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# PRIMARY PROCESSES IN PHOTOSYSTEM I

## IDENTIFICATION AND DECAY KINETICS OF THE P-700 TRIPLET STATE

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**ESR studies at 4.2 K have been conducted in Photosystem I particles prepared with SDS (CP 1 particles) which were not poised at a low redox potential. Continuous illumination induces the appearance of a triplet state, spin-polarized according to a charge-recombination process. The decay kinetics of the triplet zero-field sublevels were derived from a study of the flash-induced ESR signals at the different triplet peaks: $k_x = 1150 \pm 350\ s^{-1}$, $k_y = 1050 \pm 350\ s^{-1}$, $k_z \leq 130\ s^{-1}$. From comparison with the absorption data and from an ESR kinetic study in the g 2.0 region, it was concluded that this triplet is the P-700 triplet whose decay accounts for the whole of the optical signal observed at low temperature with $k \approx 900\ s^{-1}$ ($t_{1/2} \approx 800\ \mu s$) and which is formed through a back-reaction between $P\text{-}700^+$ and the reduced primary acceptor $A_1^-$. This interpretation of the observed optical decay was recently proposed (Setif, P., Hervo, G. and Mathis, P. (1981) Biochim. Biophys. Acta 638, 257–267), contrasting with a previous assignment to the $P\text{-}700^+\text{-}A_1^-$ decay (Mathis P., Sauer, K. and Remy, R. (1978) FEBS Lett. 88, 275–278). A prolonged illumination induces an irreversible charge separation in about half the reaction centers. This was interpreted as indicating the presence in these centers of an electron acceptor following $A_1$, which is reduced with a very weak quantum efficiency, whereas the back-reaction between $P\text{-}700^+$ and $A_1^-$ is the major decay process. The chemical nature of this acceptor is unknown.**

## Introduction

A few years ago, studies on photosynthetic bacteria revealed, by combination of ESR and of absorption spectroscopy, that when the early electron acceptor, the iron-ubiquinone complex $Q_A$, is either absent or chemically reduced, illumination induces the formation of the primary donor triplet state. The first ESR observation of this state was made by Dutton et al. [1]. In the magnetic field of an ESR experiment, this state exhibits a polarization pattern which results from unusual populations of the spin sublevels [2]. This spin polarization was recognized as resulting from an electron-hole recombination [2] which is the reversal of the photoinduced electron transfer from the primary donor to the so-called intermediate acceptor. Later on, this subject was extensively studied by ESR spectroscopy and much information gained on the partners implicated in the primary photochemistry (for recent reviews, see Refs. 3–6).

In plant photosystems (PS I and PS II), under

Abbreviations: PS, photosystem; DCIP, dichlorophenolindophenol; Chl, chlorophyll.

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conditions where an electron was supposed to migrate, after light excitation, from the primary donor (P-700 or P-680) to the primary acceptor only [7–9], a triplet state has been observed with a pattern of polarization similar to the bacterial triplet and interpreted in the same manner. In PS I, this interpretation is strengthened by the observation of a magnetic field effect on either fluorescence [10] or delayed luminescence [11] with PS I particles under highly reducing conditions.

The primary electron transfer reactions have been extensively studied in PS I particles in which the electron acceptors $A_2$ (X) and P-430 (corresponding probably to the iron-sulfur centers A and B) were absent or chemically reduced. It is now widely accepted that another acceptor $A_1$, presumably a chlorophyll molecule, mediates the electron transfer from P-700 to $A_2$ (X) (see Ref. 12 for a recent review). CP I particles prepared with SDS are devoid of active iron-sulfur centers but they keep a functional electron acceptor $A_1$, as first shown by a study of the flash-induced absorption changes [13]. In a recent work [14], we further studied these absorption changes and discussed the two most probably hypotheses for the observed species: a state ($P\text{-}700^+\text{-}A_1^-$) lasting for a few microseconds at room temperature and about 800 $\mu$s below 60 K, or a P-700 triplet state formed through a rapid (submicrosecond) back-reaction between $P\text{-}700^+$ and $A_1^-$. Our data favored the last hypothesis which nevertheless needed confirmation.

This work presents additional support to the previous suggestion. ESR results were obtained with CP I particles under mildly reducing conditions. Under continuous illumination, a spin-polarized triplet spectrum was observed ($\Delta m_s = \pm 1$ transitions) which is apparently identical to the spectrum obtained by other groups [7,9] under either different [7,9] or identical [9] redox conditions. The decay rates were derived and were found to be in fair agreement with the optical data. Under the same conditions, we were unable to detect any signal corresponding to the $P\text{-}700^+$ radical which would decay with a 1 ms lifetime at 10 K.

## Material and Methods

CP I particles were purified by polyacrylamide gel electrophoresis in the presence of SDS, as previously described [15]. For ESR experiments, the solution was concentrated to 1 mg Chl/ml by dialysis against poly(ethylene glycol) 20000. An aliquot of this solution, after addition of 10 mM sodium ascorbate and 0.5 mM DCIP, was diluted 2-fold with glycerol before introduction in the ESR tube (2 mm internal diameter). The sample was then degassed and sealed under vacuum. After being kept in the dark, the sample was introduced into a helium cryostat (SMC, France). The temperature was measured with an As-Ga probe and could be controlled to within 0.5 K by heating the helium gas and by changing its flow. The temperature probe was placed outside the sample tube, the temperature of which was thus not precisely known. In control measurements made with a different set up and a sample tube of 3 mm internal diameter, permitting to lodge a carbon resistor, it appeared that the outside probe gives a correct indication, within less than 1 K. The temperature difference most likely increases during a continuous illumination, whereas sample heating was probably negligible in flash experiments (see Results and Discussion). ESR spectra were recorded with a Varian E-109 spectrometer operating at 100 kHz modulation frequency. The instrumental response time was 150 $\mu$s for kinetic traces. For triplet and $P\text{-}700^+$ measurements, the field modulation was 20 and 4 G, respectively. Continuous illumination was provided either by a mercury (high pressure) Philips SP 500 lamp (500 W) whose emission was filtered with a water cuvette and a blue filter (Corning 5-57) (ESR measurements) or by an 800 W tungsten-iodine lamp (absorption measurements). For kinetic measurements, the sample was excited by a xenon flashlamp (10 $\mu$s; half-width, 20 J; pulse frequency about 1 Hz) which was unattenuated, unless otherwise indicated. Kinetic traces were averaged (128–4096 scans, depending on microwawe power) and spectra were recorded with a Hewlett-Packard E-900 acquisition system. The time scan and the flash were sequentially triggered by an external clock. For absorption measurements, we used the same setups as described in (Ref. 14). The photodiode

amplifier was slightly modified so as to record signals with a decay time up to 10 ms without a significant distortion due to a.c. coupling.

## Results and Discussion

*ESR spectrum induced by continuous illumination*

Illumination of CP I particles at 4.2 K induces an ESR spectrum which is shown as a 'light' minus 'dark' difference spectrum (Fig. 1). The main feature is a six-line spectrum characteristic of a triplet state for $\Delta m_s = \pm 1$ transitions [16] and displaying the aeeaae polarization pattern (a, absorption of microwave power; e, emission) which arises from a charge recombination [2]. In addition, a large signal appears in the $g = 2.0$ region.

The triplet spectrum is similar to that previously reported [7,9] under either different [7,9] or identical [9] redox conditions in PS I particles. It exhibits the same zero-field splitting parameters ($|D| = 0.0280 \pm 0.0005$ cm$^{-1}$, $|E| = 0.0038 \pm 0.0002$ cm$^{-1}$). In Fig. 1, the different peaks are labelled as for chlorophyll in vitro, assuming $D > 0$ and $E < 0$ [17]. In the particles under study, highly reducing conditions are not necessary to observe this triplet state, probably because the electron cannot go further than the primary acceptor, as the more remote acceptors are either absent or inactivated. The six lines attributed to the triplet state were absent when P-700 was oxidized by 5 mM potassium ferricyanide prior to freezing.

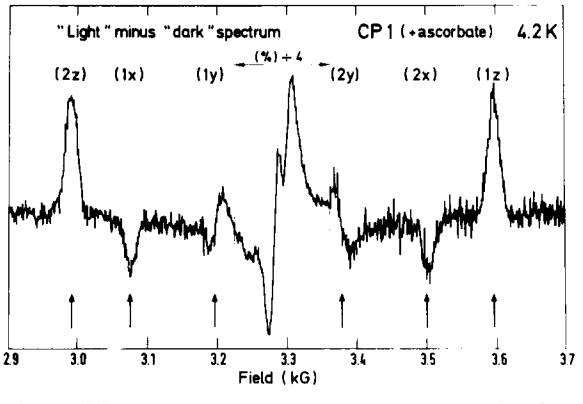


Fig. 1. Light minus dark ESR spectrum observed with CP I particles poised with ascorbate and DCIP. The light spectrum was recorded first, the dark spectrum was then recorded and subtracted. A subsequent illumination produced the same light spectrum. The triplet peaks are indicated by the six arrows and are labelled successively $2z$, $1x$, $1y$, $2y$, $2x$ and $1z$ with increasing field. In the central $g$ 2.0 region the recorder sensitivity is 4-times smaller. Instrument settings: microwave power 0.05 mW, modulation amplitude 20 G, frequency 9.22 GHz, instrument gain $5 \cdot 10^4$.

Before being illuminated at low temperature, the sample does not show any signal detectable under the conditions of Fig. 1. After illumination with continuous light, a large radical signal remains in the dark (dark spectrum). After a sufficient time of exposure to light, this dark signal does not increase any more following further illumination, but a difference subsists around $g = 2.0$ between light and dark conditions. This difference signal is larger than the triplet signal (Fig. 1) but appears very weak when compared to the radical signals in the individual spectra, either light or dark. It has a complex line shape and may be composed of two signals (see below). We could not detect any transient signal in this field range at 4.2 K following a flash excitation. Our results do not permit an unambiguous interpretation of these signals. It is quite possible that the difference spectrum around $g$ 2.0 arises from a temperature increase during continuous illumination.

*Decay kinetics of the triplet state measured by ESR*

ESR kinetic traces for CP I particles excited by xenon flashes were averaged at 4.2 K for the $2z$, $1x$ and $1y$ peaks at different microwave powers.

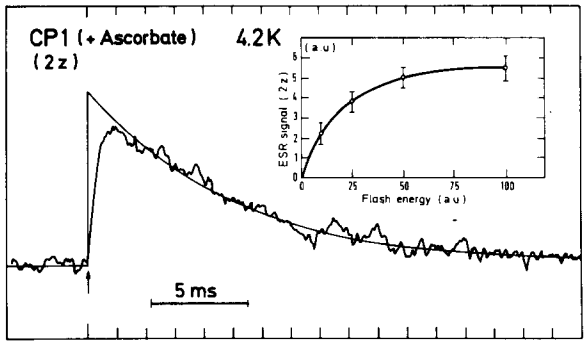


Fig. 2. Rise and decay of the flash-induced ESR signal at the ($2z$) peak of the triplet in CP I particles at 4.2 K. The rise time is instrument limited. Instrument settings: microwave power 2 $\mu$W, modulation amplitude 20 G frequency 9.22 GHz, instrument gain $5 \cdot 10^4$. The drawn line is calculated for a monoexponential decay with $k = 130$ s$^{-1}$. Inset: ESR signal at the $2z$ peak vs. flash energy. An energy of 100 corresponds to no attenuation. Same instrument settings, except microwave power: 0.05 mW.

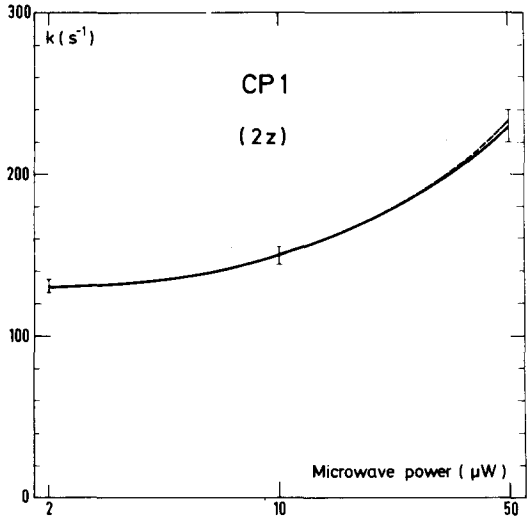


Fig. 3. Decay rate constant of the flash-induced $2z$ signal in CP I particles at 4.2 K as a function of microwave power ($x$-axis: logarithmic scale). The drawn curves which fit the experimental bars correspond to the two following sets of parameters: (———) $kz = 30\ s^{-1}$, $1/T_1 = 100\ s^{-1}$, $P = 5.5\ s^{-1}$ at 2 μW ($k_x = 1100\ s^{-1}$, $k_y = 1000\ s^{-1}$; (------) $kz = 115\ s^{-1}$, $1/T_1 = 10\ s^{-1}$, $P = 5\ s^{-1}$ at 2 μW ($k_x = 1200\ s^{-1}$, $k_y = 1100\ s^{-1}$).

To calculate the depopulation rates $k_x$, $k_y$ and $k_z$ of the three zero-field sublevels, we use a treatment similar to that of Ref. 34, except that the relaxation between the $|+1>$ and $|-1>$ levels is also taken into account (same spin-lattice relaxation time $T_1$). The trace obtained at 2 μW for the $2z$ peak is shown in Fig. 2 with the calculated monoexponential decay curve corresponding to a rate constant of $130\ s^{-1}$. As shown in the inset, the $2z$ signal was practically saturated at our maximum flash intensity. For a 2-fold attenuation, the triplet signal decreased only by about 10%. At 2 μW, the signals appear to decay almost monophasically with decay rates of $130 \pm 5$, $1200 \pm 300$ and $1100 \pm 300\ s^{-1}$ for the $2z$, $1x$ and $1y$ peaks, respectively. The poor precision for the two last figures results from the rather large instrument response time (approx. 150 μs) and from the low signal-to-noise ratio in the tail of the curve. $K_x$ and $k_y$ can thus be estimated from a monoexponential decay fitting in the intermediate time domain (after the initial instrument-limited rise and before the signal becomes too small). For each peak the decay rate is approximately the same at 2 and 10 μW. This suggests that these microwave powers do not affect the decays, as confirmed by the decay rate dependence upon microwave power at the $1z$ peak (Fig. 3). A value of $5\ s^{-1}$ can thus be estimated for the probability of the transition $P$ induced by the microwave field at 2 μW. Hence the measured rates correspond to the following parameters:

$$P = 5 \pm 0.5\ s^{-1} \text{ at } 2\ \mu W$$

$$k_z + 1/T_1 = 130 \pm 5\ s^{-1}$$

$$k_x + 1/T_1 = 1200 \pm 300\ s^{-1}$$

$$k_y + 1/T_1 = 1100 \pm 300\ s^{-1} \qquad (1)$$

From our data, we cannot determine the spin-lattice relaxation time $T_1$ but only a superior limit can be deduced from the second formula in Eqn. 1, i.e., $1/T_1 \leqslant 130\ s^{-1}$. The depopulation rates of the three zero-field sublevels can be estimated: $k_x = 1150 \pm 350\ s^{-1}$, $k_y = 1050 \pm 350\ s^{-1}$, $k_z \leqslant 130\ s^{-1}$, from which we deduce: $\Sigma k_i/3 = 750 \pm 250\ s^{-1}$ or, for the corresponding $t_{1/2}$: $700\ \mu s \leqslant t_{1/2} \leqslant 1400\ \mu s$.

*Kinetics of absorption changes at low temperature*

We previously found [14] that the absorption changes induced by a laser flash with CP I particles decay quasi-exponentially with $k \approx 900\ s^{-1}$ at 10 K. This was a somewhat rough estimation, as the a.c. coupling of the amplifier would have prevented us from measuring precisely a decay phase with $t_{1/2} > 2$ ms. We attempted to make a more accurate kinetic analysis at low temperature on a longer time scale with a modified setup (see Material and Methods). The absorption changes induced in CP I particles by a 1 μs dye laser flash were measured at $7 \pm 1$ K and $30 \pm 2$ K at two different wavelengths (700 and 820 nm), for which the same temperature effect was observed. Semi-logarithmic plot analysis indicates (not shown) that the decay is quasi-monoexponential at 30 K with $k \approx 900\ s^{-1}$ (Fig. 4a). This is consistent with the preceding decay rates of the three triplet peaks, since at 30 K, the relaxation time between the triplet sublevels is expected to be considerably smaller than at 4.2 K. This would thermally mix the three zero-field triplet state sublevels, resulting in a nearly monophasic absorption decay with a rate constant of $\Sigma k_i/3$, $i = x, y, z$. The ESR results

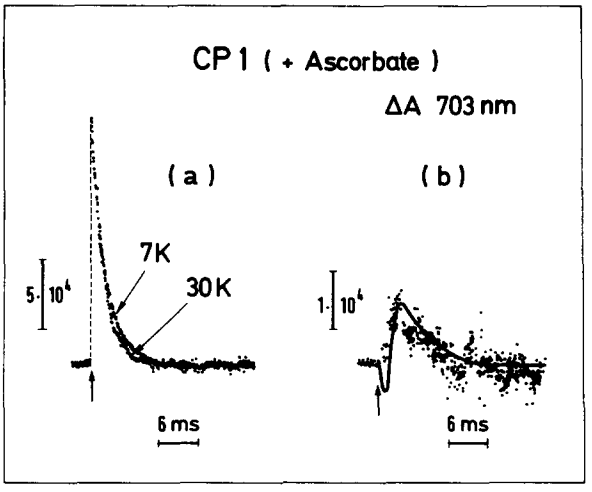


Fig. 4. (a) Kinetics of $\Delta A$ at 703 nm induced in CP I particles ($A_{678} = 1.2$) by a nearly saturating 1 $\mu$s dye laser pulse at 7 and 30 K. An upward signal corresponds to an absorption decrease. The two traces are superposed just after the flash but they differ later (lower trace at 30 K and upper trace at 7 K). Each trace is the average of 16 experiments. (b) Difference between the two traces at 7 and 30 K. The drawn curve is plotted for the following values: 7 K, two exponential components with $k = 300$ $s^{-1}$ (25%) and $k = 1300$ $s^{-1}$ (75%); 30 K, a single exponential with $k = 900$ $s^{-1}$.

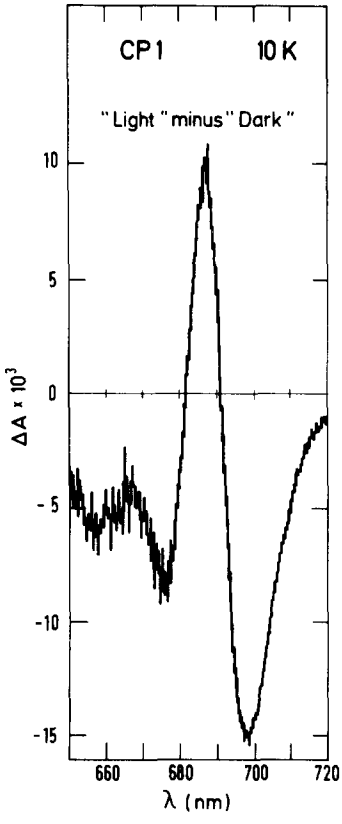


Fig. 5. Difference spectrum obtained with CP I particles ($A_{678} = 1.5$) at 10 K. After cooling in the dark, the spectrum was recorded before and after a continuous illumination (10 s) and the former was substrated from the latter. The maximum of $\Delta A$ at 700 nm corresponds to an $A_{678}/\Delta A$ ratio of about 100.

are hence compatible with the optical data within the experimental uncertainties. As shown in Fig. 4a, the decay at 7 K has a trailing tail when it is compared to that at 30 K (Fig. 4a), indicating the appearance of a polyphasic decay when the temperature decreases down to 7 K. Both $t_{1/2}$ and contributions of the two main phases at 7 K are fairly difficult to evaluate because the decay rates are not different enough and because a much slower phase ($t_{1/2} > 10$ ms) of weak magnitude (about 1% of the whole signal) is also present. However, we attempted such an estimation in Fig. 4b where a tentative simulation of the difference between the decay curves at 7 and 30 K is given, with values grossly consistent with the triplet decay, considering that the three zero-field triplet sublevels equilibrate slowly at 7 K.

*Irreversible signals induced by continuous illumination at low temperature*

When the CP I particles are illuminated at low temperature (tested for $T \leq 100$ K) with a continuous actinic light, some P-700 becomes irreversibly oxidized. This was tested both by ESR and by absorption spectroscopy. About half of the chemically oxidizable P-700 is finally oxidized and then a further illumination has no additional irreversible effect (after about 2 min in the EPR experiments and less than 10 s in the absorption experiment: the difference between the two durations is most likely due to very different conditions of illumination and absorbance in the two kinds of experiment). The absorption difference spectrum thus obtained is similar to the chemically induced ($P\text{-}700^+$-P-700) spectrum from 550 to 750 nm (Fig. 5; 650–720 nm; cf. Fig. 7 in Ref. 13), so that the contribution of the irreversibly reduced acceptor molecule appears negligible in this wavelength region. We cannot exclude that a chlorophyll acceptor could give fortuitously the same difference spectrum as P-700. However, our interpretation is further supported by a quantitative com-

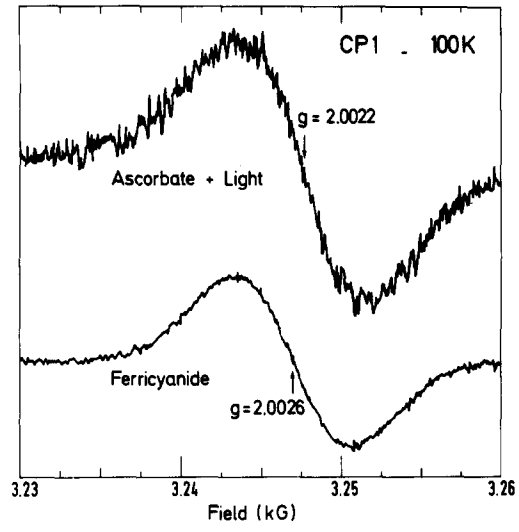


Fig. 6. ESR spectra at 100 K of CP I particles. Upper trace: after equilibration at room temperature with sodium ascorbate and DCIP, the sample was cooled in the dark down to 100 K. It was then illuminated with continuous light and the spectrum subsequently recorded in the dark. Instrument gain: $3.2 \cdot 10^4$. Lower trace: the sample was equilibrated with a mixture of potassium ferri- and ferrocyanide (0.5 mM ferricyanide, 5 $\mu$M ferrocyanide) at room temperature and cooled in the dark. Instrument gain: $1.25 \cdot 10^4$. Instrument settings: microwave power 10 $\mu$W, modulation amplitude 4 G, frequency 9.10 GHz.

parison of the effect of illumination at low temperature on the flash-induced ESR triplet signal and on irreversible signals (see Conclusion).

The light-induced ESR spectrum in CP 1 particles is shown in Fig. 6 together with the spectrum of the chemically oxidized P-700 obtained under identical conditions of temperature (100 K) and of nonsaturating microwave power (10 $\mu$W). A close examination reveals that the two spectra are not identical: in the light-induced spectrum, the line shape is broader (8.5 G instead of 7.5 G for P-700) and the $g$ value is slightly smaller ($g$ 2.0022 if we assume $g$ 2.0026 for P-700$^+$). These differences are hardly out of the noise level, but thet were consistently observed and we suggest that they result from the presence, in the light-induced spectrum, of a signal due to a reduced electron acceptor, in addition to the signal due to P-700$^+$. This proposal is supported by the observation that the shape of the light-induced signal is not modified during its growth when a weak actinic light is applied at low temperature. As the absorption changes induced by a laser flash on a dark-adapted sample are almost completely reversible, we conclude in favor of the existence of an acceptor molecule which can be irreversibly reduced in about half of the reaction centers with a very weak quantum efficiency. This electron acceptor may be either nonphysiological or an altered form of a physiological acceptor resulting from the purification procedure. It looks different from the radical attributed to the primary acceptor and reported in Refs. 18 and 19 as these radicals have $g$ values larger than the $g$ value of P-700$^+$. From the absorption data (Fig. 5) it seems doubtful that it could be a chlorophyll molecule and its chemical nature is unknown at this moment.

*ESR kinetics in the g 2 region*

The preceding light-induced signal is broader at 10 K than at 100 K (about 10 G, see Fig. 7a) probably due to a saturation effect even at the lowest microwave power available with our spectrometer (2 $\mu$W). At 10 K, the spectrum shows more clearly than at 100 K the presence of two radicals, which are probably P-700$^+$ and a reduced acceptor. We observed (not shown) a rough parallelism between the appearance of the light-induced irreversible signal and the decrease of the triplet signal elicited by a series of flashes. When the sample exhibits a maximum light-induced irreversible signal, the triplet signal is about 2-fold smaller than before the continuous illumination and is no more affected by a subsequent illumination.

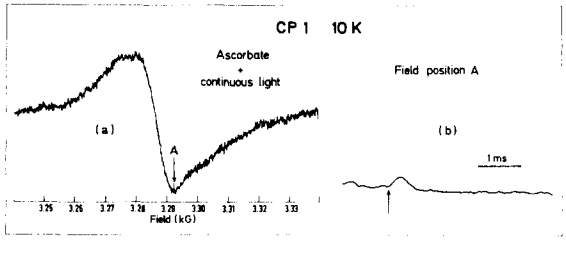


Fig. 7. ESR spectrum and kinetic trace at 10 K with CP I particles (ascorbate and DCIP). (a) The spectrum was obtained as in Fig. 6 (upper trace) by illumination at 10 K. (b) The kinetic trace is recorded for a field of 3292 G (arrow A in a). Average of 1024 experiments. The flash is indicated by the arrow. The instrument settings are identical for a and b: microwave power 0.2 mW, modulation amplitude 4 G, frequency 9.22 GHz, instrument gain $2 \cdot 10^4$.

This light-induced irreversible signal could serve as a reference of the P-700$^+$ signal to evaluate quantitatively on the same sample the flash-induced ESR signal that is elicited at 10 K around $g$ 2.0. As is shown for the high-field peak of P-700$^+$ (Fig. 7b, the signal magnitude is directly comparable with that of Fig. 7a), practically no transient signal was found in this region. At 10 K, $T_1$ was assumed to be about 900 $\mu$s for P-700$^+$ [20] and since we used a microwave powder (0.2 mW) that is well above the minimal saturating power, we estimated that $P \gg 1000\ s^{-1}$ ($P \gg 1/T_1$). Thus, we have $(2P + 1/T_1 + k) > 4000\ s^{-1}$ and (see Appendix) it would be easy to observe any chemical P-700$^+$ decay with $k \approx 900\ s^{-1}$ and to extrapolate it to zero time. The absence of such a signal under conditions where the flash is saturating (Fig. 2, inset) and where the P-700$^+$ signal is readily observable (Fig. 7a) shows that a hypothetical back-reaction between P-700$^+$ and a reduced acceptor (with $k \approx 900\ s^{-1}$) occurs in a minor part, if any, of the reaction centers (less than 10% within the experimental uncertainties). This lack of flash-induced P-700$^+$ ESR signal, together with the previous absorption results, reinforces the assignment of the whole optical decay to the triplet state decay that we observed by ESR kinetics.

## Conclusion

The first part of the present results show that a polarized triplet can be elicited by continuous illumination at 4.2 K of CP I particles whose only photochemical activity is PS I and which are not poised at low redox potential. In previous studies on that subject, a triplet with similar ESR characteristics had been obtained with other PS I particles poised at different potentials [7,9]. This different requirement is predicted on the basis of the hypothesized presence of only the primary acceptor $A_1$ in CP I particles [13], of a more complete sequence of acceptors in the other particles and of the hypothesis that the polarized triplet is formed in a back-reaction between P-700$^+$ and $A_1^-$ when the electron cannot migrate away from $A_1$. CP I particles thus behave like reaction centers of purple bacteria treated to remove the primary ubiquinone [21].

In this work, we report the first measurement of the kinetic parameters of this polarized triplet. At 4.2 K and at low microwave power, under conditions where the triplet sublevels are almost isolated, the three different peaks (at low field) decay with rate constant of $130 \pm 5$, $1200 \pm 300$ and $1100 \pm 300\ s^{-1}$. These decay rates are perfectly compatible with those obtained by flash absorption spectroscopy. In a recent work, using flash absorption spectroscopy, we already concluded that the signals elicited in CP I particles were largely due to a triplet state of P-700. The present work proves that this conclusion is correct, in agreement with an interpretation proposed by other groups [9,11]. This convergence between our absorption and ESR data leads to an additional conclusion: the triplet state which is detected by ESR is that of P-700, since the absorption difference spectrum has a well defined negative peak at 700 nm. This conclusion could not be reached by ESR alone. Structural information on P-700 should be deducible from the zero-field splitting parameters and the intersystem crossing rates of $^3$P-700. This may be somewhat premature, however, since the dimeric nature of P-700 is not as certain as for the bacterial primary donor [22] and since both $D$ and $E$ values for chlorophyll in vitro exhibit some variations depending on the solvent (for some values, see Ref. 3) and on the concentration of chlorophyll [23,24]. The kinetic properties of $^3$P-700 vary largely with temperature, contrarily to the chlorophyll triplet in vitro [13,25]. The law of variation is essentially the same for $^3$P-700 and for the primary donor of photosynthetic bacteria [13,26]. At low temperature the lifetime of $^3$P-700 is not too different from that of the Chl *a* triplet state, either monomeric or aggregated, in vitro [25], whereas the triplet of the bacterial primary donor decays significantly faster than the triplet state of bacteriochlorophyll [3].

After prolonged illumination at low temperature, the size of the flash-induced ESR triplet signal decreases progressively to about 50% of its initial value and then remains unaffected by more prolonged illumination. Absorption data, on the other hand, indicate that in about half of the reaction centers, P-700 can be photooxidized irreversibly. The best interpretation of these data is that, in 50% of the reaction centers, an electron acceptor different from $A_1$ is present. It may accept

an electron from $A_1^-$ and lead with a low quantum efficiency to a stable charge separation. This acceptor is neither an iron-sulfur center (none of their characteristic ESR lines were detected in our experiments; the CP I particles contain less than one Fe atom per reaction center [27] nor a Chl *a*, the reduction of which would probably distort the red difference spectrum of P-700. After reduction, its ESR spectrum is slightly different from that of $P\text{-}700^+$.

Fig. 8 summarizes the kinetic pathways for electron flow between the primary partners of PS I at low temperature ($T \leq 60$ K), as they can be extracted from this and other recent studies [11,14,28,29]. The primary acceptor $A_1$ may be a chlorophyll molecule [12] but definite arguments are lacking for this assignment to date. The energy differences between the state $^1$(P-700) and $^{1,3}[(P\text{-}700)^+\text{-}A_1^-]$ and between $^3$(P-700)-$A_1$ and the ground state are taken, respectively, from Ref. 11 and from a phosphorecence wavelength of 960 nm, which was found for chlorophyll both in vitro and in vivo [30–32].

Sonneveld et al. [11], from their luminescence results, proposed that the lifetime of the radical

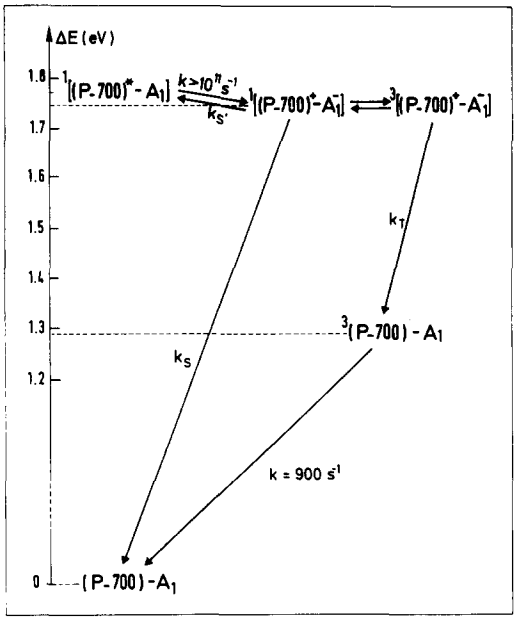


Fig. 8. Kinetic pathways for the different states involving the primary partners P-700 and $A_1$ in CP I particles at low temperature.

pair is largely determined by the recombination $^3[(P\text{-}700)^+\text{-}A_1^-] \rightarrow {}^3(P\text{-}700)\text{-}A_1$. This is consistent with a yield of P-700 triplet formation, compared to the yield of biradical formation, approaching unity, whereas we found [14] values of 0.7 and 0.5, respectively, at 10 K and 21°C. This discrepancy may be due to different interactions between the primary partners in the two kinds of PS I particles.

## Appendix

*Kinetics of the radical decay*

This section describes the hypothetical decay of the $P\text{-}700^+$ radical formed after an actinic flash in order to decide if such a decay occurs at 10 K in the CP I particles by back-reaction between $P\text{-}700^+$ and an electron acceptor. It follows a treatment similar to that used by McElroy et al. [35]. We consider that the actinic flash populates instantaneously (time zero) the two Zeeman sublevels with probabilities $p$ and $(1-p)$. The value of $p$ is ordinarily 0.5 but may differ in the process under question due to spin polarization. The various processes involved in the population changes of the $P\text{-}700^+$ sublevels are depicted in Fig. 8. $n$ is the concentration of $P\text{-}700^+$ produced at zero time by the actinic flash and $N^+(t)$ and $N^-(t)$ describe this concentration of $P\text{-}700^+$ in its respective spin states. $k$, $w_{+-}(w_{-+})$ and $P$ are, respectively, the rate constant of $P\text{-}700^+$ chemical decay, the spin-lattice relaxation rates ($w_{+-}+w_{-+}=1/T_1$) and the probability of the transition induced by the microwave field. The $P\text{-}700^+$ population $N(t) = (N^+(t), N^-(t))$ varies with time as follows:

$$\frac{dN}{dt} = AN$$

with

$$A = \begin{pmatrix} -(k+P+w_{+-}) & w_{-+}+P \\ w_{+-}+P & -(k+P+w_{-+}) \end{pmatrix} \qquad \text{(A1)}$$

The ESR signal is proportional to the population difference between the two Zeeman levels, which has the following expression, taking $(N(0) = (pn, (1-p)n)$:

$$(N_- - N_+)(t) = \frac{n(w_{+-}-w_{-+})}{2P+w_{+-}+w_{-+}} \left( \exp(-kt) \right.$$

$$+\frac{2[(1-p)(P+w_{-+})-p(P+w_{+-})]}{w_{+-}-w_{+-}}$$

$$\times\exp[-(k+2P+w_{+-}+w_{-+})t]\Bigg) \quad \text{(A2)}$$

The kinetics are a superposition of two exponential decays with rate constants $k$ and $(k+2P+1/T_1)$. So the major part of the measured decay corresponds to the chemical decay whether or not spin polarization is induced by the charge separation process, provided that $(k+2P+1/T_1) \gg k$.

As the first term of Eqn. A2 gives the population difference between the two Zeeman levels of a stable radical with concentration $n$, we can readily compare the populations $n$ of P-700$^+$ in both kinds of experiments (kinetics of decay and irreversible signal) under identical experimental conditions of temperature and microwave power, provided that $(k+2P+1/T_1) \gg k$.

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