ESRF	**Experiment title:** Crystallographic Studies of the H369A Mutant of Nitrite Reductase from *Pseudomonas aeruginosa*	**Experiment number:** LS1657
Beamline: BM14	**Date of experiment:** from: 3-3-00 to: 4-3-00	**Date of report:** Aug00
Shifts: 3	**Local contact(s):** Andy THOMPSON	*Received at ESRF:*

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Report:

We have successfully managed to crystallise the mutant enzyme H369A in the space group $P4_12_12$ with cell dimensions a=b=94.74, c=159.9Å. A dataset to 2.8Å was collected on ID14-2 (see table 1), and attempts made to solve the structure by molecular replacement using the reduced, wild-type structure as starting model (Nurizzo *et al.* 1998). This allowed only the d_1-heme domain to be placed, while the *c*-heme domain could not be located.

A MAD experiment was then performed on BM14, exploiting the heme Fe iron anomalous absorption. This low resolution dataset (3.8Å) enabled the d_1-heme Fe ion to be located, from which experimental phases could be calculated. After improvement by solvent flattening, these phases were combined with the calculated phases from the molecular replacement solution, and extended to higher resolution. After iterative rounds of model building and refinement by CNS, the c-heme Fe ion was located using difference Fourier maps. The observed phases were recalculated, the improved map quality then enabled most of the *c*-heme domain to be constructed.

The refined crystal structure shows that the *c*-heme domain, whilst preserving its classical c-type cytochrome fold, has undergone a 60° rigid-body rotation about an axis parallel to the pseudo 8-fold axis of the β-propellor, and passing through residue Gln 115 (figure 1). Even though the distance between the Fe ions of the *c* and d_1-heme is remains 21Å, the edge-to-edge distance between the two hemes has increased by 5Å. A significant change has also taken place in the ligation of the c-heme Fe ion, where the H369A mutant enzyme has lost the Met88 distal ligand. Furthermore the distal side of the d_1 heme pocket appears to have undergone structural re-arrangement, with the Tyr 10 having been retracted from the expected reduced conformation.

A B

Figure 1. Top (A) and side (B) view of the c-heme domain of the mutant structure H369A (green), compared with the wild-type (red). The d1-heme domain is coloured in grey.

Table 1 Structural Statistics

Data Collection

	f^-_{max}	f^-_{min}	remote	high resolution
Beamline		BM14		ID14-EH2
Space group		P41212		
λ (Å)	1.7389	1.7401	0.9918	0.9326
Resolution (Å)	3.9	3.9	3.9	2.8
R_{sym} (%)	7.7 (37.2)	9.2 (49.9)	7.4 (40.0)	3.8 (36.0)
R_{anom} (%)	6.8 (24.8)	5.5 (24.7)	5.7 (29.6)	-
I/σ	6.2 (1.2)	4.7 (0.8)	7.4 (1.6)	13.1 (2.1)
Completeness	95.3 (96.1)	96.1 (96.2)	96.9 (97.5)	97.7 (99.8)
Anomalous completeness	79.0 (94.6)	98.7 (96.5)	98.3 (91.3)	-
Redundancy	2.9	4.2	3.2	4.6

Refinement Statistics

Resolution (Å)	20-2.8	Mean B fact. (Å²) : main/side/solvent (Val22-Pro114) : 139.89/128.57/51.92 (Gln115-Tyr 543) : 76.30/81.20/51.92
Total reflections	6816	Rmsd B fact. : main/side 5.5/7.6
Total atoms/AU (protein/water)	4110/130	Rmsd bonds (Å) 0.013
R_{work}/R_{free} (%)	21.9/28.5	Rmsd angles,dihedrals,impropers (°) 2.2/25.4/2.08

References

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

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

Publications arising from this work

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

Cutruzzolla F., Brown K., Wilson E.K., Bellelli A., Arese M., Tegoni M., Cambillau C., Brunori M. Nitrite Reductase from Pseudomonas aeruginosa : Essential Role of Two Histidine Residues in Catalysis, PNAS (submitted)