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Forum Review

Participation of Photosynthetic Electron Transport in Production and Scavenging of Reactive Oxygen Species

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ABSTRACT

The photosynthetic electron transport chain (PETC) is the principal place of appearance of reactive oxygen species (ROS) in plants under illumination. The peculiarities of this process in different segments of the PETC are discussed. Oxygen uptake observed under impaired electron donation to photosystem II is attributed mainly to hydroperoxide formation by reaction of oxygen with organic radicals generated after detachment of electrons by $P680^+$. Oxygen reduction in the plastoquinone pool is suggested to start with the reaction of O_2 with plastosemiquinone, and to be followed by reduction of superoxide to hydrogen peroxide by plastohydroquinone. The distribution of plastoquinone throughout the thylakoid membrane interior provides for the generation of ROS by this route all along the membrane surface. O_2 reduction at the acceptor side of photosystem I remains poorly understood. The regeneration of antioxidants is stated to be a priority task of photosynthetic electron transport in view of the effectiveness of monodehydroascorbate as electron acceptor. We propose that ROS generation in the plastoquinone pool and the possible formation of hydroperoxides in the vicinity of photosystem II are key processes participating in the primary stages of redox signaling. *Antioxid. Redox Signal.* 5, 43–53.

INTRODUCTION

MOST COMPONENTS of the photosynthetic electron transport chain (PETC) of higher plants have redox potentials implying that their reduced forms can reduce dioxygen (Fig. 1). The article in which it was first stated that dioxygen can be a final acceptor of electrons from PETC appeared 50 years ago (49), and "Mehler reaction" is now an accepted name of this process.

Mass spectrometric analyses of simultaneous $^{16}O_2$ photoevolution from $H_2{}^{16}O_2$ and photoconsumption of $^{18}O_2$ in both algae and higher plants have demonstrated conclusively the presence of a Mehler reaction *in vivo* even during maximal CO_2 photoassimilation (for reviews, see 8, 59, 63). The authors of a recent analysis (9) stated that the contribution of electron transport to oxygen in total electron transport through PETC *in vivo* is no more than 10% even under stress in C_3 plants, and appreciably higher in C_4 plants, algae, and cyanobacteria. Although even 10% of electrons passing through the PETC provides considerable reduction of O_2 molecules, the capacity of this process possibly varies with growth conditions and age, and additional data are needed to obtain a complete picture (47, 65). The existence of dioxygen reduction in PETC at maximal rates of $NADP^+$ reduction was repeatedly shown *in vitro*. The electron flow to O_2 was found to be between 5 and 30% of the sum of electron flows to O_2 and $NADP^+$ in isolated thylakoids of spinach (1, 22) and pea (61) in the presence of added ferredoxin (Fd). In the thylakoids from oat (C_3 plant), the electron flow to O_2 in the same conditions was as high as 80% of the sum of electron flows to O_2 and $NADP^+$ (66).

The superoxide radical ($O_2^{\cdot -}$) is the product of one-electron O_2 reduction. *In vivo* superoxide is rapidly converted by superoxide dismutase (SOD) into hydrogen peroxide (H_2O_2). The reactions of H_2O_2 with transition metals (Fe, Cu) or with $O_2^{\cdot -}$ can produce hydroxyl radicals ($HO^{\cdot}$). The $O_2^{\cdot -}$,

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H_2O_2, and $HO^{\cdot}$ belong to reactive oxygen species (ROS) that also include peroxyl radicals and hydroperoxides of organic molecules and singlet oxygen. Here we give more attention to the production of reduced forms of dioxygen. The exact reactions leading to the appearance of such ROS in chloroplasts remain obscure. The production of singlet oxygen is not a direct result of photosynthetic electron transport, although it depends on electron outlet from primary acceptors of photosystems, because this in turn determines the concentration of chlorophyll triplets. The interaction of dioxygen with these triplet states is the main pathway of singlet oxygen generation in chloroplasts (for review, see 44).

Because of its specific structure of outer electron orbitals, dioxygen itself can oxidize the majority of biological substances only at low rates. However, reduced forms of oxygen, $O_2^{\cdot -}$, H_2O_2, and $HO^{\cdot}$, easily oxidize proteins, lipids, and nucleic acids, depriving them of their functions. Thus, light energy produces reductants in PETC, and their reaction with O_2 produces the active oxidants. We must distinguish the redox status of chloroplast stroma from its ROS status. In the light, an averaged redox potential of stroma can decrease, whereas the averaged ability of stroma to oxidize the biological molecules can rise.

Chloroplasts have powerful ROS scavenging systems, which is a trenchant argument for significant O_2 reduction there. Particular systems for ROS scavenging are found in chloroplasts for $O_2^{\cdot -}$, H_2O_2, peroxyl radicals, and hydroperoxides (6, 56). $HO^{\cdot}$ is so reactive that it is eliminated in the course of reactions. The role of electron transport in the scavenging systems is to regenerate the specific reductants of ROS, first of all ascorbate and glutathione. However, changes in amounts of ROS and, tightly connected with them, low-molecular antioxidants, ascorbate and glutathione, can carry out the function of a signal for induction of the cell components, unconnected with ROS annihilation (56). So, we will briefly discuss the use of ROS to convey signals that defend the cell from themselves, and als\o regulate the PETC.

PRODUCTION OF ROS IN PHOTOSYSTEM II (PS II)

The PETC from the water-oxidizing complex to the secondary quinone acceptor Q_B is known to be very vulnerable to damage. The role of ROS in the photodamage processes in PSII has been discussed (27, 46). Several ROS can appear in PSII. The singlet oxygen is produced mostly through the triplet state of P680, ^{3}P680, which in its turn results from charge recombination within the radical pair $P680^+Pheo^-$ when PSII acceptors are reduced. Excessive light can generate double reduced primary quinone acceptor Q_A and that increases the probability of acceptor-side-induced photoinhibition (25, 27).

The thermodynamic characteristics of the components of PSII acceptor side permit oxygen reduction as a one-electron process (Fig. 1). The circumstances—that an electron transfer from pheophytin (Pheo) to Q_A is very fast [<200 ps (16)], as well as that the prompt recombination between $Pheo^-$ and $P680^+$ takes place in the presence of reduced Q_A (67)—imply that the oxidation of $Pheo^-$ by O_2 is unlikely. From their E_m values (Fig. 1), the reduction of O_2 by reduced Q_A is possible, but again fast electron transfer to Q_B [200–500 μs (16)] may compete with it. Photoproduction of H_2O_2 at low rates, close to 1 μmol (mg Chl h)$^{-1}$, was found in the fragments of thylakoid membrane enriched PSII (PSII particles) and was suppressed there by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (70). Superoxide production in the presence of DCMU in isolated whole thylakoids has been found to be almost absent (7). In PSII particles, some pool of plastoquinone (PQ) is present (45) and the secondary quinone acceptor Q_B can emerge. The latter seems to point to Q_B as the electron donor to O_2 in spite of the E_{m7} of the pair Q_B/Q_B^- (Fig. 1). The participation of Q_B^- in oxygen reduction might be the result of its relatively long lifetime. Double reduced quinone acceptors of PSII also may be considered as species that are able to reduce O_2. However, the appearance of Q_A^{2-} under normal conditions is an unlikely event; rather it leads to the release of Q_A from its binding site. The E_{m7} for the pair Q_B^-/Q_B^{2-} is favorable for O_2 reduction, but the existence of unprotonated forms is possible only under extremely alkaline conditions (values of both pKs > 10). Really, some increase of H_2O_2 formation at alkaline pH was shown in PSII particles (70).

Recently, we found stimulation of oxygen uptake in PSII particles placed in the medium with pHs higher than 8.5 (Fig. 2). It is known that such conditions lead to impairment of electron donation to $P680^+$ from water, and this was proved in our preparations by the measurements of fluorescence induction (40). The direct disruption of water-oxidizing complex by Tris treatment also increased the rate of oxygen uptake (Fig. 2). The artificial donors to PSII, $MnCl_2$ and 1,5-diphenylcarbazide (DPC), added to these particles suppressed an oxygen uptake (Fig. 2) and restored the form of the fluorescence induction curve (data not shown). All the above data indicated that the stimulation of oxygen uptake rate is the result of events on the donor side of PSII under conditions when the donation of electrons was impaired.

What mechanism underlies the observed phenomenon? An increase of singlet oxygen production followed by its reaction with organic molecules could theoretically lead to an increase in oxygen uptake. However, it has been found in experiments with thylakoids that the formation of singlet oxygen did not occur when the water-oxidizing complex was damaged by Tris treatment (25). The observed increase in oxygen uptake rate was partly conditioned by an increased rate of oxygen reduction as catalase decreased this rate at alkaline pH, whereas the effect was much lower at neutral pHs (Fig. 2, inset). However, as the presence of artificial donors diminished the effect of catalase, an increase in O_2 reduction did not arise solely from a decrease of redox potential of immediate donor to O_2 at alkaline pHs. Possibly, an increase in O_2 reduction is an indirect result of impairing electron donation to $P680^+$. This possibility is supported by the finding that treatments of PSII particles with either $CaCl_2$ or tetracyane ethylene (which destroy the water-oxidizing complex) induces cytochrome *c* photoreduction (4). Also, light intensity-dependent generation of superoxide in such particles with destroyed water-oxidizing complex was observed (14), whereas in unmodified particles such generation was not detected (26). The immedi-

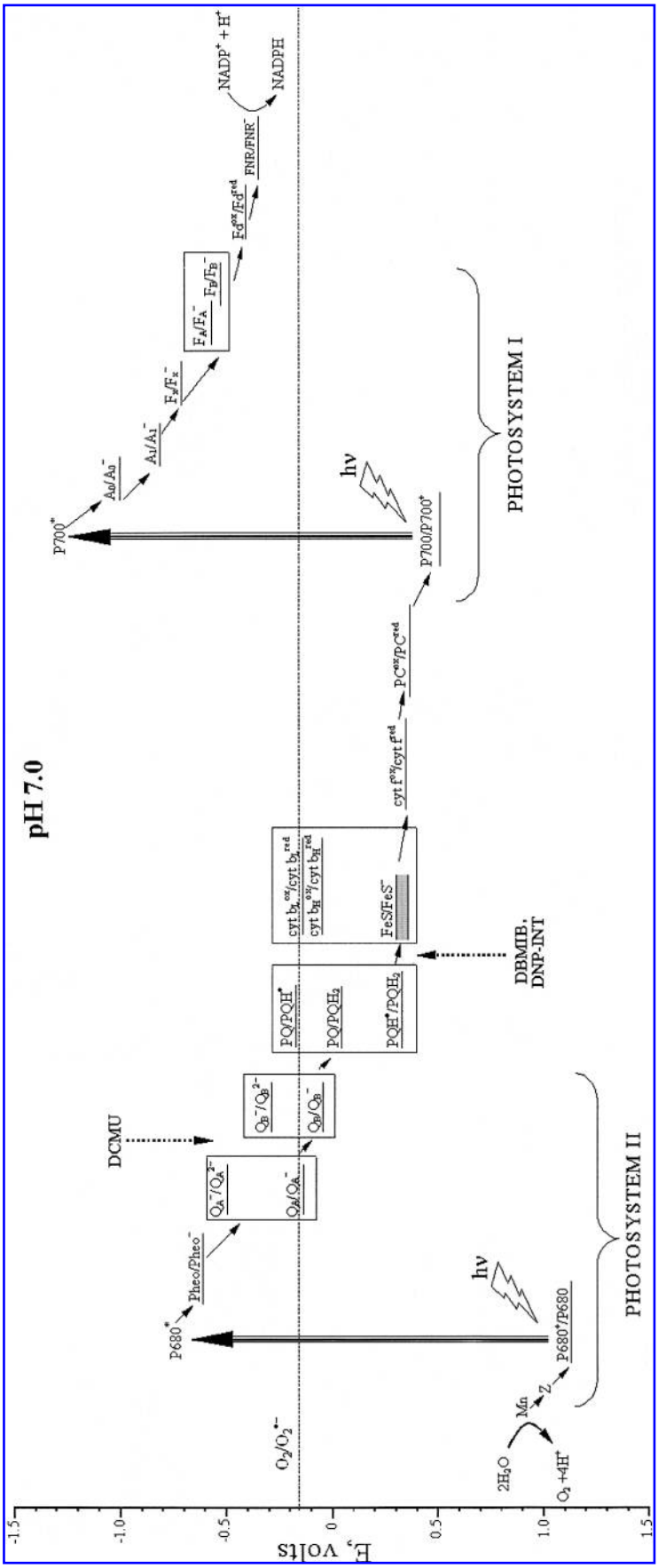

FIG. 1. Midpoint redox potentials of components of photosynthetic electron transport chain of higher plants and pair $O_2/O_2^{\bullet-}$ at pH 7.0. Mn, manganesian cluster of water-oxidizing complex; Z, redox-active tyrosine-161 of D_1 polypeptide; P680, primary electron donor in photosystem II (PSII); Pheo, pheophytin; Q_A and Q_B, primary and secondary quinone acceptors in PSII; PQ, PQH_2, PQH•, plastoquinone, plastoquinol, and plastosemiquinone, respectively; cyt b_L, and cyt b_H, low- and high-potential forms of cytochrome b_6; FeS, the Rieske iron-sulfur center; cyt f, cytochrome f; PC, plastocyanin; P700, primary electron donor in photosystem I (PSI); A_0 and A_1, primary and secondary electron acceptors in PSI; F_X, F_A, and F_B, tertiary iron-sulfur acceptors in PSI; Fd, ferredoxin; FNR, ferredoxin-NADP reductase; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-6-isopropyl-3-methyl-*p*-benzoquinone; DNP-INT, dinitrophenyl ether of 2-iodo-4-nitrothymol. Dotted arrows show the sites of actions of electron transfer inhibitors. The data are from references 16, 24, and 30.

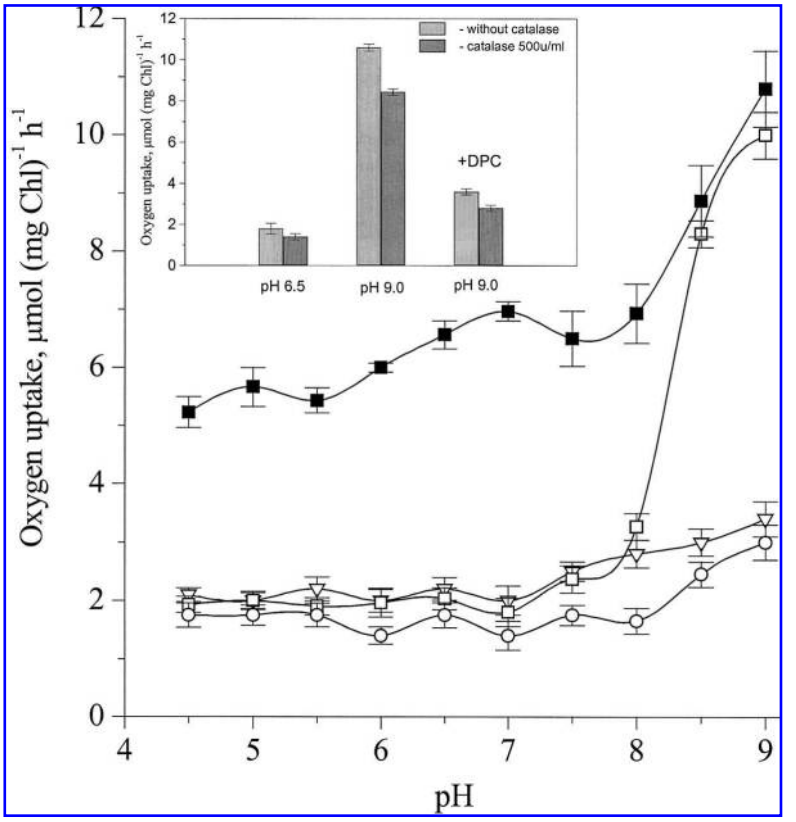

FIG. 2. Light-induced oxygen uptake rates in the fragments of thylakoid membrane enriched PSII. □, in the absence of additions; ○, in the presence of 5 μ*M* DCMU; ▽, in the presence of 1 m*M* DPC; ■, in Tris-treated fragments in the absence of additions. **Inset:** Effect of catalase on oxygen uptake rates in untreated fragments at pH 6.5 and pH 9.0. The electron donor for PSII, DPC, was added where indicated at 1 m*M*. The data are from reference 40.

ate donor of electrons to O_2 under impaired electron donation to $P680^+$ is not yet known.

The considerable part of increasing oxygen uptake at high pHs cannot be explained by an increase in oxygen reduction. We have suggested that the main reason for the oxygen uptake stimulation was an appearance of the radicals of organic molecules on the donor side of PSII, caused by oxidation by long-lived $P680^+$, the strongest biological oxidant known so far (41), followed by an interaction of these radicals with oxygen. The latter could lead to peroxyl radical and hydroperoxide formation. The possibility of transfer of the detached electrons to O_2 under aerobic conditions prevents prompt recombination of $P680^+$ with primary acceptors. Inhibition of oxygen uptake rate by DCMU (Fig. 2), which accelerates charge recombination between $Pheo^-$ and $P680^+$, is explained then by the decrease of the $P680^+$ lifetime. It may be relevant that in Tris-treated PSII particles the photobleaching of carotenoids was almost completely inhibited by DCMU, but increased in the presence of ferricyanide (42). $P680^+$ has been proposed as the major active species, which causes photoinhibition *in vivo* (5). Our data imply that the emergence of the hydroperoxides accompanies the destructive events detonated by $P680^+$.

PRODUCTION OF ROS IN PQ POOL

The role of the PQ pool in the production of ROS is often ignored. Although the thermodynamic possibility to reduce dioxygen by components of the PQ pool is recognized, the direct experimental data about this process are rare. The possibility of this reaction in the light is indicated by oxygen uptake in the presence of the PQ analogues 2,5-dibromo-6-isopropyl-3-methyl-*p*-benzoquinone (DBMIB) and 2,3-dimethyl-5,6-methylenedioxy*p*-benzoquinone(DIMEB), which are inhibitors of plastoquinol (PQH_2) oxidation by the cytochrome b_6/f complex (17). The authors concluded, however, that these inhibitors mediate electron transport between components of the PQ pool and O_2.

Figure 3 shows the pH dependence of the oxygen uptake rate in illuminated pea thylakoids in the presence of dinitrophenyl ether of 2-iodo-4-nitrothymol (DNP-INT), a nonautooxidable inhibitor of PQH_2 oxidation by the cytochrome b_6/f complex (69), in comparison with such a dependence in untreated thylakoids. DCMU (Fig. 3) and catalase (data not shown) inhibited oxygen uptake in the presence of DNP-INT, indicating that O_2 was reduced by electrons from water with H_2O_2 as a final product (39). Above we suggested that the components of PSII acceptor side do not add appreciably to an overall oxygen reduction in thylakoids. Thus, the observed oxygen uptake (to be correct, its DCMU-dependent part) in the presence of DNP-INT is the result of oxygen reduction by the PQ pool.

One-electron reduction of dioxygen in the PQ pool can occur with participation of the plastosemiquinone radical ($PQH^{\cdot}$). The thermodynamic properties of the couples $PQ/PQH^{\cdot}$ and $O_2/O_2^{\cdot -}$ satisfactorily explain the increase in oxygen uptake up to pH 6.5 observed in Fig. 3. In the pH range studied, the redox potential of $O_2/O_2^{\cdot -}$ does not change (p*K* 4.8), whereas that of $PQ/PQH^{\cdot}$ decreases up to pH close

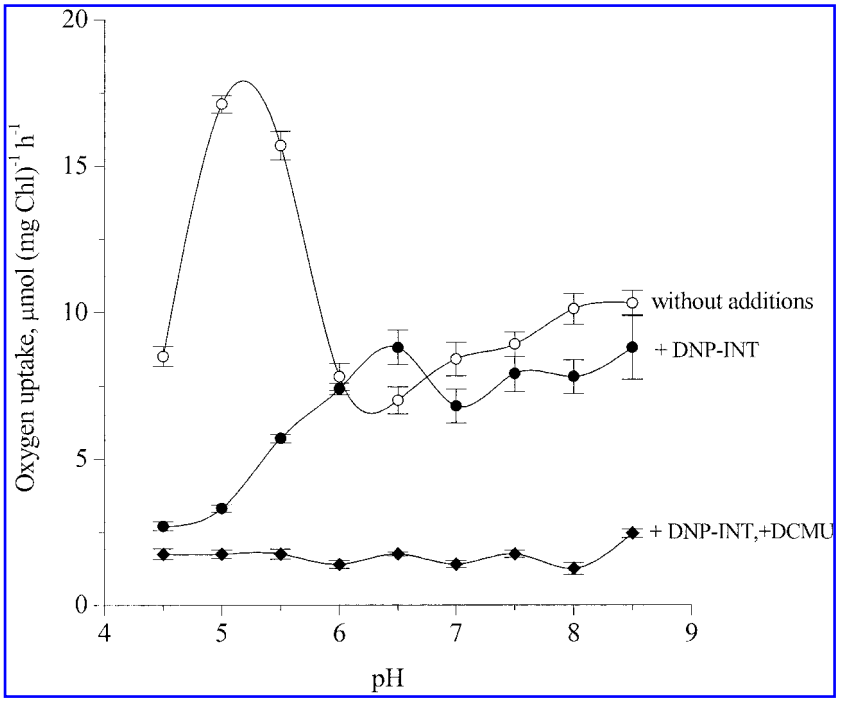

FIG. 3. Light-induced oxygen uptake rates in thylakoids under inhibition of PQ pool oxidation via cytochrome complex. ○, in the absence of additions; ●, in the presence of 5 μ*M* DNP-INT; ◆, in the presence of 5 μ*M* DNP-INT plus 20 μ*M* DCMU. The data are from reference 39.

to the $PQH^{\cdot}$ p*K* (6.0) (24), which may be higher in a membrane. One-electron reduction of O_2 in the PQ pool was confirmed by the facts that, in the presence of ascorbate as the superoxide trap, the rate of oxygen uptake increased, and the increment was completely suppressed by SOD. The superoxide formation in the presence of DNP-INT was shown in the work (15) as well.

The rate of $O_2^{\cdot -}$ formation in the PQ pool, calculated from the rate of oxygen uptake in Fig. 3, was ~25 μmol (mg Chl)$^{-1}$ h^{-1} at pH above 6.5. The initial rate of dark oxidation of the reduced PQ pool after switching off the light, which we calculated using previously published data (48), turned out to be almost the same. We found (39) that superoxide radicals detected in the medium averaged 20–25% of those potentially formed, the quantity of which was calculated from the oxygen uptake rate. To overcome such a discrepancy, we proposed that oxygen reduction in the PQ pool occurs inside a membrane as a two-stage autocatalytic process (Fig. 4). It is important that reduction of superoxide in the reduced PQ pool is extremely favorable owing to high difference in E_{m0} values of the pairs $PQH^{\cdot}/PQH_2$ (370 mV) and $O_2^{\cdot -}/H_2O_2$ (940 mV) (6). The newly generated $PQ^{\cdot -}$ can reduce a new molecule of O_2, and so on. The concentration of $PQ^{\cdot -}$ in the PQ pool will be low if the dismutation reaction (equilibrium constant is ~10^{-10}) is the sole means of its formation. An additional mechanism of $PQH^{\cdot}$ generation, such as the one that we propose, can increase the steady-state $PQH^{\cdot}$ concentration and, accordingly, oxygen reduction.

One important and distinctive feature of oxygen reduction in the PQ pool has to be noted. The number of PQ molecules in the thylakoid membrane is an order of magnitude greater than that of the reaction centers of the photosystems, and their diffusion inside the membrane may be rather quick (for reviews, see 28, 62). PQ molecules are distributed throughout the thylakoids with somewhat higher concentration in stroma-exposed membranes (36). Thus, under illumination, some level of PQ pool reduction will spread throughout the thylakoid membrane, and $O_2^{\cdot -}$ and H_2O_2 may appear even where the complexes of PSII and of photosystem I (PSI) are absent.

What contribution does oxygen reduction in the PQ pool make to the Mehler reaction *in vivo*? It is seen from Fig. 3 that the rates of oxygen uptake in thylakoids in the absence and in the presence of DNP-INT are not considerably different at pH higher than 7.2, *i.e.,* in the range of physiological pHs in chloroplasts. It is noteworthy that at these pHs the rate in the absence of DNP-INT was weakly stimulated by uncoupler as was the rate in the presence of inhibitor (39). We have also found that at pH higher than 7.0 the rate of oxygen uptake increased with temperature more in the presence of DNP-INT than in its absence (34). It was proposed that the increase of the rate of oxygen reduction in the PQ pool was responsible for the increase of oxygen uptake in untreated thylakoids.

Thus, we think that under some conditions the reduction of O_2 in the PQ pool may contribute appreciably to the Mehler reaction.

OXYGEN REDUCTION AT ACCEPTOR SIDE OF PSI

The components of the PSI acceptor side, which have the lowest redox potentials among PETC carriers, are considered to be major O_2 reducing agents in chloroplasts. It was shown in the experiments with spinach thylakoids that illumination led to generation of superoxides as the primary product of O_2 reduction (7). In this work, the authors did not observe the stimulation of superoxide production by Fd, and suggested

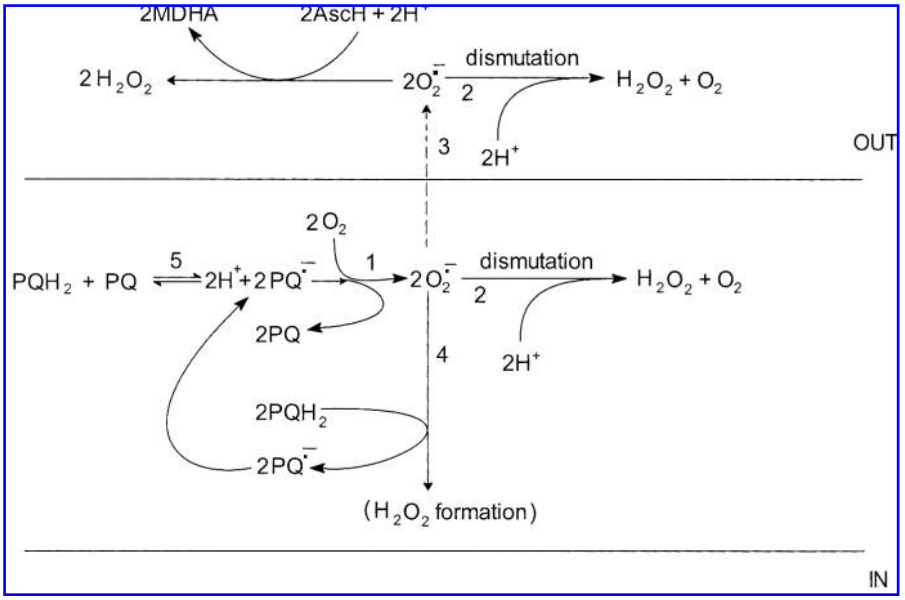

FIG. 4. Oxygen reduction in PQ pool of thylakoid membrane. 1, reduction of O_2 by PQH^-; 2, dismutation of formed superoxides; 3, release of superoxides to stroma; 4, reduction of superoxides by PQH_2 accompanied by formation of H_2O_2 and new PQH^- molecule; 5, PQH^- dismutation. Interaction of superoxides with the trap (ascorbate) outside the membrane is shown.

that $O_2^{\cdot-}$ generation occurred in the course of oxidation of the primary PSI acceptors. Takahashi and Asada (68) presented evidence supporting the location of that process in the lipophilic interior of the membrane.

It was recently observed (6) that the rates and other characteristics of photoreduction of dioxygen (K_m for O_2 and saturated light intensity) in washed thylakoids are highly different from those in isolated intact chloroplasts and in leaves; the rates are much higher in intact systems. Thus, the participation of some stromal components in the photoreduction of dioxygen *in vivo* is required. Fd, a water-soluble protein with low (−420 mV) redox potential, was most often taken as a major O_2 reducing agent in chloroplasts. The high stimulation of oxygen uptake by the added Fd was shown repeatedly in isolated thylakoids (1, 18, 22, 32, 64). A two-step oxygen reduction scheme proposed by Allen (2) stated that Fd reduced both dioxygen to superoxide and then superoxide to H_2O_2. This scheme adequately explained the peculiarity of Fd-stimulated oxygen uptake, namely, its inhibition by SOD, as well as the failure of the stimulation of superoxide production by Fd (see above).

However, the rate of oxidation of artificially reduced Fd by oxygen was found to be low (23, 29). The value of the second-order rate constant of O_2 reduction by Fd, $10^3\ M^{-1}\ s^{-1}$ (23), provides the rate of this process of the same order of magnitude as ones observed *in vivo*, if Fd in the chloroplast would be totally reduced. A direct oxidation of Fd hardly could totally explain the O_2 reduction rates observed *in vivo*, when a major physiological electron acceptor CO_2 is available (for reviews, see 59, 63), and *in vitro* under the maximum rates of $NADP^+$ reduction (1, 22, 61). Ivanov *et al.* (32) in experiments with isolated thylakoids identified two reactions of Fd-dependent O_2 reduction, and the K_m for Fd of one of them, minor, turned out to be close to that of the $NADP^+$ reduction under the same experimental conditions (61). This finding could explain not only the existence of O_2 reduction in the presence of $NADP^+$, but also that this reduction occurred with participation of Fd, which was oxidized by O_2 in a reaction possibly competing directly with $NADP^+$ reduction.

Robinson (63) had proposed that the immediate electron donors to O_2 are Fd-dependent reductases located in the stroma, nitrite reductase and glutamate synthase, whose prosthetic groups being reduced by Fd can be readily oxidized by oxygen under a deficiency of specific substrates. Recently, it was shown (52) that an isolated FAD-containing enzyme, monodehydroascorbate reductase, when added into thylakoid suspensions in the absence of Fd, becomes directly reduced at the PSI acceptor side and then reduces dioxygen at a high rate. Monodehydroascorbate reductase is situated in the stroma and possibly attached to thylakoid membrane. Other isolated flavoenzymes, glutathione reductase, Fd-$NADP^+$ reductase, glycolate oxidase, as well as free FAD possessed the same capability (52). However, the authors of this work noted that the autooxidation of MDA reductase and glutathione reductase, if they were reduced by NADPH, was slow. In our opinion, it may point to the necessity of participation of some thylakoid component(s) in fast O_2 reduction. Thus, in spite of its long history, there is no consensual view on the mechanism of dioxygen reduction at the acceptor side of PSI.

THE PARTICIPATION OF PHOTOSYNTHETIC ELECTRON TRANSPORT IN DETOXIFICATION OF ROS

Besides dismutation, nonspecific reactions of superoxides with reductants situated in chloroplast, *e.g.,* with ascorbate,

produce H_2O_2. The scavenging of H_2O_2 in chloroplasts of higher plants and algae is performed by peroxidases, the major example of which is ascorbate peroxidase (APX) (for reviews, see 6, 56). The enzyme has stromal and thylakoid-bound (tAPX) forms. The cohesion of tAPX to membrane, mainly in the PSI region (50), provides an interception of most H_2O_2 molecules in the vicinity of thylakoid membrane as the cohesion of SOD to membrane, also in the PSI region, was shown (58). As a result of the peroxidase reaction

$$H_2O_2 + 2Asc = 2MDHA + 2H_2O$$

monodehydroascorbate radical (MDHA) arises. It can dismutate to form a relatively stable product, a dehydroascorbate (DHA). Both MDHA and DHA are reduced to ascorbate by electrons coming from the PETC. In the chloroplast stroma, MDHA radical can be reduced by a specific reductase with NADPH as electron donor, and DHA by glutathione followed by rereduction of the latter also by NADPH with participation of the corresponding reductase. The sequence of events, from superoxide generation up to ascorbate regeneration, in both cases occurs finally at the expense of electrons coming from water, and results on the whole in photoreduction of dioxygen to water with no change in oxygen balance. It was named the water–water cycle and described in detail by Asada (6).

In the context of the present work, a fact of fundamental importance is the capacity of photosynthetic electron transport to reduce MDHA by reduced Fd 34-fold faster than $NADP^+$ (51). In these experiments, MDHA was generated in the suspension by added ascorbate oxidase, *i.e.,* in the bulk solution. If MDHA appears in the reaction catalyzed by tAPX, it localizes near the membrane surface, and stimulation of MDHA reduction by Fd was not observed when the generation of MDHA occurred as the result of H_2O_2 addition to thylakoids, which preserved active tAPX (20). However, under the latter conditions, the competition between MDHA and the effective electron acceptor at PSI, methyl viologen (Mv), was observed (33). Figure 5 shows that oxygen uptake accompanying Mv reduction (due to autooxidability of Mv) decreased after appearance of MDHA, the reduction of which is accompanied by oxygen evolution. The competition was also found in intact chloroplasts, and in the latter case MDHA accepted almost 20% of electrons even at 200 μ*M* Mv in the absence of other acceptors (31). Analysis of these data has shown that the competition can be explained by prompt arrival of MDHA to the site of its reduction. The data implied that if MDHA was able to compete with Mv, then it could compete with Fd *in vivo*. The decrease of $NADP^+$ reduction in (20) was obviously the effect of just this competition. The properties of MDHA as electron acceptor can explain the temporary cessation of photosynthesis in intact chloroplasts having a functional scavenging system until H_2O_2 added into a suspension was exhausted (55).

Thus, the maintenance of the system controlling the level of H_2O_2 in chloroplasts is a top-priority task for photosynthetic electron transport. One more point must be taken into account. As MDHA is a very effective electron acceptor, its appearance in the vicinity of thylakoid membrane may diminish the oxygen reduction.

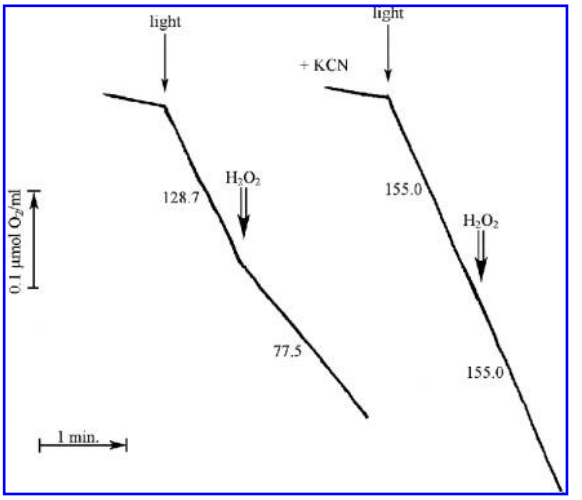

FIG. 5. Effects of H_2O_2 addition on the rate of light-induced oxygen uptake in the presence of 5 μ*M* Mv in thylakoids with active and KCN-inhibited tAPX. The media contained 5 μ*M* Mv, 0.1 m*M* NaN_3, 0.5 μ*M* nigericin, 10 m*M* ascorbate, and where indicated 1 m*M* KCN, pH 7.9. The addition of 430 μ*M* H_2O_2 is shown by arrow. The data are from reference 32.

Electron transport maintains a system controlling the level of hydroperoxides through the reduction of these species to harmless alcohols. The task of reducing hydroperoxides is executed by recently described enzymes, phospholipid hydroperoxide-dependent glutathione peroxidase (53) and 2-cysteine peroxiredoxin (10). The first enzyme uses reduced glutathione, and the chain of demand for reducing equivalents from PETC finishes in NADPH. The immediate supplier of electrons for the reaction catalyzed by the second enzyme is not defined. If thioredoxin is the donor as it is supposed (11), then the chain of demand turns out to be even shorter.

Finally, the detoxification of organic peroxyl and alkoxyl radicals that is carried through by α-tocopherol produces poorly reactive radical tocopherol-$O^{\cdot}$, which in its turn is reduced at the membrane surface by ascorbate. The ascorbate regeneration again requires the electrons from PETC.

ROS LEVELS AND REDOX SIGNALING

PETC has capacities both to produce and to scavenge ROS. It is accepted now that the response of plants to any environmental factor deviating from its optimal value, as well as to wounding, includes an increased production of the ROS (13). The control of the ROS level is necessary both to prevent oxidative stress or, more accurately, oxidative damage of cell components, and to provide some developmental processes and the response in incompatible plant–pathogen interactions (56). Taking into account the priority of usage of reducing equivalents from PETC for ROS scavenging (see above), the regulation must be directed firstly to enzymatic antioxidative systems.

H_2O_2, a rather stable substance that easily crosses biological membranes, is considered a signal-transduction agent

providing the system for the biosynthesis of enzymatic components of antioxidation with information about ROS status in the chloroplast and the cell (21, 43). The low-molecular components of this system, glutathione and ascorbate, also appear to accomplish such a task (57). In mammals and bacteria, the direct influence of H_2O_2 and glutathione on gene transcription is established (35). The immediate mechanisms for transmitting information to antioxidative genes about the status of ROS and antioxidant pools are not fully known in higher plants. However, the content of any component of these pools reacting with each other may be the primary signal that influences gene expression.

It was recently shown with transgenic *Arabidopsis* that a deficiency of 2-cysteine peroxiredoxin, which protects the photosynthetic apparatus from damage by alkyl hydroperoxides, resulted in photosynthesis impairing and in decreased levels of D1 protein and protein of light-harvesting complex associated with PSII (11). At the same time, the activities of tAPX and stromal APX and particularly of MDHA reductase, as well as transcripts for these enzymes, increased (12). Possibly, the suppression of 2-cysteine peroxiredoxin led to an increased expression of other antioxidative genes due to an increased oxidation state of the leaf ascorbate pool that was found in the same study. Thus, an ascorbate pool redox state could be the signal to gene expression in response to the emergence of hydroperoxides situated inside the membrane. The oxidation of the ascorbate pool might, in its turn, be brought about by its increased usage to regenerate α-tocopherol, which reduces peroxyl radicals. We connect these data with our finding of increased oxygen uptake and possible emergence of peroxyl radicals and hydroperoxides in the vicinity of PSII (40). These events may lead to the observed (11) degradation of components of PSII and other protein complexes connected with thylakoid membrane.

It has been found in several laboratories that gene expression in some way depends on the redox state of the PQ pool. The genes whose expression revealed such a feature were: the nuclear genes encoding light-harvesting complex proteins (19); the chloroplast genes, which regulate the stoichiometry of PSII to PSI (3, 60); and nuclear *APX1* and *APX2* genes encoding cytosolic forms of ascorbate peroxidase of *Arabidopsis* (37, 38).

We propose, based on our finding of generation of ROS in the PQ pool, that ROS participate in signals transmitted to adjust photosystem stoichiometry. The advantage of redox signaling through ROS generation in the PQ pool may be the possibility of such generation not only at the PSI where the ROS scavenging system is concentrated, but along the total membrane surface as PQs are almost evenly distributed in thylakoid membranes (see above).

The rapid expression of genes *APX1* and *APX2* that was induced by transfer of *Arabidopsis* grown in moderate light to intensive light could be induced in moderate light by treatment of leaves with DBMIB, but inhibited in excessive light by treatment with DCMU (38, 54). This result implies that the redox status of the PQ pool is a factor that controls *APX1* and *APX2* gene expression. Additional findings in these works were that *APX1* and *APX2* expression could be induced by treatment of leaves with H_2O_2 in low light, but not in the dark, and that a decrease of the gene expression in excessive light by DCMU could not be prevented by treatment with H_2O_2. From these findings, the authors suggest that "ROS and direct redox sensing of the PQ pool, independent of ROS generation, have to work concomitantly" (54). Here we suggest, instead, that ROS production in the PQ pool may be sufficient to explain the effects of PQ redox signals in photosynthetic systems, removing the need for additional assumptions The delocalized generation of these signals in thylakoid membranes may be essential.

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ABBREVIATIONS

APX, ascorbate peroxidase; Chl, chlorophyll; DBMIB, 2,5-dibromo-6-isopropyl-3-methyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DHA, dehydroascorbate; DIMEB, 2,3-dimethyl-5,6-methylenedioxy-*p*-benzoquinone; DNP-INT, dinitrophenyl ether of 2-iodo-4-nitrothymol; DPC, 1,5-diphenylcarbazide; Fd, ferredoxin; H_2O_2, hydrogen peroxide; $HO^{\cdot}$, hydroxyl radical; MDHA, monodehydroascorbate radical; Mv, methyl viologen; $O_2^{\cdot -}$, superoxide radical; PETC, photosynthetic electron transport chain; Pheo, pheophytin; PQ, PQH_2, and $PQH^{\cdot}$, plastoquinone, plastoquinol, and plastosemiquinone, respectively; PSI and PSII, photosystem I and photosystem II, respectively; Q_A and Q_B, primary and secondary quinone acceptors in PSII; ROS, reactive oxygen species; SOD, superoxide dismutase; tAPX, thylakoid-bound ascorbate peroxidase.

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