Biochimica et Biophysica Acta, 1015 (1990) 61–68
Elsevier

BBABIO 43111

Selective destruction of iron-sulfur centers by heat/ethylene glycol treatment and isolation of Photosystem I core complex

Satoshi Hoshina [1], Reiko Sakurai [1], Naoki Kunishima [1], Keishiro Wada [1] and Shigeru Itoh [2]

[1] *Department of Biology, Faculty of Science, Kanazawa University, Marunouchi, Kanazawa and* [2] *National Institute for Basic Biology, Myodaiji, Okazaki (Japan)*

(Received 7 August 1989)

Key words: Iron-sulfur center; P-700; Photosystem I; Ethylene glycol; Heat stress; (Spinach)

Heat treatment of spinach thylakoids or Photosystem I particles in the presence of ethylene glycol caused the selective destruction of iron sulfur centers F_A, F_B and F_X as judged by low-temperature EPR and flash spectroscopy. Incubation of thylakoids or Photosystem I particles in the presence of 50% ethylene glycol for 5 min selectively destroyed F_A and F_B at temperatures between 50 and 55 °C, and F_X between 60 and 70 °C. P-700 was destroyed only above 70 °C, and electron transport from ascorbate-dichlorophenolindophenol to methyl viologen was inhibited between 35 and 50 °C by the same treatment. The destruction of F_A and F_B occurred at the same temperature range and resulted in the decrease of the decay phase with a 30 ms half-time in re-reduction kinetics of flash-oxidized P-700. This was accompanied by the appearance of the 1 ms decay phase, which reflects reduction of P-700$^+$ by F_X^-. The 1 ms phase disappeared when F_X was destroyed. Photosystem I core complex was isolated by solubilizing the heat/ethylene glycol-treated Photosystem I particles with Triton X-100, followed by sucrose density gradient ultracentrifugation. The complex obtained from 60 °C/ethylene glycol-treated preparation lacked the 8 kDa F_A/F_B polypeptide and was composed of the polypeptides of apparent molecular weight 63, 60 and 5 kDa. This complex showed a small and broadened $g = 1.77$ F_X signal. It is concluded that the heat/ethylene glycol treatment gives a simple and selective degradation-isolation method of Photosystem I reaction center complex.

Introduction

Exposure of photosynthetic tissue to high temperature has dramatic effects on photosynthetic activities and membrane organization [1]. Heat treatment of chloroplasts causes the inactivation of PS II-mediated electron transport [2,3]. Gounaris et al. [3] suggested that heat treatment led to a phase separation of nonbilayer-forming lipids in thylakoid membranes, resulting in a dissociation of antenna pigment-protein complexes from the core complex of the PS II. On the other hand, the rate of PS M-mediated methyl viologen photoreduction was stimulated by treatment at temperatures above about 35 °C [3,4]. Thomas et al. [4] suggested that the stimulation of PS I activity which occurred in heat-stressed chloroplasts reflected a reorganization of thylakoid membranes, which exposed new donor sites within the cytochrome b_6/f complex.

The reducing side of Photosystem I reaction center of oxygenic photosynthetic organisms is known to consist of five different electron acceptors [5–8]. Upon the illumination an electron is liberated from reaction center chlorophyll *a*, P-700 to the electron acceptor chlorophyll *a*-690 (A_0), to the secondary acceptor phylloquinone (A_1) and then to the iron sulfur centers F_X, F_A and F_B [5–8]. F_X is proposed to be either one [4Fe-4S] or two [2Fe-2S] clusters per P-700 [6–10] associated with the two core polypeptides of about 80 kDa molecular mass [11–13] together with A_0, A_1, P-700 and about 50 molecules of antenna chlorophyll *a* [14–16]. F_A and F_B can be assumed to be a [4Fe-4S] center, respectively, located together on a 9 kDa polypeptide [9,17–20] which is isolated and characterized by Oh-oka et al. [20–22] and by Wynn and Malkin [23]. F_X

Abbreviations: Chl, Chlorophyll; DCIP, 2,6-dichlorophenol indophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; heat/EG treatment, heat treatment in the presence of ethylene glycol (typically for 5 min at 50% (v/v) concentration if not otherwise mentioned); PS, Photosystem; SDS, sodium dodecyl sulphate; MV, methyl viologen.

Correspondence: S. Hoshina, Department of Biology, Faculty of Science, Kanazawa University, Marunouchi 1-1, Kanazawa 920, Japan.

is assigned to the species A_2 observed by the flash spectroscopy and F_A/F_B to P-430 which is the terminal electron acceptor in the isolated PS I particles [12,24].

In this study, we demonstrate that heat treatment of the PS I particles or thylakoid membranes in the presence of ethylene glycol (heat/EG treatment) selectively destroys F_A, F_B and F_X. This method allows a simple and efficient isolation of the PS I core complex lacking F_A and F_B remaining virtually intact charge separation activity between P-700 and A_0.

Materials and Methods

Chloroplasts were isolated from fresh spinach leaves as described previously [25] and washed in a medium containing 10 mM NaCl and 50 mM Tricine-NaOH (pH 7.8). PS I particles (PS I-200) were prepared by solubilizing spinach thylakoids with Triton X-100 as previously described [25].

For the heat treatment, thylakoids or PS I particles (1 mg Chl/ml) were resuspended in a medium containing 0.1 M sorbitol, 10 mM NaCl and 50 mM Tricine-NaOH (pH 7.8) in the presence or absence of 50% (v/v) ethylene glycol unless otherwise noted. The samples were incubated at various temperatures for 5 min, and then rapidly cooled to 4 °C.

Isolation of PS I core complex described below were performed at 4 °C unless otherwise stated. Heat/EG-treated PS I particles were diluted with about 35 volumes of distilled water and centrifuged at $35\,000 \times g$ max for 20 min. The resulting pellet was resuspended in 0.8% Triton X-100 at a chlorophyll concentration of 800 μg/ml and incubated at 20 °C for 30 min with stirring. The sample was centrifuged at $52\,000 \times g$ max for 30 min and the supernatant was loaded onto a linear sucrose density gradient (0.1–1 M) overlaid on a 2 M sucrose cushion. The gradient also contained 0.02% Triton X-100. The gradients were centrifuged for 15 h in a Hitachi RPS-27 rotor at 24000 r.p.m. (maximal $100\,000 \times g$). The lower green band appeared between 1 and 2 M sucrose was collected using a syringe and dialyzed against distilled water for 5 h before centrifuging at $35\,000 \times g$ max for 20 min. The precipitate (PS I core complex) was resuspended in a medium containing 0.1 M sorbitol, 10 mM NaCl, 4 mM 2-mercaptoethanol and 50 mM Tricine-NaOH (pH 7.8) and stored at −80 °C until use.

PS I-dependent electron-transport activity was measured in a reaction mixture containing 250 μM methyl viologen, 20 μM DCIP, 1 mM sodium ascorbate, 1 mM NaN_3, 10 μM DCMU, 5 mM NH_4Cl, 0.1 M sorbitol, 10 mM NaCl, 50 mM Tricine-NaOH (pH 7.8) and thylakoids (10 μg Chl/ml) with a Clark-type oxygen electrode [26]. Photooxidation and difference absorption spectrum of P-700 were measured with a Shimadzu UV-300 spectrophotometer as described previously [25]. The P-700 concentration was calculated using the $\Delta A_{698-725}$ and an extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ [27]. EPR spectra were determined with an X-band EPR spectrometer (Model ER-200, Bruker, F.R.G.), equipped with a liquid-helium cryostat (Model ESR-900, Oxford Instruments, England) as described previously [28]. Reaction mixture contained 0.1 M glycine/0.1 M 2-amino-2-methyl-1,3-propanediol-NaOH buffer (pH 10.0), 50 μM methyl viologen, 50 μM DCIP, 0.7% (w/v) sodium dithionite, and thylakoids (about 2 mg Chl/ml) or PS I complex (about 1 mg Chl/ml). The reaction mixture, contained in a quartz tube (i.d., 3 mm) was illuminated at room temperature for 1 min and then frozen in liquid nitrogen under continuous illumination with white light supplied by a projector lamp. Flash-induced absorbance changes were measured using a split beam spectrophotometer at 10 °C [29]. The actinic flash was a 10 ns (half-width) laser pulse at 532 nm from the second harmonic of a Nd-YAG laser (Quanta-Ray DCR 2-10) of nearly saturating intensity. The reaction mixture contained 10 μM DCIP, 1 mM sodium ascorbate, 100 mM sorbitol, 10 mM NaCl, 50 mM Tricine-NaOH (pH 7.8) and PS I particles. Signals (32–128 measurements) were averaged in each case.

Analysis of polypeptide was done by SDS-polyacrylamide gel electrophoresis [25]. Electrophoresis was performed in a slab gel apparatus using a 5% (w/v) polyacrylamide stacking gel and a 15% (w/v) polyacrylamide separating gel. Both gels contained 6 M urea. PS I complex was solubilized for 1 h at room temperature in 0.12 M Tris-HCl (pH 6.8) containing 2 M urea, 2% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, 0.3 M sucrose, and 0.05% (w/v) bromphenolblue. Electrophoresis was carried out at a constant current of 5 mA [25]. Gels were stained with Coomassie brilliant blue R-250 as described previously [25]. Molecular weight determinations were performed on the basis of electrophoretic mobility using molecular mass markers, Sigma MW-SDS-70L (14.2–66.0 kDa) and MW-SDS-17 (2.5–17.0 kDa). Acid-labile sulfide was determined as previously reported [30]. Spinach soluble ferredoxin served as a standard for acid-labile sulfide. Chlorophyll was determined in 80% acetone [31].

Results

Effects of heat/EG treatment on PS I electron-transfer activity

Fig. 1 shows the effect of 5 min heat treatment on the rate of PS I-dependent electron transfer (ascorbate-DCIP to methyl viologen) in spinach thylakoids membranes. In the absence of ethylene glycol, the rate increased with increasing the treatment temperature as reported [3,4], and then decreased from a maximum to the original level at 55 °C. On the other hand, presence of 50% (v/v) ethylene glycol during the 5 min heat

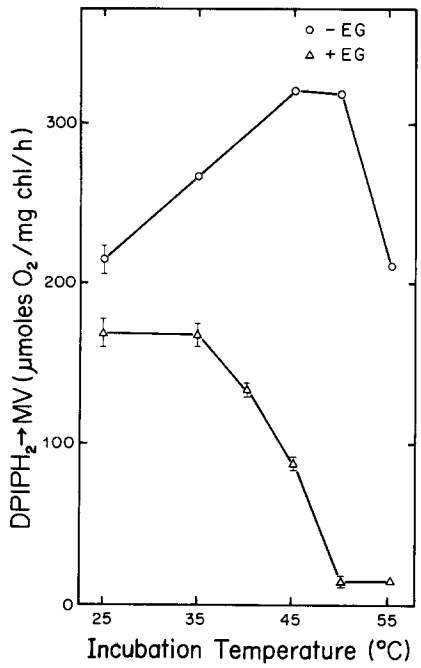

Fig. 1. Effect of heat-treatment on the methyl viologen-mediated PS I electron transport activity of thylakoid membranes. Thylakoid membranes were incubated at various temperatures in the presence (Δ———Δ) or in the absence (o———o) of 50% (v/v) of ethylene glycol. See Materials and Methods for details.

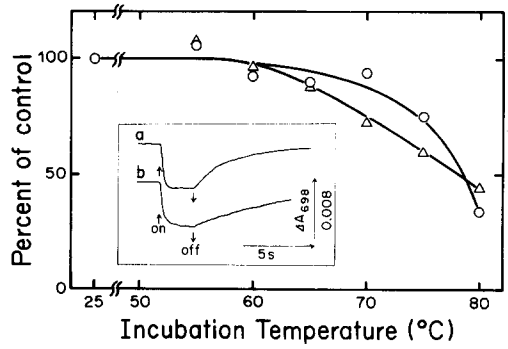

Fig. 2. Effect of the incubation temperature on the P-700 content of PS I particles in the presence of 50% (v/v) ethylene glycol. Extent of photochemically oxidizable P-700 (Δ———Δ) was measured as described in Ref. 25. The activity was measured at room temperature in a reaction mixture containing 1 mM sodium ascorbate, 2 μM DCIP, 0.5 mM methyl viologen, 100 mM sorbitol, 10 mM NaCl, 50 mM Tricine-NaOH (pH 7.8) and PS I particles. Chemically determined P-700 (o———o) was measured by the 1 mM ferricyanide minus 2 mM ascorbate difference spectrum. Inset: time-course of P-700 photooxidation in PS I particles preincubated at 25 °C (a) and 60 °C (b) in the presence of 50% (v/v) ethylene glycol.

treatment (heat/EG treatment) caused a decrease in the activity at temperatures above 40 °C, with nearly total inhibition at 50 °C. This indicates that the presence of ethylene glycol during the heat treatment caused irreversible damage on the electron-transport chain even when ethylene glycol is removed by washing. When the heat treatment was done at 55 °C the rate decreased more severely with increasing concentration of ethylene glycol. The activity was 77% of control after the 55 °C treatment at 13% ethylene glycol and the maximum inhibition (5–10% of control rate) was obtained at concentrations above 50% ethylene glycol (data not shown). Therefore, further study of heat/EG treatment was done at the concentration of 50% ethylene glycol in this study.

Fig. 2 (inset) shows the kinetics of the continuous light-induced P-700 absorption change in PS I particles. The magnitude of P-700 photooxidation was not altered by the 60 °C/EG treatment, although the rate of dark reduction was greatly slowed down. As shown in Fig. 2, little decrease in the extent of P-700 oxidation was observed after the heat/EG treatment below 60 °C. At temperatures above 65 °C a progressive loss of P-700 photooxidation was induced. However, P-700 extent assayed by chemical oxidation-reduction showed little decrease even when the particles were incubated at 70 °C where F_X was completely inactivated (described below). This indicates that the heat/EG treatment induces inactivation of some component other than P-700 in the electron transfer from ascorbate DCIP to methyl viologen.

Effects of heat/EG treatment on the flash-induced absorption changes of P-700 and P-430

In the absence of external electron acceptor, $P\text{-}700^+$ induced by laser excitation is re-reduced by iron sulfur centers. Reduction of P-700 by reduced F_B/F_A can be assumed to proceed with a half-time of about 30 ms, while that by reduced F_X, which occurs when F_B/F_A are eliminated or reduced, with an about 1 ms half-time [11,12]. PS I particles after the 25 °C/EG treatment

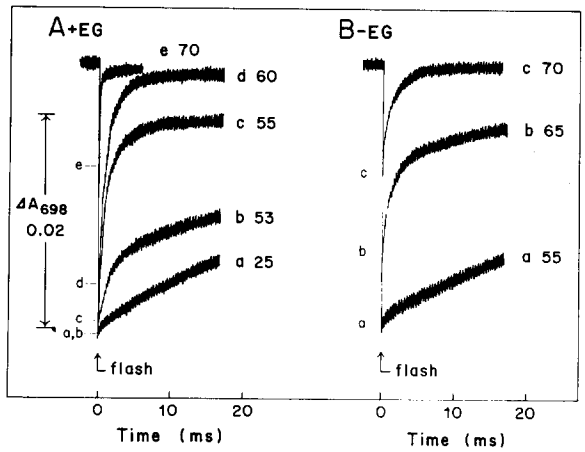

Fig. 3. Flash-induced absorption changes at 698 nm in PS I particles incubated at various temperatures. (A) PS I particles were preincubated with 50% (v/v) ethylene glycol at 25 °C (a), 53 °C (b), 55 °C (c), 60 °C (d) and 70 °C (e), respectively. (B) PS I particles were preincubated in the absence of ethylene glycol at 55 °C (a), 65 °C (b) and 70 °C (c). Horizontal lines shown at left hand indicate the maximum absorption changes after flash. The flash-induced absorption changes were measured at 10 °C.

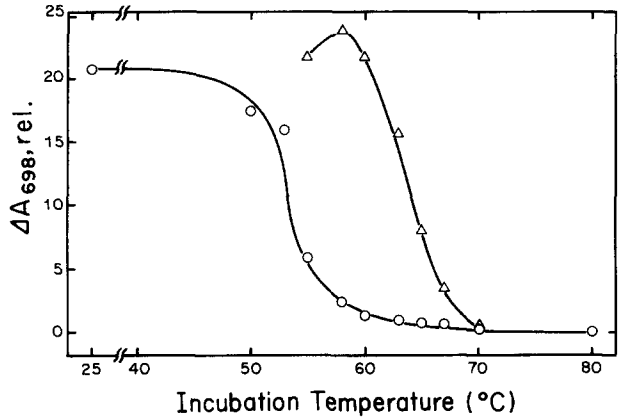

Fig. 4. Effect of incubation temperatures on the flash-induced transitions at 698 nm in PS I particles in the presence of 50% (v/v) ethylene glycol. The amplitude of the 1 ms decay component (Δ———Δ) was calculated from the semilogarithmic plot of the absorption change shown in Fig. 3A. Absorption change at 15 ms after the flash which represents the slow 30 ms phase (○———○) was determined from the data shown in Fig. 3A.

decayed with a half time of about 30 ms indicating no damage. After the heat/EG treatment above 53°C, P-700$^+$ decayed in a biphasic manner with a 'fast' ($t_{1/2}$ = 1 ms) and a 'slow' ($t_{1/2}$ = 30 ms) decay phases (Fig. 3A b,c.). The 1 ms phase is estimated to reflect the reduction of P-700$^+$ by F_X^-. After 70°C/EG treatment, PS I particles lost both the 30 ms and 1 ms decay phases and showed a decay with a half-time of about 20 μs (Fig. 3A e).

Fig. 4 shows the effect of incubation temperature of heat/EG treatment. The extent of the slow 30 ms phase represented as the extent of the absorption change, was 50% inhibited at about 53°C and totally lost at 60°C. After 60°C/EG treatment the decay consisted of only the 1 ms decay phase. The 1 ms phase, then, was 50% depressed at about 64°C and completely lost at 70°C. After the 70°C/EG treatment, PS I particles showed a decay phase with a half time of about 20 μs (Fig. 3A, e). This rapid decay is mainly due to the decay of P-700 triplet state produced in the charge recombination between P-700$^+$ and A_0^- [5]. From the curves in Fig. 4, we can assume that F_B/F_A is destroyed between 50 and 55°C and F_X between 60 and 70°C by the heat/EG treatment. P-700, on the other hand, was destroyed only above 70°C (see Fig. 2). When the PS I particles were heated in the absence of ethylene glycol, the disappearance of the 30 ms and 1 ms decay phases was seen at temperatures about 10°C higher than those in Fig. 4 (Fig. 3B, cf. Fig. 3A and 4).

Fig. 5 shows the effect of electron acceptor benzyl viologen on the flash-induced absorbance changes at 430 and 447 nm in the 60°C/EG-treated PS I particles. The decay of absorption change at 430 nm is estimated to reflect mainly the re-reduction of P-700$^+$, and partially re-oxidation of F_X^-, while the change at 447 nm, isosbestic wavelength of P-700/P-700$^+$ [32], reflects the reduction and re-oxidation of only F_X^-. In the absence of benzyl viologen, electrons return almost completely from F_X^- to P-700$^+$ with a half-time of 1 ms in this preparation. In the presence of 500 μM benzyl viologen, which rapidly oxidizes F_X^-, the 430 nm signal returned to an intermediate level decaying with a long life time, showing the stabilization of P-700$^+$ by the faster oxidation of F_X^- by benzyl viologen. A part of the absorption change at 430 nm decayed more rapidly with benzyl viologen. This is interpreted by the fast oxidation of F_X^- by benzyl viologen as confirmed by the fast decay of absorption change at 447 nm. These results confirm the rapid turnover of F_X but not of F_A/F_B in the 60°C/EG-treated preparation.

Effects of heat/EG treatment on the F_A, F_B and F_X content determined by EPR and on the content of acid-labile sulfide

The bound iron sulfur centers in thylakoid membranes were measured by EPR spectroscopy at 8 K as shown in Fig. 6. Signal intensities of F_A and F_B at g = 1.94 and 1.92, respectively, were slightly decreased by the 50°C/EG treatment. Above 52°C, pronounced losses of the signals of F_A and F_B, with no change in the signal positions, were observed. The band of F_X at g = 1.77 was broadened above 55°C at which F_A and F_B signals were almost eliminated, with no shift of the peak position.

Fig. 7 shows the dependencies of the amount of F_A, F_B and F_X on the temperature of the heat/EG treat-

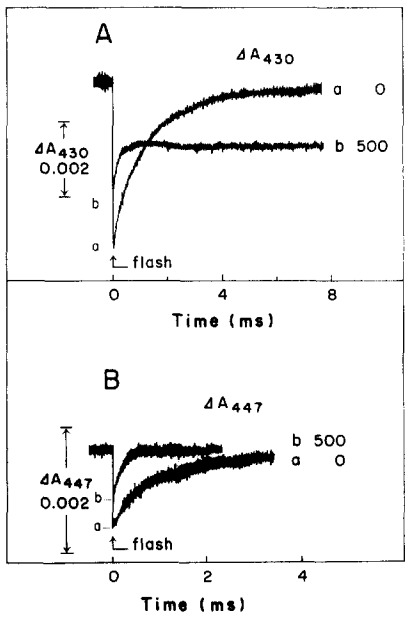

Fig. 5. Effect of benzyl viologen on the flash-induced absorption changes at 430 nm (A) and 447 nm (B) of PS I particles. PS I particles were preincubated at 60°C in the presence of 50% (v/v) ethylene glycol. (a) No Benzyl viologen added; (b) 500 μM benzyl viologen added.

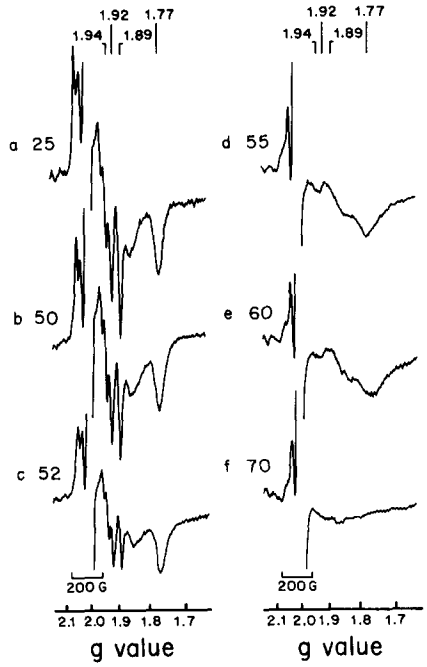

Fig. 6. EPR spectra of the iron sulfur centers in thylakoid membranes. Thylakoids were preincubated with 50% (v/v) ethylene glycol at 25 °C (a), 50 °C (b), 52 °C (c), 55 °C (d), 60 °C (e) and 70 °C (f) for 5 min. EPR experimental conditions: temperature, 8 K; microwave frequency and power of 9.69 GHz and 100 mW, respectively; gain, $1.0 \cdot 10^5$; modulation amplitude, 20 G; scan width, 3200–4200 G; time constant, 320 ms. EPR spectra were normalized by Chl concentration.

ment. The amounts of F_A and F_B were expressed as the amplitudes from peak to trough at $g = 1.94$ and 1.92, respectively. The amount of F_X was estimated more carefully by calculating the area of the band at $g = 1.77$ to minimize the error when the bandwidth was increased. The amounts of F_A and F_B were reduced to one-half by the treatment at 52 °C and to zero at 60 °C. It is also noted that F_A and F_B were destroyed at the same temperature (Fig. 7). F_X was less susceptible than

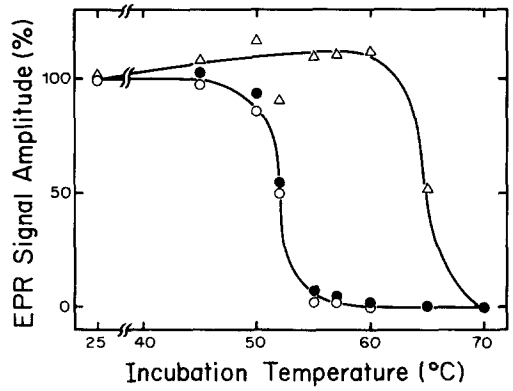

Fig. 7. Effect of heat treatment on the EPR signals of three iron sulfur centers in thylakoid membranes in the presence of 50% (v/v) ethylene glycol. ●, center A at $g = 1.94$; ○, center B at $g = 1.92$; △, center X at $g = 1.77$. See Text for the method of estimating of EPR signal intensities. EPR conditions were identical to those of Fig. 6.

TABLE I

Effect of heat/EG-treatment on the acid-labile sulfide content in thylakoid membranes.

After incubation, the samples were centrifuged at 30000 × *g* for 15 min and resuspended in 0.1 M sorbitol, 10 mM NaCl and 50 mM Tricine-NaOH (pH 7.8). Chlorophyll/P-700 ratio of 450 was used to calculate the numbers in third column. Each value is the mean of three determinations.

Thylakoid membranes incubated	Acid-labile sulfide	
	(nmol per mg Chl)	(mol per mol P-700)
Without EG at 25 °C	34.6 ± 2.9	14.0 ± 1.2
Without EG at 55 °C	34.5 ± 1.1	14.0 ± 0.4
With EG [a] at 25 °C	35.0 ± 1.7	14.2 ± 0.7
With EG [a] at 55 °C	21.6 ± 0.8	8.7 ± 0.3

[a] 50% (v/v) ethylene glycol.

F_A and F_B to the heat/EG treatment, and was reduced to be 50% at 65 °C and to be zero at 70 °C (Fig. 7).

The temperature dependencies in Fig. 7 almost agree with those in Fig. 4 and confirm that the fast and slow decay phases of $P\text{-}700^+$ re-reduction kinetics correspond to the reduction of $P\text{-}700^+$ by F_X^- and F_A/F_B^-, respectively.

Table I shows the effect of heat/EG treatment on the acid-labile sulfide content of thylakoid membranes. Untreated thylakoids had 14.0 μmol per mol of P-700 if a chlorophyll/P-700 ratio of 450 is assumed. This value may correspond to the three to four [4Fe-4S], or to the two or three [4Fe-4S] and two [2Fe-2S] iron sulfur centers as expected for F_A, F_B, F_X and Rieske-FeS centers each of which presumably exists about an equimolar as P-700. The amount was reduced to 48–62% by the 55 °C/EG treatment. This may explain the loss of about 2 [4Fe-4S] clusters and seems to be consistent with the loss of F_A/F_B determined by EPR or flash spectroscopy. No change of the amount was detected in samples heated at 55 °C in the absence of ethylene glycol. Similar experiment in Photosystem I particles or in core complex are now under progress.

Dissociation of polypeptides from PS I particles by the heat / EG treatment: isolation of PS I core complex

Heat/EG-treated PS I particles were solubilized in 0.8% Triton X-100 and the PS I core complex was prepared as described in Materials and Methods. Fig. 8 shows the EPR spectra of the PS I core complex thus prepared. PS I core complex isolated from the 55 °C/EG-treated particles (55 °C/EG core complex) showed a broadened F_X band and very small signals of F_A and F_B (Fig. 8b). PS I core complex from the 60 °C/EG-treated particles (60 °C/EG core complex) showed a small F_X band broader than that in the 55 °C/EG core complex. The signal intensity, as measured by area, decreased by about a half (Fig. 8c). No F_A and F_B signals were detected.

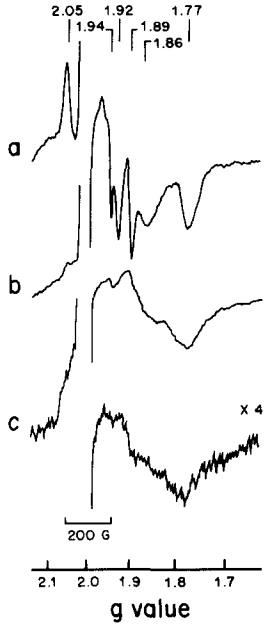

Fig. 8. EPR spectra of the iron sulfur centers of PS I particles and PS I core complex. (a) Untreated PS I particles; (b) 55 °C/EG core complex; (c) 60 °C/EG core complex. EPR experimental conditions were identical to those described in Fig. 6.

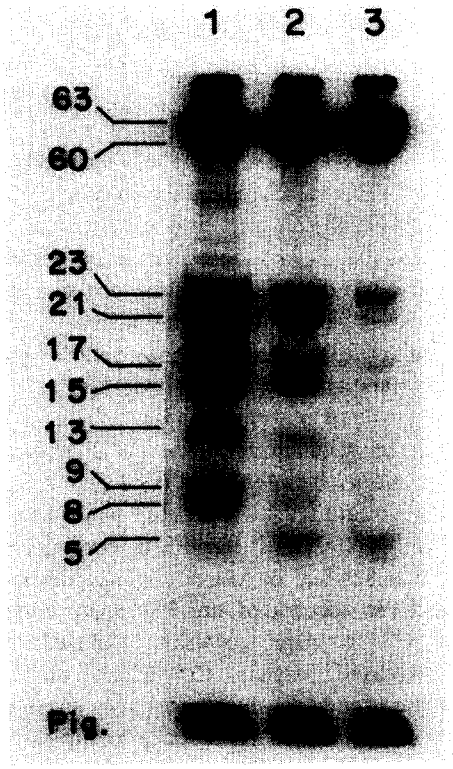

Fig. 9. Polypeptide compositions of PS I core complex obtained by Triton X-100 treatment of PS I particles after incubation at various temperatures in the presence of 50% (v/v) ethylene glycol. Lane 1, complex prepared from 25 °C/EG-treated particles; lane 2, 55 °C/EG core complex; lane 3, 60 °C/EG core complex.

Fig. 9 shows the polypeptide compositions of the core complexes. Heat/EG treatment facilitated the removal of the low-molecular weight polypeptides including the 8 kDa F_A/F_B protein from the high molecular polypeptides (60 and 63 kDa in the gel used here) carrying P-700 and F_X [11–13]. Traces of the 8 kDa polypeptide were detected in the 55 °C/EG core complex but were completely lost in the 60 °C/EG core complex. A slight cross reaction of the 8 kDa polypeptide in our gel was detected in the 55 °C/EG core complex but not in the 60 °C/EG core complex when the immunoblotting utilizing an antibody against the 9 kDa protein prepared by Oh-oka et al. [20] was carried out (Hoshina et al., unpublished data). The 60 °C/EG core complex contained polypeptides of about 5, 23, 60 and 63 kDa polypeptides and trace amounts of 21, 17 and 15 kDa polypeptides. The 23 kDa polypeptide is probably one of the LHC I apoprotein. The 5 kDa polypeptide seems to be closely associated with the 60 and 63 kDa polypeptides because almost the same amount of the 5 kDa band was detected in the 60 °C/EG core complex as that in the 25 °C/EG-treated PS I particles on the basis of 60 and 63 kDa polypeptides.

Discussion

The heat/EG treatment selectively destroyed F_A/F_B between 50 and 55 °C and F_X between 60 and 70 °C as shown by the change of $P\text{-}700^+$ re-reduction kinetics and by EPR spectroscopy in this study. The destruction of F_A and F_B centers seems to be accompanied by the denaturation of the 9 kDa protein and probably by its dissociation from the large core polypeptides. Destruction of P-700, on the other hand, occurred at the higher temperature range above 70 °C. The selective degradation of iron sulfur centers developed in this work gives a new type of PS I core or reaction center complex preparation.

Methyl viologen photoreduction by continuous light in thylakoid membranes was more sensitive to the heat/EG treatment (between 35 and 50 °C). This suggests another heat/EG sensitive site, which is probably on the PS I oxidizing side as suggested from the slower rate of P-700 reduction as shown in Fig. 2 inset. This may also be accompanied by the release or reorganization of some peripheral polypeptides bound to the intrathylakoidal surface (oxidizing side) of PS I reaction center complex, although it remains to be studied.

The heat/EG treatment, thus, is concluded to give a selective and simple method for the limited selective destruction of PS I complex. It is very interesting that the destructions of F_A/F_B, F_X and P-700 (and probably the oxidizing side) occurs in separate temperature regions which do not overlap each other in the presence

of ethylene glycol. This may reflect structurally independent nature of these components.

Ethylene glycol and glycerol are widely used as cryoprotectants to study membrane fluidity at low temperature [33,34] and are thought to disrupt hydrophobic interactions between or within proteins and perturb protein structure [35]. They are known to affect the redox potential of F_A and F_B determined by EPR [36]. Ethylene glycol accelerated the destruction of soluble spinach ferredoxin (Hoshina et al., unpublished data), which is known to be heat labile [37]. After the 55 °C/EG treatment for 5 min, the absorbance of ferredoxin at 420 nm was reduced to about 84% of control and the peak shifted to 417 nm. The 55 °C treatment without EG gave only a negligible effect. This result suggests that the high sensitivity to ethylene glycol (during the heat treatment) is a common feature among the proteins carrying iron sulfur center(s).

After heating at 55 °C, loss of acid-labile sulfide was 40–50% (Table I) and the loss of F_A and F_B detected by EPR or flash kinetics was more than 90% whereas F_X was not destroyed (Fig. 7). Thylakoids also contain [2Fe-2S] cluster in the Rieske-type protein which is present in about equimolar amounts with P-700 [38–40]. The loss of F_A and F_B (8 S^{2-}) roughly agrees with the decrease of 40–50% (6–7 S^{2-}/P-700) of acid-labile sulfide if no destruction of Rieske protein occurred (Table I).

Golbeck et al. [11,12] reported that the treatment of cyanobacterial PS I particles with lithium dodecyl sulfate removed F_A and F_B centers and changed the decay half-time of flash-induced P-700$^+$ from 30 ms to 1.2 ms. These two half-times were concluded to correspond to the reduction rates by F_A/F_B^- and F_X^-, respectively. The results in the present study also showed such change of P-700 reduction rate when F_A/F_B were selectively destroyed. This indicates that the heat/EG treatment selectively destroy iron-sulfur centers in a similar way as that by the detergent treatment and gives the core complexes depleted of F_A/F_B.

The bound iron-sulfur centers in PS I are reported to be inactivated by exposure to reagents such as urea-ferricyanide [41], sodium thiocyanate [42] or mercurials [43]. Urea-ferricyanide was the most potent inhibitor of these reagents, and also inhibited P-700 photooxidation in spinach PS I particles [41]. The heat/EG treatment used in this study seems to be milder than this treatment and can easily control the extent of destruction. Golbeck et al. [44] reported that PS I reaction center preparation from a cyanobacterium *Anacystic nidulans*, isolated by the treatment with 6.8 M urea at pH 10.0, contains P-700 and F_X but totally lacks F_A and F_B and that F_X in cyanobacteria is more stable to this treatment than that in spinach [44]. The PS I complex prepared by the heat/EG treatment of spinach PS I particles retains F_X but almost no F_A and F_B as judged by EPR spectrum, SDS-polyacrylamide gel electrophoresis and immunoblotting analysis. This complex seems to be comparable to the cyanobacterial complex [44], although F_X in our preparation deficient in F_A and F_B was partially lost and shows the broadened EPR lineshape (Fig. 8). However, the F_X EPR signal in the core complex isolated from cyanobacteria also shows the line broadening [44]. It is recently reported by Golbeck et al. [45] that the reconstitution of the cyanobacterial core complex with the spinach F_A/F_B protein, isolated according to Wynn and Malkin [23] reverted the EPR spectra of F_X, F_B and F_A to their original shapes [45]. Reconstitution of the heat/EG core complex with the F_A/F_B protein, therefore, may revert the line shape of F_X. This remains to be studied.

It is emphasized that little or no decrease of 5 kDa polypeptide was detected in the core complex from 55–60 °C/EG-treated particles. The role and characteristics of this polypeptide, which have not been characterized yet, are now under investigation.

Acknowledgements

We wish to thank: Professors K. Nishida and Y. Fujita for their support and helpful comments; the Analytical Center of National Institute for Basic Biology (NIBB) for the help in EPR measurement; Dr. H.Oh-oka of the Osaka University for immunoblotting analysis; Miss. M. Iwaki of the NIBB for the help in flash-induced spectroscopy; Dr. J.J. Brand of the University of Texas and Dr. W.P. Williams of King's College London, University of London for reviewing this manuscript; and Mr. M. Kawamoto for drawing the figures. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (62621004, 63621003) from the Ministry of Education, Science and Culture, Japan to KW.

References

1 Berry, J. and Björkman, O. (1980) Annu. Rev. Plant Physiol. 31, 491–543.
2 Katoh, S. and San Pietro, A. (1967) Arch. Biochem. Biophys. 122, 144–152.
3 Gounaris, K., Brain, A.P.R., Quinn, P.J. and Williams, W.P. (1983) FEBS Lett. 153, 47–52.
4 Thomas, P.G., Quinn, P.J. and Williams, W.P. (1986) Planta 167, 133–139.
5 Rutherford, A.W. and Heathcote, P. (1985) Photosynth. Res. 6, 295–316.
6 Sétif, P. and Mathis, P. (1986) in Encyclopedia of Plant Physiology, New Series Vol. 19, Photosynthesis III (Staehelin, L.A. and Arntzen, C.J., eds.), pp. 476–486, Springer-Verlag, Berlin.
7 Malkin, R. (1987) in Topics in Photosynthesis, Vol. 8, The Light Reactions (Barber, J., ed.), pp. 495–525, Elsevier, Amsterdam.
8 Andréasson, L.-E. and Vänngård, T. (1988) Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 379–411.
9 Golbeck, J.H., McDermott, A.E., Jones, W.K. and Kurtz, D.M. (1987) Biochim. Biophys. Acta 891, 94–98.

10 Bertrand, P., Guigliarelli, B., Gayda, J.-P., Sétif, P. and Mathis, P. (1988) Biochim. Biophys. Acta 933, 393–397.
11 Golbeck, J.H. and Cornelius, J.M. (1986) Biochim. Biophys. Acta 849, 16–24.
12 Warden, J.T. and Golbeck, J.H. (1986) Biochim. Biophys. Acta 849, 25–31.
13 Høj, P.B. and Møller, B.L. (1986) J. Biol. Chem. 261, 14292–14300.
14 Mathis, P., Sauer, K. and Remy, R. (1978) FEBS Lett. 88, 275–278.
15 Gast, P., Swarthoff, T., Ebskamp, F.C.R. and Hoff, A.J. (1983) Biochim. Biophys. Acta 722, 163–175.
16 Vierling, E. and Alberte, R.S. (1983) Plant Physiol. 72, 625–633.
17 Cammack, R. and Evans, M.C.W. (1975) Biochem. Biophys. Res. Commun. 67, 544–549.
18 Evans, E.H., Ruth, J.D., Johnson, C.E. and Evans, M.C.W. (1979) Biochem. J. 182, 861–865.
19 Høj, P.B., Svendsen, I., Scheller, J.V. and Møller, B.L. (1987) J. Biol. Chem. 262, 12676–12684.
20 Oh-oka, H., Takahashi, Y., Wada, K., Matsubara, H., Ohyama, K. and Ozeki, H. (1987) FEBS Lett. 218, 52–54.
21 Oh-oka, H., Takahashi, Y., Kuriyama, K., Saeki, K. and Matsubara, H. (1988) J. Biochem. 103, 962–968.
22 Oh-oka, H., Takahashi, Y., Matsubara, H. and Itoh, S. (1988) FEBS Lett. 234, 291–294.
23 Wynn, R.M. and Malkin, R. (1988) FEBS Lett. 229, 293–297.
24 Sétif, P., Mathis, P. and Vänngård, T. (1984) Biochim. Biophys. Acta 767, 404–414.
25 Hoshina, S. and Itoh, S. (1987) Plant Cell Physiol. 28, 599–609.
26 Hoshina, S. (1979) Plant Cell Physiol. 20, 1107–1116.
27 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171.
28 Ikegami, I. and Itoh, S. (1987) Biochim. Biophys. Acta 893, 517–523.
29 Itoh, S., Iwaki, M. and Ikegami, I. (1987) Biochim. Biophys. Acta 893, 508–516.
30 Golbeck, J.H. and San Pietro, A. (1976) Anal. Biochem. 73, 539–542.
31 Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
32 Koike, H. and Katoh, S. (1982) Photochem. Photobiol. 35, 527–531.
33 Cox, R.P. (1975) Biochim. Biophys. Acta 387, 588–598.
34 Murata, N. and Fork, D.C. (1977) Plant Cell Physiol. 18, 1265–1271.
35 De Kok, J., Braslavsky, S.E. and Spruit, C.J.P. (1981) Photochem. Photobiol. 34, 705–710.
36 Evans, M.C.W. and Heathcote, P. (1980) Biochim. Biophys. Acta 590, 89–96.
37 Wada, K. (1979) J. Biochem. 86, 1747–1752.
38 Malkin, R. and Aparicio, P.J. (1975) Biochem. Biophys. Res. Commun. 63, 1157–1160.
39 Hauska, G. (1986) in Encyclopedia of Plant Physiology, New Series Vol. 19, Photosynthesis III (Staehelin, L.A. and Arntzen, C.J., eds.), pp. 496–507, Springer-Verlag, Berlin.
40 Cramer, W.A., Black, M.T., Widger, W.R. and Girvin, M.E. (1987) in Topics in Photosynthesis, Vol. 8, The Light Reactions (Barber, J., ed.), pp. 447–493, Elsevier, Amsterdam.
41 Golbeck, J.H. and Warden, J.T. (1982) Biochim. Biophys. Acta 681, 77–84.
42 Bonnerjea, J., Ortiz, W. and Malkin, R. (1985) Arch. Biochem. Biophys. 240, 15–20.
43 Kojima, Y., Niinomi, Y., Tsuboi, S., Hiyama, T. and Sakurai, H. (1987) Bot. Mag. Tokyo 100, 243–253.
44 Golbeck, J.H., Parrett, K.G., Mehari, T., Jones, K.L. and Brand, J.J. (1988) FEBS Lett. 228, 268–272.
45 Golbeck, J.H., Mehari, T., Parrett, K.G. and Ikegami, I. (1988) FEBS Lett. 240, 9–14.