Evidence for the Involvement of Cyclic Electron Transport in the Protection of Photosystem II against Photoinhibition: Influence of a New Phenolic Compound†

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ABSTRACT: Organisms that perform oxygenic photosynthesis are subjected to inhibition of their photosynthetic functions when they are exposed to excessive illumination. Photoinhibition occurs mainly at the level of photosystem II, where a cyclic electron transport has often been proposed to be involved in photoprotection. However, a demonstration of direct protection by cyclic photosystem II against photoinhibitory damage has been lacking. In this report, we used the newly characterized compound 4-[methoxybis(trifluoromethyl)methyl]-2,6-dinitrophenylhydrazine methyl ketone (K-15), known to stimulate cyclic electron transport between the acceptor and donor sides of the photosystem [Klimov, V. V., Zharmukhamedov, S. K., Allakhverdiev, S. I., Kolobanova, L. P., & Baskakov, Y. A. (1993) *Biol. Membr. 6*, 715−732], to verify if photosystem II is significantly protected by cyclic electron transport against aerobic and anaerobic photoinhibitory damage. The photoinhibitory quenching of the maximal level of fluorescence and the decrease of the absorbance change at 685 nm related to pheophytin photoreduction observed during photoinhibitory illumination of untreated or Mn-depleted photosystem II submembrane fractions are significantly attenuated in the presence of K-15. The photodegradation of cytochrome *b*559 and the photobleaching of β-carotene and chlorophyll-670 measured in Mn-depleted photosystem II preparations are also strongly retarded when K-15 is present. The detection, by photoacoustic spectroscopy, of the energy stored during the cyclic electron transport is also reported in Mn-depleted photosystem II submembrane fractions and in photosystem II reaction center complexes. This reaction is also gradually photoinhibited due to the progressive photodegradation of the required electron transport intermediates but is significantly more stable in the presence of K-15. It is deduced that cyclic electron transport around photosystem II constitutes an effective protective mechanism against photoinhibitory damage.

Exposure of organisms possessing oxygenic photosynthesis to irradiance exceeding that required for normal growth leads to inactivation of their photosynthetic functions. The photoinactivation of the electron transport reactions, known as photoinhibition, primarily affects photosystem II (PSII)[1] (Powles, 1984). This photosystem constitutes a water−plastoquinone oxidoreductase imbedded between two integral membrane polypeptides named D1 and D2 (Debus, 1992). PSII electron transport is initiated by a photoinduced charge separation that produces the couple [$P680^+Pheo^-$]. Stabilization of the charge-separated state occurs through electron transfer from reduced pheophytin ($Pheo^-$) to the primary and secondary quinone acceptors Q_A and Q_B (Diner et al., 1991). $P680^+$ is reduced by Y_Z, which is believed to be tyrosine-161 of the polypeptide D1 (Debus et al., 1988; Metz et al., 1989), which in turn oxidizes the Mn cluster of the oxygen-evolving complex (Boska et al., 1983; Renger, 1993).

Photoinhibition is thought to involve at least two mechanisms of photosystem II inactivation that affect the acceptor and donor sides, respectively (Barber & Andersson, 1992). In the acceptor-side mechanism, the plastoquinone pool is kept in the fully reduced state by strong illumination. In that case, the Q_B site remains unfunctional due to the lack of oxidized plastoquinone, which leaves the primary quinone acceptor in the reduced state (Q_A^-) (Vass et al., 1992). The latter most probably becomes protonated and may leave its binding site in the reaction center complex after a second reduction (Styring et al., 1990). However, even if reduced Q_A remains at its binding site, its protonation should allow a significant yield of charge separation owing to the removal of the electrostatic repulsion between $Pheo^-$ and reduced Q_A (Van Mieghem et al., 1992; Vass & Styring, 1992; Setlik et al., 1990). The recombination between the primary charge separation products [$P680^+Pheo^-$] leads to the formation of P680 in the triplet state (Takahashi et al., 1987). Under aerobic conditions, the triplet states are rapidly quenched by oxygen with the consequent appearance of highly reactive singlet oxygen (Durrant et al., 1990), which, in contrast with anaerobic photoinhibition (Vass et al., 1992; Kirilovsky & Etienne, 1991), makes the inactivation irreversible due to a preferential destruction of P680 (Telfer et al., 1990).

Though acceptor-side-induced photoinhibition is considered as the main mechanism of PSII photoinactivation, donor-

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[1] Abbreviations: PSII, photosystem II; Pheo, pheophytin; Chl, chlorophyll; cyt *b*559, cytochrome *b*559; F_m, maximal yield of fluorescence; Q_m, maximal yield of thermal dissipation.

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side photoinhibition occurs independently from the presence of oxygen when the water-splitting complex is not functioning properly (Callahan et al., 1986; Jegerschöld et al., 1990; Jegerschöld & Styring, 1991). This leads to the formation of strongly oxidizing radicals such as $P680^+$ and Y_Z^+ that can be the source of photodamage (Blubaugh & Cheniae, 1990; Blubaugh et al., 1991; Klimov et al., 1990). Donor-side photoinhibition is also known to result in the irreversible photobleaching of β-carotene and Chl670 (also named Chl_Z), an accessory chlorophyll (Chl) of the reaction center complex occuring with a Chl670/P680 ratio of 1 (Thompson and Brudwig, 1988; Klimov et al., 1990; Telfer et al., 1991).

Cyclic electron transport around PSII has been suggested as a photoprotection mechanism that could retard both acceptor- and donor-side photoinhibition (Telfer et al., 1991; Thompson & Brudwig, 1988). In fact, the cycle would allow the reoxidation of reduced intermediates on the acceptor side of the photosystem and the reduction of oxidized species on the donor side. Recently, this cycle was proposed to include the reduction of cytochrome *b*559 (cyt *b*559) by $Pheo^-$ and reoxidation of the cytochrome by $P680^+$, providing a concrete role to cyt *b*559 in photoprotection (Barber & De Las Rivas, 1993; Poulson et al., 1995). However, a direct demonstration that cyclic PSII really protects against photoinhibitory illumination has not been provided. In this paper, we use the newly characterized compound 4-[methoxybis(trifluoromethyl)methyl]-2,6-dinitrophenylhydrazine methyl ketone (K-15) [see Klimov et al. (1993) and references therein], which has been shown to accept electrons from $Pheo^-$ and to reduce the donor side of PSII, thus stimulating cyclic electron transport (Klimov et al., 1993). It is demonstrated that the increased cyclic PSII electron transport activity efficiently protects against aerobic or anaerobic photoinhibitory damage in PSII submembrane fractions (depleted or not in Mn cluster) or in PSII reaction center complexes.

MATERIALS AND METHODS

Chloroplasts were prepared from deveined spinach leaves according to Whatley and Arnon (1963) and were used for isolation of the "heavy" oxygen-evolving PSII submembrane fractions, designated as DT-20, following the procedure previously described (Klimov et al., 1982) with some modifications (Allakhverdiev et al., 1994). Chl was determined according to Arnon (1949). The preparations exhibited an oxygen evolving activity of 250−300 μmol/mg of Chl h under saturating illumination with 0.3 mM potassium ferricyanide and 0.2 mM phenyl-*p*-benzoquinone as artificial electron acceptors. They retained their full complement in extrinsic polypeptides associated with the oxygen-evolving complex and their activity was independent from the addition of exogenous Ca^{2+} or Cl^-. The concentration of PSII reaction centers was calculated from the value of photoinduced absorbance changes at 685 nm (ΔA_{685}) related to the photoreduction of Pheo and from that at 680 nm related to photooxidation of the primary electron donor P680 (Klimov et al., 1980). The DT-20 samples were composed of 80−100 Chl molecules per one P680 or one photoreducible Pheo and contained 15 500−16 000 Chl molecules per one P700. The complete (>95%) extraction of Mn from the DT-20 samples was performed using Tris−EDTA treatment as described previously (Klimov et al., 1982; Allakhverdiev et al., 1994).

The phenolic compound 4-[methoxybis(trifluoromethyl-)methyl]-2,6-dinitrophenylhydrazine methyl ketone, named K-15, was synthetized jointly with the laboratory of Dr. Yu. A. Baskakov, Institute of Chemical Protection of Plants, Moscow, following the procedure described previously (Konstantinova et al., 1980).

The PSII reaction center complex (D1−D2−cyt *b*559 complex) was isolated from DT-20 preparations by the method of Nanba and Satoh (1987). The stoichiometry of the complex was about 5−6 Chl *a*/2 Pheo/1 cyt *b*559. The complex was suspended at a Chl concentration of 4 μg/mL in 50 mM Mes (pH 6.5), 35 mM NaCl, and 2 mM $MgCl_2$.

Photoinhibitory treatments were performed in a glass cuvette (10 mL) with a 10-mm light path which was irradiated from one side (400−700 nm, 140 W/m^2) passed through a heat-reflecting filter (Allakhverdiev et al., 1987). The reaction medium was composed of PSII submembrane fractions or reaction center complexes at a Chl concentration of 10 μg/mL, 20 mM Tris-HCl (pH 7.8), 35 mM NaCl, and 2 mM $MgCl_2$. Anaerobic conditions were obtained by adding glucose (10 mM), glucose oxidase (50 units/mL), and catalase (1000 units/mL), followed by a 5-min incubation before light treatment.

Photoinduced changes of Chl fluorescence (ΔF) and absorbance (ΔA) at various wavelengths (490, 559, 680, and 685 nm) were measured in a 10-mm path length sample cuvette (2 mL) using a single-beam differential spectrophotometer with a phosphoroscope assembly similar to that described previously (Klimov et al., 1980). Chl fluorescence (λ > 660 nm) was induced by a weak measuring beam (480 nm, 0.15 W/m^2). An actinic beam (λ > 600 nm, 10 W/m^2) was used to saturate the photoreactions (Klimov et al., 1980). The cyt *b*559 content was estimated from reduced (dithionite, 1 mg/mL) minus oxidized (ferricyanide, 1 mM) absorbance difference spectra using an extinction coefficient of 17.5 mM^{-1} at 559 nm (Cramer et al., 1986). Absorption spectra were recorded using Perkin-Elmer (Model 553) and Hitachi (Model 557) UV−Vis spectrophotometers.

In some experiments, fluorescence and thermal deactivations were measured simultaneously using a laboratory-constructed photoacoustic spectrophotometer in conjunction with a PAM-101 chlorophyll fluorometer (Walz, Effeltrich, Germany) as previously described (Allakhverdiev et al., 1994). For these experiments, the samples were aspirated onto a nitrocellulose filter using a gentle vacuum (Carpentier et al., 1989). The photoacoustic measuring beam (680 nm, 35 Hz) was set at an intensity of 2.2 W/m^2. The maximal yield of fluorescence (F_m) and thermal (Q_m) emissions were obtained by the addition of the nonmodulated background illumination of saturating intensity from the Walz KL1500 illuminator.

RESULTS AND DISCUSSION

Photosystem II submembrane fractions were subjected to photoinhibitory illumination (140 W/m^2) during various periods of time. The maximal yield of fluorescence (F_m) and the photoinduced absorbance change at 685 nm (ΔA_{685}) related to Pheo photoreduction were measured in the presence of 1 mg/mL sodium dithionite added after the photoinhibitory treatments. At this low potential, Q_A becomes reduced even in the dark and F_m is readily obtained upon illumination with a weak measuring light (Klimov et al., 1980). A sharp

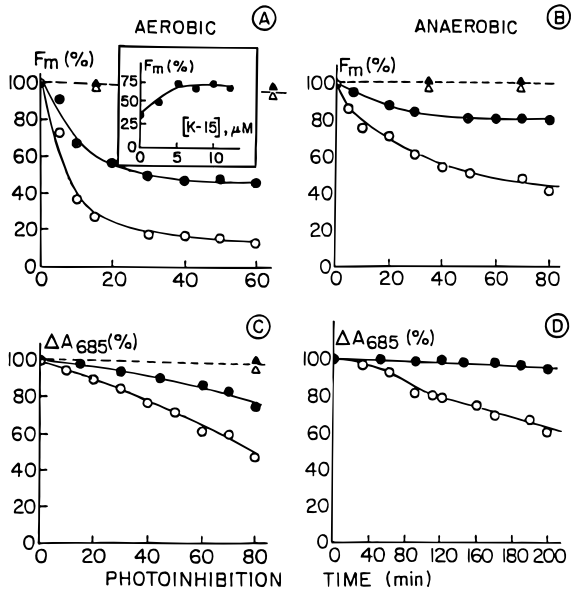

FIGURE 1: Quenching of F_m and decrease of ΔA_{685} during aerobic (panels A and C) and anaerobic (panels B and D) photoinhibitory treatment of photosystem II submembrane fractions. The photoinhibitory illumination (white light, 140 W/m^2) was performed either in the absence (open symbols) or in the presence (closed symbols) of 6 μM K-15. The values represented by triangles were obtained from control samples kept in the dark. The initial value (100%) for ΔA_{685} was 3.1×10^{-3}. The Chl concentration was 10 μg/mL. Anaerobic conditions were obtained with the system glucose/glucose oxidase in the presence of catalase (see Materials and Methods). Inset: Influence of K-15 concentration on the value of F_m measured after 10 min of photoinhibitory treatment.

decline of F_m was observed during the first 15 min of aerobic photoinhibitory illumination, after which some stabilization occurred (Figure 1A). After 40 min of photoinhibition, F_m was reduced to 15% of its initial value. The half-time for this fluorescence quenching ($t_{1/2}$) was 8−9 min.

In the present experiments, which were performed in the absence of artificial electron acceptor, both donor- and acceptor-side photoinhibition are expected to occur. Strong fluorescence quenching at F_m has been inferred to originate from conditions that promote donor-side photoinhibition where strongly oxidizing species (Y_Z^+ and/or $P680^+$) can possibly induce the formation of an oxidized β-carotene that may act as a fluorescence quencher (Blubaugh et al., 1991). When the photoinhibitory treatment was performed in the presence of 6 μM 4-[methoxybis(trifluoromethyl)methyl]-2,6-dinitrophenylhydrazine methyl ketone (also named K-15), a compound known to inhibit linear electron transport by preventing Q_A photoreduction (Klimov et al., 1993), this process was significantly attenuated (Figure 1A). Under these conditions, F_m was only reduced to 45% of its initial value after a 40-min aerobic photoinhibitory treatment and $t_{1/2}$ increased to 25 min. The optimal effect of K-15 was obtained at concentrations above 5 μM (Figure 1A, inset). The action of K-15 was limited to the period of photoinhibitory illumination; during measurements, the presence of dithionite keeps K-15 in a reduced form that does not inhibit electron transfer in PSII (Klimov et al., 1993). It was previously deduced from fluorescence and electron transport measurements that the mechanism of action of the newly characterized K-15 implies the oxidation of $Pheo^-$ and the induction of a cyclic electron transport around PSII [see Klimov et al. (1993) and references therein]. Hence, cyclic electron transport can improve the reduction of the donor side of the photosystem and prevent the formation of the fluorescence quencher.

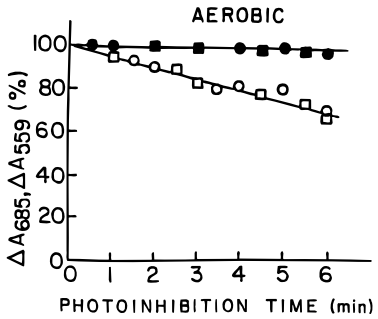

FIGURE 2: Decrease of ΔA_{685} (circles) and of the amount of cytochrome *b*559 (ΔA_{559}) (squares) during aerobic photoinhibitory treatment of Mn-depleted PSII submembrane fractions. The initial value (100%) for ΔA_{559} was 1.6×10^{-3}. The photoinhibitory illumination was performed either in the absence (open symbols) or in the presence (closed symbols) of 6 μM K-15. The Chl concentration was 10 μg/mL.

The amount of photoreducible Pheo measured as ΔA_{685} was less sensitive to aerobic photoinhibition in comparison with F_m. After 60 min of photoinhibitory illumination, the absorbance change at 685 nm decreased by only 50% in the absence of K-15 and by less than 25% in its presence (Figure 1C).

The protective action of K-15 was also demonstrated under anaerobic conditions. In that case, $t_{1/2}$ for the photoinhibitory quenching of F_m was already increased to 55 min even in the absence of K-15, due to partial impairment of acceptor-side photoinhibition, which is an oxygen-dependent photoinhibitory reaction (Metz et al., 1989; Setlik et al., 1990; Jegerschöld & Styring, 1991). F_m declined by more than 50% after an 80-min illumination in the absence of K-15, whereas in its presence, F_m decreased by only 20% (Figure 1B). Again, ΔA_{685} was much less affected in comparison with F_m and this reaction was almost fully protected in the presence of K-15 (Figure 1D).

Donor-side photoinhibition can be amplified in Mn-depleted PSII preparations where the inhibition of water splitting constitutes a condition known to specifically generate such photoinhibition (Callahan et al., 1986; Jegerschöld et al., 1990; Jegerschöld & Styring, 1991; Klimov et al., 1990). Experiments similar to those shown in Figure 1 were performed on these preparations together with cyt *b*559 measurements (Figures 2 and 3). The total amount of cyt *b*559 present and the extent of Pheo photoreduction were not affected by the photoinhibitory illumination period used (up to 8 min) under anaerobic conditions (results not shown). However, F_m did decline by 40% after only 2 min of illumination (Figure 3A), possibly due to the formation of oxidized β-carotene molecules (Blubaugh et al., 1991; Klimov et al., 1990). This decline was reduced to 20% in the presence of K-15 (Figure 3A). The changes in F_m were much more pronounced under aerobic conditions and more than 70% of F_m was quenched after 6 min of photoinhibition (Figure 3B). Further, cyt *b*559 concentration declined simultaneously with the amount of photoreducible Pheo, and both parameters decreased by about 30% after 6 min of aerobic photoinhibition (Figure 2A). Thus, the data obtained from the Mn-depleted PSII preparations are in agreement with the results obtained from untreated PSII preparations and demonstrate that F_m is affected by photoinhibition well

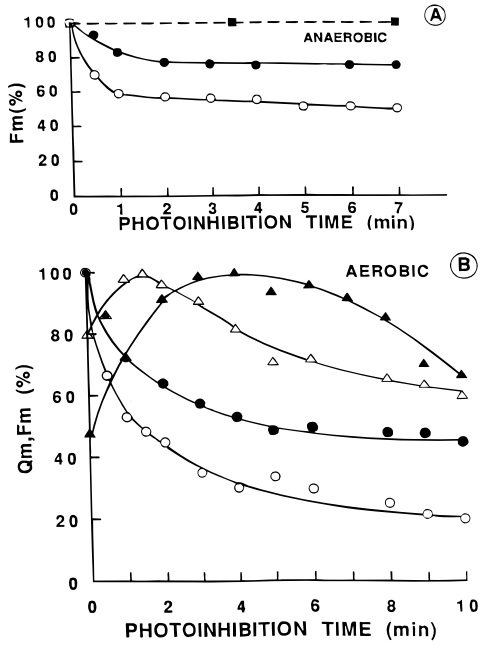

FIGURE 3: Quenching of F_m (circles) and Q_m (triangles) during anaerobic (A) and aerobic (B) photoinhibitory treatment of Mn-depleted PSII submembrane fractions either in the absence (open symbols) or in the presence (closed symbols) of 10 μM K-15. Values represented by squares were obtained from control samples kept in the dark. The preparation (100 μL) at a Chl concentration of 100 μg/mL was aspirated onto a nitrocellulose filter for simultaneous measurement of F_m and Q_m, an operation that also removes dithionite from the sample. Q_m values were normalized to 100% at their maximal value obtained with or without K-15.

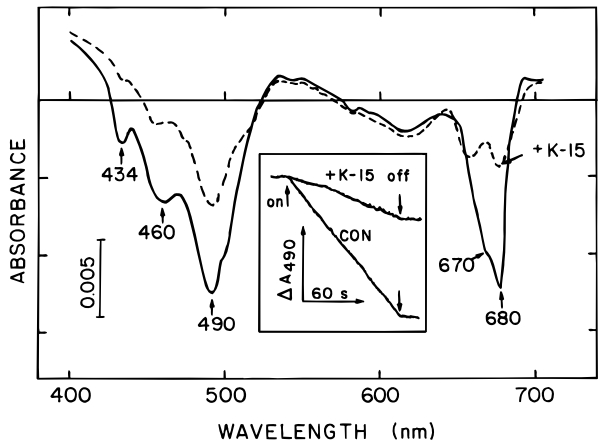

FIGURE 4: Absorption difference spectrum obtained between samples kept in the dark and samples exposed to aerobic photoinhibitory illumination during 2 min in the absence (solid line) or presence (dashed line) of 6 μM K-15. Inset: Kinetics of the absorbance change at 490 nm during photoinhibitory illumination in the absence or presence of K-15.

before Pheo photoreduction and the amount of measurable cyt *b*559 are affected (Blubaugh et al., 1991; Allakhverdiev et al., 1987).

The protective action of K-15 related to its activation of cyclic electron transport around PSII can be generalized to the irreversible bleaching of β-carotene molecules (490 and 460 nm), Chl670, and P680 also studied in Mn-depleted PSII preparations (Figure 4). The bleaching of these pigments is strongly retarded in the presence of K-15 as more specifically illustrated in Figure 4 (inset) for the bleaching of β-carotene molecules.

The possible detection by photoacoustic spectroscopy of the energy stored during cyclic electron transport around PSII in photoinhibited leaves and thylakoid membranes has been discussed previously (Jansen et al., 1989; Lapointe et al., 1993). In this technique, the sample is introduced in a closed cell and illuminated with an intensity-modulated light. The thermal deactivations in the sample generate a periodic heat flow in the gas phase that produces a pressure wave monitored by a sensitive microphone as a photoacoustic signal. In active photosynthetic materials, part of the absorbed energy is stored in photochemical intermediates and therefore not released as heat. This energy storage results in a reduced thermal dissipation yield detected as a lower photoacoustic signal in comparison with the signal obtained from a photochemically inactive sample (Braslavsky, 1986). Here, we used photoacoustic spectroscopy to monitor thermal energy dissipation in Mn-depleted PSII preparations subjected to various periods of photoinhibitory treatments either in the absence or in the presence of K-15 to verify if cyclic electron transport can be detected (Figure 3B).

The yield of thermal dissipation in Mn-depleted PSII preparations measured as the photoacoustic signal, Q_m, significantly increased during the first minute of photoinhibitory treatment (Figure 3B). During Q_m measurements, the primary quinone acceptor of PSII, Q_A, was kept in the reduced state due to the continuous application of a saturating background illumination; thus linear electron transport was blocked. Therefore, the increase of Q_m is not related to a progressive photoinhibition of energy storage during linear electron transport to the acceptor Q_B. Alternatively, the increase of Q_m could be due to the increased yield of thermal deactivation processes expected from the quenched values of F_m found under the same conditions or to a progressive decrease of energy storage in consequence of the photoinhibition of an endogenous cyclic electron transport activity. However, the rise of Q_m was much more pronounced when K-15 was present during the photoinhibitory illumination (about 50% of maximal Q_m value in comparison with a rise of only 20% observed when K-15 was absent) but the quenching of F_m was weaker (Figure 3B). Thus, the large increase of Q_m is not directly related to the quenching of F_m. The main origin for the increasing thermal dissipation yield is more likely the gradual photoinhibition of cyclic PSII electron transport. This interpretation is in agreement with the lower value of Q_m before the start of photoinhibition when K-15 is present. This lower Q_m represents a stronger energy storage due to the K-15-stimulated cyclic electron transport. We propose, following the idea that cyclic PSII electron transport may include the photoreduction and photooxidation of cyt *b*559 (Barber & De Las Rivas, 1993), that the energy storage monitored by the initially low level of Q_m (Figure 3B) involves the photoreduction of this cytochrome. The above is possible even in the presence of saturating illumination because of the constant availability of oxidized cyt *b*559, taking into account the facts that in the Mn-depleted PSII preparations (that are inactive in oxygen evolution) the photoreduction of photooxidized cyt *b*559 is quite slow and the photooxidation is enhanced [see Barber and De Las Rivas (1993) and references therein; Buser et al., 1992].

Q_m reached its maximal value of 100% after 1.5 min of photoinhibitory treatment in the absence of K-15 but only after 4 min in its presence. The above demonstrates the

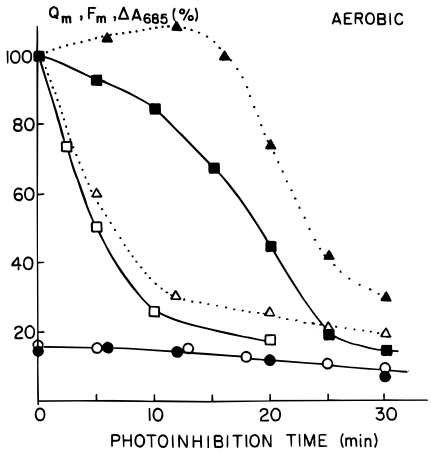

FIGURE 5: Quenching of F_m (circles) and Q_m (triangles) and decrease of ΔA_{685} (squares) during aerobic photoinhibitory illumination of PSII reaction center complexes either in the presence (closed symbols) or in the absence (open symbols) of 6 μM K-15. The preparation at a Chl concentration of 4 μg/mL in the presence of 1% lauryl maltoside was either aspirated onto a nitrocellulose filter (100 μL) as in Figure 3 for simultaneous measurements of F_m and Q_m or kept in suspension (2 mL) for ΔA_{685} measurements. The initial value (100%) for ΔA_{685} was 1.7×10^{-2} at 682 nm, which was in fact the wavelength used for measurements and corresponds to the maximal ΔA value in these preparations.

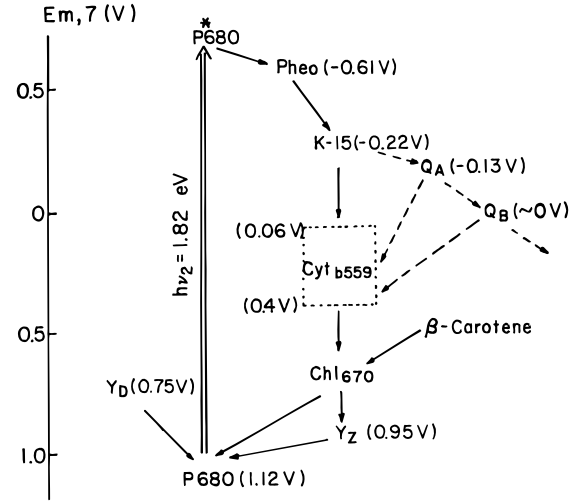

FIGURE 6: Schematic representation of the proposed photoprotective cyclic electron transport pathway around PSII, including the involvement of the phenolic compound K-15. The possibility of redox potential shifts in the case of cyt *b*559 is indicated. The dotted lines represent the main PSII electron transport pathway inhibited in the presence of K-15.

retarded photoinhibition of cyclic electron transport in PSII in the presence of K-15. The maximal value of Q_m is followed by a decline. The Q_m values found after 6 min of photoinhibitory treatment (Figure 3B) indicate that during this period Q_m decreases by 30% from its maximal value in the absence of K-15 and by 3−5% in its presence, which corresponds with the decrease in the yield of Pheo photoreduction during the same period (Figure 2).

The protective influence of K-15 against photoinhibition was also verified in the D_1−D_2−cyt *b*559 complexes. The fluorescence yield was very low in these preparations and slowly declined during the photoinhibitory treatment (Figure 5). In contrast, the amount of photoreducible Pheo decreased by more than 75% after 10 min of photoinhibition in the absence of K-15. Under these conditions, the photoacoustic signal Q_m was devoid of the initial rise found in Mn-depleted PSII preparations. Instead, it started to decline immediately after the onset of photoinhibitory treatment at a rate similar to the decline of ΔA_{685} (Figure 5). The above indicates that cyclic electron transport, if present, was not measurable as energy storage in the isolated reaction center complexes without K-15. An inefficient rate of cyclic electron transport in the reaction center complex may explain the rapid decline of ΔA_{685}.

However, cyclic electron transport was more active in the reaction center complexes exposed to photoinhibitory conditions in the presence of K-15 as indicated by the initially rising Q_m. As in Mn-depleted PSII preparations, this rise of Q_m reached a maximal value followed by the usual decline (Figure 5). The retarded decay of Q_m was paralleled with a retarded decrease of ΔA_{685}, thus confirming the equivalent results found in Mn-depleted PSII preparations (Figures 2A and 3B). This progressive reduction of the thermal dissipation yield is attributed to the similar quenching of thermal dissipation previously reported in anaerobic or sodium dithionite-treated PSII preparations kept under saturating illumination (Allakhverdiev et al.,1994). The latter conditions lead to the conversion of the reaction centers into a photochemically inert state [P680 $Pheo^-$] Q_A^- where the yield of charge separation in the primary photoreaction is strongly decreased (Allakhverdiev et al., 1994), a situation that resembles the state of PSII reaction centers where the Pheo photoreduction activity has been photoinhibited.

A role of cyclic electron transport around PSII involving the photoreduction and photooxidation of cyt *b*559 in the protection against photoinhibition has been recently proposed from experiments using the D_1−D_2−cyt *b*559 complex (Barber & De Las Rivas, 1993). Even though an endogenous cyclic electron transport could not be detected in terms of energy storage in the photoinhibited reaction center complexes, after the addition of K-15 this activity was indicated by the delayed quenching of Q_m. As discussed above, K-15-mediated cyclic electron transport involves the oxidation of $Pheo^-$ by K-15 followed by its subsequent reoxidation by a component of the donor side of PSII (Klimov et al., 1993). The seemingly inefficient cyclic electron transport found in the reaction center complexes in the absence of K-15 may indicate that quinone acceptors are required to complete a more active endogenous cyclic electron transfer pathway that could provide an efficient protection against photoinhibition, the direct reduction of cyt *b*559 by Pheo being slower (Blubaugh et al., 1991; Arnon & Tang, 1988; Buser et al., 1992).

Several different schemes representing cyclic electron transport in PSII were suggested (Blubaugh et al., 1991; Barber & De Las Rivas, 1993; Arnon & Tang, 1988; Buser et al., 1992). A proposed simplified scheme that could illustrate the cyclic electron transport demonstrated above and the site of K-15 stimulation of this cycle is presented in Figure 6. It was previously proposed by Nedbal et al. (1992) that low-potential cyt *b*559 can oxidize $Pheo^-$. It is possible that the high-potential form of cyt *b*559 is converted to the low-potential form upon reduction of the acceptor side by strong illumination (Styring et al., 1990); it has been shown that part of the cyt *b*559 is transformed to the low-potential form during photoinhibition (Tamura & Cheniae, 1987). Dithionite, but not ascorbate, can reduce K-15; therefore, its redox potential must be more negative than −0.2 V

(Klimov et al., 1993). Furthermore, electrochemical measurements have shown that the midpoint potential is equal to −0.53 V in aprotic solvents, −0.22 V in Tris-HCl buffer (pH 8.0), and −0.35 V in Tris-HCl buffer supplemented with 0.1% sodium dodecyl sulfate (Kiselev et al., 1993). Accordingly, it has been recently demonstrated that K-15 added to the D_1-D_2-cyt *b*559 complex activates both photoreduction of cyt *b*559 and dark reoxidation of its reduced form (Klimov et al., 1995). Thus, K-15 could take part as an activator in a cycle similar to that proposed in Figure 6. In Mn-depleted PSII preparations, an increased oxidation rate of $Pheo^-$ in the presence of K-15 would effectively prevent the reduction of Q_A and thus delay acceptor-side photoinhibition. Transformation of the low-potential cyt *b*559 to its high-potential form, an event that may depend on the ambient redox system as discussed by Barber and De Las Rivas (1993), would be followed by the reoxidation of the high-potential cyt *b*559 by Chl670 (Thompson & Brudwig, 1988). In fact, photooxidation of cyt *b*559 in PSII units where the oxygen-envolving complex is impaired has been the subject of early demonstration (Knaff & Arnon, 1969). Cyt *b*559 oxidation would protect against donor-side photoinhibition by keeping Chl670 and β-carotene in their reduced state. This would also lead to a more efficient reduction of P680 by Y_Z or directly by Chl670. All these protective actions were observed during the experiments presented above and are consistent with a photoprotective function of a cyclic electron transport around PSII.

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