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Photosystem I and II Reaction Centers in a New Type Oxygenic Photosynthesis of *Acaryochloris marina* Based on Chlorophyll *d*: Studies of Delayed Fluorescence and Triplet State ESR

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A cyanobacteria-like organism *Aaryochloris marina* performs oxygenic photosynthesis with near far-red light by the use of chlorophyll *d*. Reaction center chlorophyll (Chl) of Photosystem (PS) II of *A. marina* was studied by analysis of millisecond-delayed fluorescence. Delayed fluorescence is emitted by Chl *d* indicating efficient energy transfer between antenna Chl *d* molecules and the unknown primary electron donor of PS II. P740 a reaction center Chl of PS I of *A. marina* is shown to give a dimer type cation, and triplet state with a D value of 245×10^{-4} cm^{-1} in contrast to the 280-290 $\times 10^{-4} cm^{-1}$ values of P700 suggesting triplet spins interacting at a 5% larger distance in P740 than in P700.

Key words: *Acaryochloris marina*, chlorophyll *d*, delayed fluorescence, electron transfer, reaction center, triplet state

INTRODUCTION

A newly found cyanobacteria-like marine organism *Acaryochloris marina* [1] undergoes oxygenic photosynthesis with chlorophyll (Chl) *d* that absorbs at 710-740 nm, as a major pigment together with a few percent of Chl *a* [2-4]. In oxygenic photosynthesis of plants and cyanobacteria, it has been known that solar energy is converted to chemical energy by a dimer of Chl *a*, named P680, in photosystem (PS) II reaction center (RC), and by a pair of Chl *a* and Chl *a'*, named P700 in PS I[5,6]. In PS I RC of *A. marina*, on the other hand, the electron donor Chl *d* that absorbs at 740 nm, designated P740, was shown to be the primary electron donor [3]. The finding was the first exception of a long-accepted rule that Chl *a* is indispensable in RCs of oxygenic photosynthesis [7].

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A. marina cells contain Chl *d* and Chl *a* at a molar ratio of 1: 0.02 with a low amount of phycobilins [2, 4]. As for PS II of *A. marina*, neither the chemical identification of RC Chl nor the isolation of the RC complex has been achieved. Oxygen evolution in intact cells proceeds efficiently with 715 nm light [8] and the fluorescence yield of Chl *d*, as well as oxygen evolution, is sensitive to an inhibitor DCMU [8, 9]. PS I is better characterized. Redox reaction of P740 gives an absorption change at 740 nm with an apparent Em of 345mV in purified PSI RC that contains less than one Chl *a* per P740. P740, thus, seems to be a special pair of Chl *d* [3]. However, little is known about the chemical properties of P740 and other PS I components. We here studied millisecond delayed fluorescence, which is emitted from Chls re-excited by the charge

recombination reaction in PS II, in fresh intact cells to know the role of Chl *d* in PS II and cryogenic ESR to elucidate the electronic spin structure of P740 cation and triplet state.

MATERIALS AND METHODS

A. marina cells were grown in IMK medium with seawater at 29 °C and thylakoid membranes were isolated as described reported [3]. Synecosystis 6803 cells were grown in a BG11 medium.

Absorption spectrum was measured by a Shimazu 3100 spectrophotometer. Delayed fluorescence was measured with a home-built Becquerel type phosophoroscope [10]. White excitation light from a 150W Xenon lamp was focused onto an entrance slit through a heat cut filter, and then, excited cell suspension in a 1cm x 1cm x 4 cm polystyrene cuvette inside the phosophroscope at 0-1.4ms for excitation and at 0.1-2.9 ms after the excitation for the measurement of delayed fluorescence. The emission was measured with a photomultiplier (R-446, Hamamatsu) through a pulse motor-driven variable linear interference filter (VLI filter, Oriel). Signal was amplified with a lock-in amplifier and fed to a chart recorder. Prompt fluorescence was measured with a Hitachi F-4500 spectrofluorometer.

For ESR measurements isolated thylakoid membranes were pre-reduced by addition of sodium dithionite and frozen under illumination with a white light from a 650W tungsten-iodine lamp through a 50 cm optical glass fiber. ESR spectra were recorded using a Bruker 300E X-band spectrometer equipped with a liquid He flow cryostat (Oxford Instruments).

RESULTS AND DISCUSSION

Function of Chlorophylls in PS II

Absorption spectrum: In intact cells of *A. marina* and Scynecosystis 6803, absorption spectra were measured at room temperature (Fig.1). *Scynecosystis* cells show absorption peak at 684 nm of Chl *a* with a shoulder peak at 634 nm of phycobilin as is well known. In *A. marina* cells,

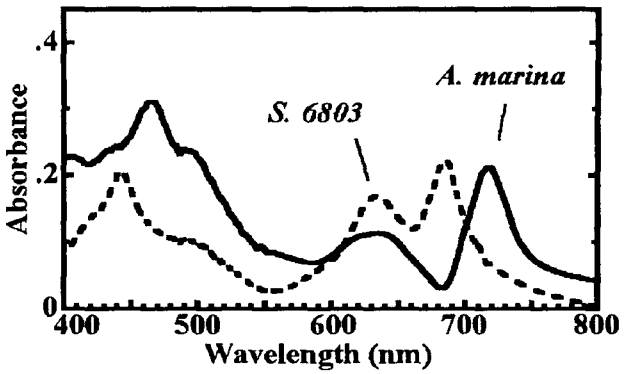

Fig. 1. Absorption spectra of intact cells of *A. marina* (solid line) and Synecosystis 6803 (broken line).

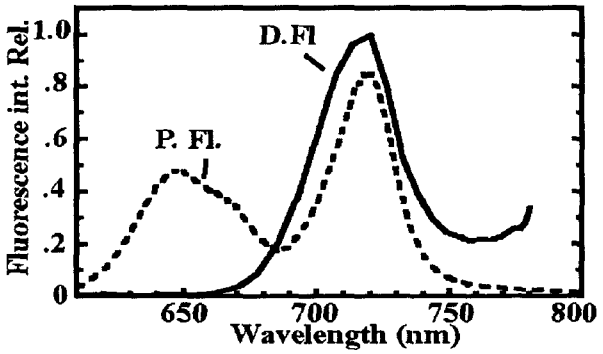

Fig. 2. Prompt and delayed fluorescence spectra of intact cells of *A. marina* at 25 oC. Broken line, prompt fluorescence measured with an excitation bandwidth of 5 nm at 430 nm and an emission bandwidth of 2 nm. Solid line, delayed fluorescence measured with a white excitation light with an emission bandwidth of 10 nm. Spectra are not fully corrected for detector sensitivities.

peak position of undeveloped phycobilin was also detected at 635 nm, and a large 718 nm peak was detected indicating that the major pigment in *A. marina* is Chl *d*. Chl *a*, which exist in *A. marina* cells at 2 % of Chl *d* [2,4] might also contribute around 660.

Prompt and delayed fluorescence: *A. marina* cells, with excitation at 435 nm, give a peak of prompt fluorescence at 720 nm of Chl *d* with an shoulder peak at 648 nm of phycobilin (Fig. 2). Some fluorescence from Chl *a* might contribute in a 670 nm range. These fluorescence bands are estimated to be emitted mainly by antenna pigments in PS II and a small fraction in the longer wavelength side may represent PSI antenna

fluorescence in analogy to the situation in cyanobacteria and plant membranes.

By the use of a Becquerel type phosphoroscope, delayed fluorescence emitted at 0.1-2.9ms after 1.9 ms excitation was repetitively measured in *A. marina* cells. The spectrum of delayed fluorescence obtained by scanning the VLI filter in Fig. 2 indicates a peak at 720 nm with almost no contribution of fluorescence of phycobilins and Chl *a*. The millisecond-delayed fluorescence is depressed by addition of DCMU indicating its production in PS II charge recombination (not shown). The emission intensity is lost after repeated freeze-and-thaws suggesting fragile nature of *A. maina* PS II. The results indicate that delayed fluorescence at this time range is emitted from Chl *d* in PS II and suggests efficient energy transfer to antenna Chl *d* from PS II reaction center chlorophyll that is a dimer of either Chl *d* or Chl *a* :P680.

Mimuro et al. reported a Chl *a*-like emission band at 670 nm that gives a fast rise followed by a 15ns decay time in the measurement within 20ns after 430 nm laser excitation at 77K in intact cells of *A. marina* and assumed this component to be delayed florescence from PS II [11]. Based on the results, they suggested PS II special pair to be Chl *a* dimer P680 as in cyanobacteria and plants and assumed a special mechanism that allows the fast uphill energy transfer from the long-wavelength antenna Chl *d* to the shorter wavelength P680, estimated the slow energy transfer from P680 to Chl *a* [11,12]. The detection of the Chl *d* delayed fluorescence in the longer time domain in the present study may not contradict to the results obtained in the faster time domain at low temperature. It is, however, clear that millisecond-delayed fluorescence is emitted from PS II charge recombination since it is sensitive to DCMU and to PS II intactness. One idea is that the 15 ns Chl *a*-like emission detected at 77K comes from a mechanism other than PS II charge recombination.

The Chl *d* delayed fluorescence does not, however, tell the chemical nature of PS II reaction center itself since the re-excited special pair of Chl *a* and also Chl *d* will transfer excitation energy to antenna Chl *d* equally well. We now are trying to get more evidence for the identity of the special pair chlorophyll in PS II RC of *A. marina*.

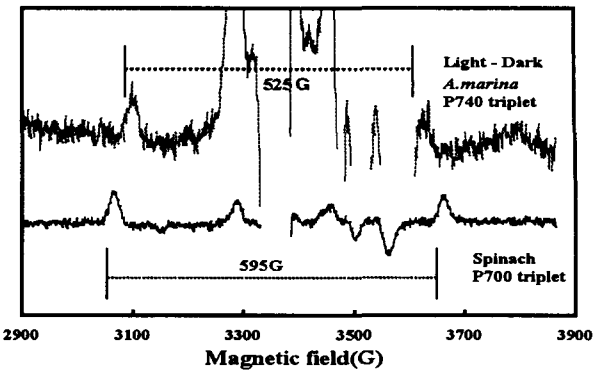

Fig. 3. Electron spin resonance spectrum of P740T measured in isolated thylakoids of *A. marina* and in spinach PS I particles. ESR conditions: microwave frequency 9.48GHz; power 0.05mW; modulation frequency 100 KHz; modulation amplitude 20 G; temperature 4.2 K.

Table 1. Zero field splitting parameters of the triplet states of P740 and P700 fromFig.3.

Triplet state	T (K)	| D | (10^{-4} cm^{-1})
Acaryochloris marina thylakoid P740	4.2	245
Spinach PS I P700	4.2	280
Synechocystis 6803 PS I P700	4.5	284
Synechococcus sp. PSI P700	4.5	289

$$D = 3/4(g\beta)^2/R^3$$

$$(R_{P740} - R_{P700})/R_{P700} \fallingdotseq 5\%$$

Characterization of PS I RC chlorophyll

Picosecond reaction of P740: Isolated PSI reaction center of *A. marina* shows absorption changes at 740 nm of P740 upon laser excitation [3]. Femtosecond time-resolved spectroscopy in isolated PS I reaction center preparation indicates a fast absorption changes of P740 together with the absorption changes at 680-720 nm with a 7.2 ps rise and a 50 ps decay [13]. The result indicates fast energy transfer between the antenna Chl *d* and P740 and the fast charge transfer from P740 to the acceptor chlorophylls, although chemical identities of the acceptor Chls are not fully clear yet.

ESR signals of iron sulfur centers, P740 cation and triplet state: Electron spin resonance of P740 cation was studied in the isolated thylakoid membranes. Photoinduced $P740^+$ signal showed an 8 gauss peak-to-peak bandwidth, which is narrower than the 10 gauss bandwidth of monomer chlorophyll cation (not shown). This strongly suggests $P740^+$ to be a cation of Chl *d* dimer. When the thylakoid membranes were frozen in the light under the strong reducing conditions in the presence of dithionite and methyl viologen, reduced iron sulfur centers F_A, F_B and F_X give ESR signals similar to those detected in spinach PS I (not shown).

Illumination at 4.2 K of this sample induced P740 triplet state signal (Fig. 3). It gives a 525-gauss axial zero field splitting that is narrower than the 595-gauss splitting of P700 triplet state in spinach PS I. The axial zero-splitting parameter D values calculated from Fig. 3 are summarized in Table I together with reported values in cayanobacterial membranes [14]. The D values are proportional to $1/R^3$, where R is an average distance between unpaired triplet spins. A 5% larger R value is calculated for P740. This indicates delocalization of triplet spins over the two halves of Chl *d* dimer in P740 and suggests either the stronger delocalization or a structure of dimer somewhat different from that of P700.

CONCLULSION

The results in the present study indicate that Chl *d* is an efficient antenna pigment in PS II of *A. marina*, although the chemical identification of the special pair chlorophyll still waits for the future analysis. PSI special pair is a Chl *d* dimer P740 based on ESR signals of a dimer-type cation radical. Triplet state of P740 gives a spin delocalizaton larger than P700.

The high efficiency reactions in *A. marina* indicate high flexibility of oxygenic photosynthesis. Even on RC poteins highly homologous to those in Chl *a*-based PS I and II of ordinary cyanobacteira [5], Chl *d* almost fully replaces the functions of Chl *a*. The Chl *d*-based system uses a photon energy intermediate between those absorbed by Chl *a* and bacteriochlorophylls so that it also can be a good tool for the study of the evolution of oxygenic photosynthesis from anoxygenic bacterial ones.

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