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], Tadatake 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 1.57Å 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