Photochemistry and Photobiology, Vol. 28, pp. 963 974

0031-8655/78/1201-0963$02.00/0

ANALYSIS OF MICROSECOND FLUORESCENCE YIELD AND DELAYED LIGHT EMISSION CHANGES AFTER A SINGLE FLASH IN PEA CHLOROPLASTS: EFFECTS OF MONO- AND DIVALENT CATIONS

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(*Received* 1 *October* 1977; *accepted* 22 *March* 1978)

Abstract—New results are presented on the effects of mono- and divalent cations on concurrent changes in the microsecond yields and kinetics of chlorophyll *a* fluorescence and delayed light emission, and the light saturation curve for the latter at 100 μs, following a 10 ns flash at 337 nm. (1) The fluorescence yield increases exponentially from 3 to 30 μs (lifetime, τ, 6.4 ± 0.6 μs), and decays biphasically between 50 and 800 μs. (2) The delayed light emission decays biphasically with two exponential phases: fast phase, τ = 7–10 μs, and slow phase, τ = 33–40 μs. (3) The light saturation curve for 100 μs delayed light emission is satisfactorily represented by a one-hit Poisson saturation curve. (4) Addition of 5 m*M* NaCl to salt-depleted chloroplasts decreases (by as much as 40%) the yields of μs fluorescence and delayed light emission, and the subsequent addition of 5 m*M* $MgCl_2$ increases the yields (≥ 2× over samples with only NaCl). (5) The fluorescence yield rise and delayed light emission decay kinetics are independent of low concentrations of cations. The lifetime of the fast phase of fluorescence decay changes from ~90 μs to ~160 μs, when Na^+ or $Na^+ + Mg^{2+}$ are added.

Based on a detailed analysis presented in this paper, the following conclusions regarding the effects of low concentrations (few m*M*) of mono- and divalent cations in sucrose-washed chloroplasts at room temperature are made: (a) Na^+ decreases (~6%) and Mg^{2+} increases (~ 20% compared with the Na^+ sample) the sensitization of photosystem II photochemistry; this effect is small, but significant. (b) Na^+ increases and Mg^{2+} decreases the efficiency for radiationless transitions in singlet excited Chl *a* in the antenna and closed reaction center of PS II; this includes non-radiative energy transfer to PS I, intramolecular intersystem crossing and internal conversion. The ratio of the sum of the rate constants for radiationless transitions to that for fluorescence increases by ~2-fold upon the addition of Na^+, and is completely reversed by the addition of Mg^{2+}. (c) The rate constant for the re-oxidation of Q^- decreases (about 50%) in the presence of Na^+ or $Na^+ + Mg^{2+}$. These conclusions imply that cations produce multiple changes in the primary photoprocesses of PS II at physiological temperatures. It is proposed that these changes are mutually independent and can co-exist.

INTRODUCTION

In order to appreciate the measurements presented in this paper, a brief background is given below. The photochemical reaction in pigment system II may be visualized as (also see Govindjee, Introduction, this volume)

$$Z\cdot P680\cdot Q \xrightleftharpoons{h\nu} Z\cdot P680^*\cdot Q \xrightleftharpoons{(1)} Z\cdot P680^+\cdot Q^- \xrightleftharpoons{(2)} Z^+\cdot P680\cdot Q^- \xrightarrow{(3)} Z^+\cdot P680\cdot Q \longrightarrow Z\cdot P680\cdot Q$$

$$\downarrow h\nu' \qquad \text{(low } \phi_f\text{)} \qquad \text{(high } \phi_f\text{)} \qquad R\ R^- \qquad M\ M^-$$

$$D\cdot P680^+\cdot Q^- \xrightleftharpoons{(2')} D^+\cdot P680\cdot Q^-$$

where Z is an electron donor to the reaction center Chl *a* of PS II (P680), Q is an electron acceptor, *hν* is a light quantum, *hν'* is a quantum of delayed light emission, P680* is an excited singlet Chl *a*, $P680^+$ is the Chl *a* cation, Q^- is a semiquinone anion, R is a secondary electron acceptor and M is the charge accumulator involved in oxygen evolution. The initial charge separation (step 1) is very rapid (< 20 ns). Step 2 may be as rapid as 30 ns, but may be much slower (μs?) depending on the structural arrangement of Z and P680; in addition, an alternate donor D may donate electrons to $P680^+$ at a lower rate constant (μs^{-1}) under special conditions. Finally, step 3

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may take 100–600 μs. It has been suggested that $P680^+$ and Q are quenchers of Chl *a* fluorescence, whereas P680 and Q^- are not. Thus, an observed fluorescence yield rise, after a 10 ns flash, may reflect step 2 or its alternative reaction with D, as mentioned above; the fluorescence decay may reflect step 3 and/or a back-reaction of the charges on the reactants in step 3. Delayed light emission (DLE), which is suggested to originate from the back-reaction of step 1 will be affected by step 2 or its alternative reaction with D.

Monovalent and divalent cations affect the yield of Chl *a* fluorescence in isolated chloroplasts; the molecular processes involved in these changes are as yet unknown. Three important mechanisms proposed to explain these cation effects are: (a) divalent cations at low concentrations (1-10 m*M*) and monovalent cations at high concentrations ($\gtrsim$ 100 m*M*) inhibit the "spill-over" of electronic excition energy from PS II to PS I, whereas, monovalent cations at low concentrations (1–10 m*M*) enhance such "spill-over" (see references in excellent reviews by Williams, 1977, and Barber, 1976; Gross and Hess, 1973; Homann, 1969); (b) mono- and divalent cations cause changes in the rate constants of non-radiative processes for de-excitation of the first excited singlet state of Chl *a* (Jennings and Forti, 1974; Malkin and Siderer, 1974); and (c) divalent cations activate PS II reaction centers, that is, cause an increase in the number of reaction centers capable of undergoing photoinduced charge separation (Li, 1975; Rurainski and Mader, 1977; Bose and Arntzen, 1978).

In this paper, we present data from *parallel* measurements, in the same chloroplast preparations, of cation-induced changes in the kinetics of fluorescence yield (3–800 μs) and delayed light emission (6–60 μs), and the light saturation curve for delayed light at 100 μs. Based on the results of these experiments, we conclude that low concentrations of mono- and divalent cations cause multiple effects at room temperature: (1) the sensitization of photosystem II is slightly decreased by monovalent cations, and increased by divalent cations; (2) monovalent cations cause a large reversible increase and divalent cations cause a decrease in the efficiency of radiationless de-excitation of singlet excited Chl *a* in the antenna and closed reaction center of PS II; and (3) that the rate constant for the re-oxidation of Q^- (the reduced form of the electron acceptor of system II) is larger in sucrose-washed chloroplasts prior to the addition of either Na^+ or $Na^+ + Mg^{2+}$.

MATERIALS AND METHODS

Chloroplast preparation. Pea (*Pisum sativum* var. Progress No. 9) seedlings were grown under cool white fluorescent light (16 h photoperiod) in vermiculite and harvested after 15–20 days. Broken chloroplasts were prepared by a modified method of Gross (1971). The leaves were homogenized for 10 s with a Waring blender in 350 m*M* sucrose buffered to pH 7.8 with 50 m*M* Tris–HCl. The slurry was filtered through 4 and 12 layers of cheesecloth and centrifuged at 500 × *g* for 1 min. The supernatant was then centrifuged at 6,000 × *g* for 10 min. The chloroplasts were osmotically shocked by resuspending the pellet in 100 m*M* sucrose solution and allowing the suspension to stand for 10 min at 4°C before re-centrifugation at 8,000 × *g* for 10 min. This salt depletion procedure (i.e. washing with sucrose) was repeated twice and the final "loose" pellet was resuspended in 100 m*M* sucrose containing 0.4 m*M* Tris–HCl at pH 7.5. Chlorophyll concentration was determined according to the method of Arnon (1949). In each series of experiments, the concentration of chloroplasts was adjusted to give equal concentrations of Chl in all samples. In each experiment a volume of concentrated stock chloroplast suspension was diluted from which 3 m/ aliquots were taken and small equal volumes of water and/or 1 *M* salt solutions were added to give the salt-depleted (control), NaCl (5 m*M*), and NaCl (5 m*M*) plus $MgCl_2$ (5 m*M*) containing samples. All measurements were made 10 min after cation addition at 23°C.

Instrumentation. The apparatus for measuring delayed light emission and fluorescence yield rise and decay kinetics has been described elsewhere (Jursinic *et al.*, 1976). The actinic light source for all measurements was an AVCO Everett Model C102 nitrogen laser (λ emission, 337 nm; pulse width at half-maximum, 10 ns). The fluorescence yield rise and decay kinetics were measured by a method similar to that of Mauzerall (1972). An actinic flash was given followed by a very weak flash (General Radio Strobotac 1538-A; two Corning CS 4-96 filters [thickness, 5 mm each] and appropriate neutral density filters) given after various delays; the fluorescence intensity in the weak flash was proportional to the fluorescence yield. Delayed light intensities were directly recorded 6–100 μs after an actinic flash; for details see Jursinic and Govindjee (1977). Both fluorescence and delayed light were detected with an EMI 9558B photomultiplier through a CS 2–64 and a RG-8 filter combination. Neutral density filters were used where needed. Steady state fluorescence was measured with a spectrofluorometer described by Shimony *et al.* (1967). Front surface fluorescence was detected with an EMI 9558B photomultiplier through a Corning CS 2-58 cut-off filter and a Bausch and Lomb monochromator (33-86-45; blazed at 700 nm) set at 685 nm with a half-bandwidth of 10 nm. Excitation was with a 750 W tungsten filament lamp through heat filters and Corning CS 4-96 and CS 3-73 glas filters. The light flux, measured with a Yellow Springs radiometer (Model No. 63), was 200 $W \cdot m^{-2}$.

Analysis of data. The normalized fluorescence yield at time $t(\Phi_n(t))$ is calculated as in Jursinic *et al.* (1976):

$$\Phi_n(t) = \frac{\Phi_{F(t)}}{\Phi_{F_0}} = \frac{S(t) - L(t)}{F_0} \tag{1}$$

where Φ_{F_0} and F_0 are the fluorescence yield and intensity from the analytic flash without prior actinic flash, and $\Phi_{F(t)}$, $S(t)$ and $L(t)$ are the fluorescence yield, signal intensity (i.e., fluorescence plus delayed light) and delayed light intensity, respectively, at time t.

Fluorescence yield rise kinetics is analysed according to the exponential relation (Jursinic and Govindjee, 1977):

$$\Phi_F(t) - \Phi_{F_0} = (\Phi_M - \Phi_{F_0})(1 - e^{-t/\tau}), \tag{2}$$

where $\Phi_F(t)$ and Φ_{F_0} are as defined for Eq. 1, Φ_M is the maximum value of $\Phi_{F(t)}$ for a particular exciting flash intensity, and τ is the lifetime of fluorescence yield rise—the time when $\Phi_M - \Phi_F(t) = e^{-1}(\Phi_M - \Phi_{F_0})$. Rewriting Eq. 2 as

$$\log\left[\frac{\Phi_M - \Phi_{F(t)}}{\Phi_M - \Phi_{F_0}}\right] = -\frac{1}{\tau \ln 10} \cdot t \tag{3}$$

shows that a plot of $\log [(\Phi_M - \Phi_F(t))/(\Phi_M - \Phi_{F_0})]$ vs t gives a straight line with a slope $= -(\tau \ln 10)^{-1}$ from which τ can be obtained.

Determination of the photosynthetic unit size is by an adaptation of the method of Weaver and Weaver (1969) to delayed light measurements. The basic assumption in this method is that the probability of hitting an open PS II reaction center during a flash obeys a Poisson distribution. Presented below is a novel approach for analysing a change in the effective absorption cross-section of a photosynthetic unit. Consider the case when an average of $\sigma \cdot n$ photons hit a photosynthetic unit, where σ is the absorption cross-section of a photosynthetic unit for 337 nm photons, and n is the number of incident photons per flash per cm^2. For generality, let c ($0 \leq c \leq 1$) denote the coupling coefficient for exciton transfer from the antenna to the reaction center; that is, c denotes the probability that a photon absorbed by the antenna will get to the trap. The situation in which $\sigma \cdot n$ photons are absorbed with probability c of being transferred to the trap is effectively the same as when $c \cdot \sigma \cdot n$ photons are absorbed with perfect transfer to the reaction center. In other words, a change in c is operationally the same as a change in the effective absorption coefficient of the pigment array serving the reaction center of PS II. The probability (P) that an open reaction center is not closed (i.e. does not undergo charge separation by a flash) is given by $P(0; c\sigma n) = (c\sigma \cdot n)^0 \cdot e^{-c\sigma \cdot n}/0! = e^{-c\sigma \cdot n}$. Therefore, the probability that charge separation occurs at an open reaction center is $1 - P(0; c\sigma n)$ or $(1 - e^{-c\sigma \cdot n})$. Thus, if the intensity of 100 μs delayed light emission is proportional to the probability of occurrence of photochemistry at a reaction center, the flash intensity saturation curve for delayed light at 100 μs would be given by the exponential rise according to the equation:

$$L(n) = L_\infty (1 - e^{-c\sigma \cdot n}), \tag{4}$$

where $L(n)$ is the 100 μs delayed light intensity and L_∞ is the intensity of delayed light emission at saturation. Equation 4 can be written as:

$$\log\left[\frac{L_\infty - L(n)}{L_\infty}\right] = -\frac{c\sigma}{\ln 10} \cdot n, \tag{5}$$

which gives a linear plot for $\log[(L_\infty - L(n))/(L_\infty)]$ vs n with $L(n) = 0$ at $n = 0$, and the slope $= -(c\sigma/\ln 10)$. The absorption cross-section of a Chl molecule, σ_m, at 337 nm was calculated from optical density measurements, and the amount of sensitization of PS II computed as $c\sigma/\sigma_m$. It must be emphasized that with 337 nm excitation an accurate estimate of σ_m in chloroplasts is difficult, as the experimentally obtained optical density at 337 nm must be corrected for absorption by molecules other than Chl. Failure to do so imposes the assumption that only Chl molecules absorb at 337 nm and would lead to an overestimated value for σ_m, and, hence, an underestimated absolute photosynthetic unit size. This error is accentuated if sample scattering is not accounted for in the optical density measurement.

Analysis of the fluorescence yield changes is as follows (see also Butler and Kitajima, 1975a). At any time, t, the fluorescence yield is given by

$$\Phi_{F(t)} = \frac{k_f}{k_f + k_h + [k_p + (1 - A)k_d]}, \tag{6}$$

with

$$k_p = k_T \cdot [T]_t = (k_T \cdot [T]_0) \cdot ([T]_t/[T]_0) = k_{p_0} \cdot A, \tag{7}$$

where k_f, k_h, k_p are the rate constants for the depopulation of the first excited singlet state of Chl a by fluorescence, non-radiative thermal processes, and photochemistry, k_d is the rate constant for energy dissipation by a closed reaction center, k_{p_0} is the maximum value of k_p, k_T is the bimolecular rate constant for energy transfer from the antenna to the reaction center (see Vredenberg and Duysens, 1963; Knox, 1973), $[T]_0$ and $[T]_t$ are the maximum concentration and concentration at any time, t, of open traps, and A is a scaling factor defined as the ratio of $[T]_t$ to $[T]_0$. In a dark adapted sample, prior to an actinic flash, the system is in a state of maximum trapping efficiency, with all traps open, and the fluorescence yield is given by:

$$\Phi_{F_0} = \frac{k_f}{k_f + k_h + k_{p_0}}. \tag{8}$$

as $A = 1$ and $(1\text{-}A)k_d = 0$. At the peak of the fluorescence yield rise after a saturating flash all the traps are closed, and the yield is:

$$\Phi_{F_M} = \frac{k_f}{k_f + k_h + k_d}. \tag{9}$$

The equality $k_d = f \cdot k_{p_0}$, where $0 \leq f \leq 1$, may be introduced without loss of generality. Since the term k_d in Eq. 9 incorporates the notion that a closed reaction center may still accept and dissipate excitation energy (Butler and Kitajima, 1975a; see also Paillotin, 1976), f is the effectiveness coefficient for energy dissipation by a closed reaction center compared to an open one. The ratio, R, of the variable fluorescence yield ($\Phi_{F_M} - \Phi_{F_0}$) to the maximum yield gives

$$R = 1 - \frac{\Phi_{F_0}}{\Phi_{F_M}} = \frac{k_{p_0} - k_d}{k_f + k_h + k_{p_0}} = (1 - f) \cdot \Phi_{p_0}, \tag{10}$$

where the maximum yield of photochemistry, $\Phi_{p_0} = k_{p_0}/(k_f + k_h + k_{p_0})$. Equations 8, 9, and 10 are independent of the model assumed for the photosynthetic unit, as they can also be derived from the puddle model formulation (see Butler and Kitajima, 1975a). However, when the exciting flash is non-saturating with respect to photochemistry, different relations for the maximum fluorescence yield for the flash, Φ_M, exist for each model. The two extreme cases are: the *puddle* model, in which a photosynthetic unit consists of one reaction center with no inter-unit energy transfer, and the *lake* model, in which numerous reaction centers share a common antenna (Robinson, 1967). In the case of the puddle model:

$$\Phi_M = A \cdot \Phi_{F_0} + (1 - A) \cdot \Phi_{F_M},$$

or,

$$\frac{\Phi_{F_0}}{\Phi_{F_M}} = \frac{1 - A}{\left(\frac{\Phi_M}{\Phi_{F_0}}\right) - A}. \tag{11}$$

In the case of the lake model: Φ_M is given by Eq. 6, and from Eqs. 6, 8 and 9, we obtain:

$$\frac{1}{\Phi_{F_M}} = \frac{1}{\Phi_{F_0}} - \frac{1}{1 - A} \cdot \left(\frac{1}{\Phi_{F_0}} - \frac{1}{\Phi_M}\right),$$

or,

$$\frac{\Phi_{F_0}}{\Phi_{F_M}} = \frac{1 - A \cdot \left(\frac{\Phi_M}{\Phi_{F_0}}\right)}{(1 - A)\left(\frac{\Phi_M}{\Phi_{F_0}}\right)}. \tag{12}$$

RESULTS

Chlorophyll a fluorescence yield rise

The normalized fluorescence yield 3 to 35 μs after an actinic flash, calculated according to Eq. 1, is plotted as a function of time in Fig. 1. The close corre-

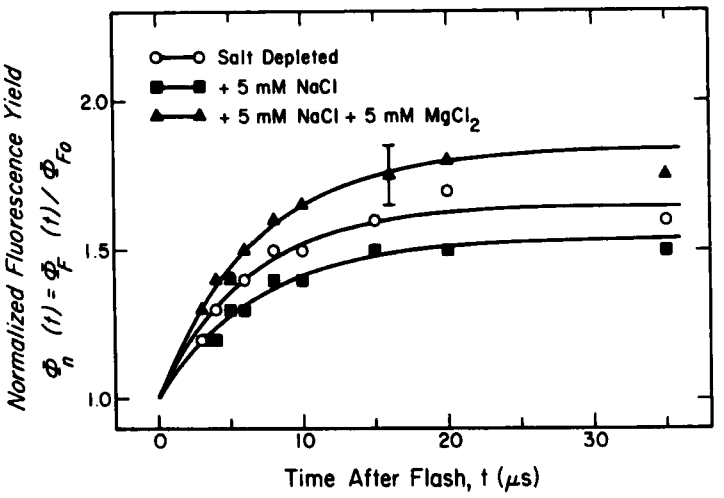

Figure 1. Chlorophyll *a* fluorescence yield rise from 3 to 35 μs, after 10 ns actinic flash, plotted as $\Phi_{F(t)}/\Phi_{F_0}$ vs time. Points represent experimental data and solid curves are least-squares fit exponential curves according to Eq. 2, $\Phi_{F(t)} - \Phi_{F_0} = (\Phi_M - \Phi_{F_0})(1 - e^{-t/\tau})$ with $[(\Phi_M/\Phi_{F_0}), \tau(\mu s)] = [1.65, 5.74]$, [1.54, 6.93], and [1.84, 6.74] for the salt-depleted, Na^+ added, and $Na^+ + Mg^{2+}$ added chloroplasts. The Φ_{F_0} values are 2.0 ± 0.1, 1.5 ± 0.1, and 2.5 ± 0.5 for the three cationic conditions. Chlorophyll concentrations were 5 μg/mℓ and the exciting intensity was 10^{14} incident photons/cm^2-flash. Typical uncertainties are given by the one error bar. For this and the following figures, see text for definition of the mathematical symbols.

spondence between the experimental points and the least-squares fit curves (solid lines) according to Eq. 2 show that the fluorescence yield rise is exponential confirming earlier findings for this time range (Mauzerall, 1972). The fluorescence yield, $\Phi_F(t)$, reaches a maximum, Φ_M, between 20 μs and 30 μs after the flash, also in accordance with previous findings (Mauzerall, 1972, 1976; Duysens *et al.*, 1975; Jursinic and Govindjee, 1977). The addition of 5 m*M* NaCl to a salt-depleted chloroplast sample causes a decrease of $\sim 30\%$ in Φ_M, while the further addition of 5 m*M* $MgCl_2$ to the NaCl sample causes an increase of $\sim 100\%$.

A plot of $\log[(\Phi_M - \Phi_F(t))/(\Phi_M - \Phi_{F_0})]$ vs t (see Eq. 3) is shown in Fig. 2. Within the limits of the experimental errors the salt-depleted, 5 m*M* NaCl replenished, and 5 m*M* NaCl plus 5 m*M* $MgCl_2$ replenished samples, from two different batches of chloroplasts, show the same lifetime of rise (τ) of 6.4 ± 0.6 μs.

Chlorophyll a fluorescence yield decay

The normalized variable fluorescence yield decay between 50 and 800 μs for samples in the three cationic conditions are plotted as $[(\Phi_{F(t)}/\Phi_{F_0}) - 1]$ vs t in Fig. 3. The kinetics of this decay is complex (Mauzerall, 1972; Zankel, 1973; Jursinic *et al.*, 1976), and an analytical description of it is as yet unavailable. However, graphical curve fitting shows that our results can be satisfactorily described by a biphasic decay given by the relation: $(\Phi_{F(t)}/\Phi_{F_0}) - 1 = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$, where A_1 and A_2 are the amplitudes of the two exponential phases with lifetimes of τ_1 and τ_2, respectively (Table 1). The fraction of fluorescence decaying by the fast phase is changed from 0.5 to 0.6 upon addition of cations. The most distinct differences are seen in the decrease in τ_1 when cations are added (~ 90 μs in the absence and ~ 160 μs in the presence of Na^+ or $Na^+ + Mg^{2+}$), and the decrease in τ_2 when Mg^{2+} is added (~ 50 ms without Mg^{2+} and ~ 4 ms with Mg^{2+}). The limited

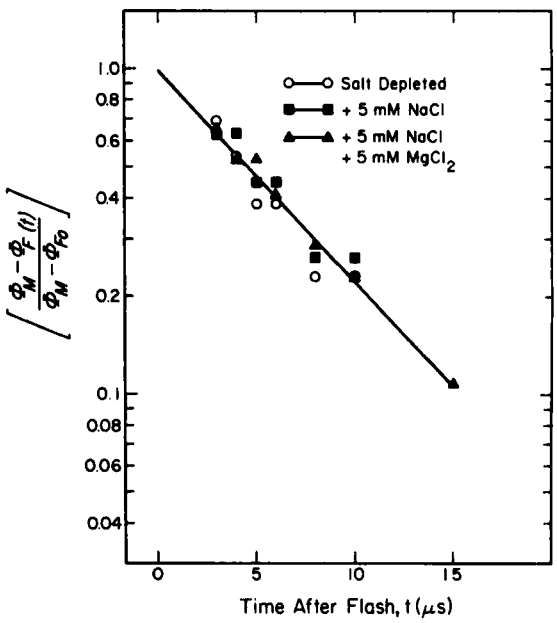

Figure 2. Chlorophyll *a* fluorescence yield rise data from Fig. 1 plotted as $\log[(\Phi_M - \Phi_{F(t)})/(\Phi_M - \Phi_{F_0})]$ vs time. The risetime calculated from the slope is 6.7 μs.

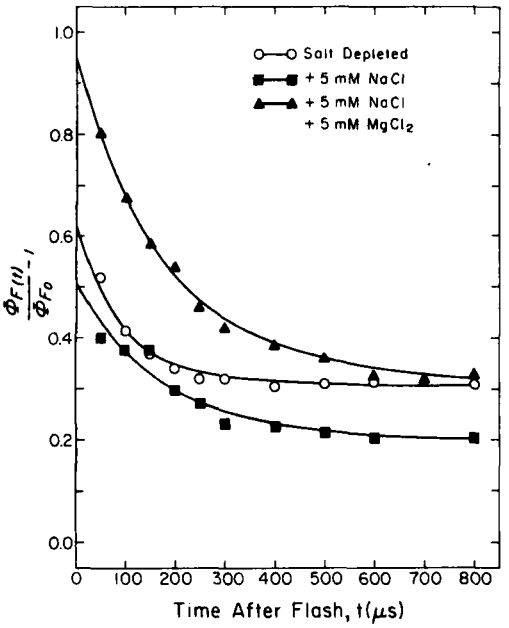

Figure 3. Chlorophyll *a* fluorescence yield decay plotted as $[(\Phi_{F(t)}/\Phi_{F_0}) - 1]$ vs time. Solid lines, theoretical curves described by the equation $(\Phi_{F(t)}/\Phi_{F_0}) - 1 = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}$ with the values for A_i and τ_i (i = 1,2) given in Table 1.

time range of our measurements < 1 ms warrants further investigation on this change in τ_2.

Delayed light emission—intensity and decay kinetics from 6 to 60 μs

The intensity of delayed light emission as a function of time from 6 to 60 μs are presented on a semilogarithmic plot in Fig. 4. When 5 m*M* NaCl is added to a salt-depleted chloroplast sample a decrease in the intensity of delayed light by as much as 40% is observed. Addition of 5 m*M* $MgCl_2$ to a sample with or without 5 m*M* NaCl causes an enhancement of the delayed light intensity (~2–3.5 times that from a sample with 5 m*M* NaCl).

The delayed light emission between 6 and 60 μs after the flash decays with biphasic kinetics. An analysis of the data by the graphical exponential peeling method (see Van Liew, 1967) is given in Figs. 4 and 5. Within experimental uncertainties, the salt-depleted, NaCl, and NaCl plus $MgCl_2$ samples show a constant lifetime of 7.2 ± 0.8 μs for the fast phase (Fig. 5). A more rigorous analysis by computer curve fitting for a sum of exponentials (Provencher, 1976) gives the results in Table 2. Here again, the lifetimes of the decays are relatively constant for the three samples: 8.8–9.6 μs for the fast phase, and 33–40 μs for the slow phase. The proportion of delayed light represented by the fast phase is slightly higher in the sample containing NaCl (76% compared to 58% in salt-depleted and $Na^+ + Mg^{2+}$ samples). We note, however, that the areas under the delayed light emission curves, calculated as $\Sigma_i A_i \tau_i$, and normalized to 1.0 for the $Na^+ + Mg^{2+}$ sample, are 0.74 for the salt-depleted sample and 0.38 for the Na^+ sample. This agrees perfectly with the values of Φ_{F_M} (see later, Table 3).

100 μs delayed light emission light saturation curve

The 100 μs delayed light is not saturated at the maximum intensity of our actinic source (Fig. 6). The light curve appears to be adequately described by a "single-hit" Poisson saturation, and the saturation intensity of delayed light emission (L_s) for each curve is obtained by iteration using Eq. 5. The value of L_s is chosen and a least-squares straight line calculated for the data plotted as $\log[(L_s - L(n))/L_s]$ vs n; the value of L_s which satisfies the condition that the least-squares line extrapolated to $L(n) = 0$ at $n = 0$ is taken as the saturation value of $L(n)$ for the light curve (Fig. 7). The slope of each least-squares line in the plot of $\log[(L_s - L(n))/L_s]$ vs n in Fig. 7 is proportional to the effective absorption cross-section of a photosynthetic unit in the samples (see Eq. 5). The average calculated values of $c\sigma$ (where c is the coupling coefficient for energy transfer from the antenna to the reaction center and σ is the absorption cross-section) for the salt-depleted, NaCl, and NaCl plus $MgCl_2$ added samples, for two batches of chloroplasts, are 144 ± 3, 136 ± 5, and 163 ± 4, respectively. It must be pointed out that no attempt is made here to remove the assumption imposed by default which leads to an overestimated σ_m, see *Analysis of*

Table 1. Parameters describing the simulated curve for variable fluorescence yield decay from 50 to 800 μs

Sample	A(a.u.)	A_1	τ_1 (μs)	A_2	τ_2 (ms)
0-salt	0.624	0.5	91	0.5	50
+5 m*M* NaCl	0.510	0.6	167	0.4	50
+5 m*M* NaCl +5 m*M* $MgCl_2$	0.960	0.6	154	0.4	4

The simulated curve assumes the fluorescence yield decay to be given by the relation: $(\Phi_F/\Phi_{F_0}) - 1 = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}$, where A_i, τ_i (i = 1,2) are the amplitudes and lifetimes of the two decay phases. In the table, A is the sum of A_1 and A_2 at $t = 0$ for the data in Fig. 3, and A_1 and A_2 are the relative fractions of A represented by the two phases. The best-fit values are within 20% of the reported values. (a.u. stands for arbitrary units.)

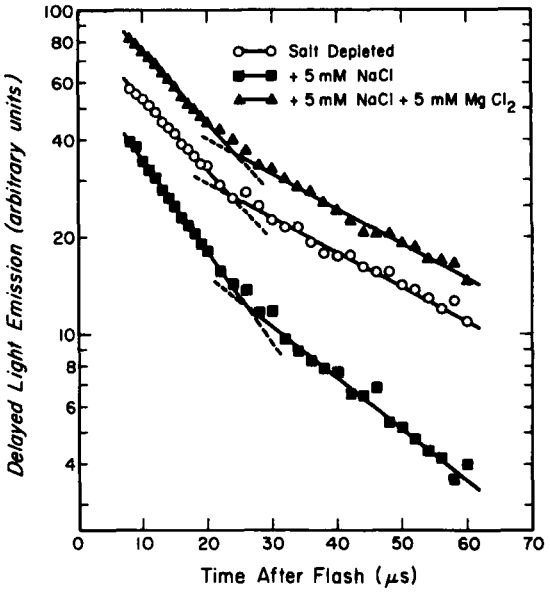

Figure 4. Semilogarithmic plot of delayed light emission decay against time. The same samples as in Fig. 1 are used.

data. However, the relative amounts of sensitization of PS II under the three experimental conditions are precise and independent of the value of σ_m, and correspond to ~0.88, 0.83, and 1.0 for the salt-depleted, + Na^+, and + Na^+ + Mg^{2+} samples.

Ratio (R) of maximum variable fluorescence to total fluorescence

Since the light saturation curve for 100 μs delayed light emission (Fig. 6) indicates that the actinic flash is non-saturating with respect to photochemistry, Φ_{F_M} is calculated for the extreme cases (puddle or lake model) using Eqs. 11 or 12 (see Table 3). The fraction of reaction centers closed, 1-A, by the flash at maximum intensity ($n = 10^{14}$ incident photons/flash/cm^2) is obtained as the ratio of the intensity of 100 μs delayed light emission produced by the flash to the saturation value of delayed light emission, L_s, obtained by linear regression. Good agreement is found between the relative maximum yields of microsecond fluorescence (Φ_{F_M}) and the relative yields of "P" level fluorescence in the steady-state (Lavorel, 1959) for the same chloroplast preparations (Table 3).

Analysis of the changes in fluorescence yield by the ratio of variable to maximum fluorescence is according to Eq. 10. The ratio, *R*, in Table 4 is calculated from the results in Table 3 by Eq. 10. The cation effects in each set of samples, under our experimental conditions, are relatively *independent* of the model assumed for the photosynthetic unit. Compared to the salt-depleted control, the sample with 5 m*M* NaCl shows a lower (12 ± 6%) value for *R*, while the sample with both 5 m*M* NaCl and 5 m*M* $MgCl_2$ present shows an enhanced (30 ± 17%) value. The results in Table 4, however, are normalized to 1.00 for *R* in the sample containing $MgCl_2$.

DISCUSSION

The analysis of the cation effects on μs fluorescence and delayed light emission, made here, is based on the following concepts (see Butler and Kitajima, 1975a; Butler, 1978): (i) that mono- and divalent cations affect the degree of coupling of energy transfer between the bulk Chl and reaction center II, (ii) that a closed reaction center of PS II can dissipate excitation energy, (iii) that there is one source for Chl fluorescence, and (iv) that the fluorescence yield decay with τ = 100–200 μs reflects the re-oxidation of the stable reduced primary electron acceptor of PS II, Q^- (Zankel, 1973).

The observed effects of NaCl and $MgCl_2$ on fluorescence and delayed light emission are not caused by the Cl^- ion, as sodium and magnesium salts with other anions are known to produce the same effects (Murata, 1971; Gross and Hess, 1973).

Sensitization of photosystem II

Depending on the coupling coefficient *c* (see description preceding Eq. 4), which defines the probability for exciton transfer between antenna and reaction center, the conceptual interpretation for the relative amounts of sensitization of reaction center II, P680, could vary. In the limit when $c = 1$ (perfect coupling) alterations in the degree of sensitization would mean true changes in the absorption cross-section, σ, of the antenna serving P680. On the other

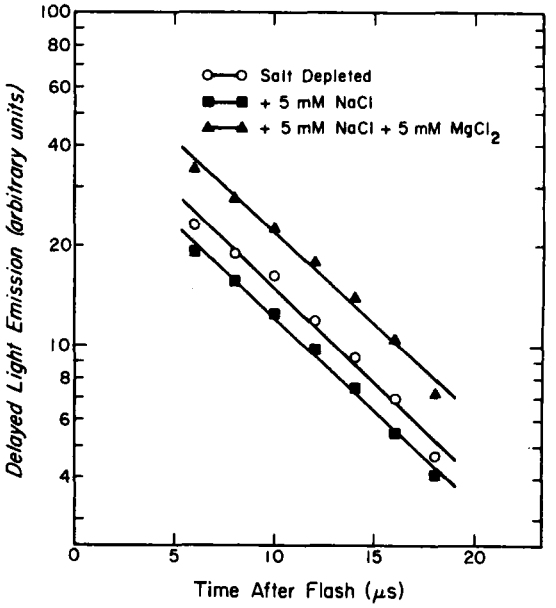

Figure 5. Kinetics of decay of the "faster" component of delayed light emission obtained by subtracting the "slower" component from the total signal in Fig. 4. Solid lines are least-squares fits to the experimental data. The decay times were 7.7 ± 0.1 μs.

Table 2. Parameters describing the best-fit curve for delayed light emission from 6 to 60 μs

Sample	L(a.u.)	L_1	τ_1 (μs)	L_2	τ_2 (μs)
0-salt	1013	0.58	9.3	0.42	40.0
+5 m*M* NaCl	777	0.76	9.6	0.24	32.8
+5 m*M* NaCl +5 m*M* $MgCl_2$	1458	0.57	8.8	0.43	36.9

The analysis by the method of Provencher (1976; version 1 a March, 1976) for a sum of exponentials is used. In all cases, the best-fit curve ("criterion", $P_{NG} > 0.99$) consists of two components, that is, $L(t) = L_1e^{-t/\tau_1} + L_2e^{-t/\tau_2}$, where $L(t)$ is the delayed light intensity at time t, and L_i and τ_i ($i = 1,2$) are the amplitudes and lifetimes of the two phases. L is the total amplitude of delayed light at $t = 0$ for the data in Fig. 4, and L_1 and L_2 are the relative proportions of the two decaying phases. The uncertainties in the above parameters range from 14 to 50% of the reported values. (a.u. stands for arbitrary units.)

hand, if c is allowed to vary ($0 \leq c \leq 1$), changes in the degree of sensitization may occur without changes in σ; that is, the degree of sensitization of P680 is defined by the degree of coupling for exciton transfer between a constant size antenna with its reaction center. Also, there are no restrictions on simultaneous variations in both c and σ. It is noted that c need not even be restricted to a one-step coupling process. For example, in the tripartite model for chloroplast fluorescence (Butler and Kitajima, 1975b, c; Butler and Strasser, 1977) c would denote the energy transfer coupling between the light-harvesting Chl-protein complex (Chl LH) and the PS II complex (Chl a_{II}). If it is also proposed that a variable coupling exists between the "bulk" and the reaction center Chls in the PS II complex, c would denote the net coupling between Chl LH and P680; that is, c is the product of the coupling coefficients between Chl LH/Chl a_{II} and Chl a_{II}/P680. Both cases may simply be referred to as the coupling of energy transfer between the antenna Chls and the reaction center. Common usage also refers to this process as the initial partitioning of absorbed quanta to PS II. Although the conceptual picture for the sensitization stays undefined, the result remains that the addition of Na^+ decreases the sensitization of P680 by ~6%, and the subsequent addition of Mg^{2+} then increases the sensitization by ~20% (cf. Butler and Kitajima, 1975b, c; Moya *et al.*, 1977). This result disagrees with the conclusion of Henkin and Sauer (1977) that the major effect of Mg^{2+} ions is to increase the effective absorption cross-section of the pigment array associated with PS II photochemistry leading to a 2-fold stimulation in total fluorescence in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

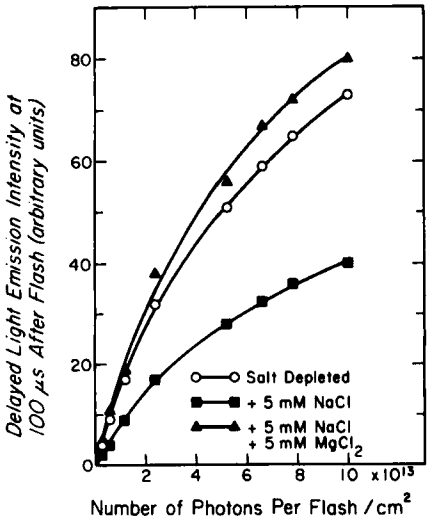

Figure 6. Flash intensity saturation curve for the 100 μs delayed light emission plotted as delayed light intensity, L(n), vs the number of incident photons/cm^2-flash, n. At full intensity, $n = 10^{14}$.

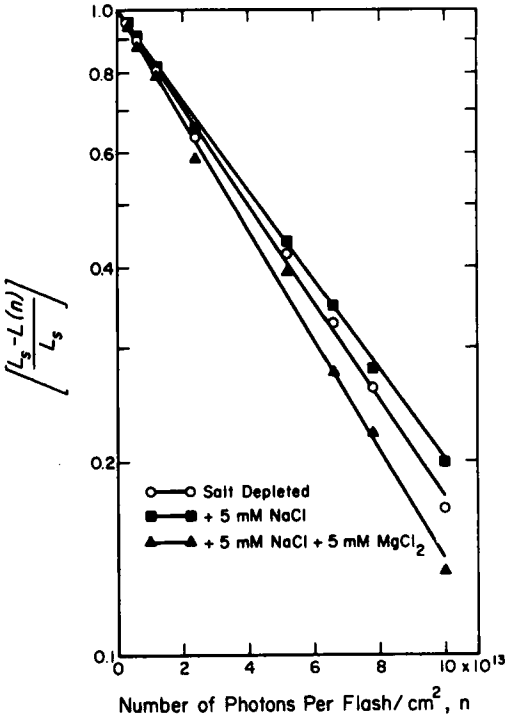

Figure 7. Light saturation data from Fig. 6 plotted as log [(L_s – L(n)/L_s)] vs n. Solid lines are least-squares fits.

Table 3. Effects of Na^+ and Mg^{2+} on the initial and maximum relative yields of Chl *a* fluorescence

Sample	$L(n)/L_s$	Φ_M/Φ_{F_0}	Φ_{F_M}/Φ_{F_0} [a]Puddle	[b]Lake	Φ_{F_0}	Φ_{F_M} (flash)	Φ_{F_M} (steady state)
Salt-depleted	0.84 ± 0.01	1.50	1.59	1.67	2.0 ± 0.1	0.66 ± 0.03	0.72 ± 0.02
+5 m*M* NaCl	0.81 ± 0.01	1.40	1.49	1.54	1.5 ± 0.1	0.46 ± 0.03	0.38 ± 0.03
+5 m*M* NaCl +5 m*M* $MgCl_2$	0.87 ± 0.01	1.81	1.92	2.04	2.5 ± 0.5	1.00 ± 0.20	1.00 ± 0.03

The fractions of active reaction centers closed by individual flashes, 1-A, in column 2 are calculated from the 100 μs delayed light emission flash intensity saturation curve as $L(n)/L_s$ for $n = 10^{14}$ incident photons/cm^2-flash. The ratios (Φ_M/Φ_{F_0}) in column 3 are obtained from the fast fluorescence yield rise curves. The quantities (Φ_{F_M}/Φ_{F_0}) in columns 4a and 4b are calculated by the use of Eq. 11 for the puddle model, and Eq. 12 for the lake model. The initial relative yields, Φ_{F_0} in column 5 are experimentally determined. The maximum relative microsecond fluorescence yields in column 6 are calculated from columns 4a, 4b and 5 and averaged. The maximum relative steady state fluorescence yields at "P" level in column 7 are obtained experimentally. The relative yields in columns 6 and 7 are normalized to 1.00 for the sample containing both NaCl and $MgCl_2$. Results presented are the average values of two separate chloroplast preparations. All experiments were done at 23°C. Notations are as given in the text. Uncertainties denote one standard deviation.

Table 4. Effects of Na^{2+} and Mg^+ on the calculated maximum yield of "primary photochemistry" in a saturating flash

Sample	$R = 1 - (\Phi_{F_0}/\Phi_{F_M}) = (1\text{-}f)\cdot\Phi_{P_0}$ Puddle model	Lake model	*R*, relative
Salt-depleted	0.37	0.40	0.77 ± 0.14
+5 m*M* NaCl	0.33	0.35	0.68 ± 0.18
+5 m*M* NaCl +5 m*M* $MgCl_2$	0.48	0.51	1.00 ± 0.05

The quantities $[1\text{-}(\Phi_{F_0}/\Phi_{F_M})]$ which according to Eq. 11 give the product of the fractional decrease in the efficiency of excitation energy dissipation by a closed PS II reaction center relative to an open one, $(1\text{-}f)$, and the values of *R* are calculated using the results in column 4a and 4b in Table 3. The values of *R* are normalized to 1.00 for the chloroplast samples to which NaCl and $MgCl_2$ are added. The results presented are the average values for two separate chloroplast preparations. Each uncertainty value presented in the last column represent one standard deviation for the samples averaged and reflect the biological variability. It is noted that for each sample series *R* is higher for the salt-depleted sample than for the 5 m*M* NaCl case.

Rate constants for fluorescence rise and delayed light decay

The fluorescence yield rise within 35 μs (Fig. 1) has been suggested to monitor the rate of disappearance of some fluorescence quencher. The $P680^+$-quencher hypothesis suggests that the quencher is the oxidized reaction center of PS II, $P680^+$ (Butler, 1972; Den Haan *et al.*, 1974, 1976; Jursinic *et al.*, 1976; Jursinic and Govindjee, 1977), and that the rise of fluorescence reflects the re-reduction of $P680^+$ to P680 by some electron donor, Z or D (see Introduction). In the alternative mechanism, the carotenoid-triplet-quencher hypothesis (Zankel, 1973; Mauzerall, 1976), the carotenoid triplets with lifetimes 3–4 μs (Chessin *et al.*, 1966; Mathis, 1966; Mathis and Galmiche, 1967; Wolff and Witt, 1969) act as the quencher. Absence of parallel measurements on $P680^+$, carotenoid triplets, and fluorescence rise in the same sample under identical conditions have precluded a choice thus far (see review by Govindjee and Jursinic, 1978). It may be possible that some linear combination of the two would provide the most satisfactory representation.

Microsecond delayed light emission has been suggested to originate from the back reaction of $P680^+$ with the reduced primary acceptor, Q^- (Van Gorkom and Donze, 1973; see also Lavorel, 1975). This implies that the disappearance of $P680^+$ would lead to a decrease in delayed light emission. The recombination hypothesis and the $P680^+$-quencher hypothesis, taken together, predict that the rate constant of decay of delayed light emission should correspond to that of the rate constant of rise of fluorescence yield. Although such an agreement is observed here—lifetimes of 6.4 ± 0.6 μs for fluorescence rise (Fig. 2) and 7.2 ± 0.8 μs for delayed light decay (Fig. 5)—it does not constitute a proof. The constancy of these lifetimes in chloroplasts with or without cations added is interpreted to mean that the rate constant of electron donation from D to $P680^+$ is unaffected by the addition of low concentrations of cations. The $P680^+$-quencher hypothesis is attractive in that it provides

the simplest unified mechanism for fluorescence yield rise and delayed light emission decay, not readily available by the carotenoid-triplet-quencher hypothesis.

The Q^- decay

Duysens and Sweers (1963) first proposed that the primary electron acceptor, Q, of PS II in its oxidized state is a quencher of Chl *a* fluorescence. In continuous light experiments, at the onset of illumination Q is in its oxidized state and the fluorescence yield is low; with prolonged illumination Q is reduced to Q^- and the fluorescence yield is high, giving the "P" level of fluorescence (Lavorel, 1959; Govindjee and Papageorgiou, 1971). Extending this hypothesis to flash excitation experiments, the maximum fluorescence yield state 20–30 μs after a short saturating flash is assumed to be one in which all Q's are in the Q^- state (equivalent to the "P" level). This suggestion is supported by our observation that the maximum fluorescence yields at $\sim 30\,\mu$s for chloroplasts in the three cationic conditions closely matched their relative "P" level yields (last two columns in Table 3). This suggestion is also consistent with the interpretation of the fluorescence yield decay. The kinetics of decay is biphasic over the interval of interest here (Mauzerall, 1972; Zankel, 1973; Jursinic *et al.*, 1976), the initial phase decays with an amplitude (relative to the total variable fluorescence) and half-time ($t_{\frac{1}{2}}$) which differs slightly between the two previous reports: 2/3 and $\sim 200\,\mu$s according to Zankel (1973) and 3/4 and $\sim 170\,\mu$s according to Mauzerall (1972). Based on the findings that the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea eliminates this phase of the decay (Zankel, 1973) and that lowering the temperature from 25 to 5°C greatly diminishes its amplitude (Mauzerall, 1972), it was suggested that the fast phase of the fluorescence yield decay reflects the re-oxidation of Q^-. The fluorescence yield decay for the salt-depleted sample has a $t_{\frac{1}{2}}$ about one-half that of chloroplasts with 5 m*M* NaCl, but the subsequent addition of 5 m*M* $MgCl_2$ produces no further change (see Table 1). This suggests that the rate constant of Q^- re-oxidation in the absence of added cations is larger than in the presence of low concentrations of mono- and divalent cations. The slow phase with $t_{\frac{1}{2}}$ in ms probably reflects the equilibrium between Q^-, the connector molecule, R (Bouges-Bocquet, 1973; Velthuys and Amesz, 1974; Diner, 1975), and the plastoquinone pool.

Amplitude of 6–100 μs delayed light emission

Assuming that delayed light emission in this time scale originates from the back reaction between $P680^+$ and Q^- (Van Gorkom and Donze, 1973; Govindjee and Jursinic, 1978), a change in its amplitude could be the consequence of one or more of the following causes: (1) a change in the quantum yield (ϕ_L) of delayed light emission; $\phi_L = L/J$, L being the intensity of delayed light emission, and J the rate of production of excited state Chl, (2) a change in the rate constant for recombination of $P680^+$ and Q^-, and (3) a change in the concentration of $P680^+$ and/or Q^-.

Figures 4 and 6 show that the intensities of delayed light, L, between 6 and 100 μs in the order of their magnitudes are $L(Na^+) < L(\text{salt-depleted}) < L(Na^+ + Mg^{2+})$. The intensity of delayed light from chloroplasts with low concentrations of NaCl and $MgCl_2$ was from 2 to 3.5 fold greater than the intensity in samples containing only NaCl. Recently, Barber *et al.* (1977) have shown that the change in intensity of ms delayed light follows qualitatively the change in fluorescence yield induced by mono- and divalent cations. Since an approximately 1.5 fold difference in fluorescence yield exists between the $+Na^+$ and the $+Na^+ + Mg^{2+}$ samples throughout the μs time range (see Figs. 1 and 3) a parallel change in the quantum yield for μs delayed light cannot be disregarded without additional information.

If, in the recombination and emission process, the delayed light photon is emitted from the vicinity of an open reaction center with yield close to Φ_{F_0} (fluorescence yield when all traps are open) (see Malkin, 1977), then changes in delayed light will have to be due to changes in the concentration of the precursors ($P680^+$ and Q^-) or the rate constant of their recombination. A Mg^{2+}-induced increase in the former, starting at 6 μs after the flash, can result from one of the two causes: (a) an increase in the initial production of $[P680^+]$ and $[Q^-]$ by the flash—the idea that Mg^{2+} ions somehow cause an increase in the number of reaction centers capable of photochemistry (Li, 1975; Rurainski and Mader, 1977; Bose and Arntzen, 1978), and (b) a Mg^{2+}-induced decrease in the rate constant for electron donation to $P680^+$ by its primary donor Z, without change in any of the other parameters affecting delayed light. Case (b) must be considered because direct monitoring of the kinetics of $[P680^+]$ changes by Van Best and Mathis (1978) suggests that the half-time for the electron transfer is about 25–45 ns. Direct measurements on $[P680^+]$ should provide the definitive test for both hypotheses. Preliminary observations of T. Wydrzynski in P. Mathis' laboratory, however, indicate that cations have no significant effect on the amplitude of X-320 (a monitor of the primary electron acceptor Q).

If the quantum yield of delayed light is unknown, the rate constant for recombination cannot be measured. Thus, the observed changes in μs delayed light could be due to any of the three changes mentioned above. However, see *NOTE ADDED IN PROOF*.

Radiationless de-excitation of singlet excited chlorophyll

Analysed according to Eq. 10, the ratio, *R*, of the variable to maximum fluorescence yield is defined by the product of two terms: (1-f), the fractional decrease in the efficiency of excitation energy dissipation by

a closed PS II reaction center compared to an open one, and Φ_{P_0}, the yield of primary photochemistry. A re-examination of Eqs. 7 and 10 shows that R is defined by five parameters: k_f, the rate constant for fluorescence; k_h, the sum of rate constants for all radiationless events in the bulk Chl; k_d, the net rate constant for radiationless transitions in the closed reaction center; k_T, the rate constant for energy transfer to the reaction center; and $[T]_0$, the maximum concentration of open traps in the sample. For our discussion it will be assumed that k_f is constant for the three different samples because of the close resemblance of their absorption and emission spectra at room temperature (see also Malkin and Siderer, 1974); only small variations in the absorption spectra have been reported by Murata (1971) and Henkin and Sauer (1977). k_T is directly related to c, the coupling coefficient for exciton transfer from the antenna to the trap. The small variations ($< 20\%$) in the sensitization of PS II suggest that changes in k_T are small. In the present discussion, a brief survey of the consequences of changes in the remaining parameters is made, which will be used later. With reference to Eqs. 7–10, a change in k_h would affect Φ_{P_0}, Φ_{F_0}, and Φ_{F_M}, a change in k_d would affect f and Φ_{F_M}, and a change in $[T]_0$ would affect f, Φ_{P_0}, and Φ_{F_0}.

Referring again to Eq. 10, one extreme possibility assumes that Φ_{P_0} is constant for all the samples, and any variations in R result from changes in f. In this case, the decrease in R with addition of Na^+ implies an increase in f; that is, Na^+ increases the efficiency for radiationless transitions at a closed reaction center. The process is reversed when Mg^{2+} is added subsequent to Na^+. The other extreme case assumes that $(1-f)$ is constant, and differences in R come about because of differences in Φ_{P_0}, so that $R(Na^+) < R(Na^+ + Mg^{2+})$ implies than $k_h(Na^+) > k_h(Na^+ + Mg^{2+})$. In other words, Na^+ increases the rate constant, and, hence, the efficiency for some radiationless transition in the bulk Chl of PS II, and Mg^{2+} reverses this change. Finally, a change in both f and Φ_{P_0} brought about by a change in $[T]_0$ may also be possible. If $[T]_0(Na^+) < [T]_0(Na^+ + Mg^{2+})$, then by Eq. 7, $k_{P_0}(Na^+) < k_{P_0}(Na^+ + Mg^{2+})$, giving $f(Na^+) > f(Na^+ + Mg^{2+})$, since $f = k_d/k_{P_0}$, and $\Phi_{P_0}(Na^+) < \Phi_{P_0}(Na^+ + Mg^{2+})$, since Φ_{P_0} is a monotonically increasing function of k_{P_0}, and Eq. 10 gives $R(Na^+) < R(Na^+ + Mg^{2+})$. However, an increase in $[T]_0$ with addition of Mg^{2+} cannot occur independently without change in one or more of the other parameters. This is because an independent change in $[T]_0$ does not lead to a 2-fold increase in Φ_{F_M} (Φ_{F_M} being independent of $[T]_0$, see Eq. 9), and even predicts a decrease in Φ_{F_0} (see Eq. 8), both cases of which are in conflict with experimental results (see Table 3). It is important to point out that fluorescence experiments do not provide the conclusive tests for possible changes in $[T]_0$, which is best tested by direct measurement of the oxidation of P680 in a saturating flash. Bose and Arntzen (1978) have shown that Mg^{2+} stimulates O_2 flash yield; this could possibly be due to activation of electron flow from Z to $P680^+$. On the other hand, the analysis of fluorescence yield changes clearly shows that changes in k_h and/or k_d must exist irrespective of changes in $[T]_0$. Therefore, these changes in the rate constants of radiationless transitions and in $[T]_0$ are not mutually exclusive. A brief comment should be made on the implications of changes in k_h and k_d. Possible radiationless transition pathways in both processes include intersystem crossing to the triplet manifold and internal conversion. In addition, k_h includes the radiationless transfer of energy from the bulk chlorophyll molecules either in the light-harvesting complex or the PS II complex to PS I, while k_d includes the direct transfer of energy from a closed reaction center II (perhaps in the state $P680 \cdot Q^-$) to PS I (cf. Butler, 1978).

An estimate of the absolute values of the rate constants of the radiationless process $(k_h + k_d)$ is made using results of Chl *a* fluorescence lifetimes at room temperature. At pH 7.6, the "P" level fluorescence lifetimes for salt-depleted, $+Na^+$, and $+Na^+ + Mg^{2+}$ samples of chloroplasts are 1.0 ± 0.1, 0.5 ± 0.1, and 1.0 ± 0.1 ns respectively (Wong, Merkelo and Govindjee, unpublished). Using an intrinsic lifetime ($\tau_0 = 1/k_f$) of 15.2 ns for Chl *a* fluorescence (Brody and Rabinowitch, 1957) and Eq. 9, the ratio of $(k_h + k_d)/k_f$ for the samples in the order given above are ~ 14, 29, and 14. This analysis implies a 2-fold decrease in $(k_h + k_d)$ upon the addition of Mg^{2+} to a $+Na^+$ sample. With these results from Chl *a* fluorescence lifetime measurements, any suggestion against a change in the rate constant for radiationless transitions is not tenable.

CONCLUDING REMARKS

Almost a decade has passed since it has been suggested that cations regulate the distribution of excitation energy between the two pigment systems (Murata, 1969a, b; Bonaventura and Myers, 1969), yet little is known about the molecular photoprocesses involved in this regulation (Barber, 1976; Williams, 1977).

Research in our laboratory has led to the following conclusions: (1) The cation effects on Chl *a* fluorescence yield require structural changes, as fixation of chloroplasts by glutaraldehyde abolishes such effects (Mohanty *et al.*, 1973); (2) both pigment systems I and II must interact to show the major effects of cations on Chl *a* fluorescence (Mohanty *et al.*, 1973); (3) the quantum yield of energy transfer from pigment system II to I is of the order of 10–20% (Mohanty *et al.*, 1973); (4) there is no direct kinetic correlation between structural changes (as measured by changes in fluorescent probes and 90° light scattering) and Chl *a* flurorescence changes upon addition of cations (VanderMeulen and Govindjee, 1974; also see Schooley and Govindjee, 1976); (5) cations affect both the constant and the variable fluorescence suggesting that they affect the antenna Chl *a*

(Wydrzynski *et al.*, 1975); (6) there is a direct effect on pigment system II—most likely on the Chl *a* complex associated with reaction center II (Wydrzynski *et al.*, 1975); (7) there are at least two separate effects of divalent cations as shown by the dependence of the fluorescence intensity at "P" level on the concentration of $MgCl_2$ (Wydrzynski *et al.*, 1975), and (8) "spill-over" of excitation energy is enhanced by the addition of low concentrations of Na^+ and decreased by the addition of low concentrations of Mg^{2+}, as indicated by the increase in the degree of polarization of PS II emission and the decrease in that of PS I upon addition of Na^+ to salt-depleted chloroplasts, and the reversal of these effects by the subsequent addition of Mg^{2+} (Govindjee and Wong, 1976).

Barber and Mills (1976) have proposed that surface charges on the membrane play an important role in the action of cations on the thylakoids. Arntzen and co-workers (see references in Arntzen, 1977) have delineated a crucial role for the light-harvesting pigment protein complex in excitation energy regulation between the photosystems. However, the molecular mechanisms which lead to the changes in the rate constants of de-excitation of the excited Chl *a* molecule, the focus of this study and that of a forthcoming publication by Wong, Merkelo and Govindjee remain unexplored.

The present analysis of microsecond delayed light emission data has brought into focus the difficulties in their interpretation without parallel measurements on the formation and the relaxation of the oxidized reaction center Chl *a*, $P680^+$, and its reduced primary electron acceptor Q^-. In spite of these difficulties, delayed light emission can be used for calculating the photosensitization of pigment system II photochemistry as shown here by the analysis of the 100 μs delayed light emission saturation curve. Although somewhat similar difficulties exist in the interpretation of the Chl *a* fluorescence yield data in the μs range, its analysis leads to the conclusion that large changes in the rate constants for radiationless transitions exist.

Acknowledgements—This research, first presented in an abstract form in *Plant Physiol.* (*Supplement*) **59,** 24, August, 1977, was supported by National Science Foundation grant PCM 76-11657. P. Jursinic was supported by a research assistantship from the University of Illinois Research Board. We thank Dr. Prasanna Mohanty for helpful discussions. D. Wong thanks Mr. John W. McKown for help in doing the Provencher analysis on the delayed light emission data.

Note added in proof—D.W. and G., in collaboration with P. Mathis, C. Vernotte and S. Saphon, have shown that the amplitudes of the absorbance changes for P680 (due to the reaction center Chl *a* of PS II) and for P515 (due to the primary charge separation) are unaffected by the cations. These experiments rule out the hypothesis that divalent cations activate the reaction center II. (It appears, however, that cations may cause some of their effects by affecting the back reaction of the primary products of light reaction II.)

Recently, Renger, Eckert and Buchwald (*FEBS Lett.* **90,** 10–14, 1978) have reported a 4–6 μs component in the $P680^+$ decay using the repetitive flash excitation. This is in agreement with our suggestion (Jursinic and Govindjee, 1977) that the lifetime of electron donation to $P680^+$ is in the range of 6–7 μs. This time was found to be 30–50 ns by van Best and Mathis (1978) after the *first* flash following 5–10 min dark adaptation. The 4–6 μs component is present after *all* the flashes, and, thus, the 6–7 μs component in Chl *a* fluorescence rise (Fig. 1, this paper) and in delayed light emission decay (Fig. 4, this paper) may indeed be attributed to the disappearance of $P680^+$ by electron donation from an endogenous donor.

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