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Crystal Structure and Stability of Red Alga *Porphyra yezoensis* Cytochrome c_6

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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 1.57Å 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

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Structure of the Intermediates in the Myoglobin-peroxide Reaction

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The biological conversions of O_2 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

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Crystal Structure of PA0740, a Novel Zinc Hydrolase of *Pseudomonas aeruginosa*

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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-homoserinelactone (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

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Crystal Structure of Carboxypeptidase 1 from *Thermus thermophilus*

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Carboxypepidase 1 from *Thermus thermophilus* (*Tth*CP1) is a metallopeptidase which hydrolyzes a peptide bond from the C-terminus of peptides and proteins and requires a divalent metal ion such as Zn^{2+} or Co^{2+} for its activity. The metal ion binding motif of TthCP1 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].

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Keywords: carboxypeptidase, structure-function protease, thermostable enzyme

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Mimicking Evolution from Inactive *Bacillus subtilis* SOD-like Protein to Active Mutants

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