DATE: Day 25 Month 07 Year 2016

SUMMARY of
2015 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Structural studies on the cold-adaptation mechanism of polar glacial microorganisms
Applicant	**Name**	Han-Woo KIM
	Affiliation	Korea Polar Research Institute
	Present Title	Principal Research Scientist
Research Collaborator (Host PI)		Atsushi Nakagawa Prof.

Summary

For life to exist in polar climates, organism must find strategies to minimize environmental stresses imposed by the harsh climatic conditions. Reactive oxygen species (ROS) are produced as a normal by-product of aerobic metabolism in living organisms. In addition, various environmental stress conditions including light/dark cycle, nutrient depletion/excess, high salinity, drought, flooding, extreme temperatures, heavy metals and UV irradiation are also known to accelerate the accumulation of ROS in plant cells, ultimately leading to death of the cells. Ascorbic acid (AsA) maintains redox homeostasis by scavenging reactive oxygen species in plants. The enzyme monodehydroascorbate reductase (MDHAR) regenerates AsA by catalysing the reduction of monodehydroascorbate, using NADH or NADPH as an electron donor. Detailed enzymatic mechanism of MDHAR remains unclear due to lack of its structural information. Here, we show the crystal structures of MDHAR in the presence of cofactors, Nicotinamide adenine dinucleotide (NAD+) and Nicotinamide adenine dinucleotide phosphate (NADP+), and complexed with AsA as well as its analogue, isoascorbic acid (ISD). The overall structure of MDHAR is similar to other iron-sulfur protein reductases, except for a unique long loop of 63-80 residues, which seems to be essential in forming the active site pocket. From the structural analysis and structure-guided point mutations, we found that Arg320 residue plays a major substrate binding role, and Tyr349 residue mediates electron transfer from NAD(P)H to bound substrate via FAD. The enzymatic activity of MDHAR favours NADH as an electron donor over NADPH. Our results show, for the first time, structural insights as a potential reason for this preference. The MDHAR-ISD complex structure revealed an alternative binding conformation of ISD, compared with the MDHAR-AsA complex. This implies a broad substrate (antioxidant) specificity and resulting greater protective ability of MDHAR.