Structure of protochlorophyllide reductase reveals a mechanism for greening in the dark

Norifumi Muraki¹, Jiro Nomata², Tomoo Shibai³, Yuichi Fujita³, Genji Kurisu⁴
¹The University of Tokyo, Life Sciences, Komaba 3-8-1, Meguro, Tokyo, 153-8902, Japan, ²Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi, 464-8601, Japan, E-mail: nmuraki@xtal.c.u-tokyo.ac.jp

Chlorophyll (Chl) is a tetrapyrrole macrocycle containing Mg and a phytol chain. The Chl biosynthetic pathway consists of the multi-enzymatic reactions. An asymmetric conjugated double bond system of Chl a, which is crucial for efficient light absorption, is formed in the penultimate step of biosynthesis, reducing protochlorophyllide (Pchlide) to form chlorophyllide a. Photosynthetic organisms adopt two different strategies for the reduction of Pchlide: one is the light-dependent Pchlide oxidoreductase that requires light for the catalysis, and the other is dark-operative Pchlide oxidoreductase (DPOR) that operates even in the dark. The greening ability of plant in the dark is attributed to the activity of DPOR. We show a crystal structure of the DPOR catalytic component NB-protein from Rhodobacter capsulatus at 2.3 Å resolution. Overall structure with two copies of homologous BchN and BchB subunits is similar to that of nitrogenase MoFe protein. Each catalytic BchN-BchB unit contains one Pchlide held without any axial ligations from amino acid residues and one Fe-S cluster (NB-cluster) coordinated uniquely by one aspartate and three cysteines. Intriguingly, NB-cluster and Pchlide are arranged spatially as almost identical to P-cluster and FeMo-cofactor in MoFe protein, illustrating a common architecture to reduce chemically stable multi-bonds such as porphyrin and dinitrogen.

Structure, stability and flexibility of a psychrophilic iron superoxide dismutase

Antonello Merlino¹, Irene Russo Krauss¹, Immacolata Castellano¹, Emmanuele De Vendittis¹, Alessandro Vergara¹, Filomena Sica¹,³
¹University of Naples ‘Federico II’, Department of Chemistry, via cintia, 80126, Napoli, Italy, ²University of Naples Biochemistry and Medical Biotechnologies, University of Naples Federico II, Napoli, Italy, ³Biostructures and Bioimages Institute, C.N.R, Napoli, Italy, E-mail: antonello.merlino@unina.it

The Antarctic eubacterium Pseudoalteromonas haloplanktis (Ph) produces a cold-active iron superoxide dismutase (SOD). PhSOD is a homodimeric enzyme, that displays a high catalytic activity even at low temperature [1-2]. The structure, stability and dynamics of PhSOD have been determined and compared with those of its mesophilic counterpart from E. coli (EcSOD). PhSOD was found to have structure and stability very similar to Ec-SOD. However, the psychrophilic protein shows an increased flexibility of the active site with respect to its mesophilic homologue. Two PhSOD mutants (C57S and C57R) have been also characterized. The C57R mutation significantly alters the half-denaturation temperature of the protein. The structural and dynamic changes induced by this mutation with respect to the C57S and wild-type structure were correlated with modifications in the thermal stability of the mutant. Altogether these data illustrate how evolution can adjust psychrophilic enzyme sequences to alter the flexibility, without compromising the overall protein structure.

Keywords: cold adapted enzymes, superoxide dismutase, protein structures

Structure of vitamin D₃ hydroxylase, a novel cytochrome P₄₅₀ from Pseudonocardia autotrophica

Yoshiaki Yasutake¹, Yoshikazu Fujii², Woo-Kwang Cheon¹, Akira Arisawa¹, Tomohiro Tamura¹,³
¹National Institute of Advanced Industrial Science and Technology (AIST), Research Institute of Genome-based Biofactory, 2-17-2-1, Tsukisamu-Higashi, Toyo-hira-ku, Sapporo, Hokkaido, 062-8517, Japan, ²National Institute of Advanced Industrial Science and Technology, 3-1-1 Koganei, Koganei-shi, Tokyo, 184-8590, Japan, ³Aichi University of Education, Department of Science, Nagakute, Aichi, 480-1192, Japan, E-mail: yasutake@coli.ri.bioj.twul.ac.jp

Vitamin D₃ hydrolase (VD₃), which catalyzes the final step of the biosynthesis of 1,25-dihydroxyvitamin D₃, is a novel P₄₅₀ that is expected to have a unique structure and function. Here we report the X-ray crystallographic structure of VD₃ hydrolase at 2.60 Å resolution. The crystal structure of VD₃ hydrolase has a novel domain organization compared to typical cytochrome P₄₅₀. We discuss the structural and functional aspects of VD₃ hydrolase, which may provide clues for the development of new drugs for vitamin D₃-related diseases.