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論文内容要旨

[1] Introduction

Fifty years after Schrödinger wrote the book entitled "*What is life*" where he tried to apply physical chemistry to biology, various vital phenomena in plants and animals in the fields of physics and chemistry have been elucidated. Photosynthesis had been also studied much enthusiastically from the physical point of view because it is identified as the driving force in the cyclic systems of organic materials in nature. Therefore at present, the study in photosynthesis begins to move from "clarifying" the function to artificially "remaking" it.

In the line of the remaking of photosynthesis, my final goal is to reconstitute of an antenna complex with chlorophyll (Chl) and bacteriochlorophyll (BChl) in order to construct an artificial photosynthesis, since the capture of the light energy in antenna complexes drives the light reaction in photosynthesis. The structure of the reconstituted or constructed antenna complex has been estimated with various measurements, however, it was hard to observe the ligation of the protein to the central Mg atom in Chl and BChl which is important structural information in vivo. In this study, I have utilized magnetic circular dichroism (MCD) spectra for Chl *a* and BChl *a*, *c* and *d* with a chlorin or bacteirochlorin ring to make a new simple method for estimating the interaction of the ligands with the center Mg atom in the Chl and BChl.

I also attempted the reconstitution of the antenna entity called chlorosome. Chlorosome, which is an antenna in green sulfur and filamentous bacteria, is much attracting many biophysicists because of the unique structure. Chlorosome can be regarded as a glycolipid micell in which the water-insoluble pigment (BChl *c*) are self-aggregated taking advantage of the internal hydrophobic area. Elucidating the aggregation properties of BChl *c* and the structure of the aggregates will give us important knowledge of the antenna function by pigment's self-assembly and the construction of pigment's self-assembled antennae. Therefore, I analyzed the formation process and the structure of the BChl *c* self-aggregates in hydrophobic environments with various spectroscopic measurements. Furthermore, I tried to reconstitute chlorosomes in an aqueous solution in order to construct an artificial pigment-assembled antenna in the condition close to that in vivo. I succeeded to construct a

pigment-lipid micell with similar absorption to that of chlorosomes.

[2] New approach of magnetic circular dichroism to clarification of the coordination states of photosynthetic pigments

Fig. 1 shows the MCD spectra of BChl *a* with a bacteriochlorin ring in diethyl in mixed solution of diethyl ether and pyridine. By titration of pyridine in the diethyl ether solution, the absorption maximum of Q_x(0-0) transition at 574 nm was red-shifted to 606 nm and the MCD intensity of Q_y(0-0) transition was increasing without red-shift. Considering that the Mg atom of BChl *a* is known to be five-coordinated in diethyl ether and six-coordinated in pyridine, the change of the coordination state influences on the transition energy of Q_x(0-0) and the MCD intensity of Q_y(0-0). The ligation to the central metal decreases the effective charge of the central metal, which causes the red-shift of Q_x(0-0). The MCD spectra of BChl *a* are dominated by the Faraday *B* term, which arises from the mixing between electronic states. Thus, the MCD intensity of Q_y(0-0) is inversely proportional to the difference in energy between Q_x(0-0) and Q_y(0-0). Therefore, the decrease of the energy difference between Q_x(0-0) and Q_y(0-0) by the change of the effective charge of the central Mg atom, can intensify the Q_y(0-0) MCD intensity. Actually when the MCD spectra of BChl *a* were measured in various solvents, there was a good correlation between the ratio of the MCD *B* term to the absorption dipole strength (*B/D*), which is often used for a quantitative argument of MCD intensity, and the energy difference between the Q_x(0-0) and Q_y(0-0) (Fig. 2). Fig. 2 indicates that the effective charge of the Mg atom is also changed by the nature of ligand molecules even for the same coordination number.

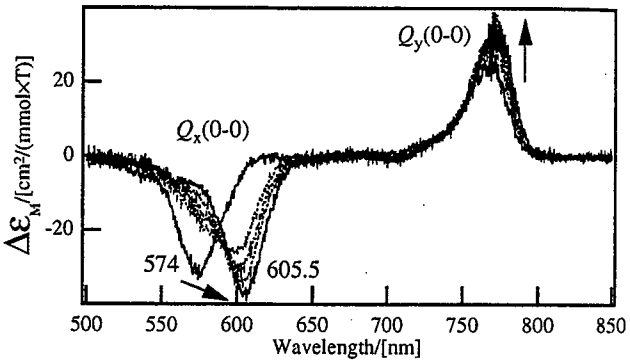

Fig. 1 MCD spectra of BChl *a* / diethyl ether solution titrated by pyridine. The volumes of pyridine added into 3 *ml* diethyl ether were 0, 10, 20, 50, and 350 μ*l*.

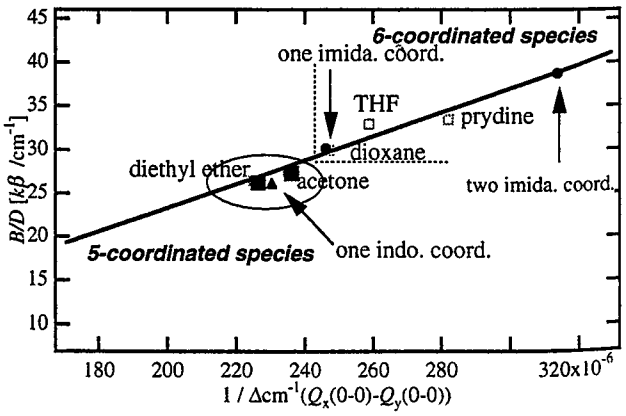

Fig. 2 Correlation between the $B/D(Q_y(0\text{-}0))$ value and the difference in wavenumber of Q_x(0-0) and Q_y(0-0).
Filled and unfilled squares: 5- and 6- coordinated states
Filled circle: imidazole ligate to the Mg atom
Filled triangle: a indole ligate to the Mg atom

For the purpose of analyzing the interaction of BChl *a* with amino acid residues, the MCD results of the five- and six- coordinated Mg atoms of BChl *a* with one and two imidazole molecules respectively, were plotted in Fig. 2. The five-coordination state where a imidazole molecule ligates to the Mg atom, locates closely to the position of the six-coordinated Mg atom with two dioxane molecules, suggesting that the coordination power of imidazole is much strong and that the effective charge of the five-coordinated Mg atom with a imidazole is similar to that of the Mg atom ligated by two dioxane molecules. I also plotted the MCD result of the five coordinated Mg atom with an indole molecule

whose structure is analogous to tryptophan, so that the position was near those of diethyl ether. Hence, indole interacts more weakly with the Mg atom than imidazole. Therefore, the correlation between the *B/D* value and the energy difference is very useful for determining not only the coordination number but also the molecules binding to the Mg atom of BChl.

[3] Spectral characteristics of the self-aggregated BChl *c*

-The formation process and structure of the BChl c self-aggregates-

There is a suggestion that BChl *c* is spontaneously aggregated in the antenna entity called the chlorosome where Q_y(0-0) transition appears around 740 nm. BChl *c* has a chiral center at the position 3^1 and *R*-type BChl *c* is major component of the BChl *c* homologs in chlorosome. Therefore, I studied the self-aggregation of the *R*-type BChl *c* in vitro and analyzed the the *R*-type BChl *c* solid high aggregates with a Q_y(0-0) band around 740nm with various spectroscopic measurements.

R-type BChl *c* formed two intermediate aggregates absorbing at 680 and 710 nm in CH_2Cl_2 and CCl_4. The 710 nm-absorbing species are known to be a piggy-back type of head-to-head dimer, where two BChl *c* molecules are strongly interacted each other and the macrocycles are parallel. In this study, it could be suggested from circular dichroism (CD), MCD and nuclear magnetic resonance (NMR) measurements that the 680 nm-absorbing species should be a loose dimer where the macrocycle planes are unparallel and the 710 nm absorbing-tight dimer is formed through the 680 nm aggregates.

The high aggregates with a Q_y(0-0) band around 740 nm have been known not to be formed in the CH_2Cl_2 and CCl_4 solution. Therefore, I attempted to make the solid state of *R*-type BChl *c* by drying the BChl *c*-CH_2Cl_2 and -CCl_4 solutions. In the process of drying the both solutions, the major Q_y(0-0) bands at 680 nm and 703-710 nm which are derived from the intermediate species, were red-shifted to 734-741 nm. The direct red-shift of Q_y(0-0) from 703-710 nm to about 740 nm had never been observed until the CH_2Cl_2 and CCl_4 solutions were dried. Therefore, *R*-type BChl c can form the solid high aggregates absorbing around 740 nm through the head-to-head dimer absorbing at 703-710 nm. Further, the solid aggregates treated with CH_2Cl_2 and CCl_4 were measrured with CD and MCD spectra, and it was indicated from the measurements that the stacking size and ordering of BChl c melecules in the solid aggregates depends on the solvent used. The X-ray diffraction was also measured on the CH_2Cl_2- and CCl_4-treated solid aggregates, and there appeared diffractions at 17.7 Å and 12.8 Å only for the sample treated with CH_2Cl_2, which showed many split carbon signals in the 2-dimentional CP/MAS ^{13}C dipolar correlation NMR spectra. From the X-ray and NMR measurements, I could propose a structural model of the solid aggregates absorbing around 740 nm where the head-to-head dimers absorbing at 710 nm are stacking (Fig. 3).

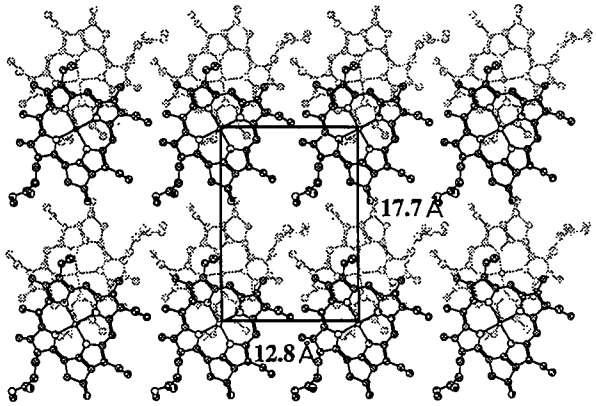

Fig. 3 The structural model of *R*-type BChl *c* solid high aggregates

[4] Reversible conversion of chlorosomes with treatments of organic solvents and reconstitution of chlorosomes

In the section 3, I maked the high aggregates of BChl *c* with the similar absorption maximum to that of chlorosomes in hydrophobic environments because the pigments can not be dissolved directly in an aqueous solution, while native chlorosomes can be dispersed in aqueous solutions. In this section, I attempted to form the high aggregates showing a Q_y transition around 740 nm in an aqueous solution with BChl *c* and other factors contained in chlorosomes.

It has been known that the Q_y(0-0) band at 740 nm of chlorosomes can be converted to a monomeric form of BChl *c* absorbing at 670 nm by the addition of 1-hexanol to a chlorosome solution and further the monomeric form of BChl *c* reverts to an aggregated state again by dilution of the hexanol-added solvent with buffer. In the reversible change, the glycolipid monolayer on chlorosomes is not broken by the treatment and most BChl *c* molecules is still confined in the chlorosome particles. In this study, I was able to convert a monomeric form of BChl *c* to an aggregated state under the treatments with the organic solvents infinitely soluble in water as well, and chlorosomes were reconstituted even on the condition that the glycolipid monolayer is broken by the treatments with the infinitely water-soluble solvents.

Further, the micell absorbing around 740 nm was constituted from BChl *c* and two kinds of lipids extracted from chlorosomes (neutral lipid and glycolipid). The each extracted lipid was dissolved in methanol containing BChl *c*, and then the concentration of methanol in the BChl *c*-lipid solutions was decreased by titration of a phosphate buffer or dialysis. In the case of the neutral lipid, both the Q_y(0-0) bands of the micelles formed by the methods of titration and dialysis appeared around 740 nm and BChl *c* formed the high aggregates in the buffer. Whereas, the micelles constituted from BChl *c* and glycolipid by the titration method show a Q_y band around 740 nm, however, the Q_y band of the micelles formed by dialysis was red-shifted only to 723 nm. Therefore, both of the lipids can make BChl *c* form the 740nm-absorbing high aggregates in an aqueous solution, however, the acceleration of the neutral lipids may be stronger than that of the glycolipids.

[5] Summary

In this study, I have developed a method to elucidate the coordination states of the central atom in Chl and BChl with MCD and also studied the reconstitution and reconstruction of chlorosomes in vitro and in vivo. The correlation between the *B/D* value of Q_y(0-0) and energy difference of Q_x(0-0) and Q_y(0-0) is very useful to determine not only the coordination number of the central metal atom of pigments but also the molecules ligating the metal atom. In the study of the BChl *c*, I was able to suggest that the 680 nm-absorbing aggregates is a loose dimer which is formed on the process of formning the 710 nm-absorbing dimer, and succeeded in forming the BChl *c*-ordered solid high aggregates absorbing around 740 nm which show X-ray diffraction and highly resolved 2-dimentional ^{13}C-^{13}C dipolar correlation NMR spectrum, so that the structural model of the BChl *c* high aggregates can be proposed. For the reconstitution of chlorosomes in an aqueous solution, the high aggregation of BChl c are easily occured by existence of the lipids contained in chlorosomes. Especially, the neutral lipid may play an important role on the aggregation of BChl *c*.

論文審査結果の要旨

本学位論文は光合成細菌の光合成器官の構造・機能解明とその人工構築を目指して光合成色素の配位構造と分子集合状態を研究したものである。資源・エネルギーの枯渇など我々を取り巻く様々な問題の解決を目指す研究の一環として光合成反応の積極的な活用方法が模索されている。このような大きな研究目標の中で、本論文は光合成明反応に必須なアンテナ色素、アンテナタンパク質、クロロゾームなど光捕集系の諸性質とその再構築を研究した論文である。

第１章は緒論であり、本研究の背景および目的について述べている。

第２章ではアンテナ色素、アンテナタンパク質、クロロゾームについてそれらを構成する色素の配位構造、結合様式を研究したものであり、これらの解析に新しい測定手段である磁気円偏光二色性が有効に用いられることを示し、その応用のための基礎を確立した重要な結果である。

第３章においては、緑色光合成細菌の集光器官クロロゾームを形成する色素バクテリオクロロフィルｃの自己会合特性に対し、２章で確立した分光学的手法ならびに固体高分解能核磁気共鳴法を用いて研究した。様々な条件での色素の会合特性とその構造を詳細に検討して、バクテリオクロロフィルｃが形成する低分子量会合体ならびに高次会合体の構造に関する詳細な知見を見い出し、天然のアンテナクロロゾームの構造解明に向けた興味深い重要な結果を得ている。

第４章においては、集光器官クロロゾームに含まれるバクテリオクロロフィルｃの様々な有機溶媒による会合状態の変化とその再会合特性を研究した。溶媒効果が溶媒の分配係数を考慮することにより統一的に理解できること、溶媒の希釈により会合体が再構成されることを見い出した。また、天然の脂質との共存下ではバクテリオクロロフィルｃは水溶液中においても、クロロゾーム類似の会合体を構築することを見出した。これらの結果はアンテナクロロゾームの構造、機能解明とバクテリオクロロフィルｃ会合体の光エネルギー捕獲素子としての応用に向けた興味深い重要な結果である。

第５章では以上の研究を総括した。

以上要するに本論文では、光生物が効率的に光エネルギーの捕獲に用いているアンテナ器官の構造と特性を光合成細菌について種々の分光法を活用することにより解明し、さらに、天然の色素および脂質を用いてクロロゾーム類似の会合体を再構築することに成功し、その工学的応用のための新しい知見を与えたものであり、生物工学の発展に寄与するところが大である。

よって、本論文は博士（工学）の学位論文として合格と認める。