

P.04.03.23*Acta Cryst.* (2005). A61, C214**Crystal Structure and Stability of Red Alga *Porphyra yezoensis* Cytochrome c_6**

Hirotaka Chida^a, Seiji Yamada^a, Hideaki Shimizu^b, Tadashi Satoh^a, Hideharu Akazaki^a, Takeshi Yokoyama^c, Yasuhiko Takayama^a, Ryu Kawachi^a, Sam-Yong Park^c, Toshiyuki Nishio^a, Tadake Oku^a, ^a*Department of Biological chemistry, Nihon University.* ^b*Lab. for Structural Neuropathology, RIKEN Brain Science Institute.* ^c*Protein Design Lab., Yokohama City University.* E-mail: oku@brs.nihon-u.ac.jp

The *c*-type cytochromes (Cyts) are characterized by consensus Cys-X-X-Cys-His heme binding motif by which the heme is covalently bonded to the two Cys residues, and the axial His and Met ligands are generally coordinated to the heme iron as its fifth and sixth ligands, respectively. In addition, conformational stability of Cyt *c* is known to be extremely high through its strong heme C-protein contacts. However, role of heme axial ligands of *c*-type Cyts in the conformational stability still remains unknown. In this work, we investigated crystal structure and the effect of heme axial ligands in the conformational stability of Cyt c_6 from the red alga *Porphyra yezoensis*. The crystal structure was determined at 1.57 Å resolution. X-ray diffraction data were collected at the BL44B2 station at SPring8, Japan. The overall structure of Cyt c_6 follows the topology of class I *c*-type Cyts in which the heme prosthetic group covalently binds to Cys14 and Cys17, and the heme iron has an octahedral coordination with His18 and Met58 as the fifth and sixth ligands, respectively. Moreover, we constructed M58C and M58H mutants of the Cyt c_6 in which sixth heme iron ligand (Met58) was replaced with Cys and His residues, respectively. The Gibbs free energy change for unfolding of the wild type, M58H and M58C were 2.43, 1.48 and 5.45 kcal/mol, respectively. These results indicate that the heme axial ligand is important key to determine the conformational stability in *c*-type Cyts.

Keywords: cytochromes, mutagenesis, structural stability

P.04.03.24*Acta Cryst.* (2005). A61, C214**Structure of the Intermediates in the Myoglobin-peroxide Reaction**

Hans-Petter Hersleth^a, Takeshi Uchida^b, Thomas Teschner^c, Åsmund K. Røhr^a, Volker Schünemann^c, Kristina Nilsson^d, Ya-wen Hsiao^d, Thomas H. Rod^d, Alfred X. Trautwein^c, Ulf Ryde^d, Teizo Kitagawa^b, Carl Henrik Görbitz^a, K. Kristoffer Andersson^a, ^a*University of Oslo, Dept. of Chemistry & Molecular Bioscience, Norway.* ^b*Okazaki National Research Institutes, Japan.* ^c*Medical University of Lübeck, Germany.* ^d*Lund University, Sweden.* E-mail: hphersle@kjemi.uio.no

The biological conversions of O₂ and peroxides to water as well as certain incorporations of oxygen atoms into small organic molecules can be catalyzed by metal-ions in different clusters or cofactors. The catalytic cycles of these reactions pass through similar metal-based complexes. We have previously reported high resolution structures of the myoglobin compound II intermediate at pH 5.2 [1], and the state has been confirmed by microspectrophotometry in the pH range 5.2 to 8.7. These structures show a relatively long Fe...O distance of 1.9 Å compared to the 1.6 Å distance of the commonly proposed oxo-ferryl [Fe^{IV}=O] species. This long Fe...O bond is supported by the newly observed Raman Fe-O mode below 700 cm⁻¹. Quantum refinement best fit either a Fe^{III}OH⁻ or a Fe^{IV}OH⁻ state [2], while the Mössbauer spectroscopy indicates a Fe^{IV}-state. From compound II we were able to generate compound III (an oxy-complex). This intermediate was reduced by the synchrotron radiation giving an equivalent of compound 0 (Fe^{III}-peroxide) for which we have solved the structure. The different states were confirmed by microspectrophotometry.

[1] Hersleth H.-P., Dalhus B., Görbitz C.H., Andersson K.K., *J. Biol. Inorg. Chem.*, 2002, **7**, 299-304. [2] Nilsson K., Hersleth H.-P., Rod T.H., Andersson K.K., Ryde U., *Biophys. J.*, 2004, **87**, 3437-3447.

Keywords: metalloproteins, crystallography, spectroscopy

P.04.03.25*Acta Cryst.* (2005). A61, C214**Crystal Structure of PA0740, a Novel Zinc Hydrolase of *Pseudomonas aeruginosa***

Gregor Hagelüken^a, Thorsten Adams^b, Lutz Wiehlmann^c, Harald Kolmar^b, Dirk W. Heinz^a, Wolf-Dieter Schubert^{a*}, ^a*German Research Center for Biotechnology, Braunschweig, Germany.* ^b*University of Göttingen, Department of Microbiology and Genetics.* ^c*Hannover Medical School, Germany.* *E-mail: wds@gbf.de

Pseudomonas aeruginosa is an opportunistic pathogen causing acute and chronic infections. During infection, *P. aeruginosa* expresses a range of virulence factors as well as proteins needed for biofilm formation. Expression of most of these proteins is primarily regulated by a sophisticated acyl-homoserine lactone (AHL) based quorum sensing system. By means of transposon mutagenesis we searched for further virulence factors of *P. aeruginosa*. During these efforts a strain in which the gene coding for the 73 kDa protein PA0740 had been knocked out, showed an increased production of AHLs. Therefore PA0740 presumably has an AHL degrading activity and may hence regulate *P. aeruginosa* quorum sensing. To further investigate the function of PA0740, we solved the crystal structure at 2.7 Å resolution. PA0740 is a symmetric dimer, exhibiting an unusual α -helical dimer interface in which the monomers are intricately intertwined. Each monomer contains an N-terminal β -sandwich domain reminiscent of class B β -lactamases. Molecular modelling indicates that 3-Oxo-C12-HSL, a putative substrate, could comfortably bind to the active site, resulting in its hydrolysis. The central domain of PA0740 is involved in dimerization, while the C-terminal domain is structurally similar to sterol carrier protein-2.

Keywords: *P. aeruginosa*, quorum sensing, zinc hydrolase

P.04.03.26*Acta Cryst.* (2005). A61, C214**Crystal Structure of Carboxypeptidase 1 from *Thermus thermophilus***

Koji Nagata^{a,b}, Shiho Tsutsui^a, Woo Cheol Lee^a, Kosuke Ito^a, Masayuki Kamo^a, Yumiko Inoue^b, Masaru Tanokura^{a,b}, ^a*Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.* ^b*RIKEN Harima Institute at Spring-8, Hyogo, Japan.* E-mail: unagata@mail.ecc.u-tokyo.ac.jp

Carboxypeptidase 1 from *Thermus thermophilus* (*Tth*CP1) is a metalloprotease which hydrolyzes a peptide bond from the C-terminus of peptides and proteins and requires a divalent metal ion such as Zn²⁺ or Co²⁺ for its activity. The metal ion binding motif of *Tth*CP1 differs from those of classical metalloproteases and a distinctive catalytic mechanism has been proposed. In this research, we have solved the crystal structure of *Tth*CP1 to analyze the structural basis of its catalytic mechanism and heat stability, and also characterized its substrate specificity.

*Tth*CP1 was crystallized using PEG8000 as the precipitant by sitting drop vapor diffusion method. A native dataset was obtained to a resolution of 2.6 Å. Diffraction data were collected using an ADSC Quantum 210 detector system at beamline PF-AR NW12 at Photon Factory (Tsukuba, Japan) [1]. The crystal structure was determined by molecular replacement using the atomic coordinates of carboxypeptidase from *Pyrococcus furiosus* (*Pfu*CP, PDB code: 1KA2 [2]). The structure, substrate specificity and thermostability of *Tth*CP1 will be presented and compared with those of *Pfu*CP [2, 3].

[1] Nagata K. et al., *Acta Cryst.*, 2004, **D60**, 1445. [2] Arndt J.W. et al., *Structure*, 2002, **10**, 215. [3] Cheng T. C. et al., *Prot. Sci.*, 1999, **8**, 2474.

Keywords: carboxypeptidase, structure-function protease, thermostable enzyme

P.04.03.27*Acta Cryst.* (2005). A61, C214-C215**Mimicking Evolution from Inactive *Bacillus subtilis* SOD-like Protein to Active Mutants**

Vito Calderone^{§‡}, L. Banci[‡], M. Benvenuti[§], I. Bertini^{*‡}, A. Fantoni[‡], S. Mangani^{§‡}, M. Migliardi[‡], M. S. Viezzoli[‡], D. E. Cabelli[§], [§]*Dept of*