Decrease of fluorescence intensity after the K step in chlorophyll a fluorescence induction is suppressed by electron acceptors and donors to photosystem 2

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Abstract

Chlorophyll a fluorescence induction measured by a fluorometer with a high temperature stressed plant material shows a new K step which is a clear peak due to fast fluorescence rise and subsequent decrease of fluorescence intensity. We focused on an explanation of the decrease of fluorescence after the K step using artificial electron acceptors and donors to photosystem 2 (PS2). Addition of the artificial electron acceptors or donors suppressed the decrease of fluorescence after the K step. We suggest that the decrease mainly reflects (by more than 81 %) an energy loss process in the reaction centre of PS2 which is most probably a nonradiative charge recombination between P680⁺ (oxidised primary electron donor in PS2) and a negative charge stored on either Pho⁻ or QA⁻ (reduced primary electron acceptor of PS2 and reduced primary quinone electron acceptor of PS2, respectively). We suggest that the energy loss process is only possible when the inhibition of both the donor and the acceptor sides of PS2 occurs.

Additional key words: charge recombination; fluorescence quenching; high temperature stress; Pisum sativum.

Introduction

In 1991-2, results of measurements of fluorescence induction using fluorometer PEA (Plant Efficiency Analyser, Hansatech, Norfolk, England) were introduced by

Received 19 April 1999, accepted 16 August 1999.
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Abbreviations: catechol - 1,2-benzenediol; DPC - 1,5-diphenylcarbazide; DQ - tetramethyl-p-benzoquinone (d duroquinone); Fl - fluorescence induction; HNQ - 2-hydroxy-1,4-naphthoquinone; OEC - oxygen evolving complex; P680 - the primary electron donor in photosystem 2; PEA - Plant Efficiency Analyser; Pho - primary electron acceptor of photosystem 2 (pheophytin); PpBQ - phenyl-p-benzoquinone; PS - photosystem; QA - primary (bound) quinone electron acceptor of PS2; QB - secondary (mobile) quinone electron acceptor of PS2.

Acknowledgement. This work was supported by grant of Ministry of Education of Czech Republic number CEZ: J14/98: N70000010.
Strasser and Govindjee (1991, 1992). Using red LED diodes for excitation, PEA allows measurements of fluorescence induction (FI) from 10 μs after the onset of excitation radiation which is characterised by the O-J-I-P pattern being clearly discriminated only when a logarithm time-axis is used (Strasser and Govindjee 1991, 1992, Strasser et al. 1995, see Fig. 1).

As fluorescence is mainly emitted by PS2 at room temperature (see Krause and Weis 1991, Dau 1994), the O-J-I-P pattern of FI also reflects the state of PS2. The O step of FI corresponds to the minimal fluorescence \( F_0 \) (also called dead fluorescence) which is obtained when all functional reaction centres of PS2 are open (Krause and Weis 1991). There is an experimental and theoretical evidence that the J step is the photochemical phase (Delosme 1967, Neubauer and Schreiber 1987, Strasser et al. 1995) connected with primary photochemistry, i.e., reduction of the primary electron acceptor in PS2, pheophytin (Pheo), and \( Q_A \) (the primary quinone electron acceptor in PS2) by charge separation and charge stabilisation, respectively (Stirbet et al. 1995, 1998, Lazár et al. 1997a, 1998). On the other hand, the J-I-P rise is the thermal phase (Delosme 1967, Neubauer and Schreiber 1987), reflecting subsequent accumulation of single or double reduced \( Q_B \) (the secondary quinone electron acceptor in PS2) (Stirbet et al. 1995, 1998, Strasser et al. 1995, Lazár et al. 1997a) but also a heterogeneity in reduction of plastoquinone pool (Strasser et al. 1995, Barthélémy et al. 1997). As there is an equivalence of the J step with \( I_1 \) step and the I step with \( I_2 \) step (Strasser et al. 1995), the \( I_1 \) and \( I_2 \) being intermediate steps of FI curve measured under extreme high irradiance (Neubauer and Schreiber 1987), the J to I rise is affected by the state of the oxygen evolving complex (OEC) as was found for the \( I_1 \) to \( I_2 \) rise by Schreiber and Neubauer (1987). The I step bears on structure of the light-harvesting complexes (Barthélémy et al. 1997). Sometimes, there is a local fluorescence minimum (dip), labelled as D, between the I and P steps. Mathematical simulation suggests that D reflects a dynamic equilibrium between PS2 and photosystem 1 (PS1) (Stirbet et al. 1995, 1998) which agrees with the same interpretation of a dip measured under low irradiance (Munday and Govindjee 1969, Schreiber et al. 1972). For the latest review on FI see Lazár (1999).

After heating plant material to about 47 °C (for 5 min or by linear heating to this temperature), a new step, K, at about 300 μs appears in FI (Guissé et al. 1995a,b, Lazár and Ilik 1997, Lazár et al. 1997b, Srivastava et al. 1997, Strasser 1997). The appearance of the K step may be caused by an inhibition of OEC (Guissé et al. 1995a,b, Srivastava et al. 1997, Strasser 1997, Lazár and Pospíšil 1999) and by an inhibition of electron transport from Pheo to \( Q_A \) (Guissé et al. 1995b). The appearance of the K step may also reflect changes in the structure of the light-harvesting complex of PS2 (Srivastava et al. 1997). The assumption that at the time at which the K step appears in FI an accumulation of \( Q_A^- \) occurs, is a basis for suggesting that a decrease of fluorescence intensity after the K step reflects an oxidation of \( Q_A^- \) via subsequent electron acceptors of PS2 (Srivastava et al. 1997, Strasser 1997). All results indicate that the appearance of the K step reflects an irreversible high-temperature induced change of PS2 (Guissé et al. 1995b, Srivastava et al. 1997, Lazár and Ilik 1997, Lazár et al. 1997b).
In this work, we focus on the decrease of fluorescence after the K step. We found that the decrease is suppressed by the addition of artificial electron acceptors and donors to PS2 and we suggest that the decrease mainly reflects a nonradiative charge recombination between P680\(^+\) and a negative charge stored on either Pheo\(^-\) or QA\(^-\).

**Materials and methods**

All measurements were done with primary leaves of two weeks old pea (*Pisum sativum*). The plants were grown at 25 °C in a growth chamber (85 % humidity) on artificial soil composed of perlit and supplied with Knop solution. The light/dark regime was 16 h light/8 h dark with continuous "white" irradiation of 90 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) of Philips SL-Prismatic bulbs (25 W). The concentration of chlorophyll \((a+b)\) in the leaves was determined according to Lichtenthaler (1987) to be about 140 mg m\(^{-2}\).

As artificial PS2 electron acceptors we used phenyl-\(p\)-benzoquinone (PpBQ) and tetramethyl-\(p\)-benzoquinone (DQ) which accept electrons next to QA\(^-\) (Henrysson and Sundby 1990) and 2-hydroxy-1,4-naphthoquinone (HNQ) which accepts electrons from Pheo\(^-\) (Renger and Kayed 1987, Nedbal et al. 1992). As artificial electron donors to PS2 we used 1,5-diphenylcarbazide (DPC) and 1,2-benzenediol (catechol - Yamashita and Butler 1968, Vernon and Shaw 1969). With the exception of HNQ which was from Lancaster (Mühlheim, Germany) all other chemicals were obtained from Sigma (Deisenhofen, Germany). The chemicals were dissolved in ethanol and then added to distilled water in a way that the ethanol formed only 1/100 of final solution volume of particular concentration. The low concentration of ethanol was chosen to avoid possible inhibition of electron transport by ethanol (Masamoto and Nishimura 1978). A concentration of a chemical which is sufficient enough to provoke an expected change in the course of FI was checked by measurements of FI with a high temperature stressed plant material upon different concentration (100, 200, 500, and 1000 \(\mu\text{M}\)) of the chemical (not shown). In this way we found for all artificial electron acceptors that the course of FI did not differ when the concentration was 200 \(\mu\text{M}\) and higher, and 500 \(\mu\text{M}\) and higher for the artificial electron donors. Thus we used the concentration of 200 \(\mu\text{M}\) for all electron acceptors and 500 \(\mu\text{M}\) for all electron donors.

The measurements of control FI were done at 25 °C after 30 min of adaptation of the leaves to darkness. When FI with the K step without any chemicals was measured, the leaves were kept for 30 min in darkness and then immersed for 5 min in a distilled water bath of 47 °C (45 °C, 49 °C) in darkness. While measuring fluorescence induction with the artificial chemical, the leaves were immersed into the solution of the chemical and kept there for 40 min in darkness before an incubation for 5 min in darkness at 47 °C. Immediately (couple of seconds) after pulling out the leaf from the water bath, FI was measured by Plant Efficiency Analyser (*PEA, Hansatech*, Norfolk, England). The irradiance was about 3 400 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) of red radiation with the maximum at wavelength of 650 nm. All particular kinds of fluorescence induction curves (room temperature, with K step, with particular artificial electron acceptor or donor) were measured five times and a typical curve
was chosen for presentation. The irradiance was measured by a quantum radiometer LI-189 (LI-COR, Lincoln, USA).

Results

A typical F1 measured by PEA fluorometer with the control (25 °C) and high temperature stressed (47 °C, 5 min) pea leaves (Fig. 1) shows the O, K, J, I, and P steps. If draining of electrons from $Q_A^-$ via subsequent electron acceptors of PS2 is only responsible for the decrease of fluorescence after the K step as suggested by Srivastava et al. (1997) and Strasser (1997) then, according to their idea, addition of an artificial electron acceptor to the acceptor side of PS2 should cause a more pronounced decrease of fluorescence after the K step. Another effect of the addition of artificial electron acceptor should be a decrease of the amplitude of the K step due to this additional path for electron transport. However, we did not find any of these effects as can be seen in Fig. 2A which shows an action of the artificial PS2 electron acceptors PbBQ, DQ, and HNQ on F1 measured at 47 °C. There was an increase of the minimal fluorescence $F_0$ by about 25% and only a very small decrease of fluorescence after the K step. The fluorescence intensity kept an almost constant value. These results show that the decrease of fluorescence after the K step need not be caused only by draining of electrons from $Q_A^-$ via subsequent electron acceptors.
of PS2 as it has been suggested by Srivastava et al. (1997) and Strasser (1997). But because there was an increase of fluorescence to the P step (Fig. 2, curves a) indicating that there still exists the electron transport behind QA (see Introduction), the suggestion of Srivastava et al. (1997) and Strasser (1997) cannot be completely ruled out. Similar results as with the artificial electron acceptors of PS2 were obtained with the artificial electron donors to P680+, DPC and catechol, whose action on Fl measured at 47 °C is shown in Fig. 2B.

![Graph showing the decrease of fluorescence intensity](image)

Fig. 2. A time course of Fl measured by PEA fluorometer with pea leaves incubated for 5 min at 47 °C in darkness (curves a), and with pea leaves which were before the high temperature incubation (A) immersed for 40 min in darkness in 200 μM solution of the artificial electron acceptor of PS2, PpBQ (curve b), DQ (curve c), and HNQ (curve d), or (B) immersed for 40 min in darkness in 500 μM solution of the artificial electron donor to P680+, DPC (curve b), and catechol (curve c). The O, K, and P steps are labelled.

One of the most general explanations of the non-zero value of F0 is that it is a consequence of the transfer equilibrium of excitons in PS2 antenna and reaction centre of PS2 (Owens 1996) as derived by Laible et al. (1994). Hence the addition of the artificial electron acceptors and donors to PS2 can somehow change the transfer equilibrium leading to the increase of F0.

Considering the suggestion by Srivastava et al. (1997) and Strasser (1997) that the decrease of fluorescence after the K step is caused by the draining of electrons from QA− via subsequent electron acceptors of PS2 and our Fig. 2, there are probably two processes responsible for the decrease of fluorescence after the K step in Fl. Thus, the decrease should be well fitted by a two-exponential decay, the first decay representing an overall rate of a process(es) which is suppressed by the addition of artificial electron acceptors and donors to PS2 (Fig. 2) and the second decay representing an overall rate of electron transport from QA− to the subsequent electron acceptors of PS2. Results of such fitting performed for the decrease of the fluorescence intensity after the K step measured for 47 °C (see inset in Fig. 1) and also for other temperatures where the K step still appears as a clear peak (45 or 49 °C) are presented in Table 1. The best fit of the decrease of fluorescence after the K
step was obtained by the two-exponential decay for 45 and 47 °C, whereas by only one-exponential decay for 49 °C. Even if Fls with the K step were measured five times for each temperature (45, 47, and 49 °C), the absolute values of fluorescence intensity and the shapes of Fl curves at a position of the P step were different at given high temperature (not shown), indicating that different inhibition of the acceptor side of PS2 occurs in different sample. Hence, we propose that this different inhibition of the acceptor side of PS2 (inhibition of electron transport from QA to QB) is expressed by the process described by $\tau_2$ because of a high scatter found for $\tau_2$. Also a decrease of the mean percentage amplitude $A_2$ with increasing temperature found for the process characterised by $\tau_2$ (Table 1) and an increased value of $\tau_2$ with increasing temperature indicate that the process describes an electron transport from QA to subsequent electron acceptors of PS2 which is more inhibited by increasing temperature (Havaux 1993). On the other hand, the time constant $\tau_1^*$ must somehow reflect a process which is suppressed by the action of artificial electron acceptors and donors to PS2 (Fig. 2). A value of the mean percentage amplitude of this process, $A_1$, and its rise with increasing temperature (Table 1) indicates that the decrease of fluorescence after the K step mainly reflects this process (by more than 81 %) and that its contribution to the decrease of fluorescence after the K step increases with increasing temperature.

Discussion

As already mentioned in the Introduction, there is sometimes a decrease of fluorescence to a local minimum (D) in Fl measured without high-temperature treatment which may reflect a dynamic equilibrium between PS2 and PS1. Thus, according to the assumptions used for the successful simulation of D (Stirbet et al. 1998), a faster oxidation of the reduced PS2 electron acceptors by PS1 is necessary. In other words, the oxidation of QA− by subsequent electron acceptors in some conditions results in the dip D. However, the same process, i.e., the oxidation of QA− by the subsequent electron acceptors may cause the decrease of fluorescence after the K step (Srivastava et al. 1997, Strasser 1997). But it implies from Table 1 that the oxidation of QA− by the subsequent electron acceptors contributes by maximally 19 % (for 45 °C) to the overall decrease of fluorescence after the K step. This small extent of a contribution of the QA− oxidation to the overall decrease of fluorescence after the K step seems to be a reasonable estimate because a rather strong inhibition of the electron transport from QA− to QB occurs at the temperature when the K step appears (Ducruet and Lemoine 1985, Bukhov et al. 1990, Cao and Govindjee 1990, Havaux 1993, Goltsev et al. 1994). Thus, we conclude that the origin of the decrease

*The value of $\tau_1$ is not the real (intrinsic) time constant of the process. The intrinsic time constant of the process could be obtained by a mathematical deconvolution of the decrease of fluorescence after the K step using an appropriate model describing all reactions occurring in the reaction centre of PS2 at high temperatures.
of fluorescence after the K step in only the oxidation of $Q_A^-$ by the subsequent electron acceptors as suggested by Srivastava et al. (1997) and Strasser (1997), i.e., in a dynamic equilibrium between PS2 and PS1, is improbable.

Table 1. Results of the best fit of the decrease of fluorescence intensity after the K step by exponential decay(s) in relation to temperature of the measurement. $A_1$ and $A_2$ are the values of average percentage amplitudes for the first and second exponential decay, respectively, whereas $\tau_1$ and $\tau_2$ are the average time constants for the first and second exponential decay, respectively. The average values of $A_i$ and $\tau_i$ and SD (standard deviation) were computed from five measurements at given temperature.

<table>
<thead>
<tr>
<th>Temperature [°C]</th>
<th>$A_1$ [%]</th>
<th>$\tau_1 \pm$ SD [ms]</th>
<th>$A_2$ [%]</th>
<th>$\tau_2 \pm$ SD [ms]</th>
</tr>
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<tbody>
<tr>
<td>45</td>
<td>81</td>
<td>1.54 ± 0.43</td>
<td>19</td>
<td>19.3 ± 18.1</td>
</tr>
<tr>
<td>47</td>
<td>84</td>
<td>1.64 ± 0.27</td>
<td>16</td>
<td>41.9 ± 24.7</td>
</tr>
<tr>
<td>49</td>
<td>100</td>
<td>1.41 ± 0.22</td>
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In addition to the inhibition of the acceptor side of PS2 mentioned above, there is an inhibition of OEC (Cramer et al. 1981, Thompson et al. 1986, Havaux 1993) at the temperature when the K step appears, both leading to an accumulation of P680+, Pheo-, and $Q_A^-$. But note, that the accumulation of these forms occurs after an absorption of minimally two photons by PS2. The first photon results in a formation of P680+ and $Q_A^-$, P680+ being immediately reduced by an electron stored on the secondary electron donor in PS2, $Y_Z$ (tyrosine 161). P680 then absorbs the second photon leading to the second charge separation resulted in a formation of P680+, Pheo-, and $Q_A^-$. If OEC is absolutely destroyed by high temperature, it cannot donate electron to $Y_Z$ which in turn cannot reduce P680+ now. However, if OEC is not absolutely destroyed by the high temperature, another photon(s) can be utilised for photochemistry. As these reactions result in a closure of the reaction centre of PS2, there is an initial increase of fluorescence from the O to the K step in FI. At the position of the K step, there should be a maximal accumulation of P680+, Pheo-, and $Q_A^-$. That is why one can suggest that the decrease of fluorescence after the K step is somehow driven by the accumulation of P680+, Pheo-, and $Q_A^-$. 

P680+ is a quencher of fluorescence (Butler 1972, Mauzerall 1972, Sonneveld et al. 1979, Deprez et al. 1983, Shinkarev and Govindjee 1993). In the case of action of the artificial electron acceptors which oxidise Pheo- and/or $Q_A^-$, due to a malfunction of OEC only P680+ accumulates. If the quenching of fluorescence by P680+ molecule itself was the case of the decrease of fluorescence after the K step, then identical FI curves should be obtained without and with the artificial electron acceptors because the quenching of fluorescence by P680+ molecule itself should not be affected by the state of the acceptor side of PS2. But we have not observed such behaviour (Fig. 24). Thus, we conclude that the quenching of fluorescence by P680+ molecule itself is probably not the origin of the process which is suppressed by the addition of artificial electron acceptors and donors to PS2, i.e., the quenching of fluorescence by P680+ molecule itself probably does not cause the main process
responsible for the decrease of fluorescence after the K step in FI as presented in Table 1. A possibility that P680+ molecule can quench fluorescence signal during FI has also been excluded by Lavergne and Trissl (1995).

Inhibition of the donor side of PS2, leading to the accumulation of P680+, is accompanied by a stimulation of a 50-μs recombination fluorescence (Schreiber and Neubauer 1989, 1990) which induces a correlated increase of fluorescence quenching (both photochemical and nonphotochemical - Schreiber and Neubauer 1989). The 50-μs recombination fluorescence has even its maximum at 46 °C (Schreiber and Neubauer 1990) which is very near the temperature of the K step appearance in our case. Thus, it is highly probable that some kind of a charge recombination process involving P680+ is responsible for the decrease of fluorescence after the K step.

As mentioned above, in addition to the inhibition of the donor side of PS2, there is an inhibition of the electron transport from QA− to QB at high temperatures leading to the accumulation of P680+ and QA−. Thus, one can suggest that a recombination between these two forms can occur. This agrees to results of Johnson et al. (1995) who have suggested that a direct charge recombination between P680+ and QA− is favoured rather than the recombination involving Pheo− in the case of PS2 with inactivated donor side. On the other hand, Briantais et al. (1996) found an increase of the rate constant of the charge recombination between P680+ and Pheo− with increasing temperature. Furthermore, as both the electron acceptor accepting electrons from Pheo− (HNQ) and the electron acceptors draining electrons from QA− (PpBQ, DQ), used in our experiments, induce a transfer of P680+Pheo−QA− to P680+PheoQA−, it is not possible to exactly discriminate on the basis of our acceptor experiments if a carrier of negative charge for the charge recombination is Pheo− or QA−.

No matter if P680+ recombines with Pheo− or QA−, the recombination results either in a direct formation of P680 or in a formation of P680 via 3P680* (see, e.g., Pospíšil 1997). The former case occurs by a nonradiative recombination where the excess energy is changed to heat. On the other hand, 3P680* can also be nonradiatively quenched to P680 by triplet oxygen or singlet β-carotene (see Pospíšil 1997 for review). The formation of P680 enables a utilisation of radiant energy for photochemistry and not for fluorescence emission thus causing the decrease of fluorescence.

In conclusion, we think that the decrease of fluorescence after the K step does not mainly result from the oxidation of QA− by the subsequent electron acceptors as suggested by Srivastava et al. (1997) and Strasser (1997). We also exclude the possibility that the decrease of fluorescence after the K step is caused by the quenching of fluorescence by P680+ molecule itself. On the other hand, we suggest that the given decrease is caused by some kind of the recombination between P680+ and a negative charge. But based on our experimental results and also on the literature, we are not able to exactly determine if the negative charge is stored on Pheo− or QA−. We can also only speculate if fluorescence is consequently quenched due to formation of P680 directly or via 3P680*.

Finally, we point out that no matter to an exact mechanism of the recombination process leading to the decrease of fluorescence after the K step, the mechanism can only occur when inhibition of both the donor and acceptor sides of PS2 happen
because both inhibitions are necessary for the accumulation of P680+, Pheo−, and QA−. The inhibition of both sides of PS2 at this temperature is reported in the literature. On the other hand, when only the inhibition of OEC occurs (it starts from about 32 °C - Havaux 1993) leading to an accumulation of P680+, and the acceptor side of PS2 is not inhibited (up to 42 °C - Havaux 1993), no accumulation of Pheo− and QA− occurs and the mechanism responsible for the fluorescence decrease after the K step cannot take place. This is also consistent with experimental FI curves lacking a decrease of the fluorescence intensity after the J step measured at temperature range where only inhibition of OEC could happen (see Fig. 1 in Lazár and Ilik 1997).

References


DECREASE OF FLUORESCENCE INTENSITY


