s1.m4.o3 **Structural Characterization of Bacterial Cytochrome *c* Peroxidases in Different Redox States: Insights on Activation and Catalytic Mechanisms.** Aude Echalier[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. E-mail: aechalier@bio.warwick.ac.uk*

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Peroxidase family is a very large group of ubiquitous proteins degrading hydrogen peroxide. They are classified depending on the electron donor partner. Yeast cytochrome c peroxidase (CCP) is the most used paradigm of this class of enzymes, having been extensively characterized [1]. Bacterial CCP are the subject of these studies. Their catalytic pathway in the lights of biochemical characterization is very different to the yeast CCP. The main differences reside in their activation and in the way the radical is stored during catalysis. These studies investigate, at a molecular level, some of these differences between yeast and *Paracoccus denitrificans* (*Pad*) CCP [2]. Pad CCP has two *c* haems covalently bound to the protein polypeptide via thioether bonds. The two haem moieties have very different properties: around 600 mV separate the two haem midpoint redox potentials. The high potential haem (HP; electron acceptor site) is coordinated with one methionine and one histidine and the low potential haem (LP; peroxidatic site) is bis-histidinyl coordinated. All the bacterial CCP are isolated in a resting state with the exception of *Nitrosomonas europaea* [3]. The HP 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 LP 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. The redox state of the crystals was followed by single crystal microspectrophotometry before and after data collection [5] and 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. 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 [6]. Since the structure determination from in situ reduced crystals was not possible, the protein was co-crystallized in the presence of reducing agent. As suggested by biochemical evidences, the structure of the activated CCP presents large structural rearrangements compared to the resting state and, in particular, a loop carrying the leaving histidine undergoes a motion of around 45° to reach the interface of the dimer. One of the coordinated histidine of the LP 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 LP 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 substrate stabilisation.

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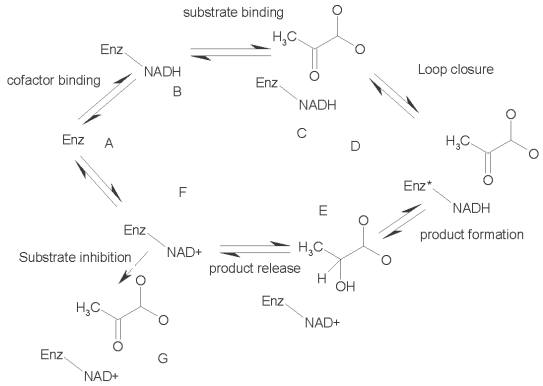
s1.m4.o4 **Catalytic Cycle of Plasmodium Falciparum Lactate Dehydrogenase.** Andrea Hadfield, Claire Limpkin, Jon Read, Camille Shammas, Debbie Shoemark, Rebecca Tranter and Leo Brady, *Department of Biochemistry, University of Bristol, School of Medical Sciences, Bristol, BS8 1TD, UK. E-mail: a.t.hadfield@bris.ac.uk*

Keywords: Time-resolved; Dehydrogenase; Protein Crystallography

Catalytic Cycle of *Plasmodium Falciparum* Lactate Dehydrogenase

Malaria is one of the major diseases of mankind, claiming 3 million lives worldwide annually. Resistance to existing antimalarial drugs is a large and increasing problem. The first high-resolution structure of an enzyme from the Plasmodium falciparum parasite, the causative agent of malaria was that of the essential glycolytic enzyme *P. falciparum lactate* dehydrogenase (*pf*LDH) (*1*). The deduction and confirmation of the precise molecular changes that accompany catalysis in this enzyme would provide an invaluable basis for the design of transition-state based inhibitors. The kinetic mechanism of action of lactate dehydrogenase has been studied at in great detail in a number of species of the enzyme (reviewed in (2)). This poster describes our progress towards elucidating the structural changes around the catalytic cycle (Figure 1) within crystals of pfLDH.



We have determined structures representing each stage in the cycle shown in figure 1, at resolutions varying from 1.2 Å (B, D, G) to 1.8 Å. Binary crystals (pfLDH + NADH) show disorder of the substrate binding loop. After soaking these crystals with substrate for 1 hour, the loop becomes ordered in the crystal (structure D) in a closed conformation. A combination of a slow turnover mutant and flash freezing has been used to capture states which we believe represent C and E with the substrate/product visible in the active site and the binding loop partially ordered. This work is being repeated using the wild-type enzyme. Structure G confirms the addition of pyruvate to NAD^+ at the C4 position in the nicotinamide ring to form a covalent adduct that has a characteristic yellow absorption.

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