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S-state distribution and redox state of Q_A in barley in relation to luminescence decay kinetics

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The decay of luminescence from barley showed a relative maximum that appeared when far-red preillumination was carried out in the presence of oxygen, but not if white light was used for pre-illumination or when oxygen was omitted. Far-red illumination kept the S-states of the water-splitting system randomized and the primary Photosystem II acceptor, Q_A, oxidized. It was furthermore found that state S_3 was longer lived in the dark after far-red excitation than after white-light excitation. The presence of O_2 during and after far-red excitation also increased the lifetime of S_3 in the dark. Q_A exhibited the most oxidized state in the presence of O_2 and immediately after far-red excitation after which it was partially reduced back to a steady-state level, in the dark, by reverse electron flow. It is concluded that the relative maximum during the decay of far-red excited luminescence was a result of preservation of S_2, together with partial reduction of Q_A by reverse electron flow, in the dark. The relative luminescence maximum was inhibited by the energy-transfer inhibitor tentoxin and its kinetics were altered by the phosphate translocator inhibitor 4,4-diisocyano-2,2′-disulfonic acid stilbene. It is suggested that the reverse electron flow was a result of reverse coupling.

Introduction

Light emission from green plants in the dark was first discovered by Strehler and Arnold during work with ATP measurements by the luceferin-luciferase method [1]. The emission, termed luminescence, was later shown to be a result of the recombination between positive charges on the donor side of PS II with electrons on the acceptor side (for a review see Ref. 2). Normally, luminescence decays asymptotically with the most light emitted during the first micro or milliseconds of the decay [3]. It was, however, observed that far-red excitation resulted in a luminescence component that during the minute range of the decay increased and resulted in a relative maximum during the decay [4]. In attempts to unravel the mechanism behind this 'far-red-induced relative maximum' several factors have been discussed. The relative maximum has been suggested to be a result of the transthylakoid ΔpH [5], PS I luminescence [6], reverse Calvin cycle reactions [7] and, recently, also suggested to be strongly affected by state 1/state 2 transitions [8]. No conclusive evidence has been presented favoring either of the suggested mechanisms. However, it was recently shown that the far-red stimulated relative luminescence maximum which originated from PS II and is dependent on reverse electron flow between the photosystems, but also on the presence of a transthylakoid ΔpH [9]. In this paper we show that the far-red-induced relative maximum during the decay of luminescence is the result of a combination of three factors: (i) a transiently very oxidized PS II acceptor side in the dark after far-red excitation in the presence of O_2; (ii) a long lifetime of state S_3 in the dark after far-red excitation in the presence of O_2; and (iii) reverse coupling, causing partial re-reduction of the PS II acceptor side in the dark.

Material and Methods

Barley leaves grown for 7–10 days in a green house were used as starting material in all experiments.

Abbreviations: PS II, Photosystem II; PS I, Photosystem I; Q_A, the primary PS electron acceptor, DIDS; 4,4′-diisocyano-2,2′-disulfonic acid stilbene; PVP, polyvinylpyrrolidone; BSA, bovine serum albumin; Hepes; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Chl, chlorophyll.

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Protoplasts were prepared as described in Ref. 9 and assayed in a medium containing 0.25 M sorbitol, 0.25 M sucrose, 10 mM KCl, 0.5 mM $MgCl_2$, 0.06% BSA, 0.2% PVP and 10 mM Hepes, all adjusted to pH 7.2. Before assays, 10 mM $NaHCO_3$ were added to the assay medium/protoplast mixture. Protoplast extracts were obtained by sequeezing protoplasts three times through a 15-μm nylon net as described in Ref. 10. Protoplast extracts were used for experiments with inhibitors, since protoplast extracts have the lowest organisation level at which the relative luminescence maximum has been shown to occur so far. Intact chloroplasts (prepared mechanically or isolated from protoplasts and capable of CO_2-dependent O_2 evolution rates above 100 μmol O_2 per mg chlorophyll per h) have so far not been shown to exhibit the relative luminescence maximum.

Luminescence, from intact barley leaves, were measured in a cylindrical plexiglass cuvette (25 cm^3). Different gas mixtures were flushed through the cuvette at a rate of 60 $l \cdot h^{-1}$. Excitation light was provided by two metal halogen lamps (Atlas 24 V, 250 W). For far-red excitation, the light was filtered through a Schott RG 715 cut-off filter.

The excitation light intensity was 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ white and 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ far-red light. Luminescence was detected with a selected Hamamatsu R374 photomultiplier and the resulting signal was amplified and recorded on a chart-strip recorder. Excitation light and luminescence emission was guided to the cuvette/photomultiplier by optical fibers. The photomultiplier during excitation was protected from the excitation light by a shutter.

Luminescence measurements from protoplast extracts were carried out in a modified Hansatech O_2 electrode (Hansatech, Norfolk, U.K.) allowing the addition of inhibitors in the dark. The chlorophyll concentration during experiments was 50 μg chlorophyll $\cdot$ ml^{-1}. Other experimental conditions were as described for intact leaves.

For fluorescence measurements, a modified Heinz Walz fluorometer (PAM 101, Heinz Walz, F.R.G.) was used allowing pulsing of a weak modulated fluorescence excitation light. For determination of possible differences in the dark redox state of Q_A after far-red excitation under aerobic and anaerobic conditions, the weak 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ modulated (100 kHz) excitation light was pulsed (pulse duration, 1 s; interval between pulses, 10 s) to the leaves in order to minimize the risk of the measuring light causing accumulation of reduced Q_A.

For determination of changes in the reduction/oxidation ratio of Q_A, a higher intensity (2.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) modulated (100 kHz) light was used for 'continous' excitation of the sample. All fluorescence measurements were carried out on intact leaves in the same cuvette as used during luminescence measurements. For a recent description of modulated fluorescence techniques see Ref. 11.

Intact barley protoplasts were used for measurements of the relative distribution of S-states in the dark after white and far-red illumination and under aerobic and anaerobic conditions. A Joliot-type O_2 electrode [12] was used for measurement of relative O_2 yield after a series of flashes obtained from a Xenon flash lamp (Heinz Walz, F.R.G.), (intensity, $2 \cdot 10^5$ $W \cdot m^{-2} \cdot s^{-1}$, $t_{1/2}$ of duration peak, 11 μs; interval between flashes, 0.7 s). In order to achieve aerobic or anaerobic conditions during S-state measurements, the flow medium (10 mM Hepes (pH 7.2) and 10 mM KCl) was bubbled with either N_2 or O_2. The protoplast samples in the assay medium were also flushed with N_2 or O_2 after application to the O_2 electrode. The number of misses, double hits and relative S-state distributions, according to the model of Kok et al. [13], was calculated from the oxygen yield on the first seven flashes using a non-linear least-squares fit procedure with the assumption that misses and double hits were the same on all flashes. The number of double hits was found to be around 10% throughout the experiments.

Results and Discussion

Fig. 1 shows that both far-red excitation and the presence of oxygen is required for the relative luminescence maximum to appear in intact barley. This is in agreement with earlier results [5]. The presence of CO_2 together with O_2 altered the maximum, but did not abolish it. To elucidate the role of the PS II donor side, in the appearance of the relative luminescence maximum, the flash-induced oxygen yield pattern was determined using isolated barley protoplasts.

As shown in Fig. 2, the oxygen yield pattern obtained after 60 s in the dark was strongly influenced by the pre-illumination conditions and the oxygen concentration; note, especially the high yield obtained on the first two flashes in the case of far-red pre-illumination. This suggests that the two higher oxidation states on the donor side, S_2 and S_3, are present in a higher proportion after far-red than after white light pre-illumination. Primarily, state S_3, but also state S_2, are known to be good 'substrates' for luminescence]2]. The unusual oxygen yield pattern obtained after white light (Fig. 2a), with a maximum on the fourth flash rather than on the third, was found to be partly due to an increased number of misses (20%), but mainly due to a high initial amount of state S_0 (49%). An alternative explanation for a high O_2 yield on the fourth flash is presented in Ref. 14 where the conclusion was reached that rapid reduction of parts of S_2 and S_3 between flashes of an unknown redox component resulted in an apparently high concentration of S_0 under reducing conditions, as shown earlier in Ref. 15. To obtain more

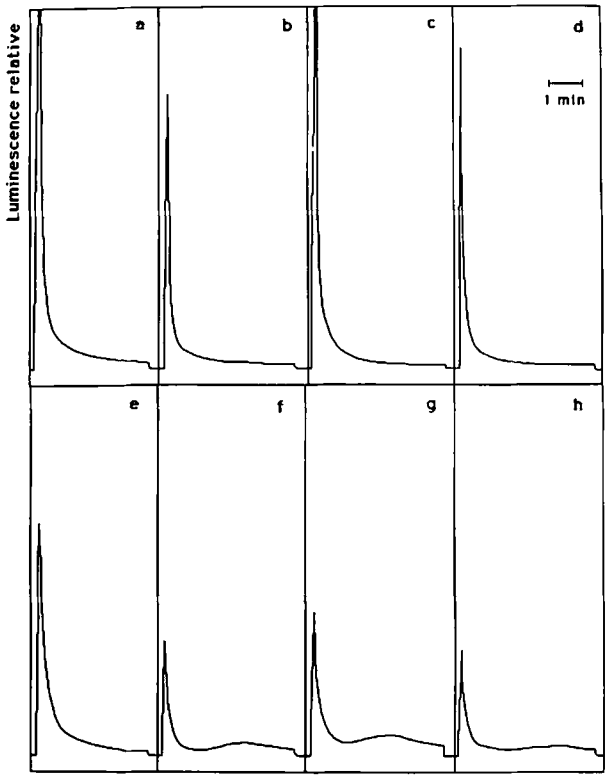

Fig. 1. Luminescence decay from intact barley leaves excited with 2 min white light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (a–d) and with 2 min white light followed by 0.5 min far-red light (5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (e–h) and flushed with different gas mixtures. a and e, N_2/CO_2 95 : 5%; b and f, O_2; c and g, air; d and h, air with 2% CO_2.

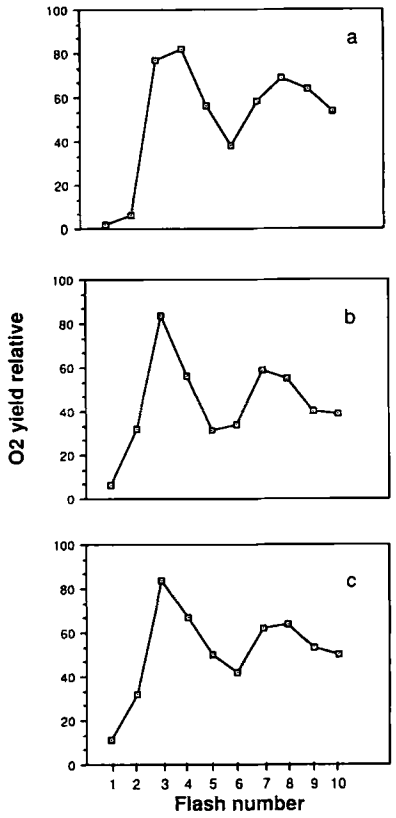

Fig. 2. Flash-induced oxygen yield pattern of barley protoplasts, dark adapted for 60 s after (a) 2-min preillumination with white light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and equilibrated with air. (b) 2-min preillumination with white light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) followed by 0.5 min of far-red light (5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and equilibrated with N_2. (c) 2-min preillumination with white light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) followed by 0.5 min of far-red light (5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and equilibrated with O_2.

detailed information, the S-state distributions were deconvoluted from the oxygen yield patterns obtained at different time intervals after the pre-illumination was ceased.

As is evident from Figs. 3 and 4, preillumination with white light resulted in a very fast deactivation of states S_2 and S_3. In contrast, far-red pre-illumination resulted in a long lifetime of S_2 and S_3. The presence of oxygen also influenced the stability. State S_2 decayed faster, but more important in this context is the increase in the stability of state S_3. The prolonged lifetime of the higher S-states after far-red illumination was likely a result of limited access to electrons for recombinations due to oxidation of the acceptor side of PS II caused by domination by PS I excitation. The lower frequency of recombinations immediately after far-red excitation compared to that after white light excitation was also manifested as a lower initial output of luminescence (Fig. 1). The effect of oxygen in these experiments is mechanistically not clear. However, the presence of oxygen decreased the initial luminescence (Fig. 1b and f) and increased the lifetime of state S_3 (Fig. 4) suggesting a decreased access to electrons on the acceptor side for recombination.

This interpretation is strenghthened by the result shown in Fig. 5. In this experiment, low intensity modulated fluorescence light was pulsed to intact barley leaves at the same time as they were sequentially treated with white light, far-red light and finally darkness. The

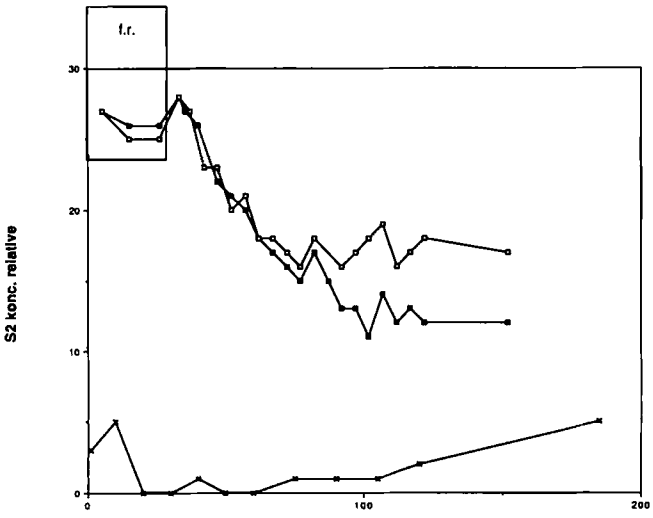

Fig. 3. Relative concentration of S_2-state. The experimental conditions were as described in Fig. 2. ■, white plus far-red preillumination equilibrated with O_2. □, white plus far-red preillumination equilibrated with N_2. ×, white preillumination equilibrated with air.

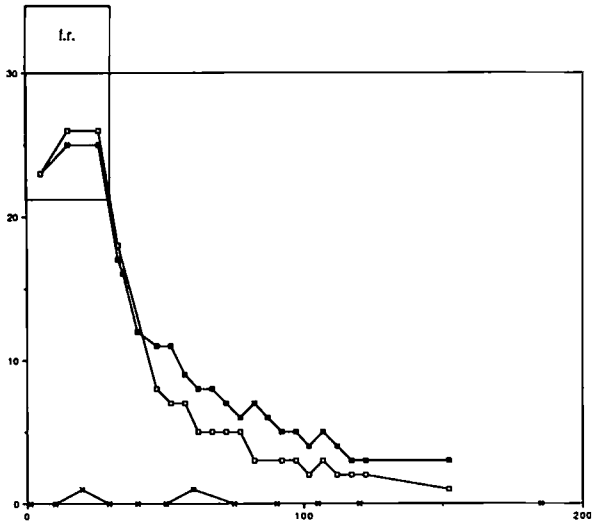

Fig. 4. Relative concentrations of S_3 (vertical axis). The experimental conditions were as described in Fig. 3.

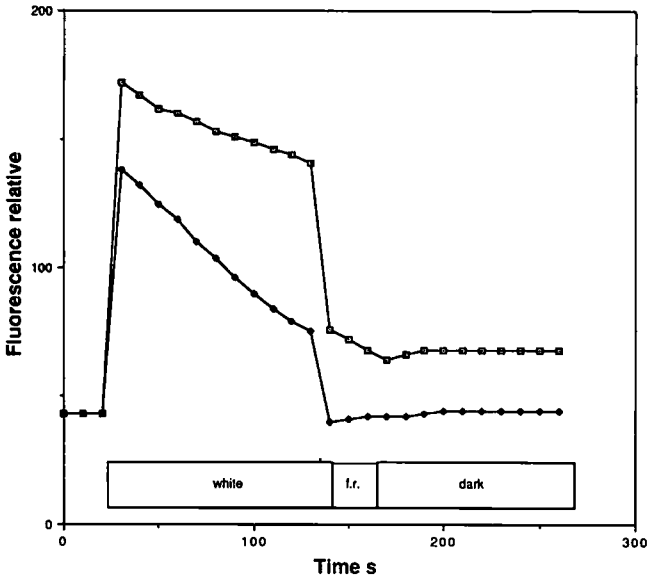

Fig. 5. Pulsed, modulated fluorescence measured during an excitation/dark sequence including both white light (600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and far-red light (5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from intact barley leaves flushed with 5% CO_2 in N_2 (□) and 5% CO_2 in O_2 (◆). Curves were normalized according to the F_0 level recorded before white light illumination.

dark level of fluorescence was substantially lower after excitation in the presence of oxygen, indicating a lower concentration of Q_A^-.

The increased stability of state S_3 after subjection to far-red light in the presence of oxygen explain in part the appearance of the relative luminescence maximum under these conditions. However, the decay of state S_3 does not show any direct correlation to luminescence and, therefore, alone cannot explain the relative luminescence maximum. In order to study more closely

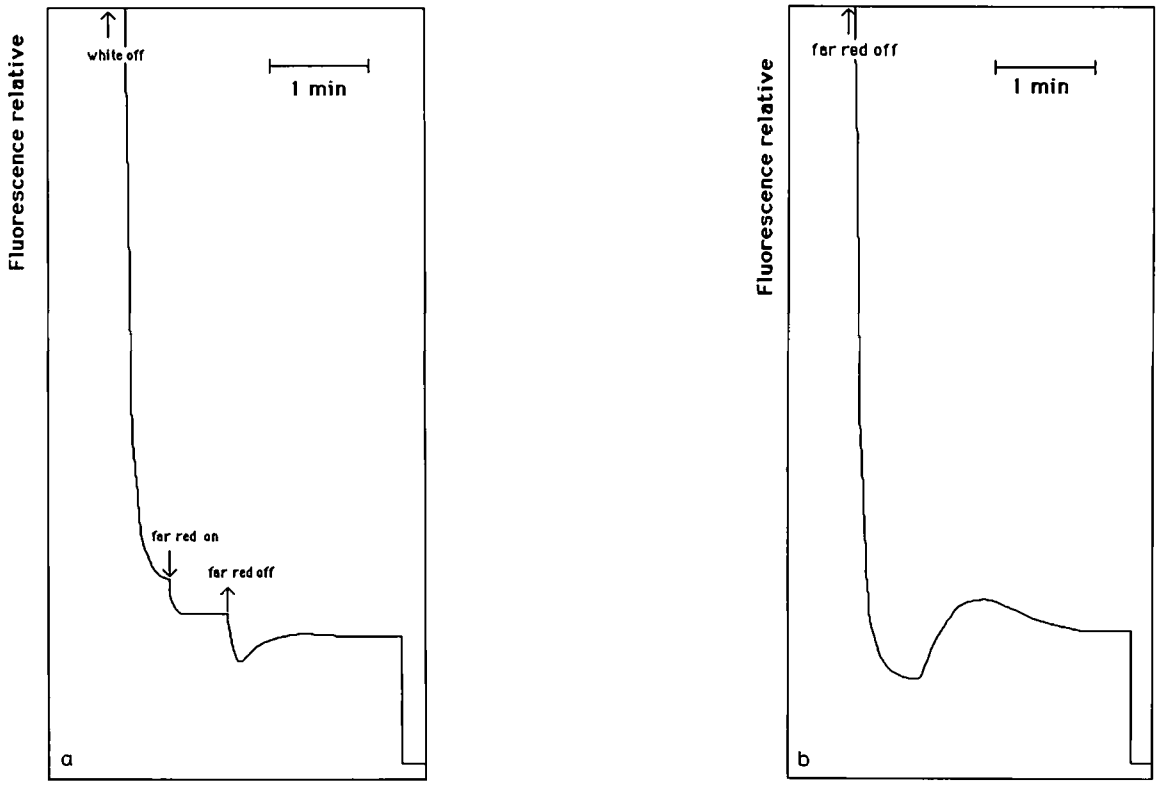

Fig. 6. (a) Modulated fluorescence from intact barley leaves in air during a light/dark sequence including white and far-red light with no interspersed dark periods. White light 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, far-red light 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, fluorescence excitation 2.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ red modulated light. (b) Modulated fluorescence from intact barley leaves after excitation with white and far-red light, with no interspersed dark periods. Other experimental conditions were as described in (a), in but with a higher amplification of the fluorescence signal.

the role of the redox state of the PS II acceptor side in this context, fluorescence was excited with 2.5 μmol · $m^{-2} \cdot s^{-1}$ red modulated light to monitor the relative levels of Q_A reduction during and after excitation with white and far-red light. The intensity of the modulated light was sufficiently high to cause, not only F_0 fluorescence but also a certain amount of variable fluorescence (5–10%). The resulting fluorescence signal observed without superimposed secondary light can thus not be considered as 'dark fluorescence'. However, the level of this fluorescence will reflect the equilibrium between a low rate of reduction and oxidation of Q_A.

As expected, white light caused a strong reduction of Q_A while far-red illumination oxidized Q_A below the level observed in the dark after white light (Fig. 6a). However, when the far-red light was switched off, the level of fluorescence dropped below the steady-state level observed during far-red illumination, indicating a transiently very oxidized Q_A. Interestingly, within 30 s after turning off the far-red light, fluorescence recovered back to a level slightly below the 'far-red steady-state level'. The transients in fluorescence observed after far-red illumination are probably a result of the push/pull nature of the Z scheme in combination with reverse electron flow. According to this theory the drop in fluorescence after far-red illumination appears when PS II excitation by the 'PS-II-contaminated' far red-light stops, but the pull for electrons from oxidized electron transport intermediates remains for 5–10 s in the dark. The recovery back to the final steady-state fluorescence level thereafter occurs as a result of both the modulated fluorescence excitation light and of reversed electron flow.

Another more pronounced manifestation of this phenomenon was observed after a white/far-red illumination sequence with no interspersed dark period (Fig. 6b). Under these conditions, after far red illumination, fluorescence transiently rose to a level above that of the final steady-state indicating an active process (additional to reduction by the modulated light), partially reducing Q_A.

The influence of the modulated light on the fluorescence transients was examined by applying a 1-s pulse immediately after far-red illumination and another 30 s after far-red illumination. The fluorescence levels obtained in this way showed the same characteristic pattern as those obtained during continuous fluorescence measurements, although the increase in fluorescence was less marked (not shown). Thus, the increase in the Q_A redox state after far-red illumination also occurred in complete darkness. The relative maximum in the decay of luminescence after far-red light in the presence of O_2 can now be explained in terms of the redox state of both the donor and the acceptor side of PS II.

During far-red PS I excitation, PS II is also slightly excited causing randomization of the S-states and a small 'trickle' of electrons through the electron transport chain. The dominating PS I excitation, however, keeps the electron carriers oxidized especially in the presence of O_2. When far-red excitation is ceased, the trickle of electrons immediately stops, although the pull for electrons from the oxidized electron carriers will continue for 5–10 s in the dark. Under these conditions, the initial output of luminescence will be low, since electrons are pulled away from the site of recombination. Consequently, the highly active luminescence substrate S_3 will be preserved in the dark. When the redox state of Q_A increases after 15–20 s in the dark from a very oxidized to a normally oxidized level, the frequency of PS II recombinations will increase when 'dark-preserved' S_3 are consumed. The increase in PS II recombinations will be evident as an increase in luminescence and thus give rise to the 'far-red-induced relative maximum'.

It has been shown previously that the far-red-induced relative maximum was dependent on both reverse electron transport between the two photosystems and on the presence of a transthylakoid ΔpH [5,9] and, thus, on the energetic state of the thylakoid. To investigate the possible influence of reversed coupling, the energy transfer inhibitor tentoxin [16] was added to a protoplast extract immediately after far-red excitation.

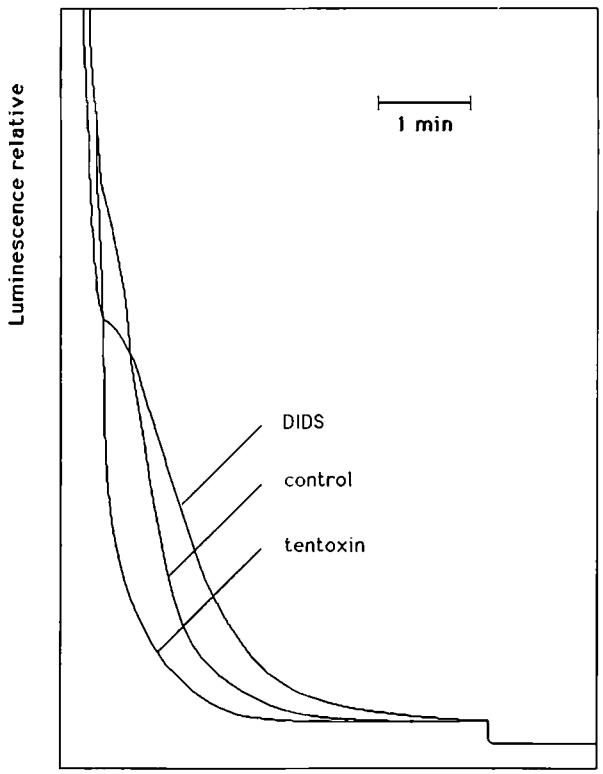

Fig. 7. Luminescence decay from protoplast extracts excited with white and far-red light as described in Fig. 5. Middle curve, control; lower curve, with 10^{-5} M tentoxin added immediately after far-red excitation, and upper curve, with 10 μM DIDS added and incubated for 2 min before excitation.

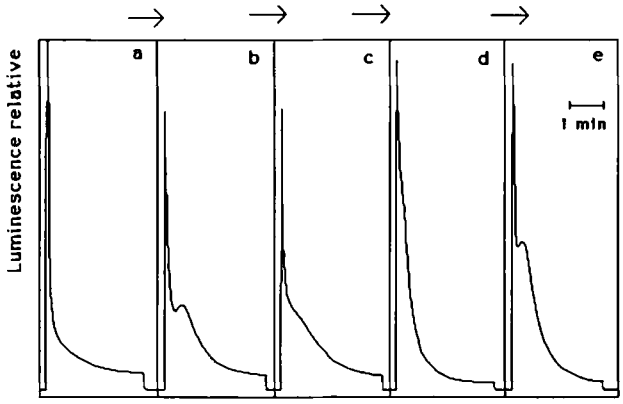

Fig. 8. Luminescence decay from intact barley leaves excited with cycles of 2 min white light and 30 s far-red light followed by luminescence measurements in the dark, and flushed with gas mixtures as indicated. The figure illustrated a sequence with the same leaves subjected to different gas mixtures. Excitation with white and far-red light was performed immediately after each luminescence measurement in b–e. (a) N_2/CO_2 95:5%; (b) N_2/O_2 80:20%; (c) N_2/O_2 80:20%; (d) N_2/CO_2 95:5%; (e) N_2/O_2 80:20%.

The far-red induced relative maximum (in this experimental system expressed as a shoulder), was completely inhibited by 10^{-5} M tentoxin (Fig. 7). This suggests that the reverse electron flow that causes an increase of the redox state of Q_A after the oxidation due to far-red excitation is an example of reverse coupling [17] and consequently, is dependent on ATP hydrolysis by the chloroplast ATPase. This conclusion is supported by the effect of the phosphate translocator inhibitor DIDS [18] on the decay kinetics of far-red excited luminescence. Addition of DIDS prior to excitation with white and far-red light stimulated the component of luminescence, expressed as a shoulder (Fig. 7). This suggests that the addition of DIDS before excitation prevents export of triose phosphates into the medium and thereby increases the concentration of substrate for reverse coupling (i.e., ATP, dihydroxyacetone phosphate) [19].

Similar results were obtained when the effect of two repeated excitations under CO_2-free, but aerobic, conditions on the decay kinetics of far-red excited luminescence were studied (Fig. 8). The far-red stimulated relative maximum was decreased after a second excitation under CO_2-free conditions. This effect was partially reversed by one excitation with CO_2 present. Excitation without CO_2 (but with O_2) will deplete the chloroplast of Calvin cycle intermediates and trioses through photorespiration. The effect on luminescence might therefore be of similar origin as the effect on luminescence from protoplast extracts by DIDS, i.e., affecting the concentration of substrate for reverse coupling.

Relative luminescence maxima have also been observed after white light illumination under low CO_2 conditions for green algal cells [20,21,22]. However, in this experimental system, luminescence maxima occurred as a result of rapid dark decrease in nonphotochemical quenching, and probably also as a consequence of a much more pronounced dark reduction of Q_A than that described in this paper.

From the results presented in this paper, it is evident that the mechanistic origin of luminescence maxima induced by far-red light is different from that induced by white light excitation in low CO_2-adapted green algal cells.

References

1 Strehler, B.I. and Arnold, W. (1951) J. Gen. Physiol. 34, 809–829.
2 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223–317, New York, Academic Press.
3 Malkin, S. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 349–431, North-Holland, Biomedical Press, Elsevier, Amsterdam.
4 Bertsch, W.E. and Azzi, J.R. (1965) Biochim. Biophys. Acta 94, 15–26.
5 Björn, L.O. (1971) Physiol. Plant. 25, 316–323.
6 Desai, T.S., Rane, S.S., Tatake, V.G. and Sane, P.V. (1983) Biochim. Biophys. Acta 724, 485–489.
7 Mellvig, S., and Tillberg, J.E. (1986) Physiol. Plantarum 68, 180–188.
8 Schmidt, W. and Senger, H. (1987) Biochim. Biophys. Acta 767, 70–79.
9 Nakamoto, H., Sundblad, L.-G., Gardeström, P. and Sundbom, E. (1988) Plant Sci. 55, 1–7.
10 Rumpho, M.E. and Edwards, G.E. (1985) Plant Physiol. 78, 537–544.
11 Schreiber, U., Schliwa, U. and Bilger, W. (1986) Photosynth. Res. 10, 51–62.
12 Joliot, P. and Joliot, A. (1968) Biochim. Biophys. Acta 153, 625–634.
13 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
14 Vermaas, W.F.J., Renger, G. and Dohnt, G. (1984) Biochim. Biophys. Acta 764, 194–202.
15 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 292, 772–785.
16 Steele, J.A., Uchytil, T.F., Durbin, R.D., Bhatnagar, P. and Rich, D.H. (1976) Proc. Natl. Acad. Sci. USA 73, 2245–2248.
17 Schreiber, U. (1984) Biochim. Biophys. Acta 767, 70–79.
18 Edwards, G.E., Robinson, S.P., Tyler, N.J.C. and Walker, D.A. (1978) Plant Physiol. 62, 313–317.
19 Schreiber, U. (1980) FEBS Lett. 122, 121–124.
20 Palmqvist, K., Sundblad, L.G., Samuelsson, G. and Sundbom, E. (1986) Photosynth. Res. 10, 113–123.
21 Sundblad, L.G., Palmqvist, K. and Samuelsson, G. (1986) FEBS Lett. 199, 75–79.
22 Sundblad, L.G., Palmqvist, K. and Samuelsson, G. (1986) FEBS Lett. 209, 28–32.