Magnetic field-induced increase in chlorophyll a delayed fluorescence of photosystem II: A 100- to 200-ns component between 4.2 and 300 K

(photosynthesis/primary acceptor W/luminescence/radical pair mechanism/primary donor P-680)

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ABSTRACT At room temperature the delayed fluorescence (luminescence) of spinach chloroplasts, in which the acceptor Q is preoxidized, consists of a component with a lifetime of 0.7 \( \mu \)s and a more rapid component, presumably with a lifetime of 100–200 ns and about the same integrated intensity as the 0.7-\( \mu \)s component. Between 4.2 and 200 K only a 100- to 200-ns luminescence component was found, with an integrated intensity appreciably larger than that at room temperature. At 77 K the 150-ns component approached 83% of saturation at roughly the same energy as the variable fluorescence of photosystem II at room temperature. At 77 K the emission spectra of prompt fluorescence but not that of the 150-ns luminescence had a preponderant additional band at about 735 nm. The 150-ns emission also occurred in the photosystem I-lacking mutant FL5 of Chlamydomonas. These experiments indicate that the 150-ns component originates from photosystem II. At room temperature a magnetic field of 0.22 T stimulated the 0.7-\( \mu \)s delayed fluorescence by about 10%. At 77 K the field-induced increase of 150-ns component amounted to 40–50%, being responsible for the observed 2% increase of the total emission; the magnetic field increased the lifetime about 20%. In order to explain these phenomena a scheme for photosystem II is presented with an intermediary acceptor W between Q and the primary donor chlorophyll P-680; recombinant of P-680+ and W- causes the fast luminescence. The magnetic field effect on this emission is discussed in terms of the radical pair mechanism.

Since the discovery of delayed chlorophyll a fluorescence (in the following we use the term "luminescence" for the sake of simplicity) by Streher and Arnold in 1951 (1) in the green alga Chlorella pyrenoidosa, it has been suggested by many workers that the emission of luminescence is caused by back reactions, which result in the reversal of the primary photochemical reaction, albeit with a low efficiency, and thus in the reexcitation of chlorophyll a and in light emission. For photosystem II (PS II) reexcitation of chlorophyll a takes place by the recombination of the oxidized primary donor P-680 and reduced acceptor. For a discussion of the so-called recombination hypothesis and evidence for it the reviews of Lavorel (2), Malkin (3), and Amesz and van Gorkom (4).

For spinach chloroplasts and the green alga Chlorella vulgaris a 1-\( \mu \)s component was reported (5, 6), which mainly seemed to originate from reaction centers which were in the so-called "closed" state P-680 -. Q is an acceptor, a plastoquinone, formerly believed to be the primary acceptor. In the presence of reduced Q, Q-, the fluorescence yield of chlorophyll a is high (7). In the light of the recombination hypothesis another acceptor W different from Q was postulated and the luminescence was postulated to be due to the charge recombination P-680+ W- Q- \( \rightarrow \) P* W Q- \( \rightarrow \) P W Q* + h\( \nu \). W might be an intermediary acceptor between P and Q analogous to I in photosynthetic bacteria, or W might be on a side path. In bacterial species in which the noniron complex X is preoxidized, a 10-ns luminescence component has been found due to the recombination of P-670+ and I*, the oxidized bacteriochlorophyll dimer and the reduced bacteriocephepthin, respectively (8–10). This luminescence slightly but progressively decreases from 300 K down to 77 K (8, 9, 11). A magnetic field of the order of 1–100 mT decreased the reaction center triplet yield and induced a luminescence increase, which was determined by measuring the fractional increase of the emission (the sum of fluorescence and luminescence) (12–14). This phenomenon was explained in terms of the radical pair mechanism (see ref. 15 for an introduction to the theory of this mechanism).

We have investigated the temperature dependence of the (sub) microsecond luminescence of PS II of spinach chloroplasts in which the acceptor Q was preoxidized. Lowering the temperature revealed a strong luminescence component with a lifetime of 100–200 ns. Surprisingly, upon cooling we found, in contrast to bacteria mentioned above, an increase of the total luminescence. We also studied the influence of magnetic fields up to 0.3 T on the chlorophyll a luminescence emission yield. These experiments were carried out in two different ways. First by measuring exclusively luminescence after a short laser flash and second by determining the total emission (the sum of fluorescence and luminescence), using a continuous lamp as excitation source, both in the absence and presence of a magnetic field.

MATERIALS AND METHODS

Chloroplasts were prepared from spinach leaves as described in ref. 16. Chlorella vulgaris strain W.T. and Chlamydomonas reinhardtii strains W.T. and FL5 were grown as described in the refs. 17 and 18, respectively. The chloroplast suspension was diluted with isolation medium [N-tris(hydroxymethyl)methyglycine, pH 7.8/0.4 M sucrose/10 mM KCl/5 mM MgCl2]. The suspensions of algae were diluted with growth medium. The diluted suspensions were mixed with a solution of glycerol, which was almost saturated with sucrose, in a ratio of 40:60 (vol/vol) in order to maintain cryostabilization upon cooling. The final absorbance of all suspensions was adjusted to 0.1 mm at 680 nm, corrected for scattering. The sample was contained in a Perspex cell of 1 mm thickness. In the case of chloroplasts a few grains of sodium dithionite (final concentration about 10 mM) were added 5 min prior to the measurements to reduce Q.

Luminescence measurements were performed with the apparatus and methods described in ref. 19. The sample was illuminated with a 505-nm line of a 15-W xenon lamp, which was combined with a 20-mW red laser (680-nm line) and the luminescence was measured by a photomultiplier (Hamamatsu R928) and a boxcar integrator (Ortec 450). The spectra were recorded with a grating monochromator (Jobin Yvon HR 800).

Abbreviations: PS II, photosystem II of photosynthesis; PS I, photosystem I of photosynthesis; P, primary donor P-680 of PS II, probably a chlorophyll dimer; W, primary acceptor of PS II; Q, electron acceptor of PS II, a plastoquinone molecule; Z, secondary donor of PS II of unknown identity; B, magnetic field in Tesla units; F, chlorophyll emission (fluorescence plus luminescence); L, chlorophyll luminescence or delayed fluorescence; a.u., energy/s in arbitrary units L and F.
placed between the poles of a small home-built electromagnet. In order to prevent orientation of the luminescing species in the magnetic field, the magnet was fed from the 50-Hz mains. The effect of the alternating magnetic field up to 0.6 T on the photomultiplier was much smaller than the accuracy of the measurement (<10⁻² of the luminescence).

RESULTS

Fig. 1A (curve a) shows the decay kinetics of the 0.7-μs luminescence component of spinach chloroplasts with prereduced Q at room temperature. Analysis of the curve indicates that a more rapid (100- to 200-ns) component also is present, for which further evidence will be given in Discussion. Curve b in this figure shows the luminescence decay kinetics under the same conditions at 77 K. It can be observed that at 77 K the lifetime of the luminescence is about 150 ns. Its amplitude is about one order of magnitude higher than at room temperature. In Fig. 1B the temperature dependence of the luminescence (lifetime and total yield) is shown for the whole region 77–300 K. Between 293 and about 200 K the 0.7-μs component disappears, and at 200 K only the 150-ns component remains. Below 200 K the lifetime of the latter component hardly changed, but the integrated luminescence increased by a factor of about 8 down to 77 K. Sometimes we found an increase by a factor of about 4 in the integrated luminescence that was accompanied by a flatter course in the region 77–150 K. Under the same conditions the fluorescence yield in this temperature range hardly changed, in agreement with ref. 20.

Fig. 2 shows the amplitudes at time zero (obtained by extrapolation) of the 150-ns luminescence component of spinach chloroplasts at 77 K as function of the energy of the laser flash. As shown in this figure, the luminescence is saturated at energies higher than 500 μJ/cm². The energy that produced 63% of the maximal luminescence amplitude is about 150 μJ/cm². At room temperature the energy for 63% saturation of the variable fluorescence of untreated chloroplasts in the same medium is also about 150 μJ/cm² when the same source of excitation is used (data not shown).

In Fig. 3 the 150-ns luminescence emission spectrum at 77 K of spinach chloroplasts is shown. The fluorescence emission spectrum is also displayed in this figure.

Fig. 4A shows the influence of a magnetic field on the amplitude of the 150-ns luminescence component at about 80

![Figure 1](https://via.placeholder.com/150)

**FIG. 1.** (A) Typical decay kinetics after a just-saturating laser flash of spinach chloroplasts in which Q was prereduced, at room temperature (curve a) and at 77 K (curve b); \( \tau \) is the lifetime of luminescence. For reasons of presentation the 0.7-μs luminescence at room temperature (a) is amplified by a factor of about 8. Luminescence is plotted as energy/s in arbitrary units (a.u.). (B) The integrated luminescence \( = \) (amplitude at zero) × \( \tau \) (○) and \( \tau \) (×) as a function of temperature. The lifetime \( \tau \) was obtained by a best-fit method assuming one exponential component, which presumably is not correct for temperatures larger than 200 K (see Discussion). Conditions were as given for A. The broken line represents the temperature dependence of the relative fluorescence yield, \( F \), measured under the same conditions. The measuring wavelength was 685 nm for both luminescence and fluorescence. Preliminary results indicate that at 4.2 K the decay time is about 200 ns and the integrated luminescence is roughly 8 a.u.

![Figure 2](https://via.placeholder.com/150)

**FIG. 2.** Amplitude of the 150-ns luminescence at 77 K of spinach chloroplasts, in which Q was prereduced, as function of the laser flash energy. Exciting wavelength was 530 nm; measuring wavelength was 685 nm.
Chlamydomonas component of the increase in magnetic field-induced luminescence, the magnitude of which was smaller than 20% of the spin-photosynthetic bacteria (cf. refs. 12–14). It was found at room temperature, which is sufficiently close to the singlet level for the magnetic field-induced luminescence-inducing flash to be appreciable when Q is reduced prior to the luminescence-inducing flash. At low temperature a strong component of about 150 ns was observed with prerduced Q. Analogously with the hypothesis proposed for the 0.7-μs luminescence component at room temperature (see Introduction), we attribute the 150-ns luminescence to recombination of \( P^+ \) and \( W^- \), which results in the excited state \( P^+W^- \). The following arguments indicate that the 150-ns component originates in PS II. The saturation of the 150-ns component at 77 K (see Fig. 2) occurs at roughly the same energy as that for the variable fluorescence of PS II at room temperature. This indicates that reaction center chlorophyll of this photosystem is the source of the luminescence. It has been proposed that the fluorescence emission of chloroplasts and algae at 77 K has maxima at 685 and 695 nm belonging to chloroplasts and algae at 77 K (26) is similar to the 150-ns luminescence spectrum, displayed in Fig. 3, which also does not contain the peak around 735 nm. From these data we conclude that the latter spectrum probably originates from the chlorophyll of PS II. This conclusion is strengthened by the presence of the 150-μs luminescence component in the PS I-lacking mutant FL5 of Chlamydomonas.

A scheme for energy and electron transfer reactions occurring in the antenna and the reaction center complex of PS II, consistent with the experiments discussed and the magnetic field-induced increases (Fig. 4) in luminescence and fluorescence, is given in Fig. 5. This scheme is similar to schemes proposed for photosynthetic bacteria (cf. refs. 12–14). It should be mentioned that the state \( P^T \), the reaction center chlorophyll in the lowest triplet state, has not been observed (possibly because of rapid decay) in PS II but was experimentally observed in bacterial mutants lacking carotenoids.

The marked magnetic field-induced increase of the amplitude and lifetime of the 150-ns luminescence component as shown in Fig. 4 may be explained by the radical pair mechanism: In a high magnetic field \( B \), only the triplet sublevel \( T_0 \) (not \( T_1 \) and \( T_-1 \)) is sufficiently close to the singlet level for the occurrence of the interconversion \( (P^+W^-)^- \rightarrow (P^+W^-)^T \). The field-reduced interconversion causes a lowering of the ratio \( (P^+W^-)^T/ (P^+W^-)^- \) and thus an increase of the concentration \( (P^+W^-)^- \). Therefore the concentration of \( P^+W^- \), and thus the luminescence intensity, is increased by the magnetic field. If the triplet formation \( (P^+W^-)^T \rightarrow P^T W^T \) is the reaction that mainly determines the decay rate of \( P^+W^- \), then the magnetic field will increase the luminescence decay time. If the decay was mainly caused by \( k_g \) (or by \( k_s \), but this is presumably rel-
FIG. 5. Hypothetical scheme for energy and electron transfer occurring in the antenna chlorophyll and the reaction center complex of PS II, in the presence of reduced acceptor Q, $k_\text{a}$, $k_\text{b}$, $k_\text{c}$, and $k_\text{d}$ are the rate constants for deexcitation of the lowest excited singlet states $A^*$ and $P^+$ by energy transfer, fluorescence, internal conversion, and intersystem crossing to the triplet states $A^*$ and $P^T$. The decay of the triplet states to the ground state occurs via $k_\text{c}$, $k_\text{d}$ is the rate constant for charge separation. $k_\text{T}$, $k_\text{f}$, and $k_\text{i}$ are the rate constants for the recombination of $P^+W^-$ to the triplet state $P^T$, the excited singlet state $P^*$, and the ground state, respectively. $\omega$ is the angular frequency for interconversion between the singlet and triplet states of $P^+W^-$. $Z$ is the secondary donor, which reduces $P^+$ with a rate constant $k_\text{d}$. For further explanation see text.


tively small), then the magnetic field would decrease the decay time because the magnetic field increases the concentration of $(P^+W^-)_S$. The observed increase in luminescence lifetime thus indicates that $k_\text{f}$ is rate determining, because the magnetic field reduces the amount of $(P^+W^-)_T$.

The fractional increase of fluorescence, $\Delta F/F$, as a function of external magnetic field closely follows the relative field-induced change of the 150-ns luminescence (Fig. 4A). We conclude that $\Delta F$ is mainly if not completely caused by a change in the 150-ns luminescence: In a magnetic field of 0.15 T we measured an increase of 40% of the total emission of the 150-ns luminescence (obtained by extrapolation to zero). The yield of the 150-ns component was established to be 20 times smaller than the fluorescence yield, which means that $\Delta F/F$ should be $0.05 \times 40\% = 2\%$ at $B = 0.15$ T, in agreement with the value of 1.7% for $\Delta F/F$ obtained by direct measurement.

At room temperature the magnetic field-induced ($B = 0.22$ T) increase of the 0.7 $\mu$s luminescence, measured at 0.4–4 $\mu$s, was about 10%. The yield of this luminescence (measured in flashes of low intensity) is about 1/160th of the fluorescence yield under the same conditions. If the 0.7-$\mu$s luminescence component were the only present, a value of 1/160 x 10% = 0.06% would be expected for $\Delta F/F$ at room temperature. We measured a value of about 0.2% for $\Delta F/F$. This difference can be explained by the presence at room temperature of a second, more rapid, luminescence component with a somewhat larger integrated luminescence than that of the 0.7-$\mu$s component. The observation of an at least 15% increase in luminescence integrated from 0.25–0.8 $\mu$s indeed shows that such a component is present. Because of the presence of the 0.7-$\mu$s component and the rapidity of this component we could not measure it with sufficient precision with the apparatus as it is at the moment. We estimate that the amplitude of this fast component was roughly 1/10th of that at 77 K, and its decay time was similar.

Corresponding curves of $\Delta F/F$ against $B$ for photosynthetic bacteria approach saturation at about $B = 150$ mT; half the effect is attained at $B_{1/2} = 25–35$ mT (14), as compared to 65 mT for half-saturation in chloroplasts (Fig. 4A). At room temperature we observed a value of $B_{1/2}$ that was about half the value of $B_{1/2} = 65$ mT at 77 K. Preliminary experiments also showed a considerable decrease (to ½ to 1/6) of $B_{1/2}$ at 80 and 290 K when the chloroplasts were suspended in isolation medium brought to pH 3.

Magnetic field modulation of the luminescence has been found in vitro not only when the luminescence is produced by radical pair recombination but also when it is produced in a process known as triplet exciton fusion (for an introduction and review see refs. 27–29). It seems to us that this mechanism might be consistent with our results if the two triplets are generated in one reaction center by heterofusion $P^C \rightarrow P^T C^T$ in which C is a carotenoid molecule and $C^T$ is the lowest carotenoid triplet state. Luminescence is generated by the reverse reaction. Such luminescence may be increased by a magnetic field (see also ref. 30). In an analogous way as for the radical pair mechanism one might assume that $P^T$ (or $C^T$) disappears in 150 ns independent of temperature. Normally the triplet decay to the ground state requires milliseconds (chlorophyll triplet) or microseconds (carotenoid triplet), but the triplet might be transferred to another molecule. For this mechanism we do not understand the observed increase in luminescence lifetime caused by a magnetic field.

Finally, we will discuss the temperature behavior of integrated luminescence and luminescence lifetime as displayed in Fig. 1. Lowering the temperature may be expected to cause a decrease in the integrated luminescence and an increase in lifetime, because of the activation energy needed for the back reaction $P^+W^+ \rightarrow P^+W$. To our surprise the opposite was observed: when the temperature was lowered from 290 to 80 K the integrated luminescence increased by a factor of 8, the 0.7-$\mu$s component disappeared, and a 150-ns component increased in amplitude but not in decay time. Even at 4.2 K this fast component exists, with a decay time of about 200 ns.

A possible explanation for the approximate constancy of the decay time of the 150-ns component with the decreasing temperature is the following. The decay time of this component may be assumed to be equal to the decay time of the radical pair $P^+W^-$, which decay time is mainly determined by the most rapid reaction, removing the state $P^+W^-$. At low temperatures the reduction of $P^+$ by the secondary donor $Z$ has a small rate constant $k_4$ of the order of 0.1–20 ms$^{-1}$ (31, 32) and therefore $k_4$ probably does not affect markedly the decay rate of the radical pair. Because a magnetic field of 0.22 T increases the luminescence lifetime by about 20% the reaction with rapid constant $k_T$ (see scheme of Fig. 5) seems to be rate determining. Recombination of $P^+W^-$ to the reaction center triplet state $P^T W$ may be a temperature-independent electron tunneling. Then the luminescence decay time will be essentially independent of temperature because, as already discussed, the magnetic field effects suggest that the reaction center triplet formation is the most rapid reaction. The luminescence intensity is determined by the rate of the back reaction $P^+W^+ \rightarrow P^+W^- (\rightarrow PW + hv)$. On the basis of the yield at 80 K of the 150-ns component, which is 0.5% (0.05 times the yield of fluorescence, which is about 10%) the rate constant $k_T$ for this back reaction may be of the order of $10^6$ s$^{-1}$ at 80 K. The sign of the magnetic field effect indicates that the reaction of $P^+W^+$ to the ground state (rate constant $k_5$ in the scheme of Fig. 5) is also rather slow. For photosynthetic bacteria $k_5$ was also found to be relatively small in comparison with the other rate constants for the decay of the radical pair (11, 33).

The 0.7-$\mu$s component at room temperature might be explained by the assumptions that at room temperature part of $Z$ is oxidized by $P^+$ in a time longer than 150 ns: $Z^+$ is reduced by a tertiary donor in 0.7 $\mu$s and at a smaller rate by the back reaction $Z^+ P^+ W^- \rightarrow Z P^+ W^-$, which causes the 0.7-$\mu$s luminescence component. The $\Delta L$ and $\Delta F$ measurements at room temperature show that a fast component, which may be the
of financed computer programs for the measurements A.S. van mutant spectroscopy.

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