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PRECURSORS TO MICROSECOND DELAYED LUMINESCENCE IN OXYGEN-EVOLVING AND INHIBITED CHLOROPLASTS

WILLIAM J. BUTTNER * and GERALD T. BABCOCK

Department of Chemistry, Michigan State University, East Lansing, MI 48824-1322 (U.S.A.)

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We have investigated submillisecond delayed luminescence in spinach chloroplasts under a variety of conditions. In Tris-washed chloroplasts, which are inhibited on the oxidizing side of P-680, the delayed light emission in the 7–200 μs time-range decayed with biphasic behavior. In fully dark-adapted samples illuminated by a single saturating laser pulse, the fast phase of delayed luminescence followed a nearly identical pH-dependent time-course as that observed optically and by ESR for P^+-680 reduction, thus verifying the recombination hypothesis for the origin of delayed light. The observed slower phase of delayed luminescence was also pH dependent, but unlike the fast phase, could not be ascribed to specific electron transfer events of PS II. This phase could be rationalized by a heterogeneity in the population of P-680. While kinetic parameters were found to be insensitive to changes in ionic strength, the overall luminescence intensity was quite sensitive to the electrical parameters, thus indicating the role of ionic strength and local charges in delayed luminescence modulation. A similar series of experiments was performed on untreated chloroplasts. The pH-dependent delayed luminescence behavior in both untreated chloroplasts and Tris-washed chloroplasts was similar despite significantly faster kinetics associated with the reduction of P^+-680 by the secondary PS II electron donor, Z, in the former preparation (e.g., Van Best, J.A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–188). Thus, it was concluded that, in untreated samples, microsecond delayed luminescence emanates primarily from centers which are not competent in oxygen evolution. The nearly identical delayed luminescence intensity in untreated chloroplasts and in Tris-washed chloroplasts was rationalized by a model which predicts modulations in delayed luminescence yield by the exciton-quenching effect of P^+-680. Computer simulations demonstrate the feasibility of this model. The previously documented flash oscillations in microsecond delayed luminescence intensity in untreated chloroplasts (Bowes, J.M. and Crofts, A.R. (1979) Biochim. Biophys. Acta 547, 336–346), which we readily observed, were attributed to alterations in delayed luminescence yield (in nonfunctional centers) by variations in charge density stored at the oxygen-evolving complex of functional centers. Taken together, our results emphasize the dependence of delayed luminescence kinetics upon electron-transfer kinetics and the dependence of delayed luminescence amplitude upon the photochemical parameters, the exciton yield and the emission yield.

* Present address: Department of Chemistry, University of Arizona, Tucson, AZ 85721, U.S.A.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; P-680, the reaction center of Photosystem II; Q, the primary stable acceptor of P-680; ϕ_L, emission yield; η, exciton yield; PS II, Photosystem II; Chl, chlorophyll.

Introduction

The electron transfer events which follow the photooxidation of the reaction center chlorophyll of Photosystem II, P-680, are a function of the integrity of the oxygen-evolving complex (see Refs. 1 and 37 for a review). In water-oxidizing chloroplasts, P^+-680 reduction occurs in less than 1 μs [2] and the secondary donor, Z, transfers its oxidizing equivalents into the oxygen-evolving complex in the μs to ms time range. For the latter reaction, the electron-transfer halftime increases as the number of oxidizing equivalents stored on previous flashes increases [3]. Upon inhibition of O_2 evolution by, for example, Tris-washing, these electron transfer times are altered. The decay of P^+-680 occurs in the 2–40 μs range and is pH-dependent [4,5]. The alterations in the kinetics of P^+-680 reduction has been ascribed to an alteration in the reduction potential of Z in inhibited preparations [6]. Moreover, in these inhibited chloroplasts, the lifetime of Z^+ is extended into the ms domain and is sensitive to the presence of exogenously added electron donors [7].

Despite these changes in electron transfer rates upon going from untreated to inhibited chloroplasts, the kinetic behavior of delayed luminescence appears to be affected only slightly by changes in the integrity of the oxygen-evolving complex. Jursinic and Govindjee [8] compared delayed light emission in intact and Tris-treated bush bean and pea chloroplasts at neutral pH. Under conditions where the donor Z was reduced prior to flash illumination, they found identical kinetic components in the decay of delayed luminescence for the two different preparations: a fast phase decayed with a halftime of approx. 7 μs and contributed about 70% of the delayed luminescence amplitude followed by a slow phase with $t_{1/2} \approx 35$ μs (see Ref. 38 for a review of submillisecond delayed light emission). Bowes and Crofts [9] investigated the flash number dependence of delayed luminescence from oxygen-evolving chloroplasts in the pH range 4.0–9.0. The results they obtained were similar to those reported by Jursinic and Govindjee in that they observed flash number independent delayed luminescence decay components with halftimes in the 10–15 μs range and in the 40–50 μs range. As opposed to the Tris-washed case, however, between pH 6 and 8, they observed distinct period 4 oscillation in the total initial intensity of delayed luminescence and in the amplitude of the fast-decay component. Oscillations in the amplitude of the slower phase were not as apparent. At pH 4, the delayed luminescence intensity was invariant to flash number and decaysed with a halftime characteristic of the 135 μs μs P^+-680Q^- recombination time. In this case, however, as well as in the Tris-washed chloroplasts experiments, significant increases in the intensity of the delayed light emission were not observed.

These results appear to be at odds with the generally accepted recombination hypothesis for delayed luminescence (see Refs. 10 and 11 for reviews). In this model, the delayed luminescence arises from a reversal of the light-induced PS II electron-transfer reactions. The immediate precursor to delayed luminescence is considered to be the P^+-680Q^- state, and decay components in the emission time-course are thought to reflect forward electron-transfer reactions which decrease the precursor concentration. Thus, both the intensity and the decay halftimes of delayed luminescence are expected to be sensitive to the rates of electron transfer on the water side of PS II. In the experiments reported here, we have examined the behavior of microsecond delayed luminescence in Tris-washed and oxygen-evolving chloroplasts as a function of flash number over the pH range 4.5–8.0. For the inhibited preparations, we find excellent agreement between the time-course of the fast delayed luminescence components and the decay of P^+-680 as determined in optical and ESR experiments [4,12]. We find the same pH-dependent phases in oxygen-evolving chloroplasts and in agreement with Bowes and Crofts [9], we observed period 4 oscillations in the amplitude of these phases. To account for these results within the recombination hypothesis, which is strongly supported by our Tris-washed chloroplast data, we discuss the likely occurrence of damaged centers in the oxygen-evolving preparations and the modulation of delayed light intensity by the photochemical parameters, the exciton yield (η) and the emission yield (ϕ_L).

Materials and Methods

Preparation of samples. Chloroplasts were prepared from market spinach by the high-salt, low-salt method of Robinson et al. [13]. Except for the presence of 1 mM EDTA during the 20 min incubation period, Tris-washed chloroplasts, which have electron transport from the oxygen-evolving complex inhibited, were prepared as described by Blankenship and Sauer [14]. The chlorophyll concentration, as determined by the method of Sun and Sauer [15], was adjusted to be about 3 mg/ml. Following isolation, the chloroplasts were frozen at −40°C and stored until needed. Freezing of the sample did not significantly affect the integrity of PS II electron transport as indicated by the rates of steady-state oxygen evolution which were in excess of 800 μequiv/mg Chl per h. In order to minimize any long-term degradation effects, chloroplasts were used within 2 weeks of isolation.

Prior to experimentation, stock solutions of chloroplasts were thawed and then stored on ice and in the dark. Aliquots of the stock solution were diluted to a final chlorophyll concentration of 5 μg/ml in a reaction media consisting of 10 mM buffer. The various buffers used were succinate (pH 4.5), Mes (pH 5.2, 6.0), Hepes (pH 7.0, 8.0) and Tricine (pH 9.0). Following a 5 min dark incubation period, the delayed light experiments were carried out. All experiments were performed at room temperature (25°C). All chemicals were of reagent grade and were used without further purification.

Experimental apparatus. The delayed luminescence experiments were performed on an apparatus designed and constructed in our laboratory. The actinic light source was the second harmonic of a Nd/YAG laser (Quanta-Ray DCR) which provided a 20 ns pulse of 532 nm light. The delayed luminescence was directed via a polished lucite light pipe to a photomultiplier tube (EMI 9558-QB) situated 90° relative to the actinic light. In order to protect the photomultiplier tube and detection circuitry during the intense laser illumination, a photomultiplier tube gating circuit similar to the design of Groves [16] was used. In addition, between the sample cell and the photocathode surface of the photomultiplier tube were a pair of protection filters (Baird-Atomic 685 nm bandpass filter and a Schott RG665 red cutoff filter). In spite of photomultiplier tube gating and use of protection filters, a small background signal was still detectable. In order to correct for this, a blank signal from chloroplasts which were completely inactivated by heating (5 min at 70°C) was subtracted from that obtained from intact samples. The intensity of this artifactual signal relative to the delayed luminescence emission obtained under low yield conditions, specifically dark-adapted tris-washed chloroplasts (pH 7.0) illuminated by a single saturating light pulse, is shown in Fig. 1. The output of the photomultiplier tube was digitized and stored in 1024 successive channels by a 7 bit analog to digital converter. The typical sampling rate was 1 point per 200 ns. The digitized signal was stored in a computer (Nicolet model 1072) in which the results of several experiments, usually ten, were averaged. The final results of an experiment was plotted on an X-Y recorder (Hewlett-Packard model 7001). A detailed description of the delayed luminescence apparatus was presented by Buttner [17].

Analysis of data. By utilizing an electronic digitizer on the kinetic traces of the delayed luminescence measurements, point values for the intensity of delayed luminescence as a function of time were obtained. The typical sampling rate corresponded

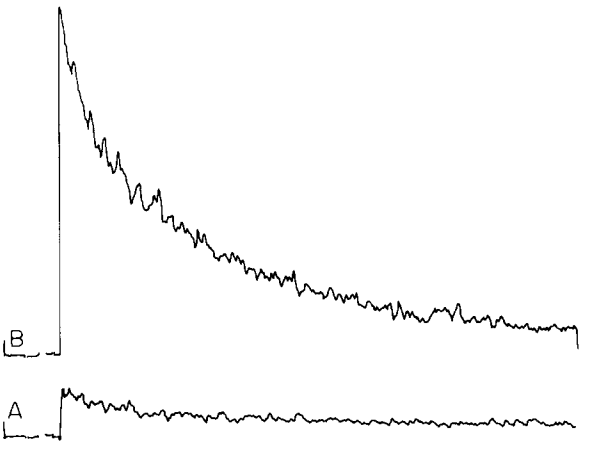


Fig. 1. A comparison of the delayed luminescence signal (B) obtained from dark-adapted, Tris-washed chloroplasts (pH 7.0) illuminated by a single laser flash with the artifactual signal (A). Aside from the use of heat-treated chloroplasts (5 min at 70°C) for trace A, trace A and B were measured under identical conditions. Each trace is the average of ten measurements.

to 1 point/μs. These data were analyzed by using a nonlinear regression analysis routine [18] on file in the Michigan State University Computer Library. Typically, the delayed luminescence analysis was in terms of a biphasic decay.

Results

Delayed light from dark-adapted, Tris-washed chloroplasts

According to the recombination hypothesis, the immediate precursor of delayed luminescence is the state P^+-680Q^-. The kinetics of P^+-680 in Tris-washed chloroplasts, as detected optically at 820 nm, were extensively studied by Mathis and co-workers [4,5]. In dark-adapted, Tris-treated samples, the kinetics of the submillisecond reduction of P^+-680 were analyzed in terms of a biphasic decay. The results indicated the presence of a dominant fast pH-dependent phase and a minor slow pH-independent phase. The fast phase varied from a reported halflife of 3.5 μs at pH 8.0 to 44 μs at pH 4.0; the halflife of the slow phase was 100–200 μs throughout the pH range studied. These workers also reported the occurrence of a minor phase with a halftime of 22 μs [19]. At pH 9.0, the P^+-680 halflife was less than 2 μs, but because of limitations in the instrument response time it could not be resolved.

Similar pH-dependent kinetics are observed for delayed luminescence following a single actinic flash on Tris-treated samples. Table I summarizes the delayed luminescence data obtained between pH 4.5 and 8.0 in dark-adapted samples. Normalized experimental traces are shown in Fig. 2. Because chloroplasts clump at low pH, no delayed luminescence experiments were performed below pH 4.5. Delayed luminescence measurements at pH 9.0 were irreproducible, presumably because of the rapid reduction of P^+-680 ($t_{1/2} < 2$ μs) coupled to the recovery time of about 7 μs that was necessary for the detection apparatus following photomultiplier tube gating and laser illumination. According to Bowes and Crofts [9], the low delayed luminescence intensity at pH 9.0 following a single flash was attributed to a parallel donor to Z which becomes dominant as a result of a high pH-induced inhibition of electron transport between the oxygen-evolving complex and P-680. Since chloroplasts suspended at pH 9.0 were still able to generate Z^+ as monitored by ESR Signal II_f (not shown, see Ref. 17), this parallel donor model is probably incorrect. Instead, the lower delayed luminescence intensity may be attributable to the acceleration in P^+-680 reduction by Z at high pH. The data summarized in Table I indicate

TABLE I

DELAYED LUMINESCENCE KINETIC BEHAVIOR INDUCED BY A SINGLE SATURATING LASER FLASH

The uncertainties represent standard deviations. The values listed in the columns A_{ext} and A_{10} were normalized to the intensity of delayed luminescence observed for Tris-EDTA washed chloroplast (5 μg/ml) at pH 7.0 10 μs after the flash. A_{ext} is the initial amplitude of delayed luminescence as obtained from the kinetic analysis. A_{10} is the delayed luminescence intensity observed 10 μs after laser illumination. $t_{1/2}$(fast) is the halflife of the fast phase. $t_{1/2}$(slow) is the halflife of the slow phase. Fraction (fast) is the fraction of the delayed luminescence which decays by the fast phase.

	pH	A_{ext}	A_{10}	$t_{1/2}$(fast) (μs)	$t_{1/2}$(slow) (μs)	Fraction (fast)
Untreated	4.5	1975±772	1642±600	18.3±1.6	89.0± 7.5	0.37±0.03
	5.2	1606±319	1254±215	17.0±4.9	73.2± 7.0	0.53±0.07
	6.0	817±258	514±118	8.9±1.7	48.8± 1.0	0.56±0.02
	7.0	1298±221	673	7.1±1.7	38.6± 4.7	0.65±0.08
	8.0	1181±240	657± 95	4.9±0.8	52.0±22.0	0.77±0.07
Tris-EDTA-washed	4.5	1467±525	1167±300	17.9±0.6	79.0± 0.8	0.49±0.08
	5.2	1604±276	1239±137	14.9±1.6	76.0±12.1	0.58±0.08
	6.0	1247±203	902±137	12.6±1.9	66.0±12.9	0.61±0.08
	7.0	1793±150	1000	7.6±1.1	45.8± 3.9	0.64±0.04
	8.0	1678±624	735±107	5.8±1.3	41.6± 8.7	0.73±0.05

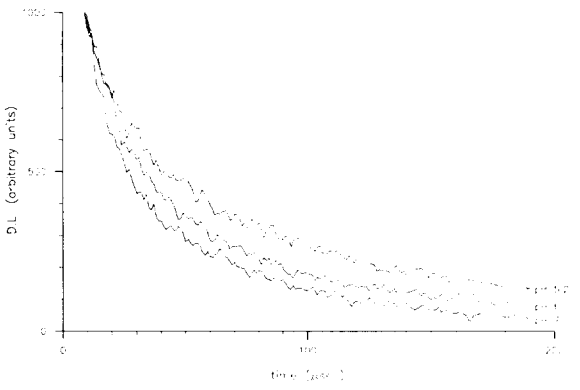


Fig. 2. Experimental delayed luminescence (D.L.) traces from 7–200 μs normalized to the initial observed delayed luminescence intensity for dark-adapted Tris-EDTA-washed chloroplasts (5 μg/ml) at the indicated pH. The reaction medium consisted of either 10 mM Mes (pH 5.2, 6.0) or 10 mM Hepes (pH 7.0). Following a single saturating laser flash, a fresh sample was introduced into the cell. Each trace is the average of ten measurements.

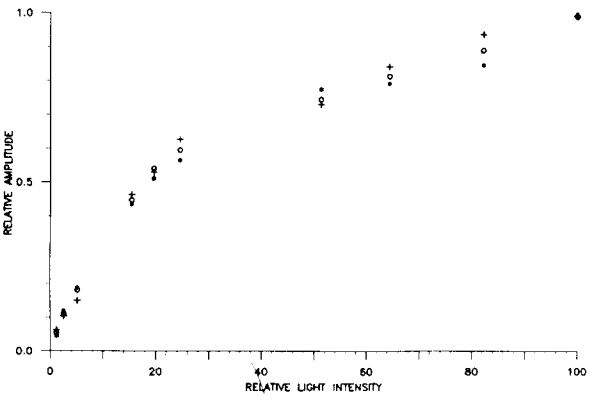


Fig. 3. Delayed luminescence light saturation behavior in dark-adapted, Tris-EDTA-washed chloroplasts (pH 7.0). Following a single laser flash, a fresh sample was introduced into the cell. ●, Total relative extrapolated delayed luminescence intensity; +, relative intensity of the 7 μs phase; *, relative intensity of the 50 μs phase.

that the fast phase of delayed luminescence under these conditions is a measure of reactions associated with P^+-680 reduction, as the kinetics of Q^- oxidation are over an order of magnitude slower [20]. That Q^- does not significantly affect the fast phase of delayed luminescence decay is further substantiated by the identical pH-dependent behavior of the fast phase of delayed luminescence observed for Tris-EDTA, DCMU-inhibited chloroplasts (data not shown) [17].

A significant difference between the kinetics of delayed luminescence and P^+-680 decay as reported by Conjeaud and Mathis [4] is the absence of a pH-independent 100–200 μs phase. The slower phase of delayed luminescence was found to be pH dependent and much faster (Table I). The relevance of this slower phase of delayed luminescence to normal PS II photochemical events is indicated by the delayed luminescence light saturation properties. Fig. 3 shows the extrapolated intensity obtained from a kinetic analysis for the total delayed luminescence, the amplitude of the fast phase, and the amplitude of the slow phase, as a function of light intensity. The identical light-saturation behavior indicates a correlation between the two observed phases of delayed luminescence and rules out heterogeneity in the primary stable acceptor (Q_α, Q_β, see Ref. 21) as an explanation for the biphasic delayed luminescence behavior. Delayed luminescence measured from chloroplasts suspended in a reaction medium consisting of 0.4 M sucrose, 10 mM NaCl, and 50 mM buffer displayed similar pH-dependent behavior (data not shown) [17].

Effect of ionic strength

The electron-transfer reactions of PS II are associated with the thylakoid membrane and thus may be affected by the membrane surface charge density and the corresponding membrane potential (e.g., Ref. 22). Since the membrane potential arises from differences in the surface charge density of the inside and outside surfaces and because the surface groups which produce this surface density are susceptible to protonation/deprotonation reactions [23], the resulting membrane potential should be pH dependent. Although in the 4.5–8 pH range there does not exist a systematic study of the membrane potential, its effects on P^+-680 reduction can be studied by monitoring the fast phase of delayed luminescence at various ionic strengths. The addition of salt should serve to neutralize the surface charge; the ionophore, gramicidin, should facilitate this effect by dissipating light-induced membrane potential gradients. The normalized results for KCl and $CaCl_2$ at pH

5.2 and 7.0 and the effect of gramicidin on delayed luminescence decay (Fig. 4) show that the decay of microsecond delayed luminescence is independent of ionic strength in the presence or absence of gramicidin for both mono- and divalent cations. These results indicate that the primary reactions of PS II are insensitive to the surface charge of the membrane and particularly that the pH-dependent fast phase of P^+-680 reduction as monitored by delayed luminescence does not depend upon pH-dependent membrane potential phenomena. These results are consistent with those of Jursinic et al. [39] who noted no effect of gramicidin on the kinetics of microsecond delayed luminescence at pH 7.8.

The amplitude of delayed luminescence, however was extremely sensitive to salt and gramicidin effects. This was particularly true for divalent cations. A 1 M Ca^{2+} concentration essentially quenched delayed luminescence completely (data not shown). Alternatively, identical concentrations of monovalent cations (K^+) caused changes of less than 10% in the delayed luminescence yield. In all cases, gramicidin resulted in a 25–40% quenching of delayed luminescence compared to samples which, other than the addition of gramicidin, were treated in an identical manner. Wong et al. [40] have also reported mono- and divalent cation effects on the intensity but not the decay time of microsecond delayed luminescence. These experiments emphasize that under certain conditions, the overall luminescence yield can be changed without causing a significant alteration in the kinetics of PS II electron-transfer reactions [11,40].

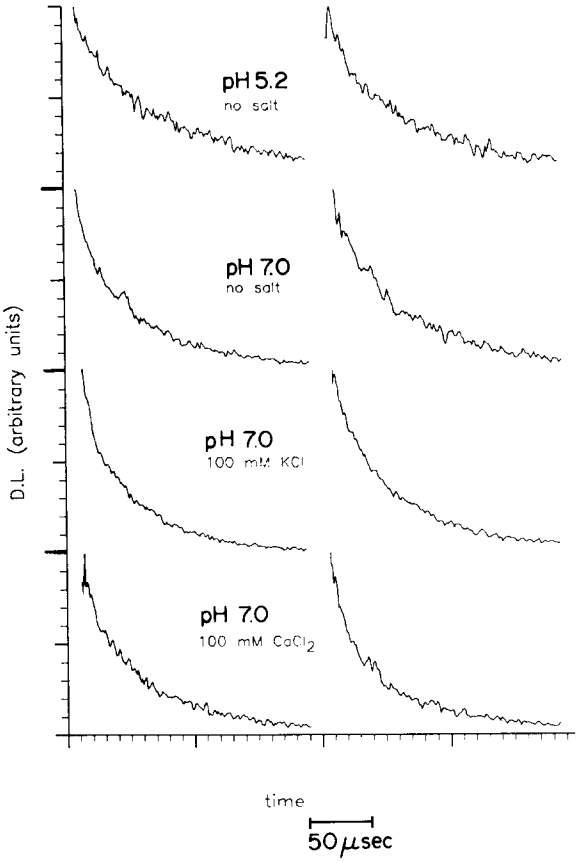


Fig. 4. Experimental delayed luminescence (D.L.) traces from 7–200 μs normalized to the initial observed delayed luminescence intensity for dark-adapted Tris-EDTA-washed chloroplasts (5 μg/ml) in the presence (right-hand side) or absence (left-hand side) of gramicidin at the indicated pH and salt concentration. Following a single saturating laser flash, a fresh sample was introduced into the cell. The initial delayed luminescence intensity in the presence of gramicidin was 50–75% of the control. Each trace is the average of ten measurements.

Delayed luminescence from dark-adapted, untreated chloroplasts

The delayed luminescence in the 7–200 μs range from untreated chloroplasts followed a biphasic pH-dependent decay similar to that observed for Tris-washed chloroplasts. The results for untreated chloroplasts are summarized in Table I and nor-

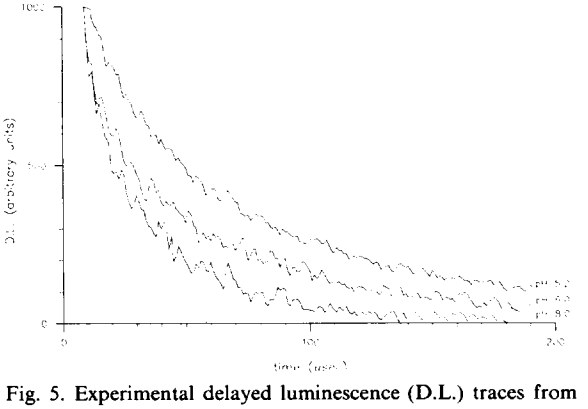


Fig. 5. Experimental delayed luminescence (D.L.) traces from 7–200 μs normalized to the initial delayed luminescence intensity for dark-adapted untreated chloroplasts (5 μg/ml) at the indicated pH. The reaction media consisted of either 10 mM Mes (pH 5.2, 6.0) or 10 mM Hepes (pH 8.0). Following a single saturating laser flash, a fresh sample was introduced into the cell. Each trace is the average of ten measurements.

malized data are shown in Fig. 5. Delayed light emission in this time range has been studied by Bowes and Crofts [9] and by Jursinic and Govindjee [8] who also noted a biphasic decay. These studies were not, however, as extensive as those summarized in Table I, and the similar pH dependence of both the fast (approx. 10 μs) and slow (approx. 40 μs) phases in untreated and inhibited chloroplasts was not noted.

The close correspondence observed between delayed luminescence phases and decay times in Tris-washed and untreated samples is anomalous, given the recombination hypothesis and the well-documented alterations in P^+-680 reduction times induced by Tris inhibition. Thus, the Tris data show that the 10 μs delayed luminescence phase and its pH dependence in Tris-washed chloroplasts are in good agreement with optical studies on P^+-680 decay times [4]. P^+-680 reduction, however, in untreated, dark-adapted chloroplasts proceeds with a submicrosecond halflife [24,25], although slower phases are consistently observed. This point is considered in more detail below.

Delayed luminescence in response to flash sequence in untreated and Tris-washed chloroplasts

In untreated chloroplasts near neutral pH, delayed luminescence follows the same four-flash oscillatory behavior as that observed for O_2 evolution [9,10,26]. This behavior is summarized in Fig. 6 where we show typical four-flash delayed luminescence traces for dark-adapted chloroplasts at several different pH values. Flash-induced oscillations in delayed luminescence amplitude were observed at pH 7.0 for flash trains of up to seven flashes [17]. Table II summarizes amplitude and decay time data for several identical experiments on different sample preparations. The variance in relative delayed luminescence intensity from one batch of chloroplasts to another is indicated by the magnitude of the standard deviations. Much higher precision was obtained for the kinetic phases. These experiments show that the amplitude of delayed luminescence at a given pH oscillates with flash number and that the extent of the oscillation pattern varies with preparations. Despite the intensity variations, the decay halftimes for both fast and slow phases are relatively constant with flash number.

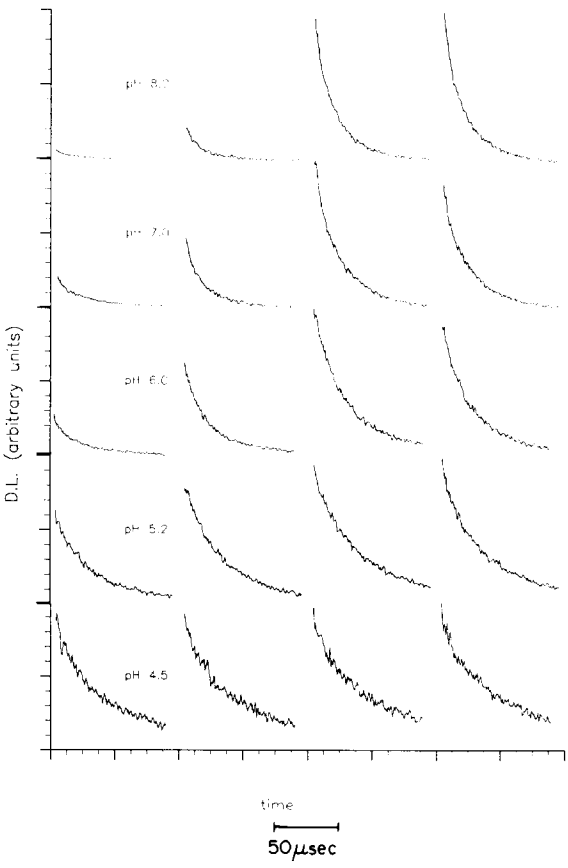


Fig. 6. Delayed luminescence (D.L.) curves induced by each of four actinic flashes in dark-adapted, untreated chloroplasts (5 μg/ml) at the indicated pH and normalized to the maximal observed intensity in a given flash train. The illumination rate corresponded to 1 flash/s. The reaction media consisted of either 10 mM succinate (pH 4.5), 10 mM Mes (pH 5.2, 6.0), or 10 mM Hepes (pH 7.0, 8.0). Following the fourth flash, a fresh sample was introduced into the cell. For pH 7.0 and 6.0, the maximum delayed luminescence intensity was induced by flash 3. At pH 4.5, 5.2, and 8.0, the delayed luminescence intensity was maximal on the fourth flash. Each trace is the average of ten measurements.

The existence of the flash oscillations has been attributed to the well-documented flash-induced changes in the rate of electron transfer on the oxidizing side of P-680 in intact, oxygen-evolving centers. Alterations in delayed luminescence kinetics as well as amplitude are predicted by such a model and this prediction is contradicted by both the results here and those obtained by Bowes and Crofts [9]. The observed oscillations in yield only

TABLE II

DELAYED LUMINESCENCE KINETIC BEHAVIOR OF UNTREATED CHLOROPLASTS

The uncertainties represent standard deviations. The values listed in the columns A_{ext} and A_{10} were normalized to the delayed luminescence intensity observed 10 μs after the third flash at pH 7.0. The flash repetition rate was 1 flash/s. For explanations of A_{ext}, A_{10}, $t_{1/2}$ (fast and slow) and fraction (fast), see legend to Table I.

pH	Flash	A_{ext}	A_{10}	$t_{1/2}$(fast) (μs)	$t_{1/2}$(slow) (μs)	Fraction (fast)
4.5	1	538 ± 15	448 –	18.3 ± 1.36	89.0 ± 7.5	0.37 –
	2	571 –	474	15.0 –	91.0 —	0.40 –
	3	587 –	497 –	13.5 –	98.0 –	0.37 –
	4	655 –	510 –	9.0 –	70.1 –	0.37 –
5.2	1	587 ± 33	459 ± 14	17.0 ± 4.9	73.2 ± 7.0	0.53 ± 0.07
	2	771 ± 91	615 ± 70	22.3 ± 0.7	87.5 ± 24.3	0.53 ± 0.1
	3	906 ± 94	772 ± 47	19.9 –	86.8 ± 1.8	0.42 ± 0.01
	4	952 ± 117	794 ± 78	15.0 ± 0.05	80.0 ± 5.0	0.44 ± 0.01
6.0	1	335 ± 29	211 –	8.9 ± 1.7	48.8 ± 1.0	0.56 ± 0.02
	2	718 –	518 –	17.8 –	84.1 –	0.80 –
	3	1088 –	875 –	24.9 –	95.1 –	0.72 –
	4	1028 –	775 –	19.8 –	68.0 –	0.62 –
7.0	1	441 ± 77	229 ± 1	7.1 ± 1.7	38.6 ± 4.7	0.65 ± 0.08
	2	781 ± 209	444 ± 82	9.2 ± 0.2	50.0 ± 7.1	0.67 ± 0.07
	3	1476 ± 155	1000	12.2 ± 3.2	44.0 ± 5.35	0.55 ± 0.06
	4	1402 ± 150	871 ± 18	8.3 ± 2.45	39.0 ± 3.45	0.48 ± 0.03
8.0	1	394 ± 240	148 –	4.9 ± 0.8	52.0 ± 22.0	0.77 ± 0.07
	2	594 –	259 –	5.7 –	24.6 –	0.64 –
	3	1813 ± 816	1039 ± 186	9.6 ± 5.0	30.5 ± 7.0	0.55 ± 0.10
	4	1980 ± 813	1163 ± 466	6.8 ± 0.7	28.0 ± 1.0	0.44 ± 0.02

suggest an S state dependence in the photochemical parameters which control delayed luminescence yield (see below). This dependence may involve membrane electrical phenomena similar to those noted for Tris-washed chloroplasts in the presence of salts or gramicidin. For example, it is now generally accepted that proton release associated with water oxidation occurs sequentially as oxidative equivalents are stored, with a stoichiometry of 1 : 0 : 1 : 2 for the transitions $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_2 \rightarrow S_4$, respectively [27,37]. For dark-adapted chloroplasts with 25% of the centers in the S_0 state and 75% in the S_1 state, there should be a substantial charge stored on the oxidizing side of PS II just prior to the second flash and maximal charge stored prior to the third flash. Thus, the oscillations in delayed light emission tracks with the proton release/charge accumulation pattern. This observation, coupled with the lack of oscillation in delayed luminescence decay halftimes with flash number and the similarity of these halftimes to those observed in Tris-washed chloroplasts, suggests that delayed luminescence behavior is negligibly low in functional centers within 1 μs of flash excitation. Only centers in which P^+-680 reduction occurs in the 2–20 μs time range contribute to the observed delayed luminescence. To account for the oscillation in delayed luminescence amplitude, but not kinetics, we postulate a modulation of photochemical yield in these centers by charge accumulation at the oxygen-evolving complex.

The addition of gramicidin to untreated samples did not affect the kinetics or relative intensity of delayed luminescence with flash number; the absolute intensity of delayed luminescence in the presence of gramicidin was about 85% of the control. This eliminates the possibility that changes in the bulk transmembrane potential are the cause for the flash-induced oscillations of delayed luminescence (see also Ref. 39). Local fields, how-

ever, such as those caused by the storage of charge, may not be affected by gramicidin. Support for this suggestion is provided by the heat inhibition experiments of Fig. 7. The delayed luminescence decay traces in Fig. 7A were obtained from samples of chloroplasts which were inhibited by a mild heating (50°C) procedure. Fig. 7B shows the effect of increasing exposure time in a 50°C water-bath on rates of oxygen evolution and the initial intensity of delayed luminescence induced by the first and third actinic light flash normalized to the sum of the initial delayed luminescence yield induced by the first four flashes. It is apparent that, although the heating procedure had an immediate effect on oxygen yields, an induction time occurs before any effect is observed on delayed luminescence and its period 4 oscillations. These results indicate that the relationship between O_2 and delayed luminescence oscillatory behavior is not strictly linear; it is possible to inhibit up to 35% of the oxygen yield without significantly affecting the

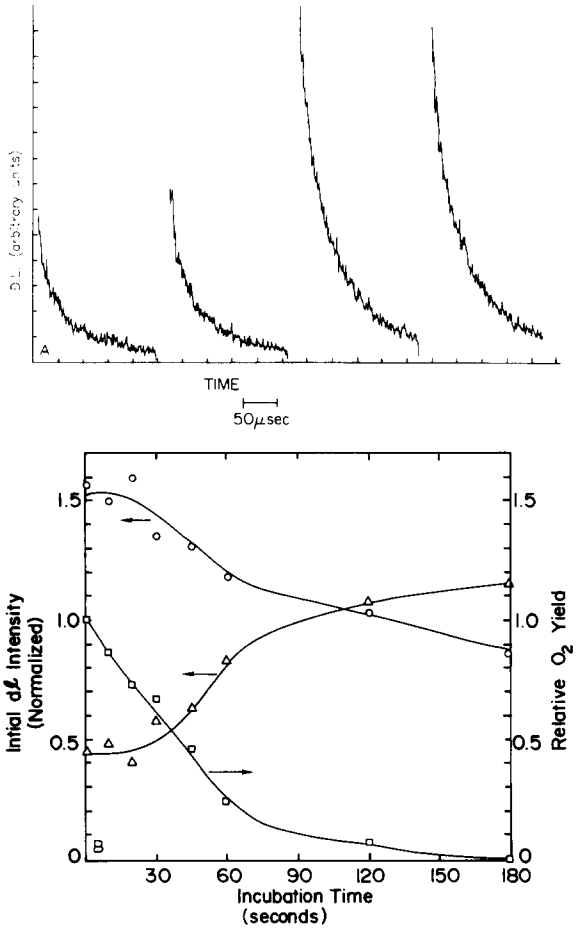


Fig. 7. (A) Delayed luminescence (D.L.) following each of four actinic flashes in untreated chloroplasts (5 μg/ml) exposed to a 50°C water-bath for 30 s which resulted in a 35% inhibition of the rate of steady-state oxygen evolution. The illumination rate corresponded to 1 flash/s. The reaction medium was 10 mM Hepes (pH 7.0). Following the fourth flash, a fresh sample was introduced into the cell. Each trace is the average of ten measurements. (B) The effect of increasing exposure time to mild heat (50°C) on delayed luminescence behavior and steady-state oxygen evolution (□). The effect of heating on delayed luminescence is plotted as the initial delayed luminescence intensity induced by flash 1 (△) and 3 (○) normalized to the sum of the initial delayed luminescence intensity induced by flash 1 through 4. The control rate of oxygen evolution was 225 μmol O_2/mg Chl per h.

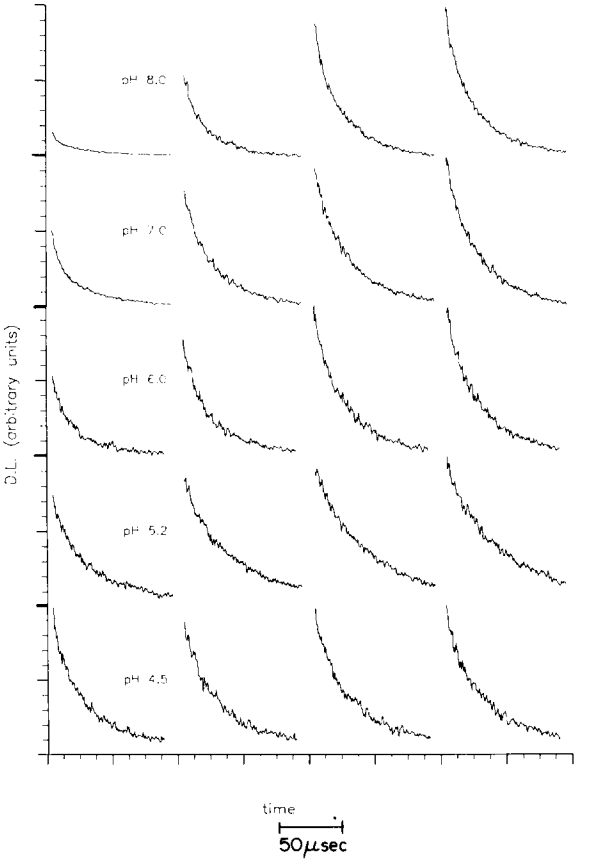


Fig. 8. Delayed luminescence (D.L.) following each of four actinic flashes in dark-adapted Tris-EDTA-washed chloroplasts (5 μg Chl/ml) at the indicated pH and normalized to the intensity induced by flash number 4. All other conditions were as described in Fig. 6.

delayed luminescence oscillations. Because delayed luminescence is expected from mild heat-inhibed, nonfunctional centers, these results suggest that the delayed luminescence emitted from these photosystems is modulated by charge accumulation in the remaining functional centers.

As reported by Bowes and Crofts [9], the flash oscillatory behavior for delayed luminescence is observed around pH 7.0 and is quenched at pH less than 6.0 and pH greater than 8.0. The delayed luminescence flash number dependence for untreated chloroplasts is shown in Fig. 9 and is summarized in Table II. A similar summary for the flash number dependence of delayed luminescence in Tris-washed samples is shown in Fig. 8 and in Table III. In order to facilitate a comparison of delayed luminescence intensities, the results summarized in Table III are normalized to the delayed luminescence intensity observed 10 μs after the third actinic flash in untreated chloroplasts. A summary of the amplitudes for flash 1 through 4 in untreated and Tris-washed chloroplasts is shown in Fig. 9. Most notable in the Tris-washed samples

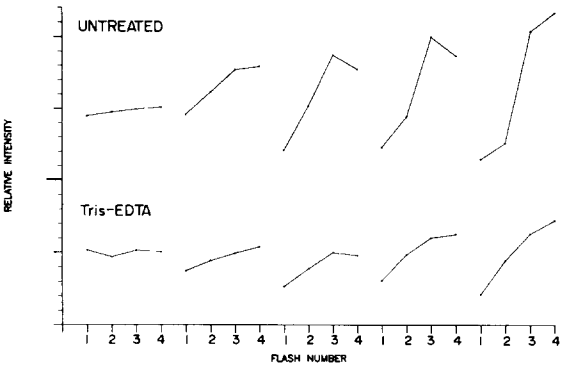


Fig. 9. Summary of the initial delayed luminescence intensity for untreated and Tris-EDTA-washed chloroplasts following each of four actinic flashes at pH 4.5, 5.2, 6.0, 7.0 and 8.0. The plots are normalized to the delayed intensity observed approx. 10 μs after the flash 3 at pH 7.0 in untreated chloroplasts.

TABLE III

DELAYED LUMINESCENCE KINETIC BEHAVIOR OF Tris-EDTA-WASHED CHLOROPLASTS

The uncertainties represent standard deviations. The values listed in the columns A_{ext} and A_{10} were normalized to the delayed luminescence intensity observed in untreated chloroplasts 10 μs after the third flash at pH 7.0. The flash repetition rate was 1 flash per s. For explanations of A_{ext}, A_{10}, $t_{1/2}$ (fast and slow) and fraction (fast), see legend to Table I.

pH	Flash	A_{ext}	A_{10}	$t_{1/2}$(fast) (μs)	$t_{1/2}$(slow) (μs)	Fraction (fast)
4.5	1	648 ± 63	516 –	17.9 ± 0.6	79.0 ± 0.82	0.49 ± 0.08
	2	491 –	469 –	32.2 –	95.2 –	0.73 –
	3	617 –	514 –	18.0 –	79.1 –	0.53 –
	4	620 –	506 –	15.9 –	71.9 –	0.45 –
5.2	1	483 ± 84	373 ± 42	14.9 ± 1.6	76.0 ± 12.1	0.58 ± 0.08
	2	545 ± 79	444 ± 59	16.4 –	75.8 –	0.35 –
	3	609 ± 147	494 ± 111	16.8 –	73.4 –	0.22 –
	4	636 ± 162	540 ± 106	12.7 –	74.0 –	0.26 –
6.0	1	368 ± 4	267 –	12.6 ± 1.92	66.0 ± 12.9	0.61 ± 0.08
	2	508 –	389 –	11.4 –	40.4 –	0.65 –
	3	754 –	500 –	6.7 –	39.8 –	0.38 –
	4	708 –	471 –	9.3 –	46.1 –	0.37 –
7.0	1	544 ± 117	307 ± 35	7.6 ± 1.1	45.8 ± 3.9	0.64 ± 0.04
	2	725 ± 71	486 ± 17	8.6 ± 3.5	45.4 ± 7.3	0.50 ± 0.09
	3	872 ± 120	602 ±	6.4 ± 0.21	45.6 ± 6.9	0.49 ± 0.01
	4	1011 ± 149	628 ± 30	8.9 ± 2.9	41.6 ± 1.2	0.40 ± 0.06
8.0	1	484 ± 314	212 ± 90	5.8 ± 1.3	41.6 ± 8.7	0.73 ± 0.05
	2	712 ± 79	445 ± 23	7.2 ± 0.55	33.2 ± 1.5	0.55 ± 0.03
	3	1077 ± 394	631 ± 101	7.9 ± 1.2	38.0 ± 1.1	0.50 ± 0.01
	4	1128 ± 380	723 ± 106	9.9 ± 1.8	44.2 ± 2.1	0.53 ± 0.01

is the absence of flash oscillations at any pH and although the intensity does increase with flash number, it becomes invariant after the third turnover.

Several points about Tables II and III and Figs. 6, 8 and 9 are apparent. The kinetic phases which show oscillatory behavior in untreated chloroplasts are present in Tris-washed chloroplasts. Additionally, even though electron transport is significantly perturbed near the reaction center in Tris-washed chloroplasts, the intensity of delayed luminescence differs by less than a factor of 2 between untreated and Tris-washed samples which are otherwise treated in an identical manner. Moreover, under certain conditions, such as delayed luminescence induced by the third actinic flash (pH 7.0), the intensity of delayed luminescence emanating from untreated chloroplasts is greater than that observed for Tris-washed chloroplasts. Below we present a model which can account for several of these observations.

Discussion

Although delayed luminescence is clearly a PS II phenomenon [10,11], it is an indirect probe of PS II that may monitor PS II events which occur in only a minority of centers. Nonetheless, the nearly identical pH-dependent kinetics for the fast phase of delayed luminescence and the reduction of P^+-680 in Tris-washed chloroplasts as determined optically [5] verifies that the fast phase of delayed luminescence arises primarily from P^+-680 reduction. Moreover, based upon the complementary measurements of P^+-680 decay and Signal II_f rise as monitored by ESR [12], this phase of delayed luminescence reflects P^+-680 reduction by Z. This observation substantiates the recombination hypothesis of the origin of delayed luminescence [10].

The slower, pH-dependent 35 μs phase of delayed luminescence decay in Tris-washed chloroplasts is more difficult to interpret. Attempts to model this phase by postulating a first-order forward reduction of P^+-680 by Z ($t_{1/2} \approx 7$ μs), a first-order back reaction between P^+-680 and Q^- ($t_{1/2} \approx 120$ μs) and a first-order back reaction between Z^+ and P-680 ($t_{1/2}$ variable) were not successful [17]. An alternate explanation for this phase attributes it to slower P^+-680 reduction in a minority of centers. Support for this suggestion exists as there are numerous reports in the literature of the detection of a 35–50 μs phase for the reduction of P^+-680 by several techniques (e.g., Refs. 9, 28 and 29). It appears, therefore, that the decay of P^+-680 is heterogeneous in these chloroplast preparations and that it consists of a major decay in the 0–10 μs range which is pH dependent and subsequent slower phases in the 20–100 μs range which are also pH dependent. This biphasic reduction may arise from a structural heterogeneity in PS II. In optical studies involving *Chlamydomonas* PS II particles, P^+-680 was observed to decay with multiphasic kinetics [30]. Subsequent EPR investigations have shown that in these particles only a fraction of P^+-680 is reduced by Z. This fraction was approximately equal to the relative contribution of the 10 μs phase to the total P^+-680 decay [31]. Apparently, in a fraction of PS II centers, electron transport between P-680 and Z is inactivated; the 35–50 μs phase of delayed luminescence might reflect P^+-680 reduction in these centers. This is supported by Eckert and Renger [28] who demonstrated that the oxidative equivalents generated by the fraction of P^+-680 which is reduced with a 35 μs halflife are not available for water oxidation. The fact that a heterogeneity exists should not be surprising. It is likely that within the leaf, there exists a distribution of PS II centers at various stages of development. The procedure to isolate chloroplasts from whole leaves is nondiscriminatory and PS II centers which do not have fully functional electron-transport chains would be obtained along with those that are fully functional. Therefore, the heterogeneity may arise from either immature or aged centers, as well as from centers which are damaged during isolation. The identical light saturation behavior in dark-adapted Tris-washed chloroplasts of the 7 and 50 μs phase (see Fig. 3) indicates that the antenna system is fully developed for both populations. This does not argue against heterogeneity, since physiological studies have demonstrated that fully competent PS II is developed in the final stage of chloroplast development [32].

Although the decay behavior of delayed luminescence in the microsecond time range in non-oxygen-evolving preparations can be reason-

ably explained, the existence of the 7–10 μs phase in oxygen-evolving chloroplasts which oscillates with a period 4 is perplexing. The currently accepted model for PS II electron transport in intact centers does not accommodate a 10 μs phase (e.g., see Refs. 1 and 37). There are, however, several reports in the literature describing the existence of a 10 μs phase for P^+-680 reduction in O_2-evolving samples. For example, Mathis and co-workers [19] reported that in untreated chloroplasts, the contribution of the 10 μs phase of the absorption change at 820 nm associated with oxidized P-680 represents about 20 to 25% of the total P-680 pool, with the majority of centers reduced by submicrosecond kinetics. Similarly, Van Best and Mathis [24] reported that P^+-680 decayed primarily by submicrosecond kinetics, but that slower phases, which represented a small fraction of the total P-680 pool, were also present. Brettel et al. [25] have recently reported similar phenomena in their optical studies of P^+-680 reduction in O_2-evolving cyanobacterial samples. Given these observations and our results above which show that the fast phase of delayed luminescence decay in untreated and Tris-washed samples have very similar pH dependencies, it appears that the 10 μs phase of delayed luminescence in untreated chloroplasts and the slower P^+-680 decay observed optically may arise from a minority of centers with inactive electron transport from the oxygen-evolving complex.

The intensity of delayed luminescence in the 7–200 μs time range from Tris-washed and untreated samples differs only slightly. To account for the similarity in delayed luminescence yield in intact and Tris-washed chloroplasts, within the context of the model introduced above, it becomes apparent that the overall luminescence yield is greater in intact samples than in Tris-washed chloroplasts. This difference in delayed luminescence yield may be quite significant, since the 10 μs phase of delayed luminescence in oxygen-evolving samples probably originates from inactive centers, as the majority of P^+-680 is a minority of reduced in less than 1 μs. Part of the apparent discrepancy of delayed luminescence intensity may be attributed to the exciton-quenching effect by P^+-680 [33]. Lavorel [10] described the origin of delayed luminescence as occurring in a sequential two-step process consisting of the chemical generation of an exciton followed by its radiative relaxation. The first step is controlled by the exciton yield (η) which represents the fraction of precursor (P^+-680Q^-) back reactions which result in the formation of an exciton. The second step is the emission yield (ϕ_L) which refers to the probability that a chemically generated exciton will relax radiatively. The observed delayed luminescence (d.l.) intensity would therefore be proportional to the product of the exciton yield, the emission yield and the net rate of recombination:

$$\text{d.l.} = \eta \phi_L k_b [P^+\text{-}680Q^-] \tag{1}$$

The chemical generation of an exciton by P^+-680Q^- back reaction results in a depletion of P^+-680. Thus, if an exciton remains localized around the reaction center of its origin, the quenching effect of P^+-680 on delayed luminescence would not be significant. Fluorescence studies have indicated, however, that excitation energy absorbed by the antenna system of one reaction center may induce photochemistry in a neighboring reaction center [34]. Thus, excitons tend to delocalize throughout the antenna system. Butler [35] derived an expression for the fluorescence yield for the connected packet model of PS II, and through a comparison with experimental results, obtained estimates for the various parameters. Once delocalized, a chemically generated exciton would be indistinguishable from a photo-generated exciton. Modifying Butler's expression to incorporate the chemical generation, as opposed to the photogeneration, of an exciton and the quenching behavior of P^+-680, the delayed luminescence (d.l.) yield would be:

$$\text{d.l.} = \frac{\psi_{FII}(1-\psi_{TII}\psi_{tII}A)}{1-\psi_{T(22)}-\psi_{TII}\psi_{tII}+\psi_{T(22)}\psi_{TII}\psi_{tII}A}\eta k_b [P^+\text{-}680Q^-] \tag{2}$$

in which the emission yield (ϕ_L) is assumed to behave similarly to the fluorescence yield. in the above expression, $\psi_{T(22)}$ is the connection parameter between PS II units, $\psi_{TII}\psi_{tII}$ is the product of the probability of the exciton being localized at the

reaction center and subsequent relocalization throughout the antenna system, and ψ_{FII} is the intrinsic fluorescence yields [35]. Originally A represented the fraction of open centers, or more specifically, the fraction of centers which will quench fluorescence. In the simulation described below and shown in Fig. 10, the exciton-quenching effect of P^+-680 is incorporated by allowing A to consist of the sum of the fraction of centers with Q oxidized and the centers with P-680 oxidized. It is assumed that P^+-680 is as efficient an exciton quencher as Q^- [36]. For Fig. 10, values for $\psi_{T(22)}$ and $\psi_{TII}\psi_{tII}$ were from Butler [35]. [P^+-680Q^-], [P^+-680], and [Q^-] were obtained from analytic expressions which were derived to describe submillisecond electron transport in Tris-washed or similarly inhibited chloroplasts [17]. A description of the derivation is presented in the Appendix. For the simulation depicted in Fig. 10, the rate constants were obtained from literature values [1,4,20], or, if not available, were estimated from the approximate potential difference between neighboring carriers. Initially, all of the centers were considered to be in the [P^+-680Q^-] state, a condition which would correspond to fully dark-adapted samples illuminated by a single saturating laser flash. Moreover, in the simulation, it was assumed that the emission yield and the prompt fluorescence yield behaved in a similar manner and all time dependencies of the prompt fluorescence were due to kinetic parameters (i.e., the instantaneous concentration of P^+-680Q^-). The exciton yield was considered to be constant and all time dependencies of the luminescence yield (i.e., the fraction of P^+-680Q^- states which back react radiatively) were attributed to variations in the emission yield induced by the depletion of quenchers. Additionally, it was assumed that for untreated chloroplasts, P^+-680 in active centers was reduced before delayed luminescence could be monitored.

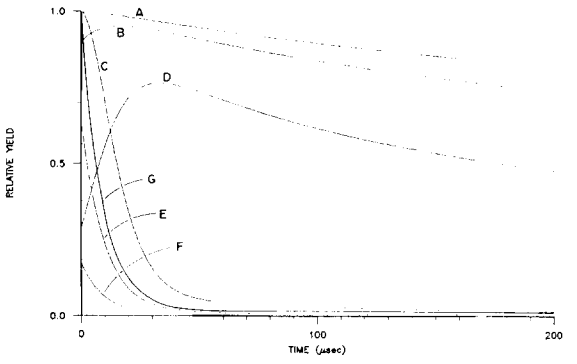


Fig. 10. Delayed luminescence and emission yield behavior as predicted by Eqn. 2 for Tris-washed chloroplasts and untreated chloroplasts with a fraction (0.05 and 0.20) of damaged centers. [P^+-680Q^-], [P^+-680], and [Q^-] were calculated as described in the text. In the simulation, k_1, k_2, k_b and k_{-1}, as defined by Eqn. A-2, were assigned values corresponding to halflives of 7, 200, 120 and 320 μs, respectively [1,4,20]. $\psi_{T(22)}$ and $\psi_{TII}\psi_{tII}$ were assigned values of 0.51 and 0.31, respectively [35]. Details given in text. The calculated emission yield and delayed luminescence profiles are plotted relative to that which is predicted by 100% inhibited centers. (A) Relative emission yield (ϕ_L) for untreated chloroplasts (5% damaged). (B) Relative emission yield (ϕ_L) for untreated chloroplasts (20% damaged). (c) Relative delayed luminescence intensity for Tris-washed chloroplasts. (D) Relative emission yield (ϕ_L) for Tris-washed chloroplasts. (E) Relative delayed luminescence intensity for untreated chloroplasts (20% damaged). (F) Relative delayed luminescence intensity for untreated chloroplasts (5% damaged). (G) Decay of P^+-680Q^- as predicted by the electron-transfer model described in the text.

The quenching effect of P^+-680 is most pronounced in Tris-washed chloroplasts in which the majority of the reaction centers are reduced with 10 μs kinetics. In untreated chloroplasts, P^+-680 is reduced mainly by submicrosecond kinetics, and the 10 μs phase was assumed to occur primarily in damaged centers. Fig. 10 depicts the relative emission yields (ϕ_L) and delayed luminescence intensity for Tris-washed chloroplasts and for untreated chloroplasts assumed to have 5 or 20% damaged centers. With only 20% of the PS II centers inhibited, the predicted initial delayed luminescence intensity is over 60% of that observed for Tris-washed chloroplasts with 100% inhibition. Thus, in this model, P^+-680 serves not only as an essential precursor to delayed luminescence, but it is also a significant quencher of delayed luminescence. It is apparent from Fig. 10 that the explicit consideration of the quenching effect of P^+-680 may account for the similar delayed luminescence intensity in untreated and Tris-washed samples. These model studies directly address the cause for the relative low yield of delayed luminescence under conditions in which the back reaction should be more facile, as in Tris-washed chloroplasts, relative to that of intact samples. Additionally, it represents an explicit consideration of the role of P^+-680 as a quencher of delayed luminescence. More im-

portantly, this model provides an explanation for the anomalous 10 μs phase of delayed luminescence in 'intact chloroplasts' and thus rationalizes delayed luminescence in terms of well-characterized electron-transfer components. The alternative to this is to introduce additional, ad hoc electron-transfer components in PS II. This treatment allows us to avoid the introduction of these additional carriers, while retaining a reasonable explanation for microsecond delayed luminescence. These studies also demonstrate that the large variance in the relative amplitude of delayed luminescence, which is observed from one preparation of chloroplasts to the next, may arise from a difference in the fraction of damaged centers.

A more subtle manifestation of this model for delayed luminescence yield is the slight difference that it predicts for the decay of delayed luminescence and the electron-transfer profiles. Although the pH dependence of delayed luminescence is nearly identical to the pH dependence of P^+-680 reduction as determined optically [4] or by ESR [12], a closer comparison of the previously reported halflives to the halflives reported here for delayed luminescence indicate that the delayed luminescence decay is slightly slower. For example, at pH 7.0, P^+-680 reduction occurs with a halflife of about 5 μs as monitored optically [19], whereas the fast phase of delayed luminescence has a halflife of 7.6 μs. Such differences are predicted by the model described above and demonstrated in Fig. 10 where the decay of P^+-680Q^- initially proceeds at a faster rate than the product $\eta\phi k_b[P^+\text{-}680Q^-]$. In the simulation, the halflife of P^+-680 was set at 7 μs (corresponding to approx. pH 6), whereas the simulated delayed luminescence curve had an apparent fast phase of approximately 9.5 μs. This retardation arises because the emission yield is time dependent and will increase as the quencher concentration, [P^+-680], decreases. Hence, as the total number of precursor states decrease, a greater fraction will relax radiatively. Thus, a secondary effect of the role of P^+-680 as an exciton quencher would be to not only decrease the overall delayed luminescence yield, but to slow down the rate of decay in delayed luminescence intensity.

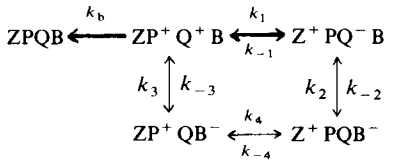
Fig. 4 demonstrates that factors which affect the membrane potential also affect the intensity but not necessarily the kinetics of delayed luminescence. The addition of a divalent cation (Ca^{2+}) or of gramicidin resulted in a quenching of delayed luminescence without appreciable effect on the kinetics. Thus, the increased delayed luminescence yield observed following multiple turnovers may not be due only to an increase in fractions of centers which back react because the stabilization pathway is blocked, but also to an electrostatic interaction associated with charge stored on the oxidizing and reducing side of P-680. This is supported by the observation that the flash-induced change in delayed luminescence yields parallel the storage of charge, as determined from the proton release pattern, on the oxidizing side of P-680 in intact, O_2-evolving chloroplasts.

In the electron-transfer model for PS II described above and in the Appendix, the natural lifetime that is observed for a given constituent is a convolution of all of the rate constants which make up the reaction scheme. In such a scheme, both the amplitude and the halftime of each phase of delayed luminescence decay are determined by these parameters; it is impossible to associate changes in delayed luminescence yield, without changes in kinetics, directly to electron transfer reactions. This analysis indicates, then, that the effect of ionic strength, ionophores and flash-induced electric fields on delayed luminescence is a reflection of alterations in either the exciton or emission yield. As indicated by the results listed in Tables II and III, however, delayed luminescence decay tends to slow down slightly as PS II undergoes multiple turnovers. Thus it appears that electrostatic interactions associated with local charge density will alter the activation energy, and hence the rate of reaction, for various electron transfers. Since the charge density is localized and, at least in the case of untreated chloroplasts, not necessarily associated with the PS II electron-transport chain which is undergoing the back reaction, the effect is small. In a subsequent report, we will demonstrate that a delocalized electric field of appropriate magnitude may significantly alter kinetic parameters.

Appendix

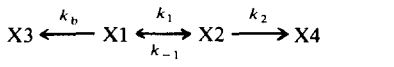
In chloroplasts which have electron transport inhibited between P-680 and the oxygen-evolving

complex, such as for Tris-washed chloroplasts, the electron-transport scheme in dark-adapted samples following a single turnover may be represented as:



(A-1)

P in the above scheme represents the P-680-pheophytin complex. If only submillisecond events are considered, the back reaction between the reduced form of the secondary quinone acceptor (B^-) and Q may be ignored, and for reasons of energetics, the P^+Q^- back reaction may be considered to be irreversible. With the added assumption that P^+ reduction precedes Q^- oxidation, the reaction scheme simplifies to:



(A-2)

In the above expression, X3 represents the fully relaxed state (ZPQB), X1, X2, and X4 represent the states ZP^+Q^-B, Z^+PQ^-B and Z^+PQB^-, respectively. The rate of change of these states is simply:

$$\frac{d[X1]}{dt} = -(k_1 + k_b)[X1] + k_{-1}[X2] \quad \text{(A-3a)}$$

$$\frac{d[X2]}{dt} = k_1[X1] - (k_{-1} + k_2)[X2] \quad \text{(A-3b)}$$

$$\frac{d[X3]}{dt} = k_b[X1] \quad \text{(A-3c)}$$

$$\frac{d[X4]}{dt} = k_2[X2] \quad \text{(A-3d)}$$

The differential Eqns. A-3a and A-3b are coupled and may be solved by standard methods for systems of linear, first-order differential equations to obtain explicit expressions for X1 and X2; these expressions may then be used in A-3c and A-3d to solve for X3 and X4. Invoking the boundary condition that at time = 0, all centers are in the P^+Q^- state, a condition which would correspond to fully dark-adapted samples illuminated by a single saturating light pulse, the solution for X1, X2, X3 and X4 is:

$$[X1] = A1_- \exp(L_- t) + A1_+ \exp(L_+ t) \quad \text{(A-4a)}$$

$$[X2] = A2_- \exp(L_- t) + A2_+ \exp(L_+ t) \quad \text{(A-4b)}$$

$$[X3] = A3_-[1 - \exp(L_- t)] + A3_+[1 - \exp(L_+ t)] \quad \text{(A-4c)}$$

$$[X4] = A4_-[1 - \exp(L_- t)] + A4_+[1 - \exp(L_+ t)] \quad \text{(A-4d)}$$

where:

$$L_\pm = \frac{-(k_1 + k_b + k_{-1} + k_2) \pm R}{2}$$

$$A1_\pm = \frac{\pm(k_{-1} + k_2) \mp (k_1 + k_b) + R}{2R}[X1]_0$$

$$A2_\pm = \frac{\pm k_1[X1]_0}{R}$$

$$A3_\pm = \frac{k_b\{R \pm (k_1 + k_b) \mp (k_{-1} + k_2)\}}{R(k_1 + k_b + k_{-1} + k_2 \mp R)}[X1]_0$$

$$A4_\pm = \frac{\pm 2k_1k_2[X1]_0}{R(k_1 + k_b + k_{-1} + k_2 \mp R)}$$

with:

$$R = \left[(k_1 + k_b - k_{-1} - k_2)^2 + 4k_1k_{-1}\right]^{1/2}$$

Thus, explicit expression for the various states (X1, X2, X3 and X4), hence the constituents, associated with electron transfer in Tris-inhibited chloroplasts are obtained. With slight modifications, the above expression may be used to describe electron transport in Tris-washed, DCMU-treated chloroplasts. In such chloroplasts, secondary electron transport on the reducing side of PS II is also inhibited; i.e., $k_2 = 0$ and the state X4 need not be considered. By utilizing these expressions, in conjunction with documented rate constants, the recombination hypothesis may be tested [17]. This simple electron-transfer model for PS II could describe the kinetic behavior of the fast phase of delayed luminescence. This model, however, could not account for the 35 μs phase, nor more importantly, the variable yield of delayed luminescence under various experimental conditions (e.g., ionic strength variations). A simple electron-transfer model for delayed luminescence

in untreated chloroplasts was found to be inadequate.

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