



Title	Structural characterization of magnesium dechelataase and chlorophyllide a oxygenase involved in the chlorophyll metabolic pathway [an abstract of dissertation and a summary of dissertation review]
Author(s)	DEY, Debayan
Citation	北海道大学. 博士(生命科学) 甲第15158号
Issue Date	2022-09-26
Doc URL	http://hdl.handle.net/2115/87445
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	theses (doctoral - abstract and summary of review)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Debayan_Dey_abstract.pdf (論文内容の要旨)



[Instructions for use](#)

学位論文内容の要旨

博士の専攻分野の名称 博士 (生命科学) 氏 名 Debayan Dey

学位論文題名

Structural characterization of magnesium dechelatease and chlorophyllide *a* oxygenase involved in the chlorophyll metabolic pathway
(クロロフィル代謝系に含まれるマグネシウム脱離酵素とクロロフィリド *a* オキシゲナーゼの構造解析)

In land plants, green algae and some cyanobacteria, chlorophyll *a* and chlorophyll *b* form the principal components of the photosynthetic machinery that play crucial role in absorption, transmission, and transformation of light energy. The difference between the two chlorophyll species is the presence of a formyl group at the C7 position in chlorophyll *b* while a methyl group occurs at the same position in chlorophyll *a*. Both chlorophylls possess distinct absorption spectra in the blue and red regions, which allows this combination of pigments to utilize a wide range of light spectra for photosynthesis. The light harvesting complexes (LHCs) of photosynthetic organisms are composed of core and peripheral antenna complexes. While chlorophyll *a* is present in the core antenna of photosystems I and II as chlorophyll-protein complexes, chlorophyll *b* mainly resides in the peripheral antenna complexes along with other pigments like fucoxanthin. Moreover, chlorophyll *a* is vital for photochemistry in oxygenic photosynthetic organisms whereas chlorophyll *b* is necessary for stabilizing the major light-harvesting chlorophyll-binding proteins and also in regulating the photosynthetic antenna size by altering the chlorophyll *a/b* ratio.

Chlorophyll biosynthesis must be finely regulated for efficient photosynthetic performance during the formation of photosystems at the greening stage and also during adaptation to various environmental conditions. Not only chlorophyll biosynthesis but also chlorophyll degradation needs to be regulated because the latter plays a crucial role in mobilizing resources from chloroplast to developing organs. In addition, chlorophyll breakdown forms a key part of nitrogen recycling and is important in avoiding cellular photodamage. Before degradation, chlorophyll *b* must be converted to chlorophyll *a* because chlorophyll *b* derivatives are not catalyzed in the later steps of the chlorophyll degradation pathway. The interconversion pathway between chlorophyll *a* and chlorophyll *b* is referred to as the chlorophyll cycle.

Chlorophyll *a* is converted to chlorophyll *b* in two successive steps by chlorophyll(ide) *a* oxygenase (CAO). In the first step of chlorophyll *b* conversion, the enzyme chlorophyll *b* reductase (CBR) reduces the formyl group of chlorophyll *b* to produce 7-hydroxymethyl chlorophyll *a*. In the final step, chlorophyll *a* is formed by the enzyme 7-hydroxymethyl chlorophyll *a* reductase (HCAR), the structure of which resembles an archaeal F₄₂₀-reducing [NiFe] hydrogenase. Chlorophyll *a* is then converted to a primary fluorescent Chl catabolite by four continuous steps. First, central magnesium (Mg) ion in chlorophyll *a* is extracted by a Mg-dechelatease enzyme encoded by the *Stay-Green* (SGR) gene to form pheophytin *a*, which is then hydrolyzed to become pheophorbide *a* and phytol by pheophytinase (PPH). As the porphyrin of pheophorbide *a* is cleaved by pheophorbide *a* oxygenase (PAO), the green color completely fades in chlorophyll catabolite, leading to the formation of red chlorophyll catabolite. Subsequently, it is turned to the primary fluorescent chlorophyll catabolite by red chlorophyll catabolite reductase (RCCR) which is transferred out of the chloroplasts and isomerized to non-fluorescent products by acidic pH in the vacuole. My PhD study provides insights into the structural characteristics of two chlorophyll metabolic pathway enzymes – SGR and CAO.

Chapter 1 deals with the Mg-dechelatease enzyme which catalyzes Mg²⁺ dechelation from chlorophyll *a*. This reaction is the first committed step of chlorophyll degradation pathway in plants and is thus indispensable for the process of leaf senescence. There is no structural information available for this or

its related enzymes. This chapter provides insight into the structure and reaction mechanism of the enzyme through biochemical and computational analysis of an SGR homolog from the Chloroflexi *Anaerolineae* (AbSGR-h). Recombinant AbSGR-h with its intact sequence and those with mutations were overexpressed in *Escherichia coli* and their Mg-dechelatase activity were compared. Two aspartates – D34 and D62 were found to be essential for catalysis, while R26, Y28, T29 and D114 were responsible for structural maintenance. Gel filtration analysis of the recombinant AbSGR-h revealed formation of a homo-oligomer. The three-dimensional structure of AbSGR-h was predicted by a deep learning based method, which was evaluated by protein structure quality evaluation programs while structural stability of wild-type and mutant forms were investigated through molecular dynamics simulations. Furthermore, in concordance with the results of enzyme assay, molecular docking concluded the significance of D34 in ligand interaction. By combining biochemical analysis and computational prediction, the study unveils the detailed structural characteristics of the enzyme, including the probable pocket of interaction and the residues of structural and functional importance.

Chapter 2 also deals with the in-depth analysis of the structure of Mg-dechelatase enzyme. The crystal structure of a highly active SGR homolog from *Anaerolineae* (AbSGR-h) bacterium at 1.75 Å resolution has been reported. Previous study revealed the catalytic significance of D34 residue in AbSGR-h protein for interaction with the central Mg of chlorophyll *a*. Therefore, recombinant WT AbSGR-h and three mutants (D34E, D34N and D34Q) were overexpressed in *E. coli* and purified by nickel column and size exclusion chromatography. Gel filtration profiles of the WT and three mutant proteins were found to be similar thus confirming the role of D34 to be solely catalytic rather than maintaining the multimeric conformation of the protein. Activity analysis revealed substantial decrease of Mg-dechelation level for the D34E mutant and loss of activity for the D34N and D34Q mutants. The kinetic parameters of WT and D34E mutant AbSGR-h were elucidated by Michaelis-Menten analysis. Furthermore, molecular docking analysis showed stable interaction of the central Mg ion of chlorophyll *a* with the carbonyl oxygen atom of D34 residue in the crystal structure of AbSGR-h monomer within a distance of 4.4 Å. Besides, the catalytic triad found in AbSGR-h was found to show high resemblance with those observed in hydrolases. This study enhances the existing knowledge about the reaction mechanism of Mg-dechelatase and also provides the first crystal structure of a homolog from the SGR family.

Chapter 3 highlights the structural characteristics of the CAO enzyme, that is responsible for converting chlorophyll *a* to chlorophyll *b*. CAO belongs to the family of Rieske mononuclear iron oxygenases. Here, the tertiary structures of CAO from the Prasinophyte *Micromonas pusilla* (MpCAO) and model plant *Arabidopsis thaliana* (AtCAO) were predicted by deep learning based methods, followed by energy minimization and subsequent stereochemical quality assessment of the predicted models. Although plant CAO structure exhibits the three-fold symmetric homotrimer form, like most other Rieske non-heme iron oxygenases, *Micromonas* CAO exist as two distinct polypeptides (MpCAO1 and MpCAO2). Thus, its heterodimeric association was computationally investigated. Furthermore, the chlorophyll *a* binding cavity on the surface of MpCAO2 was predicted and molecular docking analysis revealed presence of the substrate at the vicinity of the mononuclear iron center. This study enables the structural visualization of the electron transfer pathway between the two distinct subunits of MpCAO.