Cyclic Electron Flow Around Photosystem II as Examined by Photosynthetic Oxygen Evolution Induced by Short Light Flashes

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Photosynthetic oxygen evolution from photosystem II particles was analyzed as consequence of a train of short (5 \mu s) flashes of different light quality and different intensities to study cyclic electron flow around photosystem II. Damped oscillations of the amplitudes of \textit{O}_2-evolution corresponding to a flash sequence were fitted numerically and analyzed in terms of a non-homogeneous distribution of misses, represented by the probability parameter \( \alpha \). Application of red light, known to promote cyclic electron flow around photosystem II (Gruszecki et al., 1995) results in a considerable increase of all \( \alpha \), indicating that at the molecular level the misses may be interpreted as resulting from a competition for the reduction of oxidized P680 between cyclic electron flow and the electron flow coming from the water splitting enzyme. In accordance with previous findings, application of light flashes of the spectrum covering the absorption region of carotenoids resulted in an inhibition of cyclic electron flow and a pronounced decrease of the level of the miss parameter. Possible molecular mechanisms for the activity control of this cyclic electron transport around photosystem II by carotenoids are discussed.

**Introduction**

Several physiological mechanisms are known to operate at different organisational levels of the photosynthetic apparatus in order to regulate its activity in dependence on the fluence rate of incident light. This optimizes the efficiency of photosynthesis and provides the photosynthetic apparatus with protection against overexcitation-related photodestruction (Horton and Ruban, 1992). A cyclic electron transfer around photosystem II is considered as such a protective mechanism maintaining the primary electron donor P680 in a reduced condition under the influence of strong light. Under this condition light-induced electron transfer from P680 to pheophytin (generation of P680\textsuperscript{+}) is significantly faster than the maximal rate of P680\textsuperscript{+} (Strzalka et al., 1990; Schulder et al., 1992) reduction by electrons transported from the water oxidizing enzyme (see also discussion in Gruszecki et al., 1995). In previous reports (Gruszecki et al., 1995, 1996) we have correlated the inhibition of activity of the cyclic electron transport around PSII with the excitation of photosynthetically active carotenoids. We have postulated that \( \beta \)-carotene which is a constituent of the PSII reaction centre, was a component of the cyclic electron transfer chain, being placed between P680 and cyt \( b_{559} \). In the present paper, photosynthetic oxygen evolution induced by short, single turnover flashes was analyzed with the aim to examine the competition for electron donation to P680\textsuperscript{+} between the postulated cyclic electron flow and the usual linear electron transport which leads to water splitting.

Photosynthetic oxygen evolution induced by short, single-turnover light flashes yields the pattern of a damped oscillation (Joliot et al., 1969). The explanation of this behaviour was proposed in 1970 by Kok and coworkers introducing a model of a four-step oxidizing cycle within the water oxidizing enzyme. To describe the experi-

**Abbreviations:** PSII, photosystem II; P680, primary electron donor in photosystem II; Cyt\( b_{559} \), cytochrome \( b_{559} \); Hepes, N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid].

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mentally observed damping of oxygen evolution, Forbush et al. (1971) introduced two perturbations into the model: double hits, understood as the transition of the system through two successive states as the consequence of a single flash and misses, representing missed transitions from one state to the other despite the flash. Misses, which are characterized by the probability $\alpha$ appear to be the main reason for the damping of $O_2$-oscillations (Lavorel, 1978). It was later demonstrated that the model which describes most closely the experimental data has to assume a nonhomogeneous distribution of misses between different states (Delrieu, 1974, 1983). One of the proposed explanations for misses relates them to the acceptor side of PSII (Shinkarev and Wright, 1993) and assumes an electron transfer back to P680$^+$, which is consistent with the proposed model of a cyclic electron transfer around PSII (Gruszecki et al., 1995).

Materials and Methods

Photosystem II particles, isolated from leaves of tobacco (var. John William’s Broadleaf) according to the procedure of Berthold et al. (1981) were suspended in 20 mM Hepes-NaOH buffer, pH 7.4, containing 15 mM NaCl, 5 mM MgCl$_2$, 2 mM CaCl$_2$ and 400 mM sucrose. An aliquot of the PSII particle preparation corresponding to 10 $\mu$g of chlorophyll and containing $5 \times 10^{-4}$ M potassium ferricyanide was deposited on a HA 0.45 $\mu$m Millipore filter and placed on a bare platinum electrode (Schmid and Thibault, 1979) with the PSII layer being in direct contact with the electrode (to ensure a short diffusion distance for oxygen to the electrode surface). All operations were carried out in weak green light. Before starting the polarographic measurements, the sample was dark adapted for 10 min.

White light flashes of 5 $\mu$s duration at half intensity and spaced 300 ms apart were generated by a Stroboscope 1539A (General Radio, Concord, Mass., USA) flash lamp. The flash intensity was evaluated by the integration of the total light quantum number of a high flash number in the spectral region between 400 and 750 nm. The intensity obtained was then divided by the flash number. A single white flash applied had the fluorescence rate corresponding to continuous light of 6000 $\mu$Em$^{-2}$s$^{-1}$. A single red flash had the fluorescence rate corresponding to 2500 $\mu$Em$^{-2}$s$^{-1}$ and was obtained by application of a cut-off filter with transmission above 600 nm. A single green flash had the fluorescence rate corresponding to continuous green light of 1300 $\mu$Em$^{-2}$s$^{-1}$ and was obtained by use of a Schott interference filter of 525 nm correspondingly. A blue flash had the intensity of 3300 $\mu$Em$^{-2}$s$^{-1}$ and was obtained by application of a Schott band-pass filter BG12, with transmission between 300 and 500 nm. Strong red light corresponding to 9200 $\mu$Em$^{-2}$s$^{-1}$ with the same spectral characteristics as weak red light was also used. The measurements of every experimental variant were repeated at least 5 times.

Results and Discussion

Original traces of polarographic measurements of photosynthetic oxygen evolution, induced by a train of 15 short light flashes, are shown in Fig. 1. Different light qualities and intensities were applied in this study (Table I): red light absorbed exclusively by chlorophylls, allowing active cyclic electron flow around PSII (Gruszecki et al., 1995, 1996); red light with addition of green light, the latter absorbed exclusively by carotenoids in the peripheral region of their absorption spectrum; blue light, covering the absorption spectra of both chlorophylls in the Soret band and carotenoids. Fig. 2 shows diagrams of amplitudes of photosynthetic oxygen evolution following separate light flashes, calculated on the basis of the recordings presented in Fig. 1 or similar ones for other variants. As it may be seen, different light qualities result in different patterns of $O_2$ evolution amplitudes. In particular, in the case of red light, promoting active cyclic electron flow around PSII, the typical damped oscillations do not occur and the obtained pattern of oxygen evolution resembles that of a system with over-reduced S-states containing a high proportion of the S$_1$ state. Such a phenomenon was described by Schmid and Thibault (1983) in dark-adapted Euglena cells and can also be induced by treatment with a strong reductant in Chlorella, which normally exhibits a typical Kok pattern of oxygen evolution. This, however, cannot be the case in the system used in our study, since the presence of the oxidizing agent, potassium ferricyanide, in the reaction medium practically excludes the overreduction of the S-state sys-
Fig. 1. Original traces of polarographic measurements of photosynthetic oxygen evolution following illumination of the photosystem II particles from tobacco with a train of 15 short light flashes of different light quality: A – white light; B – red light; C – simultaneous flashes of red light and green light.

Table I. Determination of misses for the S-state transition $S_i$ to $S_{i+1}$ and determination of the initial S-state distribution in dependence on the light quality of the flashes in photosystem II particles of *Nicotiana tabacum*.

<table>
<thead>
<tr>
<th>Flash colour</th>
<th>Number of the S-state $i$</th>
<th>$S_i$ miss</th>
<th>Average miss $\alpha_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>0</td>
<td>0.17</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.77</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.06</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.009</td>
<td>0.017</td>
</tr>
<tr>
<td>Red</td>
<td>0</td>
<td>0.165</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.78</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.056</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.001</td>
<td>0.18</td>
</tr>
<tr>
<td>Strong</td>
<td>0</td>
<td>0.165</td>
<td>0.53</td>
</tr>
<tr>
<td>Red</td>
<td>1</td>
<td>0.78</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.047</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.008</td>
<td>0.44</td>
</tr>
<tr>
<td>Red+Green</td>
<td>0</td>
<td>0.165</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.78</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.052</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.003</td>
<td>0.30</td>
</tr>
<tr>
<td>Blue</td>
<td>0</td>
<td>0.169</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.796</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.031</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.004</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Values are normalized to 1. Light intensities in $\mu$Em$^{-2}$ s$^{-1}$: white, 6000; red, 2500; strong red, 9200; red 2500 + green 1300; blue 3300. The determination of the miss parameter contains an error considerably below 1 percent.

The explanation for the observed pattern can only be the reduction of the rate of successful transitions between successive S-states under red light flashes (see below), i.e. an increase of the miss parameter $\alpha_i$. Table I presents the best least-square-fitted parameters corresponding to the experimental patterns depicted in Fig. 2 and also to patterns of other variants. The method of numerical fitting applied was that described by Burda and Schmid (1996). As it may be seen in Table I, independently of the light quality and intensity the initial distribution of states is such that about 77% of the reaction centres remain in the $S_1$ state and about 17% in $S_0$ state, the $S_2$ and $S_3$ states being populated to a much lower extent. In the sample illuminated with strong white light the miss probability is only significant in the $S_2$ to $S_1$ transition ($\alpha_2 \approx 0.57$) which is characteristic for this variant. In the case of red light, all $\alpha_i$ parameters are significantly higher, so the average value of $\alpha$ ($\alpha_m$), calculated per single flash, increases by a factor of 4.5 when compared to the white light experiments. In our opinion this increase of misses is directly related to the activity of the cyclic electron flow around PSII and only indirectly, as it will be discussed below, with the light intensity. In fact, the experiments with considerably higher red light intensity result in a similar oxygen evolution pattern (not shown) and also in a relatively high value of $\alpha_m$ (0.54, see Table I). In these experiments all $\alpha_i$ parameters appear to be relatively high, indicating that the cyclic electron flow is active from the first flash. Possibly, the electron removed from P680 does not immediately return to P680$^+$. It looks as if rather another electron, and this could come from the $\beta$-carotene of the reaction centre (Gruszczki *et al.*, 1995), is used for reduction of P680$^+$, creating at the same time a $\beta$-carotene cation radical. Light induced creation of this molecular spe-
Fig. 2. Flash-induced oxygen yield pattern in photosystem II particles of tobacco expressed as amplitudes of the polarographic signal (rel. units) per amount of total chlorophyll deposited on the electrode (in μg). Illumination: A – with white light, B – with red light, C – with red light + green light, D – with blue light. The parameters of the mathematical fits corresponding to the patterns A – D are presented in Table I.

As concluded from our previous study (Gruszecki et al., 1995, 1996) the present findings support that the β-carotene in the PSII reaction centre plays a central role in the regulation of the activity of cyclic electron flow. According to the recent report (Bialek-Bylka et al., 1995) this particular molecule remains in the 15–15'-cis configuration in darkness and isomerizes to the all-trans form upon illumination. The blue light-induced isomerization of the PSII reaction centre β-carotene would be a good candidate for a molecular mechanism of regulation of cyclic electron flow.

Excitation related changes in polarizability of the pigment molecule may be responsible for decreasing the energetic barrier for isomerization. The carotenoid triplet state provides the most favourable conditions for the cis-trans isomerization (Koyama and Hashimoto, 1993), however, formation of such a state is not possible via blue light absorption and intersystem crossing. Therefore, we assume that the electric charge delocalization in the β-carotene cation radical combined with an excitation of the molecule may provide the conditions for an efficient cis-trans isomerization, as observed experimentally (Bialek-Bylka et al., 1995). The alternative pathway for the formation of the triplet state of the reaction centre β-carotene by triplet – triplet excitation energy transfer from P680 is considered not to be effective (Telfer and Barber, 1995).

Further studies are necessary, however, to elucidate the eventual role of the cis-trans isomerization of the reaction centre β-carotene in a molecular mechanism engaged in the regulation of cyclic electron transport around PS II.

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