REPORT FOR EXPERIMENT 30-01-558 HELD ON BM30A

Mechanism of superoxide reduction in *Desulfoarculus baarsii* superoxide reductase investigated by kinetic crystallography.

Superoxide reductases (SOR) form a newly discovered class of antioxidant enzymes by which some anaerobic or microaerophilic organisms eliminate superoxide, $O_2^{-\cdot}$. Contrary to superoxide dismutase (SOD), the SOR catalysed reaction does not produce O_2, but instead reduces $O2^{-\cdot}$ to form H_2O_2 exclusively: $O_2^{-\cdot} + 1\ e^- + 2H^+ \rightarrow H_2O_2$.

Our ultimate goal is to contribute unravelling the mechanism of $O_2^{-\cdot}$ reduction by SOR by using kinetic crystallography. Pulse radiolysis experiments and resonance Raman spectroscopy have suggested that reduction of $O_2^{-\cdot}$ by SOR proceeds through the formation of a Fe^{3+} (hydro)peroxo species. We wish to directly observe this proposed intermediate, which would be of considerable value to confirm the hypothesis and to visualise associated conformational changes.

During last experiment, we have solved, as planned, the structure of the oxidised form of the *Desulfoarculus baarsii* SOR E47A mutant (this mutant is thought to stabilise the iron peroxo intermediate) in complex with ferrocyanide to 1.5 Å. This allowed us to identify ferrocyanide as a competitive inhibitor of the enzyme, the first ever discovered. We observed that ferrocyanide is perfectly tuned to "plug" the SOR active site and prevent superoxide from binding. The sixth coordination to the active site Fe^{3+} centre (which adopts an unusual [His_4,Cys_1] square pyramidal pentacoordination) was found to be provided by one of the cyanide "arm" in a non canonical bent geometry. It was also confirmed by microspectrophotometry that, as anticipated, the oxidised form of the enzyme was quickly reduced by x-ray induced photo-electrons when high brilliance beamlines (ID14/EH4) were used, showing the importance of using bending magnet beamlines for this kind of experiments. These results will be reported in a publication under preparation.