s8a.m7.p5 ***Ab initio*** **crystal structure of low potential cytochrome c_{549} from *Synechocistis* sp. PCC6803.** F.J. Enguita[1], C. Frazão[1], R. Coelho[1], J. Navarro[2], M. Hervás[2], M. De la Rosa[2], G.M. Sheldrick[3], and M.A. Carrondo[1], [1]*Instituto de Tecnologia Química e Biológica, P-2781-901 Oeiras, Portugal*, [2]*Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla y CSIC, Apartado 11113, E-41080 Sevilla, Spain*, [3]*Institut für Anorganishe Chemie der Universitat Göttingen, Tammannstraβe 4, D-37077 Göttingen, Germany.*
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In macromolecules the phase problem is usually solved using either difference diffraction data (from derivatives or anomolous scatterers) or knowledge of similar molecules. The very high resolution diffraction data enabled by the use of synchrotrons sources, cryo-stabilization of crystals, and automatic data collection procedures have prompted the development of approaches to solve the phase problem using exclusively single wavelength native data, that is, employing *ab initio* methods.

Mono-hemic cytochromes *c* structures typically display a well known cytochrome *c* fold and present positive reduction potentials. Low potential (LP) cytochromes *c* form a sub-family with their relatively negative reduction potentials, but no 3D structure is yet available. They seem essential for photosystem II (PSII) activity, the source of photosynthetic oxygen evolution in cyanobacteria, algae and plants. In cyanobacteria, low potential cytochrome c_{549} (LPc_{549}) is an extrinsic protein of PSII.

LPc_{549} from *Synechocystis* sp. PCC6803 is a low-spin bis-histidinyl mono-hemic cytochrome *c* of 15 kDa with a reduction potential around –250 mV. Crystals were obtained by vapour diffusion from sitting drops containing 16% PEG 8k, 0.2 M calcium acetate and 0.1 M Tris/HCl pH = 8.5. They diffracted to 1.21 Å at BW7B beamline of EMBL outstation in DESY, Hamburg.

The LPc_{549} structure was obtained *ab initio*, using the native data and knowledge of Cys-X-X-Cys-His plus heme stereochemistry, the characteristic heme-*c* binding motif: a rotational search of the heme and its binding motif anchored at the iron position, known from a Patterson analysis, originated an initial seeding model that was expanded by SHELXC to a 669 point atoms model (60% of LPc_{549}). The point atom model was smoothed through atomic B refinement and completed by Fourier maps analysis. The refinement was performed by SHELXL, with atomic anisotropic displacements (a.d.p.) and including hydrogens in a riding model, to R=14.9% and R_{free}=20.8%.

The LPc_{549} structure shows the typical cytochrome *c* arrangement of α-helices around the hydrophobic pocket that harbours the heme, and is similar to c_6 and c_{551} SCOP sub-families, although bis-histidinyl heme coordination is unprecedent within the mono-hemic cytochromes *c* family. The a.d.p. distribution for heme axial histidines reveals a conspicuous asymmetry.

s8a.m7.p6 **Structural analysis of active site mutants of horseradish peroxidase C (HRP C)**. K. Meno, O. Mirza, A. Henriksen, M. Gajhede, *Protein Structure Group, Dept. of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*.
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Peroxidases are widely distributed throughout the plant and animal kingdoms. Plant peroxidases are heme enzymes that catalyze the reduction of hydrogen peroxide to water, oxidizing a suitable hydrogen donor such as a phenol, an indole or an aniline in the process. The hydrogen donor is released as a free radical, which can participate in further biochemical reactions[1]. Peroxidases show highly diverse substrate specificity and they can therefore participate in a wide range of biological functions. These include polymerization and depolymerization reactions, electron transport and a range of biosynthetic pathways. However, it has proven impossible to determine the specific *in vivo* function of this family of enzymes, in part due to the presence of several isoenzymes in the same cell and the broad substrate specificity.

This study aims at contributing to the elucidation of the reaction mechanism of HRP C. This is accomplished by crystallization of recombinantly expressed HRP C (rHRP C) with single site mutations in the distal heme cavity, in which the catalytic reaction takes place. Diffraction data was collected to 1.48, 2.50 and 2.00 Å respectively on the three mutants Phe41Ala, His42Glu, and His42Glu + Arg38Ser. The rHRP C structure has previously been determined[2] so the mutant structures were solved by molecular replacement. Phe41Ala and the double mutant were co-crystallized with ferulic acid (FA) in the orthorhombic space-group $P2_12_12_1$. His42Glu was co-crystallized with benzhydroxamic acid (BHA) in the monoclinic space-group $P2_1$. FA and BHA are reducing substrates, which can be processed by this enzyme. The structures of the rHRP C–substrate complexes with FA and BHA are known[3,4], so the comparison with the mutants reveals the role of the mutated amino acid residues in the binding of substrates.

The structures show that Phe41 is important in defining the area of the distal heme cavity where certain reducing substrates bind. Glu42 can because of the position of its side chain partly replace His as a proton acceptor in the reaction explaining the preservation of some activity in this mutant. Arg38 seems to be important in the reaction mainly because of its positive charge and hydrogen bonding ability.

[1] Dunford H.B. One-electron oxidations by peroxidases., Xenobiotica, (1995), 25: 725-733.
[2] Gajhede M., et al. Crystal structure of horseradish peroxidase C at 2.15 Å resolution., Nat. Struc. Biol., (1997), 4: 1032-1038.
[3] Henriksen A., et al. The structures of the horseradish peroxidase C-ferulic acid complex and the ternary complex with cyanide suggest how peroxidases oxidize small phenolic substrates. J. Biol. Chem., (1999), 274: 35005-35011.
[4] Henriksen A., et al. Structural interactions between horseradish peroxidase C and the substrate benzhydroxamic acid determined by X-ray crystallography. Biochemistry, (1998), 37: 8054-8060.