Keywords: heme, transport, bacteria

P04.03.192

Acta Cryst. (2008). A**64**, C291

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

Norifumi Muraki[1], Jiro Nomata[2], Tomoo Shiba[1], Yuichi Fujita[2], Genji Kurisu[1]
[1]The University of Tokyo, Life Sciences, Komaba 3-8-1, Meguro, Tokyo, 153-8902, Japan, [2]Nagoya 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.

Keywords: chlorophyll synthesis, nitrogenase-like enzyme, iron-sulfur cluster

P04.03.193

Acta Cryst. (2008). A**64**, C291

Structure, stability and flexibility of a psychrophilic iron superoxide dismutase

Antonello Merlino[1], Irene Russo Krauss[1], Immacolata Castellano[2], Emmanuele De Vendittis[2], Alessandro Vergara[1,3], Filomena Sica[1,3]
[1]University of Naples 'Federico II', Department of Chemistry, via cinthia, 80126, Napoli, Italia, Napoli, Italy, I-80126, Italy, [2]Department of Biochemistry and Medical Biotechnologies, University of Naples Federico II, Napoli, Italy, [3]Biostructures 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.

[1] Castellano, I. et al. (2006) *Biochimie* 88, 1377-89.
[2] Merlino, A. et al. (2008) *Protein and Peptide Letters*, 4, in press.

Keywords: cold adapted enzymes, superoxide dismutase, protein structures

P04.03.194

Acta Cryst. (2008). A**64**, C291

X-ray structures of redox partner proteins for *Hyphomicrobium* Cu-containing nitrite reductase

Daisuke Hira, Masaki Nojiri, Kazuya Yamaguchi, Shinnichiro Suzuki
Osaka University, Department of Chemistry, Graduate School of Science, 1-1 Machikaneyama, Toyonaka, Osaka, 560-0043, Japan, E-mail：flat@ch.wani.osaka-u.ac.jp

Cu-containing Nitrite Reductase (NIR) is a soluble enzyme involved in bacterial denitrification, catalyzing one-electron-reduction of nitrite into nitric oxide. Crystal structure of novel hexameric NIR (HdNIR) from *Hyphomicrobium denitrificans* has been reported [1]. The overall structure of HdNIR shows a trigonal prism-shaped molecule, in which a monomer is organized into a unique hexamer. Each monomer is composed of an N-terminal region like a blue copper protein and a C-terminal region having homology with well-known trimeric NIRs. In the case of trimeric NIRs the type 1 Cu site buried within each monomer relays an electron from the redox partner protein to the type 2 Cu site where is catalytic site. On the basis of several data for NIRs from various organisms, a hydrophobic patch region of the surface on the type 1 Cu of NIR has been proposed as the binding site for the partner protein. While, in the case of HdNIR, the hydrophobic patch region were covered by its N-terminal region. Recently, we have revealed that two electron transfer proteins, a cytochrome *c* (HdCyt c_{550}) and a blue copper protein, pseudoazurin (HdPaz), act as electron donor for HdNIR in the periplasm [2]. To study the interactions between two proteins and HdNIR, we determined the crystal structures of HdCyt c_{550} and HdPaz at resolutions 1.50 and 1.18 Å, respectively. HdCyt c_{550} exhibits the typical cytochrome *c* folding having five α-helices. While, HdPaz possesses eight β-strands, forming two β-sheets, and two C-terminal α-helices. We discuss the interactions of HdNIR with HdCyt c_{550} and HdPaz by comparing their structures.

[1]. M. Nojiri, *et al., Proc. Natl. Acad. Sci. USA* (2007) 104, 4315.
[2]. D.Hira, *et al., J. Biochem.* (2007) 142, 335.

Keywords: electron transfer, blue copper protein, cytochromes

P04.03.195

Acta Cryst. (2008). A**64**, C291−292

Structure of vitamin D_3 hydroxylase, a novel cytochrome P450 from *Pseudonocardia autotrophica*

Yoshiaki Yasutake[1], Yoshikazu Fujii[2], Woo-Kwang Cheon[1], Akira Arisawa[2], Tomohiro Tamura[1,3]
[1]National Institute of Advanced Industrial Science and Technology (AIST), Research Institute of Genome-based Biofactory, 2-17-2-1, Tsukisamu-Higashi, Toyohira-ku, Sapporo, Hokkaido, 062-8517, Japan,