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Universal Existence of One Chlorophyll *a'* Molecule in Photosystem I of Oxygenic Photosynthetic Organisms

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Chlorophyll (Chl) *a'* is the C13^2-epimer of Chl *a* which is the constituent of P700, the primary electron donor of Photosystem (PS) I, of a thrmophilic cyanobacterium, *Synechococcus elongatus*, whose structure was recently determined by X-ray crystallography. To determine whether PS I of diverse oxygenic photosynthetic organisms universally contain one molecule of Chl *a'*, pigment compositions of thylakoid membranes and PS I complexes isolated from cyanobacteria, green algae, red algae and higher plants were determined by reversed-phase HPLC. The results show that involvement of one Chl *a'* molecule in PS I is the universal feature for Chl *a*-based PS I of oxygenic photosynthetic organisms.

Keywords: Photosynthesis, Chlorophyll *a'*, Phylloquinone, Photosystem I, HPLC

Introduction

Chlorophyll (Chl) *a'* is the C13^2-epimer of Chl *a*, which was found by our HPLC analyses at the core part of Photosystem (PS) I of higher plants with a1:1 stoichiometry between Chl *a'* and P700 [1, 2]. Though, the presence of one Chl *a'* molecule in P700, the primary electron donor of PS I, was confirmed recently by X-ray crystallographic studies of a thermophilic cyanobacterium, *Synechococcus elongatus* PS I crystals [3], Chl *a'* contents of other organisms are yet to be determined. There are few reports on detailed pigment composition analysis for organisms frequently employed for biophysical and biochemical studies of PS I, such as a mesophilic cyanobacterium *Synechocystis* PCC6803 and a green alga, *Chlamydomonas reinhardtii*. To examine whether existence of one Chl *a'* molecule in P700 is the universal feature for PS I of oxygenic photosynthetic organisms, we isolated PS I complexes from cyanobacteria, higher plants, green algae and red algae, and determined the Chl *a'* contents on the basis of P700 and phylloquinone (PhQ), the secondary electron acceptor of PS I (two molecules per PS I), by simultaneous determination of Chl *a'* and PhQ with reversed-phase HPLC [2], and compared the HPLC results with spectrophotometrically determined P700.

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Materials and Methods

Thyalkoid membranes, native PS I and PS I core complexes were isolated from *Thermosynechococcus elongatus* and *S.* PCC 6803 (donation by. M. Ikeuchi, Univ. Tokyo), green algae *C. reinhardtii* IAM C-9 and *Chlorella vulgaris* IAM C-29, and a red alga, *Porphyridium purpureum* IAM R-1 cultured in our laboratory. Spinach was purchased from local market. Details of preparation methods will be described elsewhere.

Pigment extraction and reversed-phase HPLC analysis were conducted as described previously [2]. P700 concentration was determined from steady-light-induced absorbance change at the maximum of the Q_Y-bleaching band or at 808 nm.

Results and Discussion

To examine whether Chl *a'* exists in the core part of PS I, pigment composition of thylakoid membranes, native PS I and PS I core complexes which have different number of Chl molecules per PS I were determined with reversed-phase HPLC (Fig. 1). In *T. elongatus*, the Chl *a*/Chl *a'* and the Chl *a*/PhQ ratios slightly decreased in going from thylakoid membranes to native PS I because of lower PS II content in cyanobacteria (Fig. 1(A)-(a), (b)). When antenna Chl *a* was removed from native PS I by harsh detergent treatment, the Chl *a*/Chl *a'* and Chl *a*/PhQ ratios were further decreased with keeping the Chl *a*/PhQ ratio of 0.5, which shows a 1:1 stoichiometry between PS I and Chl *a'* by taking into account the 1:2 stoichiometry between PS I and PhQ. Existence of one Chl *a'* molecule in crystallographic PS I structure of the same cyanobacterium

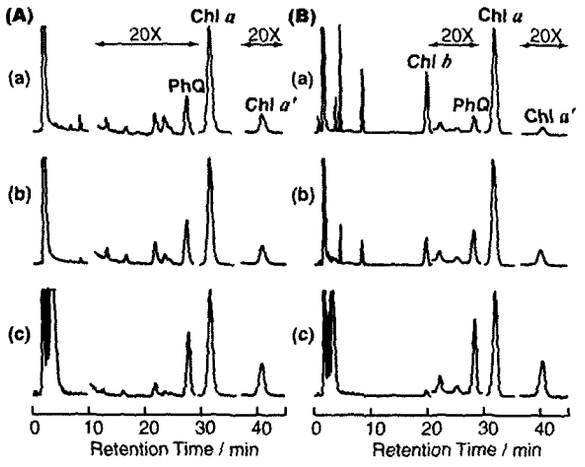

Fig. 1 Reversed-phase HPLC traces of pigments extracted from (a) thylakoid membranes, (b) native PS I and (c) PS I core complexes of (A) *T. elongatus* and (B) spinach. Detection wavelength was 266 nm. The Chl *a* peak areas are arbitrarily scaled to a common intensity.

[3] is further confirmed by HPLC analysis. The Chl *a*/Chl *a'* and Chl *a*/PhQ ratios determined by HPLC were 91 ± 1 and 46 ± 1 (n = 5), respectively, which also agree well with the result of X-ray crystallographic analysis, the Chl *a*/Chl *a'* ratio of 96 and the Chl *a*/PhQ ratio of 48 [3]. In a mesophilic cyanobacterium, *S.* PCC6803, almost the same relation was also found in native PS I and PS I core complexes, though the Chl *a'*/PhQ ratio of thylakoid membranes were 0.3 (Fig. 2).

In spinach (Fig. 1(B)), the Chl *a*/Chl *a'* and the Chl *a*/PhQ ratios largely decreased from thylakoid membranes to PS I core complexes with a decrease of light-harvesting pigments, Chl *b*. The Chl *a'*/PhQ ratio was about 0.5 for all the samples as in *T. elongatus*. The same stoichiometric relation was also found in native PS I and PS I core complexes of a red alga, *P. purpureum* (Fig. 2), though the pigment composition of thylakoid membranes was ambiguous, due to alteration of Chl *a* during disruption of algae (data not shown).

Though PhQ was not detected in green algae, *C. reinhardtii* and *C. vulgaris*, the Chl *a'*/Chl *a* ratio was also decreased from thylakoid membrane to PS I core complexes as in *T. elongatus* and spinach. Modification of HPLC conditions is now under way for detection of quinone molecules in PS I of green algae.

Fig. 2 summarizes the results of HPLC analysis and P700 determination. The PhQ/P700 molar ratios were about 2 in all sample isolated from cyanobacteria, spinach, and PS I of *P. purpureum* as expected. The Chl *a'*/P700 ratio was about 1 for all samples examined here. These results confirm that PS I of photosynthetic organisms examined here contain one Chl *a'* molecule at the core part of PS I on the basis of PS I concentration determined by two entirely different methods, both PhQ and P700 determination.

X-ray crystallographic analysis of PS I of *T. elonagtus* revealed the structure of P700 and the surrounding protein environment at atomic resolution [3]. P700 is a heterodimer of Chl *a* and Chl *a'*. The surrounding protein environment of the Chl *a* and Chl *a'* is also "heterodimeric", that is, Chl *a* buried within hydrophobic aromatic residues, and polar residues such as Thr 743, Ser 607, Tyr603 of PsaA and one water molecule exist in the vicinity of Chl *a'*. These hydrophilic residues and water molecule provide hydrogen bonds to Chl *a'*, and are well conserved within PsaA of diverse organisms. The existence of Chl *a'* in P700 may be required, among others, by stereochemical requirements for properly binding to or for creating such a characteristic environment of PsaA. In this view, the results obtained here and conservation of the surrounding amino acid residues indicates the universal existence of one Chl *a'* molecule within P700 of Chl *a*-based PS I.

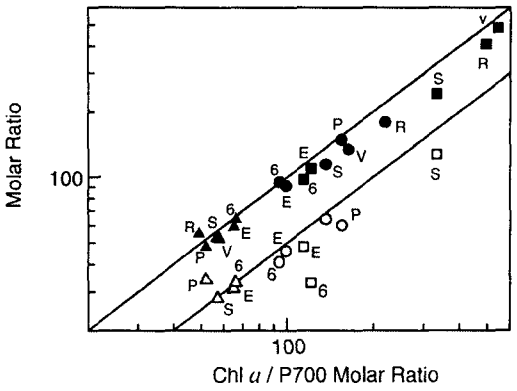

Fig. 2 Relationship between the Chl *a*/P700 molar ratio, and the Chl *a*/ Chl *a'*, and Chl *a*/PhQ ratios. Open symbols: Chl *a*/PhQ ratios, closed symbols: Chl *a*/Chl *a'* ratios. squares: thylakoid membranes, circles: native PS I, triangles: PS I core. Characters beside each marker are abbreviated name of organisms, 6: *S.* PCC6803, E: *T. elongatus*, P: *P. purpureum*, R: *C. reinhardtii*, S: spinach, V: *C. vulgaris*. Each point represents the mean of three to five different samples.

References

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