Structure of Protochlorophyllide Reductase Reveals a Mechanism for Greening of Plants in the Dark

The greening ability of plants in the dark is attributed to the activity of dark-operative protochlorophyllide (Pchlide) oxidoreductase (DPOR) catalyzing the stereo-specific reduction of the C17=C18 double bond of Pchlide to form chlorophyllide *a*, the direct precursor of chlorophyll *a*. Here we show a crystal structure of the DPOR catalytic component from *Rhodobacter capsulatus* at 2.3 Å resolution. The overall structure with two copies each of homologous BchN and BchB subunits is similar to that of nitrogenase MoFe protein. Each catalytic BchN-BchB unit contains one Pchlide and one iron-sulfur cluster coordinated uniquely by one aspartate and three cysteines. Through X-ray analysis of the catalytic component of DPOR, we propose a novel mechanism for the stereo-specific reduction of Pchlide.

The greening ability of photosynthetic organisms is attributed to the activity of protochlorophyllide (Pchlide) oxidoreductase catalyzing the stereo-specific reduction of the C17=C18 double bond of Pchlide to form chlorophyllide *a*, the direct precursor of chlorophyll *a*. There are two distinct types of enzymes catalyzing the Pchlide reduction, light dependent type and dark-operative type enzymes. Dark-operative Pchlide oxidoreductase (DPOR) is a nitrogenase-like enzyme composed of electron transfer and catalytic components. However, it is not clear how DPOR catalyzes the stereo-specific reduction of the stable porphyrin Pchlide and how it resembles nitrogenase. Here we show the structural basis of the greening ability of plants in the dark, through X-ray crystallographic analysis of the catalytic component of DPOR from *Rhodobacter capsulatus*, and propose a novel mechanism for the stereo-specific reduction of Pchlide [1].

The catalytic component, NB protein composed of BchN and BchB, of DPOR was crystallized in the Pchlide-bound and Pchlide-free forms, and the structures were solved to a resolution of 2.3 Å, and 2.8 Å, respectively. The NB protein is a $(BchN)_2(BchB)_2$ heterotetramer associated with a dimer of BchN-BchB heterodimer (Fig. 1). A BchN-BchB hetero-dimer is related by a non-crystallographic two-fold axis. Each contains one iron-sulfur cluster at the subunit interface. The subunit arrangement and molecular dimensions are similar to those of nitrogenase MoFe protein, a NifD-NifK heterotetramer [2]. A unique feature of NB protein different from MoFe protein is an extensive domain swapping between the functional BchN-BchB units. The unique C-terminal domain of BchB is intercalated into the other BchN-BchB (BchN'-BchB') unit contributing to the accommodation of Pchlide. Both intercalating C-terminal regions are located within the cleft formed by the BchN-BchB heterotetramer.

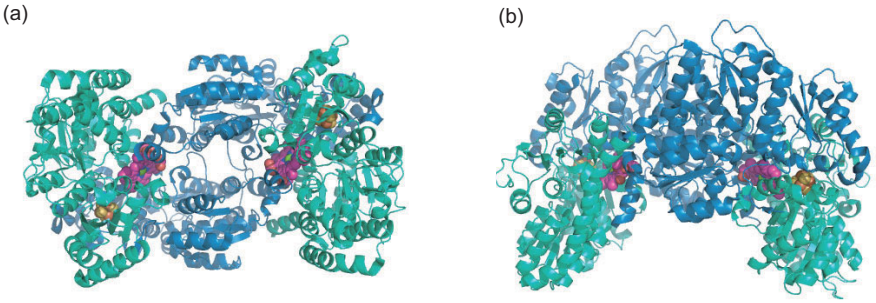

Figure 1
Crystal structure of the DPOR catalytic component.
(a) Top view and (b) side view of the structure. BchN and BchB subunits are colored in dark-blue and dark-green, respectively. The [4Fe-4S] clusters and Pchlide molecules are shown in the CPK model.

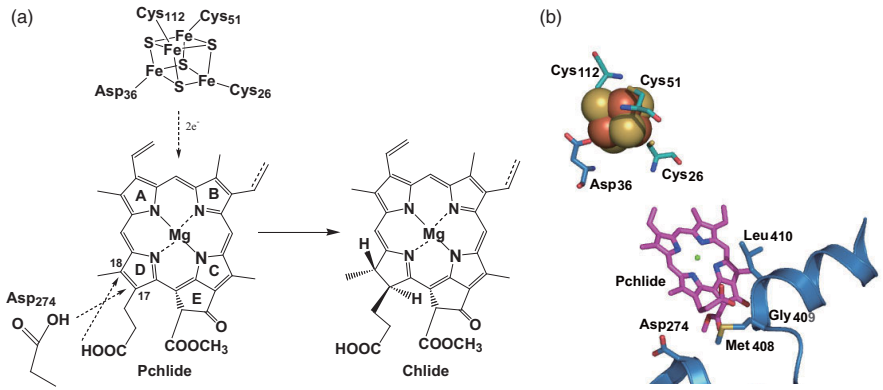

Figure 2
Pchlide reduction and Pchlide-binding cavity.
(a) The C17=C18 double bond of the Pchlide D ring is trans-reduced by DPOR.
(b) Pchlide-binding cavity showing the C-terminal helix from the next protomer is partly unwound upon Pchlide binding.

Two iron-sulfur clusters are each coordinated by the side chains of three BchN residues and one BchB residue, and prove to be a [4Fe-4S] type cluster. A surprise of the cluster is the direct coordination from BchB-Asp^{36} to the cluster together with three Cys residues of BchN. BchB-Asp^{36} is completely conserved in all known BchB/ChlB but not at all in NifK. To evaluate the importance of the Asp ligation of the NB-cluster, we examined the enzymatic activity of BchB-D36C variant, and determined its crystal structure. BchB-D36C variant almost eliminated the activity. The crystal structure of D36C had the conventional [4Fe-4S] cluster coordinated by four Cys residues including the substituted Cys, suggesting that the Asp ligation is essential for the catalysis.

The substrate, Pchlide, found in the native form is accommodated in the cavity surrounded by many hydrophobic residues. Comparison of the cavity structure of the Pchlide-binding form with that of the Pchlide-free form suggested that the middle part of the C-terminal helix of the intercalating BchB (BchB') is partly unwound and serves as a lid of this cavity upon Pchlide binding. Displacement of the terminal helix allows Met^{408}, Gly^{409} and Leu^{410} of BchB' to interact with Pchlide, enforcing the conformational distortion of the C17-propionate of Pchlide, almost perpendicular to the porphyrin plane (Fig. 2).

The NB-cluster and the bound Pchlide are aligned in tandem along the pseudo two-fold axis formed by BchN and BchB. The distance between the NB-cluster and the Pchlide rings is close enough, and the environment is hydrophobic enough for the through space electron transfer. To accomplish trans-specific reduction of the C17=C18 bond of the Pchlide D-ring, two protons should attack from opposite directions from the porphyrin plane concomitant with the electron transfer events. The most probable candidate for the proton donor for C17 carbon is BchB'-Asp^{274}, just 4.9 Å away beneath the D-ring of Pchlide. Because of the unique distorted configuration of the propionate, a proton from the propionate itself is close enough (4.8 Å) to attack the C18 carbon. Thus, the distorted configuration of the propionate could provide a structural basis for the trans-protonation of the C17=C18 double bond.

REFERENCES

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