ESRF	**Experiment title:** Crystallographic Studies of the CO-Bound form 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). In line with results in solution, preliminary experiments on crystals indicate that the complex can be formed where a molecule of CO is bound to the Fe[d1-heme] and that this CO molecule can be dissociated upon irradiation by visible light of wavelength 632nm. The present experiment was undertaken to confirm that a CO-bound complex can be obtained.

A dataset was collected on ID14-EH1, from crystals of the CO-bound complex with nitrite reductase (Table 1). These crystals belonged to the space group C2 with cell dimensions a=172.1, b=115.25, c=88.02Å, β=103.89°.

Using the reduced nitrite reductase structure as search model (Nurizzo *et al.* 1998), the structure of the CO-bound complex was solved by molecular replacement using AmoRe. After preliminary minimisation and B-factor refinement using bulk-solvent correction by CNS, the Fourier difference map clearly showed the presence of the CO molecule.

Figure 1. 2FoFc electron density map in the region of the d1-heme, clearly showing the bound CO molecule.

Table 1 Structural Statistics

Data Collection	
Beamline	ID14-EH1
Space group	C2
λ (Å)	0.9326
Resolution (Å)	1.95
R_{sym} (%)	16.0 (52)
I/σ	11.1 (1.6)
Completeness	99.7 (99.8)
Redundancy	6.6

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

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