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Thermoluminescence evidence for light-induced oxidation of tyrosine and histidine residues in manganese-depleted photosystem II particles

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In the thermoluminescence (TL) glow curve of photosystem II, particles depleted of manganese, a tyrosine modifier, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD) abolishes the TL band appearing around −55°C (TL_{-55}). Addition of a histidine modifier, diethylpyrocarbonate results in the disappearance of the band peaking around −30°C (TL_{-30}). NBD treatment also abolishes the EPR signal II_{fast} of oxidized tyrosine donor, Y_z, and inhibits the electron transport from diphenylcarbazide to 2,6-dichlorophenol-indophenol. It is concluded that the TL_{-55} and TL_{-30} bands can be assigned to oxidized tyrosine (Y_z^+) and histidine (His^+) residues, respectively, which participate in electron transfer from manganese to the reaction center of chlorophyll, $P680^+$.

Histidine; Tyrosine; Photosystem II; Electron transport; Manganese-depletion; Thermoluminescence

1. INTRODUCTION

In photosystem II (PS II) the primary charge separation is stabilized by a very fast electron donation from the water-splitting side to the oxidized reaction center of chlorophyll, $P680^+$. EPR measurements demonstrate that a redox-active intermediate component revealed as a so-called Signal II_{vf} transfers electrons between the manganese cluster and $P680^+$ [1]. This component, which is also known as Z or Y_z [1,2], has been identified as an oxidized tyrosine (Tyr-161) of the D1 polypeptide [1,3]. The reduction kinetics of Y_z^+ suggests that an additional electron carrier may function either between Y_z and manganese [4] or on a parallel pathway [5]. It was proposed by a theoretical model for the molecular mechanism of water oxidation that histidine residues as ligands for manganese are involved in the electron transfer between the water-oxidizing complex and Y_z [6,7]. Consistent with this model NMR [8], EPR [9], X-ray [10], as well as thermoluminescence [11] studies indicate that the Mn cluster is not oxidized on each of the four charge-accumulation steps but that in the S_2 to S_3 transition a histidine radical of D1 protein is oxidized [12].

Abbreviations: DCPIP, 2,2-dichlorophenol-indophenol; DEPC, diethylpyrocarbonate; DPC, diphenylcarbazide; DT-20, oxygen evolving photosystem II particle; FeCy, ferricyanide; His^+, redox active histidine residue; NBD, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole; Q_A, primary quinone acceptor of photosystem II; SiMo, silicomolybdate; PS II, photosystem II; Y_z, tyrosine, donor of PS II.

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Thermoluminescence (TL) of photosynthetic organisms originates from charge recombination between positively charged donors and negatively charged acceptors of PS II [13]. If histidine and tyrosine are redox-active components of PS II, one can expect their light-induced oxidation and participation in charge recombination, especially in Mn-depleted PS II particles in which the natural ultimate donor component, Mn, is absent. Indeed, using a histidine modifier, diethylpyrocarbonate (DEPC) in NH_2OH treated PS II membranes which are partially depleted of Mn, it has been shown that the A_T TL band appearing at −20°C can be assigned to a ligand histidine residue on the D1 protein which interacts with the Mn functioning in water oxidation [14].

Earlier we have found that in the glow curve of PS II particles, completely depleted of Mn, three unidentified TL bands can be observed around −30 (TL_{-30}), at −55 (TL_{-55}) and −75°C (TL_v) [15]. In the present work these TL investigations are extended. By applying a tyrosine modifier, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD) [16] and a histidine modifier, DEPC [17] to various PS II particles (completely depleted of Mn and depleted of Mn and non-heme iron) the TL_{-55} and TL_{-30} thermoluminescence bands were assigned to redox active tyrosine (Y_z) and histidine residues, respectively, which participate in electron transfer from manganese to $P680^+$.

2. MATERIALS AND METHODS

Oxygen evolving PS II particles (DT-20) were isolated from pea leaves and characterized as described in [18]. The removal of Mn

(more than 95%) from DT-20 preparations was carried out as in [15,18]. Extraction of iron was performed as in [19]. The activity of PS II was measured in a dual-beam spectrophotometer at 590 nm following the rate of 2,6-dichlorophenol-indophenol (DCPIP) photoreduction. The EPR Signal II_{fast} was recorded at +20°C as described previously [20]. Modification of histidine and tyrosine residues in the PS II particles was carried out in 50 mM sodium phosphate buffer (pH 6.5) at 100 μg Chl/ml in the presence of DEPC or NBD at +20°C. The reaction was stopped at a given time by the addition of histidine and tyrosine solution (at a final concentration of 20 mM) and the particles were resuspended in measuring buffer after centrifugation.

TL was measured in an apparatus described in [11,15]. 0.4 ml aliquots of samples (50 μg Chl/ml) were illuminated with white light at −80°C for 1 min and heated at a rate of 20°C/min.

3. RESULTS AND DISCUSSION

Fig. 1 shows the TL curves of Mn-depleted and of Mn/Fe-depleted particles. The glow curves exhibit three TL bands characteristic of Mn-depleted DT-20 particles [15]. Two bands, designated according to their peak positions, appear at −30 (TL_{-30}) and at −55°C (TL_{-55}). The peak position of the third band (TL_v) which is also known as Z_v band is observed at about −75°C [11]. Treatment of Mn-depleted DT-20 particles with a tyrosine modifier, NBD [16], results in a complete abolishment of the TL_{-55} band, but it does not change the TL_{-30} band (Fig. 1, curve 2). On the other hand, the histidine modifier, DEPC [17], considerably diminishes the TL_{-30} band (Fig. 1, curve 3) in agreement with the observation of Ono and Inoue [14]. DEPC has a negligible effect on the TL_{-55} band (Fig. 1, curve 3). Such dependence of TL_{-55} and TL_{-30} bands on NBD and DEPC treatment is observed in a wide concentration range (Fig. 2). Since the effects of DEPC and NBD are similar in Mn-depleted as well as in Mn and non-heme iron-depleted PS II particles (Fig. 1, curves 2 and 3) we can exclude any disturbing effect of the lower-S states and the acceptor side histidines which are involved in the binding of the non-heme iron [21]. On the basis of our observations (Figs. 1 and 2) we suggest that the TL_{-55} and TL_{-30} bands are associated with charge re-

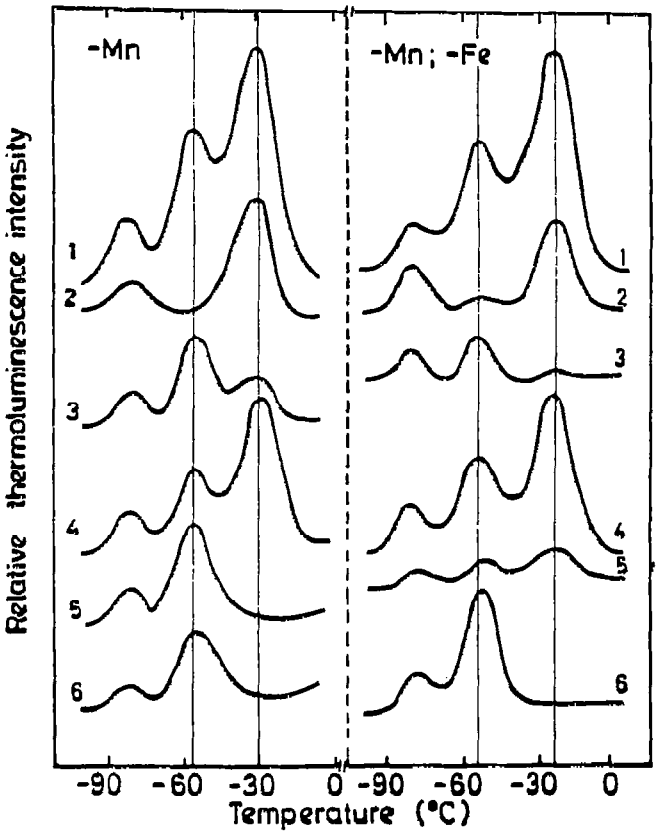

Fig. 1. Effect of various chemicals on thermoluminescence of PS II particles (DT-20) depleted of manganese (left-hand side) or manganese and non-heme iron (right-hand side). Left-hand side: (1) no addition; (2) 4 μM NBD; (3) 8 μM DEPC; (4) 5 μM DCMU; (5) 1 mM FeCy; (6) 4 μM $MnCl_2$. Right-hand side: (1) no addition; (2) 4 μM NBD; (3) 8 μM DEPC; (4) 300 μM orthophenanthroline; (5) 20 μM SiMo; (6) 300 μM benzidine. The PS II particles were suspended in 50 mM HEPES buffer (pH 7.5) and 35 mM NaCl at a chlorophyll concentration of 50 μg/ml. Thermoluminescence was excited by white light at an intensity of 10 $W \cdot m^{-2}$ for 1 min.

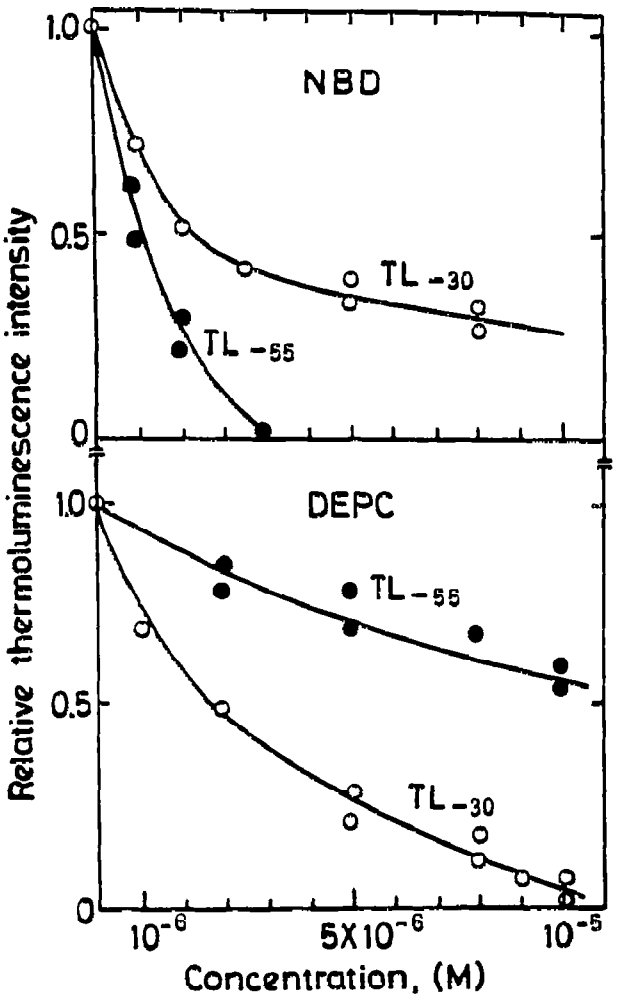

Fig. 2. Effect of the tyrosine modifier, NBD and histidine modifier, DEPC on the amplitude of the TL_{-30} and TL_{-55} thermoluminescence bands. Measuring conditions as in Fig. 1.

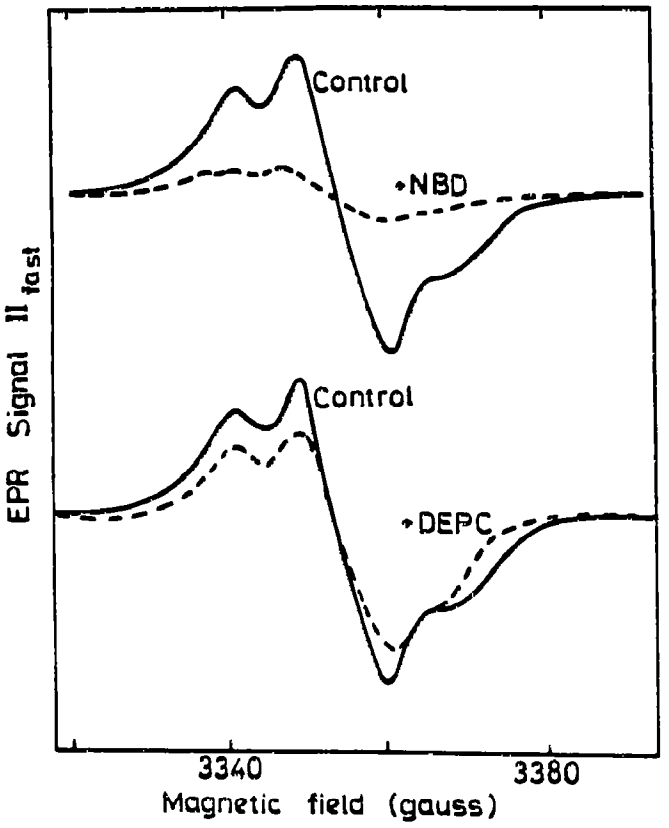

Fig. 3. Effect of NBD and DEPC on the EPR Signal II_{fast} of tyrosine donor, Y_z^+. Additions: 4 μM NBD; 8 μM DEPC. Experimental conditions were the same as in [20].

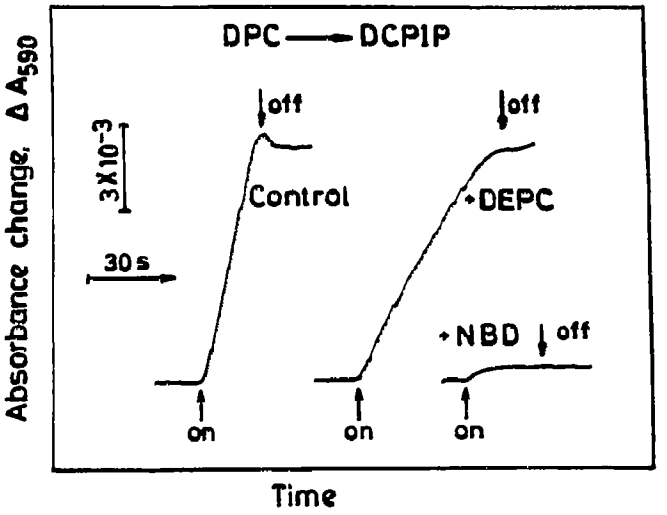

Fig. 4. Effect of NBD and DEPC on the electron transport rate measured from 1 mM diphenylcarbazide (DPC) to 100 μM 2,6-dichlorophenol-indophenol (DCPIP). The photoreduction of DCPIP was followed at 590 nm. Chlorophyll concentration of the sample was 10 μg/ml.

combination between oxidized tyrosine (Y_z^+) and histidine (His^+) residues, respectively, and reduced acceptors of PS II.

It has been shown that exogenous reductants, including Mn^{2+} and benzidine, can donate electrons to the tyrosine radical, Y_z^+ in Mn-depleted PS II particles [22]. Since both Mn^{2+} and benzidine abolish the TL_{-30} band but not the TL_{-55} band (Fig. 1, curve 6) we conclude that these donors donate electrons to Y_z^+ directly or via redox-active histidine.

Separate experiments have demonstrated that NBD treatment abolishes the EPR signal II_{fast} while DEPS-treatment has only a slight effect on this signal. These data support our suggestion that the TL_{-55} band is associated with the oxidation of Y_z (compare Figs. 2 and 3).

The question arises: what is the interacting negatively-charged acceptor counterpart of His^+ and Y_z^+ in the charge recombination? Since depletion of non-heme iron as well as addition of DCMU and orthophenanthroline had no effect on TL_{-55} and TL_{-30} (Fig. 1, curves 1 and 4) the reduced acceptor undergoing charge recombination with Y_z^+ and His^+ is either Q_A^- or another acceptor located between Q_A and P680. Electron acceptors of PS II, like FeCy and SiMo almost completely abolish the TL_{-30} band, but SiMo has also a significant effect on the TL_{-55} band (Fig. 1, curve 5). This indicates the participation of two different acceptors in the generation of the two bands. Since FeCy, which can accept an electron from Q_A^- has a drastic effect on TL_{-30} it can be assumed that this band originates from charge recombination between an oxidized donor-side histidine and the reduced acceptor, Q_A^-. Considering that the TL_{-30} band (at −30°C) probably corresponds to the A_T band (at −20°C) our conclusion concerning the origin of TL_{-30} band is in accordance with the suggestion of Ono and Inoue [14] given for the origin of the A_T band. However, their PS II particles were not completely devoid of Mn (ca. 90% depletion) and the involvement of acceptor-side histidines was not excluded (in contrast to our Fe-depleted PS II particles).

The generation of the TL_{-55} band should involve the participation of another acceptor than Q_A. This acceptor is probably not identical with the reduced pheophytin *a* acceptor because the midpoint potential of the latter (E_m ~ −610 mV) is much lower than that of Q_A/Q_A^- (E_m ~ −130 mV; [23]). Consequently, the corresponding TL band should appear at much lower temperature than −55°C. However, if one considers that the midpoint potential of protonated pheophytin (PhH_2) is more positive than −610 mV [24], its involvement in the generation of TL_{-55} cannot be completely excluded. Thus, the identification of the acceptor interacting with Y_z^+ requires further investigation.

Although the oxidation of histidine at a graphite electrode occurs at almost the same potential (~ +1100 mV [25]) as the estimated midpoint redox potential of Y_z^+/Y_z redox couple (E_m ~ +1000 mV; [26]) partial electron transport measurements (Fig. 4) suggest a more positive in vivo redox potential for the Y_z^+/Y_z redox couple than

that for the His^+/His pair. While the histidine modifier, DEPC, only slightly influences the electron transport from diphenylcarbazide to DCPIP (Fig. 4), NBD completely blocks the electron flow. This observation is consistent with the appearance of the tyrosine TL band (TL_{-55}) at lower temperature than the band associated with the charge recombination of the oxidized histidine (TL_{-30}). It is intriguing that modification of tyrosine by NBD abolishes the TL_{-55} band but does not result in the disappearance of the histidine band, TL_{-30} (Figs. 1 and 2). If the histidine and tyrosine are arranged in series, the inhibition of tyrosine should block the oxidation of histidine as well, and in turn quench the TL_{-30} band. The resistance of TL_{-30} against NBD treatment indicates that the histidine residue may be located on another pathway to P680 than the Y_z^+ radical (see also [4,5]). An alternative possibility is that although the tyrosine and histidine residues function in series, after modification of Y_z by NBD, the histidine residue is still capable of oxidation and charge recombination with a lower efficiency.

The resistance of the third band (TL_v) against the histidine (DEPC) and tyrosine modifier (NBD) indicated the involvement of a third donor in the charge recombination. Due to its low peak temperature this donor should have a more positive midpoint potential than His^+/His or Y_z^+/Y_z. A computer-predicted three-dimensional structure around Y_z and Y_D suggested that the tyrosines form hydrogen bonds with nearby histidine residues (His-190) of the D1 and D2 proteins, respectively [27], and a phenylalanine (Phe 186) which is situated between tyrosine and P680, and may be involved in electron transfer between them. The three low-temperature TL bands (TL_{-30}; TL_{-55} and TL_v) observed in manganese-depleted DT-20 particles may correspond to the three oxidized aromatic residues (histidine, tyrosine and phenylalanine).

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