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BRIEF COMMUNICATION

Relation between the heat-induced increase of F_0 fluorescence and a shift in the electronic equilibrium at the acceptor side of photosystem 2

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Abstract

F_0 fluorescence and thermoluminescence (TL) were recorded simultaneously on various dark-adapted leaf samples. Above 40 °C, a sharp peak of TL coincided with the onset of the heat-induced F_0 rise. It results from a back-transfer of an electron from the secondary Q_B^- to the primary acceptor Q_A of photosystem 2, followed by a luminescence-emitting recombination with Tyr-D^+. This demonstrates that the critical temperature at which the F_0 starts rising also corresponds to a shift towards the left of the $Q_A \leftrightarrow Q_B^-$ equilibrium.

Additional key words: Q_A and Q_B sites; temperature; thermoluminescence; *Zea mays*.

An increase of the F_0 basal fluorescence upon a progressive warming starts at critical temperatures above 38 °C. It occurs in algae (Lavorel 1969), leaves (Schreiber and Berry 1977), and isolated thylakoids (Schreiber and Armond 1977). It is correlated with a drop in electron transport activity (Schreiber and Berry 1977, Berry and Björkman 1980) and with the development of leaf necrosis within 2-4 d (Bilger *et al.* 1984). This destructive phenomenon has to be distinguished from grana unstacking (conversion to state 2) triggered at lower temperatures around 30 °C, which corresponds to a physiological protection of photosystem 2 (PS2) against high irradiance under warm conditions (Weis 1985, Sundby *et al.* 1986, Havaux and Lannoye 1987).

The F_0 rise has been ascribed to a block of PS2 centres, followed by a disconnection of the light-harvesting complex causing a F_0 decrease at higher temperatures (Schreiber and Armond 1978, Armond *et al.* 1980). Both effects are accompanied by a heat-induced phase separation of thylakoid membrane lipids (Gounaris *et al.* 1983). Time-resolved analysis of fluorescence decays have

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confirmed that heat causes a disconnection of a peripheral component of the antenna above 40 °C and a decrease of PS2 photochemistry starting at 30 °C (Briantais *et al.* 1996).

In triazine-resistant plants, the F_0 rise is shifted towards lower temperatures by about 3 to 5 °C. This shift allows that a temperature-dependent decrease of the $Q_A \leftrightarrow Q_B^-$ equilibrium constant can be revealed as a monophasic DCMU-like fluorescence induction (Ducruet and Lemoine 1985, Ducruet and Ort 1988, Havaux 1989). Bukhov *et al.* (1990) provided further evidence that an increase in the redox potential of Q_A, leading to its reduction by secondary donors, would contribute to the F_0 rise.

In order to obtain a direct evidence of a heat-induced formation of Q_A^- at the acceptor side of PS2, we recorded simultaneously the TL and F_0 emissions in dark-adapted leaves from maize (Fig. 1) or different other species (not shown). TL and F_0 fluorescence were measured as described by Ducruet and Miranda (1992) at a 0.1 °C s^{-1} heating rate. F_0 was excited by an ultra-low blue LED through a 480 nm interference filter, and pulsed once every 5 sampling steps. The F_0 emission superimposed to the TL signal was separated from it by interpolation.

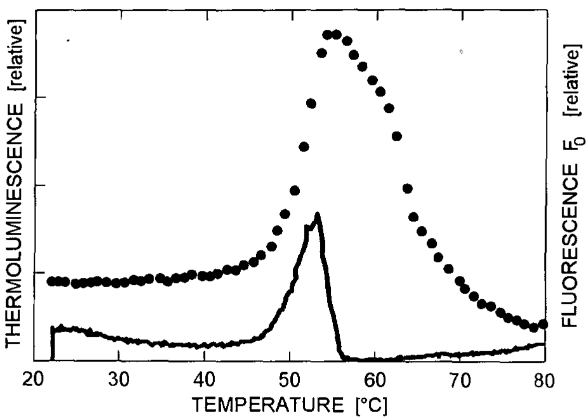

Fig. 1. Thermoluminescence (*line*) and F_0 fluorescence (*dots*) from a dark adapted *Zea mays* leaf recorded at a 0.1 °C s^{-1} heating rate.

Fig. 1 shows an example of a sharp TL peak growing proportionally to the initial rise of F_0 before dropping at *ca.* 52 °C (Fig. 1). This TL emission was most frequently observed in dark-adapted leaves, exhibiting, however, various amplitudes and shapes in different leaf samples. A steep initial F_0 rise corresponded to a sharp TL peak, whereas occurrence of a F_0 pretransition corresponded to a broader TL band. This 52 °C TL band can be identified as a C band usually observed in the presence of DCMU (Demeter *et al.* 1984) which results from a radiative recombination of Q_A^- with Tyr-D^+ (Johnson *et al.* 1994). Tyr-D 161 on the D2 protein is a side-path electron donor to $P680^+$. However, this heat-induced C band could not be fitted by a TL simulation, even though activation energies higher than 2 eV were assumed, which contrasts with the 1.32 eV found for a classical C band recorded in the presence of DCMU (Tatake *et al.* 1981). Therefore, this band cannot originate from a classical recombination between stabilized charge pairs and we propose that it reflects a heat-induced shift of the $Q_A \leftrightarrow Q_B^-$ equilibrium towards the left. Indeed, energetic considerations deduced from the difference between a Q band (S_2/Q_A^-, *ca.* 5 °C) and a B band (S_2S_3/Q_B^-, *ca.* 38 °C) imply that a Tyr-D^+/Q_B^- band could not peak at the same temperature as the Tyr-D^+/Q_A^- C band. Considering that approximately half of the PS2 centres contain a reduced Q_B^- in a dark-adapted material with an apparent equilibrium constant $[Q_B^-]/[Q_A^-]$ in the range 15-20 (Robinson and Crofts 1983), heat causes a reverse electron transfer from Q_B^- to Q_A, representing the kinetically limiting step. The recombination of Q_A^- with Tyr-D^+ gives rise to the TL emission, which falls abruptly when these charge pairs are depleted. Variable amounts of Q_B^- and Tyr-D^+ in dark-adapted leaves as well as the simultaneous heat-induced disruption of the thylakoid membrane can explain the variations in the intensity of this 52 °C TL emission, which is approximately one tenth of that of a B band induced by one flash. An irradiation given just before TL recording strongly reduces this 52 °C emission by generating S_2/S_3 states which recombine with Q_B^- as a B band at lower temperature, thus depleting the reduced Q_B^- pool (Demeter *et al.* 1984).

Above the critical temperature at which F_0 starts rising, a back transfer of an electron from Q_B^- to Q_A is revealed through its radiative recombination with Tyr-D^+. The temperature-induced shift of the $Q_A \leftrightarrow Q_B^-$ equilibrium towards the left can contribute to the initial rise of F_0 by (*a*) a backward transfer of an electron from Q_B^- to Q_A, generating stable Q_A^- in those PS2 centres where no Tyr-D^+ is present; (*b*) additionally, a forward reduction $Q_A \rightarrow Q_A^-$ driven by charge separation at higher intensities of the F_0 exciting beam, as it occurs in DCMU-inhibited centres.

References

Armond, P.A., Björkman, O., Staehelin, A.: Dissociation of supramolecular complexes in chloroplast membranes. A manifestation of heat damage to the photosynthetic apparatus. - Biochim. biophys. Acta **601**: 433-442, 1980.

Berry, J., Björkman, O.: Photosynthetic response and adaptation to temperature in higher plants. - Annu. Rev. Plant Physiol. **31**: 491-543, 1980.

Bilger, H.-W., Schreiber, U., Lange, O.L.: Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. - Oecologia **63**: 256-252, 1984.

Briantais, J.-M., Dacosta, J., Goulas, Y., Ducruet, J.-M., Moya, I.: Heat stress induces in leaves an increase of the minimum level of chlorophyll fluorescence, F_0: A time-resolved analysis. - Photosynth. Res. **48**: 189-196, 1996.

Bukhov, N.G., Sabat, S.C., Mohanty, P.: Analysis of chlorophyll *a* fluorescence changes in weak light in heat treated *Amaranthus* chloroplasts. - Photosynth. Res. **23**: 81-87, 1990.

Demeter, S., Vass, I., Horváth, G., Läufer, A.: Charge accumulation and recombination in photosystem II studied by thermoluminescence. II. Oscillation of the C band induced by flash excitation. - Biochim. biophys. Acta **764**: 33-39, 1984.

Ducruet, J.-M., Lemoine, Y.: Increased heat sensitivity of the photosynthetic apparatus in triazine-resistant biotypes from different plant species. - Plant Cell Physiol. **26**: 419-429, 1985.

Ducruet, J.-M., Miranda, T.: Graphical and numerical analysis of thermoluminescence and fluorescence F_0 emission in photosynthetic material. - Photosynth. Res. **33**: 15-27, 1992.

Ducruet, J.-M., Ort, D.R.: Enhanced susceptibility of photosynthesis to high leaf temperature in triazine-resistant *Solanum nigrum* L. Evidence for photosystem II D_1 protein site of action. - Plant Sci. **56**: 39-48, 1988.

Gounaris, K., Brain, A.P.R., Quinn, P.J., Williams, W.P.: Structural and functional changes associated with heat-induced phase-separations of non-bilayer lipids in chloroplast thylakoid membranes. - FEBS Lett. **153**: 47-52, 1983.

Havaux, M.: Comparison of atrazine-resistant and -susceptible biotypes of *Senecio vulgaris* L.: Effects of high and low temperatures on the *in vivo* photosynthetic electron transfer in intact leaves. - J. exp. Bot. **40**: 849-854, 1989.

Havaux, M., Lannoye, R.: Reversible effects of moderately elevated temperature on the distribution of excitation energy between the two photosystems of photosynthesis in intact avocado leaves. - Photosynth. Res. **14**: 147-158, 1987.

Johnson, G.N., Boussac, A., Rutherford, A.W.: The origin of 40-50 °C thermoluminescence bands in Photosystem II. - Biochim. biophys. Acta **1184**: 85-92, 1994.

Lavorel, J.: On the relation between fluorescence and luminescence in photosynthetic systems. - In: Metzner, H. (ed.): Progress in Photosynthesis Research. Vol. II. Pp. 883-898. Tübingen 1969.

Robinson, H.H., Crofts, A.R.: Kinetics of the oxidation-reduction reactions of the photosystem II quinone acceptor complex, and the pathway for deactivation. - FEBS Lett. **153**: 221-226, 1983.

Schreiber, U., Armond, P.A.: Heat-induced changes of chlorophyll fluorescence in isolated chloroplasts and related heat damage at the pigment level. - Biochim. biophys. Acta **502**: 138-151, 1978.

Schreiber, U., Berry, J.A.: Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. - Planta **136**: 233-238, 1977.

Sundby, C., Melis, A., Mäenpää, P., Andersson, B.: Temperature-dependent changes in the antenna size of Photosystem II. Reversible conversion of Photosystem II_α to Photosystem II_β. - Biochim. biophys. Acta **851**: 475-483, 1986.

Tatake, V.G., Desai, T.S., Govindjee, Sane, P.V.: Energy storage states of photosynthetic membranes: activation energies and lifetimes of electrons in the trap states by thermoluminescence method. - Photochem. Photobiol. **33**: 243-250, 1981.

Weis, E.: Light- and temperature-induced changes in the distribution of excitation energy between Photosystem I and Photosystem II in spinach leaves. - Biochim. biophys. Acta **807**: 118-126, 1985.