| ESRF | **Experiment title:** STRUCTURAL DYNAMICS OF NITRITE REDUCTASE | **Experiment number:** LS - 603 |
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| **Beamline:** ID9 | **Date of Experiment:** from: 05-June-1997 to: 07-June-1997 | **Date of Report:** |
| **Shifts:** | **Local contact(s):** Dominique BOURGEOIS | *Received at ESRF :* 29 AOUT 1997 |

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## Report:

We have used 9 shifts beam time, plus an extra 3 shifts, normally of Laue, which have been converted to monochromatic data collection. Despite two series of problems with the line shutter (1st shift) and with the beam stability (2d shift), 2550 CCD frames have been collected on 8 crystals, leading to 6 usable data sets.

Oxidized NiR-Pa

Data on the oxidized NIR-Pa were collected at 2.15Å resolution at 100K (1) , whereas on other sources, diffraction limit was about 3.3-3.5Å resolution for this crystal form. Data were reduced by a combination of DENZO and of a de-overlapping program written by D.Bourgeois at ESRF, PROW (2), leading to a completion of 98% with the same data quality. Such a resolution has been obtained on the CCD with a large cell, thanks to PROW, making it possible to deconvolute overlapped reflections. The model of Pa NiR, issued the model refined against the ESRF 2.9 Å data was then refined against the new data using X-PLOR 3.8 Engh and Huber parameters. The map improvement was considerable; it allowed to build a few missing parts (loops, N-term) and further confirm a unique feature of Pa NiR, domain swapping of the N-termini of subunits A and B. Pa and Thiosphaera pantotropha (Tp) NiR (4,5) have a completely different structure of the heme ligands. In Pa, the N-terminus of one monomer is contacting the other monomer, permitting thus cooperativity. A paper on the oxidized NiR-Pa is in press in Structure (1).
The final integration and refinement parameters are given in the table.

Reduced NiR-Pa

- reduced form alone:

NiR-Pa, in the presence of a reducing agent (Ascorbate, 100 mM) is very rapidly reduced at the c-heme (less than I'), but even after 40', is not fully reduced at the d-heme, as seen from the spectra. The X-ray data show very little change.

- complex with NO:

On contrary, NiR-Pa, in the presence of a reducing agent (Ascorbate, 100 mM) and of the substrate (K+N02-, 100 mM), is able to perform only one catalytic turn at basic pH; it then stops, because the product NO has a high affinity to the reduced Fe(II). We have generated crystals of the reduced enzyme with bound product (NO). This reaction is rather slow (20-40'), as seen from the spectral changes, followed in the crystal, using ID9's microspectrophotometer. The structure of the reduced forms of NiR-Pa in complex with NO (the product) is now solved at 2.6 Å resolution (3), and exhibits changes at the reaction site (Tyr 10 movement) and at the c-heme site (a loop is displaced, permitting the movement of Tyr 10).

- complex with CN:

CN is a potent inhibitor (Kass= 1011) of the reduced form. The structure of the reduced forms of NiR-Pa in complex with CN is available at 2.6 Å resolution (3). It exhibits the same kind of structural changes as with NO bound.

Conclusion: the different redox states and complexes demonstrate the great flexibility of this enzyme upon changing the redox state or the type of ligand.

references

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