ESRF	**Experiment title:** Crystallographic Studies of the Reaction Intermediates of Nitrite reductase from *Pseudomonas aeruginosa*	**Experiment number:** LS1657
Beamline: ID14-1	**Date of experiment:** from: 9-6-00 to: 11-6-00	**Date of report:** Aug00
Shifts: 6	**Local contact(s):** Stephanie MONACO	*Received at ESRF:*

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Report:

The dissimilatory nitrite reductase catalyses *in vivo*, the reduction of nitrite to nitric oxide according to the reaction $NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O$. Nitrite reductase is a homodimer of 120 kDa, carrying one *c* heme and one d_1 heme per monomer. The *c* heme is the electron acceptor pole and is reduced by cytochrome c551. The *d1* heme is the catalytic site. The crystal structure of nitrite reductase from *Pseudomonas aeruginosa* has previously been solved in both the reduced and oxidised state (Nurizzo et al. 1997, Nurizzo et al. 1998) with major structural re-arrangements being observed for Tyr 10 and the loop region 56-62 in the c-heme domain . These conformational changes are not provoked by the reduction of the c-heme (Nurizzo *et al.* 1999), and therefore the present study was undertaken in order to establish whether the conformational changes are concomitant with the reduction of the d1-heme.

Datasets were collected on ID14-EH1, from crystals of nitrite reductase cryo-quenched at different intermediate stages of d1-heme reduction (Figure 1, Table 1). These crystals belonged to the space group $P2_12_12$ with cell dimensions a=165.2, b=89.9, c=112.2Å.

Each structure was solved by molecular replacement using AmoRe and the reduced nitrite reductase structure as starting model. Difference Fourier maps were calculated by CNS in the region of the loop 56-62, and near Tyr10. Analysis of the results is still underway.

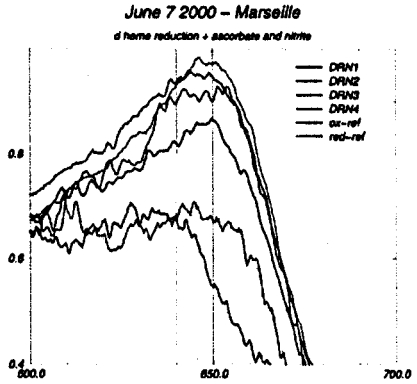

Figure 1. Visible absorption spectre of quenched intermediates of d1-heme reduction. The displacement of the peak between 640 and 650nm is clearly visible.

Table 1 Structural Statistics

Data Collection	**DRN1**	**DRN2**	**DRN3**	**DRN4**
Beamline		ID14-EH2		
λ (Å)		0.9326		
Resolution (Å)	2.4	2.7	3.1	2.6
R_{sym} (%)	10.0 (34.3)	7.5 (27.2)	11.1 (34.3)	7.5 (26.4)
I/σ	3.2 (1.8)	7.1 (2.7)	5.7 (2.2)	8.2 (2.5)
Completeness	91.3 (91.3)	96.7 (96.7)	98.3 (98.4)	98.2 (99.1)
Redundancy	2.9	4.0	3.4	5.8

References

Nurizzo D, Silvestrini MC, Mathieu M.,Cutruzzolla F., Bourgeois D., Fulop V., Hajdu J.,Brunori M., Tegoni M., Cambillâu C (1997) Structure, **5**, 1157

Nurizzo D, Cutruzzolla F., Arese M., Bourgeois D., Brunori M., Cambillau C., Tegoni M. (1998) Biochemistry, **37**, 13987

Nurizzo D, Cutruzzolla F., Arese M., Bourgeois D., Brunori M., Cambillau C., Tegoni M. (1999) J. Biol. Chem., **274**, 14997

Publications arising from this work

Brown K, Cutruzzolla F., Wilson E.K., Bellelli A., Arese M., Brunori M., Tegoni M., Cambillau C (in preparation)