THERMOLUMINESCENCE AS A PROBE OF PSII IN LEAVES

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1. INTRODUCTION

Recently several advances have been made in the understanding of luminescence phenomena. 1) In agreement with previous hypothesis (reviewed by Lavorel, 1975) it seems clear that thermoluminescence (TL) and delayed luminescence (DL) are a result of recombination of photoinduced charge pairs (i.e. Lavergne Etienne, 1980; Rutherford et al, 1982, Demeter, 1982; Rutherford, Inoue 1983). 2) This recombination is probably an important deactivation process (Rutherford et al, 1982). 3) Some phases of slow DL and certain TL bands have now been demonstrated to be different manifestations of the same phenomena (Desai et al, 1982; Rutherford, Inoue, 1983).
These developments have recently been applied to the study of PSII in leaves (Rutherford et al, 1983). In this report the new data obtained in leaves is discussed along with some experimental rationale that may be of use to further studies on this system.

2. METHODS

Thermoluminescence and delayed luminescence were recorded as described previously (Ichikawa et al, 1975; Rutherford, Inoue, 1983). Leaf discs of market spinach were used in all experiments. Other details were as in (Rutherford et al, 1983) or as described in the text.

3. RESULTS AND DISCUSSION

3.1 Charge storage on the donor and acceptor side of PSII

Period of four oscillations of the 30°C TL band and of slow DL have been obtained with a series of flashes. In chloroplasts this TL and this phase of DL have both been associated with $S_2Q_B^-$ and $S_3Q_B^-$ recombination (Rutherford et al 1982; Rutherford, Inoue, 1983). By analogy, these phenomena are similarly assigned in leaves. The oscillation pattern is different to that reported in dark adapted chloroplasts but is similar to that obtained in chloroplasts where the population of stable Q_B^- in the dark is increased by preillumination (Rutherford et al, 1982). Thus the pattern of oscillations in leaves may also be explained by the existence of a larger amount of stable Q_B^- in leaves than in dark adapted chloroplasts.

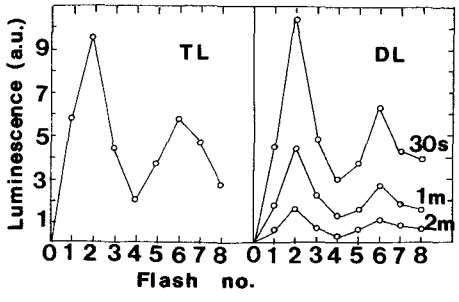

FIGURE 1. Oscillations of thermoluminescence (TL) and delayed luminescence (DL) after a series of flashes in leaves.

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More information onthe redox state of Q_B^- in the dark can be obtained using a trick developed in chloroplast TL studies. A period of continuous illumination given at 77K introduces a single electron into the quinone acceptor complex at the expense of cytochrome b_{559} without significantly changing the S states. Warming of the sample after such a treatment results in the inversion of the $Q_B:Q_B^-$ ratio. Since dark adapted chloroplasts typically show $Q_B:Q_B^-$ ratios of 70:30, this treatment results in a marked decrease of TL on the 1st flash with an increase of the 2nd flash (Rutherford et al, 1982). In leaves however, very little effect is observed after 77K illumination indicating that $Q_B \sim Q_B^-$. This agrees with the explanation of the oscillation pattern above and also with the measurement of a $Q_B:Q_B^-$ ratio of 50:50 in whole cells of algae (Wollman, 1980). It has been suggested that the increased amount of Q_B^- in whole cells, leaves and recently illuminated chloroplasts is a reflection of the presence of reduced PQ pool while dark adaptation is taking place (Rutherford et al, 1983). Previously a correllation between stable Q_B^- and the amount of stable S states present at the beginning of dark adaptation has been assumed (Rutherford et al, 1982; Velthyus, 1980). This relationship increased if recombination deactivation (i.e. $S_2Q_B^- \rightarrow S_1Q_B$ and $S_3Q_B^- \rightarrow S_1Q_B$) were in competition with non-recombination deactivation (i.e. $S_2Q_B^- \rightarrow S_1Q_B^-$ and $S_3Q_B^- \rightarrow S_1Q_B^-$) or if reduced PQ from the pool was involved in one electron recombination deactivation with S_2Q_B (i.e. $S_2Q_B + PQH_2 \rightarrow S_1Q_B^- + PQ + 2H^+$). The latter reaction would provide an increased Q_B^- population under conditions where the PQ pool was more reduced.

In dark adapted chloroplasts the amount of stable Q_B^- can be decreased further by giving one flash or two flashes and then allowing dark adaptation in accordance with the recombination deactivation theory. In leaves this does not occur. Again this could be due to a one electron back reaction of PQH_2 with S_2Q_B. The very slow decrease in Q_B^- level in the dark in chloroplasts could be due not only to the slow oxidation of the pool but also to the requirement for reaction centre turnovers so that recombination deactivation can take place.

To test these ideas preillumination of chloroplast in the presence of an electron acceptor like methyl viologen should result in the PQ pool staying oxidized. Dark adaptation under these conditions should not involve the formation of increased Q_B^- and so an increase in 2nd and 6th flash TL relative to 1st and 5th would not be expected in this case. Our preliminary attempts to oxidize the PQ pool in leaves by preillumination of leaves infiltrated with methyl viologen did not modify the relative extents of the glow peaks on the first and second flashes. However, complementary measurement of the redox state of the pool by fluorescence induction is required to verify that the pretreatment worked.

3.2. Deactivation measured by thermoluminescence

TL can be used to measure the deactivation of S_2 in chloroplasts (Rutherford, Inoue, 1983b; Rutherford et al, 1983). The technique is of particular interest because it divides the measurement up into two kinds of centres: a) those in the state $S_2Q_B^-$ and b) those in the state S_2Q_B. The experimental protocol is explained schematically on the following page.

Results of such experiments show that S_2 decays faster in centres where Q_B^- is present than in those where Q_B is present. For centres in the state $S_2Q_B^-$ two possible deactivation routes can be considered: 1) $S_2Q_B^- \longrightarrow S_1Q_B$ and 2) $S_2Q_B^- \xrightarrow{e^-} S_1Q_B^-$. The results obtained here indicate that the first of these, the recombination deactivation, dominates. For the centres in the state S_2Q_B two possible mechanisms can also be considered 1) $S_2Q_B \xrightarrow{e^-} S_1Q_B$ 2)

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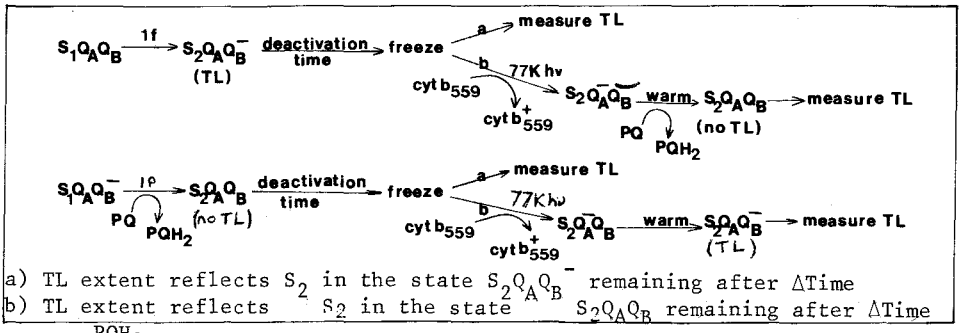
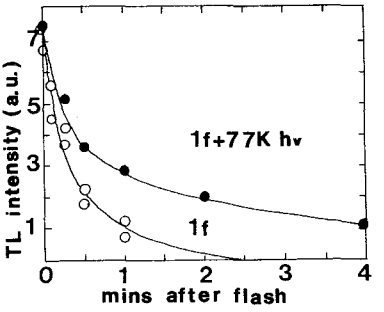
. This relationship results in a predicted 25% Q_B^- stable in the dark. This number would be increased if recombination deactivation (i.e. $S_2Q_B^- \rightarrow S_1Q_B$ and $S_3Q_B^- \rightarrow S_1Q_B$) were in competition with non-recombination deactivation (i.e. $S_2Q_B^- \rightarrow S_1Q_B^-$ and $S_3Q_B^- \rightarrow S_1Q_B^-$) or if reduced PQ from the pool was involved in one electron recombination deactivation with S_2Q_B (i.e. $S_2Q_B + PQH_2 \rightarrow S_1Q_B^- + PQ + 2H^+$).



a) TL extent reflects S_2 in the state $S_2Q_AQ_B^-$ remaining after ΔTime
b) TL extent reflects S_2 in the state $S_2Q_AQ_B$ remaining after ΔTime

$S_2Q_B \xrightarrow{PQH_2} S_1Q_B^-$. The second of these possibilities is of interest not only as a possible source of Q_B^- stable as described above but also because such a reaction may be luminescent, although its kinetics and emission peak will depend upon the redox state of the pool.

FIGURE 2. Deactivation of S_2 measured in leaves at room temperature. The experimental rationale is as described in the scheme above.



In principle S_3 deactivation can be monitored in experiments analogous to those outlined above but after 2 flashes (Rutherford, Inoue, 1983b). However, since $S_2Q_B^-$ and $S_3Q_B^-$ TL emission temperatures are the same, and since S_3 probably decays via S_2 , things start to get rather complicated.
Consider S_3 deactivation in centres in the state $S_3Q_B^-$. If we take two possible reactions into account, 1) $S_3Q_B^- \longrightarrow S_2Q_B$ and 2) $S_3Q_B^- \xrightarrow{e^-} S_2Q_B^-$, $S_2Q_B^-$ TL would be observed also, confusing the S_3 deactivation kinetics. In fact, the data obtained in leaves is suspiciously similar to the decay rate for $S_2Q_B^-$.
Deactivation experiments should be carried out on chlorella cells to see if the TL measurement is really due to S_3 or to S_2. At the same time, this may distinguish between the two possible mechanisms for $S_3Q_B^-$ deactivation suggested above. It is of note that, at this time, all "$S_3Q_B^-$ TL" could in fact, result from this reaction: $S_3Q_B^- \xrightarrow{e} S_2Q_B^- \xrightarrow{TL} S_1Q_B$.
The higher yield of luminescence from S_3 than from S_2 which has been observed in chloroplasts, could be explained in the context of this scheme if it is assumed that the two $S_2Q_B^-$ deactivating reactions (see above) are normally in competition with each other but, when $S_2Q_B^-$ is formed from $S_3Q_B^-$, the non-recombination deactivating electron has been used up and so now $S_3Q_B^-$ deactivates only by recombination - hence an apparent increase in yield of luminescence.
Weighing against this hypothesis is the observation that $S_3Q_B^-$ and $S_2Q_B^-$ TL can be separated from one another by pH (Rutherford, Inoue, 1983b). At pH 5.5 the $S_3Q_B^-$ luminescence remains at around 25°C but the $S_2Q_B^-$ band is shifted to lower temperatures. This is also the case in leaves infiltrated with pH 5.5 buffer.

An increase in stability as the pH is lowered indicates an acceptor side effect (e.g. Q_B/Q_BH, Em shifts higher as the pH is lowered). However, the same acceptor is involved in both kinds of centres, so an increase in emission would be expected for both peaks. The absence of shift in the $S_3Q_B^-$ peak implies the presence of an equal and opposite effect on donor side. The observation can be rationalised if the S_2 to S_3 change involved loss of a proton while the S_1 to S_2 change does not. This indeed, is what has been observed by direct measurements of proton release. This observation by TL can be considered confirmation of these previous measurements.
The ability to distinguish between S_2 and S_3 TL is potentially very useful. The deactivation of S_3 via S_2 can be observed in chloroplasts (Rutherford, Inoue, 1983) and preliminary data from leaves infiltrated with pH 5.5 buffer may be similarly interpreted. Deactivation experiments at pH 5.5 with and without low temperature illumination deserve further study since they might distinguish between some of the possible deactivation mechanisms discussed above.

3.3. The effect of DCMU on leaves

Infiltration of leaf discs with DCMU results in a shift of the flash induced TL from around 30°C to around 0-10°C. This is interpreted as a loss of $S_2Q_B^-$ recombination with instead the formation of $S_2Q_A^-$ recombination luminescence. Similar data has been obtained in chloroplasts and has been interpreted in the same way (Rutherford et al, 1982). This kind of effect is as predicted in recent theoretical work (DeVault et al, 1983).

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