

Structural characterization of bacterial cytochrome *c* peroxidases in different redox states: insights on activation and catalytic mechanisms.

Aude Echalié^a, Graham W. Pettigrew^b and Vilmos Fülöp^a

^a Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

^b Division of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, Scotland

Keywords: peroxidase; catalytic mechanism; activation

Peroxidase family is a very large group of ubiquitous proteins degrading hydrogen peroxide through different means. They are classified depending on the electron donor partner. Yeast cytochrome *c* peroxidase is the most used paradigm of this class of enzymes, having been extensively biochemically and structurally characterized [1]. Bacterial cytochrome *c* peroxidases are the subject of these studies. Their catalytic pathway in the lights of biochemical characterization is very different to the yeast cytochrome *c* peroxidase. The main differences reside in their activation and in the way the radical is stored during catalysis. These studies investigate at a structural and molecular levels some of these differences between yeast and *Paracoccus denitrificans* cytochrome *c* peroxidase [2]. *Paracoccus denitrificans* cytochrome *c* peroxidase has two *c* haems covalently bound to the protein polypeptide via thioether bonds. The two haem moieties have very different electrochemical and biochemical properties: around 600 mV separate the two haem midpoint redox potentials. The high potential haem ($E_0 = +320$ mV; electron acceptor site) is coordinated with one methionine and one histidine and the low potential haem ($E_0 = -320$ mV; peroxidatic site) is bis-histidiny coordinated. All the bacterial cytochrome *c* peroxidases are isolated in a resting state with the exception of *Nitrosomonas europaea* [3]. The high potential haem needs to be reduced by one electron to allow the conversion from resting to active protein, triggering the loss of one of the coordinants from the low potential haem allowing the hydrogen peroxide to bind. It is this conversion that we have studied in detail by cryocrystallography. First, the X-ray structure of the resting (oxidized state) protein [4] was determined to 2.0 Å at 100K. Several studies [5,6] have shown that X-ray can cause damages and induce changes to protein crystals. The redox state of the crystals was followed by single crystal microspectrophotometry before and after data collection [7] and it seems that very early in the data collection, X-rays start to induce the reduction of the crystals. Different strategies were used to minimise the X-ray-induced reduction (among them: co-crystallisation with mild oxidative agent, composite data collection). But it is believed that because the data collection and all the crystal manipulations were done at 100K, the structure obtained is quite close to the real oxidized structure and no major structural changes took place in the crystals [8]. Oxidised crystals were subjected to reducing conditions to determine the structure of the activated protein but the crystals failed to diffract X-rays. The protein was co-crystallized in the presence of reducing agent. The crystallization conditions being completely different to the one for the oxidized state and the fact that the *in-situ* reduction causes the loss of diffraction may indicate large

conformational changes in the protein upon activation. The structure of the activated cytochrome *c* peroxidase presents effectively large structural rearrangements compared to the resting state and in particular a loop carrying the leaving histidine undergoes a motion of around 45° to joint the interface of the dimer. One of the coordinated histidine of the low potential haem dissociates, leaving solvent access for substrate binding. In order to understand how the first part of the catalysis is carried out and how the substrate binds to the low potential haem, the reduced protein was co-crystallised in the presence of a substrate analogue, cyanide. The position and the orientation of the cyanide bound and the hydrogen bonding pattern may give insights on the potential hydrogen donor entities needed for the reaction. Further catalytic intermediates will be presented to give an overview of the complex activation and catalytic mechanism which occur in the protein.

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