	Experiment title:	Experiment number:
	Structure determination of the Nickel containing Superoxide Dismutase from *Streptomyces seoulensis*	**LS1803**
Beamline: BM14	**Date of experiment**: from 27-11-2000 to 29-11-2000	**Date of report**: 12-07-01
Shifts to BAG: 6	**Local contact(s)**: Andrew THOMPSON	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(*) Jochen Wuerges*, Kristina Djinovic-Carugo
Sincrotrone ELETTRA, Area Science Park, S.S. 14 km 163.5, I-34012 Trieste, Italy

Ni containing superoxide dismutase

The Ni containing superoxide dismutase (NiSOD) is a metallo-enzyme that catalyses the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. It plays thus an important role in the protection of biomolecules from oxidative damage caused either by the radicals itself or by reactive oxygen species formed by superoxide. NiSOD was purified from *Streptomyces seoulensis* sp. nov. and from *Streptomyces coelicolor* (ref. 1). It was biochemically studied and compared to the two already superoxide dismutases families of superoxide dismutases - one containing copper and zink (CuZnSOD), and the other one having iron (FeSOD) or manganese (MnSOD) in its redox-active metal centre. The NiSOD could not be assigned to any of the two groups and shows a distinct amino acid composition and sequence. Moreover, NiSOD shows a coordination environment of the metal centre which is unlike any other SOD, being composed of 3 S-ligands, one N-ligand and one other N- or O-ligand, as determined by X-ray absorption spectroscopy (ref. 3). In the course of this spectroscopic investigation it remained unclear whether the metal centre is mono or dinuclear and which residues are involved in the coordination of the metal ion.

We obtained crystals of NiSOD useful for X-ray diffraction in three different crystal forms. In April 2000 MAD data of high quality were collected at BM14, however, it was not possible to derive initial phases to interpret the electron density maps. This is most probably due to the presence of translational non-crystallographic symmetry in the used crystal form. At ID14-4 in July, another crystal form lacking the translational pseudosymmetry was used for MAD data collection from which the peak and inflection point data recently provided us with an interpretable

map. SAD and 3λ MAD phasing were not successful, the latter due to severe radiation damage during the 'remote' data collection. In November data on all crystal forms were to be collected at BM14. On the orthorhombic crystals we collected data on a native and a NaBr-cryosoaked crystal. On small needle-shaped rhombohedral crystals no data were collected because of a too low signal to noise ratio and low beam intensity. In June 2001 a data collection at 2Å wavelength was performed at ID29 to utilise the anomalous signal of Sulphur (data processing in progress).

Model building into the electron density maps obtained with the MAD dataset on the crystal form lacking the translational pseudosymmetry is currently in progress.

References:

1.Youn, H.-D., Kim, E.-J., Roe, J.-H., Hah, Y.C., Kang, S.-O. (1996). A novel nickel-containing superoxide dismutase from Streptomyces spp. Biochem. J. **318**, 889-896.

2.Kim, E.-J., Chung, H.-J., Suh, B., Hah, Y. C. and Roe, J.-H. (1998). Transcriptional and post-transcriptional regulation by nickel of sodN gene encoding nickel-containing superoxide dismutase from Streptomyces coelicolor Mueller. Molecular Microbiology **27**(1), 187-195.

3. Choudhury, S. B. et al. (1999). Examination of the Nickel Site Structure and Reaction Mechanism in Streptomyces seoulensis Superoxide Dismutase. Biochemistry **38**, 3744-3752.

Table 1: Summary of data collections on **BM14 beamline**:
Compound: Ni-containing superoxide dismutase (NISOD)

Crystal	NiSOD native	NiSOD NaBr cryosoaked
Space Group	P212121	P212121
Unit cell	a=66.3 Å, b=119.2 Å, c=120.5 Å	a=112.2 Å, b=114.4 Å, c=128.7 Å
Resolution	2.0 Å	3.78 Å
N° measurements	219589	42869
N° reflections	58180	15126
Completeness	95.0%	90.0%
Rsym	6.4%	7.5%

	Experiment title:	Experiment number:
	Heme containing Cu,Zn SOD	**LS1803**
Beamline:	**Date of experiment**:	**Date of report**:
ID14-4	from 11-02-2001 to 12-02-2001	12-07-01
Shifts to BAG: 2	**Local contact(s)**:	*Received at ESRF:*
	Hassan BELRHALI	

Names and affiliations of applicants (* indicates experimentalists):

(*) Cristiana Petruz*, Kristina Djinovic-Carugo*
Sincrotrone ELETTRA, Area Science Park, S.S. 14 km 163.5, I-34012 Trieste, Italy

Heme containing Cu,Zn SODs

Superoxide dismutases (SOD) are enzymes catalyse the dismutation of superoxide to molecular oxygen and hydrogen peroxide, and are essential for protection from radicals. In the context of studies of periplasmic Cu,Zn SODs from pathogenic bacteria, we have undertaken structural characterisation of the enzyme from *Haemophilus ducreyi*, the etiological agent for the sexually transmitted human genital disease chancroid. The bacterium is unable to synthesise heme, and the role attributed to its Cu,Zn SOD is dual: protection from oxidative damage, and transport of heme. The characterisation of the enzyme namely suggest that a single heme moiety is bound to the functional Cu,Zn SOD dimer. We have successfully crystallised the enzyme in the apo and holo form (with and without the heme moiety) from PEG solutions in hanging drop conditions, and collected diffraction data on both enzyme forms on ID14-1 beamline. Both structures have been solved with molecular replacement techniques. In the case of holo enzyme, the electron density maps clearly show the presence of one heme moiety per dimer, as anticipated from biochemical studies of the enzyzme.

Compound: heme containing Cu,Zn SOD

Crystal	Native apo enzyme	Native holo enzyme
Space Group	C2	P1
Unit cell	a= 71.4 Å b= 64.0 Å c=.74.0 Å β = 118.0	a= 37.3 Å b=66.2 Å c=.69.2 Å α= 66.3 β= 89.7 γ= 75.8
Resolution	1.5 Å	1.5 Å
Redundancy	3.9	1.5 Å
Completeness	99.4%	91.7%
Rsym	4.5%	6.7%
I/σ (overall)	24.2	50.4

	Experiment title: **Myosin subfragment S2**	Experiment number: **LS1803**
Beamline: ID14-1	**Date of experiment**: from 11-02-2001 to 12-02-2001	**Date of report**: 12-07-01
Shifts to BAG: 2	**Local contact(s)**: Hassan BELRHALI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(*) Kristina Djinovic-Carugo*, Nico Thomae*#
Sincrotrone ELETTRA, Area Science Park, S.S. 14 km 163.5, I-34012 Trieste, Italy
#Max-Planck Institut Fuer Molekulare Physiologie Rheinladdamm 201, 44139 Dortmund, Germany

Myosin subfragment S2

The contraction of striated muscle is the function of a complex macromolecular machine in which the sliding of thin filaments (composed of F-actin) and thick filaments (composed of myosin) past each other is responsible for muscle shortening and force generation. The thick filaments contain a family of associated proteins, the myosin-binding proteins MyBP-C and H. MyBP-C is arranged regularly along the thick filament in positions coinciding with every third level of myosin heads (ref 1, 2). C-terminal part of MyBP-C binds the light meromyosin segment of myosin, while N-terminal phosphorylation domain binds to 126 residues of myosin subfragment S2 (ref 3). Mutations in a number of sarcomeric proteins have been indentified that cause familial hypertrophic cardyomyopathy (FHC), and MyBP-C together with cardiac β-myosin are among affected genes (ref 4). Construct containing 126 residues of human cardiac β-myosin segment S2 (containing E18K mutation) was crystallised (anisotropic diffraction to 2.5 Å). In spring 2001 we collected datasets on: native crystals, native crystals co-crystallised with thiomersal, and on platinum soaked crystals of the E18K mutant. These experiments were performed on ID14-1 beamline. The datasets are not ideally complete because of radiation damage. Due to un-isomorphism between datasets, the data could not been successfully used for phasing, although we could identify heavy atom positions is the case of mercury derivatised crystals.

1. Bennett P, Craig R, Starr R, Offer G. The ultrastructural location of C-protein, X-protein and H-protein in rabbit muscle. J Muscle Res Cell Motil 1986, 6, 550-67.
2. Bonne G, Carrier L, Bercovici J, Cruaud C, Richard P, Hainque B, Gautel M, Labeit S, James M, Beckmann J, et al. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. Nat Genet 1995, 4, 438-40.
3. Gruen M, Prinz H, Gautel M. cAPK-phosphorylation controls the interaction of the regulatory domain of car diac myosin binding protein C with myosin-S2 in an on-off fashion. FEBS Lett 1999, 453, 254-9.
4. Gruen M, Gautel M. Mutations in beta-myosin S2 that cause familial hypertrophic cardiomyopathy (FHC) abolish the interaction with the regulatory domain of myosin-binding protein-C. J Mol Biol 1999, 286, 933-49.

Summary of data collections on **ID14-1** beamline:
Compound: myosin subfragment (S2)

Crystal	Native	Hg derivative	Pt-soaked E18K mutant
Space Group	P1	P1	P1
Unit cell	a= 33.5 Å α = 90.1	a= 41.4 Å α = 96.6	a= 40.1 Å α = 96.6
	b= 41.8 Å β = 95.4	b= 41.7 Å β= 95.2	b= 41.7 Å β= 95.2
	c=.111.6 Å γ= 109.6	c=.97.4 Å γ= 104.6	c=.97.4 Å γ= 104.6
Resolution	2.7 Å	2.6 Å	2.6 Å
Redundancy	2.2	1.9	1.5
Completeness	90.4%	82.5%	70.0%
Rsym	6.0%	9.8%	7.4%
I/σ (overall)	10.4	10.1	13.7

Postal address: User Office, ESRF, B.P. 220, F-38043 GRENOBLE Cedex, France
Street address: 6 rue Jules Horowitz, F-38043 GRENOBLE Cedex
Telephone: +33 (0)4 7688 2552; Fax: +33 (0)4 7688 2020; e-mail: useroff@esrf.fr