Volume 278, number 2, 183–186 FEBS 09330 January 1991

ADONIS 0014579391001014

Biochemical evidence for histidine oxidation in photosystem II depleted of the Mn-cluster for O_2-evolution

Taka-aki Ono and Yorinao Inoue

Solar Energy Research Group, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan

Received 30 October 1990; revised version received 29 November 1990

Flash excitation of Tris-treated photosystem II (PSII) membranes generated a thermoluminescence band peaking at ~20 °C, indicating that a positively charged oxidizing equivalent is stably accumulated on donor side of PSII even in the absence of the Mn-cluster. This oxidizing equivalent was sensitive to low concentrations of exogenous Mn^{2+}, and its stable accumulation was reversibly inhibited by diethylpyrocarbonate treatment that modifies histidine residues with high specificity. It was inferred that there is a photooxidizable histidine residue(s) on donor side of PSII and it provides a redox-active ligand for Mn.

Histidine oxidation; Photosystem II; Mn cluster; Tris treatment; O_2 evolution

1. INTRODUCTION

Photosystem II (PSII), a pigment protein complex in plant thylakoids, undergoes two notable functions, charge separation and O_2 evolution. After the structural visualization by X-ray crystallography of the reaction center of non-sulfur purple bacterium [1], the molecular device of higher plant PSII for primary charge separation and following electron transfer to stabilize the separated negative charges is assumed to be analogous to that in bacterial system. On the other hand, the knowledge about transfer and stabilization of the positive charges in PSII remains still limited, although it has been established that they are finally stabilized on the Mn-cluster via Z, a tyrosine residue in D1 protein [2,3]. Besides the tyrosine, histidine has been proposed to function as a redox-active residue in mediating the positive charge transfer between Z (tyrosine-161) and Mn-cluster by providing a ligand for Mn binding [4]. In fact, Tamura et al. [5] observed that chemical modification of histidine residue(s) by diethylpyrocarbonate (DEPC) led to the loss in capability of Mn photoligation in NH_2OH-treated PSII. Recently, several groups found new EPR signals and/or thermoluminescence (TL) components in Ca^{2+}-depleted PSII which are not apparently ascribable to oxidized Mn [6–8], and suggested the possibility that they arise from oxidized histidine residues [6,8]. More recently, Boussac et al. [9] correlated their EPR signal to histidine oxidation by use of transient spectroscopy during S_2 to S_3 transition.

If a histidine residue is really functional as a redox-active ligand for the Mn-cluster, we shall be able to detect its oxidation in Tris-treated PSII. Since Tris-treated PSII is depleted of the Mn-cluster [10], the oxidizing equivalent will be stably trapped on the histidine residue due to the loss of its sink, the Mn-cluster. In this study, we examined the properties of such positive equivalent stabilized in Tris-treated PSII by means of TL measurement and chemical modification. We found that a single flash excitation creates a stable oxidizing equivalent on donor side of Tris-treated PSII, and its stable accumulation was reversibly suppressed by treatment with a histidine modifier. It was also revealed that the positive equivalent is lost on reacting with exogenous Mn^{2+}. These results are interpreted and discussed in line with the view that a histidine residue is a putative redox-active ligand for Mn-binding.

2. MATERIALS AND METHODS

Tris-treated PSII membranes were prepared from BBY-type O_2-evolving PSII membranes [11] by incubation with 0.8 M Tris·HCl (pH 8.7) for 30 min in darkness. The treated membranes were washed twice and then suspended in 400 mM sucrose, 20 mM NaCl, 40 mM Mes-NaOH (pH 6.5). These preparations were used either directly or after storage in liquid N_2. Chemical modification by DEPC of Tris-treated PSII membranes was carried out at 20°C in 0.1 M sodium phosphate buffer (pH 6.5) at 200 μg Chl/ml as described in [5] with slight modifications. The reaction was stopped at a given time by dilution with an ice-cold histidine solution (pH 6.5, 40 mM final). The

Correspondence address: T. Ono, Solar Energy Research Group, The Institute of Physical and Chemical Research, Wako, Saitama 351-01, Japan

Abbreviations: PSII, photosystem II; Mes, 4-morpholineethanesulfonic acid; DEPC, diethylpyrocarbonate; DPC, diphenylcarbazide; DCIP, 2,6-dichlorophenolindophenol; Q_A, secondary quinone acceptor in photosystem II; Z, secondary electron donor in photosystem II; D, auxiliary second donor of photosystem II; Chl, chlorophyll; TL, thermoluminescence

modified membranes were washed twice and suspended in 400 mM sucrose, 20 mM NaCl, 40 mM Mes-NaOH (pH 6.5). For restoration from modification, the modified membranes were incubated overnight at 4°C in 400 mM sucrose, 200 mM NH_2OH, 20 mM NaCl, 40 mM Mes-NaOH (pH 7.0). After three washes with the same medium (pH 6.5) omitting NH_2OH, the restored membranes were suspended in the washing medium.

TL glow curves were measured as described previously [11]. Samples were excited with saturating Xe flashes or continuous light for 30 s at −23°C, cooled rapidly in liquid N_2, and the light emission during warming was recorded against sample temperature. DCIP photoreduction was measured spectroscopically as described in [5]. EPS signals II_f and II_s were recorded at 20°C as described previously [12].

3. RESULTS

When Tris-treated PSII membranes are illuminated at low temperature (−20 to −50°C), a TL band peaking at around −20°C is observed. On excitation with flashes, the peak height increased with flash number showing saturation after 4 flashes (Fig. 1A). The height after the 1st flash amounted to about 60% of the saturation level, indicative of relatively high quantum yield of the light process. This TL component has been called A_T-band [13]. The charge pair responsible for this component has not been identified yet, although Q_A^- is proposed to be the plausible negative counterpart [13]. However, the fact that this TL component can be observed in Tris-treated PSII devoid of O_2-evolving activity indicates that a positively charged oxidizing equivalent can be stably accumulated on donor side of PSII even in the absence of the Mn-cluster. We attempted to characterize the chemical entity of this positive equivalent by use of an amino acid modifier, DEPC.

As shown by the glow curves in Fig. 1B, this TL component (A_T-band) is reversibly affected by a histidine modifier. When Tris-treated PSII membranes were further treated with DEPC at slightly acidic pHs where this modifier exhibits high specificity for histidine [14], the TL band was almost completely abolished (Fig. 1B,a). It is established that NH_2OH removes the carbethoxy group from modified histidine or tyrosine residues [14]. Consistent with this, the lost A_T-band was restored by about 30% on treating the modified membranes with NH_2OH (Fig. 1B,b). These results are interpreted that a histidine residue(s) is involved in charge trapping, probably for positive charge stabilization for this TL component, and its modification with DEPC inactivates this capability, whereas removal of carbethoxy group from modified residues reactivates the capability. The relatively low yield of reactivation may be due to detrimental effects of NH_2OH on Q_A [15], since the intensity of TL from non-modified Tris-treated PSII membranes was appreciably suppressed by the same NH_2OH treatment (Fig. 1B,c).

Lower panel of Fig. 1 shows the pH dependence of A_T-band suppression induced by DEPC-modification. The apparent first-order rate constants for A_T-band

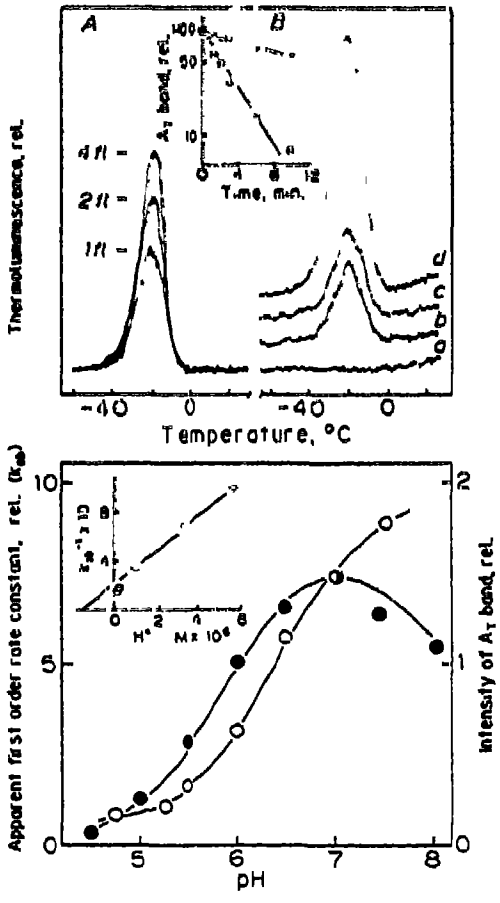

Fig. 1. Effect of DEPC modification on TL A_T-band. Upper panel (A) A_T-bands induced in non-modified Tris-treated PSII by 1, 2 and 4 flashes (*fl*) given at −23°C. (B) DEPC-modified Tris-treated PSII (a), modification was restored by NH_2OH treatment (b), non modified Tris-treated PSII before (d) and after NH_2OH treatment (c), course of A_T band suppression during DEPC modification at pH 7.5 (□) and 5.0 (○) (inset). TL was induced by continuous illumination at −23°C for 20 s for panel B measurements. Lower panel pH dependence of the rate of DEPC modification (○) compared with that of A_T-band height in non-modified Tris treated PSII (●). Tris treated PSII was modified at 20°C with 3.5 mM DEPC in 0.1 mM Na/P_i-buffer adjusted at various pHs, and apparent first-order rate constants calculated from the course of A_T-band suppression shown by panel B inset were plotted as a function of pH (○) or proton concentration (inset), in comparison with the height of A_T-band in non modified Tris-treated PSII (●), suspended in citrate (pH 3.75), Mes (pH 5.0-6.5) and Hepes (pH 7.0-8.0). TL induction was done as in panel (B).

suppression were estimated at various pHs from the time courses of suppression as shown in the inset of Fig. 1 (upper panel), and plotted as a function of pH. The rate of suppression was very low below pH 5.5, but increased steeply above pH 5.5 to reach plateau at around pH 7.5. By use of the reported relationships that the increase in observed rate constant is directly proportional to proton concentration [5,16], the pK_a value for the putative histidine residue in this experiment was estimated to be 6.2. This value agrees with those reported for functional histidine residues in various en-

zymes [16-18]. The lower panel also shows the effect of pH on the amplitude of the A_T-band induced in non-modified Tris-treated PSII membranes. The height of A_T-band was very low below pH 5.0, progressively increased with pH to reach maximum at pH 7.0, which was then followed by gradual decrease at higher pHs. The decrease in amplitude above pH 7.5 will be due to some pH effect on the acceptors. Notably, this pH dependence in acidic region coincides well with the DEPC-induced suppression of A_T-band. This implies that unprotonated histidine(s) is responsible for stabilization of the positive equivalent for A_T-band in Tris-treated PSII membranes.

Table I shows the effects of histidine modification on various activities of PSII. Despite the strong suppression of A_T-band, EPR signal II_f was only slightly affected and signal II_s not at all. Photooxidation of DPC or Mn with DCIP as electron acceptor was appreciably affected, but their inhibition extent was much less than that of A_T-band. These results imply that the modification of this histidine residue(s) suppresses the A_T-band by preferentially affecting the function of the positive charge accumulator, but not so much the function of the negative charge carrier, Q_A^-.

Tris-treated PSII membranes are devoid of the Mn-cluster, but a functional cluster can be reconstituted by photoactivation, which involves photooxidation by PSII of Mn^{2+} followed by incorporation of the oxidized Mn^{3+} into a tetranuclear cluster through ligation to proper amino acid residues [19,20]. It is thus expected that the positive equivalent on the histidine residue(s) in Tris-treated PSII may be reduced by Mn^{2+}. As Fig. 2 shows, exogenous Mn^{2+} markedly suppressed the A_T-band amplitude, and the double reciprocal plot in the inset revealed that the dissociation constant for this suppression was about 18 μM. Notably, this value agrees well with the value reported for Mn^{2+} requirement in photoactivation [21,22]. In view of the fact that DEPC-modified PSII membranes are no more capable of photoactivation [5], the histidine residue(s) will probably play a role in photoactivation, oxidation of Mn^{2+} to Mn^{3+} by use of its oxidizing equivalent.

Table I

Effect of DEPC treatment on PSII activities

PSII activity	Before DEPC treatment	After DEPC treatment
A_T-band	100	13
Signal II_s	100	100
Signal II_f ($Z \rightarrow P680^+$)	100	83
DPC → DCIP	100 (270)	64 (172)
Mn^{2+} → DCIP	100 (66)	62 (41)

Tris-treated PSII membranes were treated with 3 5 mM DEPC at pH 6 5 DCIP photoreduction was assayed in 400 mM sucrose, 20 mM NaCl, 40 mM Mes/NaOH (pH 6 5) supplemented with 50 μM DCIP and 1 mM DPC or 100 μM $MnCl_2$ as electron donor, and expressed in μmol DCIP/mg Chl h (in parentheses) Electron transport from Z to $P680^+$ was estimated from the amplitude of EPR signal II_f in the presence of 10 mM potassum ferricyanide

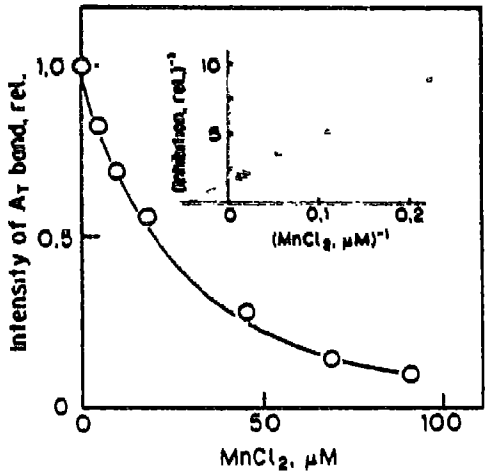

Fig 2 Inhibition of flash-induced A_T-band by Mn^{2+}. Tris-treated (non-modified) PSII membranes suspended in 400 mM sucrose, 20 mM NaCl, 40 mM Mes/NaOH (pH 6.5) were supplemented with various concentrations of $MnCl_2$ and A_T-band height was measured after dark incubation at 0°C for 1 min The inset indicates the double reciprocal plot of the inhibition extent

4. DISCUSSION

The present study revealed that the TL A_T-band is reversibly affected by treatment with DEPC, a specific modifier of histidine residues. The fact that the A_T-band is emitted from Tris-treated PSII devoid of the Mn-cluster implied that the emission arises from recombination of Q_A^- with some unknown positive charge other than Mn. Our present results suggested that in the PSII reaction center devoid of the functional tetranuclear Mn-cluster, a histidine residue(s) is photooxidized and the resulting oxidizing equivalent is stabilized in a manner which allows radiative charge recombination to emit TL A_T-band. The results also indicated that the histidine residue(s) looses these functions when modified by DEPC.

There is, however, a general ambiguity as to the specificity of the modifier employed. Although DEPC is known to specifically attack histidine residues at slightly acidic pHs, it has a potential capacity to modify tyrosine and lysine residues in the same pH ranges [14]. However, based on the following observations we can preclude the possibility that tyrosine and/or lysine residues are responsible for the phenomenon. (i) The DEPC-induced suppression of A_T-band was reversed by NH_2OH treatment that removes carbethoxy group from modified histidine or tyrosine [14]. (ii) The pK_a value (6 2) determined for DEPC-induced suppression of A_T-band agrees with the pK_a of histidine residues in various enzyme proteins, and does not much differ from the pK_a values reported for their modification [16-18]. (iii) EPR signals II_f and II_s that arise from ox-

idized form of tyrosine-161 and tyrosine-162 of D1 and D2 proteins, respectively, were insensitive to DEPC. (iv) Histidine-specific modification has been reported for NH_2OH-treated PSII at pH 6.5 [5]. We may thus conclude that the suppression of A_T-band is due to modification of histidine residue(s), but not of other amino acid residues.

The mechanism of A_T-band suppression may be interpreted in two alternative ways. (i) The positive charge for the A_T-band is stabilized as an oxidized histidine residue, so that its modification results in loss of positive charge and thereby the A_T-band. (ii) The positive charge is stabilized in a redox component other than the histidine residue, and histidine modification indirectly influences its oxidation and/or stabilization. In general, we can list four PSII redox components, Z, D, Mn-cluster and cytochrome b_{559}, as candidates for the positive charge stabilizer. Among these, Mn-cluster and cytochrome b_{559} are unlikely, since in Tris-treated PSII, the former is absent [10] and the latter takes a low potential inactive form [22]. Z and D are also unlikely, since we could experimentally confirm that EPR signals II_f and II_s were not much affected by DEPC modification. Oxidized chlorophyll may be an additional candidate. However, this is also unlikely in view of the observation by Koike et al. [13] that the maximal induction of A_T-band by illumination at around $-20°C$ is accompanied by only a weak EPR signal ($g = 2$) due to chlorophyll cation radical. All these informations and considerations appear opposite to the latter possibility, but rather appear to support the former view that the histidine residue(s) itself is the carrier of the oxidizing equivalent. Notably, the oxidation potential of this positive equivalent is assumed to be as high as that of S_3-state judging from the peak temperature of the A_T-band [13]. It is also of note that histidine is the only possible amino acid that suffices such a high oxidation potential [23]. Based on these considerations, we prefer to assume that in Mn-depleted PSII, a histidine residue(s) is oxidized in place of Mn and the stabilized oxidizing equivalent undergoes radiative charge recombination with Q_A^- to emit TL A_T-band.

There are several indications that a histidine residue(s) provides a ligand(s) for the Mn-cluster [4,5]. As shown by Fig. 2, exogenous Mn^{2+} was accessible to the oxidizing equivalent stabilized in the putative histidine residue, and the dissociation constant for this reaction was similar to that determined for Mn ligation during photoactivation. This may imply that the oxidized histidine residue acts as a redox-active ligand for Mn ligation. We note in this context that this view is exactly consistent with the results by Tamura et al. [5] that DEPC treatment abolishes the capability of Mn photoligation. If we further speculate by taking into account such properties of the A_T-band as strong dependence on pH of the suspension medium, relatively high quantum yield and requirement of Z^+ (unpublished data), the likely location of the histidine residue is on the lumenal side of the D1 protein.

Very recently, Boussac et al. [9] detected an oxidized histidine in Ca^{2+}-depleted PSII and claimed that it is the chemical entity of the S_3-state. Since their experiments were done with PSII containing the Mn-cluster, their histidine may differ from the one we discussed in this report. However, if we take into account the fact that the Mn-cluster in Ca^{2+}-depleted PSII is inactivated as evidenced by the loss of O_2 evolution [6–8], it might be possible to assume histidine oxidation as an auxiliary reaction in their case either.

Acknowledgements: This study was supported by grants for Solar Energy Conversion by Means of Photosynthesis and for 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 Nos. 0132064 and 01300009

REFERENCES

[1] Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
[2] Rutherford, A.W. (1989) Trends Biochem. Sci. 14(6), 227–232.
[3] Babcock, G.T., Barry, B.A., Debus, R.J., Hoganson, C.W., Atamian, M., McIntosh, L., Sithole, I. and Yocum, C.F. (1989) Biochemistry 28, 9557–9565.
[4] Kambara, T. and Govindjee (1985) Proc. Natl. Acad. Sci. USA 581, 228–236.
[5] Tamura, N., Ikeuchi, M. and Inoue, Y. (1989) Biochim. Biophys. Acta 973, 281–289.
[6] Boussac, A., Zimmerman, J.-L. and Rutherford, A.W. (1989) Biochemistry 28, 8984–8989.
[7] Sivaraja, M., Tso, J. and Dismukes, G.C. (1989) Biochemistry 28, 9459–9464.
[8] Ono, T. and Inoue, Y. (1990) Biochim. Biophys. Acta (in press).
[9] Boussac, A., Zimmermann, J.-L., Rutherford, A.W. and Lavergne, J. (1990) Nature 347, 303–306.
[10] Yamashita, T. and Tomita, G. (1976) Plant Cell Physiol. 17, 571–582.
[11] Ono, T. and Inoue, Y. (1986) Biochim. Biophys. Acta (1986) 850, 380–389.
[12] Ono, T. and Inoue, Y. (1989) Biochim. Biophys. Acta 973, 443–449.
[13] Koike, H., Siderer, Y., Ono, T. and Inoue, Y. (1986) Biochim. Biophys. Acta 850, 80–89.
[14] Miles, E.W. (1977) Methods Enzymol. 47, 431–442.
[15] Sivaraja, M. and Dismukes, G.C. (1988) Biochemistry 27, 6297–6306.
[16] Cousineau, J. and Maighen, E. (1976) Biochemistry 15, 4992–5000.
[17] Holbrook, J.J. and Stinson, R.A. (1973) Biochem. J. 131, 739–748.
[18] Forsman, C., Jonsson, B.-H. and Lingskog, S. (1983) Biochim. Biophys. Acta 748, 300–307.
[19] Tamura, N. and Cheniae, G.M. (1987) Biochim. Biophys. Acta 809, 245–259.
[20] Ono, T. and Inoue, Y. (1987) Plant Cell Physiol. 28, 1293–1299.
[21] Miller, A.-F. and Brudvig, G.W. (1989) Biochemistry 28, 8181–8190.
[22] Ono, T. and Inoue, Y. (1983) Biochim. Biophys. Acta (1983) 723, 191–201.
[23] Erixon, K., Lozier, R. and Butler, W.L. (1972) Biochim. Biophys. Acta 267, 375–382.
[24] Brabec, V. and Mornstein, V. (1980) Biophys. Chem. 12, 159–165.