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Photosynthetic properties of a cyanobacterium, *Gloeobacter violaceus* PCC7421.

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Introduction

While the origin of oxygenic photosynthetic organisms is unknown, phylogenetic analyses of reaction centre (RC) complexes in photosynthetic bacteria and prokaryotic oxygenic photosynthetic organisms could suggest a pathway for that origin, namely, a co-existence of two kinds of RC complexes in one organism through unknown process(es). The appearance of the oxygenic photosynthetic organisms represents the largest discontinuous process in the evolution of photosynthetic organisms. The appearance of oxygenic photosynthetic organisms determined the direction of global biological evolution through an increase in the oxygen concentration on the Earth. While an experimental approach to understanding this process has recently begun (Satoh et al. 2001), there are many challenges to understanding the discontinuity of the reaction processes of photosynthesis. One possible approach is to search for an organism(s) that has retained primitive properties. It was the adherence to this approach that led us to focus on the cyanobacterium *Gloeobacter violaceus* (Rippka et al. 1974).

According to phylogenetic analyses based on the 16S rRNA sequence (Nielissen et al. 1995, 1996), *G. violaceus* branched off from the main cyanobacterial tree at a very early stage, which suggests that it may retain some of the primitive properties of early cyanobacteria. In fact, it displayed many uncommon properties, such as lack of thylakoid membrane development (Guglielmi et al. 1981), having its photosynthetic and respiratory systems located on the cell membranes. Phycobilisomes attach to the cell membranes from the cytoplasmic site (Guglielmi et al. 1981), and oxygen evolution is thus mediated in the periplasmic space. The regulation of genes related to photosynthesis is unknown. There is no photosystem (PS) I chlorophyll (Chl) *a* fluorescence at the liquid nitrogen temperature in this alga (König and Schmidt, 1995). Taken together, these properties indicate that *G. violaceus* is a suitable target for analyses of the possible primitive reaction processes in cyanobacteria. We measured the photosynthetic properties of *G. violaceus*. We also discuss the significance of our findings for the evolution of cyanobacteria.

Materials and methods

Gloeobacter violaceus PCC7421 was grown autotrophically in the BG11 medium under a very low light intensity (10 μE/m^2/sec) at 25°C and 35°C. For the 35°C growth, cells grown at 25°C for two weeks were transferred to the 35°C condition and kept growing for two weeks. *Synechocystis* sp. PCC6803 grown in the BG11 medium was used as a reference.

An oxygen evolution activity was measured with a Pt-electrode (Rank Brothers, England) at 25°C. Temperature was controlled by running water.

Absorption and fluorescence spectra were measured with a Hitachi 557 dual wavelength spectrophotometer and a Hitachi F4500 spectrofluorometer, respectively. For the low temperature measurements, a custom-made Dewar bottle was installed. The time-resolved fluorescence spectrum was measured with the apparatus reported previously (Mimuro et al., 1999). A light source was a Ti-sapphire laser with a frequency doubler. The excitation pulse duration was 250 fs at 440 nm.

A content of P_{700} was estimated by a difference spectrum after oxidation-reduction reaction with a Hitachi 557 dual wavelength spectrophotometer; an extinction coefficient of P_{700} reported by Hiyama and Ke (1972) was used. Chl *a*' (an epimer of Chl *a* at the position of 13^2 on the macrocycle) and pheophytin (Pheo) *a* was estimated by HPLC as reported previously (Kobayashi et al. 1991). Chl *a* content was spectrally estimated after extraction with methanol.

Results

1. Growth and oxygen evolution activity

Cells were grown only under a very low light condition and thus growth was slow; a doubling time at 25°C was approximately 14 days. Cells contain three-peaked phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC) and Chl *a*. A unique carotenoid oscillaxanthin was also present. A relative content of Chl *a* to that of phycobiliproteins were low, thus cell suspension looked violet.

Oxygen evolution activity was saturated under a low light intensity. Compared with that of *Synechocystis* sp. PCC6803, an initial slope of the light saturation curve was approximately 30%, and the saturation point was approximately at 40 μE/m^2/sec, which was less than 20% of that of *Synechocystis* sp. These indicated that light absorption by antenna and energy transfer to PS II was inefficient compared with *Synechocystis* sp., and oxygen evolution itself was also inefficient under a high light condition. The actual reason(s) for these was not clear, however photoinhibition either or both of the donor and acceptor sides might be responsible for the low activity.

2. Fluorescence properties

It is known that *G. violaceus* cells do not show PS I Chl *a* fluorescence at the cryogenic temperature (König and Schmidt, 1995). We have confirmed this property by the time-resolved fluorescence spectrum. Even by the apparatus with a time resolution of 1.5 ps, we could not detect the PS I Chl *a* fluorescence in the wavelength region between 650 and 760 nm. A very fast energy transfer within PS I Chl *a* was not responsible for the absence of PS I Chl *a* fluorescence. Therefore we concluded that absence of PS I Chl *a* fluorescence is a typical feature of this species. The sequence data of *psaA/B* of *G. violaceus* indicate a relatively low similarity to those of *Synechocystis* sp. PCC6803 (unpublished), thus variation of sequence might be responsible for the absence of PS I Chl *a* fluorescence.

A loss of energy at the terminal of phycobilisomes was detected by the steady-state fluorescence spectrum. We observed that the peak location was different depending on the excitation conditions; by excitation of PE at 560 nm, the peak was located at 684 nm, and by Chl *a* excitation at 440 nm, it was shifted to 686 nm. It is known that the former came from a terminal emitter of phycobilisomes, usually known as the anchor polypeptide, and the latter, from PS II Chl *a*. It is natural to interpret that the energy flow from phycobiliproteins to PS II Chl *a* was partly impaired at the terminal of phycobilisomes. This might be one of reasons for a low oxygen evolution activity in *G. violaceus*.

3. RC content

Constitution of photosynthetic system of *G. violaceus* was analyzed. We estimated the RC content by plural methods; as for the P_{700} content, a content of Chl *a*' and P_{700} itself was measured. Since the Chl *a*' content is shown to be 1 molecule per 1 P_{700}, this can be an index for the P_{700} content (Kobayashi et al. 1991). The P_{680} content was estimated by a content of Pheo *a* because Pheo *a* is present in a molar ratio 2 molecules per 1 P_{680} (Nanba and Satoh, 1987). The PS II activity was confirmed by delayed fluorescence in the ns time range.

The P_{700} content in *Synechocystis* sp. PCC6803 was 1 per 130 Chl *a*, and this was consistent with other reports (Kawamura et al. 1979, Murakami and Fujita 1991). The P_{700} content of the 25°C-grown *G. violaceus* cells was estimated to be 1 molecule per 107 ± 9 (n=19) Chl *a* by Chl *a*' content and 1 per 122 ± 6 (n=18) Chl *a* by the oxidation-reduction method. It is natural to assume that all P_{700} are not necessarily chemically active, thus a content of P_{700} estimated chemically was lower than that by Chl *a*'. Even by this assumption, two values were close to each other, indicating the consistency and validity of two kinds of measurement. On the other hand, P_{680} content was low. Pheo *a* was found 1 per 193 ± 14 (n=16) Chl *a*, indicating that P_{680} was present 1 per 386 Chl *a*. The PS I/II ratio was, thus estimated to be from 3.16 to 3.61. These values were somewhat larger than that reported previously (Murakami and Fujita 1991), however it was in a range of normal growth. A lifetime of delayed fluorescence was 14 ns, comparable to that of spinach chloroplasts and green alga (Mimuro 1988).

At 35°C, the P_{700} content was significantly increased; 1 Chl *a*' per 80 ± 3 (n=16) Chl *a*, and 1 P_{700} per 105 ± 4 (n=20) Chl *a*. On the other hand, the Pheo *a* content was 1 per 250 ± 22 (n=14) Chl *a*. Thus, the PS I/II ratio was in a range of 4.76 to 6.25. These values were extraordinary large, and exceeded the previously reported values (Murakami and Fujita 1991). At 35°C, Chl *a* content per cells was almost a half that at 25°C, therefore, under this condition, the cell membranes were rich in the PS I complexes. A lifetime of delayed fluorescence was prolonged to 22 ns, indicating the modified PS II activity. A slow lifetime of delayed fluorescence was observed in the isolated D_1-D_2-cyt b_{559} complex (Mimuro et al. 1988).

Discussion

G. violaceus was able to grow only under a very low light intensity. Under a light-limiting condition, an oxygen evolution activity was lower by 70% than that found in *Synechocystis* sp. PCC6803. A partial loss of energy was observed at the terminal of phycobilisomes, therefore this might be responsible for difference in the initial slope of light saturation curve. The lifetime of delayed fluorescence in *G. violaceus* was comparable to that found in spinach chloroplasts, thus it seems that constitution of RC II itself is comparable to other oxygenic photosynthetic organisms. However a light saturation occurred in a low light intensity. It is therefore natural to interpret that photoinhibition occurs in a low light intensity either or both of the donor and acceptor sides. Based on these observations, the constitution of PS II in *G. violaceus* was postulated to be rather primitive or incomplete. On the other hand, the

difference absorption spectrum of P_{700} and the P_{700} content in *G. violaceus* were almost the same as those of *Synechocystis* sp. PCC6803, indicating that the constitution of PS I has already been close to that of other oxygenic photosynthetic organisms. It is reasonable to infer that in *G. violaceus*, PS I is rather close to a complete machinery but the PS II still contains a defect(s) in the constitution.

Absence of the PS I Chl *a* fluorescence can be explained by alternation of pigment binding site or amino acid sequences. A low sequence homology of the *psaA/B* genes in *G. violaceus* to those of other cyanobacteria might be a reason for the absence of the PS I Chl *a* (unpublished). As indicated by Satoh et al (2001), a part of binding sites of Chl *a* in PsaA/B is flexible, thus Chl *a* in one site does not turn to the fluorescent form at low temperature.

In conclusion, *G. violaceus* is a good target to study the possible primitive reaction processes in cyanobacteria, especially in PS II, and this leads to consideration of the evolution of oxygenic photosynthetic organisms.

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