Scavenging of Superoxide Generated in Photosystem I by Plastoquinol and Other Prenyllipids in Thylakoid Membranes†

Jerzy Kruk,*,‡ Małgorzata Jemioła-Rzemińska,‡ Kvetoslava Burda,§ Georg H. Schmid,|| and Kazimierz Strzałka‡

Department of Plant Physiology and Biochemistry, Faculty of Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Kraków, Poland, Institute of Nuclear Physics, ul. Radzikowskiego 152, 32-342 Kraków, Poland, and Fakultät für Biologie, Lehrstuhl Zellphysiologie, Universität Bielefeld, D-33501 Bielefeld, Germany

Received January 9, 2003; Revised Manuscript Received May 20, 2003

ABSTRACT: We have examined scavenging of a superoxide by various prenyllipids occurring in thylakoid membranes, such as plastoquinone-9, α-tocopherolquinone, their reduced forms, and α-tocopherol, measuring oxygen uptake in hexane-extracted and untreated spinach thylakoids with a fast oxygen electrode under flash-light illumination. The obtained results demonstrated that all the investigated prenyllipids showed the superoxide scavenging properties, and plastoquinol-9 was the most active in this respect. Plastoquinol-9 formed in thylakoids as a result of enzymatic reduction of plastoquinone-9 by ferredoxin-plastoquinone reductase was even more active than the externally added plastoquinol-9 in the investigated reaction. Scavenging of superoxide by plastoquinol-9 and other prenyllipids could be important for protecting membrane components against the toxic action of superoxide. Moreover, our results indicate that vitamin K_1 is probably the most active redox component of photosystem I in the generation of superoxide within thylakoid membranes.

The main source of oxygen uptake in chloroplasts that is dependent on thylakoid-bound components is chlororespiration and photoreduction of oxygen in photosystem I (PSI).[1] Only the superoxide generation in PSI is directly light-dependent, whereas chlororespiration reactions are light-independent. The main component associated with PSI contributing to a generation of superoxide in intact chloroplasts under strong light illumination was identified as monodehydroascorbate reductase (*1*), containing FAD as a redox cofactor. However, this enzyme is loosely bound to thylakoid membranes and is not present in isolated thylakoids (*1*, *2*). It was suggested that within PSI, superoxide is produced in the aprotic, hydrophobic interior of the thylakoid membrane by Fe−S centers: F_X and $F_{A/B}$ (*3*, *4*). After diffusing to the membrane surface, the superoxide undergoes protonation and dismutation to hydrogen peroxide and oxygen. It was suggested that superoxide is able to reduce oxidized cytochrome *f* and PC (*5*), forming superoxide-mediated cyclic electron transport around PSI. When superoxide is formed in the aprotic interior of the membrane, its lifetime is extended, and it can directly induce membrane lipid peroxidation or destruction of membrane proteins. In the water phase, after dismutation to H_2O_2 and oxygen, it is especially toxic to Calvin cycle enzymes and also to the oxygen-evolving complex (*4*). Therefore, there exists special enzymatic systems protecting chloroplast components against toxic action of superoxide (*6*). However, within thylakoid membranes, where the $O_2^{\bullet-}$ lifetime is extended, superoxide and H_2O_2 scavenging enzymes cannot be active. This function could fulfill thylakoid prenyllipids such as plastoquinol (PQH_2-9), α-tocopherol (α-Toc), or α-tocopherolquinol (α-TQH_2), which are well-known antioxidants (*7*−*10*). Until now, no systematic study was performed on these reactions.

For the measurements of superoxide production in photosynthetic systems, mass spectrometry of oxygen isotopes was applied (*11*), as well as superoxide-dependent cytochrome *c* reduction that can be followed spectrophotometrically (*1*, *3*, *12*). The first method requires special and expensive instrumentation, while the latter is indirect and can be unspecific. In our present experiments, we applied flash-light illumination and used a fast oxygen electrode for measurements of superoxide generation in thylakoid membranes. To avoid interference of oxygen formed photosynthetically, the oxygen evolution was inhibited by DCMU, and hydroquinone (HQ) was used as an artificial electron donor. It is known that under flash illumination, production of superoxide by PSI is considerably increased as compared to continuous illumination (*3*), where it can reach up to 20−30% of total electron flux under strong light in intact leaves (*11*). The reason for this difference is probably the fact that under continuous illumination, the generation of the proton gradient lowers the quantum yield of photosystem II and suppresses the electron donation from photosystem II to PSI. In the presence of HQ, an artificial electron donor, the electron donation to PSI should not be limited.

In the present work, we measured selectively the effect of various thylakoid prenyllipids on superoxide generation

† This work was supported by KBN Research Grant 6 P04A 031 20.

* Corresponding author. Tel.: (48 12) 252 6361. Fax: (48 12) 252 6902. E-mail: jkruk@mol.uj.edu.pl.

‡ Jagiellonian University.

§ Institute of Nuclear Physics.

|| Universität Bielefeld.

[1] Abbreviations: PSI, photosystem I; PQ, plastoquinone; PQH_2, plastoquinol; α-Toc, α-tocopherol; α-TQ, α-tocopherolquinone; α-TQH_2, α-tocopherolquinol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HQ, hydroquinone; Chl, chlorophyll; PC, plastocyanin; DNP-INT, dinitrophenyl ether of iodonitrothymol; DBMIB, dibromothymoquinone.

10.1021/bi034036q CCC: $25.00
Published on Web 06/20/2003

in isolated thylakoid membranes, following oxygen uptake with the fast oxygen electrode, and the significance of the observed effects was discussed.

MATERIALS AND METHODS

Spinach thylakoids were isolated according to the method described in ref *13*. The oxygen uptake measurements were performed on dark adapted thylakoids (150 μg/mL Chl, 600 μL sample volume) in 50 mM Hepes buffer (pH 7.5), containing 10 mM NaCl and 5 mM $MgCl_2$ using a three electrode system in the presence of 50 μM DCMU and 10 mM HQ as an electron donor. The electrode system is described in ref *14* in detail. The signal of the oxygen level presented in the figures is the direct amplified amperometric current due to the reduction of oxygen on the electrode. Saturating light flashes of 5 μs (full width at half-maximum) were provided by a xenon lamp (Stroboscope 1539A from General Radio). The samples were illuminated by 15 flashes spaced 300 ms apart after 10 min of sedimentation and dark adaptation time. The oxygen uptake for single flashes was measured from the obtained traces taking the difference between the minimum in the signal after a flash and the signal level just before the flash. The samples where plastocyanin (PC) was released from thylakoids were prepared by repeated (5-fold) freeze−thawing of thylakoids, whereas the sample where PC was removed from thylakoids was obtained as the previous samples with an additional centrifugation and suspension of the sedimented thylakoids in the buffer. Mercuric chloride treatment was performed by incubation of thylakoids at $HgCl_2$/Chl = 1:1 (mol/mol) for 40 min at 0 °C (*15*) and KCN treatment (KCN/Chl = 1:1, mol/mol) was performed for 60 min at 0 °C before the measurements (*16*). The lyophilized thylakoids (1 mg of Chl) were extracted three times using 1 mL of hexane for each extraction (20 min). After centrifugation and removal of the supernatant, thylakoids were dried in a rotatory evaporator and suspended in the buffer, giving a final concentration of Chl of 1 mg/mL. This suspension was used as a stock solution. PQ-2 was obtained from Hoffman-La Roche (Basel, Switzerland), and PQ-9 was obtained as described in ref *17*. Prenylquinols were prepared from the corresponding oxidized forms according to the method described in ref *18*. Vitamin K_1 was from Aldrich, spinach ferredoxin from Sigma, and α-Toc from Merck. All prenyllipids were added to thylakoid membranes as ethanol solutions.

RESULTS

To measure superoxide generation in thylakoid membranes with the oxygen electrode under illumination, which manifests in oxygen uptake, it is necessary to inhibit photosynthetic oxygen evolution by DCMU and to use an artificial electron donor. The appropriate electron donor should be neither lipid-soluble nor a superoxide scavenger to avoid interference of a donor with the measured reaction. Among many compounds tested (e.g., ascorbate, duroquinol) that were able to donate electrons to PSI, the most suitable for our purposes turned out to be hydroquinone (HQ). Figure 1 shows that the control sample evolves oxygen with the oscillation period of four under flash-light illumination, which is consistent with the four-state model of the oxygen evolving complex (*19*). The addition of DCMU abolishes completely

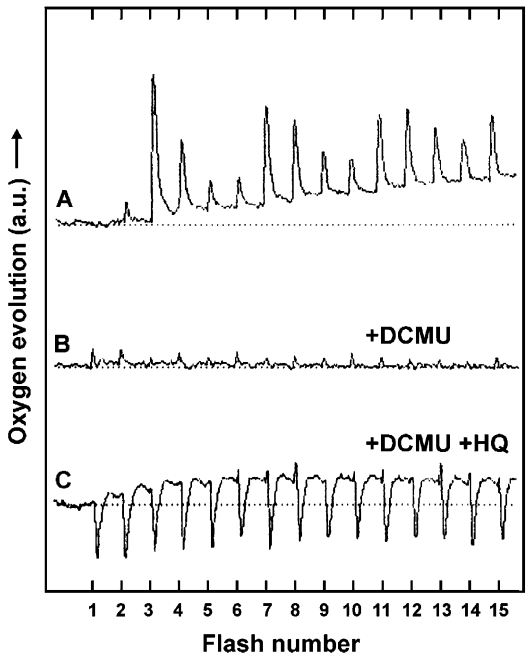

FIGURE 1: Oxygen evolution pattern of spinach thylakoids under short saturating light flashes (A); panel B as panel A after addition of 50 μM DCMU; and panel C as panel B after addition of 10 mM HQ.

oxygen evolution, while the subsequent addition of HQ stimulates oxygen uptake without any clear oscillations. If we compare the total oxygen evolution at the first four flashes, when the full Kok cycle is completed (*19*), for the control sample with the oxygen uptake at a single flash, we can estimate that the oxygen uptake in the presence of HQ corresponds to about 25% of the total electron flux. The positive drift of the baseline in trace C is probably due to oxygen released in the dismutation reaction of superoxide, the release of oxygen from the formed H_2O_2 as a result of the activity membrane-bound ascorbate peroxidase (*20*), and the instrument response.

To examine the electron donation site of HQ to the electron transport chain, we measured the influence of different inhibitors and treatments on the oxygen uptake by PSI in the presence of HQ. Plastocyanin can be released from thylakoids by a repeated freeze−thawing procedure (*21*) and can be removed from thylakoids by centrifugation. Figure 2 shows that the release of PC from thylakoids inhibited the oxygen uptake by 23%, while the removal of PC by centrifugation inhibited it by as much as 73%. The use of PC inhibitors (KCN and $HgCl_2$) had also pronounced effects on the inhibition of the investigated reaction. The measurements of oxygen uptake in the presence of cytochrome b_6f inhibitors (DNP-INT and DBMIB) (*22*) show that the inhibition was negligible (Figure 2). The obtained results indicate that the site of electron donation by HQ is at cytochrome *f* of the cytochrome b_6f complex or at PC. It is known that HQ is able to reduce both of these components and cannot reduce cytochrome b_6 because of the low-redox potential of this cytochrome. We were not able to find whether the HQ reduction site is at cytochrome *f* or PC because there are no specific inhibitors of the cytochrome *f* function.

To obtain the control sample free of prenyllipids, we performed lyophilization and hexane extraction of thylakoids.

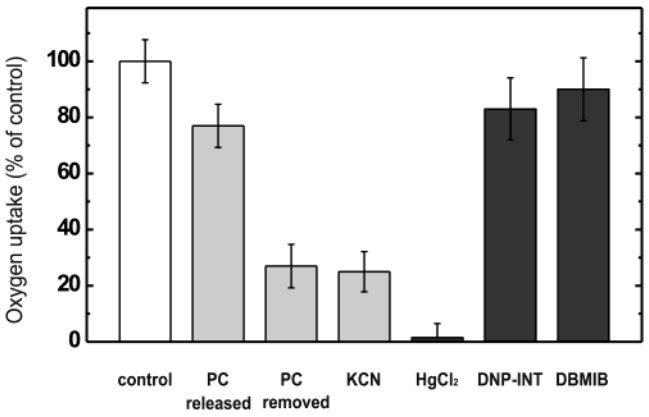

FIGURE 2: Influence of cytochrome b_6f inhibitors (20 μM DNP-INT, 5 μM DBMIB), PC inhibitors (KCN, $HgCl_2$), and PC release and removal from thylakoids on the oxygen uptake in flash-illuminated spinach thylakoids in the presence of 50 μM DCMU and 10 mM HQ. The given oxygen uptake values are the average for all 15 flashes applied and from three to five replicates. The oxygen uptake for single flashes was measured as described in Materials and Methods.

Table 1: Effect of the Hexane Extract and Vitamin K_1 on Oxygen Uptake in Hexane-Extracted Spinach Thylakoids (150 μg/mL Chl, 50 mM Hepes, pH 7.5, 10 mM NaCl, 5 mM $MgCl_2$) in the Presence of 10 mM HQ and 50 μM DCMU[a]

sample	average oxygen uptake (mV)	Y_1/Y_2 signal ratio[b]
control	15 ± 1	1.06
+ extract (1:1)	12 ± 1	1.30
+ extract (5:1)	5 ± 1	2.02
+ 1 μM vit. K_1	15 ± 1	1.26
+ 10 μM vit. K_1	16 ± 2	1.71

[a] The numbers in brackets denote the amount of the added extract in relation to its original amount in thylakoids. [b] Y_1 and Y_2 refer to the oxygen uptake intensity at the first and second flash, respectively. The given oxygen uptake values are the average for all 15 flashes applied and from three replicates. The oxygen uptake for single flashes was measured as described in Materials and Methods.

This procedure removes selectively prenyllipids from thylakoid membranes preserving their structure and function (*23*). In the first approach, we added the total hexane extract containing different membrane prenyllipids back to hexane-extracted thylakoid membranes. In the presence of DCMU and HQ, apart from the inhibition of oxygen evolution, a considerable change in the ratio of the first two oxygen uptake signals was observed with the increase in the amount of the added extract (Table 1). Since hexane extraction removes also partially vitamin K_1 from PSI (*24*), we tested the effect of this vitamin on oxygen uptake by illuminated thylakoids. It was found that adding relatively low concentrations of vitamin K_1 changed the ratio of the first two signals (Figure 3, Table 1) similarly to the effect of the added extract but without any pronounced effect on the average oxygen uptake. The other membrane prenyllipids that could be present in the extract, like $PQ(H_2)$-9, α-$TQ(H)_2$, and α-Toc, had no effect on the ratio of the first two signals (data not shown), indicating that autoxidation of vitamin K_1 in PSI is mainly responsible for the observed oxygen uptake in our system. The effect of other membrane prenyllipids and the synthetic $PQ(H_2)$-2 couple on the oxygen uptake in hexane-extracted thylakoids is illustrated in Figure 4A. The results show that oxygen uptake was inhibited by all the

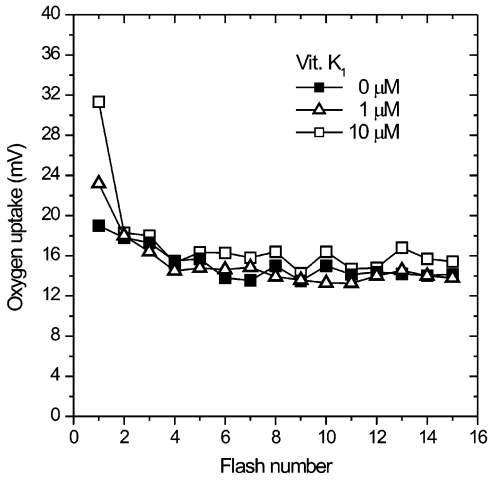

FIGURE 3: Oxygen evolution pattern of hexane-extracted thylakoids under flash-light illumination and the effect of vitamin K_1. The oxygen uptake for single flashes was measured as described in Materials and Methods.

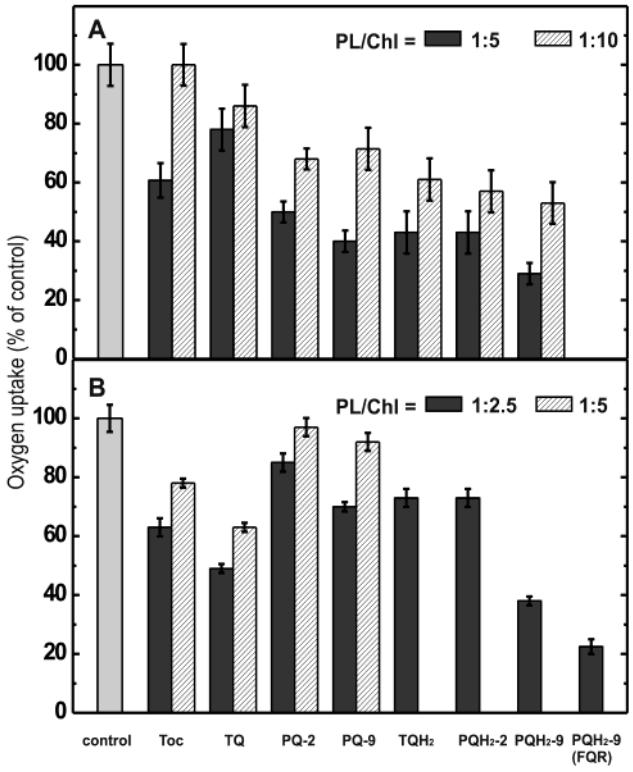

FIGURE 4: Influence of different prenyllipids (PL) on oxygen uptake in hexane-extracted (A) and untreated thylakoids (B) under flash-light illumination in the presence of 50 μM DCMU and 10 mM HQ at a PL/Chl molar ratio of 1:5 and 1:10 (A) or 1:2.5 and 1:5 (B). The given oxygen uptake values are the average for all 15 flashes applied and from three to five replicates. The oxygen uptake for single flashes was measured as described in Materials and Methods. The inhibition was uniform on every flash within given errors, and the positive drift of the baseline in the original traces, like those in Figure 1C, was proportional to the extent of the oxygen uptake signal. FQR stands for ferredoxin-PQ reductase.

investigated prenyllipids. The well-known antioxidant α-Toc was relatively inactive in the measured reaction, as well as α-TQ. The other prenyllipids showed similar inhibition efficiency with the highest effect for PQH_2-9. Generally, the reduced forms were more active than the corresponding oxidized forms in the inhibition of oxygen uptake. The inhibitory effect of both PQ-2 redox forms, PQ-9 and

α-TQH_2, was lower for hexane-untreated thylakoids than in the case of extracted samples even though higher prenyllipid concentrations were used in this experiment (Figure 4B). In contrast to the extracted membranes, α-TQ was the most active in the inhibition among the investigated prenylquinones. Similarly to the extracted thylakoids, PQH_2-9 showed the highest inhibition of the oxygen uptake for untreated thylakoids either when it was added externally or when it was obtained by reduction of the native PQ-9 by a membrane-bound ferredoxin-PQ reductase in the presence of ferredoxin and NADPH (Figure 4B) (*25*). The control experiments showed no significant influence of ferredoxin or NADPH added alone on the measured reaction. The reduction of the PQ-pool in thylakoid membranes was evidenced by measurements of fluorescence induction kinetics (data not shown). Taking into account the strongest inhibitory effect of PQH_2-9 and that PQ-9 together with PQH_2-9 dominate among prenyllipids of thylakoid membranes (*7*, *26*−*28*), it can be deduced that both PQ-9 redox forms contributed mainly to the inhibitory effect of the hexane extract on the average oxygen uptake shown in Table 1.

DISCUSSION

The presented data show that the fast oxygen electrode can be successfully applied to the measurements of light-induced, PSI-related oxygen uptake in the presence of photosynthetic oxygen evolution inhibitor (DCMU) and an appropriate electron donor. Under our experimental conditions, the only reason for the observed oxygen uptake can be superoxide generation in PSI and its dismutation to hydrogen peroxide and oxygen. The observed inhibition of oxygen uptake by thylakoid prenyllipids was not affected by externally added superoxide dismutase (data not shown), so it suggests that the superoxide was generated by PSI components located within the thylakoid membrane and not on its surface, such as monodehydroascorbate reductase or ferredoxin-$NADP^+$ reductase.

The stimulatory effect of vitamin K_1 on the oxygen uptake, at very low concentrations, is interesting in respect to its possible contribution to the superoxide generation in PSI. Originally, it was suggested that the main sites of superoxide generation from which it is released to thylakoid membrane interior are Fe−S centers: F_X and $F_{A/B}$ (*3*, *4*). In light of the currently known 3-D structure of PSI and kinetics of the electron-transfer reactions (*29*, *30*) it seems that the vitamin K_1 radical is the main source of superoxide generated in PSI. Both F_A and F_B centers are localized outside the membrane surface at the stroma exposed side, and their redox potentials are not much lower than that of ferredoxin, which is known for its very low autoxidation rate (*31*). It suggests that even if F_A and F_B centers are the sites of superoxide generation, this reaction is of low efficiency, and the formed superoxide will be released to stroma rather than to the membrane interior. The F_x center is located near the stromal surface at the edge of the membrane plane and shows considerably lower redox potential than the $F_{A/B}$ centers (*30*). Thus, it could be the effective source of superoxide that could partially diffuse toward the membrane interior. Vitamin K_1 is located deeper within the membrane; at about one-fourth of the membrane thickness from the stromal side, it shows lower redox potential than F_x, and its reoxidation is slower than that of the F_x center (*29*). All these facts suggest that

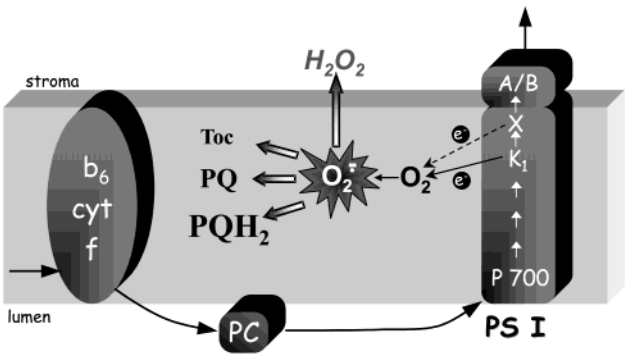

FIGURE 5: Model showing generation sites of superoxide radical in PSI and its scavenging by membrane prenyllipids. The size of the prenyllipid symbols reflects their reactivity with superoxide (based on results from Figure 4A, prenyllipid/Chl = 1:5).

vitamin K_1 should be the main source of superoxide released into the membrane interior. It is known that there are two vitamin K_1 molecules within PSI responsible for two active branches with two different electron-transfer rates (*32*). Hexane extraction removes one of the two vitamin K_1 molecules (*24*), probably the one responsible for the slower component. The increased oxygen uptake after reincorporation of the extracted vitamin K_1 molecule suggests that its autoxidation could be the reason for the observed effect, while the tightly bound, extraction-resistant vitamin K_1 molecule is the main source of the superoxide generation and oxygen uptake in the extracted thylakoids. The question arises as to the reason the addition of vitamin K_1 stimulates oxygen consumption only at the first flash (Figure 3). The reason for this could be that the added vitamin K_1 molecule incorporates into its pocket within the PSI protein, and after one-electron reduction to the polar semiquinone form it changes the surrounding protein conformation because of strong electrostatic interactions in the way that its subsequent photoreduction is not possible anymore. Possibly, hexane extraction of the loosely bound vitamin K_1 modifies irreversibly the protein pocket of this molecule so that its reincorporation in the fully active form is not possible.

Among the investigated thylakoid membrane prenyllipids, PQH_2-9 showed the highest inhibitory effect on the oxygen uptake both in the extracted thylakoids as well as in the untreated membranes, and it was especially active when it was reduced in situ enzymatically. The higher PQH_2-9 activity than that of other prenyllipids is probably connected with their different localization in thylakoid membranes. The headgroups of PQ-2, α-TQ, their reduced forms, and α-Toc are supposed to be located close to the membrane surface (*18*, *33*, *34*), while the headgroups of the reduced and oxidized form of PQ-9 are supposed to reside closer to the interior of the thylakoid membrane (*18*, *33*, *35*) where the superoxide is generated. The lower activity of PQ-9 than that of PQH_2-9 is probably due to a different mechanism of reaction of these prenyllipids with superoxide. Considering the superoxide scavenging effectiveness of the investigated thylakoid prenyllipids, we should also take into account their relative proportions in the membrane where $PQ(H_2)$-9 are the most abundant prenyllipids (*7*, *26*−*28*). The relative superoxide scavenging activity of major thylakoid prenyllipids is illustrated in Figure 5.

The question arises as to the mechanism of superoxide scavenging reactions by the prenyllipids. Prenylquinones (Q)

could be reduced by superoxide to the corresponding semiquinones:

$$Q + O_2^{-\bullet} \rightarrow Q^{-\bullet} + O_2 \quad (1)$$

and the formed semiquinone can undergo dismutation to the corresponding quinone, and the quinol anion and the latter after protonation is converted to the hydroquinone form. When prenylquinols or α-Toc reacts with superoxide, the reaction probably follows the mechanism suggested for 3,5-di-*tert*-butyl alcohol in aprotic media (i.e., by proton abstraction from the prenylquinol or α-Toc molecule (*36*)).

It is important to realize that electrons transferred from superoxide to PQ-9 via the semiquinone form of PQ-9 (reaction 1) may reenter the PQ-pool in a form of PQH_2-9. When PQH_2-9 is further oxidized by the cytochrome b_6f complex in a linear electron transport chain, electrons are transferred to PSI forming superoxide-mediated cyclic electron flow around PSI.

The results of our experiments indicate that the PQH_2-9/PQ-9 couple, as well as other natural membrane prenyllipids, such as α-Toc or α-$TQ(H)_2$, plays an important role in scavenging the superoxide radical formed in PSI, preventing thylakoid membrane components from deleterious effects of the superoxide. Moreover, the superoxide-dependent reactions of prenyllipids reduce the level of superoxide diffusing toward the membrane surface and inhibit formation of toxic hydrogen peroxide in chloroplasts.

ACKNOWLEDGMENT

We would like to thank Prof. Walter Oettmeier for a gift of DNP-INT. The authors wish to thank Robert N. Corning for correcting the manuscript.

REFERENCES

1. Miyake, C., Schreiber, U., Hormann, H., Sano, S., and Asada, K. (1998) *Plant Cell Physiol. 39*, 821−829.
2. Miyake, C., and Asada, K. (1992) *Plant Cell Physiol. 33*, 541−553.
3. Takahashi, M., and Asada, K. (1988) *Arch. Biochem. Biophys. 267*, 714−722.
4. Asada, K. (1994) in *Causes of Photooxidative Stress and Amelioration of Defence Systems in Plants* (Foyer, C. H., and Mullineaux, P. M., Eds.) pp 77−104, CRC Press, Boca Raton, FL.
5. Takahashi, M., Kono, Y., and Asada, K. (1980) *Plant Cell Physiol. 21*, 1431−1438.
6. Asada, K. (1999) *Annu. Rev. Plant Mol. Biol. 50*, 601−639.
7. Kruk J., and Strzałka, K. (1995) *J. Plant Physiol. 145*, 405−409.
8. Munne-Bosch, S., and Allegre, L. (2002) *Crit. Rev. Plant Sci. 21*, 31−57.
9. Kruk, J., Strzałka, K., and Schmid, G. H. (1994) *Free Radical Res. 21*, 409−416.
10. Kruk, J., Jemioła-Rzemińska, M., and Strzałka, K. (1997) *Chem. Phys. Lipids 87*, 73−80.
11. Osmond, C. B., and Grace, S. C. (1995) *J. Exp. Bot. 48*, 1351−1362.
12. Asada, K., Kiso, K., and Yoshikawa, K. (1974) *J. Biol. Chem. 249*, 2175−2181.
13. Robinson, H. H., and Yocum, C. F. (1980) *Biochim. Biophys. Acta 590*, 97−106.
14. Schmid, G. H., and Thibault, P. (1979) *Z. Naturforsch. 34*, 414−418.
15. Kimikura, M., and Katoh, S. (1972) *Biochim. Biophys. Acta 283*, 279−292.
16. Ouitrakul, R., and Izawa, S. (1973) *Biochim. Biophys. Acta 305*, 105−118.
17. Kruk, J. (1988) *Biophys. Chem. 32*, 51−56.
18. Kruk, J., Strzałka, K., and Leblanc, R. M. (1992) *Biochim. Biophys. Acta 1112*, 19−26.
19. Kok, B., Forbush, B., and McGloin, M. (1971) *Photochem. Photobiol. 14*, 307−321.
20. Asada, K. (2000) *Philos. Trans. R. Soc. London. B 355*, 1419−1431.
21. Hager, A., and Holocher, K. (1994) *Planta 192*, 581−589.
22. Oettmeier, W., Johanningmeier, U., and Trebst, A. (1982) in *Function of Quinones in Energy Conserving Systems* (Trumpower, B. L., Ed.) pp 425−441, Academic Press, New York.
23. Magree, L., Henninger, M. D., and Crane, F. L. (1966*) J. Biol. Chem. 241*, 5197−5200.
24. Malkin, R. (1986) *FEBS Lett. 208*, 343−346.
25. Endo, T., Mi, H., Shikanai, T., and Asada, K. (1997) *Plant Cell Physiol. 38*, 1272−1277.
26. Barr, R., and Crane, F. L. (1971) *Methods Enzymol. 23A*, 372−408.
27. Kruk, J., and Strzałka, K. (1998) *Phytochemistry 49*, 2267−2271.
28. Kruk, J., Burda, K., Schmid, G. H., Radunz, A., and Strzałka, K. (1998) *Photosynth. Res. 58*, 203−209.
29. Fromme, P., Jordan, P., and Krauss, N. (2001) *Biochim. Biophys. Acta 1507*, 5−31.
30. Chitnis, P. R. (2001) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 52*, 593−626.
31. Hosein, B., and Palmer, G. (1983) *Biochim. Biophys. Acta 723*, 383−390.
32. Guergova-Kuras, M., Boudreaux, B., Joliot, A., Joliot, P., and Redding, K. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 4437−4442.
33. Jemioła-Rzemińska, M., Kruk, J., and Strzałka, K. (2003) *Chem. Phys. Lipids 123*, 233−243.
34. Wang, X., and Quinn, P. J. (2000) *Mol. Membr. Biol. 17*, 143−156.
35. Kruk, J., Strzałka, K., and Leblanc, R. M. (1993) *J. Photochem. Photobiol. 19*, 33−38.
36. Nanni, E. J., Stallings, M. D., and Sawyer, D. T. (1980) *J. Am. Chem. Soc. 102*, 4481−4485.

BI034036Q