Biochimica et Biophysica Acta, 636 (1981) 39–49
Elsevier/North-Holland Biomedical Press

BBA 48062

SUB-MICROSECOND CHLOROPHYLL *a* DELAYED FLUORESCENCE FROM PHOTOSYSTEM I

MAGNETIC FIELD-INDUCED INCREASE OF THE EMISSION YIELD

ARIE SONNEVELD, LOUIS N.M. DUYSENS and ADRI MOERDIJK

Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands)

(Received December 2nd, 1980)

Key words: Photosystem I; Chlorophyll a; Luminescence; Magnetic field effect; Radical pair mechanism; Fluorescence yield

(1) In photosystem I (PS I) particles in the presence of dithionite and intense background illumination at 290 K, an external magnetic field (0–0.22 T) induced an increase, ΔF, of the low chlorophyll *a* emission yield, F ($\Delta F/F \simeq 1-1.5\%$). Half the effect was obtained at about 35–60 mT and saturation occurred for magnetic fields higher than about 0.15 T. In the absence of dithionite, no field-induced increase was observed. Cooling to 77 K decreased ΔF at 685 nm, but not at 735 nm, to zero. Measuring the emission spectra of F and ΔF, using continuous excitation light, at 82, 167 and 278 K indicated that the spectra of F and ΔF have about the same maximum at about 730, 725 and 700 nm, respectively. However, the spectra of ΔF show more long-wavelength emission than the corresponding spectra of F. (2) Only in the presence of dithionite and with (or after) background illumination, was a luminescence (delayed fluorescence) component observed at 735 nm, after a 15 ns laser flash (530 nm), that decayed in about 0.1 μs at room temperature and in approx. 0.2 μs at 77 K. A magnetic field of 0.22 T caused an appreciable increase in luminescence intensity after 250 ns, probably mainly caused by an increase in decay time. The emission spectra of the magnetic field-induced increase of luminescence, ΔL, at 82, 167 and 278 K coincided within experimental error with those of ΔF mentioned above. The temperature dependence of ΔF and ΔL was found to be nearly the same, both at 685 and at 735 nm. (3) Analogously to the proposal concerning the 0.15 μs luminescence in photosystem II (Sonneveld, A., Duysens, L.N.M. and Moerdijk, A. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5889–5893), we propose that recombination of the oxidized primary donor P-700$^+$ and the reduced acceptor A$^-$, probably A_1^-, of PS I causes the observed fast luminescence. The effect of an external magnetic field on this emission may be explained by the radical pair mechanism. The field-induced increase of the 0.1–0.2 μs luminescence seems to be at least in large part responsible for the observed increase of the total (prompt + delayed) emission measured during continuous illumination in the presence of a magnetic field.

Introduction

In addition to the primary donor P-700, the reaction center complex of PS I probably contains three electron acceptors, A_1, A_2 and P-430; the complex may be written as P-700 → A_1 → A_2 → P-430 [1–3]. The arrows indicate electron transfer starting after excitation of P-700: P-700 + $h\nu$ → P-700*. As reviewed in Ref. 4, optical and EPR spectroscopy indicate that P-430 is an iron-sulfur protein. Recently, experimental evidence was presented indicating that A_2 is probably some kind of an iron-sulfur center [5] identical to X, which was postulated to be an electron acceptor between P-700 and P-430 [6–8]. From optical and EPR spectra, Shuvalov et al. [5,9,10] concluded that A_1 was a chlorophyll *a* dimer, but on the basis of less

Abbreviations: PS, photosystem; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea.

extensive evidence, other authors concluded that A_1 is a chlorophyll or pheophytin monomer [11–13].

If P-430 and A_2 (X) are reduced by dithionite and intense continuous light (e.g., see Refs. 1, 2 and 14), the state P-700 $A_1A_2^-$ P-430$^-$ presumably will persist for some time after darkening (see Discussion). In the presence of A_2^-, after a laser flash, the following reactions may be expected to occur: P-700 $A_1 + h\nu \rightarrow$ P-700$^*A_1 \rightarrow$ P-700$^+A_1^- \rightarrow$ P-700$^*A_1 \rightarrow$ P-700 A_1 + delayed fluorescence (luminescence). In addition, non-emissive back reactions may occur such as P-700$^+A_1^- \rightarrow$ P-700$^TA_1$, leading to the formation of the triplet state of P-700.

In the literature, there are many reports about luminescence from photosynthetic bacteria and PS II of a variety of species (see Refs. 15–17 for a survey). Papers concerning luminescence from PS I are scarce and in a number of studies on PS I particles and/or mutants which lack PS II it is reported that PS I luminescence is absent or negligible [15–17].

Shuvalov [18] concluded that dark recombination of P-700$^+$ and P-430$^-$ was accompanied by a weak luminescence component with a lifetime of 20 ms at room temperature. Furthermore, Shuvalov suggested that sub-millisecond chlorophyll *a* delayed fluorescence under reducing conditions arose from thermal activation of a (reaction center) chlorophyll triplet at 293 K and from triplet-triplet annihilation at 77 K. We have made luminescence measurements on PS I in a 1000-times shorter time range using a 15 ns laser flash as excitation source. Between 77 and 290 K, we have observed an 0.1–0.2 μs luminescence component under conditions in which P-430 and probably also A_2 were prereduced. From the luminescence enhancement by a magnetic field, we have concluded that the sub-microsecond luminescence was caused by charge recombination, probably at P-700$^+$, from A_1^-. We also found a magnetic field-induced increase in the total PS I emission, fluorescence plus luminescence, measured in continuous light. It is concluded, from the emission spectra and temperature dependence, that the magnetic field-induced increase in sub-microsecond luminescence and fluorescence both have the same origin, probably the PS I reaction center.

Magnetic field-induced changes in luminescence and total emission and reaction center triplet yield were observed earlier under reducing conditions for several photosysnthetic bacteria [19–27] and PS II [22,24,28,29]. The magnetic field effect was explained by the so-called radical pair mechanism. Shortly before our investigations on PS I were completed, Voznyak et al. [30] reported some data on the fluorescence yield dependence on external magnetic fields for this photosystem (see Discussion).

Materials and Methods

PS I particles were isolated from spinach chloroplasts with the aid of 5% Triton X-100 and sucrose gradient centrifugation [31]. They were kept frozen at −20°C until needed. P-700 was determined as described in Ref. 32; the preparation contained about 80 chlorophyll molecules per P-700 molecule.

The suspension of PS I particles was diluted with 10 mM Tris-HCl buffer (pH 8) and mixed in a ratio of 40 : 60 (v/v) with an almost saturated solution of sucrose in glycerol that prevented crystallization upon cooling. PS I acceptors were reduced (see Discussion) about 10 min prior to the measurements by the addition of solid sodium dithionite (about 2 mg/ml) and strong preillumination (400–600 nm, 40 mJ $\cdot$ cm^{-2} $\cdot$ s^{-1}) of 5–10 min duration. In most experiments air was present before dithionite was added. In some experiments no glycerol was added and the air was removed by bubbling with oxygen-free nitrogen. In this case, the buffer contained 0.2 M glycine (pH 10), 2 mg/ml sodium dithionite and 20 μM neutral red and the period of preillumination was 1–2 min; this buffer (pH 10) is called the low redox buffer; the neutral red contribution to the fluorescence was subtracted.

The sample of absorbance 0.2 at 680 nm was contained in a perspex cell of 1 mm thickness, placed in a dewar vessel in which the temperature was varied by blowing through cold nitrogen (gas). The temperature was monitored with a copper/constantan thermocouple extending into the cell and could be kept constant to within 1–2 degrees Celsius.

The luminescence and fluorescence were measured using the apparatus and methods described in Ref. 33. These quantities have been expressed in arbitrary units (A.U.) of energy/s. The continuous excitation light for recording the (magnetic field-induced)

fluorescence emission (spectra) passed through a filter combination, Schott KG 1/5 and Corning CS 4-96, and a perspex light guide (400–600 nm, 10–50 mJ · cm^{-2} · s^{-1}); the spectra were corrected for the spectral sensitivity of the apparatus. Because of the low emission yield of the PS I particles, the slitwidth of the monochromator was set at 7 mm resulting in a transmitted bandwidth of 11 nm.

In order to study the effect of an external magnetic field on the emission yield, the sample was placed between the poles of a small home-built electromagnet. In order to prevent orientational effects on the emission due to the magnetic field, the current and thus the field of the magnet were alternating with the mains frequency (50 Hz); the current was varied by means of a Variac transformer and the maximum value of the field was 0.3 T (3 kG). The magnetic field strength was measured with an 811 AB Bell Incorp. Gauss meter using an HTJ 8 transversal probe. The direction of the field was perpendicular to the measuring and laser beams and to the cathode of the photomultiplier (see also Ref. 24). It was ensured (see Ref. 29) that magnetic fields up to 0.25 T did not affect the photomultiplier which was placed at a distance of about 1 m from the magnet gap.

Results

At room temperature we found practically the same results for PS I particles diluted in the low redox buffer (without glycerol) and samples containing Tris-HCl buffer and glycerol (see Materials and Methods for further details). However, the time of preillumination, to reach the maximal magnetic field-induced fluorescence increase, ΔF, was 5-times longer (about 3 min) for the latter mixture. The results described hereafter have all been obtained with samples containing Tris-HCl and glycerol.

Fig. 1 shows the effect of a 50 Hz magnetic field on the relative fluorescence yield of PS I particles, after the addition of dithionite and in the presence of strong background illumination, at different wavelengths and temperatures. Due to the magnetic field B, a stimulation of the fluorescence emission of maximally about 1.5% was observed that appeared to be independent of the direction of B. In the presence of ferricyanide (10 mM), which oxidizes

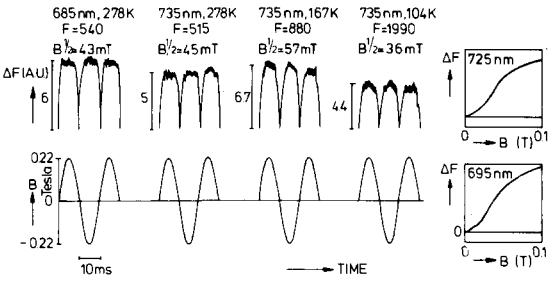

Fig. 1. Typical fluorescence changes (ΔF) of PS I particles in the presence of dithionite (about 2 mg/ml) and strong continuous background illumination caused by a sinusoidal magnetic field B (50 Hz, bottom curve) of 0.22 T peak value. ΔF is averaged over 500 periods and given for different wavelengths and temperatures as indicated; F is the relative value of the fluorescence at zero field and expressed in the same units as used for ΔF. The value of B producing half ΔF, $B_{1/2}$, is also shown; $B_{1/2}$ was obtained by plotting ΔF vs. B using a simple computer program: see the two plots on the right (278 K). A.U., arbitrary units.

P-700 chemically, no field-induced fluorescence increase could be detected. The same result was obtained in the absence of dithionite and when Triton X-100 was added (8%, v/v) to the the reaction medium. Heating the sample for 5 min at about 90°C also led to $\Delta F \simeq 0$. However, 5 min heating at 60–65°C decreased ΔF by about 50%. All these phenomena indicate that the reaction center of PS I is involved and that reducing conditions are necessary.

Fig. 1 further shows that ΔF at 735 nm was not lowered with decreasing temperature and that the value of B which gives half-stimulation, $B_{1/2}$, was rather temperature insensitive. A computer analysis showed that in the temperature range 77–290 K, $B_{1/2}$ varied between 35 and 60 mT and that saturation of the stimulation occurred (see flattening of the (sinusoidal) traces near the maximum) at fields higher than about 0.15 T. This behavior is completely different from that found for PS II in intact systems [28,29] and in isolated particles (Sonneveld, A. and Moerdijk, A., unpublished observations) where a strong increase of both ΔF and $B_{1/2}$ was observed upon cooling. Moreover, as shown by Fig. 2, ΔF of PS I at 685 and 735 nm as a function of temperature in the region 77–290 K was found to be completely different for the two wavelengths: ΔF(685) decreases to zero from 290 to about 150 K, but

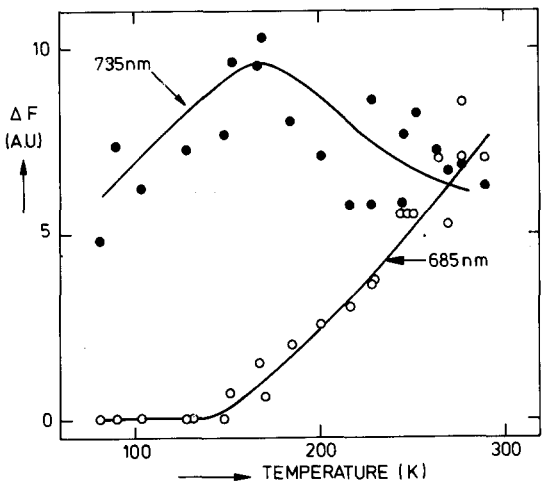

Fig. 2. Magnetic field-induced increase of fluorescence, ΔF, as a function of temperature for the wavelengths 685 (○) and 735 nm (●). Obtained from measurements as shown in Fig. 1. A.U., arbitrary units.

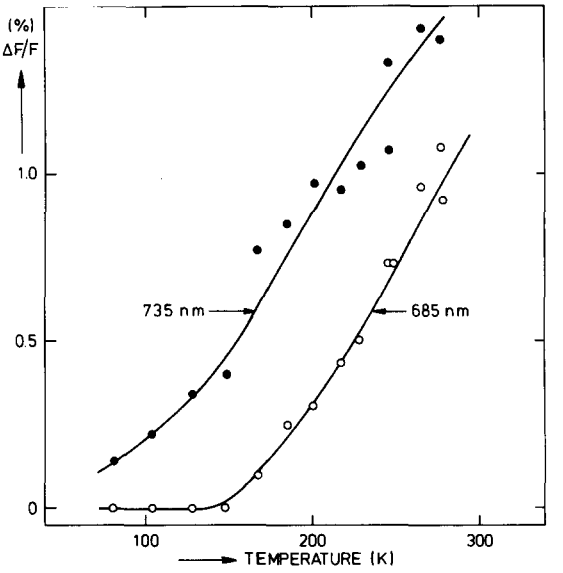

Fig. 3. Magnetic field-induced fractional increase of fluorescence, $\Delta F/F$, as a function of temperature at 685 (○) and 735 nm (●), obtained from measurements as shown in Fig. 1.

$\Delta F(735)$ has about equal values at 77 and 290 K with a small maximum at about 170 K *. However, $\Delta F/F$ decreases with temperature both at 685 and at 735 nm (Fig. 3): $F(735)$ increases upon lowering the temperature, whereas $F(685)$ is almost constant.

The results presented in Figs. 2 and 3 suggest that the emission spectra for F and ΔF are not proportional to each other at these temperatures. Indeed, these spectra (Fig. 4A–C) show considerable differences between F and ΔF, but the two emissions have maxima at about the same wavelengths: 700, 725 and 730 nm at 278, 167 and 82 K, respectively. For the purpose of comparison, also sub-microsecond luminescence emissions as a function of wavelengths are presented in Fig. 4A–C, but these data will be discussed later in this section.

Using a fast electronic gate for switching off the photomultiplier during a 15 ns actinic laser flash, the luminescence decay kinetics of PS I particles after the flash were measured under conditions as mentioned above for ΔF. Fig. 5 (curve a) shows these kinetics in the time range 0.25–20 μs, at room temperature, after a flash of about 500 $\mu J/cm^2$ in the absence of a magnetic field. The luminescence decay kinetics were described with good precision by a single exponential with a characteristic time of 110 ns. Curve b of Fig. 5 shows that the luminescence intensity after 250 ns is appreciably enhanced by a magnetic field of 0.22 T. Although the response time of 10 ns of our apparatus is not a limiting factor in recording such fast decaying signals, the time-lag of about 200 ns between the flash and the moment at which the photomultiplier is fully active made extrapolation to shorter times uncertain. Nevertheless, computer simulation showed that a magnetic field increases the lifetime of the decay by about 20% in all experiments, e.g., from 110 to 130 ns, the amplitudes at time zero being 40 and 41, respectively (Fig. 5). In other experiments the amplitudes sometimes tended to be somewhat larger in the presence of the magnetic field.

After the intense background illumination was switched off, the approx. 0.1 μs luminescence intensity remained almost constant for tens of minutes; at longer periods, in the absence of back-

* Some experiments have been performed on the temperature dependence of emission with a suspension of absorbance 0.1 with essentially similar results to those for an absorbance of 0.2. This indicates that the results are not markedly affected by changes in self-absorption upon cooling.

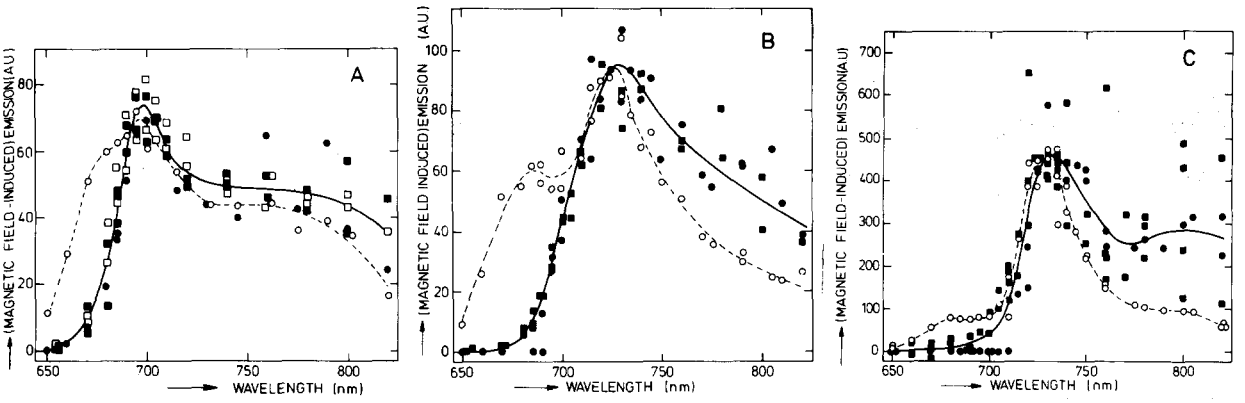

Fig. 4. Fluorescence and luminescence emission spectra of PS I particles in the presence of dithionite (about 2 mg/ml) and with (fluorescence) or after (luminescence) strong continuous illumination. Fluorescence, F (○); approx. 0.1 μs luminescence (only shown in A), L (□); magnetic field (B)-induced increase, ΔF ($\Delta F = F(B = 0.22\ \mathrm{T}) - F(B = 0)$) (●); magnetic field-induced increase of 0.1 μs luminescence, ΔL ($\Delta L = L(B = 0.22\ \mathrm{T}) - L(B = 0)$) (■). The luminescence was measured using repetitive 15 ns laser flashes (530 nm, 3 Hz) and L was determined by integration of the signal (see Fig. 5) between 0.25 and 0.8 μs. The points of ΔF, ΔL and L strongly suggest roughly similar spectra within the wide limits of accuracy and were represented by the solid line. The dashed line is drawn through the points of F. The spectra are normalized at the peak. The units for F are the same for A–C. Values of ΔF and ΔL for these figures can in principle be obtained from Figs. 2 and 8, respectively. Temperature: 278 (A), 167 (B), and 82 K (C). A.U., arbitrary units.

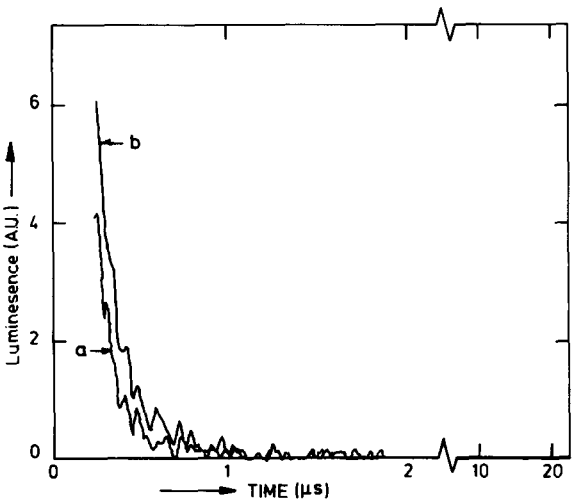

Fig. 5. Typical luminescence decay kinetics of PS I particles at 695 nm in the presence of dithionite (about 2 mg/ml) and continuous illumination, measured using repetitive just-saturating laser flashes (15 ns, 530 nm, 3 Hz); 36 signals were averaged. B is the strength of the external magnetic field (in T). Each signal was fitted with good precision by a single exponential with decaytime τ and amplitude A_0 at time zero. The temperature was 278 K. (a) $B = 0$, $A_0 = 40$, $\tau = 110$ ns; (b) $B = 0.22$ T, $A_0 = 41$, $\tau = 130$ ns. A.U., arbitrary units.

ground illumination, it gradually decreased. In that case, the original maximal luminescence intensity was obtained again after a few minutes of strong background illumination. In the absence of dithionite or in the presence of dithionite but without preillumination, the 0.1 μs luminescence was negli-

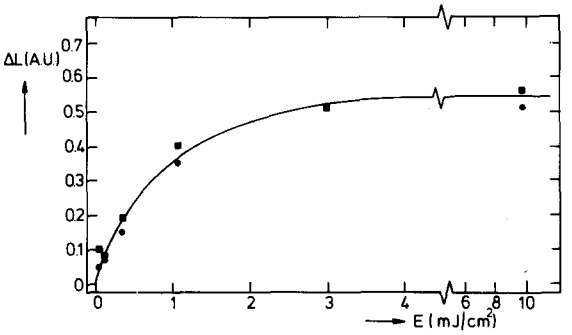

Fig. 6. The magnetic field (B)-induced increase of 0.1 μs luminescence of PS I particles (conditions as given in Fig. 5), ΔL, as function of the laser excitation energy for the emission wavelengths 685 (■) and 735 (●) nm. ΔL is determined by taking the integral of the luminescence signal $L(B = 0.22\ \mathrm{T})$ minus that of $L(B = 0)$ in the time region 0.25–0.8 μs; ΔL for 685 nm is multiplied by a factor of about 2. A.U., arbitrary units.

gible. After other treatments such as 5 min heating at 90°C, or the addition of ferricyanide (final concentration 10 mM) or Triton X-100 (8%, v/v), no luminescence could be observed after addition of dithionite and in strong light. About 50% luminescence remained after 5 min heating at 60–65°C. All these results strongly suggest that the PS I reaction center is involved in the emission of the about 0.1 μs luminescence component.

Fig. 6 gives the magnetic field-induced luminescence increase, ΔL, in the 0.25–0.8 μs time region as a function of the laser excitation energy. The luminescence amplitude as a function of the laser energy was about proportional to that of ΔL (data not shown). For the emission wavelengths 685 and 735 nm, almost equal saturation characteristics were found: at an energy of approx. 900 $\mu J/cm^2$, the 0.1 μs luminescence amounted to 63% of the maximum value attained at about 3 mJ/cm^2 or greater. By linearly extrapolating the initial slope of the saturation curve, it was estimated that the 'saturation energy' of the 0.1 μs component corresponds to about one photon absorbed per 50–60 chlorophyll molecules, i.e., about one photon per P-700 molecule. This estimate is in reasonably good agreement with the determination of about 80 chlorophyll molecules per P-700 molecule as reported in Materials and Methods, especially if one takes into consideration the possibility that a certain amount of chlorophyll *a* may not transfer its energy with good efficiency to the reaction center.

The lifetime of the approx. 0.1 μs component increased to about 0.2 μs upon lowering the temperature to 80 K (see Fig. 7). Because the luminescence lifetime, τ, was about equal at 685 and 735 nm, the average value of τ for both wavelengths is displayed in Fig. 7. Below 167 K, only the data for 735 nm are given in Fig. 7 because the luminescence at 685 nm was small at such low temperatures which made accurate curve fitting impossible. The lifetime is lengthened at all temperatures when a magnetic field of 0.22 T is applied. Cooling decreased the amplitude of luminescence at 685 nm, but at 735 nm there was little or no decrease (data not shown). For the purpose of comparison with ΔF, Fig. 8 shows the field-induced increase of luminescence, ΔL, as function of temperature; ΔL was obtained by taking the difference of the integrals of $L(B = 0.22\ T)$ and $L(B = 0)$ between 0.25 and 0.8 μs (see Fig. 5). A reasonable coincidence between the curve of ΔF (Fig. 2) and that of ΔL (Fig. 8) as

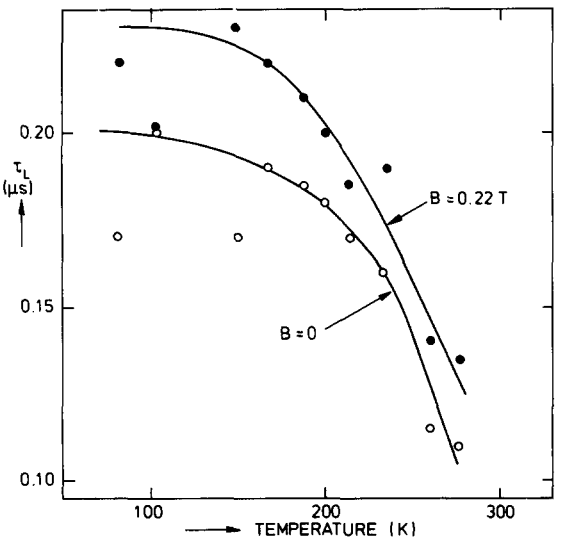

Fig. 7. Lifetime of luminescence, τ_L, of PS I particles in the presence of dithionite (about 2 mg/ml) and after strong preillumination, as function of temperature in the absence (○) and presence (●) of a magnetic field $B = 0.22$ T. For further conditions and explanation see Fig. 5.

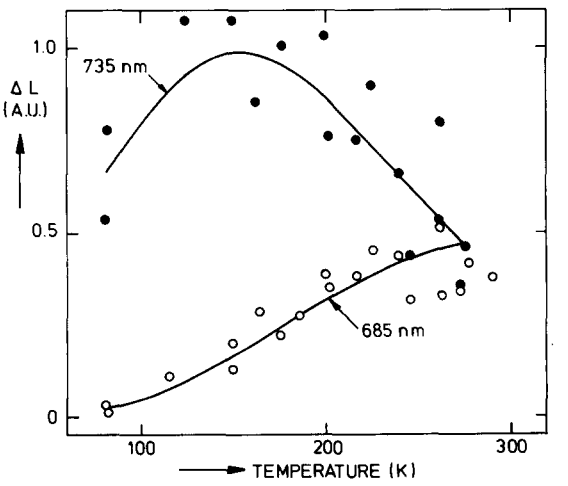

Fig. 8. Magnetic field-induced luminescence increase, ΔL, of PS I particles at 685 nm (○) and 735 nm (●) as a function of temperature. For further explanation see Fig. 5 and text. A.U., arbitrary units.

functions of temperature for both 685 and 735 nm is observed. Moreover, ΔL as a function of B at 278 K almost followed the course of ΔF vs. B that is shown in Fig. 1 on the right (ΔL data not shown).

In order to show further the identical origin of ΔL and ΔF, we measured the emission spectra of ΔL at 278, 167 and 82 K. Although the measuring points beyond 730 nm scatter (the fluorescence yield of the PS I preparation is about 10-times lower than that of intact chloroplasts), the data shown in Fig. 4A–C (the solid lines are drawn through about the average value of the points of ΔF and ΔL) strongly suggest that the spectra of ΔF and ΔL are proportional to each other. As shown in Fig. 4A, the points of the approx. 0.1 μs luminescence, L, coincide rather well with those of ΔL and ΔF. All these data indicate that L, ΔL and ΔF have the same origin for which the reaction center of PS I is the most likely candidate in view of the effect of the different treatments mentioned earlier and saturation of ΔL (Fig. 6).

Discussion

The 0.1–0.2 μs photosystem I luminescence

In the presence of dithionite and in continuous light, the PS I acceptors P-430 and A_2 (or X) will be reduced [1,2,5,14]. Excitation of the complex P-700 A_1A_2P-430$^-$ leads to the formation of the radical pair P-700$^+A_1^-$, which will decay by three decay pathways: (1) recombination to the first excited singlet state P-700$^*A_1$; (2) recombination to the reaction center triplet state P-700$^TA_1$; (3) recombination to the ground state P-700 A_1. The approx. 0.1 μs luminescence shown in Fig. 5 is most likely due to recombination of P-700$^+$ and A_1^- to the state P-700$^*A_1$ from which emission can occur directly or via back transfer to the antenna. The following arguments support this hypothesis:

(1) The saturation characteristics of this component (Fig. 6) indicate that the PS I reaction center is involved.

(2) The magnetic field effect indicates that the luminescence is caused by charge recombination, probably at P-700$^+$, and that the decay of this luminescence is probably mainly caused by recombination to the state P-700$^TA_1$ (see below).

(3) No microsecond luminescence was observable under ambient redox conditions with air (P-430 oxidized) or in the presence of dithionite without (pre) illumination (P-430 reduced but A_2 oxidized).

(4) Heating the sample for 5 min at 60–65°C, which is sufficient to destroy P-430 [18,34] and probably also A_2 [13], did not abolish the 0.1 μs luminescence, suggesting also that P-700 and A_1 play a role. However, the 0.1 μs component was almost absent if dithionite and preillumination were omitted after heat treatment.

(5) Contaminating PS II remains are probably not the source of the observed luminescence, because the procedure of PS I particle preparation using 5% (w/w) Triton X-100 would probably destroy PS II. Furthermore, ΔF of PS II is about maximal at 685 nm at 77 K [29] where the observed ΔF is zero. Also, the magnetic field dependence and the emission spectra are much different from those of PS II [28,29]. Moreover, in the presence of hydroxylamine and DCMU, which would give maximal luminescence in the case of PS II [29], the 0.1 μs luminescence was not observable.

After switching off the background illumination, we observed almost no decrease in luminescence intensity for tens of minutes. This indicates that A_2^- is reoxidized very slowly in our samples. The about 10 s decay of A_2^- reported by Shuvalov et al. [5] may be caused by the presence of certain mediators that were absent in our reaction mixture. We also found in the range of tens of minutes no decrease of the PS I fluorescence emission yield after switching off the background illumination. This would be expected if A_2^- (X^-) became reoxidized [14].

As shown by Fig. 5 (curve b), the approx. 0.1 μs luminescence after 250 ns is appreciably increased by a magnetic field and the luminescence decay time is increased by about 20%. These phenomena are characteristic for the radical pair mechanism (see Ref. 35 for an introduction). The radical pair P-700$^+$-A_1^-, initially present in a singlet state, will, because of different hyperfine or other magnetic interactions and different g values of the electron spins, oscillate between singlet and triplet states: $(\text{P-700}^+A_1^-)^S \rightleftharpoons (\text{P-700}^+A_1^-)^T$. The state $(\text{P-700}^+A_1^-)^S$ will directly recombine to P-700$^*A_1$ (the rate constant for the recombination reaction to the ground state P-700 A_1

is assumed to be relatively small, cf. Refs. 24 and 28) and the state $(P\text{-}700^+A_1^-)^T$ to the reaction center triplet state $P\text{-}700^TA_1$; generation of $P\text{-}700^T$ has been observed at 11 K [36]. In the presence of a sufficiently high external magnetic field, the ratio $(P\text{-}700^+A_1^-)^S/(P\text{-}700^+A_1^-)^T$ will increase because the triplet substates of $(P\text{-}700^+A_1^-)^T$ will split up so that practically only one triplet level (T_o) instead of three will be available [19,35]. This causes an enhancement of the $(P\text{-}700^+A_1^-)^S$ concentration and thus of the recombination rate to the excited singlet state $P\text{-}700^*A_1$, resulting in an increase of luminescence, as observed experimentally. The lengthening of the lifetime of 0.11 to 0.13 μs (Fig. 5) can be explained by assuming that the lifetime of the radical pair is largely determined by the recombination $(P\text{-}700^+A_1^-)^T \rightarrow P\text{-}700^TA_1$ [24], as has been concluded for photosynthetic bacteria [26] and PS II [28,29].

From sub-nanosecond absorbance difference spectroscopy data at room temperature, it was concluded by Shuvalov et al. [9] that $P\text{-}700^+$ and A_1^- recombine with two lifetimes, $\tau_1 = 10$ ns and $\tau_2 \simeq 3$ μs, where τ_2 is the decay into a 'state with some triplet character'. Under similar conditions, decay times of about 3 μs were found earlier by Sauer et al. [1,2] using the same method; these decays were ascribed to the back reaction of $P\text{-}700^+$ and A_1^-. A corresponding ΔA decay time of 5–10 μs was observed in sodium dodecyl sulfate-treated and heated PS I complexes at room temperature [3,12,13]. Our approx. 0.1 μs decay time for $P\text{-}700^+A_1^-$ at room temperature differs from those mentioned above. The failure to observe the 0.1 μs component may be caused by the response time of the apparatus mentioned in Refs. 1–3, 12 and 13, which is too slow, whereas the ΔA transients of Ref. 9 do not give kinetic information in the time region 3.6 ns–1 μs. A 10 ns and 3 μs decay of $P\text{-}700^+A_1^-$ might be only weakly emissive or absent in our particles due to different techniques of sample preparation (cf. Refs. 1–3). However, the following assumptions and arguments might be reconcilable with the three different observed decay times: (a) From the state $(P\text{-}700^+A_1^-)^S$, an effective back reaction of 10 ns to $P\text{-}700^*A_1$ may occur that was observed by Shuvalov et al. [9] via the $P\text{-}700^+/P\text{-}700$ absorption change at 694.3 nm. (b) The 0.1 μs luminescence decay may indirectly reflect the loss caused by the recombination reaction $(P\text{-}700^+A_1^-)^T \rightarrow P\text{-}700^TA_1$, as proposed above for the explanation of the magnetic field effect. From time-resolved EPR spectroscopy at room temperature, Dismukes and Tycko [37] estimated a recombination halflife of about 0.5 μs for the pair $P\text{-}700^+A_1^-$. Because the time resolution of their apparatus was about 200 ns, the true halflife may be shorter, and perhaps be the same as the value of 0.1–0.2 μs reported by us. (c) When the state $P\text{-}700^TA_1$ decays in about 3 μs to the ground state P-700 A_1, this reaction might be responsible for the 3 μs ΔA decay kinetics reported in Refs. 1–3 and 9. Sauer et al. [2] mentioned that they could not exclude a chlorophyll *a* triplet being responsible for the 3 μs decay. In addition, the microsecond difference absorption spectrum at 25°C of Baltimore and Malkin [12,13] is not very different from that of a chlorophyll *a* triplet (e.g., see Ref. 38).

Down to 77 K, the lifetime of luminescence increases to about 0.2 μs. This can be explained by assuming a slight increase of recombination time of $(P\text{-}700^+A_1^-)^T$ to $P\text{-}700^TA_1$; the recombination may occur via an electron-tunnelling process. However, ΔA data at liquid nitrogen temperature give the much longer lifetimes of approx. 100 μs [39,40] and approx. 1 ms which were attributed to the decay of $P\text{-}700^+$ [3,5,39,40]. At low temperatures there may be a multiphasic decay pattern of $P\text{-}700^+A_1^-$ as found also at room temperature [9], and we may observe a rapid phase which is missed in the ΔA measurements. Part of the 77 K absorption kinetics, attributed to $P\text{-}700^+$ reduction, might be also caused by decay of the reaction center triplet $P\text{-}700^T \rightarrow$ P-700, although the in vivo difference spectra observed are different from the triplet spectrum observed in vitro. In bacterial reaction centers of *Rhodopseudomonas sphaeroides* R-26, analogous decays of 3 μs at room temperature and of about 120 μs at liquid nitrogen temperature are caused by the reaction center triplet [41]. Our hypothesis that the 0.1 μs decay of luminescence is caused by the reaction $(P\text{-}700^+A_1^-)^T \rightarrow P\text{-}700^TA_1$ can be tested by measuring the kinetics of the absorption changes caused by $P\text{-}700^T$ formation.

The common origin of ΔL and ΔF

If the luminescence L contributes significantly

to the total emission F, consisting of prompt fluorescence and luminescence, one would expect an enhancement of this emission, ΔF, due to the application of an external magnetic field B as a result of the stimulation of luminescence, ΔL. Such magnetic field-induced PS I emission changes of about 1–2% have indeed been found by Voznyak et al. [30] and by us (see Fig. 1). Analysis of the sinusoidal ΔF curves of Fig. 1 (see ΔF vs. B plots on the right) revealed values of 35–60 mT for $B_{1/2}$, comparable to values of 5–50 and 25–65 mT observed for several bacterial preparations [19–25,27] and PS II [22,24, 28,29], respectively; ΔF in all preparations saturated for $B \gtrsim 0.2$ T.

The following experimental results provide evidence for the suggestion that magnetic field-induced changes in the 0.1–0.2 μs luminescence are, at least for an appreciable fraction (preliminary experiments, which lack precision because of small L and ΔL at low flash energy, indicate at least 40%), responsible for those of ΔF measured under the same conditions: (i) Comparison of Figs. 2 and 8 shows that ΔF and ΔL as functions of temperature are roughly proportional to each other, both at 685 and 735 nm. The differences between ΔL and ΔF in these figures may be due to the fact that ΔF contains all the field-induced luminescence changes from time zero to 'infinity', whereas ΔL only those in the time region 0.25–0.8 μs. A preliminary attempt to extrapolate the luminescence decay curves (see Fig. 5) to time zero gave a better correspondence between ΔL and ΔF than that of Figs. 2 and 8, but still there may be other magnetic field-affected luminescence components in other time regions (below 200 ns and above 1 μs) contributing to ΔF. (ii) The course of ΔF as a function of the magnetic field strength at 278 K (Fig. 1) was found to be equal to that of the magnetic field-induced increase in 0.1–0.2 μs luminescence (data not shown). (iii) As shown in Fig. 4A–C, the emission spectra of ΔF and ΔL are roughly proportional to each other at different temperatures, which also supports the idea that both emissions have the same origin. (iv) As mentioned in detail in Results, under conditions in which $\Delta F = 0$ we also found $\Delta L = 0$ and when ΔL was maximal, ΔF was also maximal.

We found almost proportional field-dependent positive ΔF changes as a function of B at different wavelengths between 650 and 820 nm as shown in Fig. 1 for 685, 695, 725 and 735 nm at 278 K. We did not observe a decrease in emission around 725 nm at low magnetic fields as reported by Voznyak et al. [30], who observed at 7 mT at room temperature a fractional ($\Delta F/F$) decrease of about 0.25% at 725 ± 5 nm, which did not occur for the complete emission above 660 nm. Such a behavior is difficult to understand, unless two different types of reaction center with different emission spectra are postulated to be present in the particles obtained by Voznyak et al. [30].

As pointed out above, ΔF is caused by luminescence changes. Thus ΔF, which is more easily and accurately determined than ΔL, may be used as a monitor of luminescence changes. Fig. 4A–C shows that the spectrum of ΔF, especially around 675 nm, does not coincide with that of F. This may be explained by the assumption that the 675 nm fluorescence band is due to a non-luminescent chlorophyll *a*, e.g., solubilized chlorophyll [32,42,43]; F(675) was nearly independent of temperature. Assuming that the location of the F(675) band is also only slightly dependent on temperature and has a small contribution above 700 nm, it can be concluded that this chlorophyll probably is not responsible for the differences between the spectra of F and ΔF (Fig. 4A–C) at wavelengths longer than the peak. Therefore, we propose as an ad hoc hypothesis that the PS I luminescence is emitted by the reaction center and, for the main part, by the chlorophyll molecules in its vicinity. Some of these have absorption and emission peaks that are relatively red-shifted compared to those of the bulk antenna chlorophyll. Appreciable prompt fluoresence is emitted by antenna molecules at shorter wavelength maxima. Upon cooling, the rate of back transfer from the reaction center and the red-shifted F(750–820) chlorophyll molecules toward the antenna chlorophylls F (730) may be strongly diminished, causing the more pronounced long-wavelength emission in the spectra of ΔF compared to those of F. At the same time, a lowering of the rate of transfer from the F(730) molecules, to those emitting at longer wavelengths, may be responsible for the about 10-fold increase in fluorescence yield at 730 nm (see Fig. 4). As expected this increase is accompanied by an increase in fluorescence lifetime [44].

The observed changes in the emission spectra (Figs. 3 and 4) as a function of temperature may be plausibly explained as follows. Several assumptions are made. One is that the magnetic field-induced fractional increase in luminescence is independent of temperature, as indicated by our experiments (see Results). If the rate of transfer of the electron from the excited reaction center chlorophyll P-700 to A_1 is more rapid than the rate of energy transfer from P-700 to surrounding chlorophyll molecules (amongst others $F(735)$), then a quasi-steady-state is established which is determined by the ratio of the rates of the forward and back reaction $P\text{-}700^*A_1 \rightleftharpoons P\text{-}700^+A_1^-$. Then the ratio of concentrations $[P\text{-}700^*A_1]/[P\text{-}700^+A_1^-]$ as a function of temperature is given by the Boltzmann distribution. If it is assumed that the rate of downhill energy transfer from $P\text{-}700^*$ to the molecules emitting at 735 nm is independent of temperature, the luminescence emitted at 735 nm will be given by $L(735) = k[P\text{-}700^*A_1] \cdot \phi(735)$, in which ϕ is the fluorescence yield of the molecules emitting the peak at 735 nm, and k is a proportionality constant. From this it follows that $[P\text{-}700^*A_1] = L(735)/(\phi(735) \cdot k) \propto \Delta F(735)/F(735)$. The latter proportionality follows from $\phi(735) \propto F(735)$ and $L \propto \Delta F$.

The energy difference between $P\text{-}700^+A_1^-$ and $P\text{-}700^*A_1$ is estimated from the plot of $\Delta F/F$ vs. temperature (Fig. 3), and was found to be about 25 meV (0.6 kcal/mol). In an analogous way it is estimated from Fig. 3 that the 'activation energy' for the excitation of the 685 nm emission between 150 and 280 K is about 65 meV (1.5 kcal/mol). We may interpret this as the energy difference between chlorophyll molecules in the lowest excited singlet state at 685 nm ($Chl^*(685)$) and $P\text{-}700^+A_1^-$. The energy difference between $P\text{-}700^*A_1$ and $Chl^*(685)$ would then be $65-25 = 40$ meV, corresponding to an emission peak of $P\text{-}700^*$ at about 700 nm. Other investigators [3,5] reported an activation energy of about 42 meV (1 kcal/mol) for the back reaction $P\text{-}700^+A_1^- \rightarrow P\text{-}700\ A_1$ (see, however, the earlier part of our discussion).

Finally, we mention that the strong fluorescence yield increase in our PS I particles at 725–735 nm upon cooling to 82 K (Fig. 4) is observed also in intact systems [32,45–47], as well as in isolated PS I complexes [45,48–50]. A great number of investigators reported more or less similar PS I fluorescence emission spectra at room temperature and at about 80 K as observed by us [32,42,45,46, 48–53]. It should be noted, however, that PS I fluorescence spectra have been measured for smaller particles, different from ours [43,54,55]. Probably in these smaller particles no energy transfer occurs to a pigment fluorescing at 730 nm; this pigment may have been lost. In fair agreement with Ref. 45, we measured at room temperature an about 10-times lower fluorescence yield at 730 nm for the PS I complex than for intact chloroplasts or isolated PS II particles prepared by Rijgersberg et al. [32].

Acknowledgements

We are indebted to Mrs. H.M. Nan for carefully preparing the PS I particles, to Mr. D. Los for writing the computer program used for the analysis of the data, to Drs. R. van Grondelle and A.J. Hoff for helpful discussions and criticism of the manuscript, and to Drs. A.P.G.M. Thielen for preparing the PS II particles and measuring several PS I absorbance changes. This work was financed by the Netherlands Foundation for the Advancement of Pure Research (ZWO) via the Netherlands Foundation for Biophysics (SvB).

References

1 Sauer, K., Acker, S., Mathis, P. and van Best, J.A. (1977) in Bioenergetics of Membranes (Packer, L., ed.), pp. 351–359, Elsevier/North-Holland, Amsterdam
2 Sauer, K., Mathis, P., Acker, S. and van Best, J.A. (1978) Biochim. Biophys. Acta 503, 120–134
3 Mathis, P., Sauer, K. and Remy, R. (1978) FEBS Lett. 88, 275–278
4 Ke, B. (1978) Curr. Top. Bioenerg. 7, 75–138
5 Shuvalov, V.A., Dolan, E. and Ke, B. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 770–773
6 Friesner, R., Dismukes, G.C. and Sauer, K. (1979) Biophys. J. 25, 277–294
7 McIntosh, A.R., Chu, M. and Bolton, J.R. (1975) Biochim. Biophys. Acta 376, 308–314
8 Evans, M.C.W., Sihra, C.K., Bolton, J.R. and Cammack, R. (1975) Nature 256, 668–670
9 Shuvalov, V.A., Ke, B. and Dolan, E. (1979) FEBS Lett. 100, 5–8
10 Shuvalov, V.A., Klevanik, A.V., Sharkov, A.V., Kryukov, P.G. and Ke, B. (1979) FEBS Lett. 107, 313–316
11 Heathcote, P., Timofeev, K.N. and Evans, M.C.W. (1979) FEBS Lett. 101, 105–109

12 Baltimore, B.G. and Malkin, R. (1980) Photochem. Photobiol. 31, 485–490
13 Baltimore, B.G. and Malkin, R. (1980) FEBS Lett. 110, 50–52
14 Telfer, A., Barber, J., Heathcote, P. and Evans, M.C.W. (1978) Biochim. Biophys. Acta 504, 153–164
15 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223–317, Academic Press, New York
16 Malkin, S. (1977) in Primary Processes of Photosynthesis, Vol. 2, Topics in Photosynthesis (Barber, J., ed.), pp. 349–431, Elsevier, Amsterdam
17 Amesz, J. and van Gorkom, H.J. (1978) Annu. Rev. Plant Physiol. 29, 47–66
18 Shuvalov, V.A. (1976) Biochim. Biophys. Acta 430, 113–121
19 Blankenship, R.E., Schaafsma, T.J. and Parson, W.W. (1977) Biochim. Biophys. Acta 461, 297–305
20 Hoff, A.J., Rademaker, H., van Grondelle, R. and Duysens, L.N.M. (1977) Biochim. Biophys. Acta 460, 547–554
21 Voznyak, V.M., Elfimov, E.I. and Proskuryakov, I.I. (1978) Dokl. Akad. Nauk. S.S.S.R. 242, 1200–1203
22 Rademaker, H., Hoff, A.J. and Duysens, L.N.M. (1979) Biochim. Biophys. Acta 546, 248–255
23 Michel-Beyerle, M.E., Scheer, H., Seidlitz, H., Tempus, D. and Haberkorn, R. (1979) FEBS Lett. 100, 9–12
24 Rademaker, H. (1980) Ph.D. Thesis, University of Leiden
25 Voznyak, V.M., Elfimov, E.I. and Sukovatitzina, V.K. (1980) Biochim. Biophys. Acta 592, 235–239
26 Van Bochove, A.C., van Grondelle, R. and Duysens, L.N.M. (1980) in Proceedings of the 5th International Congress on Photosynthesis, in the press
27 Kingma, H., Duysens, L.N.M. and Peet, H. (1980) in Proceedings of the 5th International Congress on Photosynthesis, in the press
28 Sonneveld, A., Duysens, L.N.M. and Moerdijk, A. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5889–5893
29 Sonneveld, A., Duysens, L.N.M. and Moerdijk, A. (1980) in Proceedings of the 5th International Congress on Photosynthesis, in the press
30 Voznyak, V.M., Ganago, I.B., Moskalenko, A.A. and Elfimov, E.I. (1980) Biochim. Biophys. Acta 592, 364–368
31 Vernon, L.P., Shaw, E.R. and Ke, B. (1966) J. Biol. Chem. 241, 4101–4109
32 Rijgersberg, C.P., Amesz, J., Thielen, A.P.G.M. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482
33 Sonneveld, A., Rademaker, H. and Duysens, L.N.M. (1980) FEBS Lett. 113, 323–327
34 Shuvalov, V.A., Klimov, V.V. and Krasnovskii, A.A. (1976) Mol. Biol. 10, 326–339
35 Adrian, F.J. (1977) in Chemically Induced Magnetic Polarization (Muus, L.T., Atkins, P.W., McLaughlan, K.A. and Pederson, J.B., eds.), pp. 77–105, Reidel, Dordrecht, The Netherlands
36 Frank, H.A., McLean, M.B. and Sauer, K. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5124–5128
37 Dismukes, G.C. and Tycko, R. (1980) in Proceedings of the 5th International Congress on Photosynthesis, in the press
38 Kiwi, J. and Grätzel, M. (1980) J. Phys. Chem. 84, 1503–1507
39 Sauer, K., Mathis, P., Acker, S. and van Best, J.A. (1978) Biochim. Biophys. Acta 545, 466–472
40 Mathis, P. and Conjeaud, H. (1979) Photochem. Photobiol. 29, 833–837
41 Parson, W.W., Clayton, R.K. and Cogdell, R.J. (1979) Biochim. Biophys. Acta 387, 265–278
42 Gasanov, R.A. and Govindjee (1974) Z. Pflanzenphysiol. 72, 193–202
43 Brown, J.S. (1976) Carnegie Inst. Washington, Yearb. 75, 460–465
44 Searle, G.F.W., Barber, J., Harris, L., Porter, G. and Tredwell, C.J. (1977) Biochim. Biophys. Acta 459, 390–401
45 Boardman, N.K., Thorne, S.W. and Anderson, J.M. (1966) Proc. Natl. Acad. Sci. U.S.A. 56, 586–593
46 Murata, N., Nishimura, M. and Takamiya, A. (1966) Biochim. Biophys. Acta 126, 234–243
47 Rijgersberg, C.P., Melis, A., Amesz, J. and Swager, J.A. (1979) in Chlorophyll Organization and Energy Transfer in Photosynthesis, CIBA Foundation Symposium 61 (new series), pp. 305–322, Elsevier/North-Holland, Amsterdam
48 Ogawa, T., Vernon, L.P. and Mollenhauer, H.H. (1968) Biochim. Biophys. Acta 172, 216–229
49 Ogawa, T. and Vernon, L.P. (1970) Biochim. Biophys. Acta 197, 292–301
50 Ogawa, T. and Vernon, L.P. (1969) Biochim. Biophys. Acta 180, 334–346
51 Anderson, J.M., Waldron, J.C. and Thorne, S.W. (1978) FEBS Lett. 92, 227–233
52 Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814–822
53 Mohanty, P., Zilinskas Braun, B., Govindjee, and Thornber, J.P. (1972) Plant Cell Physiol. 13, 81–91
54 Brown, J.S. (1977) Photochem. Photobiol. 26, 519–525
55 Vacek, K., Wong, D. and Govindjee (1977) Photochem. Photobiol. 26, 269–276