel flavodoxine-like protein, WrbA, - on the way to three-dimensional structure.** Julie Wolfova,[a] Ivana Kuta Smatanova,[a'], Rita Grandori,[b] Neal Chatterjee[c] and Jannette Carey[c], [a]*Institute of Physical Biology, University of South Bohemia at Ceske Budejovice, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic.* [a']*Institute of Physical Biology, University of South Bohemia at Ceske Budejovice and Institute of Landscape Ecology, Academy of Science of the Czech Republic, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic.* [b]*Institute of Chemistry, Johannes Kepler University, Altenbergerstrasse 69, A-4040 Linz, Austria.* [c]*Chemistry Department, Priceton University, Washington Rd and William St, Princeton, NJ 08544-1009, USA. E-mail: julinka.w@tiscali.cz*

Keywords: Flavodoxine-like protein; WrbA apoprotein; Standard and advanced crystallization techniques

Tryptophan (W)-repressor binding protein A, WrbA, 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 WrbA unknown. According to sequence analysis and homology modeling [3] WrbA 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 WrbA 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 WrbA 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 WrbA for FMN. Due to these peculiarities the structural analysis may aid in understanding the physiological roles of WrbA family members. These factors motivated our research for diffraction-quality crystals.Purified WrbA apoprotein and holoprotein were used for crystallization trials. Standard and advanced crystallization techniques were applied to crystallize mentioned proteins. WrbA apoprotein crystals grown in capillaries were measured directly at synchrotron DESY (beamline X13) in Hamburg (Germany). Crystals diffracted to a resolution of 2.2Å. Attempts with variable growing conditions are performed to improve quality of apo- and holoprotein crystals.

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References:

[1] Yang W., Ni L. & Somerville R.L., *Proc. Natl. Acad. Sci*., USA **90** (1993), 5796-5800.
[2] Grandori R., Khalifah P., Boice J.A., Fairman R., Giovanielli K. & Carey J., *J. Biol. Chem.*, **273** (1998), 20960-20966.
[3] Grandori, R. & Carey, J., *Trends Biochem. Sci*., **19**(1994), 72.
[4] Burnett, R.M., *et al.*, *J. Biol. Chem*.,. **249**(1974), 4383-4392.

s1.m8.p1 **Structure of superoxide reductase bound to ferrocyanide and active site expansion upon X-ray induced photo-reduction.** V. Adam[1,2], A. Royant[1], V. Niviere[3], F.P Molina Heredia[3] and D. Bourgeois[1,2]. [1]*LCCP, UMR 9015, IBS-CEA/CNRS/UJF, 41 avenue Jules Horowitz, 38027 Grenoble, Cedex 1, France,* [2]*ESRF, BP 220, 38043 Grenoble Cedex, France,* [3]*CBCRB, UMR 5047, DRDC-CEA/CNRS/UJF, 17 avenue des Martyrs, 38054 Grenoble, Cedex 9, France. E-mail: bourgeoi@esrf.fr*

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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.

[1] Jenney, F.E., Jr., Verhagen, M.F., Cui, X., and Adams, M.W. (1999). Anaerobic microbes: oxygen detoxification without superoxide dismutase. *Science* **286**, 306-309.
[2] Lombard, M., Fontecave, M., Touati, D., and Niviere, V. (2000). Reaction of the desulfoferrodoxin from Desulfoarculus baarsii with superoxide anion. Evidence for a superoxide reductase activity. *J Biol Chem* **275**, 115-121.
[3] Yeh, A.P., Hu, Y., Jenney, F.E., Jr., Adams, M.W., and Rees, D.C. (2000). Structures of the superoxide reductase from Pyrococcus furiosus in the oxidized and reduced states. *Biochemistry* **39**, 2499-2508.
[4] Coelho, A.V., Matias, P., Fülöp, V., Thompson, A., Gonzalez, A., and Carrondo, M.A. (1997). Desulfoferrodoxin structure determined by MAD phasing and refinement to 1.9 A resolution reveals a unique combination of a tetrahedral FeS4 centre with a square pyramidal $FeSN_4$ centre. *JBIC* **2**, 680-689.
[5] Auchere, F., Raleiras, P., Benson, L., Venyaminov, S.Y., Tavares, P., Moura, J.J., Moura, I., and Rusnak, F. (2003). Formation of a stable cyano-bridged dinuclear iron cluster following oxidation of the superoxide reductases from Treponema pallidum and Desulfovibrio vulgaris with K(3)Fe(CN)(6). *Inorg Chem* **42**, 938-940.
[6] Berglund, G.I., Carlsson, G.H., Smith, A.T., Szoke, H., Henriksen, A., and Hajdu, J. (2002). The catalytic pathway of horseradish peroxidase at high resolution. *Nature* **417**, 463-468.