Separation and Determination of Minor Photosynthetic Pigments by Reversed-phase HPLC with Minimal Alteration of Chlorophylls

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Reversed-phase HPLC conditions for separation of chlorophyll (Chl) *a*, Chl *a'* (the C13²-epimer of Chl *a*), pheophytin (Pheo) *a* (the primary electron acceptor of photosystem (PS) II), and phylloquinone (PhQ) (the secondary electron acceptor of PS I), have been developed. Pigment extraction conditions were optimized in terms of pigment alteration and extraction efficiency. Pigment composition analysis of light-harvesting complex II, which would not contain Chl *a'* nor Pheo *a*, showed the Chl *a'*/Chl *a* ratio of $3-4 \times 10^{-4}$ and the Pheo *a*/Chl *a* ratio of $4-5 \times 10^{-4}$, showing that the conditions developed here were sufficiently inert for Chl analysis. Preliminary analysis of thylakoid membranes with this analytical system gave the PhQ/Chl *a'* ratio of 0.58 ± 0.03 ($n = 4$), in line with the stoichiometry of one molecule of Chl *a'* per PS I.

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Conversion of solar to chemical free energy in higher plant photosynthesis is achieved by cooperation of hundreds of light-harvesting pigments and redox cofactors, which are bound to two large pigment-protein complexes, photosystem (PS) I and II, embedded in thylakoid membranes. In thylakoid membranes, there are 300 to 450 molecules of chlorophyll (Chl) *a* and about 170 molecules of Chl *b* in the minimal functional unit called one photosynthetic unit.¹ Most of Chl *a* molecules and all of Chl *b* molecules are the light-harvesting pigments which funnel excitation energy to the reaction center where photochemical reactions take place.

In PS I and PS II, it has been known that one to two molecules of Chl derivatives are involved in the respective reaction center and participate in the light-induced charge separation.² In PS II, two molecules of pheophytin (Pheo) *a*, the demetallated form of Chl *a*, act as the primary electron acceptor.³ We previously detected one or two molecules of Chl *a'*, the C13²-epimer of Chl *a*, in the core part of PS I.⁴⁻⁶ Though the amount of Chl *a'* within PS I has not been finally determined yet, one possibility for the function of Chl *a'* is a constituent of P700, the primary electron donor of PS I.⁴⁻⁶ Presence of the "prime-type" pigments in the reaction center complexes of heliobacteria⁷ and green sulfur bacterium, *Chlorobium tepidum*,⁸ the evolutionary counterparts of PS I,⁹ suggests that "prime-type" pigments play essential roles in the photochemical reaction. Recently, Chl *d'* was also detected in PS I of a Chl *d*-containing prokaryote, *Acaryochloris marina*.¹⁰

To clarify the *in vivo* function of Chl *a'*, its abundance within thylakoid membranes and PS I should be precisely determined in the first place. For this purpose, HPLC analysis of Chl *a'* in pigment extract solutions is currently the sole reliable way, because absorption and fluorescence spectra of Chl *a* and Chl *a'* are mutually almost indistinguishable, but their

chromatographic behaviors are different.¹¹ Comparing the Chl *a'*/Chl *a* ratio obtained by HPLC with the amounts of cofactors in PS I, such as the primary electron donor, P700 (one unit per PS I),¹² and the secondary electron acceptor, phylloquinone (PhQ) (two molecules per PS I),¹² would enable precise determination of Chl *a'* within PS I. To achieve this, *in vitro* alteration of Chls must be suppressed to a minimum during pigment extraction and HPLC. After extraction from protein matrices, Chl *a* is very labile in organic solvents. Epimerization, demetallation and oxidation (allomerization) of Chl *a*¹³ would readily proceed, and render the analytical data of Chl *a'* and Pheo *a* ambiguous. In practice, considerable amounts of Chl *a* allomers (sometimes incorrectly assigned to Chl *a'*) are frequently found in the HPLC traces. Conditions for HPLC and pigment extraction, which keep Chls intact during experiments, are hence highly desirable for the determination of Chl *a'* and Pheo *a*. In previous studies on Chl *a'* determination, pigments were extracted with acetone in the presence of an excess amount of a desiccant, Na₂HPO₄, to suppress pigment alterations by water-soluble acidic/basic compounds in the samples.⁶ However, it is possible that weakly basic Na₂HPO₄ itself base-catalyzes the epimerization of Chl *a*. Choice of acetone for the extraction solvent also seems to be inadequate for quantitative extraction of thylakoid membrane pigments, because acetone is known to show lower extractability of Chls from protein matrices.¹⁴ Whether the previous results obtained by the acetone extraction with Na₂HPO₄ (Ref. 6) reflect the *in vivo* pigment compositions must be re-examined under strict control of pigment alterations and extraction efficiency of Chls.

In our previous works, normal-phase HPLC was used for analyses of Chl *a'*.^{5,6} Though normal-phase HPLC showed high resolution for epimeric mixtures of Chl *a* and Pheo *a*,¹¹ stoichiometric comparison of Chl *a'* with PhQ required two HPLC runs under different conditions.¹⁵ Establishment of HPLC conditions which can separate PhQ, Chl *a'*, and Chl *a* is highly desirable for precise determination of Chl *a'* within PS I,

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because direct comparison of the amount of Chl *a'* with that of PS I is possible on a single HPLC trace. However, no such HPLC conditions were reported so far.

In this work, we developed reversed-phase HPLC conditions which can resolve Chl *a'*, Pheo *a*, PhQ and Chl *a* within appropriate time. Pigment extraction efficiencies were examined under these HPLC conditions. The chlorophyllous pigment alterations were kept minimal in the present analytical system, and the Chl *a'*/PhQ ratios in thylakoid membranes correspond to a 1:1 stoichiometry of Chl *a'* and PS I.

Experimental

Preparation of thylakoid membranes and light-harvesting complex II

Thylakoid membranes and light-harvesting complex (LHC) II were prepared from spinach. Thylakoid membranes were prepared as described previously.⁴ LHC II was extracted from PS II membranes¹⁶ by treatment with dodecyl- β -D-maltoside (Dojin Chemical) at a detergent/Chl ratio (w/w) of 10 in water for 15 min on ice. Insolubilized materials were removed by centrifugation at 100000g for 15 min, then the supernatant was subjected to sucrose gradient centrifugation in 0.2 to 0.6 M sucrose gradients containing 0.02% (w/v) Triton X-100 at 140000g for 10 h. The upper red fluorescent band of the sucrose gradient was recovered, and then loaded onto an anion exchange HPLC (POROS HQ/L, 7.5 mm ϕ $\times$ 100 mm, Perceptive Biosystems). The column temperature was maintained at 10°C. The column was washed for 10 min with 0.025% Triton X-100, 5 mM MgSO₄, 50 mM Tricine-NaOH (pH = 7.8) at a flow rate of 5 mL/min. LHC II was eluted by raising the MgSO₄ concentration to 25 mM. LHC II was precipitated from the eluent by addition of 0.1 M KCl and centrifugation at 10000g for 15 min. Sample preparations were conducted at 4°C under dim green light, unless otherwise noted.

Pigment extraction

Ten microliters of thylakoid membranes or LHC II suspension was added to 1 mL of acetone/methanol = 7/3 (v/v) stored at -20°C, then the mixture was sonicated in an ice-water bath for 30 s. The pigment solution was filtered through a 0.45 μ m PTFE filter, Millex FH₁₃, Millipore. The solvents were removed in a rotary evaporator. The dried pigments were dissolved in acetone or the HPLC eluent, and then injected onto HPLC with an injector, Rheodine Model 7125, equipped with a 20 μ L sample loop. To reduce pipetting error during sample injection, in particular for construction of calibration curves, excess volume of the solution (60 to 100 μ L) was injected. Pigment extraction was done under dim green light. The samples and pigment extracts were on ice during the experiments.

HPLC conditions

A polyetheretherketone (PEEK) column (4.6 mm ϕ $\times$ 150 mm) packed with Nucleosil ODS100 was employed (ODS 1151N-PEEK, Senshu Science). Pigments were eluted by a step gradient method, acetonitrile/methanol/ethanol/water = 85/9/3/3 (v/v) before elution of Chl *a'* and acetonitrile/ethanol = 85/15 (v/v) after elution of Chl *a'* at a flow rate of 1.2 mL/min. The column temperature was maintained at 20°C by circulating thermostated water around the column. The eluents were degassed by an on-line degasser, JASCO DG 980-50.

The elution profile was monitored by two UV-Vis detectors, Shimadzu SPD 10-AV, connected in series or a fluorescence

detector, JASCO FP-920. The signals from the detectors were acquired on a Macintosh computer via an interface, Mac Integrator I (EYELA) and processed with the packed software. Differences in the extinction coefficients of each pigment at the detection wavelength were calibrated by constructing calibration curves for the standard samples of each pigment. Standard samples of Chl *a*, Chl *b* and Pheo *a* were prepared as described previously.^{11,21} A standard sample of PhQ was purchased from Sigma and used without further purification. The purity of the standard samples determined by reversed-phase HPLC was as follows: Chl *a*, 98.6% (impurities were Chl *a* allomers and Chl *a'*); Chl *b*, 98.7% (impurities were Chl *b* allomers and Chl *b'*); Pheo *a*, 99.3% (Pheo *a'* was the sole impurity); PhQ, 95.7%. Extinction coefficients in acetone as described in Ref. 11 were employed for Chl *a*, Pheo *a* and Chl *b*. An extinction coefficient, 18.9 mM⁻¹ cm⁻¹ in ethanol at 248 nm¹⁷ was used for PhQ. The *r*² value after least-squares fitting is above 0.999 for all the calibration curves (data not shown). The Chl *a'*/Chl *a* ratio was deduced from direct comparison of peak areas, because the extinction coefficients of Chl *a'* and Chl *a* are the same.¹¹

The absorption spectrum of each peak was obtained with a photodiode array detector, Shimadzu SPD M10Avp.

Reagents

All the organic solvents used here were of HPLC grade

Fig. 1 (a) HPLC trace of thylakoid membrane extract detected at 266 nm. (b) Absorption spectra of the peaks in the HPLC trace obtained by photodiode array detection. See text for peak assignments.

(Wako). Buffers and salts were of reagent grade (Wako). Water has been purified with a Milli-Q-SP-TOC system (Millipore).

Results and Discussion

Separation and identification of photosynthetic pigments

Methanol has been frequently used for eluent of reversed-phase HPLC analyses.¹⁸⁻²⁰ Since Chl *a* is known to undergo several alterations in methanol,¹³ we sought for an eluent containing a minimal amount of methanol. After examination of several mixtures of acetonitrile, alcohols and water, the eluent described in Experimental gave satisfactory resolution for PhQ, Chl *a* and Chl *a'*. Figure 1 shows an HPLC trace of thylakoid membrane pigments detected at 266 nm, and absorption spectra of the peaks. Peaks 1, 2 and 3 showed xanthophyll-like absorption spectra. Detailed assignment of these peaks is out of the scope of this paper. Absorption spectra and retention times of peaks 4, 6, 8, 9 agreed well with the standard samples of Chl *b*, Chl *a*, β -carotene, Pheo *a*. The retention time and absorption spectrum of peak 5 were matched

well with the standard sample of PhQ. Its absorption spectrum indicates no contamination from Chls, carotenoids and other quinones. Peak 7 was assigned to Chl *a'* from the absorption spectrum and retention time comparison with the standard sample.²¹

In our previous work, reversed-phase HPLC analysis of Chl *a'* purified by normal-phase HPLC, containing less than 0.1% of Chl *a* as the sole impurity, showed 3.9% of Chl *a'* allomers. In contrast, the same sample on the normal-phase medium showed practically no allomers.²¹ This indicates that Chl *a* was oxidized during reversed-phase HPLC, probably due to the polar nature of the eluent. Under the HPLC conditions developed here, Chl *a* purified by normal-phase HPLC showed less than 0.1% of Chl *a'* and Chl *a* allomers; this confirms the inertness of the HPLC conditions to Chl *a* (Fig. 2). It was noted that the use of a conventional stainless steel column induced considerable allomerization of Chl *a* (data not shown). PEEK columns and a titanium column did not show such allomerization.

Alteration of Chl *a* during pigment extraction with desiccants

In our previous analyses, pigments were extracted from fresh leaves or thylakoid membranes with acetone after grinding with a weakly basic desiccant, Na₂HPO₄, to reduce alteration of Chls by water soluble acidic and basic compounds in the samples.^{5,6} Though weakly basic compound might cause epimerization of Chl *a*, the effect of desiccants on the Chl *a* alteration was not studied in detail. The effect of desiccants on the pigment composition of thylakoid membranes was examined with the present reversed-phase HPLC. The Chl *a'*/Chl *a* ratio for thylakoid membranes by acetone extraction without desiccant was $0.28 \pm 0.02\%$ ($n = 3$) (Fig. 3(a), left). The Chl *a'*/Chl *a* ratio increased to $0.43 \pm 0.03\%$ ($n = 3$) by co-extraction with anhydrous Na₂HPO₄ (Fig. 3(b), left). The Chl *a'*/Chl *a* ratio in the presence of Na₂HPO₄ agrees with the previous result, 0.47%.⁶ When Na₂HPO₄ was replaced with a weakly acidic desiccant, anhydrous Na₂SO₄, the Chl *a'*/Chl *a* ratio of acetone extract was reduced to $0.33 \pm 0.03\%$ ($n = 3$), a level similar to

Fig. 2 Analysis of highly purified Chl *a* by reversed-phase HPLC. Chl *a* was purified by preparative normal-phase HPLC.²¹ Detection was done with a fluorescence detector ($\lambda_{\text{ex}} = 425$ nm, $\lambda_{\text{em}} = 660$ nm).

Fig. 3 HPLC traces of thylakoid membrane pigments extracted with acetone in the presence of desiccants. A 20- μ L aliquot of thylakoid membrane suspension was ground with 20 g of desiccants for 1 min, and then pigments were extracted with 20 mL of acetone.⁶ The solvent was removed in a rotary evaporator prior to injection onto HPLC. (a) extracted with acetone without desiccant, (b) extracted with acetone and Na₂HPO₄, (c) extracted with acetone and Na₂SO₄. Detection was done at 266 nm (left) and at 469 nm (right). Peak 1, Chl *b*; peak 2, PhQ; peak 3, Chl *a*; peak 4, Chl *a'*; peak 5, Pheo *a*; peak 6, Chl *b'*. A small peak before peak 6 exhibited a carotenoid-like absorption spectrum (data not shown). The peak areas of Chl *a* (left) and Chl *b* (right) are arbitrarily scaled to a common intensity.

Fig. 4 HPLC traces of thylakoid membranes extracted with acetone or methanol. (a) acetone extract, (b) methanol extract. No scaling was done between traces (a) and (b). Detection was done at 266 nm (left) and at 469 nm (right). Peak assignments are the same as in Fig. 3.

Fig. 5 Relative amounts of Chl *a*, Chl *a'*, PhQ extracted with an acetone/methanol mixture with varying mixing ratio, deduced from HPLC analyses. For each pigment, the amount is normalized by the amount extracted by methanol. Each point represents a mean of two different samples. ●: Chl *a'*; ○: Chl *a*; ◇: PhQ.

that by extraction without desiccant (Fig. 3(c), left). HPLC traces detected at 469 nm, which primarily detects Chl *b* and carotenoids, enhanced the difference between the three extraction conditions (Figs. 3(a) – (c), right). The Chl *b'*/Chl *b* ratio was increased by extraction with Na_2HPO_4 . The Chl *b'*/Chl *b* ratio of extraction with Na_2SO_4 was slightly larger than that by extraction without desiccant. The pronounced epimerization of Chl *b* seen in Fig. 3(b) is due to 7-fold higher epimerization rate of Chl *b* as compared to Chl *a*.²² These results show that Na_2HPO_4 induces epimerization of Chl *a* and Chl *b* during acetone extraction. A small amount of water in acetone might promote base-catalyzed epimerization in the presence of weakly basic desiccant. The Pheo *a*/Chl *a* ratios by acetone extraction with Na_2HPO_4 and Na_2SO_4 were also higher than that without desiccants. No significant difference of the Pheo *a*/Chl *a* ratio was observed between the extractions with Na_2HPO_4 and Na_2SO_4 . The increase in the Pheo *a*/Chl *a* ratio by extraction with Na_2HPO_4 and Na_2SO_4 might occur during grinding the samples. We conclude that the results of acetone extraction with Na_2HPO_4 (Ref. 6) did not reflect the actual pigment composition of thylakoid membranes, and was contaminated by the epimerization of Chl *a* by Na_2HPO_4 .

Effect of extraction solvent on pigment composition of thylakoid membranes

For quantitative extraction of thylakoid membrane pigments, mixtures of acetone/methanol have been frequently employed.^{4,20,23} It was reported that an acetone/methanol = 8/2 mixture could liberate 95–98% of Chl *a* and *b*.²⁰ To avoid potential alteration of Chls, methanol in the extraction solvent should be kept to a minimum. Effect of the acetone/methanol mixing ratio on the pigment composition of thylakoid membranes was examined by the reversed-phase HPLC. HPLC traces of thylakoid membrane pigments extracted with acetone and methanol are shown in Fig. 4. No apparent differences were observed for Chl *b* and PhQ between the two traces. The peak area of Chl *a* slightly increased, and the peak area of Chl *a'* significantly increased, in the methanol extract. The relative amounts of Chl *a'*, Chl *a* and PhQ extracted with acetone/methanol mixtures with varying mixing ratios are summarized in Fig. 5. The amount of each pigment extracted with methanol is taken as a standard. The relative amount of Chl *a'* was significantly affected by the composition of extraction solvent. Acetone extraction liberated only 40% of Chl *a'*. The relative amount of Chl *a'* increased by addition of methanol to extraction solvent, and reached a constant level above 30% of methanol.

Acetone extracted only 85% of Chl *a*, in line with the report by Sane and Park that *ca.* 10% of Chl *a* remained in thylakoid membranes after acetone extraction.²⁴ The amount of Chl *a* also increased with an increase in methanol as in the case of Chl *a'*, but the trend was less conspicuous.

The amount of PhQ was almost independent of the acetone/methanol mixing ratio. The constant value of the relative amount of PhQ matched well with the fact that PhQ can be completely extracted by relatively non-polar solvents, such as hexane containing 0.3% methanol²⁵ or diethyl ether.²⁶

The amount of Pheo *a* was also affected by the acetone/methanol mixing ratio in a manner similar to that in Chl *a'* (data not shown). Based on the results in Fig. 5, we chose acetone/methanol = 7/3 as extraction solvent.

A reason for the significant difference in the amount of Chl *a'* obtained in acetone extractions and that obtained in methanol extractions may be the epimerization of Chl *a* in methanol-rich solvent during extraction, because the epimerization rate of Chl *a* in methanol is about 10-fold faster than in acetone (Mazaki and Watanabe, unpublished result). However, this is not the case for the present experiment. HPLC traces detected at 469 nm showed no difference in the Chl *b'*/Chl *b* ratio between

Fig. 6 HPLC traces of LHC II (right) and thylakoid membranes (left) detected at (a) 266 nm, (b) 660 nm and (c) 469 nm. Pigments were extracted by acetone/methanol = 7/3. On traces (a) and (b), the peak areas of Chl *a* are arbitrarily scaled to a common intensity. In traces (c), the peak areas of Chl *b* are arbitrarily scaled to a common intensity. Peak assignments are the same as in Fig. 3.

Table 1 Pigment compositions determined by reversed-phase HPLC analyses

	Chl <i>a</i> '/Chl <i>a</i> , %	PhQ/Chl <i>a</i> , %	Pheo <i>a</i> /Chl <i>a</i> , %	Chl <i>a</i> '/PhQ	Chl <i>a</i> /Chl <i>b</i>
Thylakoid membranes	0.45 ± 0.01	0.78 ± 0.03	0.98 ± 0.03	0.58 ± 0.03	2.66 ± 0.06
LHC II	0.03 – 0.04	trace	0.04 – 0.05	n.d.	1.23 ± 0.01

All values are mean ± S.D. of four different samples. The PhQ/Chl *a* ratios were deduced from HPLC traces detected at 266 nm, while other ratios were from HPLC traces detected at 660 nm.

acetone and methanol extractions. In contrast to Fig. 3, the increase in Chl *a*' (Fig. 4, left) did not accompany an increase in Chl *b*' (Fig. 4, right), and this rules out the Chl *a* epimerization during extraction as a possible cause. Though the peak area of Chl *b*' changed in different preparations (*cf.* Fig. 6), the Chl *a*' peak area was almost constant. The small amount of Chl *b*' detected in Fig. 4 might have been produced during sample preparation. The different extractability of Chl *a*' and Chl *a* between acetone and methanol might arise from a difference in their local environments in the protein matrices. In this context, we note that acetone treatment of thylakoid membranes can enrich the PS I reaction center, P700, up to the Chl *a*/P700 ratio of *ca.* 16.²⁴ The lower extractability of Chl *a*' with acetone might indicate the association of Chl *a*' with the very core part of PS I.

Pigment composition analysis of thylakoid membranes and light-harvesting complex II

Figure 2 clearly demonstrates the inertness of the reversed-phase HPLC developed here for isolated Chl *a* molecules. Determining if this is also the case for biological materials is of much importance. LHC II of higher plants, containing only Chl *a* and Chl *b* as chlorophyllous pigments and probably free from Chl *a*' and Pheo *a*, thus would be useful to evaluate the inertness of the analytical conditions. Figure 6 shows HPLC traces of an LHC II extract detected at 266, 469 and 660 nm. The insignificant level of PhQ on the HPLC trace of 266 nm detection confirms the absence of PS I in the LHC II preparation (Fig. 6(b), right). β -Carotene, a carotenoid located in the reaction center complexes and minor LHCs,²⁷ was not

found in the HPLC trace of 469 nm detection (Fig. 6(c), right). The absence of PS I and PS II proteins was also confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (data not shown). The Chl *a*/Chl *b* ratio was 1.23, in line with a previous result, 1.2.²⁸ The Chl *a*'/Chl *a* ratio and the Pheo *a*/Chl *a* ratios of LHC II were very low as expected (Table 1). A very low amount of Chl *b*' was observed in the HPLC trace of 469 nm detection (Fig. 6(c), right). The very low levels of Chl *a*', Pheo *a* and Chl *b*' well indicate that the analytical conditions developed here are sufficiently inert for Chls to determine precisely Chl *a*' and Pheo *a* within thylakoid membranes. Parts of these small amounts of Chl *a*' and Pheo *a* in LHC II might have been formed during purification of LHC II, detergent extraction, overnight sucrose gradient centrifugation and anion exchange HPLC. In fact, LHC II sometimes showed extensive allomerization of Chl *a* which did not take place with thylakoid membranes (data not shown).

The analytical conditions developed here were applied to thylakoid membranes (Fig. 6, left and Table 1). The PhQ/Chl *a* ratio was 0.76%, in line with a previous result (0.62 to 0.74%).²⁹ The Pheo *a*/Chl *a* ratio was 0.98%. Since about 190 molecules of Chl *a* are present per PS II of spinach thylakoid membranes, as reported from the determination of Q_A, the secondary electron acceptor of PS II,³⁰ the Pheo *a*/Chl *a* ratio agrees with the well-known stoichiometry of 2 Pheo *a*/PS II.³ The Chl *a*'/Chl *a* ratio of thylakoid membranes was 0.45%. The Chl *a*'/Chl *a* and Pheo *a*/Chl *a* ratios obtained by acetone/methanol extraction were similar to those obtained by acetone extraction with Na₂HPO₄.⁶ However, the agreement between the two methods must be accidental, because a significant amount of

Chl *b'* was detected by the acetone extraction with Na₂HPO₄ (Fig. 3(b), right), whereas only a tiny amount of Chl *b'* was found in acetone/methanol extraction (Fig. 6(c), left). Analyses of different preparations would give different values between the two methods.

The reversed-phase HPLC conditions developed here can compare the amounts of Chl *a'* and PhQ on the same HPLC trace. Using these conditions, we obtained a Chl *a'*/PhQ ratio of 0.58 in thylakoid membranes. When PhQ, two molecules of which exist per PS I, is taken as an index for the amount of PS I, the Chl *a'*/PhQ ratio of 0.58 supports the stoichiometry of one molecule of Chl *a'* per PS I. However, comparisons of the Chl *a'*/Chl *a* ratio with the P700/Chl *a* ratios determined previously by several workers, 0.26%³⁰ to 0.34%,³¹ lead to the stoichiometry of 1.32 to 1.74 molecules of Chl *a'* per PS I. To reach a final conclusion as to the Chl *a'*/PS I stoichiometry, we need to conduct detailed pigment composition analysis of intact PS I preparations and to compare the results of spectrophotometric determination of P700. Such experiments are currently under way. The analytical conditions developed here would prove useful for pigment composition analyses of oxygen-evolving photosynthetic apparatus, in particular for PS I.

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