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Photosystem I and II reaction centers of a new oxygenic organism Acaryochloris marina that use chlorophyll *d*.

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Introduction

A newly found cyanobacteria-like unicellar prokariote, *Acaryochloris marina*, contains Chls *d* and *a* in a molar ratio of 30:1 and udergoes oxygenic photosynthesis(Miyashita et al. 1996,1997). Chl *d* absorbs at 700-720 nm light and has never been identified to be active in photosynthesis. It is now shown to function as efficient light harvesting pigment. The action spectra for PS I and PS II of this organism (Miyachi et al. 1997) indicated that Chl *d* function as efficient antena and the quantaum energy required for the oxygenic photosynthesis is by 10% lower than that ever assumed.

PS I RC of this organism contains a special pair of Chl *d*, named P740 after its absortion peak, per 145 Chl *d* (Hu et al., 1998), instead of P700. P740 shows an Em of +335 mV that is more negative than that of P700 and produce strong redox power compensating the lower quantum energy.

The chemical identity of PS II special pair is not known yet in this organism. Two possibilitis can be assumed : the first is the function of Chl *a* -dimer P680 in combination with a unknown mechaism to prevent energy dissipation from P680 to antenna Chl *d*, the second is the function of Chl *d* -based special pair that can accept excitation energy from antenna Chl *d*, in combination with the modified redox level(s) of donor/acceptor molecules to compensate low quantum energy absorbed by the special pair. Detection of long lived-fluorescence at 680 nm in membranes of this organism lead Mimuro et al (2000) to assume the function of P680, although the mechanism to prevent energy dissipation from P680 to Chl *d* was not proposed.

We report here the evidence for the second mechanism, suggesting the function of Chl *d*-electron donor in PS II, as was the case in P740 in PS I. Phtoreactions in thylakoid membranes, PS I RC complex and PS II enriched preparations of this organism are presented.

Materials and Methods

Acaryochloris marina, grown in K medium (Keller et al. 1987) at 25 ^{o}C and pH 7.0 was harvested and thylakoid membranes were isolated after disruption by a Bead-Beater (Edmund Buhler, Germany) at 4 ^{o}C, as described in Hu. Et al (1998). The green pellets of ontained thylakoids were resuspended in Bis-Tris buffer and further used.

Thylakoid membranes at 1 mg Chl *d* ml^{-1} were stirred on ice with 0.5 % (w/v) -DM for 25 min in the dark, and then layered onto a 10-30 % (w/v) linear sucrose density gradients and centrifugated overnight at 40,000 rpm (198,600 x g, rav, Hitachi P40ST swing-out rotor) at 4 ^{o}C. Five distinct and well-separated green bands were resolved on the gradients. The band 2 and 3 from top which contained PSII particles, were collected and stored at -80 ^{o}C and further purified by column chromatography to obtain crude PS II RC complex. The bottom band 5 was collected as PS I RC and stored. Measurements of absorption spectra and laser-induced absorption changes were done as described previously (Hu et al., 1998).

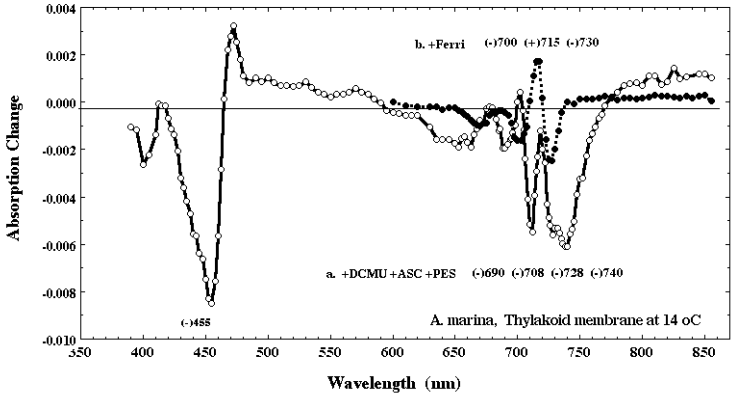

Fig.1 Difference spectrum induced by 10 ns laser excitation in thylakoid membranes of *A. marina*. a, in the presence of 1 mM ascorbate, 1µM PES, 10 µM DCMU. b , in the presencef 1 mM ferricyanide. At 15oC

Results and Discussion

Flash-induced absorption change was measuerd in the thylakoid membranes of *A. marina*. The membranes contained Chls *d* and *a*, and no phycobilisomes. The difference spectrum obtained in the presence of 1 mM ascorbate, 1 µM phenazine ethosulfate and 10 µM DCMU, which was added to supress turnover of PS II RC, showed +/- peaks at 455(-), 472(+), 690(-), 700(+), 708(+), 718(+), 728(-) and 740(-) nm, respectively. The spectrum resembles that deteted in the purified PS I RC complex (Hu et al., 1998) and can be attributed to P740 with characteristic peaks at 450 and 740 nm and can be attributed netither to P700 nor to P680. The decay time of P740 was about 30 ms and was accelerated by phenazine ethosulfate.

As expected from the reported 380 mV E_m of P740, addition of ferricyanide abolished the absorption change of P740 remaining smaller (about one third at the negative peak), reversible absorption change that showed a faster 100 µs decay time. The remaining component, thus, seems to be associated with PS II. The difference spectrum showed peaks at 685(+), 700(-), 715(+) and 730(-) nm, respectively, and is different from that of P740. The spectrum may be interpreted as an mixture of shift of pigments in the shorter wavelength side and a broad bleach around 720 nm and absorption increase above 740 nm. The absorption change was found to be overlapping on P740 if measurement was done without redox reagents in thylakoids. The difference spectrum, thus, seems to be associated with PS II reaction center. Relative extent of absorption changes at 670-80 nm was small suggesting small contribution of Chl *a*.

Absorption spectra at 77K of isolated PS I RC and PS II enriched fraction.

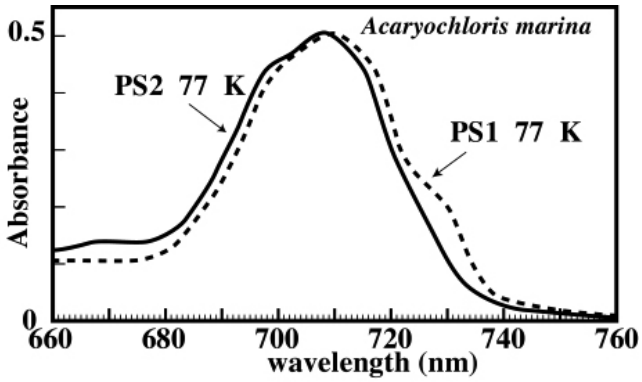

Fig.2 Absorption spectra of PS I RC and crude PS II-RC fraction of *A. marina* at 77K.

PS I RC complex was isolated as a heavy (band 5) fraction in sucrose density gradient after β-dodecyl maltoside treatment. PS II enriched fraction was obtained as the light (band 3 and 4) fractions and was further purified to deplete residual PS I. The obtained crude PS II RC was almost free from PS I polypeptides. Fig. 2 shows the absorption spectra of PS I RC and crude PS II-RC at 77 K. The PS I RC showed a peak at 710 nm with a marked shouder at 730 nm, less marked shoulders at 700 and 715 nm and a small peak around 740 nm containing P740. The crude PS II RC showed a peak at 708 nm, sholders at 696, 714 and 725 nm. Boichenko et al (2001) reported that PS I and PS II action spectra overlaps each other with PS II a few nm shifted to the shorter wavelength side peaking at 695 nm and PS I with characteristic 735 nm shoulder band in intact cells of *A. marina*. Larger contribution of Chl *a* to PS II was also suggested. The spectra of PS I RC and crude PS II RC in Fig 2 seem to well interpret the action spectra of PS II (measured by oxygen evolution) and PS I (measured by suppression of respiration).

The PS I RC showed difference absorption sepctrum similar to that of P740 in Fig. 1 measured in the thylakoid membranes indicating almost intact PS I activity. Function of phylloquinone was confirmed by Iwaki et al (paper in preparation) by the extraction/reconstitution of phylloquinone in the RC. FTIR measument indicated similar but partially different nature of $P740^+$ suggesting its environment to be similar to that of P700. ENDOR of $P740^+$ was also similar to those of cyanobacterial $P700^+$, and lacked sginal of one methyl group next to formyl group. It was thus, concluded that P740 resides in PS I RC in a environments essentially similar to that of P700.

RC chlorophyll of PS II

Laser flash induced difference absorption spectum was measured in the PS II-enriched fractions that lacked oxygen evoluving activity. Fig. 3 compares the absorption changes in the thylakoids, in the PS II enriched fraction (fraction 3) at 15 °C. The latter gave changes at 650-750 nm with a peak at 725 nm but lacked the sharp shift-like features. Similar spectrum was detected in the crude PS II RC at 77K. The spectrum seems to reflect light-induced triplet state of Chl *d* since it gave the decay time siginificantly faster than that of fraction 3, and had smaller positive

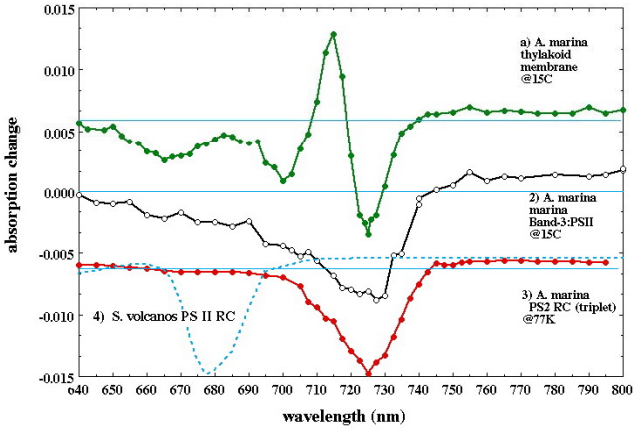

Fig.3 Flash-indcued difference spectra measured (1) in thylakoid membranes in the presence of 1 mM ferricyanide at 15°C, (2) PS II-enriched fraction-3 and at 15°C and (3) purified PS II-enriched faction at 77K and (4) in purified PS II RC of S. volcanus.

change above 745 nm that indicates cationic nature. None of these three spectra suggested the contribution of Chl *a* that are contained at 6-10% in these preparations suggesting no function of P680.

Conclusion

Both in thylakoid membranes and in the purifed PS I RC complex, function of P740 was characterized. P740 seems to function in an environment similar to that of P700, although specific molecular feature of Chl *d* may require some modifications of RC proteins. If we compare type-I RCs and their special pair Chls (Fig.4), *A. marina* PS I fills the gap between anoxygenic and oxygenic type-I RCs and indicates that type-I RCs forms a continuous line that represents the sequential exchanges of Chls on the essentially homologous type-I RC polypeptides.

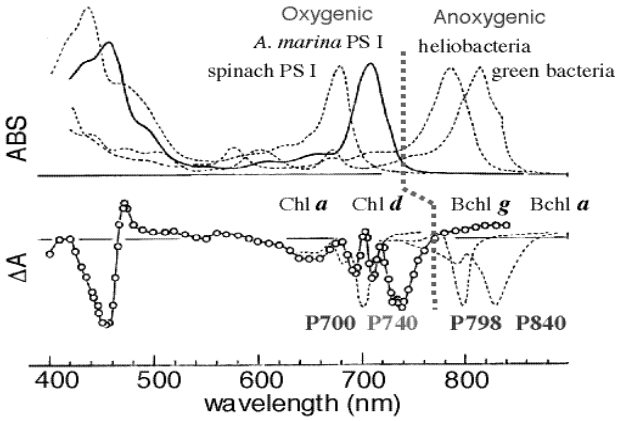

Fig.4 Absorption spectra of type-I RC complexes and the diffrence absorption spectra of special pair Chls in each RC

PS II of this organism also seems to use Chl *d* in the special pair P720 but does not seem to use P680 made of Chl *a*. The action of P720 in PS II RCC does not seem to be in cntradiction to the observation of the long-lived Chl *a*-like fluorescence, since if the Chl *a*-like pigment is well coupled to P720 in energy transfer this pigment, as well as Chl *d,* can be an emitter of delayed fluorescence as is actually seen in PS II with P680 in which most of prompt/delayed fluorescence are emitted by shorter wavelength antenna pigments but not by P680 itself. It is very interesting how *A. marina* efficiently accomplishes oxygen evolution with low energy 700-720 nm light absorbed by Chl *d*. Modification of E_m of pheophytin (we do not know yet whether phephytin *d* or *a* functions in PS II) might be also possible to compensate the shortage of input energy. Isolation of the D1/D2 type PS II RC complex from *A. mairna*, now attempted, seems to be essential.

Acknowledgements

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