Blue Light-Enhanced Photosynthetic Oxygen Evolution from Liposome-Bound Photosystem II Particles; Possible Role of the Xanthophyll Cycle in the Regulation of Cyclic Electron Flow Around Photosystem II?

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Light-driven electron transport in liposome-bound photosystem II (PS-II) particles between water and ferricyanide was monitored by bare platinum electrode oxymetry. The modification of the experimental system with the exogenous quinones α-tocopherol quinone (α-TQ) or plastoquinone (PQ) resulted in a pronounced effect on photosynthetic oxygen evolution. The presence of α-tocopherolquinone (α-TQ) in PS-II samples decreased the rate of red light-induced oxygen evolution but increased the rate of green light-induced oxygen evolution. Blue light applied to the assay system in which oxygen evolution was saturated by red light resulted in a further increase of the oxygen signal. These findings are interpreted in terms of a cyclic electron transport around PS-II, regulated by an excitation state of β-carotene in the reaction centre of PS-II. A mechanism is postulated according to which energetic coupling of β-carotene in the reaction centre of PS-II and that of other antenna carotenoid pigments is regulated by the portion of the xanthophyll violaxanthin, which is under control of the xanthophyll cycle.

Introduction

Photosynthetic oxygen evolution is the direct consequence of vectorial electron transport in photosystem II (PS-II) from water, via chlorophyll P 680, pheophytin, the first quinone electron acceptor Q_A to the second quinone electron acceptor Q_B (for a review see Govindjee and Coleman, 1993). There are several reports supporting the hypothesis according to which a fraction of the electrons flowing from water to Q_B is turned back to P 680 (Mende, 1980; Heber et al., 1979; Falkowski et al., 1986; Thompson and Brudvig, 1988; Canaani and Havaux, 1990; McNamara and Gounaris, 1992; Lapointe et al., 1993). According to these reports cytochrome b_{559} is involved in such a cyclic electron transport around PS-II and the electrons which are used to reduce cyt b_{559} and which back react with P 680 are diverted from the vectorial electron flow at the level of Q_A (Yamagishi and Fork, 1987; Gounaris et al., 1988; Mathis et al., 1989; Satoh et al. 1990; Sinclair and Kelley, 1992) or Q_B (Mende, 1980; Heber et al., 1979; Thompson et al., 1988; Canaani and Havaux, 1990; McNamara and Gounaris, 1992; Tsujimoto and Arnon, 1985; Whitmarsh and Cramer, 1978; Cramer et al., 1992; Barabás et al., 1993). On the other hand Maurier and Bendall express the view that cyclic electron transport around PS-II, if existing at all, has no significance in the photosynthetic process (Meunier and Bendall, 1993). In the present study the modulation of photosynthetic oxygen evolution by light quality in the presence of exogenous quinones is interpreted in terms of a cyclic electron transport around PS-II which is controlled by an excitation state of the reaction center β-carotene, energetically coupled to PS II antenna carotenoids.

Abbreviations: PS-II, Photosystem II; SiMo, Silicomolybdate; cyt b559, cytochrome b_{559}; Q_A, the first quinone acceptor in the reaction center of photosystem II; Q_B, the second quinone electron acceptor in the reaction center of photosystem II; α-TQ, α-tocopherolquinone; PQ, plastoquinone.

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Materials and Methods

PS-II particles were isolated from leaves of tobacco (var. John William's Broadleaf) as described elsewhere (Berthold *et al.*, 1981) and suspended in a 30 mM Tricine-NaOH buffer (pH 7.5) containing 60 mM KCl. Small unilamellar liposomes were prepared with egg yolk lecithin (Sigma) with the incubation buffer as described above. An amount of 10 mg of lipids was dried under a nitrogen stream and then sonicated with 1 ml buffer. In some cases α-Tocopherol quinone (α-TQ), purified as described elsewhere (Kruk, 1988), or plastoquinone-9 (PQ-9), which was a gift from Hoffmann La Roche, Basel, were used in the lipid fraction in a concentration of 1 mol%. PS-II particles were incorporated to liposomes by shaking and brief pulses of sonication. Excess PS-II particles, not incorporated to lipid vesicles were removed from the samples by centrifugation at $12000 \times g$. The concentration of chlorophyll in the PS-II particles incorporated into liposomes was usually 20 μg per ml of liposome suspension or slightly higher. Oxygen evolution measurements were based on chlorophyll, i.e. any differences in the chlorophyll concentration of the assay were carefully taken into account. Directly before oxygen measurements, ferricyanide was added to each sample to give a final concentration of 1 mM. Oxygen evolution was measured with a bare platinum electrode described in detail by Schmid and Thibault (1979). Light of modulated intensity was provided by a halogen projector lamp equipped with a set of different filters: green light - 500 nm interference filter, blue light - 350 nm to 550 nm band-pass filter and red light - 600 nm - 750 nm cut-off filter. Spectrophotometric measurements were carried out with an UV/Vis Spectrophotometer, Lambda 3 (Perkin Elmer).

Results and Discussion

Fig. 1 shows the original traces of oxygen measurements of PS-II samples illuminated with red or green light. Two kinds of samples were examined: samples containing exogenous α-TQ or samples without additions. As it is seen, the modification of isolated PS-II particles with exogenous quinone results in a decrease of the initial rate of photosynthetic oxygen evolution if induced by red light which is exclusively absorbed by chlorophyll pigments. On the other hand, the addition results in an increase of oxygen evolution if induced by green light, exclusively absorbed by carotenoids. Since exogenous quinones are electron acceptors that are artificially added to the samples one might expect an enhancement of vectorial electron transfer from water, i.e. an enhancement of oxygen evolution. This obviously is not the case with red light-induced oxygen evolution; α-TQ rather lowers oxygen evolution. One possible explanation might be that the reconstitution of the Q_A function and/or that of Q_B in the PS-II sample leads to electron transport in which a fraction of electrons transferred from P 680 to the electron acceptor ferricyanide goes back to P 680$^+$ by the pathway of a cyclic electron transfer around PS-II. In this context it should be noted that the reduction of cyt b_{559} was postulated to be part of a cyclic electron transport which was reported to be dependent on the reconstitution of quinones in PS-II reaction centre preparations (McNamara and Gounaris, 1992; Satoh *et al.*, 1990; Sinclair and Kelley, 1992; Tsujimoto and Arnon, 1985).

The effect of the quinone-induced increase of photosynthetic oxygen evolution in the sample illuminated with green light may be explained in terms of the presence of an additional electron acceptor in the system, under conditions in which cyclic electron transfer is inactive. The fact that the green light applied is exclusively absorbed by carotenoids might suggest an interdependence between carotenoid absorption and inactivation of the pathway of cyclic electron transport around PS-II. Thus, the β-carotene, present in reaction centre of PS-II (Kruse *et al.*, 1994) might be the candidate involved in the regulation of a cyclic electron flow .

Fig. 2 shows traces of measurements of oxygen evolution induced by red light of saturating intensity. This oxygen evolution was substantially enhanced when samples were illuminated with blue light in addition to the saturating red light. Blue light is absorbed by both carotenoids and chlorophylls whereas red light is exclusively absorbed by chlorophyll pigments. The effect of carotenoid excitation-dependent enhancement of photosynthetic oxygen evolution in PS-II particles might be interpreted as being due to an inhibition of cyclic electron flow around PS-II just as in the case of the green light effect, shown above.
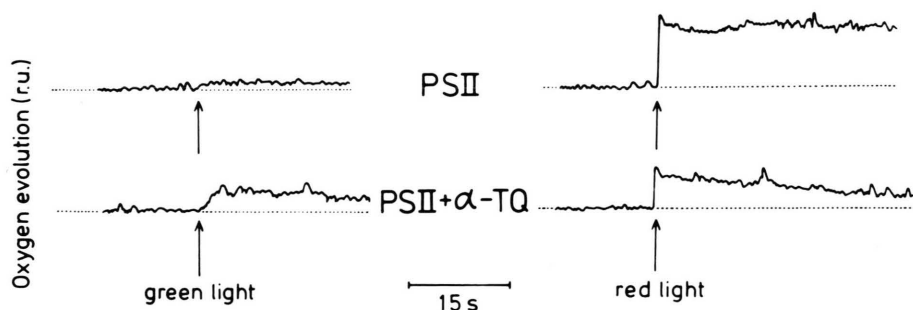
Fig. 1. Original traces of the oxygen signal from PS-II particles incorporated into liposomes, containing α-TQ when indicated. Arrows indicate the onset of green light ($7 \mu E\ m^{-2}s^{-1}$) or red light ($15\ \mu E\ m^{-2}s^{-1}$) irradiation.

A similarly strong effect was observed in samples of liposome-bound PS-II in the presence of α-TQ or PQ. In the absence of exogenous quinones the effect of blue light was also observable but about 50% less intensive (not shown). On the basis of these experiments it is difficult to decide whether reconstitution of the Q_A or Q_B function is essential in the effect observed. Silicomolybdate (SiMo), an electron acceptor of PS-II (Barr *et al.*, 1975), with its binding site close to the non-heme iron, accepts electrons between Q_A and Q_B (Schansker and Van Rensen, 1993). This electron acceptor completely inhibits the blue light effect on photosynthetic oxygen evolution described in this paper (see Fig. 3). It looks as if the state in which both quinones (Q_A^-, Q_B^-) are reduced, represents the necessary condition for an efficient reduction of cyt b_{559}. In the electron transfer scheme shown in Fig. 4 linear and cyclic electron transfer pathways separate at a level close to the none-heme iron between Q_A and Q_B. In order to explain the experimental results described above, as the decisive element of cyclic electron flow between cyt b_{559} and P 680 one β-carotene molecule was hypothetically introduced into the scheme. This localization is supported by the finding that the presence of β-carotene in the reaction center of PS-II was necessary to oxidize cyt b_{559} (Cox and Bendall, 1974) and that generation of a radical cation of β-carotene was reported (Schenck *et al.*, 1982; Telfer *et al.*, 1991; De Las Rivas *et al.*, 1993) as a consequence of electron transfer to P 680$^+$ under conditions of SiMo-induced stabilization of oxidized cyt b_{559}. These results imply that the level of the redox potential of the β-carotene molecule in the reaction center of PS-II should lie between 1.2 V (because β-carotene is oxidized by P680$^+$) and 0.15 V which is the redox potential of low-
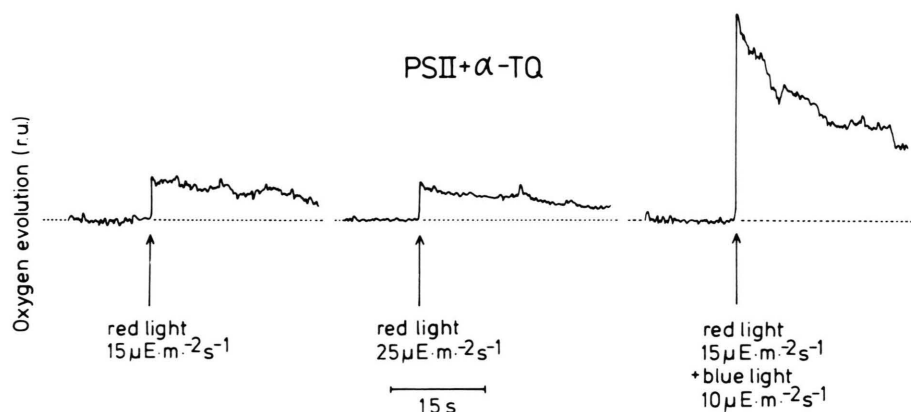

Fig. 2. Original traces of the oxygen signal from PS-II particles incorporated into liposomes containing α-TQ. Arrows indicate the onset of light. Light quality and intensity are indicated.
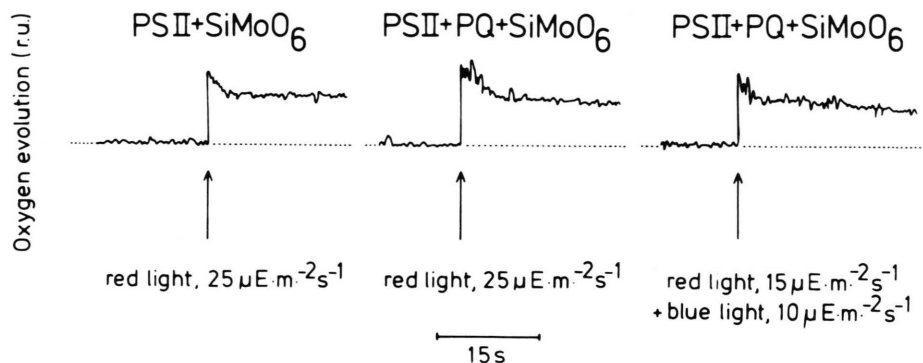
Fig. 3. Original traces of the oxygen signal from PS-II particles incorporated into liposomes containing PQ when indicated. The electron acceptor SiMo was present in all samples in a concentration of 5 μM. Arrows indicate the onset of light. Light quality and intensity are indicated.

potential cyt b_{559} (Shuvalov *et al.*, 1994). The act of light absorption by β-carotene would decrease its potential by about 2.58 V, corresponding to the energy of a 0-0 transition to the ^{1}Bu state (480 nm). The resulting potential obviously has to be below the level of low-potential cyt b_{559} or even of the extra low-potential cyt b_{559} (-0.049 V, (Shuvalov *et al.*, 1994) which should make it impossible to transfer electrons via β-carotene to P 680 in the direction indicated in Fig. 4. Electron transfer from β-carotene in the opposite direction (β-carotene oxidation by cyt b_{559}) was demonstrated in PS-II samples in the presence of the charged reagent tetraphenylboron (Velthuys, 1981). In addition to the reasons pointed out above there is an additional argument in favour of direct participation of β-carotene in the chain of cyclic electron transport around PS-II. β-carotene is a long rod-

like molecule (long about 30 Å (Milon *et al.*, 1986)), able to span the hydrophobic core of a thylakoid membrane (thick about 30 Å (Deisenhofer and Michel, 1989)) thus connecting electrically, due to the conjugated double bound system, like a "molecular wire" even electron donors and acceptors separated by long distances such as the porphyrin center of cyt b_{559} and P 680.

Fig. 5 shows the effect of blue light on the absorption spectrum of the PS-II sample in the presence of SiMo, which is supposed to block cyclic electron transport. Three observed spectral features should be noted: i) the negative absorbance values in the difference spectrum in the region
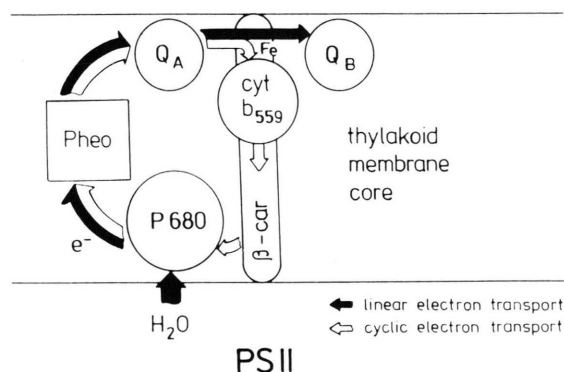
Fig. 4: Hypothetical scheme of a cyclic electron flow around PS-II.
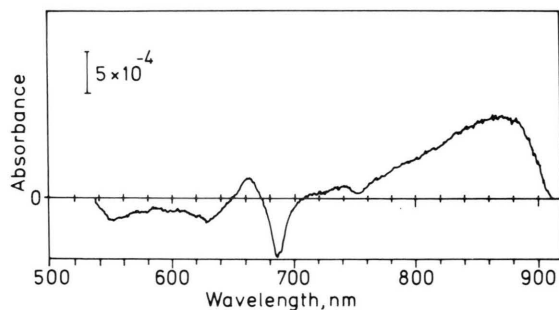
Fig. 5: Difference absorption spectrum of PS-II in the presence of exogenous α-TQ. The following spectra were subtracted in order to see an effect of blue light in the presence of the electron acceptor SiMo: ((PS-II + SiMo + BL) minus (PS-II + BL)) minus ((PS-II + SiMo) minus (PS-II). Where: (PS-II + SiMo + BL) denotes the PS-II sample containing 5 μM SiMo illuminated with blue light of 10 μE m^{-2}s^{-1} during the absorbance measurements. A cut-off filter (500 nm) was placed in front of the detector during measurements.

corresponding to cyt b_{559} (see at 560 nm) which are probably related to its oxidized state in the presence of SiMo; ii) the hypsochromic shift of the Qy-band of chlorophylls, demonstrated by the appearance of negative and positive bands in the corresponding region of the difference spectrum; and iii) finally the appearance of a broad positive band in the near-infrared spectral region which may be interpreted as being related to a carotenoid cation radical formation (Schenck *et al.*, 1982). This spectral analysis additionally supports the concept presented above, namely that excitation of β-carotene promotes its oxidized state that directly inactivates cyclic electron flow back to P 680. SiMo alone has no effect on the PS-II spectrum in the near-infrared region (not shown).

The weak point of the model, according to which regulation of the cyclic electron flow around PS-II by β-carotene would be realized by its excitation, is that a number of light quanta absorbed by two β-carotene molecules in the reaction center of PS-II in a given time unit is not enough to keep them in a preponderantly excited state. This, however, may be easily overcome if light quanta absorbed by numerous antenna carotenoids were funneled to the reaction center β-carotene(s) what should promote its (their) excited state. The assumption is based on the physical principles of excitation energy transfer, considering that singlet-singlet excitation energy transfer between states of similar energies (carotinoid-carotinoid singlet energy transfer) should be more efficient than energy transfer between molecules whose singlet energy levels are separated by a certain gap (carotenoid-chlorophyll singlet energy transfer). If the flux density of light quanta is assumed to be 500 $\mu E \cdot m^{-2} \cdot s^{-1}$, with the absorbance due to antenna carotenoids of photosystem II being 1 and the cross section of the photosystem complex being 14 300 nm^2 (estimated according to Dainese *et al.*, 1992), then the rate of excitation of a β-carotene molecule which would collect all carotenoid antenna excitations, may be estimated to be approx. 4 x 10^6 s^{-1}. This process would not be fast enough to compete with the decay of the carotenoid singlet state ^{1}Car* which is 1 x 10^{11} s^{-1} according to Cogdell *et al.* (1994). Due to this estimation it is rather the lowest triplet level of β-carotene which has a decay rate of 1.67 x 10^5 s^{-1} according to Mathis and Schenck (1982) which

might be the candidate responsible for the energy storage in the reaction center β-carotene, thereby serving as a switch of cyclic electron transport around photosystem II. Carotenoid triplet states are formed via intersystem crossing and were detected in photosynthetic material by means of Raman spectroscopy and EPR-techniques (for a review see Mathis and Schenck, 1982). There are several lines of support for the hypothesis that light absorbed by carotenoids may inactivate cyclic electron flow around PS-II. As a result of such an inactivation a fraction of cycling electrons being directed to vectorial electron flow should increase photosynthetic oxygen evolution. Such a mechanism might explain the effect of monospecific antisera to seveal carotenoid pigments (violaxanthin in particular) on photosynthetic electron transport leading to a decrease of the rate of photosynthetic oxygen evolution (Lehmann-Kirk *et al.*, 1979b). It is not probable that antisera to violaxanthin considerably decrease the total cross-section of antenna pigments nor that violaxanthin participates directly in electron transfer resulting in 30% inhibition of light-induced oxygen evolution (Lehmann-Kirk *et al.*, 1979 a + b; Radunz and Bader, 1982). Pigment-antiserum interaction may, however, affect molecular distances in the antenna system network decreasing the efficiency of excitation energy transfer towards the reaction center on the energetic level of carotenoids and in consequence "feeding" it into the reaction center β-carotene. A decreased rate of excitation of this β-carotene should make cyclic electron flow possible, thus decreasing photosynthetic oxygen evolution. A correlation between the rate of photosynthetic oxygen evolution and the concentration of carotenoid pigments in different species was recently reported (Sandmann *et al.*, 1993). A central role of the xanthophyll pigment violaxanthin in the proposed mechanism may be also concluded on the basis of the correlation reported between the state of epoxidation of xanthophyll cycle pigments and photosynthetic oxygen evolution (Thayer and Björkman, 1990). Violaxanthin, being the epoxidized derivative of zeaxanthin, is de-epoxidized under conditions of overexcitation of the photosynthetic apparatus in the xanthophyll cycle (Yamamoto, 1979; Siefermann-Harms, 1977; Hager, 1980), which operates in the thylakoid membrane (Gruszecki and Krupa, 1993). Zeaxan-

thin was concluded to be present directly within the lipid phase of the thylakoid membrane (Gruszecki and Strzalka, 1991; Havaux and Gruszecki, 1993) whereas violaxanthin was shown to act as an antenna pigment (Siefermann-Harms, 1984; Havaux *et al.*, 1991). The importance of violaxanthin in stabilizing the native structure of pigment-protein antenna complexes (Plumley and Schmidt, 1987; Paulsen *et al.*, 1990; Paulsen and Hobe, 1992; Paulsen *et al.*, 1993) may lie in the mechanism of violaxanthin-dependent regulation of the flow of excitation energy from the antenna to the reaction center (β-carotene). This is consistent with the finding that under physiological conditions a major fraction of chloroplast violaxanthin is bound to three minor pigment-protein complexes CP-24, CP-26 and CP-29, proposed to play a role in the regulation of excitation energy transfer from the main antenna complexes towards the reaction center of PS-II (Dainese *et al.*, 1992; Dainese *et al.*, 1993; Bassi *et al.*, 1993). In the present study the equivalent of 1 μl of a monospecific antiserum to violaxanthin (for the methodology see Lehmann-Kirk *et al.*, 1979b) was found to inhibit completely the blue light-induced enhancement of photosynthetic oxygen evolution. The control serum gave 30% of inhibition under the same conditions indicating that it is just the interaction to exogenous proteins that leads to an effect on the mechanism responsible for the action of light absorbed by carotenoid pigments. If one compares the amount of oxygen evolved as the consequence of a blue light pulse with that evolved under steady state conditions in red light (Fig. 2), the conclusion is that cyclic electron transport might account for 70% of the total flow via P_{680}. This clearly, implies that cyclic electron flow is of the same order of magnitude as that vectorial one.

The discussion presented above may be concluded with a model of the control of cyclic electron flow around PS-II via the activity of the xanthophyll cycle. De-epoxidation of violaxanthin which is a light-dependent process and essentially operates under overexcitation conditions results in inhibition of efficient energy transfer from antenna carotenoids towards the reaction center, thus decreasing the rate of excitation of the reaction center β-carotene. β-carotene in its ground state facilitates and opens the possibility to efficient cyclic electron transfer around PS-II back to P 680 via cyt b_{559} whereas β-carotene in the reaction center in its excited state makes cyclic electron flow impossible (for the energetic reasons pointed out above). A modulation of the excitation state of the reaction center β-carotene via the xanthophyll cycle is proposed in order to control the efficiency of the photoprotective mechanism represented by the cyclic electron flow around photosystem II.

Acknowledgements

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