Structure of protochlorophyllide reductase reveals a mechanism for greening in the dark
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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. Each monomer is organized into a unique hexamer, which is well-known trimeric NIRs. In the case of trimeric NIRs the type 1 Cu site buried within each monomer relays an electron from the Cu-containing Nitrite Reductase (NIR) 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

Structure of vitamin D₃ hydroxylase, a novel cytochrome P₄₅₀ from Pseudonocardia autotrophica
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The Antarctic eubacterium Pseudonocardia 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
Vitamin D₃ (VD3) is a steroid hormone that plays a crucial role in bone metabolism, control of cell proliferation and differentiation in mammals. VD3 is activated by sequential hydroxylation at C25 and C1α, catalyzed by cytochrome P450 monooxygenases. In humans, three species of P450, CYP27A1, CYP27B1 and CYP2R1, are involved with the activation of the VD3 in liver and kidney. The activated form of VD3 (1α,25-dihydroxy VD3) is currently used as a pharmaceuticals for osteoporosis, psoriasis, rickets and hypoparathyroidism. We have recently isolated the VD3 hydroxylating enzyme (VDH), a novel cytochrome P450 from actinomyces *Pseudonocardia autotrophica*. To investigate the substrate binding mechanisms enabling the sequential hydroxylation of VD3, we have undertaken the crystallographic studies. The recombinant VDH was overexpressed using *Rhodococcus erythropolis* and purified and crystallized. The crystals belong to the trigonal space group P3₁ with unit-cell parameters a = b = 61.7 Å and c = 98.8 Å. The structure of VDH in substrate-free form was solved by molecular replacement at 1.75 Å resolution, using the P450eryF structure as a search model. The VDH exhibits a typical P450 fold, and the clear electron density indicates that polyethylene glycol is bound to the active site. Possible substrate-binding residues of VDH were identified, which could be targeted for protein engineering to enhance the substrate affinity and catalytic activity. The crystallization experiments for the substrate complexes are currently under way. This work was supported by the Project on Development of Basic Technologies for Advanced Production Methods Using Microorganism Functions by the New Energy and Industrial Technology Development Organization (NEDO).

**Keywords:** crystal structure, cytochrome P450, vitamin D3