ESRF	**Experiment title:** Cryocrystallography of cytochrome c oxidase from *Paracoccus denitrificans*	**Experiment number:** LS-664
Beamline: ID02-BL4	**Date of experiment:** from: 02-Mar-97 to: 10-Mar-97	**Date of report:** 22-Feb-97
Shifts: 12	**Local contact(s):** Jonathan Grimes	*Received at ESRF:* 28 FEV. 1997

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Preliminary report:

Cytochrome c oxidase (ferrocytochrome c:oxygen oxidoreductase, EC 1.9.3.1), a membrane protein complex is the terminal enzyme of most respiratory chains. It catalyses the final electron transfer steps from cytochrome c to molecular oxygen. In addition to the four protons consumed in water formation per oxygen molecule, up to four protons are electrogenically translocated across the membrane.

We have obtained good crystals of cytochrome c oxidase from *Paracoccus denitrificans*. They belong to space group P4 with a=b=206.7 Å, c=83.5 Å. The protein complex is composed of four subunits (total molecular mass is 125 kDa). Subunit I contains two haem A molecules, called haem a and haem a_3, and copper B (Cu_B). Haem a_3 and Cu_B form the binuclear centre where molecular oxygen is reduced to water. Electrons from cytochrome c are first translocated to the copper A centre (Cu_A), which is located in the protein subunit II, and from there via haem a to the binuclear centre.

The structure of cytochrome c oxidase from *Paracoccus denitrificans* has been solved (Iwata, et al. Nature 376: 660-669), but there remain a lot of questions to be studied, especially the mechanism of proton pumping.

Because beam time was allocated from 02 Mar to 10-Mar only the experiments can be described:

We intend to collect the following data sets at 100 K.

(A) *The fully reduced enzyme:* Reduction with dithionite generates the four electron reduced enzyme (all metal sites get reduced)

(B) *Azid inhibited* enzyme: Azide binds to cytochrome c oxidase with a stoichiometry of 1:1. The binding of azid in haeme-copper oxidases may cause displacement of another nitrogenous ligand from Cu_B.

The published structure of cytochrome c oxidase from *Paracoccus denitrificans* (measured at room temperature) shows no electron density associated with the Cu_B ligand His325. In the new structural model based on cryo-data measured at ID02/BL04 (L-545) this residue is now well defined. With data sets (A) and (B) we want to show if absence of electron density is an effect of reduction in the X-ray beam (= redox dependent His-ligand switching) or of azide contamination (= ligand displacement).

(C) Format inhibited enzyme: Format inhibits cytochrome c oxidase and induces the conformational conversion of the „fast“ form to the „slow“ form in the case of beef heart cytochrome c oxidase. The binding site and the basis of the conformational change should be examined.