Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis, 2005

Volume 49(1-2):181-183, 2005
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Alternative electron donors to photosystem II play an important role in the reduction of the electron transport chain in heat-treated barley leaves

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ABSTRACT Electron donation to Tyr_Z in heat-inactivated photosystem II (PSII) reaction centers by alternative donors was evaluated. Chl *a* fluorescence transients indicate that in the 0.1-3 ms range (K-step) only one electron is available, but that in the 0.2-2 s range accumulation of Q_A^- took place that coincided with the re-reduction of plastocyanin PC^+ and $P700^+$. Using Chl *a* fluorescence emission induced by short repetitive pulses and simultaneous 820 nm transmission and Chl *a* fluorescence transient measurements we show that electron donation to photosystem II takes place with a halftime of ~10 ms. There is a large pool of such alternative donors and they significantly contribute to the reduction of the linear electron transport chain since inhibition of PS II by DCMU prevented the re-reduction of PC^+ and $P700^+$.
Acta Biol Szeged 49(1-2):181-183 (2005)

KEY WORDS

alternative electron donors
DCMU
Chl *a* fluorescence
heat treatment
photosystem II
820 nm transmission

Exposure of plants to high temperatures inactivates the oxygen-evolving complex (OEC) of photosystem II (PSII). Under in vitro conditions, several reductants (ascorbate, Mn^{2+}, hydroxylamine) have been shown to be able to donate electrons to PSII with an inactive OEC. However, up to now it has not been shown to what extent alternative donors are available in the lumen under in vivo conditions and if they are able to reduce (at least partially) the electron transport chain. We studied this by Chl *a* fluorescence emission induced by short repetitive pulses and simultaneous 820 nm transmission and Chl *a* fluorescence induction kinetic measurements.

Materials and Methods

Measurements were carried out on 7-day-old barley seedlings. Oxygen-evolution was completely eliminated by a heat pulse (48°C, 40 s, in water; Tóth et al. 2005). For DCMU+heat-treatments, leaf segments were placed in DCMU-solution for 5 hours and then heat-treated.

Chl *a* fluorescence emission was measured with a new, "fast" Handy PEA instrument (Hansatech Instruments, UK) with which short light pulses (minimum 300 µs) with short time intervals (2.3 ms, 100 ms and longer) can be given. Light (650 nm peak wavelength, 3000 µmol photons m^{-2} s^{-1} light intensity) is provided by 3 light-emitting diodes. The first reliably measured point of the fluorescence transient is at 20 µs.

The 820 nm transmission measurements paralleling Chl *a* fluorescence were carried out using a PEA Senior instrument (Hansatech Instruments, UK). The excitation light intensity was 1800 µmol photons m^{-2} s^{-1}, produced by 4 LEDs. Far-red light and modulated far-red measuring light (820 nm peak wavelength) are provided by two additional LEDs (see also Schansker et al. 2005).

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Results and Discussion

In the inset of Figure 1A Chl a fluorescence transients of untreated and heat-treated barley leaves are presented. In heat-treated samples, the K-step ($F_{\sim 300\mu s}$) appeared reflecting partial Q_A reduction due to electron donation by Tyr_Z. Next, the fluorescence level decreased and then a second peak emerged at around 1 s, reflecting further Q_A^- accumulation. Apart from heat-stimulated non-photochemical reduction of the plastoquinone pool (Bukhov et al. 1999; Tóth et al. in preparation) we also considered re-reduction of Tyr_Z^+ by alternative electron donors.

The regeneration of the K-step, *i.e.* the re-reduction of Tyr_Z^+ by either recombination or alternative donors was studied by giving 5 ms light pulses spaced 2.3 ms (Fig. 1A) or 100 ms apart (Fig. 1B). No fluorescence increase could be observed after the 2.3 ms time interval. After 100 ms darkness a considerable part of the K-step was regenerated. After another 98 pulses, still 30-40% of the original K-step could be regenerated. Therefore, re-reduction of Tyr_Z^+ occurred in the 2.3-100 ms range. The recombination reaction between Q_A^- and Tyr_Z^+ has a halftime of ~120 ms (Dekker et al. 1984) and can only explain part of the re-reduction. This means that a considerable pool of alternative donors must have been available.

Forward electron transport can be blocked by DCMU. Figure 2A shows that DCMU+heat-treated samples exhibited a P-level that was close to the F_M value of untreated leaves

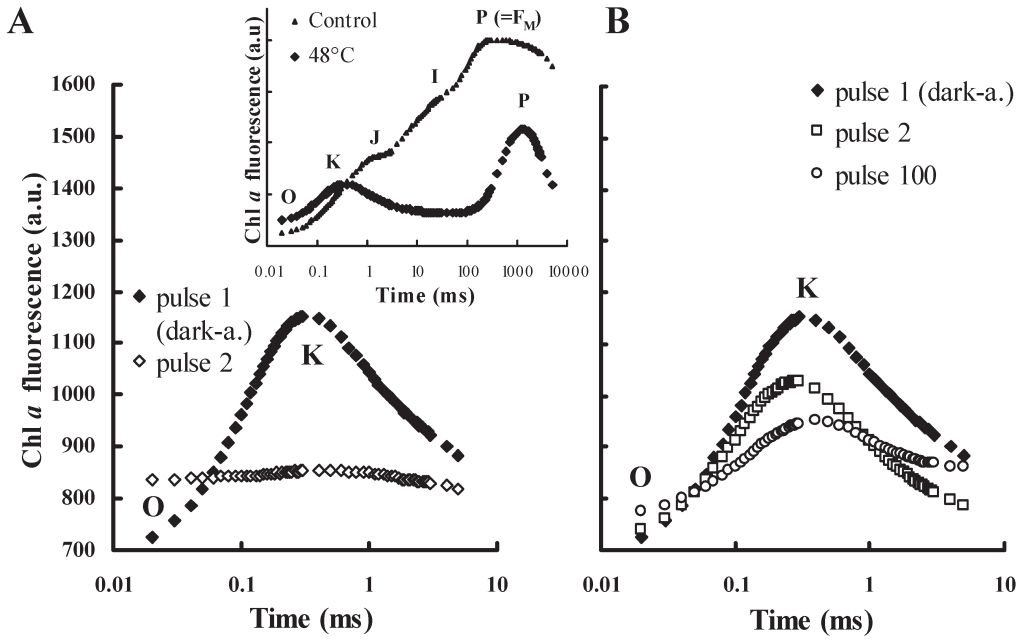

Figure 1 A: Chl *a* fluorescence transients of heat-treated barley leaves induced by two 5 ms light pulses spaced 2.3 ms apart. Inset: Chl *a* fluorescence (OJIP) transients of untreated and heat-treated leaves. **B:** Chl *a* fluorescence transients of heat-treated barley leaves induced by 100 5 ms-long light pulses spaced 100 ms apart. The 1st, 2nd and 100th transients are shown.

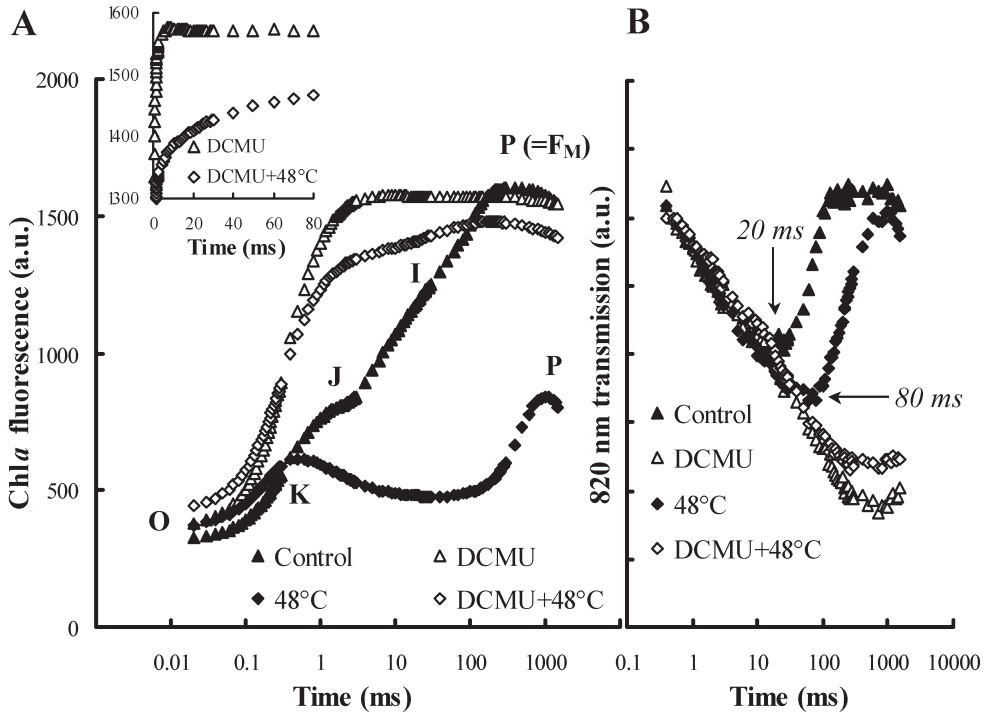

Figure 2. Chl *a* fluorescence transients (**A**) and simultaneously measured 820 nm transmission signals (**B**) of untreated, heat-treated, DCMU-treated and DCMU+heat-treated barley leaves.

and the shape of the fluorescence transient was biphasic. The small, slow component had a halftime of about 10 ms and the F_M was reached at about 100 ms (inset Fig. 2A). We ascribe the slow component to recombination between Q_A^- and Tyr_Z^+ that was gradually blocked by re-reduction of Tyr_Z^+ by alternative electron donors.

Simultaneously measured 820 nm transmission was used to track the redox state of P700+PC in order to monitor electron flow through PSI. In control leaves, a red light pulse induced an initial oxidation of P700 and PC followed by a re-reduction when electrons arrive from PSII. Re-reduction took place also in heat-treated samples, but it occurred later (control, 20 ms; heat-treated, 80 ms) and it was much slower (the corresponding $t_{1/2}$ values were 40 ms and 140 ms, respectively).

In the case of DCMU-treated samples, the 820 nm transmission signal continued to decrease and there was no re-reduction phase. For heat+DCMU-treated samples, there was no re-reduction, either. This means that in heat-treated leaves, electron donation to PSII by alternative donors is an important component of the residual electron transport activity in the thylakoid membrane.

Acknowledgements

Financial support by the Swiss National Science Foundation (grant No. 3100-057046.99/1) and Federal Office for Education and Science (grant No. C990075) are gratefully acknowledged.

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