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Photosystem I is not solely responsible for oxygen reduction in isolated thylakoids

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Abstract

It was found that the contribution of segments of photosynthetic electron transport chain (PETC) besides Photosystem I (PSI) to oxygen reduction increased with increase in light intensity, and at high intensities achieved 50% at pH 5.0, and was higher than 60% at pH 6.5 and pH 7.8. The data are explained as the result of O₂ reduction in plastoquinone (PQ) pool as well as in PSI followed by reduction of superoxide radicals generated in both processes by plastohydroquinone.

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Plastoquinone (PQ)-pool participation in oxygen reduction was rarely discussed, and at all events it was not regarded as significant. We recently found [1] that in the presence of DNP-INT, the inhibitor of PQH₂ oxidation by *b₆f* complex, the reasonable rate of light-induced oxygen consumption was observed. The effects of pH, catalase, DCMU, and ascorbate on this rate as well as the measurements of SOD-dependent cyt *c* reduction revealed that the oxygen consumption was the result of initial dioxygen reduction in PQ-pool by PQH• molecules. These data also allowed suggesting that most of superoxides before leaving the membrane were reduced by PQH₂ to H₂O₂. PQ-pool is now considered as that segment of photosynthetic electron transport chain (PETC), the redox state of which regulates not only the state transition but also the expression of chloroplast and nuclear genes coding some PETC components [2,3]. Moreover, the redox state of PQ-pool was found to be a key in triggering the so-called ‘systemic acquired

acclimation’ [4,5]. How the signal is transmitted from thylakoid membrane, where PQ-pool is situated, to the system of gene expression remains unknown. We have proposed that the reactive oxygen species generated in PQ-pool might be the physical carriers of the signal [6]. The goal of the presented work was to evaluate the role of PQ-pool in oxygen reduction when an entire PETC operates and O₂ is a sole acceptor of electrons.

Thylakoids were isolated from pea leaves as described previously [1], suspended in a medium containing 0.4 M sucrose, 20 mM NaCl, 5 mM MgCl₂, 10 mM HEPES-KOH (pH 7.6) and stored on ice. The preparations were essentially catalase-free that were checked with the measurements of H₂O₂ decomposition. Oxygen concentration changes in a stirred thylakoid suspension (3.2 ml) were measured at 21 °C in glass vessel with a Clark-type oxygen electrode, using the basic reaction mixtures containing 0.1 M sucrose, 20 mM NaCl, 5 mM MgCl₂, 1 μM gramicidin D (Gr D), 50 mM Mes-KOH/Glycine (pH 5.0), or 50 mM Mes-KOH (pH 6.5), or 50 mM HEPES-KOH (pH 7.8), and thylakoids with 45 μg of Chl. Separate Photosystem I (PSI) operation was achieved by additions of 10 μM DCMU to block the electron transfer from Photosystem II (PSII), and 5 mM ascorbate plus 0.1 mM TMPD (donor pair) to provide electron donation to plastocyanin/P700 [7]. Stock solutions of DCMU and Gr D were in dimethyl sulfoxide. To prevent the reaction of superoxides with ascorbate, a saturating

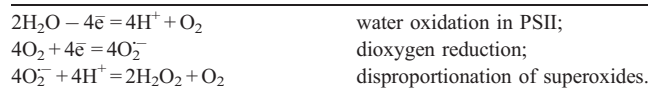
Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DNP-INT, dinitrophenylether of 2-iodo-4-nitrothymol; Gr D, gramicidin D; Mv, methyl viologen; PETC, photosynthetic electron transport chain; PQ, plastoquinone; PQH•, plastosemiquinone; PQH₂, plastohydroquinone, plastoquinol; PSI, photosystem I; PSII, photosystem II; SOD, superoxide dismutase; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylene diamine

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amount (that was chosen in separate experiments) of SOD was added into reaction mixture in experiments with donor pair; SOD was also added without this pair to correct for its unspecific influence. Light from slide projector was filtered through a red cutoff ($\lambda > 600$ nm) and heat absorbing filters. Light intensity was varied using neutral filters, and was measured as photon flux density using Li-Cor quantum meter (model LI-250). Chlorophyll concentration was determined in 95% ethanolic extracts [8]. DCMU, methyl viologen (Mv), HEPES, Mes, and catalase were purchased from Sigma; SOD was from Sigma and TRIS (Russia); Gr D was from Calbiochem. All other chemicals were of analytical grade.

Fig. 1 shows that the rate of light-induced oxygen consumption under operation of the entire PETC increased with an increase of light intensity at pHs 5.0, 6.5, and 7.8. Only at pH 5.0 this rate was saturated, although at rather high intensity close to $500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, while at higher pHs the saturation was not achieved even at $800 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The rates of oxygen consumption when only PSI operated in thylakoids also increased with light intensity (Fig. 1), but at pH 6.5 and pH 7.8 to a lesser extent than the rates of oxygen consumption in the entire PETC. The rates of electron transfer to oxygen were calculated from the oxygen consumption rate. Under operation of the entire PETC the oxygen balance resulted from the following reactions:



In this case the stoichiometry between electrons and oxygen is one O_2 molecule consumed per 4e^- transferred to oxygen. Under operation of separate PSI, not water but ascorbate was the source of electrons in the reaction, which did not affect the oxygen balance. In this case the stoichiometry is one O_2 molecule consumed per 2e^- transferred to oxygen. Such stoichiometry under similar electron transport conditions, but with DPIP instead of TMPD, in the presence of saturating amount of SOD was proved by Allen and Hall [9,10]. When catalase was added into reaction mixture, the residual oxygen consumption was close to zero (lesser than 10% at all pHs) for the entire PETC operation, and was almost exactly 50% for separate PSI operation (not shown). This evidenced that H_2O_2 was the final stable product in both the cases.

Fig. 1. The dependencies of the oxygen uptake rates in isolated thylakoids on light intensity at pH 5.0 (A), pH 6.5 (B), and pH 7.8 (C); (1) the entire PETC, (2) separate PSI. In the insert at corresponding pH, the contribution of PSI into oxygen reduction in the entire PETC is shown as percentage of total amount of electrons reducing oxygen in the entire PETC. (For color see online version).

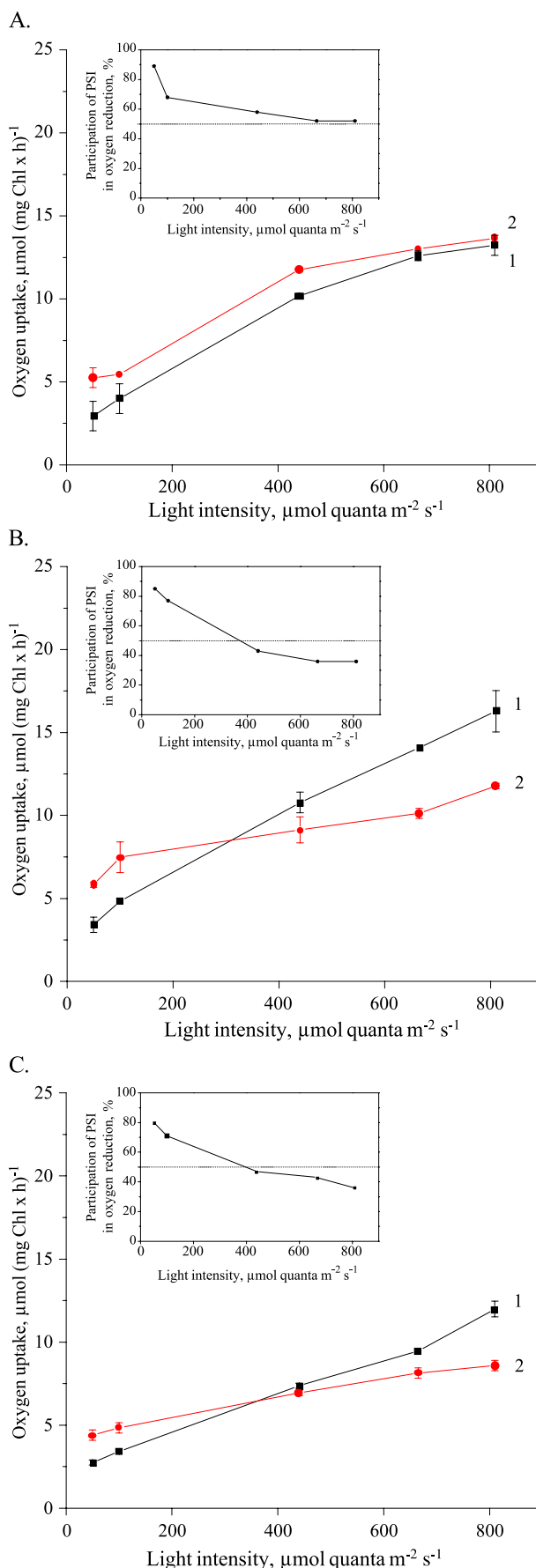


Table 1
Influence of Mv on the light-induced oxygen uptake rates in an entire PETC and in separate PSI

Additions to thylakoids	Oxygen uptake, $\mu\text{mol O}_2/\text{mg Chl h}$		
	pH 5.0	pH 6.5	pH 7.8
–	9.2 ± 0.8	9.6 ± 0.7	9.0 ± 0.8
Mv	41 ± 4	149 ± 8	170 ± 7
TMPD/ascorbate, DCMU	9.5 ± 0.7	10.6 ± 0.7	7.7 ± 0.6
TMPD/ascorbate, DCMU, Mv	57 ± 2	160 ± 9	183 ± 8

SOD, 500 u ml^{-1} , was present in all the mixtures. Where indicated: $10 \mu\text{M}$ DCMU, 5 mM ascorbate, 0.1 mM TMPD, and 0.2 mM Mv. Light, $500 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$.

The comparison between the rates of electron transfer to oxygen in the entire PETC and in separate PSI is possible only when the step of oxygen reduction is the rate-limiting step in both cases. Since we had found that under some conditions Gr D increased the oxygen consumption rate [1], this uncoupler was present in all reaction mixtures so that the electron donation to P700 in both cases did not limit the electron transfer. Mv, which effectively accepts electrons at PSI and is promptly oxidised by oxygen, increased the rates of oxygen consumption in the light under all the experimental conditions (Table 1). This evidenced that in the absence of Mv, only oxygen reduction limited oxygen consumption.

Thus, the rate of electron transfer to oxygen calculated from the rate of oxygen consumption under separate PSI operation may be taken as the rate of this electron transfer through PSI under operation of the entire PETC. In the inserts in Fig. 1, at corresponding pHs the contribution of PSI to oxygen reduction is expressed as a percentage of total number of electrons reducing O_2 in the entire PETC. This contribution decreased with an increase of light intensity and achieved steady value close to 50% at pH 5.0, while at both pH 6.5 and pH 7.8 at high intensities it was about 35%. It may be noted that if, in spite of SOD presence, ascorbate and/or a reduced TMPD had some capacity to reduce superoxides, the above contributions were overestimated.

The acceptor part of PSI is considered as the main site of oxygen reduction in PETC (see Refs. [6,11] for more discussion). The presented results showed that during the entire PETC operation without added electron acceptors, the segments of PETC from water to plastocyanin did contribute significantly to oxygen reduction, and this contribution increased with an increase of light intensity. A thermodynamically possible oxygen reduction at the acceptor side of PSII is thought to be negligible in intact thylakoids [11], and it was found to be very low even in membrane fragments containing PSII [12]. The low-potential components of b_6f complex are not usually suspected of participation in the oxygen reduction. High-potential cyt b_6 , where electron comes after first PQH_2 oxidation, has redox midpoint potential at pH 7 of -45 mV [13], rather high as compare with the potential of O_2^-/O_2 , -160 mV [11]. This potential of low-potential cyt b_6 was estimated as -150 mV [13] or

even -170 mV [14], and if, under some circumstances, electron can stay here for noticeable time its transfer to O_2 cannot be excluded. The finding that inhibition of cyt b oxidation by antimycin in mitochondrial and yeast bc_1 complexes induced oxygen reduction, supplemented by inhibitor analysis, led to a conclusion that the ubisemiquinone formed at the quinol oxidising site of both complexes was a reductant of O_2 [15,16]. Our data [1] also showed the perceptible oxygen reduction under inhibition of ingoing electron into b_6f complex in the presence of DNP-INT. Thus, PQ appears as that segment of PETC where, besides PSI, dioxygen reduction occurs.

The significant participation of the segments of PETC from water to plastocyanin in oxygen reduction at pH 5.0, averaging 50% at high light intensities (insert in Fig. 1A), seems to contradict our previous data about low oxygen reduction in PQ-pool at this pH even at $500 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ [1]. This seeming contradiction can be explained, however, from our hypothesis [1] stating that PQ-pool can participate in two reactions of oxygen reduction, namely, in the reduction of molecules O_2 by PQH^\cdot and in the thermodynamically favourable reduction of O_2^- by PQH_2 , with additional assertion that PQH_2 can reduce the superoxides generated not only in PQ-pool but also in PSI. To interact with PQH_2 , the superoxides in PSI must be generated inside the thylakoid membrane, and this was repeatedly proposed [17,18]. The immediate reductant of O_2 in PSI in washed thylakoids is not exactly established, and F_A and F_B , FeS clusters located at the interface of membrane and stroma, as well as F_x , an intramembrane FeS cluster, may be such reductants. The participation of F_x , and even of A_1 , phylo-

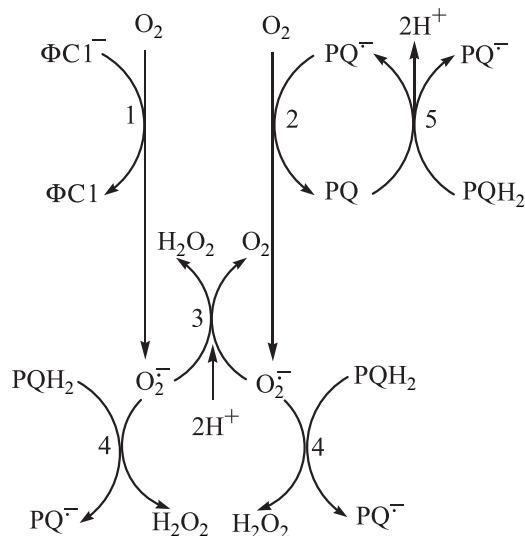


Fig. 2. A tentative scheme of oxygen reduction in the thylakoid membrane. 1—Superoxide generation due to O_2 reduction in PSI; 2—superoxide generation due to O_2 reduction in PQ-pool by PQH^\cdot ; 3— H_2O_2 formation due to disproportionation of superoxides; 4— H_2O_2 formation and generation of new PQH^\cdot due to superoxide reduction by PQH_2 ; 5— PQH^\cdot appearance due to reaction between PQH_2 and PQ^\cdot , reversion of dismutation reaction.

quinone, a secondary electron acceptor with redox potential of -820 mV [19], in the reduction of dioxygen in the membrane, where O_2 concentration is higher than in aqueous phase, can increase in strong light when these carriers become highly reduced because of limited outflow of electrons to dioxygen.

At pH 5.0 when the reduction of O_2 by PQH^{\cdot} is low, PQ-pool can contribute to oxygen reduction mainly through reduction by PQH_2 of $O_2^{\cdot-}$ generated in PSI. Such collaborative participation of PQ-pool and PSI in oxygen reduction we named ‘co-operative oxygen reduction’. If PQ-pool participates only in such co-operative oxygen reduction then its contribution cannot exceed 50% that we observed at pH 5.0. At higher pHs, the reduction of O_2 by PQH^{\cdot} is thermodynamically possible [1]. PQH^{\cdot} is always present in PQ-pool as the result of reversion of dismutation reaction. Additional PQH^{\cdot} molecules could be generated in the course of oxidation of PQH_2 by both $O_2^{\cdot-}$ and b_6f complex (see above). A high extent of PQ-pool reduction under our experimental conditions (estimated from chlorophyll fluorescence yield, not shown) was possibly conducive to the second way owing to a decrease in amount of PQ capable to oxidise a high potential cyt b_6 . This simulated in a certain sense antimycin effect in bc_1 complexes. In accordance with the scheme in Fig. 2, the participation of PQH^{\cdot} in oxygen reduction during the entire PETC operation may result in PQ-pool contribution to this process of more than 50%. This was really observed at maximal used light intensities when oxygen reduction in separated PSI was apparently close to saturation (Fig. 1).

Thus, we propose that under some conditions PQ-pool can significantly contribute to oxygen reduction and production of ROS. The evident advantage of this pool as place of production of the signalling ROS is its prevalence along the entire thylakoid membrane while the powerful ROS scavenging systems are concentrated in close proximity to PSI.

Acknowledgements

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