A Possible Role of Redox-Active Histidine in the Photoligation of Manganese into a Photosynthetic O_2-Evolving Enzyme†

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Received January 15, 1991; Revised Manuscript Received March 25, 1991

ABSTRACT: Our previous experiments with a histidine modifier suggest that in Mn-depleted photosystem (PS) II a histidine residue is photooxidized and charge recombination between the oxidized histidine and Q_A^- emits the thermoluminescence A_T-band [Ono, T., & Inoue, Y. (1991) *FEBS Lett. 278*, 183–186]. By use of the A_T-band as an index for histidine oxidation, and EPR signals II_f and II_s as indexes for tyrosine oxidation, we studied the role of this putative redox-active histidine in the photoactivation of the O_2-evolving enzyme in NH_2OH-treated PSII. The following results have been obtained. (i) Strong-light photoinhibition of NH_2OH-treated PSII quickly impaired both capabilities of photoactivation and A_T-band emission with almost the same half-inhibition time of 1–2 s, while signal II_f was well retained and signal II_s was not affected at all after complete loss of photoactivation capability. (ii) The capability of exogenous Mn^{2+} photooxidation was relatively sensitive to strong-light photoinhibition, but DPC photooxidation was highly resistant. (iii) Weak-light photoinhibition simultaneously impaired the capabilities of photoactivation, A_T-band emission, and signal II_f with the same half-inhibition time of 1 min, leaving signal II_s unaffected. (iv) It was inferred that the putative redox-active histidine is essential for the photooxidation of coordinated Mn^{2+}, the probable initial step of photoactivation, and its photodamage results in the loss of the capabilities of photoactivation and A_T-band emission. Based on these, a scheme of electron transfer on the donor side of PSII involving histidine oxidation via Z^+ is proposed.

The tetrameric Mn cluster essential for photosynthetic O_2 evolution is assembled in PSII[1] through photoactivation. Kinetic analyses have indicated that at least two sequential photochemical events are involved in the process with a rate-limiting dark reaction in between. Kinetic features of photoactivation are universal for dark-grown algal cells and gymnosperm leaves (Cheniae & Martin, 1973; Oku & Tomita, 1976), intermittently flashed angiosperm leaves (Remy, 1973; Inoue et al., 1974), algal cells grown under a Mn deficiency (Cheniae & Martin, 1971), or chloroplasts and PSII membranes depleted of Mn by various means (Yamashita & Tomita, 1976; Tamura & Cheniae, 1986). The primary step of photoactivation is considered to be the oxidation of a coordinated Mn^{2+} atom by PSII photochemistry followed by subsequent oxidation of the other Mn^{2+} atoms through unknown dark reactions (Ono & Inoue, 1987; Tamura & Cheniae, 1987).

On illumination of Mn-depleted PSII in the absence of externally added Mn^{2+}, the donor side of PSII is selectively photodamaged (photoinhibition). When this photodamage occurs, the capability of photoactivation is most preferentially lost among other PSII reactions (Blubaugh & Cheniae, 1990a). This suggests that a redox component essential for photoactivation is selectively impaired by photoinhibition. As for photoinhibition under weak light, Blubaugh and Cheniae (1990b) reported that the course of photodamage of Z, tyrosine-160 of the D1 protein, coincides with the loss of photoactivation capability. This implies that oxidized Z (Z^+) is responsible for Mn^{2+} oxidation as the initial step of photoactivation. This view is consistent with the general consensus that Z^+ functions as the terminal oxidant on the donor side of PSII and oxidizes various externally added electron donors when the Mn cluster is disrupted. As for photoinactivation under strong light, on the other hand, Klimov et al. (1990) have suggested that the photoactivation capability is markedly inhibited while the EPR signal II_f arising from Z^+ remains photoinducible. This implies that Mn^{2+} oxidation in photoactivation is mediated by an unknown redox component other than Z^+.

Recently, we reported biochemical evidence suggesting that a histidine residue is stably photooxidized in Tris-treated PSII membranes that are completely depleted of Mn atoms and that charge recombination between the oxidized histidine and Q_A^- gives rise to a thermoluminescence (TL) band peaking at −20 °C (A_T-band) (Ono & Inoue, 1991). It was furthermore found that low concentrations of Mn^{2+} markedly suppress the A_T-band probably by rereducing the oxidized histidine. These results led us to hypothesize that this putative histidine residue is located in close vicinity of the Mn^{2+}-ligating site and functions as an intrinsic oxidant for Mn^{2+} oxidation in photoactivation. To test this hypothesis, we compared the effects of photoinhibition on the capability of photoactivation and A_T-band formation as well as those on various PSII activities in NH_2OH-treated PSII membranes. The results showed that stable oxidation of the putative histidine residue as monitored by the TL A_T-band was impaired in a manner parallel with the loss of photoactivation capability under both weak- and strong-light photoinhibition while photodamage of other PSII

† This work was supported by a grant for Photosynthetic Sciences and the Biodesign Research Program (to T.O.) at the Institute of Physical and Chemical Research (RIKEN) given by the Science and Technology Agency (STA) of Japan and partly by MESC Grants-in-Aid for Cooperative Research No. 0132064 and No. 0130009.

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[1] Abbreviations: Chl, chlorophyll; D, auxiliary electron donor of photosystem II; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DPC, diphenylcarbazide; EPR, electron paramagnetic resonance; Mes, 4-morpholineethanesulfonic acid; PS, photosystem; Q_A, primary quinone acceptor of photosystem II; Q_B, secondary quinone acceptor of photosystem II; TL, thermoluminescence; Z, secondary electron donor of photosystem II.

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activities including signal II_f and II_s formation proceeded with respectively different sensitivities. On the basis of these results, a possible role of the putative redox-active histidine in photoactivation and O_2 evolution is discussed.

Materials and Methods

PSII Membranes Preparation. Triton X-100 solubilized BBY-type PSII membranes were prepared from spinach as described (Ono & Inoue, 1986) and stored in liquid N_2. After thawing, the membranes (0.5 mg of Chl/mL) were incubated in 2 M NaCl/400 mM sucrose/20 mM NaCl/40 mM Mes–NaOH (pH 6.5) for 20 min at 0 °C in the dark for complete depletion of 16- and 24-kDa extrinsic proteins. The NaCl-washed PSII membranes (0.5 mg of Chl/mL) were then incubated in 400 mM sucrose/20 mM NaCl/1 mM NH_2OH/40 mM Mes–NaOH (pH 6.5) at 0.5 mg of Chl/mL for 1 min at 0 °C in the dark to deplete Mn, followed by one wash with 400 mM sucrose/20 mM NaCl/0.5 mM EDTA·2Na/40 mM Mes–NaOH (pH 6.5). The NH_2OH-treated membranes were then washed twice with 400 mM sucrose/20 mM NaCl/40 mM Mes–NaOH (pH 6.5) and suspended in the same medium. The resulting membranes were either used immediately or used after storage in liquid N_2. Photoactivation capability was not affected by the storage.

Photoinhibition. NH_2OH-treated PSII membranes (NH_2OH-PSII) were photoinhibited as described (Blubaugh & Cheniae, 1990; Klimov et al., 1990), with modifications. An aliquot (1 mL) of NH_2OH-PSII (0.25 mg of Chl/mL) suspended in 400 mM sucrose/20 mM NaCl/40 mM Mes–NaOH (pH 6.5) in a glass vial was illuminated with weak light from a fluorescent lamp (0.7 mW/cm^2) or with strong yellow light (300 mW/cm^2) from a 500-W tungsten lamp through a layer of cutoff filter (>500 nm) and three layers of heat-cut filters. After illumination for a given time, the sample was quickly cooled on ice and subjected to such assays as TL, DCIP photoreduction, and EPR spectra with or without condensation by centrifugation. Duration of exposure to strong light was controlled with a mechanical shutter placed before the sample.

Photoactivation. Photoactivation was effected according to the method of Tamura and Cheniae (1986) with modifications (Miyao et al., 1990). The photoinhibited NH_2OH-PSII (0.3 mg of Chl/mL) was suspended in 400 mM sucrose/20 mM NaCl/40 mM Mes–NaOH (pH 6.5) supplemented with 100 mM NaCl/20 mM $CaCl_2$/2 mM $MnCl_2$/0.01 mM DCIP and illuminated at room temperature with a fluorescent lamp (0.7 mW/cm^2). After illumination for 20 min, the samples were directly subjected to the O_2-evolution assay.

Activity Assays. O_2-evolving activity was measured with a Clark-type oxygen electrode as described (Ono & Inoue, 1986) at 25 °C in 400 mM sucrose/20 mM NaCl/20 mM $CaCl_2$/40 mM Mes–NaOH (pH 6.5) supplemented with 0.8 mM phenyl-*p*-benzoquinone as the electron acceptor. DCIP photoreduction at room temperature was measured spectroscopically at 600 nm in the same medium ($CaCl_2$ omitted) supplemented with 1 mM DPC or 0.1 mM $MnCl_2$ as the electron donor as described (Ono & Inoue, 1987). EPR signals II_s and II_f were recorded at 20 °C with a JEOL X-band EPR spectrometer Model JES FE1XG as described (Ono & Inoue, 1989). For TL measurements, samples were excited by continuous light (>500 nm) at −23 °C unless otherwise noted and cooled rapidly in liquid N_2, and the light emission during warming was recorded against the sample temperature as described (Ono & Inoue, 1989). DCMU was not included in the sample to allow multiple turnovers of PSII reaction center unless otherwise noted.

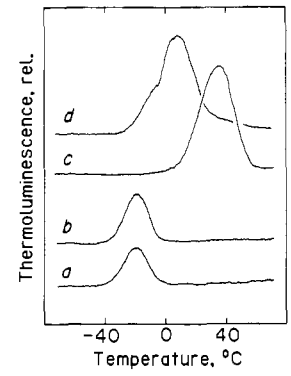

FIGURE 1: Effect of NH_2OH treatment on TL glow curves from PSII membranes. NH_2OH-treated PSII membranes were excited by continuous light for 15 s at −23 °C in the absence (a) or presence (b) of 10 μM DCMU. Control PSII membranes were excited by a single flash at 0 °C (c) or by continuous light for 15 s at −23 °C in the presence of 10 μM DCMU (d).

Results

Figure 1 shows TL glow curves induced by continuous illumination at −23 °C of normal (untreated) and NH_2OH-treated PSII membranes (NH_2OH-PSII). The normal O_2-evolving membranes exhibited TL bands at around 10 °C (d, +DCMU) and 35 °C (c, −DCMU) arising from $S_2Q_A^-$ and $S_2Q_B^-$ charge recombination, respectively (Rutherford et al., 1982). Note that the sample for curve c was excited by a single flash at 0 °C, since continuous illumination at −23 °C induced both $S_2Q_B^-$ and $S_2Q_A^-$ bands due to the low temperature induced partial suppression of electron tranfer between Q_A and Q_B. NH_2OH-PSII, on the other hand, showed a TL band peaking at around −20 °C in both the presence and absence of DCMU but had no band above 0 °C, in accordance with the absence of the Mn cluster in NH_2OH-PSII. On the basis of its peak temperatures and dependencies on pH and excitation temperature (data not shown), the TL band at −20 °C was identified as the A_T-band, which had been observed in Tris-washed PSII (Koike et al., 1986; Ono & Inoue, 1991). By use of a histidine modifier, diethyl pyrocarbonate, this band was suggested to arise from the recombination between oxidized histidine and Q_A^- (Ono & Inoue, 1991).

Figure 2 shows the effect of strong-light photoinhibition on the A_T-band. The capability of A_T-band formation was rapidly lost by photoinhibition: the band height was suppressed to about 10% of the original after 15 s of illumination with strong light. The shape of the band, however, did not change, and no new band was induced in place of the A_T-band. These results imply that photoinhibition impairs the capability of charge pair formation without any changes in the thermodynamic parameters of the charge pair due to photodamage. Since the TL band in normal PSII arising from the $S_2Q_A^-$ charge pair was totally resistant to the same strong illumination (data not shown), we can conclude that the high sensitivity of the A_T-band to strong light is due to the absence of electron donation from the Mn cluster in NH_2OH-PSII.

Figure 3 shows the effects of strong-light photoinhibition of Z^+ and D^+ in NH_2OH-PSII, the oxidized forms of secondary and auxiliary electron donors of PSII, respectively. EPR signals were measured in the absence of DCMU so as to allow multiple turnovers of PSII photoreaction. Before photoinhibition, the EPR signal II_f arising from Z^+ was photoinduced by being superimposed on the dark stable signal II_s arising from D^+, since quick rereduction of Z^+ is blocked by the absence of the Mn cluster in NH_2OH-PSII. When photoinhibited, a free radical signal from an unidentified source became pronounced after 2 s of photoinhibition and was almost

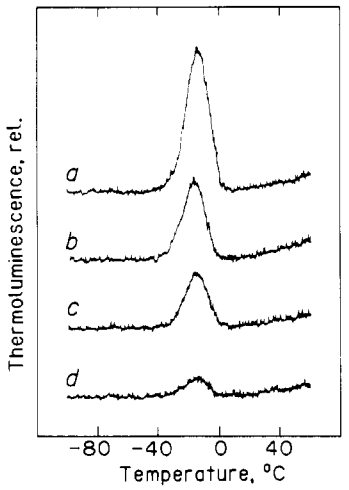

FIGURE 2: Effects of strong-light photoinhibition on the TL A_T-band. NH_2OH-treated PSII membranes were illuminated with strong light (>500 nm, 300 mW/cm^2) for 0 s (a), 2 s (b), 4 s (c), or 15 s (d) and then subjected to TL measurement. TL excitation was done as in Figure 1 (a).

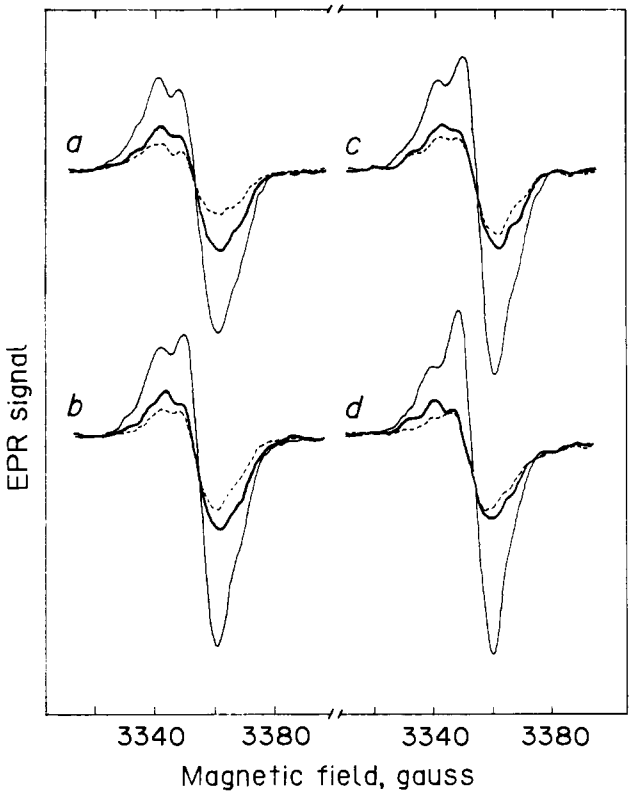

FIGURE 3: Effect of strong-light photoinhibition on EPR signals II_f and II_s. NH_2OH-treated PSII membranes were photoinhibited for 0 s (a), 2 s (b), 4 s (c), or 15 s (d) as described in the Figure 2 legend. Dashed curves indicate the dark spectra before illumination; thin curves indicate the light spectra during illumination, and bold curves indicate the dark after light spectra recorded after 1 min of dark relaxation. Chl concentrations were 3.3 (a), 3.3 (b), 3.1 (c), and 3.0 (d) mg of Chl/mL. Instrumental settings: microwave power, 5 mW; modulation frequency and amplitude, 100 kHz and 4 G, respectively; microwave frequency, 9.43 GHz; temperature, 20 °C.

fully developed after 15 s. Notably, however, signal II_f could still be largely induced even after 15 s of photoinhibition. This indicates that the capability to oxidize Z is well retained while the capability to induce the A_T-band is severely impaired by photoinhibition (cf. Figure 2). If we assume that the terminal oxidant that will be accumulated upon the illumination of Mn-depleted PSII is not Z^+ but oxidized histidine, the putative

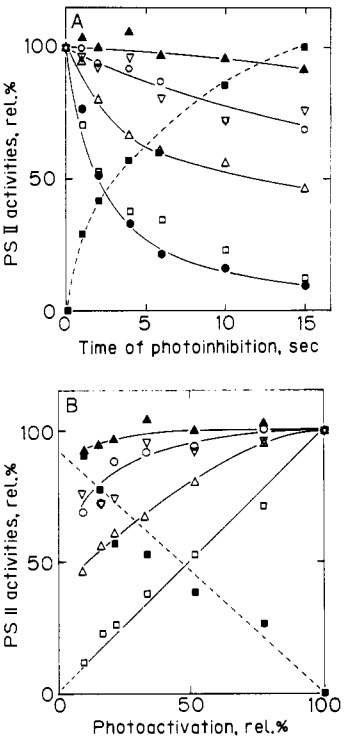

FIGURE 4: Time course of strong-light photoinhibition in NH_2OH-treated PSII membranes. (Panel A): Photoactivation (●), TL A_T-band (□), EPR signal II_f (○), EPR signal II_s (▲), g = 2.0 radical (■), and photooxidation of DPC (▽) or Mn^{2+} (△). Photoactivation capability was expressed by O_2 evolution recovered by 20 min of illumination. The amplitudes of signal II_f, signal II_s, and g = 2.0 radical were estimated from the peak heights at 3340 (signal II) and 3345 (g = 2.0 radical) G on the derivative spectra shown in Figure 3. For g = 2.0 radical signal, the contribution of signal II_{s+f} was estimated from the peak height at 3340 G and subtracted. All signal amplitudes were normalized on an Chl basis. Instrumental settings for EPR measurement are the same as described in the Figure 3 legend. O_2 evolution before and after photoactivation was 27 and 385 μmol of O_2/mg of Chl per h. Panel B is a variation of panel A data, in which various activities were replotted with respect to the photoactivation capability. As for the ordinate scale, 100% stands for 1432, 378, and 78 microelectron equivalents/mg of Chl per h for photoactivation, DPC oxidation, and Mn^{2+} oxidation, respectively. DPC-photooxidation and Mn^{2+}-photooxidation activities were presented after the subtraction of basal activity (25 microelectron equivalents/mg of Chl per h) measured without supplement of DPC or Mn^{2+}. For g = 2.0 radical, the EPR signal intensity after 15 s of photoinhibition was assumed as 100% in Panel A but as 92% in panel B, taking into account the 8% active O_2 centers remaining in NH_2OH-treated PSII.

positive charge for the A_T-band, we may expect that the redox-active histidine will easily degrade. It also appears likely that the unidentified free radical will originate from the species that is oxidized in place of the redox-active histidine. Notably also, the amplitude of signal II_s was not much affected by the photoinhibition. It is thus inferred that the oxidation of tyrosine residues responsible for Z^+ and D^+ is not much impaired by strong-light photoinhibition but that the capability of histidine oxidation as monitored by the capability of A_T-band formation is selectively photodamaged.

Figure 4A shows the changes of various PSII activities during strong-light photoinhibition: DCIP photoreduction supported by DPC or Mn^{2+}, signals II_s and II_f formation, A_T-band formation, and photoactivation of O_2 evolution enzyme. These activities were measured with use of the same batch of photoinhibited NH_2OH-PSII. The capability of photoactivation as expressed by the recovery of O_2-evolving activity was rapidly lost with an apparent half-time of about 2 s; only 10% capability remained after photoinhibition for

Table I: Half-Inhibition Times of Various PSII Activities during the Strong-Light Photoinhibition of NH_2OH-Treated PSII[a]

	half-inhibition time (s) (relative amplitude (%))			
components	photoactivation	A_T band	Mn → DCIP	signal II_f
fast	1.4 (55)	1.5 (50)	1.7 (22)	– (0)
slow	6.4 (45)	8.4 (50)	17 (78)	22 (100)

[a] Relative amplitudes of fast and slow components are indicated in parentheses. The photoactivation capability was estimated from O_2 evolution recovered by 20 min of illumination with continuous light. The signal II_f amplitude was estimated as described in the Figure 4 legend.

15 s. This inactivation course (●) agreed with that of A_T-band suppression (□). The activity of Mn^{2+} photooxidation was also susceptible to photodamage, but its inhibition course was slower than those of photoactivation and A_T-band capabilities. DPC photooxidation and signal II_f were rather insensitive to photoinhibition: 70–80% capabilities remained after photoinhibition for 15 s. Signal II_s was almost completely resistant to photoinhibition, while the free radical signal increased rapidly with an apparent half-rise time of about 2.5 s (■). These results indicate that a specific component on the donor side of PSII is selectively impaired by strong-light photoinhibition, while the acceptor side of PSII is not much affected.

Figure 4B is a variation of Figure 4A data, in which various PSII activities were plotted against the photoactivation capability remaining after photoinhibition. The A_T-band formation (□) exhibited a directly proportional relationship with photoactivation capability, showing an intersect at the origin, while the other activities did not show such a relationship. This strongly suggests that the oxidation of the putative redox-active histidine residue assumed to be responsible for the A_T-band plays an essential role in photoactivation. The unidentified free radical signal (■) was inversely proportional to the photoactivation capability, showing an intersect at around 90% of PSII activities. From this inverse relationship, we may deduce that this radical originates from some redox species close to the putative histidine and is formed when the A_T-band capability is impaired. We tentatively presume that the radical is likely due to the Chl cation.

As shown in Figure 4A, the inactivation courses of photoactivation capability, A_T-band capability, and Mn^{2+} oxidation are biphasic. The half-times of the fast and slow components for the two inactivation processes were calculated from respective semilogarithmic plots of the data in Figure 4A and listed in Table I, together with their amplitudes. Inhibition courses of photoactivation capability and A_T-band capability were similarly decomposed into fast ($t_{1/2}$ = 1.5 s) and slow ($t_{1/2}$ = 6–8 s) components with a relative amplitude of 1:1, suggesting that these two inhibition processes consist of two respective mechanisms. For the inactivation of Mn^{2+} photooxidation, a fast component having a similar short half-time ($t_{1/2}$ = 1.7 s) was found, but its relative amplitude was as large as 20%. The loss of signal II_f exhibited only one very slow component of $t_{1/2}$ = 22 s. This half-time was comparable to the slow component found for Mn^{2+} oxidation ($t_{1/2}$ = 17 s).

Figure 5 shows the effect of weak-light photoinhibition on the A_T-band and signals II_s and II_f. Weak-light illumination markedly diminished the A_T-band capability: the amplitude of the A_T-band was suppressed to less than 15% of that of control membranes after photoinhibition for 15 min (panel A). As shown in panel B, the same weak-light illumination concomitantly suppressed signal II_f capability. This contrasts to the very slight effect of strong-light photoinhibition on signal II_f (see Figures 2 and 3). Signal II_s, however, was not affected

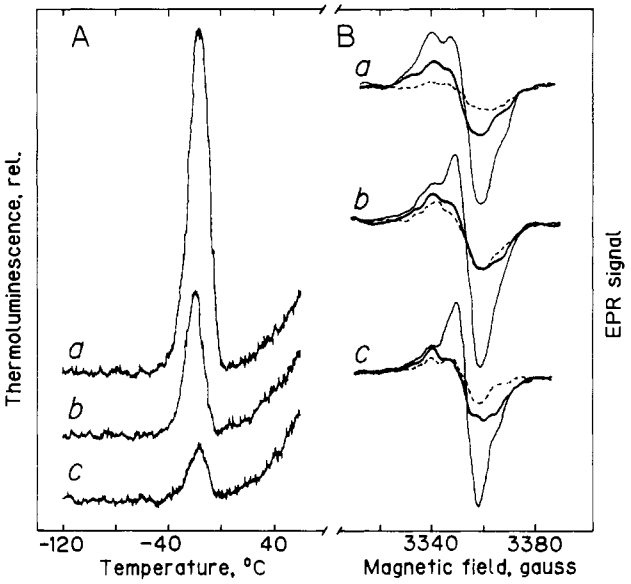

FIGURE 5: Effects of weak-light photoinhibition on the TL A_T-band (A) and EPR signals II_f and II_s (B). NH_2OH-treated PSII membranes were photoinhibited for 0 min (a), 2 min (b), or 10 min (c) (white light, 0.7 mW/cm^2). TL excitation was done as in Figure 1 (a). In panel B, dashed curves indicate the dark spectra, thin curves indicate the light spectra during illumination, and bold curves indicate the dark after light spectra recorded after 1 min of dark relaxation. Instrumental settings for EPR measurements were the same as described in the Figure 3 legend, and Chl concentrations were 4.2 (a), 3.9 (b), and 4.0 (c) mg of Chl/mL.

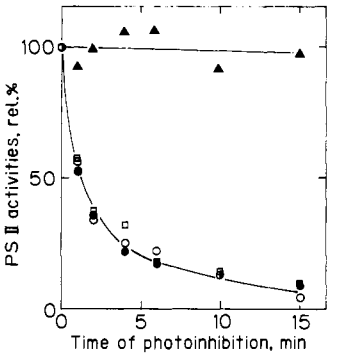

FIGURE 6: Time course of weak-light photoinhibition in NH_2OH-treated PSII membranes: photoactivation (●), TL A_T-band (□), EPR signal II_f (○), and EPR signal II_s (▲). The photoactivation capability was represented by O_2 evolution recovered by 20 min of continuous illumination. O_2 evolution before and after photoactivation was 20 and 348 μmol of O_2/mg of Chl per h in non-photoinhibited membranes. Amplitudes of signals II_f and II_s were estimated from the peak heights at 3340 G of the spectra as shown in Figure 5.

by weak-light photoinhibition, although the free radical signal presumed to arise from Chl^+ was appreciably induced.

Figure 6 shows the time course of weak-light photoinhibition. The capabilities of both photoactivation (●) and signal II_f (○) were markedly suppressed after weak illumination for 10 min, while signal II_s (▲) was not affected, in agreement with the results of Blubaugh and Cheniae (1990b). The A_T-band capability (□) was photoinhibited with the same course as that of photoactivation. This is consistent with the conclusion derived from strong-light photoinhibition that the A_T-band capability is closely correlated with photoactivation capability.

Table II compares the two protocols of photoinhibition by strong light and weak light. After photoinhibition by 15 s of strong light or 15 min of weak light, recovery of O_2 evolution by photoactivation was suppressed to about 10% of the original level, and the A_T-band capability was impaired to similar

Table II: Effects of Strong-Light and Weak-Light Photoinhibition on the Capabilities of Photoactivation, Thermoluminescence A_T-Band Formation, and Induction of EPR Signals II_f and II_s in NH_2OH-Treated PSII[a]

activities	non-photoinhibited control	photoinhibited: strong light (15 s)	photoinhibited: weak light (15 min)
photoactivation	100 (380)	8 (30)	9 (35)
A_T-band (histidine oxidation)	100	12	8
signal II_f (D1 tyrosine-160 oxidation)	100	70	4
signal II_s (D2 tyrosine-161 oxidation)	100	90	95

[a] The numbers in parentheses represent the O_2-evolving activity in micromoles of O_2 per milligram of Chl per hour. The photoactivation capability was represented by O_2 evolution recovered by 20 min of illumination with continuous light. The amplitudes of EPR signals II_f and II_s were measured as described in the legends of Figures 4 and 6.

extents by both protocols. In contrast, signal II_f was markedly photodamaged by weak-light photoinhibition but not much by strong-light photoinhibition, whereas signal II_s was highly resistant to both photoinhibitions. These results indicate that the donor side of PSII is differently damaged by strong- and weak-light illumination, although the capabilities of photoactivation and A_T-band formation are equally inhibited.

Discussion

The present results showed that the donor side of PSII is differently photodamaged when NH_2OH-PSII is illuminated under weak light or strong light. Under weak light, the capability of photoactivation of O_2 evolution diminished in a manner parallel with the loss of signal II_f capability, in agreement with the results reported by Blubaugh and Cheniae (1990a,b). On the basis of these results, they proposed that the oxidized tyrosine-160 of the D1 protein is involved in photoactivation presumably as a mediator for Mn^{2+} oxidation. Under strong-light illumination, on the other hand, photoactivation capability was inhibited with higher specificity, leaving signal II_f unaffected. This implies that the photodamage of Z observed under weak-light photoinhibition is not always the direct cause for the loss of photoactivation capability by photoinhibition. In contrast, the acceptor side of PSII is not much impaired by photoinhibition under both strong light (Figure 4) and weak light (Blubaugh & Cheniae, 1990; T.O. and Y.I., unpublished data).

Our results in this study suggest that the photodamage of photoactivation capability is more closely correlated with the loss of TL A_T-band capability than with the loss of signal II_f (Z^+). In our previous report (Ono & Inoue, 1991), we demonstrated that a histidine residue is indispensable for A_T-band formation and proposed that the positive charge for the A_T-band is an oxidized histidine residue. If we extend this idea to interpret the present results, we may consider that the redox-active histidine is essential for photoactivation. It is generally considered that in photoactivation a Mn(II) atom coordinated to its binding site is photooxidized to Mn(III) before being assembled to an active Mn cluster (Ono & Inoue, 1987; Tamura & Cheniae, 1987). Taking into account the finding in our previous paper that exogenously added Mn^{2+} is accessible to the putative histidine residue with an affinity similar to that for photoligation of Mn (Ono & Inoue, 1990), we assume that exogenous Mn^{2+} coordinates directly to this histidine residue or to a close vicinity of the residue to be subsequently photooxidized to Mn(III).

The course of strong-light photodamage of Mn^{2+}-photooxidation capability exhibited a component that apparently corresponds to the fast components found for the loss of photoactivation and A_T-band capabilities (Table I). Although this component accounts for only 20% of the total activity, we assume that this component corresponds to Mn^{2+} oxidation mediated by oxidized histidine: photodamage of this redox-active histidine results in the inhibition of Mn^{2+} photooxidation. The other slow component that dominates during strong-light photoinhibition may be ascribed to oxidation by Z^+ and/or D^+ as suggested by Blubaugh and Cheniae (1990a,b), since its $t_{1/2}$ roughly agrees with that of signal II_f photoinhibition (Table I). Tamura et al. (1989) have reported that chemical modification of histidine residues by diethyl pyrocarbonate inhibits photoactivation capability. Since diethyl pyrocarbonate selectively inhibits the A_T-band formation (Ono & Inoue, 1991), it may be reasonable to assume that the inhibition of photoactivation by diethyl pyrocarbonate is ascribed to modification of the histidine residue responsible for the A_T-band. If we further speculate by taking into account the finding that Mn^{2+} specifically suppresses radiolabeling of the D1 protein by [^{14}C]diethyl pyrocarbonate (Tamura et al., 1989), we may infer that our histidine responsible for the A_T-band is located on the D1 protein.

As shown in Figure 6, the loss of A_T-band capability under weak-light photoinhibition proceeded in a manner parallel with the loss of signal II_f capability, not only with respect to its inactivation course but also with respect to its extent. This indicates that in weak-light photoinhibition the loss of signal II_f capability is responsible for the suppression of A_T-band formation: the putative histidine is oxidized by $P680^+$ probably via tyrosine-160 (Z). This idea is consistent with the observation that the PSII photoreaction as monitored by the electron transfer from DPC and DCIP is not much damaged under this photoinhibition protocol (Blubaugh & Cheniae, 1990a; T.O. and Y.I., unpublished data). We may thus consider that the putative histidine residue functions in photoactivation as a redox mediator between Z and coordinated Mn^{2+}. This view is also consistent with the predicted three-dimensional structure model of the PSII reaction center in which the two tyrosine residues for Z and D are located to form hydrogen bonds to nearby histidine residues (Svensson et al., 1990).

An additional issue in this study is that the redox properties of the putative histidine suffice the conditions required for a redox-active ligand for the Mn cluster as proposed by Kambara et al. (1985). They postulated that a histidine residue ligating Mn mediates electron transfer between Z^+ and the Mn cluster in S-state transition (Padhye et al., 1986). Recently it was reported that illumination of Ca-depleted PSII membranes induces new EPR and TL signals, which are likely to be assumed to arise from an oxidized amino acid (histidine) (Boussac et al., 1989; Sivaraja et al., 1989; Ono & Inoue, 1990), and Boussac et al. (1990) correlated this new oxidizing equivalent to the formal S_3-state. We do not have sufficient evidence that this oxidized histidine is identical with the redox-active histidine discussed in this study because of the differences in experimental conditions: their formal S_3-state histidine is oxidized in Ca-depleted PSII retaining the Mn cluster, while the A_T-band histidine is oxidized only after depletion of the Mn cluster. It is, however, worthwhile to note that the lower limiting temperature for A_T-band induction (Koike et al., 1989; T.O. and Y.I., unpublished data) coincides with that of histidine oxidation in Ca-depleted PSII (Ono & Inoue, 1990) or with that of the S_2 to S_3 transition (Brudvig et al., 1983). Furthermore, the peak temperature of the A_T-band is similar to the emission temperature of the TL A-band arising from $S_3Q_A^-$ charge recombination (Koike et

al., 1986), indicating that the oxidized histidine assumed as the positive charge for the A_T-band has an oxidation potential roughly the same as that of the S_3-state. These considerations lead us to speculate that the same histidine is oxidized in both Mn-depleted PSII and Ca-depleted PSII.

The histidine oxidation in Ca-depleted PSII may also be interpreted in a different way: it does not correspond to the formal S_3-state but is an auxiliary reaction due to the inhibition of the S_2 to S_3 transition by Ca depletion (Ono & Inoue, 1989). This view may be supported by a more recent paper by Baumgarten et al. (1990) that a $g = 2$ EPR signal appeared upon the illumination of Cl^--depleted PSII in which the S-state transition was blocked at the S_2-state. Since their signal resembles those found in Ca^{2+}-depleted PSII, this may be another observation of oxidized histidine in the presence of the Mn cluster. Boussac et al. (1990) proposed that oxidized histidine will be magnetically coupled with the multiline center of the Mn cluster to yield their $g = 2$ EPR signal. The magnetic coupling will be reasonably assumed in view of the interpretation that the redox-active histidine is likely to be located in close vicinity of the Mn cluster. Further studies are required to determine the functional relationships between the two redox-active histidine residues suggested in Mn-depleted and Ca-depleted PSII.

Throughout this paper, we simply assumed that the positive charge responsible for the A_T-band is an oxidized histidine, mostly on the basis of our previous results (Ono & Inoue, 1991) that a histidine modifier prevented the capability of A_T-band formation. However, if one scrutinizes our previous results, one may alternatively assume that the positive charge carrier is not the histidine residue itself but something else and that the histidine residue is subsidiarily needed for its redox reaction. This may certainly be a matter of argument in view of the situation that the evidence for histidine oxidation is not always completely unequivocal. Practically, however, we feel it difficult to conceive any more likely candidates other than histidine, since we have to exclude Z^+, D^+, cytochrome b_{559}, and chlorophyll from the possible candidates for positive charge carrier for the A_T-band (Ono & Inoue, 1991). It has to be also argued how the strong-light photoinhibition inactivates the redox histidine while leaving Z unaffected.

Shelving these arguments and questions for the present, our results can be speculatively summarized as follows. On the donor side of Mn-depleted PSII, electrons are transferred from the redox-active histidine residue to the reaction center (P680) via Z (the secondary donor of PSII) and the positive charge accumulated on the histidine residue oxidizes exogenous Mn^{2+} to initiate the assembly of the Mn cluster. The oxidized forms of histidine and tyrosine residues are unstable and are degradated or inactivated during illumination with strong and weak light, respectively, unless rereduced quickly, resulting in the loss of the capabilities of photoactivation and A_T-band formation. When these residues are inactivated, chlorophyll molecules are oxidized by either Z^+ under strong light or $P680^+$ under weak light. Among the redox-active components on the donor side of PSII, P680 is a dimer of chlorophyll *a* coordinating to both D1 and D2 proteins (Michel et al., 1986) and Z is tyrosine-160 of D1 protein (Debus et al., 1988). Taking these facts into account, we tentatively hypothesize that the putative histidine is an amino acid residue of the D1 protein. Further studies are obviously needed to confirm the involvement of the histidine residue in the electron-transfer reaction on the donor side of PSII.

Registry No. L-His, 71-00-1; Mn, 7439-96-5.

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