FA1-MS14-P01

Insight into the Reduction Potentials of *Allochromatium Vinosum*-like Ferredoxins.
Emmanuel Saridakis[a], Petros Giastas[a], Jean-Marc Moulis[b], Panayotis Kyritsis[c], Irene M. Mavridis[a]. [a]*National Center for Scientific Research "Demokritos", Athens, Greece.* [b]*DSV, IRTSV, Laboratoire de Chimie et Biologie des Métaux, CEA-Grenoble, France.* [c]*Department of Chemistry, University of Athens, Greece.*
E-mail: esaridak@chem.demokritos.gr

The 2[4Fe-4S] Ferredoxins (Fds) are a type of electron transfer proteins found in anaerobic microorganisms. Two main families can be distinguished: The first includes the clostridial Fds with two isopotential [4Fe-4S] clusters (~ –400 mV), whereas the second is characterized by very negative and widely different reduction potentials of the two clusters (~ -670 to ~ -430 mV). The *Allochromatium vinosum* ferredoxin (AlvinFd, structure solved to a resolution of 2.1Å) is the prototype of the second family [1]. The crystal structures of the C57A and V13G molecular variants of AlvinFd and of the Alvin-like ferredoxin from *Escherichia coli* (EcFd) recently determined (at 1.05, 1.48 and 1.65 Å resolution, respectively), as well as the Alvin-like ferredoxin from *Pseudomonas aeruginosa* (1.32Å resolution) [2] indicate that polar interactions of side chains and water molecules with cluster II sulfur atoms, which are absent in the environment of cluster I, are correlated to the *ca*. 180-250 mV difference between the reduction potentials of clusters I and II. Moreover, the structures combined with cyclic voltammetry studies establish clear effects of the degree of exposure of the cluster with the lowest reduction potential (cluster I) towards less negative reduction potentials (E°). This is better illustrated by V13G AlvinFd (high exposure, E° = −594 mV) and EcFd (low exposure, E° = −675 mV). In C57A AlvinFd, the movement of the protein backbone, as a result of replacing the non-coordinating Cys57 by Ala, leads to a +50 mV upshift of the potential of the nearby cluster I, by removal of polar interactions involving the thiolate group and adjustment of the H-bonds network involving the cluster atoms. These findings provide insight into the significant effects of subtle structural differences of the protein and solvent environment around the clusters of [4Fe-4S] ferredoxins on their electrochemical properties.

[1] Moulis J.-M., Sieker L.C., Wilson K.S., Dauter Z. *Protein Sci.* **1996**, 1765. [2] Giastas P., Pinotsis, N., Efthymiou, G., Wilmanns M., Kyritsis P., Moulis J.-M., Mavridis I. M. *J. Biol. Inorg. Chem.* **2006**, 445.

Keywords: ferredoxins; redox proteins; electron transfer proteins

FA1-MS14-P02

High Resolution X-Ray and Neutron Data Collection on Antifreeze Protein.
Andre Mitschler[a], Matthew Blakeley[b], Isabelle Petit-Hartlein[c], Christoph Mueller-Dieckmann[d], Alexandre Popov[d], Eduardo Howard[e], Michael Haertlein[c], Alberto Podjarny[a]. [a]*IGBMC,CNRS, INSERM, UdS, Illkirch, France.* [b]*Institut Laue Langevin, Grenoble, France.* [c]*PSB Deuteration Laboratory, Grenoble, France,* [d] *ESRF , Grenoble, France.* [e]*IFLYSIB , CONICET, La Plata, Argentina.*
E- mail: podjarny@titus.u-strasbg.fr

Type III AntiFreeze Protein (AFP, 7kDa) from the polar fish *Macrozoarces Americanus*, inhibits ice growth in vivo at subzero temperatures. As crystals of this protein could not be grown to sizes corresponding to very large crystal volumes (>>1mm3), the complete deuteration protocol has been performed at the ILL/EMBL Perdeuteration Facility. Several "tiny" crystals of AFP(D) with volumes around0. 13 mm^3 could be grown in D2O media. The quality of these crystals and their structural identity vs. the hydrogenated protein AFP(H) was checked by Synchrotron X-Ray data collection up to a resolution of 1.05Å, also at 293K, on Beamline ID29 at ESRF. Two neutron Laue diffraction data sets were collected up to a resolution of 1.85Å at 293K on the improved new LADI-III detector at ILL with two distinct orientations of the quartz capillary containing the crystal. Exposure time per frame could be reduced to 24 hours without decreasing the overall data accuracy. Following our previous work on human-Aldose Reductase (h-AR(D), 36kDa) [1,2] this second example highlights again the major capability of the perdeuteration procedure, which allows to overcome the crucial need for larger crystals of several cubic millimeters often difficult to grow. Thus, neutron protein crystallography should become moreaccessible tothe structural biology community.For recent reviews, see [3,4]. Data collection details together with data processing statistics will be shown [5].

[1] Hazemann et al.,**2005**, *Acta Cryst. D61, 1413 - 1417*. [2] Blakeley et al., **2008**, *Proc. Natl. Acad. Sci. USA, 105, 1844 - 1848*. [3] Niimura & Bau, **2008**, *Acta Cryst. A64, 12 - 22*. [4] Blakeley et al., **2008**, *Curr. Opin. Struct. Biol., 18, 1-8.* [5] Haertlein et al., **2009**, *Acta Cryst. F, in press.*

Keywords : antifreeze proteins; neutron crystallography; data collection processing

FA1-MS14-P03

Quantitative Analysis of Atomic Polarization in Protein Human Aldose Reductase.
Munshi Parthapratim[a], Guillot Benoit[a], Liebschner Dorothee[a], Jelsch Christian[a]. [a]*Nancy Universit, Laboratoire de Cristallographie, Resonance Magnetique & Modelisation, CRM2, CNRS, Vandoeuvre les Nancy, France.*
E-mail: munshi.parthapratim@crm2.uhp-nancy.fr

The protein human aldose reductase (h-AR) is an excellent system to investigate, as this enzyme is extremely important and claims to be a pharmaceutical target due to its involvement in diabetes complications [1]. With