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# Thermodynamics of electron transport in Photosystem I studied by electric field-stimulated charge recombination

Marten H. Vos and Hans J. van Gorkom

*Department of Biophysics, Huygens Laboratory of the State University, Leiden (The Netherlands)*

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**The kinetics of chlorophyll luminescence induced by externally applied electric-field pulses in osmotically swollen thylakoid systems are known to consist of a fast and a slow phase, attributed to charge recombinations in Photosystem I and II, respectively (Symons, M., Korenstein, R. and Malkin, S. (1986) Biochim. Biophys. Acta 806, 305–310). We confirm this assignment and show that conditions can be created under which the phases can be studied separately. A previously unresolved 80 μs decay of the precursor of Photosystem II electroluminescence is attributed to manganese oxidation. After illumination the precursor of Photosystem I electroluminescence decayed biexponentially with a major component with a half-time of 70 ms and a minor component with a half-time of about 15 ms at room temperature. The same kinetics were observed in the absorbance difference at 700 nm and in 'normal' Photosystem I luminescence. On the basis of these findings the Photosystem I electroluminescence is attributed to charge recombination between the oxidized primary donor P$^+$-700 and the reduced electron acceptor F$_A^-$ (70 ms component) and possibly F$_B^-$. The enthalpy of activation of the first reaction was determined at 460 ± 30 meV. Field-induced absorbance changes showed that charge recombination occurred in up to 20% of the reaction centers. It is further shown that in Photosystem I, in contrast to Photosystem II, the luminescence yield is enhanced by an external electric field with at least a factor of 150 at higher field strengths. The results are discussed in terms of a thermodynamical model. It is concluded that in PS I the primary charge separation, P-700A$_0$ → P$^+$-700A$_0^-$, as well as subsequent electron transport to F$_A$ and F$_B$ are electrogenic.**

## Introduction

Reversal of photosynthetic electron transport may result in photon emission, i.e., delayed luminescence. The intensity and kinetics of delayed luminescence depend on various parameters, such as redox and spin states and reaction rates in the reaction center. External modification of these parameters results in a change in luminescence intensity. A very large enhancement can be obtained if, after illumination, the back reaction is stimulated by an electric field, externally applied to the photosynthetic membrane. Sufficiently large

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Abbreviations: A$_0$ and A$_1$, primary and secondary electron acceptor of Photosystem I; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DMSO, dimethylsulphoxide; $E_m$, midpoint potential; F$_X$, F$_A$ and F$_B$, secondary electron acceptors of Photosystem I; I, primary electron acceptor of Photosystem II; Mops, morpholinepropanesulphonic acid; P-430, optically observed terminal electron acceptor of Photosystem I, presumably corresponding to F$_A$/F$_B$; P-680 and P-700, primary electron donors of Photosystem II and I; PS, Photosystem; Q$_A$, secondary electron acceptor of photosystem II; TPB, tetraphenylboron; Z, secondary electron donor of Photosystem II.

Correspondence: H.J. van Gorkom, Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden, The Netherlands.

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electric fields can be generated in a macroscopic aqueous sample by using osmotically swollen thylakoid systems. Upon dilution of chloroplasts in a hypotonic medium the outer membrane and stroma, if still present, are lost and the thylakoid system unfolds to form a single large vesicle, a 'bleb'. The electric field which can be generated in the bleb membranes by an externally applied field pulse has been quantitatively characterized [1]. Until recently electroluminescence signals were used to study charge recombinations in Photosystem II [2-6], which has a much larger fluorescence yield than Photosystem I. It is known, however, that two phases in the kinetics of electroluminescence during a pulse can be distinguished [3]. In recent studies by Symons et al. [7,8] the rapid and slow phases in electroluminescence were attributed to PS I and PS II, respectively, on the basis of their action spectra and of their sensitivity to pH, temperature and added chemicals. The precursors of PS I electroluminescence have not yet been identified.

Knowledge of electron transport in Photosystem I is still relatively restricted. For reviews, see Refs. 9 and 10. In brief, the primary charge separation between P-700 and $A_0$, both believed to be chlorophyll *a* species, is stabilized by electron transport to a secondary acceptor $A_1$, possibly phylloquinone, with a half-time of 20 ps [11], and further to one of three iron-sulfur clusters $F_X$, $F_A$ and $F_B$, which can be distinguished by EPR spectroscopy. The identity of the optically observed electron acceptor 'P-430' [12] is not well defined and may contain contributions of all three iron-sulfur clusters, depending on the conditions of measurement [9]. The precise electron-transfer pathways between $F_X$, $F_A$ and $F_B$ are not clear [9]. $F_A$ and $F_B$ differ only about 40 mV in midpoint potential, $F_A$ having the least negative potential (−550 mV). Some evidence has been found that there is no direct electron transport between $F_A$ and $F_B$. $F_X$ has a much lower potential (−705 mV). The role of this iron-sulfur center as a redox intermediate in vivo is still uncertain. In vivo $P^+$-700 is rereduced by plastocyanin and the final acceptor is reoxidized by ferredoxin. Both plastocyanin and ferredoxin are water-soluble proteins. Our chloroplast preparations are diluted several hundred times, so these redox reactions may be disregarded.

From recent photovoltage measurements by Trissl et al. [13] it was concluded that PS I electrogenicity occurs within 50 ps and that no electrogenic charge-transfer steps occur in the next 50 ns. This shows that electron transfer to $A_0$ and/or $A_1$ is electrogenic; electron transfer from $A_0$ to $A_1$ could not be resolved in those measurements.

We have studied the two electroluminescence phases under conditions in which either of the photosystems is selectively blocked. The rapid (PS I) phase kinetics and its kinetic correlation with 700 nm absorbance difference and 'ordinary' luminescence were used to obtain information on the electroluminescence precursors, thermodynamic properties and electrogenicity of the electron-transfer steps of PS I. With our analysis the electrogenicity of electron transfer to $A_0$ and that of subsequent electron transport could be distinguished.

## Materials and Methods

Chloroplasts from laboratory-grown, about 30 days old, spinach were isolated as described previously [5] in a buffer containing 0.4 M sucrose, 50 mM tricine, 10 mM KCl and 5 mM $MgCl_2$ (pH 7.8). 5% DMSO was added and the preparations were stored at 77 K until use [14]. Before measurement a suspension of chloroplasts in isolation buffer with a chlorophyll concentration of 1 mg/ml was diluted 200-fold in 1 mM Mops buffer (pH 6.6). The pH of the final suspension was 6.6. If needed chemicals (10 μM DCMU and either 50 μM ferricyanide for PS II measurements or 5 μM TPB for PS I measurements) were added. The sample was diluted at least 10 min before measurement.

The experimental set-up was as described before [5] with some modifications. In brief, a 1 × 1 × 1 cm cuvette was used of which two opposite sides consisted of platinum electrodes; one side was used for in- and outlet of a stopped-flow system and the remaining sides were used for illumination and detection of luminescence and/or absorbance. Electric pulses of up to 1500 V (slightly less in ferricyanide containing suspensions because of the enhanced conductivity) with a 90% completion risetime of 1 μs were generated with a home-built pulse generator. Saturating flashes at 532 nm, 15 ns half-width, were obtained with a Nd-YAG laser (JK Lasers). Measuring light for absorbance-difference measurements was supplied by a 250 W tungsten halogen lamp. Luminescence and absorbance were observed with S20, red extended, photomultipliers. The luminescence photomultiplier was shut off during the flash by a home-built electronic gate. Absorbance and luminescence signals were recorded by either a Datalab 905 or a Biomation 8100 transient recorder and fed into a microcomputer for averaging. The cuvette and the sample stock were thermostatted to the same temperature. Measurements were performed at 20 °C, unless stated otherwise.

## Results

A typical example of an electroluminescence signal is shown in Fig. 1A. In the preparation used here only DCMU was added, which prevents PS II electron transport after $Q_A$ and the associated decrease of electroluminescence. Two different phases are clearly visible in the decrease of the signal during the pulse. The kinetics of both phases depend on the external field strength, higher field yielding faster times (not shown). To check the proposal by Symons et al. [8] that the fast and slow phases have their precursors in PS I and PS II, respectively, conditions were created in which recombination in either of the two photosystems was inhibited. When ferricyanide was added the primary donor of PS I, P-700, remained in the oxidized state after preillumination, so that no flash-induced charge separation took place in PS I. Tetraphenylboron (TPB) is a charged lipid-soluble molecule, which specifically rereduces the donor side of PS II [15]. So preillumination in the presence of TPB and DCMU yields the redox state P-680I$Q_A^-$, in which, after a subsequent flash, no charge recombination can occur on a timescale larger than nanoseconds. Experiments under those conditions (Fig. 1B, C) showed that the two phases can be well separated and showed that the assignment of the fast phase to PS I [8] is correct. It is remarkable that, although the fluorescence yield of PS II is much higher than that of PS I (see, for example, Ref. 17), the electroluminescence yield of PS I may be of the same order as that of PS II at sufficiently high electric fields. When charge recombination in PS I as well as PS II was blocked by the presence of DCMU, TPB and ferricyanide no electroluminescence was observed (Fig. 1D). Spectral characterization of the two electroluminescence phases in our bleb suspensions yields clearly distinct emission spectra (not shown), which are similar to recently reported [16] electroluminescence spectra in chloroplasts subjected to very high electric field strengths in an isotonic medium.

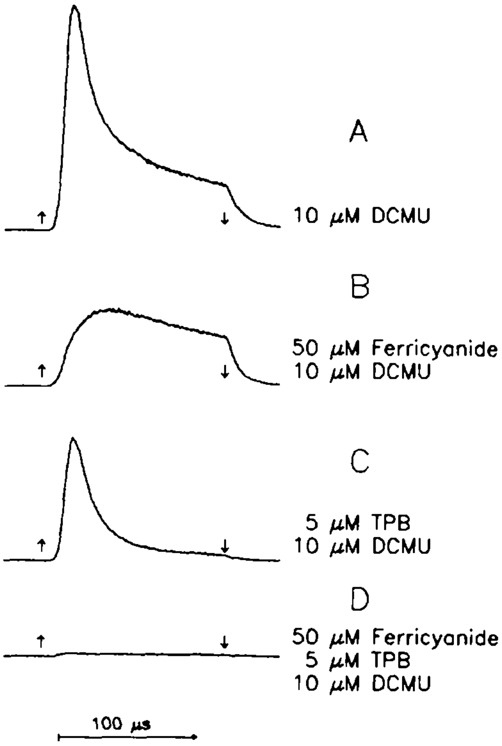

Fig. 1. Electroluminescence signals measured 10 ms after a flash. Under all conditions four preflashes were given. Luminescence transmitted by a Schott RG 665 cut-off filter was measured. External field strength: 1300 V/cm. Each recording is the average of 10 traces. The arrows indicate the onset and offset of the pulse.

Another clear difference between PS I and PS II electroluminescence is observed in the dependence of their peak intensities on the time between the flash and the pulse (Fig. 2A and B). For PS II the kinetics consisted of a fast monoexponential component with a half-time of 80 μs and a much slower component, which could not be fitted with a single exponent over the complete time range studied. Its initial decay half-time was about 3 ms. The amplitude ratio of the fast and slow components was about 5. The characteristic polyphasic slow component, which has been observed before [5], presumably reflects the charge recombination between the reduced secondary acceptor $Q_A^-$ and oxidized manganese in the oxygen evolving complex. We ascribe the fast component to rereduction of the oxidized secondary donor $Z^+$ by manganese, because its decay time corresponds to the rise time of the corresponding ultraviolet absorbance increase [18]. This fast component was not well resolved in earlier experiments [5] due to the use of xenon instead of laser flashes.

The PS I peak emission displayed a very different dependence on the time between the flash and the pulse. It decayed biexponentially in the millisecond time range, whereas no decay component on the microsecond time scale was observed. It had a major component with a half-time of about 70 ms and a minor component with a half-time of approx. 15 ms. The same kinetics were observed for $P^+$-700 decay by absorbance difference spectroscopy at 700 nm and in the decay of delayed fluorescence emitted by PS I after a flash in the absence of an electric field (Fig. 2C and D). These absorbance and luminescence signals, like the PS I electroluminescence (Fig. 1D), were absent in the presence of ferricyanide (traces 2 of Fig. 2C and D). The time of the observed decay is similar to the decay time found for $P^+$-700$P^-$-430 recombination in digitonin-treated PS I particles [12].

The amplitude of the minor decay component varied from 0 to 30% of the total amplitude, both of the electroluminescence and of the absorbance change in different chloroplast batches and its decay time varied from 5 to 20 ms. The amplitude and decay kinetics of the 700 nm absorbance change were found to be the same after a pair of flashes, spaced at 50 ms, as after a single flash

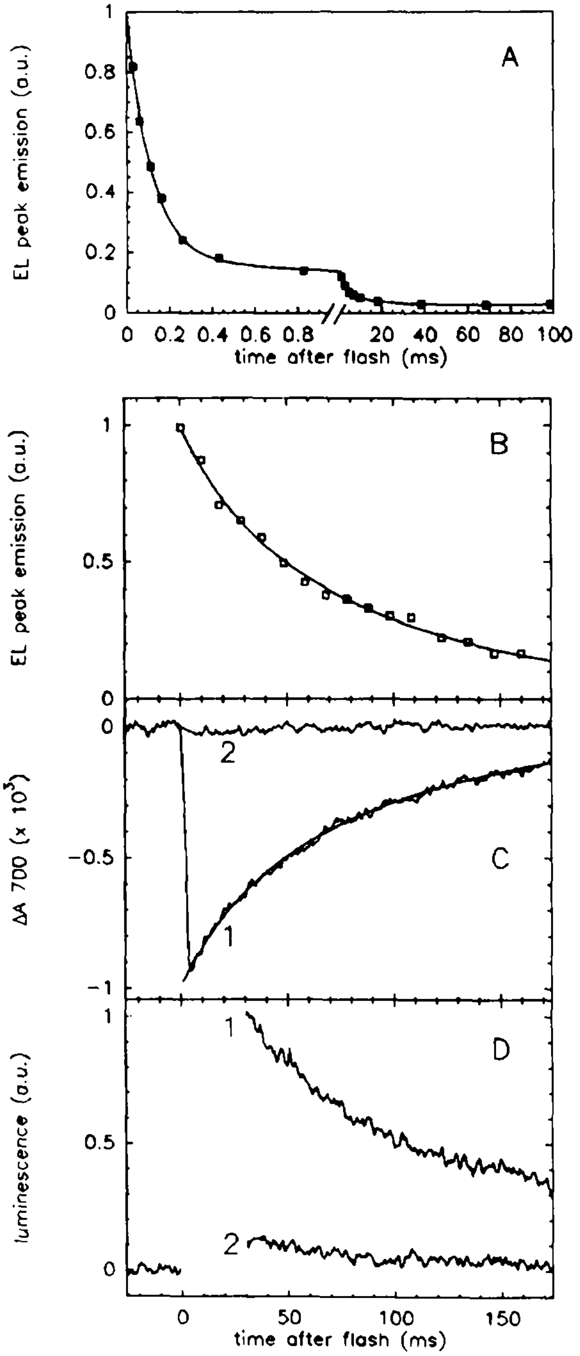

Fig. 2. (A and B) Decay of the peak intensity of the PS II and PS I electroluminescence as a function of time between flash and pulse. (A) PS II; conditions: 10 μM DCMU, 100 μM ferricyanide, *E*-field = 1000 V/cm, measured through RG 665 and AL 685 filters. (B) PS I; conditions: 10 μM DCMU, 5 μM TPB, four preflashes, *E*-field = 1400 V/cm, measured through RG 695 filter. (C) Absorbance difference kinetics measured at 700 nm. Additions: 1, as in B; 2, as in B with 50 μM ferricyanide. Average of 20 traces. (D) Luminescence kinetics after a flash in the absence of an electric field measured with a RG 645 filter. Additions as in C. Average of 40 traces. The first 30 ms after the flash could not be recorded due to flash and gate artifacts. Curves in B and C are biexponential with a slow phase of $t_{1/2} = 70$ ms and a fast phase of $t_{1/2} = 16$ ms and an amplitude of 0.3 relative to the slow phase. They are a best fit to the measured absorbance difference kinetics.

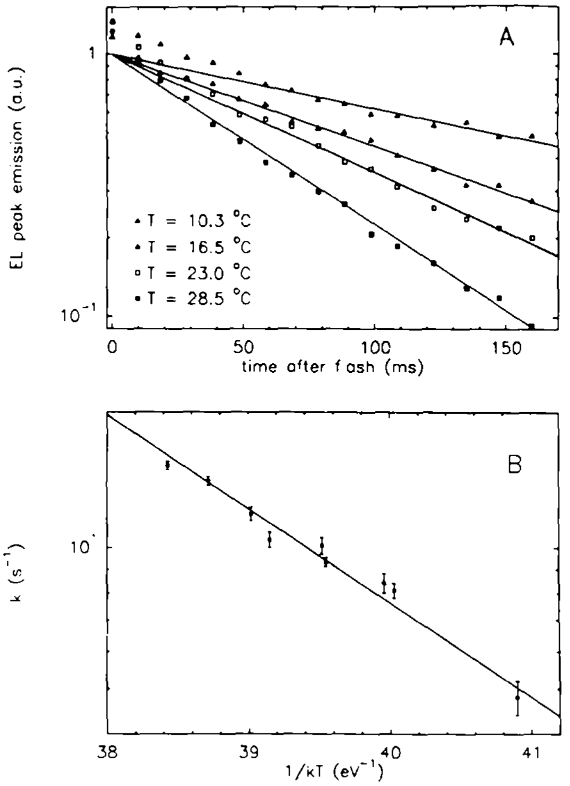

Fig. 3. (A) Temperature dependence of PS I electroluminescence decay. For the shown monoexponential fits only the points after $t = 45$ ms were used. (B) Arrhenius plot of the slow component of the rapid phase of electroluminescence decay. The fit corresponds to an activation enthalpy of 460 meV. Conditions as in Fig. 2B.

(not shown). This indicates that the two decay phases must both be due to charge recombination, in two different reaction center populations.

From the temperature dependence of the slow component of this decay the activation enthalpy for the back relation can be obtained. The electroluminescence kinetics were measured at different temperatures. The results are shown in Fig. 3. Luminescence and absorbance difference kinetics displayed similar temperature dependences (data not shown). The activation enthalpy was determined at 460 ± 30 meV.

For the first time, pulse-induced charge recombination was detected directly in absorbance kinetics (Fig. 4, trace 1). This signal was superimposed on a field-induced bleaching observed without flash preillumination as well; the traces in Fig. 4 are the difference between measurements with and without a flash at 60 ms before the pulse. The field-induced bleaching, which was proportional to the pulse duration, decayed in the seconds time range and is presumably due to a field-induced movement of the absorbing material. With the 100 μs pulses of 1400 V/cm used here the subtracted absorbance changes were of the same order of magnitude as the absorbance changes due to charge recombination. The same kinetics were observed at other wavelengths. In the presence of ferricyanide, when no PS I charge separation takes place, no pulse-induced absorbance changes remained after the subtraction (Fig. 4, trace 2). A maximum field-induced change in the $P^+$-700 concentration of about 20% was observed. The field-strength dependence of the absorbance increase at 700 nm is shown by solid triangles in Fig. 5. A luminescence decrease was also observed, but was complicated by photomultiplier gate recovery effects.

The detection of the non-stimulated PS I luminescence allows direct comparison of its yield with that of field-stimulated luminescence. This was not possible for PS II, where delayed luminescence caused by the electrogenic charge recombination could not be measured, due to the presence of a strong luminescence signal caused by non-electrogenic charge recombination, presumably in PS $II_\beta$ [5]. Solid circles in Fig. 5 show the field dependence of the ratio of the amount of PS I luminescence induced by an 800 μs pulse, applied at 60 ms after the flash, over the amount of PS I

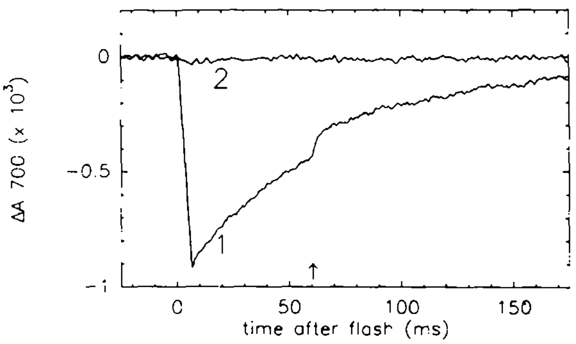

Fig. 4. Absorbance changes at 700 nm (average of 20) caused by a 100 μs electrical field pulse of 1400 V/cm (arrow). The field-induced change without illumination was subtracted (see text). Conditions: 1, as in Fig. 1C; 2, as in Fig. 1D.

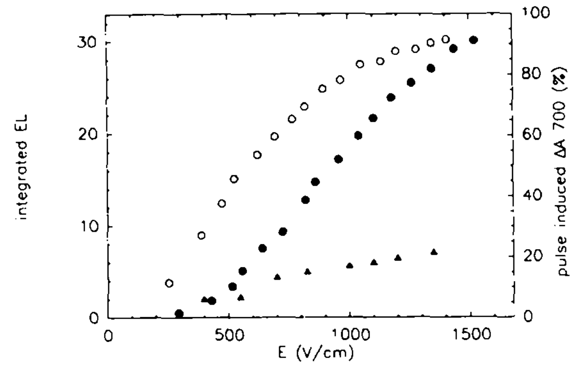

Fig. 5. Time-integrated electroluminescence as a function of external electrical-field strength for a 800 μs pulse given 60 ms after the flash (PS I, solid circles) or a 4 ms pulse given 8 ms after the flash (PS II, open circles). Further conditions as in Fig. 1B and C. The PS I values are given relative to the time-integrated PS I luminescence in the absence of a field as described in the text. Absorbance changes at 700 nm induced by a 200 μs pulse (solid triangles) are plotted relative to the total absorbance difference at the time of the pulse. Further conditions as in Fig. 4.

luminescence emitted at times longer than 60 ms after the flash if no field is applied. The luminescence without a pulse was calculated from the detected intensity at 60 ms and the rate constant of its decay, which is monophasic after 60 ms. The electroluminescence was calculated by integration of the intensity during the pulse, corrected for its anisotropy by measuring the intensity polarized parallel ($EL_{\parallel}$) and perpendicular ($EL_{\perp}$) relative to the field, and taking $2/3(EL_{\parallel}+2EL_{\perp})$. For comparison similar unscaled data obtained under PS II conditions are shown (open circles). Here a 4 ms pulse was used, because PS II electroluminescence decays much slower during the pulse than PS I electroluminescence (see Fig. 1B and C). If the luminescence yield of PS II is field independent [4], this curve reflects the fraction of charge pairs recombining during the 4 ms pulse used and rises only because the recombination rate increases. The amplitude of the P-700 absorbance change (triangles) shows the fraction of PS I charge pairs recombining during the 800 μs pulse. PS I recombination during the pulse is probably more than 5-times faster than PS II recombination (Fig. 1B and C) and a saturation at lower field strengths than PS II electroluminescence might be expected. The P-700 data are not conclusive, but seem to be consistent with this expectation. The integrated PS I electroluminescence, however, clearly does not saturate at low field, but increases with field strength until it is 30-times higher than the integrated PS I luminescence observed without field pulse. This shows that in PS I, in contrast to PS II, not only the recombination rate, but also the luminescence yield is enhanced by an external electrical field. Theoretically, in at most 50% of the reaction centers which are in the charge-separated state charge recombination can take place, because only in one-half of a bleb recombination is stimulated; in the other charge separation is stabilized. In our preparation it is about 20% (Fig. 5, see above), suggesting that 40% of the PS I reaction centers was exposed to a sufficiently high field (cf. Ref. 4). Thus, at the maximum available field strength of 1500 V/cm the PS I luminescence yield is enhanced by a factor of 150.

## Discussion

The assignment of the fast and slow phases of electroluminescence in osmotically swollen chloroplasts to PS I and PS II, respectively, by Symons and coworkers [8] has been clearly confirmed. Additional evidence was found by the separation of the phases in the presence of ferricyanide or TPB (Fig. 1) and by the kinetic correlation of the fast phase peak intensity decay with $P^+$-700 decay and a PS I luminescence phase (Fig. 2). Inhibition of either of the phases allowed a study of the PS I and PS II components separately.

The assumption in earlier publications from this laboratory [4–6] that all detected electroluminescence originated in PS II is not at variance with our present observations. In Ref. 4 all electroluminescence was correlated with variable fluorescence and therefore with PS II. In Ref. 5 the time scale of the kinetics of the electroluminescence precursor (microseconds) excludes a significant contribution by PS I electroluminescence. In Ref. 6 low field strengths were used and no signal was observed after one flash under conditions where PS I electroluminescence must have been at least as big after one as after two flashes. The absence of a significant PS I electroluminescence in those earlier studies may be explained by the relatively low field strengths used and by the wavelengths of detection (680 nm in Ref. 4, 685 nm in Refs. 5 and 6).

The kinetics during the pulse and the field strength dependence of the electroluminescence signal may yield information on the system it originates from. The risetime of the signal depends on the voltage at which back reaction occurs and on the risetime of the field over the bleb walls. This risetime is linearly related to the bleb radius [4] and was about 20 μs in our preparations. The decay kinetics during the pulse reflect the exhaustion of the precursors. A striking difference between PS I and PS II electroluminescence is the much faster rise and especially decay time of the former (Fig. 1). This feature can be explained qualitatively by the fact that in the PS I measurements no reactions on the donor side take place and $P^+$-700 remains available for recombination, whereas in PS II $P^+$-680 is already reduced by secondary electron donors when the pulse is given. These secondary reactions have been shown to be non-electrogenic by electrochromic [19] and photovoltage [20] measurements.

In PS II electroluminescence a fast decay (80 μs half-time) of the peak intensity as a function of the delay between flash and pulse was observed, followed by a slow decay with a relative amplitude of 20% (Fig. 2A). Assuming charge transfer between Z and manganese to be non-electrogenic [19,20], all electroluminescence at the time of the emission peak (about 30 μs after the start of the pulse) can be attributed to centers that were in the state $Z^+Q_A^-$ at the onset of the pulse. The 80 μs phase is interpreted to reflect the reduction of $Z^+$ by manganese ($Z^+S_1 \rightarrow ZS_2$); the slower multiexponential phase the recombination $S_2Q_A^- \rightarrow S_1Q_A$. This interpretation implies that the equilibrium constant $[ZS_2]/[Z^+S_1]$ is 5 and the corresponding midpoint potential difference about 35 mV at short times after the flash. It should be kept in mind, however, that the recombination of $S_2Q_A^-$ is progressively slower at longer times after the flash.

The decay of the PS I electroluminescence peak intensity and the kinetically identical luminescence and absorbance difference decay (Fig. 2B–D) correspond in time scale with the decay of $P^+$-700$P^-$-430 measured in PS I particles by Hiyama and Ke [12] and in pretreated chloroplast fragments by Shuvalov et al. [21,22]. This decay was interpreted as a second-order process with equal amounts of $P^+$-700 and $P^-$-430 present. However, since it seems unlikely that this kind of reaction would occur in reaction centers, and since we observed different ratios of the two components in different preparations, we interpret our data as a back reaction from a heterogeneous population of reaction centers. We attribute the 70 ms decay component to charge recombination between $P^+$-700 and $F_A^-$. The observed decay time of 70 ms compared with the recently observed decay time of $P^+$-700$F_X^-$ of 250 μs [23] predicts a midpoint potential difference of 140 mV between $F_A/F_A^-$ and $F_X/F_X^-$, assuming that both recombinations proceed via thermal equilibrium with the same high-energy intermediate, presumably $P^+$-700$A_0^-$. This fits well with the titrated midpoint potential of $F_X/F_X^-$ of −705 mV [9]. The minor component, with a half-time of 5–20 ms, which was present to different extents in different preparations, can be attributed to centers in which for some reason a conformational change had taken place. A possible explanation is that in those centers photoreduction of $F_A$ is inhibited, and recombination from $F_B^-$ is observed. The time scale of this recombination, about 5 times faster than the 70 ms component, corresponds with the known midpoint potential difference between $F_A/F_A^-$ and $F_B/F_B^-$ of 40 mV [9].

The PS I electroluminescence was of the same order of magnitude as PS II electroluminescence when measured under the conditions of Fig. 1, whereas normal PS I luminescence is very weak compared to PS II luminescence [22]. A rough estimate shows that this is reasonable. $P^+$-700$F_A^-$ decays in 70 ms, partially via a route involving decay of the singlet excited state to the ground state, with some probability of photon emission. The life time of the excited state in the absence of photochemistry or quenching by $P^+$-700 may be of the order of 1 ns; much shorter times would prevent efficient photosynthesis. For the same reason the excited state must be in equilibrium with $P^+$-700$F_A^-$. The equilibrium constant between $P^+$-700$F_A^-$ and the excited state may be estimated from the energy of a 700 nm photon and the midpoint-potential difference between $P^+$-700/P-700 and $F_A/F_A^-$. A potential energy map of 740 meV is obtained and hence an equilibrium con-

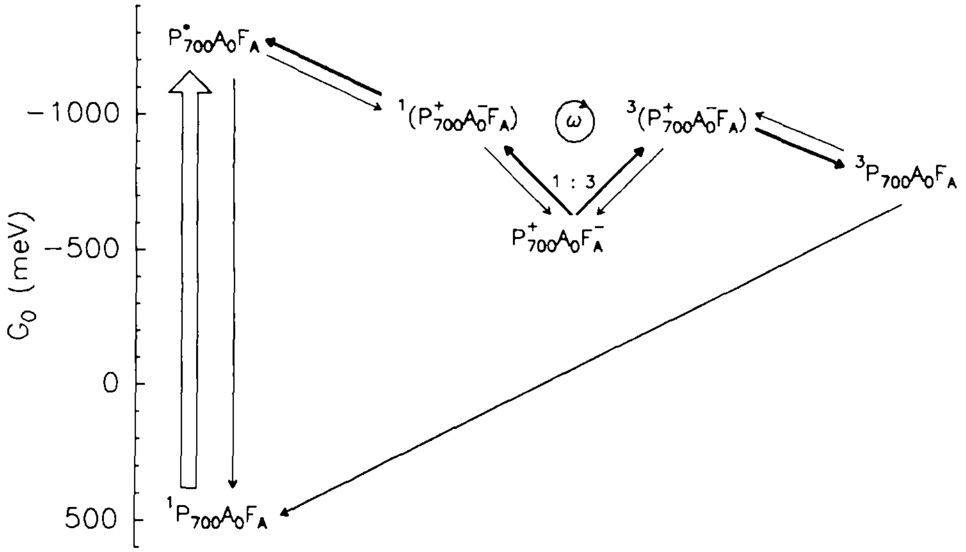

Fig. 6. Simplified scheme of electron transfer in Photosystem I, considering only $F_A$ as a final acceptor. Standard free-energy levels are given relative to the standard hydrogen electrode. Bold arrows indicate the direction of the electric field-induced equilibrium change.

stant of about $10^{12}$. Decay of $P^+$-700$F_A^-$ via this route should take $10^3$ s, and thus with the observed decay time of 70 ms, account for 0.007% of the total decay. Stimulation by a factor 150 would raise this value to 1%. In PS II this value is 3% [4]. It is concluded that the observed relative amplitudes of PS I and PS II electroluminescence are indeed reasonable. Since in the absence of an electric field 150-times less PS I luminescence was emitted about 1000-times slower, the signal amplitude was about $10^5$ times lower than the PS I electroluminescence peak and should be more than 100-times lower than PS II luminescence. A much lower luminescence yield is indeed observed in PS I compared to PS II.

The main decay route of $P^+$-700$F_A^-$ may be via the triplet state of P-700, as indicated in the scheme of Fig. 6, and explained in more detail for PS II in Ref. 24. We explicitly assume that the charge recombination takes place via reversal of the normal electron-transfer path and requires activation up to the energy level of the primary radical pair $P^+$-700$A_0^-$. This state will be generated three times more often in an electron spin configuration allowing recombination to the triplet state P-700 (indicated by $^3(P^+$-700$A_0^-)$) than in a configuration allowing recombination to the singlet excited state $P^*$-700 or to the ground state P-700 (indicated by $^1(P^+$-700$A_0^-)$). The last mentioned decay path was omitted in Fig. 6 because we have no evidence for it and it cannot be substantially faster than the triplet decay route, as will be discussed below. A change of electron spin configuration in $P^+$-700$A_0^-$ can occur during a field pulse but is normally about $10^3$ times slower [25] than the 30 ps $A_0^-$ oxidation by secondary electron acceptors [11].

The measured activation enthalpy of 460 meV of $P^+$-700$F_A^-$ recombination (Fig. 3B) suggests that the midpoint potential of $A_0/A_0^-$ is at least 460 mV more negative than that of $F_A/F_A^-$ ($-550$ mV [9]), corresponding to an equilibrium constant of at least $10^8$. The unknown entropy contribution more likely increases than decreases this value, but cannot increase it very much if the midpoint potential of $P^+$-700/$P^*$-700 is 740 mV more negative than that of $F_A/F_A^-$ (see above). An equilibrium constant between $P^+$-700$A_0^-$ and $P^+$-700$F_A^-$ of at least $10^8$ implies that during this equilibrium $A_0$ reduction takes at least $10^8$ times longer than its measured 30 ps (1/e time) oxidation [11]; at least 3 ms. The much faster electroluminescence rise time (Fig. 1C) demonstrates an electric-field effect on this equilibrium.

The same equilibrium constant, combined with the measured 70 ms decay time, also implies that the charge recombination time of the activated state $P^+$-$700A_0^-$ should be less than $10^{-8} \cdot 70$ ms = 700 ps. The reported much longer life times of the primary radical pair $P^+$-$700A_0^-$ when subsequent forward electron transport is blocked (of the order of 30 ns [26–28]) imply that direct decay to the ground state (not shown in Fig. 6) is much slower than 700 ps. We conclude that charge recombination from the state $^3(P^+$-$700A_0^-)$ occurs in less than 700 ps and recombination from $^1(P^+$-$700A_0^-)$ directly to the ground state can be neglected (see above).

When the charge recombination of $P^+$-$700F_A^-$ is accelerated by an electric field the equilibria in the scheme of Fig. 6 are expected to shift in the direction indicated by the bold arrows. An acceleration of the recombination is caused by an increase of the equilibrium concentration of $P^+$-$700A_0^-$, as discussed above, and possibly also by preventing charge separation in the state $^3$P-$700A_0$. The stimulation of recombination of $^1(P^+$-$700A_0^-)$ to the singlet excited state is quantitatively insignificant in this respect, but does explain the 150-fold enhancement of the luminescence yield. Without stimulation of electron transfer from $A_0^-$ to $P^+$-700 the luminescence enhancement cannot be explained. Also in chromatophores of purple bacteria an (ATP-induced) electric field over the primary radical pair results in an enhancement of the luminescence yield [29].

Summarizing, from the electroluminescence rise time and from the electrical field-induced luminescence-yield increase we conclude that electron transport both from P-700 to $A_0$ and from $A_0$ to further acceptors is electrogenic. Time-resolved electric measurements [13] suggest that electrogenicity of the secondary redox reactions is restricted to electron transfer between $A_0$ and $A_1$ if subsequent reactions occur within 50 ns. Our own analysis does not allow a discrimination between secondary reactions.

Shuvalov et al. [21,22] reported that in chloroplast fragments, which were detergent and heat-treated to remove PS II activity, $P^+$-$700A_0^-$ recombination occurred with a (second-order) half-time of 20 ms at 20 ° C and an activation enthalpy of 200 meV. These data deviate somewhat from our data, probably due to the analysis in terms of a second-order process (see above) and/or to a decrease in the energy level of $P^+$-$700A_0^-$ caused by the pretreatment. In the latter case the back reaction may have occurred in equilibrium with the triplet state $^3$P-700.

In PS II, comparison of fluorescence changes with integrated electroluminescence [4] showed that the luminescence yield was virtually unaffected by an external electric field. The saturation with field strength of the integrated PS II electroluminescence (Fig. 5) also points to this direction. Taking in mind a scheme similar to that in Fig. 6, with P-700, $A_0$ and $F_A$ replaced by P-680, I and $Q_A$ respectively, this is in accordance with the conclusion from field-induced fluorescence changes [17] that electron transfer between I and $Q_A$ accounts for at least 90% of the electrogenicity in PS II. It is at variance, however, with recent photovoltage measurements by Trissl et al. [20], who found a considerable electrogenicity of electron transfer from P-680 to I. The origin of this discrepancy remains to be clarified.

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