Effects of hydroxylamine and silicomolybdate on the decay in delayed light emission in the 6–100 μs range after a single 10 ns flash in pea thylakoids

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(Received 22 April 1982)

Key words: Delayed light Emission, Hydroxylamine, Photosynthesis, Photosystem II, Silicomolybdate

Abstract. Measurements are reported on μs delayed light emission, following a single 10 ns excitation flash, in Alaska pea thylakoids treated with hydroxylamine (NH_2OH) or with silicomolybdate.

1. In thylakoids treated with 2 mM NH_2OH *in the light,* or *in the dark,* the quantum yield of delayed light emission is considerably enhanced. A 10 μs lifetime component of delayed light emission is not significantly changed, whereas a 50–70 μs lifetime component is increased. $MnCl_2$ and diphenylcarbazide are unable to reverse the above effects of NH_2OH treatment. Thus Mn^{2+} and diphenylcarbazide must not donate electrons directly to reaction center II but on the oxygen-evolution side of the NH_2OH block.

2. When the closed form of photosystem II reaction centers ($P_{680}Q^-$), where P_{680} is the reaction center chlorophyll and Q is a 'stable' electron acceptor, is generated by preillumination of NH_2OH-treated thylakoids with diuron present, the μs delayed light emission is inhibited, *but* a low level residual delayed light emission remains. Possible origins of this emission are discussed. It is believed that the best explanation for residual DLE is the existence of another acceptor besides Q that partakes in charge separation and rapid dissipative recombination when the reaction center is in the $P_{680}Q^-$ state.

3. The quantum yield of delayed light emission from 'closed' reaction centers ($P^+_{680}Q^-$) that have all charge stabilization reactions (i.e., flow of electrons to P^+_{680} and out of Q^-) blocked by NH_2OH treatment and addition of diuron is 1.1×10^{-3} for components measured in a range from 6 to 400 μs and extrapolated to zero time.

4. The addition of silicomolybdate, which accepts electron from Q^-, causes delayed light emission in the μs range to be greatly inhibited.

Introduction

The reaction center complex of photosystem II includes two components: P_{680}, the reaction center chlorophyll *a* or the primary electron donor, and a quinone molecule Q (or X-320), a 'stable' electron acceptor. Upon absorption of a quantum of light, the reaction center undergoes a charge separation with P_{680} becoming oxidized [4, 12] and Q reduced [9]. It has been suggested

[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Abbreviations: DCMU = diuron = 3–(3,4–dichlorophenyl), 1,1 dimethylurea; DLE = delayed light emission; DPC = diphenylcarbazide.

Photosynthesis Research 3, 161–177 (1982) 0166–8595/82/03/0161–17 $00.20/0

that μs delayed light emission (DLE) is generated by the charge recombination of P^+_{680} and Q^- [24, 37]. Loss of photosystem II oxidation-reduction energy by DLE is prevented through stabilization of the charge by the following reaction [25]: $ZP^+_{680}Q^- \rightarrow Z^+P_{680}Q^-$, where Z is a secondary electron donor on the oxygen-evolving side of photosystem II. It has also been suggested that the early phases of the μs DLE decay should be related to steps in this charge stabilization [16, 25].

Hydroxylamine (NH_2OH) affects photosystem II activity and, in particular, it inhibits oxygen evolution [5]. If thylakoids are treated with 2 mM NH_2OH in the dark, then oxygen-evolving capacity is destroyed, but NH_2OH can still act as an electron donor to photosystem II. However, if thylakoids are treated with 2 mM NH_2OH in the light, both oxygen-evolving activity and the ability for NH_2OH to donate electrons to photosystem II are eliminated [5]. On the basis of the μs rise of chlorophyll *a* (Chl *a*) fluorescence yield measured in intact *Chlorella* cells treated with NH_2OH in the dark [7, 8, 10], Den Haan and coworkers proposed that NH_2OH eliminates the $ZP^+_{680} \rightarrow Z^+P_{680}$ charge stabilization reaction, and in place of Z, another donor, D, reduces P^+_{680} with a halftime (t 1/2) of 25 μs. Lavorel [25] measured the μs decay of DLE in *Chlorella* and found a component with 5–10 μs lifetime (τ) to decrease upon NH_2OH addition, whereas components with τ of 50–70 μs and 110–300 μs increase. Most, although not all, of the early work was restricted to intact algal cells.

In this paper, we compare the effects of NH_2OH treatment *in light* and *in darkness* on the decay of DLE in the μs range in pea thylakoids. Furthermore, we report, in NH_2OH-treated samples, the effects of various electron donors to PS II:$MnCl_2$, diphenylcarbazide (DPC), benzidine, and phenylenediamine and of the electron acceptor silicomolybdate, which accepts electrons from Q^- [13, 39], on μs DLE. This was done to test their sites of action and to better understand the nature of DLE. Since $MnCl_2$ and DPC could not restore the DLE to the control value, it was suggested that they donate electrons farther away from P_{680} on the water side. Silicomolybdate appears to decrease μs DLE by removing electrons from Q^-. Measurements on a residual DLE when reaction center II is presumed to be closed by treatment of thylakoids with NH_2OH, diuron and preillumination and on the quantum yield of μs DLE are also reported.

Materials and methods

Pea plants (*Pisum sativum*, var. Alaska) were grown in the laboratory. Leaves harvested 10 days after germination were used to prepare chloroplasts. The procedure for preparation of thylakoid membranes (thylakoids) was identical to that previously described [19]. In all experiments, thylakoids were diluted immediately before use to a chlorophyll concentration of

5 μg/ml with a medium containing 0.4 M sucrose, 40 mM KCl and 50 mM sodium phosphate (pH = 7.8). All experiments were carried out at 25 °C.

Hydroxylamine treatments of chloroplasts were as follows: (a) thylakoids at a chlorophyll concentration of 300 μg/ml were incubated with 2 mM NH_2OH in white light (2 mW cm^{-2}) for 15 min at 25 °C; these treated thylakoids were centrifuged and washed twice with buffer and resuspended to a chlorophyll concentration of 5 μg/ml (see ref. 5); or (b) 2 mM NH_2OH was added to thylakoids as in (a) except that the treatment was in complete darkness.

The effectiveness of 2 mM NH_2OH treatment was tested by measuring, under saturating continuous illumination, the rate of oxygen evolution with ferricyanide as an electron acceptor, using a Yellow Springs Instrument Clark electrode and a Model 53 oxygen monitoring system.

The apparatus used for measuring μs DLE has been described elsewhere [19, 20]. Measurements were made after a single saturating 10 ns nitrogen laser pulse (λ, 337 nm). Various preillumination conditions are indicated in the Results.

The protocol of Zankel [38] was followed for measurements of the quantum yield of DLE (Φ_{DLE}). This is essentially a comparison of the light emitted as DLE to that emitted as fluorescence in the presence of diuron during the time it takes one quantum to arrive per reaction center. The chlorophyll *a* fluorescence rise measurement, needed for the Φ_{DLE} calculation, was made by replacing the nitrogen laser with light from an incandescent lamp passed through a 2-inch water filter, a Corning CS 4-96 glasss filter and a camera shutter. The intensity of this continuous broadband blue excitation was 1.5 mW cm^{-2} incident at the sample. As required for determining Φ_{DLE}, all experimental conditions, except the excitation source, were kept identical for both DLE and chlorophyll *a* fluorescence measurements.

For experiments in which silicomolybdate was present, the following protocol was used: (a) chloroplasts were illuminated for 10 s either with the nitrogen laser at a flash rate of 32 Hz or with continuous illumination; (b) during this illumination, either silicomolybdate or ferricyanide was added, followed by the addition of diuron; and (c) after 1 min dark adaptation, a single laser flash was given followed by measurements of the DLE decay. This particular procedure for the use of silicomolybdate was followed because it was reported earlier [39] that silicomolybdate accepted electrons before the diuron block most efficiently when it was added in the light prior to diuron. For measuring electron flow in these samples, a Clark oxygen electrode (described above) was used.

Recrystallized NH_2OH was used in this work, and the pH of its solution was adjusted to a value of 7.8 at 25 °C. Fresh solutions of NH_2OH were prepared on the day of the experiment. Silicomolybdate obtained from K & K Laboratories was dissolved in warm water and filtered before use.

Results

Treatment of thylakoids under illumination with 2 mM NH_2OH (6.6 NH_2OH per chlorophyll) destroys oxygen evolution and inhibits electron donation by NH_2OH to photosystem II [5]. To test the effects of the NH_2OH treatment under illumination on electron flow in photosystem II, the chlorophyll *a* fluorescence transient under continuous illumination with diuron present was measured [29]. Treatment under illumination almost completely eliminated the fluorescence rise and thus photosystem II electron flow. Oxygen evolution was entirely stopped (data not shown), so the very low level of residual electron flow must be due to endogenous donors. Addition of 1 mM NH_2OH (200 NH_2OH per chlorophyll) restored a small amount of the fluorescence transient in these samples (Fig. 1). This indicates that

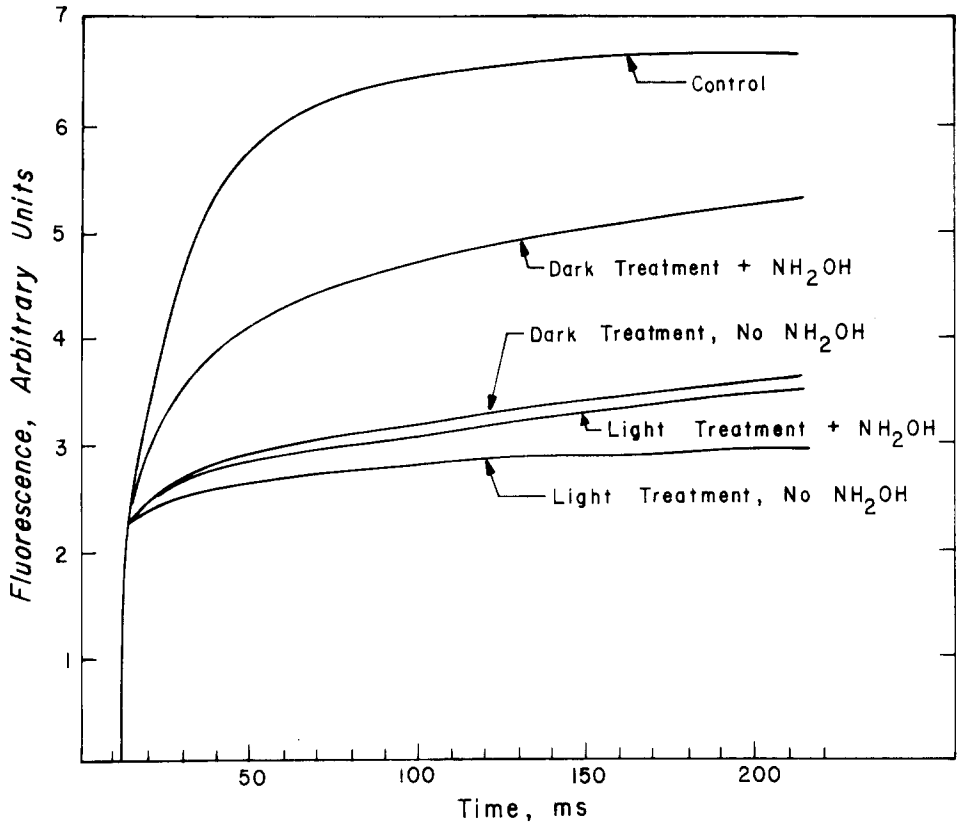

Figure 1. Chlorophyll *a* fluorescence rise as a function of time of continuous illumination. Thylakoids received pretreatments as indicated and were dark adapted for 10 min prior to the measurement. Diuron was present at a concentration of 4 μM, and NH_2OH when present was at a concentration of 1 mM. The shutter was opened and the change in fluorescence intensity was continuously monitored. Thylakoids were at a chlorophyll concentration of 5 μg/ml

NH_2OH acted as an inefficient electron donor to photosystem II in this sample. The decay of DLE is shown as a semi-logarithmic plot in Figure 2 and in terms of exponential lifetimes and amplitudes in Table 1, line 2. The rapidly decaying component with $\tau \sim 10\,\mu s$ is not significantly changed by NH_2OH treatment in the light, but a slower component of $52\,\mu s$ lifetime in the control is enhanced by over four fold and now decays with a lifetime of $77\,\mu s$. Also, the yield of DLE, represented by the area under the decay curve, is increased by 3.5 fold.

Since a high level of DLE is indicative of blocked electron flow from Z to P_{680}^+, the effects of various photosystem II electron donors ($MnCl_2$, reduced benzidine, reduced phenylenediamine and diphenylcarbazide) on DLE were studied to determine if any of them donated electrons to P_{680}^+. $MnCl_2$ partially reversed the effects of NH_2OH, and the other electron donors were unable to significantly reverse the effects of NH_2OH (in light) on DLE (see lines 3–6 of Table 1).

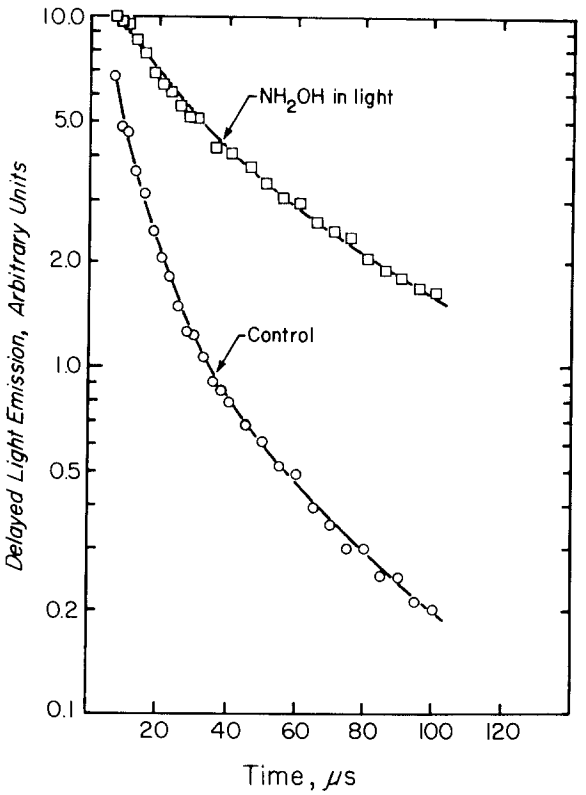

Figure 2. Plot of delayed light emission for 6 to $100\,\mu s$ after a single 10 ns excitation following 5 min dark adaptation. Decays are shown for control Alaska pea thylakoids (○—○—○) and those treated with 2 mM NH_2OH per $300\,\mu g$ chlorophyll/ml in the light for 15 min (□—□—□). The NH_2OH was then washed out and the delayed light measurements were made on samples having a chlorophyll concentration of $5\,\mu g$/ml

Table 1. Decay characteristics of delayed light emission in the 6–100 μs range standard graphical procedures were applied to semilog plots of delayed light emission decays to calculate amplitudes (α) and lifetimes (τ). Various components are extrapolated to zero time, and component amplitudes have been put on the same scale and are given in relative units. τ values are given in microseconds. Typical measurement errors are given as ± one standard deviation unit. The areas under the curve are normalized to one for the control sample. Alaska pea chloroplasts at a chlorophyll concentration of 5 μg/ml were used, and specific treatment procedures are given in Materials and methods

Line	Sample conditions	α_1	τ_1, μs	α_2	τ_2, μs	Area
1	Control	8.5	10	1.5	52	1.0
2	Treated with 2 mM NH_2OH in light	6.5 ± 0.6	10 ± 1	6.5 ± 0.6	77 ± 7	3.5
3	Treated with 2 mM NH_2OH in light plus 10 μM $MnCl_2$	6.4	12	5.6	64	2.7
4	Treated with 2 mM NH_2OH in light plus 1 mM ascorbate and 10 μM benzidine	7.1	10	6.0	81	3.5
5	Treated with 2 mM NH_2OH in light plus 1 mM ascorbate and 10 μM phenylenediamine	7.0	9	5.8	81	3.3
6	Treated with 2 mM NH_2OH in light plus 1 mM diphenyl carbazide	6.0	10	6.4	67	3.0
7	Treated with 2 mM NH_2OH in dark	6.9	10	5.8	67	2.8
8	Treated with 2 mM NH_2OH in dark plus 1 mM NH_2OH	7.8	9	5.2	58	2.3

In thylakoids treated with 2 mM NH_2OH *in darkness,* the fluorescence transient under continuous illumination is restored to a large extent when 1 mM NH_2OH is added (Fig. 1). Thus, under these conditions, NH_2OH can donate electrons to the photosystem II reaction center.

Treatment with NH_2OH in the dark produces changes in DLE decay similar to NH_2OH treatment under illumination. The 10 μs component is not significantly changed, but the slower decay component is enhanced, as is the yield of DLE. However, here the presence of 1 mM NH_2OH as an electron donor causes small decreases in the amplitude of the slow component and in the yield of DLE (see Fig. 3 and lines 7 and 8 of Table 1). This corresponds with NH_2OH acting as a donor and causing a partial reversal of effects of NH_2OH dark-treatment.

Elimination of the NH_2OH enhancement of DLE. Treatment of thylakoids in the light or dark with 2 mM NH_2OH results in a significant enhancement of DLE (Figs. 2 and 3, Table 1). If 0.1 μM diuron (1 diuron per 50 chlorophyll) is added to samples treated with 2 mM NH_2OH in either the

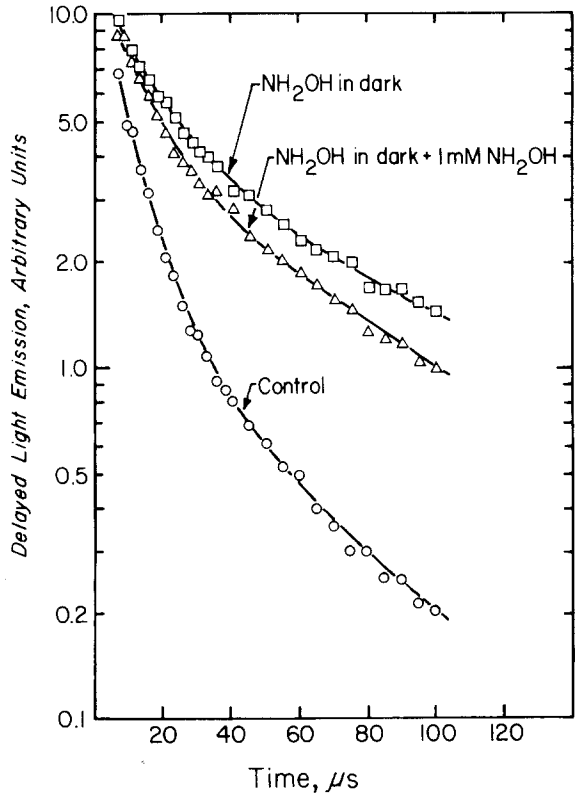

Figure 3. Plot of delayed light emission for 6 to 100 μs after a single flash following 5 min dark adaptation. Decays are shown from thylakoids treated with 2 mM NH_2OH per 300 μg chlorophyll/ml for 15 min in the dark. The NH_2OH was washed out and the delayed light measurements were made on samples having a chlorophyll concentration of 5 μg/ml with (△—△—△) or without (□—□—□) 1 mM NH_2OH added as an electron donor and control (○—○—○)

dark or the light, and if these samples are preilluminated with saturating continuous white light for 2 min (solid squares, Fig. 4), or with 2 or more flashes of laser light (see below), then the NH_2OH-induced enhancement of DLE is eliminated. This preillumination effect on the NH_2OH-treated samples occurs only if diuron is present. (For the loss of DLE in the millisecond and second ranges in samples having both NH_2OH and diuron present, see refs. 3, 29, 34.) This effect was further characterized by observing the rate of onset of the preillumination effect. We measured DLE intensity at 60 μs after an excitation pulse following preillumination of the sample for various lengths of time, with bursts of excitation laser pulses given at a frequency of 32 Hz (Fig. 5). Samples treated with 2 mM NH_2OH in the dark show an onset of the preillumination effect with a τ 1/2 of 2.2 s, which is accelerated (τ 1/2 = 0.8 s) by the addition of 1 mM NH_2OH. The rate of onset of the

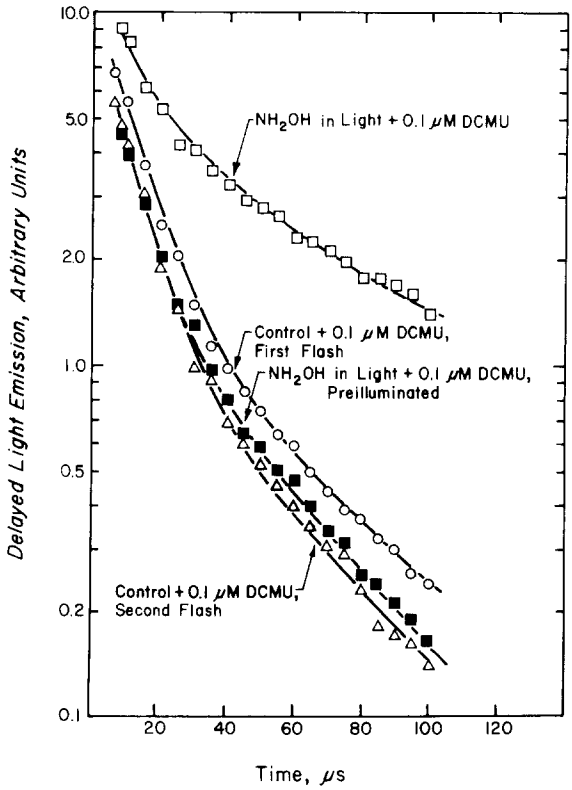

Figure 4. Plot of delayed light emission for 6 to 100 μs after one or two flashes in Alaska pea thylakoids treated as indicated in the legend of Fig. 2. The various sample conditions are as follows: control plus 0.1 μM diuron and decay after a single flash (○—○—○) and after a second flash given 66 ms after the first (△—△—△); treated with NH_2OH in the light plus 0.1 μM diuron (□—□—□), preilluminated for 2 min in saturating continuous light and the decay after a single flash (□—□—□)

preillumination effect in thylakoids treated with 2 mM NH_2OH in the light (also shown in Fig. 5) is slower (τ 1/2 = 5 s) and unaltered by the addition of 10 μM $MnCl_2$ or 1 mM NH_2OH (data not shown). The more rapid onset of preillumination inhibition of DLE in samples treated with NH_2OH in the dark is consistent with the higher rates of electron flow to the reaction center of these types of samples, which was observed in Figure 1. The elimination of the enhancement of DLE by preillumination is not reversed even in samples kept in darkness for as long as 2 h at room temperature.

A residual amount of DLE remains however, even after many minutes of preillumination, and is of comparable amplitude to that seen in control samples on the first flash with diuron present. This residual DLE signal is not artifactual since it can be completely eliminated by heating thylakoids for 10 min at 100 °C. The ratios of emission at 686 nm (mostly from photosystem II) to that at 715 nm from both photosystem I and II appear to be

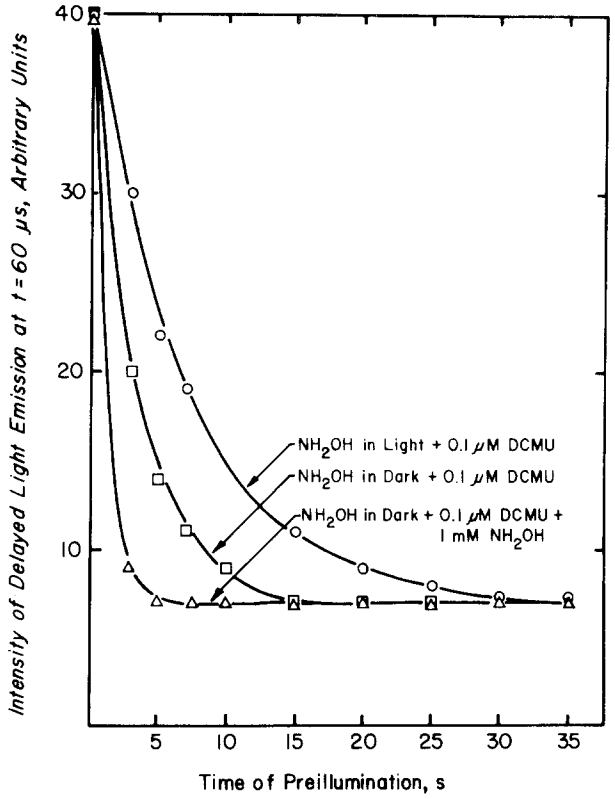

Figure 5. The intensity of delayed light emission at 60 μs after a single flash in Alaska pea thylakoids following preillumination with the excitation laser operating at 32 Hz. The samples are as follows: treated with NH_2OH in the light as in the legend of Fig. 2, plus 0.1 μM diuron (○—○—○); treated with NH_2OH in the dark as in the legend of Fig. 3 plus 0.1 μM diuron (□—□—□) and plus 0.1 μM diuron and 1 mM NH_2OH (△—△—△)

the same for both the residual DLE (see above) and for 2 mM NH_2OH-treated thylakoids. Thus, the source of emission seems to be from the same photosystem, which is believed to be photosystem II.

In control thylakoids treated with 0.1 μM diuron, the halftime of the Q^- oxidation rate, monitored by following the decay in Chl *a* fluorescence yield after a single excitation flash, is 1.0 ± 0.3 s (upper curve, Fig. 6). Therefore, if two flashes are given with a time separation of much less than 1 s, the reaction centers will be in the closed form $P_{680}Q^-$ at the time the second flash is given. As the delay time between the first and second flashes is decreased from ~2 s to ~130 ms, the DLE intensity at 60 μs after the second flash decreases to about 70% of that occurring after the first flash (lower curve, Fig. 6). After the second flash, the level of DLE returns to the first flash level with a recovery halftime of 0.7 ± 0.4 s (lower curve, Fig.

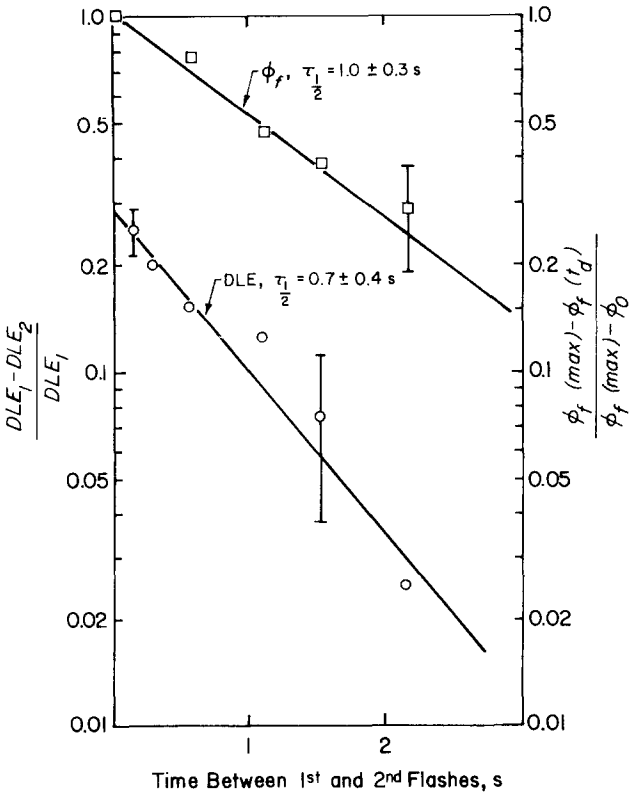

Figure 6. The delayed light emission intensity at 60 μs after the first flash (DLE_1) and the second flash (DLE_2) are plotted as

$$\frac{DLE_1 - DLE_2}{DLE_1}$$

versus the time between the first and second flash. The chlorophyll *a* maximum fluorescence yield, $\Phi_f(max)$, constant fluorescence yield, Φ_0, and fluorescence yield, $\Phi_f(t_d)$ at time t_d, after the first excitation are plotted as

$$\frac{\Phi_f(max) - \Phi_f(t_d)}{\Phi_f(max) - \Phi_0}$$

versus the delay time between the first excitation flash and a second weak analytic flash. Samples were Alaska pea thylakoids at a chlorophyll concentration of 5 μg/ml

6), which is about the same (in view of the errors) as the Q^- oxidation as indicated by the fluorescence yield decay. So again, a large amount of residual DLE is observed under conditions when the reaction center is in a state ($P_{680}Q^-$) that is closed to normal photochemistry.

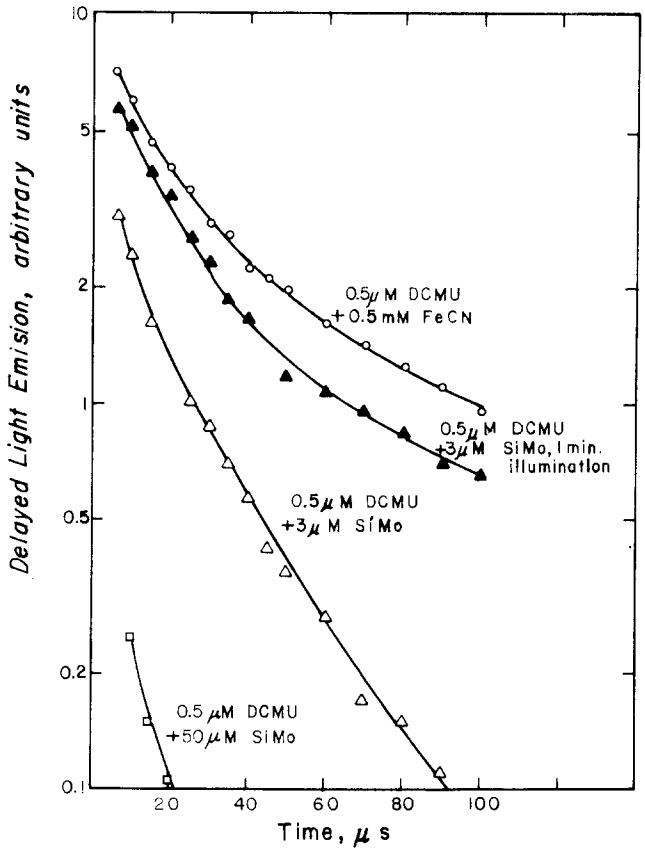

Figure 7. Plot of delayed light emission in the 6–100 μs time range after a single excitation flash following 1 min dark adaptation. Thylakoids were at a chlorophyll concentration of 5 μg/ml in the following media: 100 mM KCl, 5 mM $MgCl_2$ and 20 mM Tricine at pH 8.1. Decays are shown for thylakoids treated as in Methods with (○—○—○), 0.5 μM diuron and 0.5 mM ferricyanide (FeCN); (△—△—△), 0.5 μM diuron + 3 μM silicomolybdate (SiMo); (□—□—□), 0.5 μM diuron + 50 μM SiMo; and (▲—▲—▲), 0.5 μM diuron + 3 μm SiMo and illuminated for 1 min with saturating light

Inhibition of μs DLE by silicomolybdate. Figure 7 shows that with diuron and ferricyanide present, DLE is high; however, with diuron and 3 μm silicomolybdate (0.6 silicomolybdate per chlorophyll) added, the μs DLE is reduced to the value of the control (-diuron), as expected. Higher concentrations of silicomolybdate (50 μM) cause a further reduction of DLE. This is believed to be due to nonspecific quenching of quantum yield, since the F_0 (dark level) of fluorescence is also reduced. In samples having diuron and silicomolybdate present, initial electron flow rate through photosystem II, determined by oxygen evolution rate within 5 s of illumination, was 60%

larger than the rate observed in thylakoids having only ferricyanide present. With 0.5 μM diuron and 3 μM silicomolybdate present, the DLE is enhanced by illuminating the sample for 1 min in saturating light (Fig. 7). This corresponds with the short-lived ability of silicomolybdate to accept electrons from Q^- in continuous light [39]. The nonspecific inhibitory effect of 50 μM silicomolybdate on DLE, noted above, is large and it cannot be reversed by illumination of the sample with continuous light.

Quantum yield of delayed light emission. As described in Materials and methods, a procedure similar to that used by Zankel [38] was followed to estimate the number of quanta emitted as DLE per quantum reaching a photosystem II reaction center. These yields were determined for all DLE decay components observed in the 6–400 μs range and extrapolated back to zero time. These are not total yields, because any components observable only at times less than 6 μs have not been included. The error caused by truncating the area under the curve after 400 μs will be only a few per cent due to the very low level of DLE in the ms and s range. For control chloroplasts, the DLE quantum yield (Φ_{DLE}) is 3.1×10^{-4} and for 2 mM NH_2OH-treated samples with diuron present, but not preilluminated, it is 1.1×10^{-3}. Both of these values for Φ_{DLE} are significantly lower than the Chl *a* fluorescence yield (Φ_f) of 2.5×10^{-2} reported for low light intensity illumination [23].

Discussion

The main new observations in this paper are: (a) differences in the μs DLE behavior in thylakoids treated with 2 mM NH_2OH *in light* or *in darkness*; (b) the decrease in μs DLE with silicomolybdate is added to the thylakoids; and (c) the presence of residual DLE in thylakoids containing both NH_2OH and diuron used to apparently close the reaction center II.

Hydroxylamine effects in light and dark. The increase in the yield (Table 1 area values; Fig. 2) upon NH_2OH treatment is well known [25] and easily explained by a block in electron flow from Z to P^+_{680}, thus increasing the latter's concentration, a component needed for DLE [15, 26]. The increase is in a 50 μs DLE component. This component may reflect charge stabilization on the heterogeneous electron acceptor side of photosystem II [6, 17, 28], since NH_2OH is known to block reactions on the donor side [7, 8]. However, evidence exists [7] that novel donor properties occur in reaction centers in which oxygen evolution has been eliminated. A donor referred to as D is known to donate electrons with a lifetime of approximately 30 μs, which is too fast to account for the DLE component observed here. After NH_2OH treatment *in light,* none of the electron donors used ($MnCl_2$, diphenyl carbazide, reduced benzidine, and reduced phenyldiamine) restored μs

DLE to the control value (Table 1), suggesting that this treatment produces an efficient block between Z and P_{680} that cannot be overcome by these donors, i.e., these donors do not donate electrons to P^+_{680} [see also refs. 19, 21, 32]; in contrast, Mn^{2+} has been suggested to donate electrons to P^+_{680} under other conditions [2]. After NH_2OH treatment *in darkness,* a block between Z and P_{680} is produced, and this block is partially overcome by added NH_2OH (Figs. 1 and 3; Table 1), i.e., the latter acts as an electron donor as already known for *Chlorella* [7, 8].

Silicomolybdate effect. μs DLE is greatly inhibited when a silicomolybdate plus diuron sample is compared with that with diuron only, the former having the ability to evolve O_2 just like a control sample [39]. Furthermore, when silicomolybdate action is stopped by 1 min postillumination, μs DLE approaches that of the diuron sample. These results are consistent with the hypothesis that when concentration of the second component Q^- needed for DLE is decreased, DLE also decreases.

The earlier work by Mohanty et al. [30] showed that millisecond DLE from chloroplasts containing diuron was enhanced when silicomolybdate was added. This result, which apparently contradicts the data presented in Figure 7, is due to the phosphoroscopic method used by Mohanty et al. to measure DLE. The presence of diuron inhibits DLE due to the build up of closed (ZPQ^-) reaction centers under the multiple phosphoroscopic-illumination. The addition of silicomolybdate relieves this diuron inhibition and enhances DLE by a greater amount than any inhibitory effect due to silicomolybdate competition for Q^-. Thus, the effect observed here after a single flash in dark-adapted thylakoids is masked when DLE is measured by the phosphoroscope method. It should also be noted that NH_2OH treatment uncouples chloroplasts [5], so differences between single and multi-excitation experiments cannot be explained by a build up in membrane potential or pH gradient.

The residual delayed light emission. If microsecond DLE is due to $P^+_{680}Q^-$ recombination, then conditions that cause the photosystem II reaction center to be in a closed state ($P_{680}Q^-$) should also result in inhibition of DLE. In our experiments, the $P_{680}Q^-$ state was brought about by adding diuron to block Q^- decay by electron flow to the intersystem electron carrier pool (A) and by giving a second flash rapidly enough so that Q^- oxidation by any other pathway(s) remains small. However, even with a second flash given as rapidly as 133 ms after the first flash, approximately 70% of DLE remains and the 30% drop in DLE disappears at approximately the same rate as Q^- becomes reoxidized (Fig. 6). This recovery time agrees well with earlier results [38]; however, a residual amount of DLE with a rapid second flash was not reported earlier. On the third and succeeding flash, the level of DLE remains the same as on the second flash. We believe this residual

DLE is the result of charge separation taking place at photosystem II even though Q is reduced. A photoreaction can take place under these conditions if a second acceptor, X_a, which has been postulated [11], exists. Then this residual DLE will be due to the recombination of P^+_{680} and X^-_a.

In NH_2OH-treated samples, the reaction centers could be put in the closed form $P_{680}Q^-$ by having diuron present and preilluminating the sample. The rate at which centers become closed, of course, depends upon the tendency for electrons to pass through the NH_2OH block from Z to P^+_{680}. The completeness of NH_2OH block is reflected in the time taken for reaction center to become closed, as indicated by inhibition of DLE. From Figures 1 and 5, it is apparent that NH_2OH treatment in the light does not completely block electron flow to P^+_{680}, but the block is much more effective than that occurring for NH_2OH treatment in the dark. A residual amount of DLE is found even after long periods of preillumination, which should put all reaction centers in the closed $P_{680}Q^-$ state. The amount of residual DLE is small compared to that from non-preilluminated samples treated with NH_2OH and diuron, but the amount of residual DLE is comparable to that found in samples with just diuron present (Fig. 4). There are several nonmutually exclusive origins for DLE when both NH_2OH and diuron are added and the sample is preilluminated: (a) An electron carrier may exist prior to Q, and DLE may occur from the recombination of oxidized P_{680} and the reduced carrier even though electron flow to P_{680} and electron flow out of Q^- is blocked. There are many reports in the literature of additional acceptors besides Q [11, 14, 18, 36]. Indeed, pheophytin (Ph) has been implicated as an active participant in photosystem II charge separation [22], but DLE emission from $P^+_{680}Ph^-$ appears with nanosecond not microsecond kinetics. (b) Residual DLE may arise from a presumably small subpopulation of reaction centers, which are capable of undergoing a rapid electron cycle around photosystem II. This cycling will be caused by a participation of various donors operating near photosystem II reaction centers. (c) A special case of this type could occur if photosystem II kinetics were dependent on the density of closed centers [27]. Thus the occurrence of DLE, after the first flash, causes opening of reaction centers, but its rate may be faster after the second and succeeding flashes (or preillumination) because of the presence of closed reaction centers in the $P^+_{680}Q^-$ state. An equilibrium is reached with a population of both open and closed reaction center II complexes, which gives rise to residual DLE. (d) This residual DLE may have a separate origin: triplet-triplet annihilation [34] or electron-hole recombination [1]. At present, we believe the best explanation for residual DLE is the existence of another acceptor besides Q that partakes in charge separation and rapid dissipative recombination when the reaction center is in the $P^+_{680}Q^-$ state.

Quantum yield of delayed light emission. The quantum yield of DLE (Φ_{DLE}) of 3×10^{-4} for decay components beginning at 6 μs after the flash, as ex-

pected, is larger than the 1×10^{-4} value Zankel [38] obtained for the 50 μs lifetime decay component. In *Chlorella,* a $\ll 1$ μs delayed light emission component has been reported [33]. If we normalize the aerobic *Chlorella* delayed light emission signal to that from thylakoids at 10 μs, then we find the Φ_{DLE} contributed by the faster component extrapolated to zero time is 9×10^{-5}. Thus, the total Φ_{DLE} may be about 5×10^{-4}. Ruby [31] measured DLE in the 1–700 ms time range after a single flash in *Chlorella* and found Φ_{DLE} for ms DLE to be 10^{-5}. Similar measurements after a single flash in the 1.5 ms–30 s range with spinach chloroplasts [35] gave a value of 4×10^{-7} for Φ_{DLE}. Our value for Φ_{DLE} in samples treated with NH_2OH in the light and having diuron present (but not preilluminated), so that all charge stabilization reactions have been inhibited, is about 4% of the Chl *a* fluorescence yield. This indicates that under conditions that will maximize the charge recombination back reaction, more than 90% of the recombinational energy is unobserved. Since components with lifetimes smaller than 10 μs do not exist with NH_2OH present [36], this must be due mainly to a large portion of the recombinational energy being dissipated by nonradiative pathways.

Acknowledgement

This research was supported by a 1980 Biomedical Research grant (PHS 2S07RR07030-15). We are very appreciative of fruitful discussions with Dr. C. Wraight concerning this work.

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