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Disintegration and reconstitution of Photosystem II reaction center core complex. II *. Possible involvement of low-molecular-mass proteins in the functioning of Q_A in the PS II reaction center

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Spinach Photosystem II (PS II) reaction center complex (RC) was disintegrated in three different ways using 0.5% *n*-dodecyl β-D-maltoside (DM) (DM particle), 20 mM *n*-octyl β-D-thioglucopyranoside (OTG) (OTG particle) or 4% Triton X-100 (Triton particle and NS particle), respectively, followed by chromatography on ion-exchange columns. The average numbers of plastoquinone A-9 (PQA) molecule retained in the DM, OTG and Triton particles were 0.88, 0.63 and 0.68 per reaction center, respectively, on the basis of two pheophytin *a* (Pheo *a*) molecules and the corresponding Q_A activities were about 80, 15 and 45% of the original RC level. A difference in the protein pattern on SDS-PAGE between the Triton and OTG particles was observed in the low-molecular-mass region, i.e., bands of the *psbL* gene product (L protein) and 4.1 kDa protein detected in the former were almost lost in the latter, while the bands for the 6.1 kDa and three small proteins appeared in the latter but not in the former. By the treatment of the RC with 20 mM OTG at 10°C for 1 h, several protein components, including the proteins in the low-molecular-mass region, were extracted. Reconstitution of the OTG particle with the crude mixture of the low-molecular-mass proteins contained in the OTG extract resulted in successful recovery of the Q_A activity in the resulting particle at about 50% of the original RC level. From the results of the reconstitution experiments and comparison of the protein compositions among the particles, we propose that, besides the D1 and D2 proteins, two subunits of cytochrome *b*-559, 47 and 43 kDa chlorophyll binding proteins and the recently discovered 4.8 kDa protein, some other small proteins including the 5.0 kDa L protein are possibly involved in normal functioning of Q_A in the PS II reaction center.

Introduction

A minimal preparation of PS II initially isolated from higher plants by Nanba and Satoh [1] as D1/D2/cytochrome *b*-559 complex was recently found by Ikeuchi et al. [2] and by Webber et al. [3] to contain another small polypeptide with molecular mass of 4.8 kDa. This complex (NS particle) contains 4–5 Chl *a*, 2 Pheo *a* [4–6] and 2 β-Car molecules [7,8] and has photochemical activity to exhibit a reversible absorbance change attributed to the reduced Pheo *a* [4–6,8]. Although there is striking similarity in the pigment

* Paper I is Ref. 14.

Abbreviations: PS II, Photosystem II; RC, reaction center complex of Photosystem II; LHCP, light harvesting chlorophyll *a*/*b* proteins; P680, primary donor chlorophyll *a* of Photosystem II; Z, primary electron donor to P680; Q_A, primary quinone acceptor of Photosystem II; Q_B, secondary quinone acceptor of Photosystem II; Chl, chlorophyll; Pheo *a*, pheophytin *a*; β-Car, β-carotene; PQA, plastoquinone A-9; DCPIP, 2,6-dichloro-phenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; OGP, *n*-octyl β-D-glucopyranoside; OTG, *n*-octyl β-D-thioglucopyranoside; DM, *n*-dodecyl β-D-maltoside; Mops, 3-(*N*-morpholino)propanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Bistris, bis (2-hydroxyethyl)iminotris(hydroxymethyl)methane; Tricine, tris(hydroxymethyl)methylglycine; HPLC, high-performance liquid chromatography; SDS-PAGE, SDS polyacrylamide gel electrophoresis; FeCN, potassium ferricyanide; ORF, open reading frame.

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compositions between the NS particle and the reaction center complex isolated from purple bacteria, the quinone molecule which is present in the latter [9] is not found at all in the former; as a matter of course, Q_A activity is not retained [4–8].

Recently, reconstitution of a reaction center complex substantially identical to the NS particle with exogenous quinone was reported by two groups. Upon addition of decylplastoquinone to the dispersion of the complex in the presence of DM, Chapman et al. observed photoreduction of cytochrome b-559 in the complex [10]. Mathis et al. reported that addition of 2,5-dibromo-3-methyl-6-isopropylbenzoquinone to the NS particle preparation induced quenching of the photoinduced primary radical pair ($P680^+$ Pheo a^-), stabilization of $P680^+$ and a rapid reduction of cytochrome b-559 in the NS particle [11,12]. Gounaris et al. also observed photoreduction of cytochrome b-559 upon addition of the naturally occurring quinone, PQA, in the complex in the presence of the detergent Triton X-100 [13]. In addition, reconstitution of PQA resulted in the ability of the complex to catalyse the photoreduction of DCPIP in the presence of the exogenous electron donor [13]. However, in order to recover quinone-mediated electron transport in the complex, very large amounts of the quinones must be added. Even then, the quantum yield for the reaction was extremely low, while Satoh et al. were unable to detect any effect of PQA on the photochemical properties of the NS particle [12].

In part I of this series, we reported isolation of a complex containing 4 Chl *a*, 2 Pheo *a*, 1 β-Car and 1 PQA molecules by means of density gradient centrifugation and examination of its photochemistry by means of a transient absorption spectroscopy [14]. Contrary to our expectations, even in this preparation only the signal attributing to the formation of the triplet state P680 as a result of recombination of photoinduced $P680^+$ and Pheo a^- was observed, instead of the signal arising from the reduction of Q_A.

These results seem to us to suggest that, besides the components comprising the NS particle and 47 kDa and 43 kDa chlorophyll-binding proteins, a certain unknown component is involved in the PS II as an essential factor for activating the quinone at the Q_A site.

In this paper, we report the evidence that, besides the 4.8 kDa protein and the α- and β-subunits of cytochrome b-559, at least one more low-molecular-mass protein possibly participates in the normal functioning of Q_A and is removed from the RC by an OTG treatment concomitant with the loss of the Q_A activity and then functionally reconstituted by dialysis.

Materials and Methods

The O_2-evolving PS II particle was prepared from fresh spinach leaves by the method of Kuwabara and Murata [15] and suspended in buffer solution A (0.2 M sucrose, 20 mM NaCl and 20 mM Mops (pH 7.0)). When stored in liquid N_2, 70% by volume of glycerol was added to the suspension.

Disintegration of the PS II particle

Disintegration of the PS II particle was carried out by three different methods. (1) The LHCP-depleted PS II particle (RC) prepared by Ghanotakis et al. [16] was washed by 0.8 M Tris-HCl to remove the three lumen surface proteins. Complete removal of the remaining LHCP and the 28, 20 and 11 kDa proteins from the resulting particle was performed as described in Ref. 17, but the final step in the procedure, repeating the column chromatography, was eliminated. Briefly, the Tris-washed RC was resuspended in solution B (20 mM NaCl, 10 mM $MgCl_2$, 1.5% taurine and 20 mM Bistris (pH 6.5)) additionally containing 0.5% DM at Chl concentration of 0.5 mg/ml and passed through an S-Sepharose column to remove the 20 and 11 kDa proteins. The flow through from the column was successively loaded onto a Q-Sepharose column and the 28 kDa protein and a trace of LHCP were washed out with solution B containing 0.03% DM and 30 mM $MgSO_4$ in the dark. Finally, the residue on the column was eluted by changing $MgSO_4$ concentration to 100 mM and collected as the DM particle. (2) The RC was solubilized in a solution of 20 mM OTG and 50 mM Tris-HCl (pH 7.5) at 0.2 mg Chl/ml. After incubation for 1 h at 10°C, under stirring, an equal volume of a solution of 35 mM OGP and 50 mM Tris-HCl (pH 7.5) was added and centrifuged at $30000 \times g$ for 5 min at 4°C. The supernatant was loaded on a DEAE-Toyopearl 650-S column equilibrated with solution C (0.05% DM and 50 mM Tris-HCl (pH 7.5)). The flow-through was collected as the OTG extract to be used for reconstitution experiments and then the column was extensively washed by recycling the equilibration solution for 3 h at 2°C in the dark and subsequently eluted with the same solution under NaCl gradient. The elution pattern showed a single peak accompanied by a shoulder and the fraction around the peak was collected as the OTG particle. (3) According to the method of Nanba and Satoh [1] with a slight modification, the PS II particle was treated with a solution of 4% (w/v) Triton X-100 and 50 mM Tris-HCl (pH 7.2) at 1 mg Chl/ml for 1 h on ice with stirring. It was then centrifuged at $100000 \times g$ for 1 h at 4°C and the supernatant solution was subjected to a DEAE-Toyopearl 650–S column preequilibrated with a solution of 0.2% Triton X-100, 5 mM NaCl and 50 mM Tris-HCl (pH 7.2) and the column was washed with the equilibration solution for 18 h at 4°C in the dark. After changing the eluent to solution C, the column was subjected to gradient elution by NaCl (5–200 mM) in solution C. The elution profiles showed two distinct peaks at NaCl concentrations of 130 mM and 170 mM,

respectively, and both of the peak fractions were collected separately. The first peak fraction contained the NS particle, while a particle consisting of many more protein components was obtained from the second peak fraction and named as the Triton particle.

Analysis of protein and pigment compositions of the five particles

The protein compositions in each particle were examined by two different SDS-PAGE systems. To cover the wide molecular mass region (3 to 50 kDa), an SDS-PAGE system 1 (16–22% polyacrylamide gel with 7.5 M urea) was used according to Ikeuchi et al. [18]. To obtain high resolution for the protein components in low-molecular-mass region, SDS-PAGE system 2 (the Tricine-discontinuous polyacrylamide gel system described in Refs. 19, 20) was used in the presence of 6 M urea.

The extraction and analysis of Chl *a*, Pheo *a* and PQA in the particles were performed as described in Refs. 14, 21. Analysis of cytochrome *b*-559 was done by measuring redox difference spectra of cytochrome *b*-559 as dithionite-reduced minus FeCN-oxidized spectra in the presence of 0.05% DM or 0.5% Triton X-100 as mentioned in Ref. 22.

Reconstitution of Q_A function on the OTG particle

The OTG extract obtained during the disintegration of the RC with OTG was dialyzed against a solution of 5 mM Tris-HCl (pH 7.5) for 3 h at 4°C and loaded on a DEAE-Sephacel column (Pharmacia) preequilibrated with solution D (17.5 mM OGP, 10 mM OTG, 0.05% DM and 5 mM Tris-HCl (pH 7.5)). The column was eluted with solution D containing 10 mM NaCl at first and then with that containing 120 mM NaCl and the elutions were collected as OTG extract-1 and OTG extract-2, respectively. The protein compositions of the OTG extracts were also examined by the SDS-PAGE systems 1 and 2.

In addition to the OTG extracts, thylakoid total lipids and PQA were isolated to be submitted to the reconstitution experiments. The former was isolated from the broken thylakoids as described in Ref. 23 and the latter was extracted from the PS II particles and purified by an HPLC with a reverse-phase column (Waters, μ-Bondapak C18) as in Ref. 14. A mixed solvent (methanol/ethanol, 65 : 35, v/v) was used as the eluent.

Functional reconstitution of Q_A on the OTG particle of which Q_A activity had been depressed at 16% of the original RC level was tried with the total thylakoid lipids, PQA and the OTG extracts. The OTG particle dispersed in solution D was divided into several aliquots and to each was added one of the test samples. After adjusting the final concentrations of the reaction center, OGP and OTG to 5 μM, 17.5 mM and 10 mM, respectively, the mixture was incubated for 30 min in ice and dialyzed against a 25 mM phosphate buffer (pH 7.5) for 16 h in ice, changing the buffer solution more than twice. At the end of dialysis the solution was diluted 5-fold with the same buffer and finally centrifuged at 280000 $\times$ *g* for 80 min at 4°C. The resulting pellet was resuspended in solution C and submitted to the flash photolysis experiment.

Assay of Q_A activity

Q_A activity was determined by measuring the transient absorbance change at every 5 nm between 260 nm and 350 nm upon single flash illumination. A single-beam flash spectrophotometer basically same as that described in part I of this series [14] was used. The measuring light was provided by a xenon lamp of 150 W instead of a halogen lamp through a suitable interference filter. The measurements were carried out in a cuvette with 5 mm light pass-length. Before the flash experiment each sample solution was kept in darkness in ice. After 5 min of additional dark adaptation in the cuvette at 25°C, the sample was illuminated by a train of laser flashes with 2.5 s intervals and the absorbance change at a given wavelength was recorded for each flash and accumulated.

Results and Discussion

Polypeptide and pigment compositions of the five particles.

Fig. 1 shows the separation profiles of the proteins in the SDS-PAGE system 1 (a) and system 2 (b) with the five particles. In the both systems, an equal amount of the samples on the basis of cytochrome *b*-559 was loaded on each lane. In the case of RC, above the band of α-subunit of cytochrome *b*-559, ten bands corresponding to 47 kDa, 43 kDa, D1 and D2 proteins, three lumen-suface proteins of 17, 23 and 33 kDa, 28 kDa antenna protein, 20 and 11 kDa proteins and a trace of LHCP appeared in the system 1 SDS-PAGE and below the band of the α-subunit of cytochrome *b*-559 at least ten bands appeared in the system 2 SDS-PAGE. Among the latter, five bands were assigned as shown in Fig. 1b by comparing them with the corresponding bands in the SDS-PAGE system 1 for the same RC preparation on the basis of the results reported by Ikeuchi et al. [2,18]. Since resolution of the SDS-PAGE system 2 for the proteins around 5.0 kDa was significantly improved, two proteins with molecular mass of 5.0 kDa were separated into two distinct bands (compare lane 1 in system 1 and 2). In order to assign these two bands, the corresponding proteins were partially purified and subjected to an amino acid sequencer (model 477A, Applied Biosystems). The N-terminal amino acid sequence of the lower band agreed with that of the L protein deduced from the tobacco *psbL* nucleotide sequence [24] at the positions between 7 and 18 except for 14th

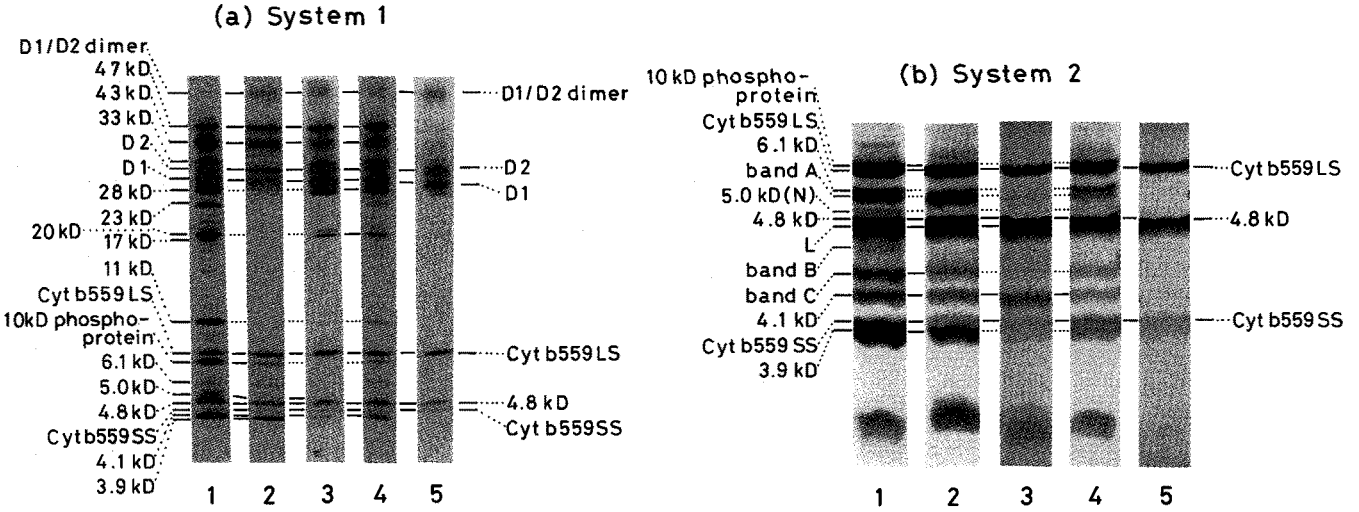

Fig. 1. SDS-PAGE profiles of the five different particles. Gels were stained with silver. Lane 1: RC, lane 2: DM particle, lane 3: Triton particle, lane 4: OTG particle, lane 5: NS particle. (a) Separation patterns in the SDS-PAGE system 1. Compositions of the gels, the buffer systems and the solution dissolving samples were the same as those used by Ikeuchi et al. [18] except for modifying the linear gradient of methylene bisacrylamide and pH in the separating gel to be 0.21–0.29% (w/v) and 8.5, respectively. Electrophoresis was carried out at 25°C with a constant current of 10 mA/cm^2. Assignment of each band was done by referring Fig. 1 in Ref. 18. (b) Separation patterns of the proteins smaller than 10 kDa in the SDS-PAGE system 2. The discontinuous buffer system in the gels, the electrode buffers and the solution dissolving the samples were the same as those described in Ref. 19. The gel was composed of three layers and their compositions were as follows: separating gel, 18.8% acrylamide, 1.2% methylene bisacrylamide and 6 M urea; spacer gel, 13.6% acrylamide and 0.4% methylene bisacrylamide; stacking gel, 3.9% acrylamide and 0.1% methylene bisacrylamide. Electrophoresis was performed at 25°C. Every run started at 30 V. After about 2 h, when the sample had completely entered the spacer gel, the applied voltage was changed to about 130 V. Assignment of each band was performed as mentioned in the text. The proteins corresponding to the bands A, B and C have not yet been identified. Cyt b559 LS and Cyt b559 SS represent α- and β-subunits of cytochrome *b*-559, respectively.

and 17th amino acids. Six amino acids starting from the N-terminal could not be determined due to interference from endogenous pigments slightly contaminating the sample. Determination of the N-terminal amino acid sequence of the upper band was not successful because of the poor purification of the sample. So the assignment of the upper band as the nuclear coding 5.0 kDa protein reported in Ref. 2 is tentative. In addition to the separation of the two 5.0 kDa proteins, three bands which were not observed in the system 1 (lane 1) appeared in system 2 and they were designated as bands A–C in order of the molecular mass on the gel. Protein compositions of the remaining four particles were also elucidated from the SDS-PAGE using two different systems and the results are presented in Table I.

Results of the pigment analysis are summarized in Table II in the form of molar ratio on the basis of 2 Pheo *a*. The molar ratio of cytochrome *b*-559 to Pheo *a* is practically 1:2 in any sample examined. The relative amount of PQA to 2 Pheo *a* is 2.1 in the RC and it decreases to 0.88 in the DM particle. The OTG and Triton particles retain 0.63 and 0.68 PQA per 2 Pheo *a* on average, respectively, and the NS particle has no PQA.

Q_A activities of the five particles

In order to examine the Q_A activity of each particle, we measured transient absorbance change at every 5 nm of wavelength from 260 to 350 nm upon illumination by a train of laser flashes, because the reduced Q_A was found to exhibit a typical semiquinone optical spectrum dominated by absorbance in this region [25]. Fig. 2a shows typical traces of the absorbance change at different wavelengths induced by the illumination in the RC in the presence of 0.2 mM FeCN. At every wavelength examined, a rapid change in the absorbance occurs within 1 μs. In Fig. 2b, the amplitude of this rapid change was plotted against the wavelength and compared with the absorbance difference spectrum for Q_A reduction in the PS II particle reported by Schatz and Gorkom [25]. The compatibility of the plots with the difference spectra of Q_A reduction seen in the figure may suggest that these initial rapid changes are attributed to a photoinduced formation of Q_A^-. As seen in Fig. 2a, the rapid changes in the absorbance are followed by decay consisting of two phases. The fast decay component with a relaxation time of about 150 μs is sufficiently smaller in magnitude than the slow component. More than 85% of the initial change decayed with a relaxation time of 5 ms. Since measurements in the present work were carried out with a flash repetition rate of 1 per 2.5 s in the presence of 0.2 mM FeCN, the reoxidation of Q_A^- by FeCN between flashes was essentially complete. Also the reduction of Z^+ was complete prior to the next flash, because the water splitting system is active in the RC. Under these conditions, the

TABLE I

Protein compositions of the four particles

+ + indicates that the stained density of the corresponding protein band in the sample was essentially same to that in the RC. + indicates that the stained density of the corresponding protein band in the sample was partially reduced compared with that of the RC. − indicates that the corresponding protein band was detected but its stained density was drastically reduced. − − indicates that the corresponding protein band was not detected at all.

Protein components of RC	DM particle	Triton particle	OTG particle	NS particle
47 kDa	+ +	+ +	+ +	− −
43 kDa	+ +	+	+	− −
33 kDa	− −	+	+	− −
D2	+ +	+ +	+ +	+ +
D1	+ +	+ +	+ +	+ +
28 kDa	− −	+	+	−
23 kDa	− −	− −	−	− −
20 kDa	− −	+	+	− −
17 kDa	− −	− −	− −	− −
11 kDa	− −	− −	−	− −
Cyt *b*-559 LS	+ +	+ +	+ +	+ +
10 kDa phospho-protein	+	− −	+	− −
6.1 kDa	−	− −	+	− −
band A	+ +	− −	+	− −
5.0 kDa	−	− −	−	− −
L protein	+ +	+	−	− −
4.8 kDa	+ +	+ +	+ +	+ +
band B	− −	− −	− −	− −
band C	+	− −	−	− −
4.1 kDa	+	+ +	−	− −
Cyt *b*-559 SS	+ +	+ +	+ +	+ +
3.9 kDa	+ +	− −	+	− −

TABLE II

The pigment compositions and Q_A activities of the RC, DM, OTG, Triton and NS particles

The pigment compositions are shown in the form of molar ratio on the basis of 2 Pheo *a* and the Q_A activities are the values relative to the RC.

Sample	Pheo *a*	Chl *a*	Cyt *b*-559	PQA	Q_A activity (%)
RC	2	42.4	0.93	2.1	100
DM particle	2	29.6	0.97	0.88	78
OTG particle	2	18.9	1.02	0.63	16
Triton particle	2	12.2	1.06	0.68	45
NS particle	2	5.6	1.11	0	0

backreaction between $P680^+$ and Q_A^- with a time constant of 150 μs was reasonably supposed to be insignificant. Thus we may conclude that the flash-induced transient absorbance changes shown in Fig. 2 reflect a photoinduced rapid formation of Q_A^- followed by its reoxidation mainly by FeCN direct and/or via Q_B. The same measurements were carried out with the other particles. Although the signal amplitude was different with each particle, the rapid absorbance change was observed in every particle except for the NS particle. Fig. 3 summarizes the results obtained with the RC, DM, Triton, OTG and NS particles as plots of the initial amplitude of the signal against the monitoring wavelength. The DM particle exhibited quantitatively a spectrum almost identical to that of the RC when the measurements were carried out at an equivalent reaction center concentration (0.8 μM). While in the OTG

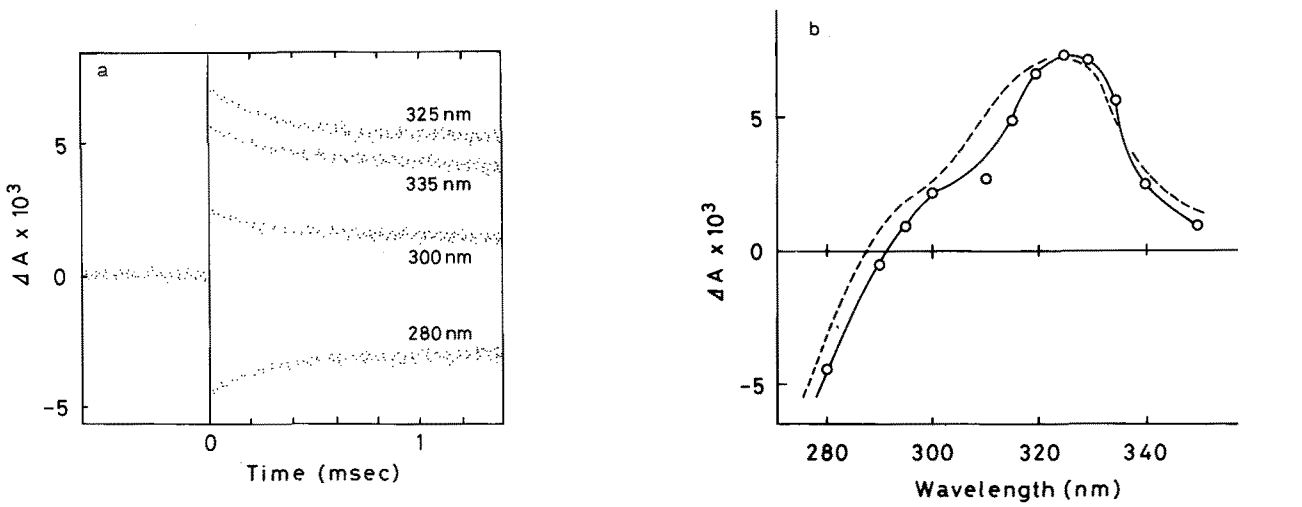

Fig. 2. Transient absorbance change at different wavelengths in the RC induced by flash illumination. Temperature, 25 ± 1°C. Sample was suspended in solution C at concentration of 0.8 μM reaction center on the basis of cytochrome *b*-559 with 0.2 mM $K_3Fe(CN)_6$ and illuminated by a train of laser flashes (pulse width, 7 ns; intensity, 30 mJ per pulse) from a Q-switched frequency-doubled Nd-YAG laser with 2.5 s intervals. The absorbance change was recorded for each flash and accumulated. (a) Time courses of the absorbance change at four wavelengths. (b) Comparison of action spectrum of the initial amplitude of absorbance change in the RC (○) with the photoinduced difference spectrum of Q_A in the PS II particle (- - - - -) reported by Schatz and Gorkom [25].

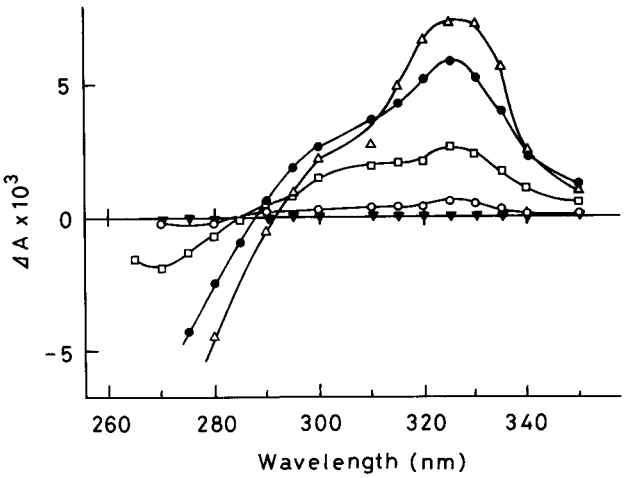

Fig. 3. Action spectra of photoinduced absorbance changes in the RC (△), DM (●), Triton (□), OTG (○) and NS (▼) particles. The experimental conditions are the same as described in Fig. 2.

and Triton particles, the spectra were essentially the same as that of the RC in shape, but their intensities were suppressed to 16 and 45% of the original RC level. No transient absorbance change upon flash illumination was detected in the NS particle by a flash spectrophotometer with the time resolution limited to about 1 μs. The results of the Q_A activity thus obtained are compared with those of the pigment analysis for the corresponding particles in Table II. With respect to the DM, Triton and NS particles, their Q_A activities are explained more or less in terms of the amount of PQA molecule retained in each complex. The number of Q_A reversibly reduced upon single flash illumination in the DM particle was 0.8 per reaction center, which is substantially in agreement with the number of PQA retained in the complex, suggesting most of the retained PQA molecules to act as an electron acceptor at the Q_A site. A large portion of PQA's preserved in the Triton particle are supposed to function as Q_A. The NS particle completely lost its Q_A activity, concomitant with the total depletion of PQA. In contrast, the number of photo-reducible Q_A's in the OTG particle is 0.16 per reaction center, which is only 25% of the number of PQA molecules retained in the particle. This discrepancy suggests that the other 75% of PQA is locate at a site other than Q_A or at the Q_A site but in an inactive form. If the latter case applies, one may suppose that some essential components activating PQA at the Q_A site were removed from the OTG particle during the preparation process. In order to investigate this possibility, we tried to reconstitute the Q_A function in the OTG particle with the OTG extract.

Reconstitution of Q_A in the OTG particle

Fig. 4 compares the transient absorbance changes at 325 nm upon flash illumination in the DM and OTG particles and in the reconstituted OTG particle with the OTG extract (see Materials and Methods). After reconstitution with the OTG extract, the Q_A activity determined from the initial amplitude of the signal was restored in the OTG particle at a level of 50% of the RC, which is about 3-times larger than that of the OTG particle itself. The crude OTG extract contains lipids, pigments, PQA and many proteins, any of which might be responsible for restoring the Q_A activity. In order to identify the components really involved in the reactivation of the Q_A function, the ability to restore the Q_A activity in the OTG particle was tested for the OTG extract-1, OTG extract-2 (see Materials and Methods), PQA-free OTG extract, heat-treated OTG extract, thylakoid total lipids and purified PQA. The PQA-free OTG extract was obtained as follows. The OTG extract eluted out from a DEAE-Toyopearl 650-S column as flow through was immediately frozen in solid CO_2/ethanol and lyophilized in the dark under reduced pressure. The resulting powder was resuspended in a hexane solution containing 0.16% methanol and incubated for 10 min in ice under stirring and centrifuged at 0°C. The pellet was dried with a rotary evaporator at 0°C and finally suspended in solution D to be used for reconstitution. Complete removal of PQA from the resulting preparation was confirmed spectroscopically. Heat treatment of the OTG extract was done by incubating it at 90°C for 10 min and immediately cooling down in ice. To be submitted to the reconstitution experiments, each of the total lipids and purified PQA was also suspended in solution D. Table III summarizes the results of the reconstitution. Reconstitution with the OTG extract-1 and PQA-free OTG extract recovers the Q_A activity in the OTG particle at about 90 and 75% of that with the OTG extract, respectively, but none of the OTG extract-2, heat-treated OTG extract, lipids and

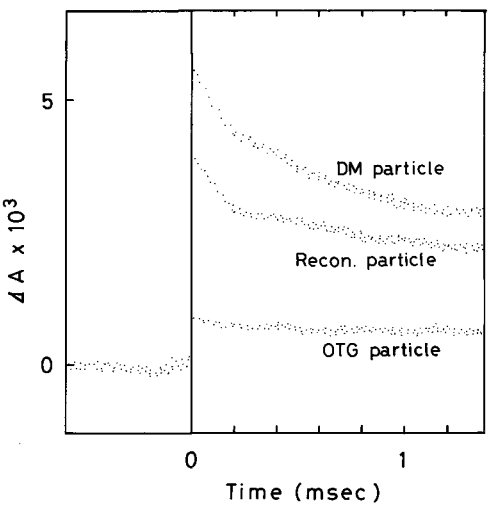

Fig. 4. Typical traces of photoinduced transient absorbance change in the DM and OTG particles and the reconstituted OTG particle with the OTG extract. The experimental conditions are the same as described in Fig. 2.

Table III

Comparison of the Q_A activities of the RC and the OTG particles reconstituted with different materials

Sample	Q_A activity (%)
RC	100
OTG particle	16
Reconstituted OTG particle with	
OTG extract	50
OTG extract-1	45
OTG extract-2	20
PQA-free OTG extract	38
heat-treated OTG extract	19
lipids	16
PQA and lipids	18
OTG particle + OTG extract [a]	16

[a] To prepare this sample, OTG extract was dialyzed against 25 mM phosphate buffer (pH 7.5) to remove OTG and OGP and added to the OTG particle suspended in solution C.

purified PQA shows any significant effect on the Q_A activity in the OTG particle. These observations indicate that the reconstitution of the Q_A activity was done in the OTG particle retaining an inactive PQA at Q_A site by reinsertion of certain proteinaceous components rather than the lipids and PQA solubilized in the OTG extract but not in the OTG particle, which had lost PQA at the Q_A site even when PQA was added in the reconstitution medium. It should be noted here that removal of the detergents from the OTG extract by dialysis before mixing with the OTG particle resulted in the loss of recovery of the Q_A activity (bottom row in Table III). This might suggest that membrane proteins rather than water-soluble proteins are responsible for recovering the Q_A activity.

The present results seem to disagree with those reported by Gouraris et al. [13]. Those researchers observed photoreduction of cytochrome *b*-559 in the NS particle upon addition of PQA and catalytic activity of the resulting NS particle to photoreduce DCPIP in the presence of exogenous electron donor. However, the quantum yields for the reactions were extremely low, even when a very high level of PQA was added. This may suggest that the added PQA actually acted as an electron mediator, but in a certain different way from that occurring in the intact PS II reaction center. For instance, the added PQA could be bound to the complex nonspecifically and reduced elsewhere other than at the Q_A site; alternatively a certain component required for normal functioning of Q_A could be lost together with PQA during the preparation of the NS complex. As pointed out by the authors themselves, the assessment of the interaction of the added quinones with the Q_A site should be done by using transient optical spectroscopy instead of the steady state optical techniques which they employed. We could not detect any effect of PQA on the photochemical property of the NS particle in the experiment done using the flash spectrophotometer.

Relationship between the protein compositions and Q_A activity

Although the possibility of certain unknown components other than proteins participating in the activation of the Q_A function in the OTG particle has not been ruled out completely at this moment, it may be worthwhile examining the relationship between the protein compositions and Q_A activity for each particle and OTG extract. Fig. 5 compares the protein pattern on SDS-PAGE of the three OTG extracts, i.e., OTG extract (lane 1), OTG extract-1 (lane 2) and OTG extract-2 (lane 3). It is shown that whole proteins with molecular mass higher than 20 kDa contained in the OTG extract are eluted in a fraction pooled as OTG extract-2, while all of the proteins smaller than 10 kDa exist in the OTG extract-1 fraction. These results suggest that certain proteins smaller than 10 kDa are responsible for recovery of the Q_A activity in the OTG particle, because the OTG extract-1 restores the Q_A activity in the reconstituted OTG particle, but the OTG extract-2 does not. This presumption can be confirmed by examining the protein compositions of the DM particle. As clearly shown in lane 2 in Fig. 1a, the DM particle which holds the Q_A activity at 78% of the original RC level consists of the 47 kDa, 43 kDa, D1 and D2 proteins, 10 kDa phosphoprotein, cytochrome *b*-559 and several low-molecular-mass proteins without any other protein of molecular mass higher than 10 kDa. Then we confined ourselves to examining the possibility of each protein in the low-molecular-mass region being involved in the normal functioning of the Q_A by comparing the pattern of the bands for the different particles and the OTG extract-1. The nuclear coding 5.0 kDa protein, the band B and 6.1 kDa proteins disappear in the DM particle, indicating that these proteins are not responsible for the Q_A function. The Triton particle, which retains the Q_A activity at 45% of the RC, additionally lost the 10 kDa phosphoprotein and the 3.9 kDa, band A and band C proteins completely. This fact may rule out the possibility of the proteins mentioned here being involved in the Q_A function. In contrast with these proteins, the bands of the 5.0 kDa L protein and 4.1 kDa nuclear coding protein are clearly detected in the DM and Triton particles. While these two bands substantially disappear in the OTG particle, they are instead observed in the OTG extract-1. The protein components consisting the NS particle are the D1, D2, α- and β-subunits of cytochrome *b*-559 and 4.8 kDa proteins and no other low-molecular-mass protein band is detected, even in the SDS-PAGE system 2.

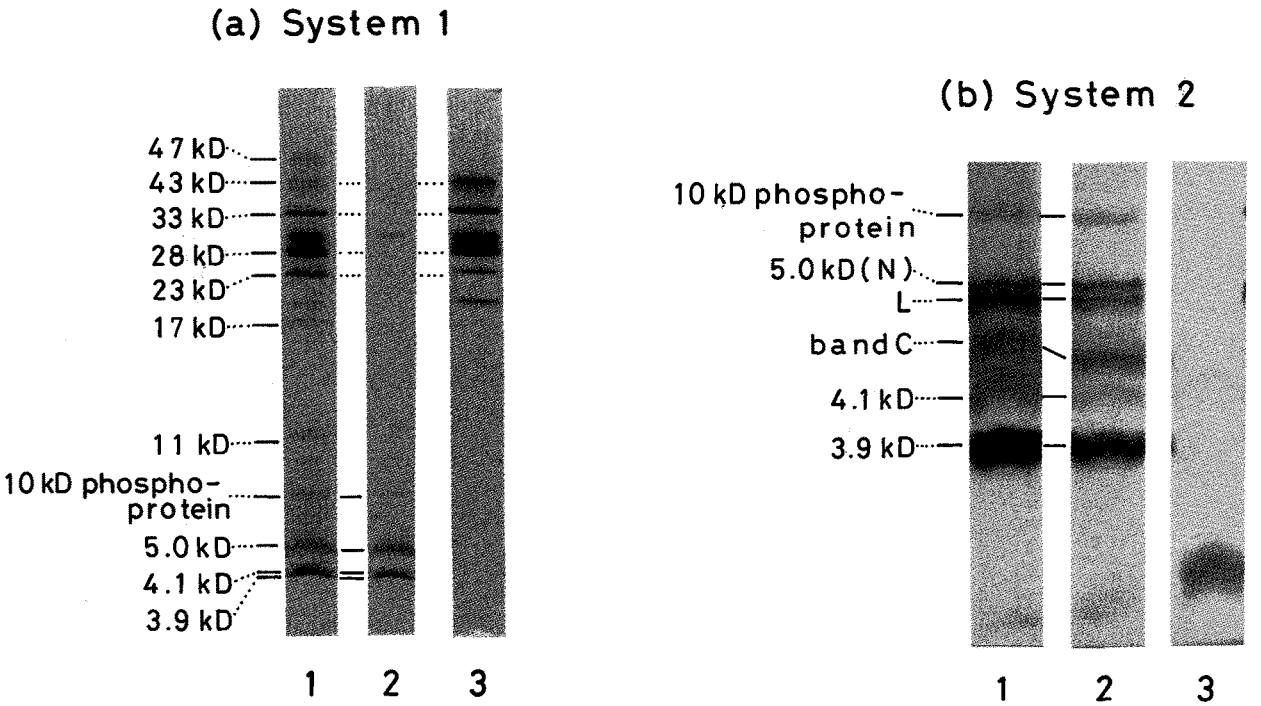

Fig. 5. SDS-PAGE profiles of the three OTG extracts. Lane 1: OTG extract, lane 2: OTG extract-1, lane 3: OTG extract-2. The experimental conditions are the same as described in Fig. 1.

Thus, the behavior of the Q_A activity of all the particles examined, including the reconstituted particles, can be explained by assuming that, besides the D1, D2, 47 kDa, 43 kDa and 4.8 kDa proteins and cytochrome *b*-559, the 5.0 kDa L protein and/or the 4.1 kDa protein are crucial for normal functioning of the Q_A in the PS II reaction center. The nucleotide sequences of the *psbL* gene encoding the L protein were reported in tobacco [24] and liverwort [26] and the proteins deduced from both the sequences were found to consist of 37 amino acid residues which contain a single membrane-spanning segment at the C-terminal. A homologous ORF has also been found in pea, spinach, wheat, *Euglena* and *Synechocystis* [27–29], suggesting the L protein to be one of the common components in the PS II.

The 4.1 kDa protein was found to be associated with the PS II complex in both higher plants (spinach and wheat) and cyanobacteria (*Synechococcus vulcanus*) and the N-terminal sequence was determined by Ikeuchi et al. [2,30]. The sequence in spinach contains one charged amino acid, Lys, at position 8 which followed by a hydrophobic amino acid stretch presumably spanning the membrane [30]. Although the sequence homology of the protein among the three species are relatively low, the lysine residue and the subsequent hydrophobic stretch appear to be conserved.

Both of the L and 4.1 kDa proteins are now being purified to be examined their abilities to restore the Q_A activity in the OTG particle.

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