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# Absorption studies of Photosystem I photochemistry in the absence of vitamin K-1

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**Flash-induced absorption changes were studied on different timescales (nanosecond to millisecond) and at different temperatures (10 to 278 K) in highly enriched spinach PS I particles lacking vitamin K-1 and in which the electron transfer from the primary acceptor to the secondary acceptors was blocked. At all temperatures, the initial absorption change at 820 nm was followed by a fast decay ($t_{1/2} \approx 47$ ns at 278 K and $\approx 82$ ns at 10 K) which is attributed to the decay of the primary radical pair ($P\text{-}700^+\text{-}A_0^-$). A slower phase of absorption decay is attributed to the P-700 triplet state, which was formed as a result of the biradical recombination, with a yield of about 30% at 278 K and about 75% at 10 K. Under air, the $^3$P-700 state decayed with a $t_{1/2}$ of about 50 $\mu$s at 278 K, whereas in the absence of oxygen it decayed with $t_{1/2} \approx 560$ $\mu$s. At 278 K, this yield was shown to depend on the presence of a magnetic field, with a maximum around 60 G. The $^3$P-700 decay halftime was nearly independent of temperature in the absence of oxygen ($t_{1/2} \approx 1$ ms at 10 K). The implications for the mechanisms involved in this decay are discussed. Addition of vitamin K-1 to these particles resulted in a decrease in the amplitude of the fast submicrosecond decay and a concomitant increase in the amplitude of a slow phase, indicating an efficient transfer from $A_0^-$ to vitamin K-1. However, most functional properties of the acceptor $A_1$ were not reconstituted under these conditions.**

## Introduction

Light excitation of Photosystem I (PS I) induces a charge separation between the primary electron donor P-700 and the primary electron acceptor, $A_0$, which is probably a chlorophyll molecule absorbing near 690 nm [1] ($P\text{-}700^*\text{-}A_0 \rightarrow P\text{-}700^+\text{-}A_0^-$). This primary charge separation is then stabilized by further electron transfer from $A_0^-$ to secondary acceptors $A_1$, $F_X$, $F_B$ and $F_A$. Whereas $F_X$, $F_B$ and $F_A$ are most probably iron-sulfur centers [2–4], it has been recently proposed that $A_1$ is a quinone molecule [5–7] and more precisely a vitamin K-1 (or phylloquinone) molecule [8], which is the only quinone present in PS I [9–11]. Vitamin K-1 can be completely removed from PS I particles by treatment with organic solvents [12,13]. Highly enriched PS I particles, from which antenna chlorophylls have been extracted by a treatment with water-saturated diethyl ether, are thus devoid of vitamin K-1 [12].

The electron transfer pathways of these particles are modified at room temperature as well as

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Abbreviations: PS I, Photosystem I; $\Delta A$, absorption change; DCIP, dichlorophenolindophenol.

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at low temperature compared to unextracted PS I particles. In particular, the electron transfer is essentially blocked at the level of the primary acceptor $A_0$. From flash absorption studies of these particles under different oxidoreduction conditions, we report here different properties of the primary radical pair ($P\text{-}700^+\text{-}A_0^-$) as well as of the triplet state of P-700.

## Material and Methods

*Biological material*

The preparation of PS I particles from spinach, including the diethyl ether treatment, has been described previously [14,15]. In brief, the PS I particles, obtained after solubilization of the thylakoid membranes with digitonin, were lyophilized (control particles) and then extracted with water-saturated diethyl ether. The ether-extracted particles were solubilized with 20 mM phosphate buffer (pH 8) containing 0.1–0.2% Triton X-100 by incubation for 15 min at 0–4°C. Insoluble greyish-white materials were removed by centrifugation and the blue-green supernatant was diluted about 2–4-times with 20 mM phosphate buffer (pH 8) or 0.1 M glycine-NaOH buffer (pH 10) according to the purpose of the experiments. The temperature was maintained between 0 and 5°C during all treatments to minimize damage of the samples.

From the chemical assay of PS I particles, using an absorption coefficient of 64 $mM^{-1} \cdot cm^{-1}$ for the red maximum of P-700 [16], a chlorophyll to P-700 ratio of 150 was found for control particles and a ratio of 13 for extracted particles. Chlorophyll concentration was determined optically in 80% acetone extracts. The vitamin K-1 content of the particles was determined as described in Ref. 12.

*Addition of vitamin K-1*

Stock solutions of vitamin K-1 were prepared in ethanol. For reconstitution experiments, vitamin K-1 was added directly to the extracted PS I particles in aqueous solution (1% ethanol, final concentration). The PS I particles were incubated for 30 min with 50 $\mu$M vitamin K-1, were diluted by about 20-times with the 20 mM phosphate buffer (pH 8) and then centrifuged at 50000 rpm for 1 h. The resultant precipitate was resolved in 0.5 ml of the same buffer containing 0.1% Triton X-100. The recovery of the PS I particles after this treatment was about 53%.

*Absorption kinetic measurements*

Nanosecond absorption changes at 820 nm were measured as in Ref. 17 with the modifications described in Ref. 18. In brief, excitation laser pulses at 532 nm were of 30 ps duration (full width) and the measuring light was detected by a rapid response silicon photodiode (Lasermetrics 3117). The signal from the diode was first amplified (10-20-1C amplifier from Nuclétudes: bandwidth 500 Hz–500 MHz) and then recorded by a Tektronix 7912 digitizer interfaced with a signal averager (Didac, Intertechnique). In some cases, the same arrangement was used for absorption experiments at 820 nm with a microsecond or millisecond time resolution. In the latter case, the output signal of the rapid photodiode was amplified using the 7A22 Tektronix plug-in amplifier (DC–1 MHz). Microsecond absorption experiments were also performed with another apparatus which has been previously described [19,20]. For all microsecond absorption experiments, the signals were recorded using a Biomation 2805 digitizer coupled to a Tracor 1710 signal averager. Room-temperature experiments were performed using either a rectangular cuvette with the first apparatus (rapid silicon photodiode) (optical path 10 mm in the direction of the measuring beam and 2 mm in the direction of the excitation beam) or a square cuvette with the second apparatus (10 mm $\times$ 10 mm). For low-temperature experiments, the PS I particles were contained in a Plexiglas cuvette which was inserted in a cryostat cooled with helium gas [21]. The pathlengths for excitation and detection were 5 and 10 mm, respectively, for nanosecond absorption measurements.

## Results

*Kinetics of flash-induced absorption changes at 5°C*

The kinetics of flash-induced absorption changes were studied at 820 nm on nanosecond to millisecond time scales with PS I particles in the presence of ascorbate and DCIP. In control particles containing two vitamin K-1 per reaction

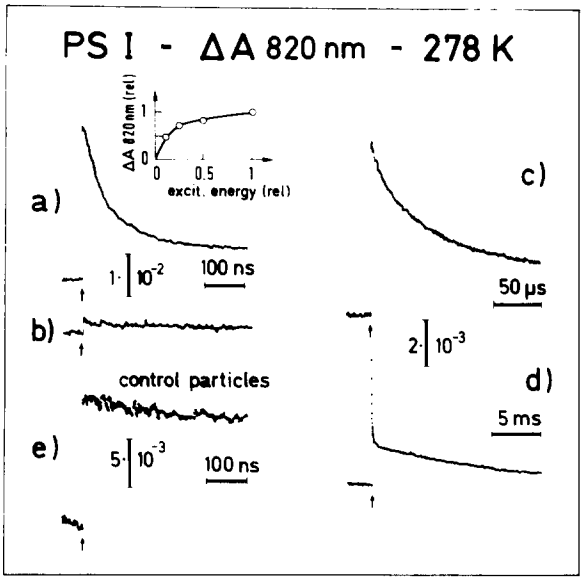


Fig. 1. Flash-induced absorption changes at 820 nm and at 5 °C in control PS I particles (e: $A_{678nm} \approx 18$; Chl/P-700 ≈ 1500 or extracted PS I particles (a–d and inset: $A_{676nm} = 4.2$; Chl/P-700 ≈ 13). Addition of 2 mM potassium ferricyanide (trace b) or 4 mM sodium ascorbate and 50 µM DCIP (a, c, d, e and inset) at pH 8. Excitation: 30 ps pulses at 532 nm; energy ≈15 mJ (a–e and inset: excitation energy of 1); 1 flash/5 s. Electrical bandwidth: 500 Hz–500 MHz (a, b, e and inset), DC–1 MHz (c) and DC-3 kHz (d). Average of 5 (e), 10 (b and c), 20 (d) or 50 (a) experiments. Inset: initial $\Delta A$ measured on a nanosecond timescale as a function of excitation energy.

center [12], a steplike signal is observed (Fig. 1, trace e). The absorption rise within 2 ns is followed by a slow decay (much longer than 1 µs). In this case, the primary charge separation is presumably followed by a very fast subnanosecond electron transfer to the secondary acceptor, $A_1$ [1,22], and further stabilized by electron transfer to iron-sulfur centers. As the secondary electron acceptors do not contribute to the absorption changes at 820 nm, the signal observed at this wavelength most probably corresponds to the photooxidation of P-700 which is followed by a very slow recombination with P-430 ($t_{1/2} \approx 30$ ms [23]).

By contrast, in the case of the extracted PS I particles devoid of vitamin K-1, the immediate absorption increase is followed by a fast submicrosecond decay which amounts to about 78% of the total amplitude of the signal (Fig. 1, trace a). A curve-fitting analysis of the fast decay was attempted on the basis of one (poor fit; $t_{1/2} = 50$ ns) or two exponential decay phases (much better fit; $t_{1/2} = 84$ ns and $t_{1/2} = 26$ ns, respectively 41 and 59% of the fast phase). In the same particles, the signal almost completely disappears in the presence of ferricyanide (Fig. 1, trace b; 8% of its value in the presence of ascorbate). This fast decay can be attributed to charge recombination between P-700$^+$ and $A_0^-$ [18,24], thus indicating that electron transfer from $A_0^-$ to secondary acceptors is blocked in these particles. The disappearance of the signal in the presence of ferricyanide confirms this assignment to PS I photochemistry.

The inset of Fig. 1 shows the initial size of the signal at 820 nm as a function of excitation energy (a relative energy of 1 corresponds to approx. 15 mJ per pulse). A 50% attenuation of the laser was accompanied by only a 20% decrease in the initial signal size. Thus, the laser pulse was almost saturating prior to attenuation. Assuming that the absorption coefficients of P-700$^+$ and $A_0^-$ are about the same at 820 nm (i.e., $\epsilon_{P\text{-}700^+} \approx \epsilon_{A_0^-} \approx 6500$ $M^{-1} \cdot cm^{-1}$ [25,26]), the initial signal amplitude without attenuation of the laser corresponds to one radical pair (P-700$^+$ – $A_0^-$) for about 25 chlorophyll molecules. However, the chemical assay of P-700 gave a chlorophyll- to P-700 ratio of 13, and the origin of this discrepancy has not yet been clarified.

The fast decay is followed by slower decay phases which represent about 22% of the total signal and which are displayed in traces c and d of Fig. 1. This slow decay was satisfactorily deconvoluted into 2 exponential phases: a component with a $t_{1/2} \approx 50$ µs (14–15% of the total signal) and a slower component with a $t_{1/2} \approx 10$ ms (7–8% of the total signal). The spectrum of the faster microsecond component ($t_{1/2} \approx 50$ µs) was measured in the red and near infrared regions (not shown) from kinetic traces similar to trace c of Fig. 1. It exhibits a negative maximum at 696 nm and a nearly flat positive band from 750 to 820 nm. These features indicate that this component probably reflects the decay of the P-700 triplet state [27], which is most likely formed from a recombination reaction between P-700$^+$ and $A_0^-$. The slower component ($t_{1/2} \approx 10$ ms) also shows a

negative maximum around 696 nm, but a detailed spectrum of this phase was not measured. This slow component may be due to reaction centers where P-700$^+$ is not rapidly reduced by a recombination reaction with $A_0^-$.

*Addition of vitamin K-1*

Addition of 50 $\mu$M exogenous vitamin K-1 on extracted PS I particles has important effects on the submicrosecond decay kinetics at 820 nm. It can be seen that the amplitude of the fast submicrosecond decay phase due to the recombination reaction between P-700$^+$ and $A_0^-$ decreases upon addition of vitamin K-1 with an increase in a slow phase (Fig. 2, left and upper right). The decrease in initial signal size can be interpreted by a subnanosecond electron transfer from $A_0^-$ to vitamin K-1. Resolution of the slow phase on longer time scales reveals a very slow component ($t_{1/2} > 500$ ms) (not shown) which has no equivalent in control particles. The kinetics of $\Delta A_{820}$ were completely conserved after dilution of the reconstituted sample by about 20-times with buffer followed by centrifugation and resuspension (see Material and Methods). However, addition of dithionite to the reconstituted particles at pH 8

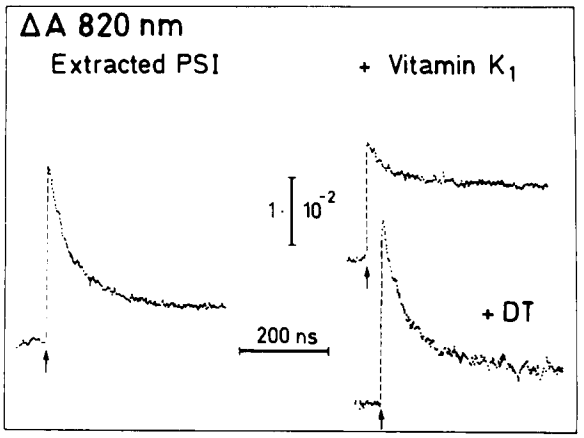


Fig. 2. Effect of the addition of vitamin K-1 on flash-induced absorption changes at 820 nm in extracted PS I particles ($A_{676nm} \approx 4.8$; Chl/P-700 $\approx$ 13). Excitation: 30 ps pulses at 532 nm; energy $\approx$ 15 mJ. Electrical bandwidth: 500 Hz–500 MHz. Left: without addition. Upper right: after addition of 50 $\mu$M vitamin K-1 and 30 min incubation. Lower right: the last sample was centrifuged and redissolved in buffer and 5 mM sodium dithionite was added. The trace was multiplied by a factor of 1.81 to compensate for the loss of chlorophyll during the centrifugation step.

(before or after the dilution treatment) resulted in a complete reversal of the effect due to vitamin K-1 addition (Fig. 2, lower right). This last observation indicates that, although exogenous vitamin K-1 can act as an efficient electron acceptor from $A_0^-$, the functional acceptor $A_1$ has not been reconstituted in these experiments, as this acceptor is not reducible by dithionite [28,29].

*Absorption study of the P-700 triplet state at 5 °C*

The microsecond component which was attributed above to the triplet state of P-700 was studied under different redox conditions. Its halftime of decay is about 50 $\mu$s in a fresh sample of highly enriched PS I particles in the presence of ascorbate and DCIP at pH 8. However, this halftime increases when the sample has been illuminated by many laser flashes or by a continuous illumination (up to values of about 100 $\mu$s). Moreover, in the presence of sodium dithionite at pH 8, the decay is considerably slowed down. In addition to a slower component similar to the one detected in the presence of ascorbate ($t_{1/2} \approx 10$ ms), a component with a $t_{1/2}$ of about 560 $\mu$s is now present. Similar kinetic behavior is also found in the presence of dithionite at pH 10 (Fig. 3, b, upper trace). Under both pH conditions, the size and the spectrum in the red and infrared regions of the 560 $\mu$s component are identical with the size and the spectrum of the 50 $\mu$s component described above, indicating that it is probably due to the decay of the P-700 triplet state. These variations in the decay halftime of the P-700 triplet state can be explained by quenching by $O_2$. $O_2$ may have been partly consumed by reduced acceptors when PS I particles are illuminated in the presence of ascorbate and is completely absent in the presence of excess dithionite.

The assignment of the microsecond decay phase to the P-700 triplet state can be further proved by a study of the effect of magnetic fields. The effect of magnetic fields of 60 G and 400 G are shown in the presence of ascorbate and dithionite in Fig. 3 (a and b) together with the curves showing the dependence of the microsecond $\Delta A_{820}$ signals as a function of magnetic field under different redox conditions (inset). Such magnetic field effects are easily explained within the framework of the radical pair mechanism [30–32]. It has been recently

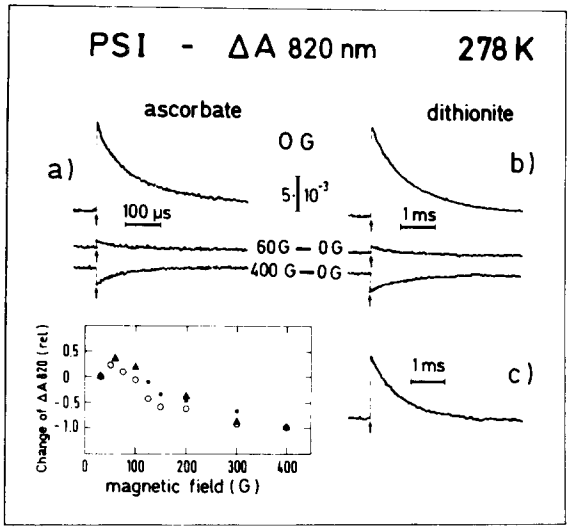


Fig. 3. Magnetic field effect on flash-induced absorption changes at 820 nm, at 5 °C, in extracted PS I particles ($A_{676nm} \approx 7.0$; Chl/P-700 ≈ 13) in the presence of 4 mM sodium ascorbate (+50 µM DCIP) (left, traces a) at pH 8 or 15 mM sodium dithionite (right, traces b and c) at pH 10. Excitation: 30 ps pulses at 532 nm; energy ≈ 15 mJ. Electrical bandwidth: DC–1 MHz. (a) and (b) Kinetic traces in the absence of external magnetic field (upper traces), differences between signals for $B = 60$ G and $B = 0$ (middle traces), differences between signals for $B = 400$ G and $B = 0$ (lower traces0. (c) Differences between signals in the absence of magnetic field (average of 50 experiments) and signals in the presence of a magnetic field (average of 5 series of 10 experiments at 125, 150, 200, 300 and 400 G). Inset (lower left), magnetic field dependence of the change of amplitude of the $\Delta A_{820}$ component measured from the initial signal in kinetic traces similar to traces a and b under different conditions: ▲, in the presence of ascorbate and DCIP at pH 8; ●, in the presence of dithionite at pH 8; ○, in the presence of dithionite at pH 10. Changes are given in relative values by setting a value of −1 for the maximum field effect at 400 G. This maximum effect corresponded in the three cases to a change in $\Delta A_{820}$ of $2.4 \cdot 10^{-3}$.

reported that in CP1-SDS particles, an increase in the yield of $^3$P-700 formation for small magnetic fields (peaking at 60 G) is followed for higher fields by a decrease well below the control without field [18]. This bell-shaped curve was tentatively explained by an exchange interaction of 60 G between P-700$^+$ and $A_0^-$ [18]. The magnetic field effects observed in the extracted PS I particles are therefore very similar to the effects observed previously on CP1-SDS particles, although the increases at low fields are relatively smaller.

Decay kinetics of the P-700 triplet state in the absence of $O_2$ are better seen in trace c of Fig. 3 where a difference between several microsecond signals recorded in the absence of magnetic field and the same number of signals recorded in the presence of magnetic fields larger than 125 G is plotted. The subtraction procedure allows us to eliminate the slower decay component ($t_{1/2} \geqslant 10$ ms) which is not magnetic-field-dependent, and the difference exhibits a monoexponential decay with $t_{1/2} \approx 560$ µs.

*Flash-induced absorption changes at 10 K*

It has been reported elsewhere that, although vitamin K-1 is absent in the extracted PS I particles, an irreversible charge separation between P-700 and $F_A$ (or $F_B$) is still quite efficient at low temperature [13]. Therefore, we studied in these particles the recombination kinetics at low temperature under conditions where $F_A$ and $F_B$ are prereduced so that no irreversible charge separation can occur (dithionite at pH 10 followed by 1 min illumination with a flashlight and 2 min dark adaptation before lowering the temperature in the dark). The immediate absorption increase at 820 nm that follows laser flash excitation is followed by a multiphasic decay.

A fast submicrosecond decay (Fig. 4, trace a) (56% of the total signal) is followed by a much slower decay (Fig. 4, traces b and c) (44% of the total decay). The fast decay is probably due to a recombination reaction between P-700$^+$ and $A_0^-$ [24]. A curve-fitting analysis has been attempted on the basis of one exponential component (poor fit; $t_{1/2} = 130$ ns) or two (much better fit; $t_{1/2} =$ 170 ns and $t_{1/2} = 34$ ns, respectively, 53 and 47% of the fast phase).

The slower decay itself can be deconvoluted into at least two exponential components, a decay phase with $t_{1/2} \approx 1$ ms (37% of the total signal) followed by a much slower component with $t_{1/2} \approx$ 200 ms (7% of the total signal). Fig. 5 shows the spectrum of the 1 ms component in the red and near-infrared regions. It exhibits a negative maximum at 700 nm and a flat positive band between 730 and 820 nm. These features indicate that the 1 ms component can be ascribed essentially to the P-700 triplet state [27], although a minor component due to antenna triplet states could also be present. The very slow component ($t_{1/2} \approx 200$ ms)

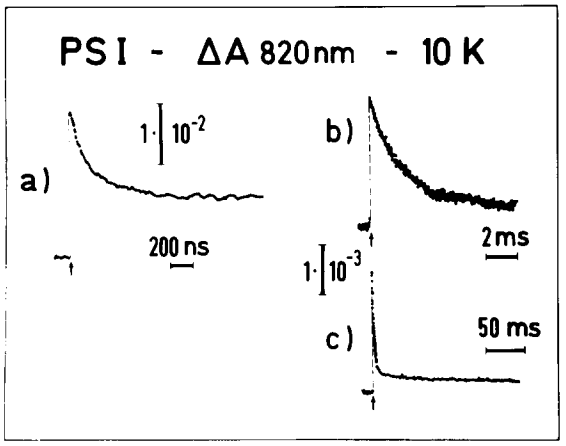


Fig. 4. Flash-induced absorption changes at 820 nm and at 10 K in extracted PS I particles (Chl/P-700 ≈ 13). Addition of 15 mM sodium dithionite at pH 10. The samples were illuminated for 1 min with a flashlight, kept in the dark for 2 min at 5 °C and frozen in the dark, so as to prereduce the iron-sulfur centers $F_A$ and $F_B$. (a): $A_{676nm}$ (along the measuring light direction) = 5.08; excitation: 30 ps laser pulses at 532 nm (10 mJ); electrical bandwidth: 500 Hz–500 MHz. (b) and (e): $A_{676nm}$ (along the measuring light direction) = 1.10; excitation: YAG-pumped dye laser; 20 ns pulses at 600 nm (10 mJ); electrical bandwidth: 100 Hz–0.3 MHz (b0 and DC–3 kHz (c).

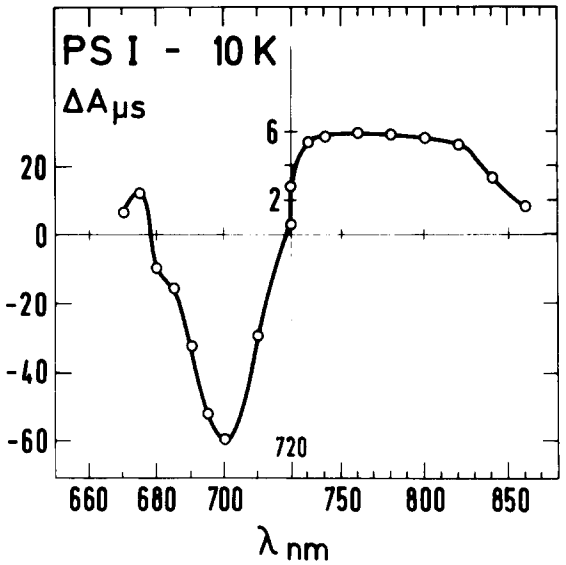


Fig. 5. Difference spectrum of flash-induced absorption changes at 10 K in extracted PS I particles ($A_{676nm}$ = 1.10; Chl/P-700 ≈ 13) on a millisecond timescale. Addition of 15 mM sodium dithionite at pH 10. The sample was prepared as in Fig. 4. Excitation, 20 ns pulses at 600 nm (10 mJ); electrical bandwidth, 100 Hz–0.3 MHz. The spectrum was drawn by taking the initial absorption changes in kinetic traces similar to trace (b) of Fig. 3. The spectrum has been arbitrarily normalized to a value of (−60) at 700 nm. The absorption change at 700 nm was $2.5 \cdot 10^{-2}$. The *Y*-scale is extended 5-times for the right part compared to the left part.

could well correspond to a recombination reaction between P-700$^+$ and $F_X^-$ in a minority of reaction centers [29,33,34], but its spectrum has not been measured. As a matter of fact, a reversible charge separation between these two species has already been reported at low temperature in the same PS I particles, as observed by EPR [13]. We found no detectable effect of a magnetic field (0–400 G) either on the submicrosecond decay kinetics at 820 nm or on the yield of $^3$P-700 formation.

*Temperature dependence of the flash-induced absorption signals at 820 nm*

Submicrosecond flash-induced absorption changes were also studied as a function of temperature at 820 nm in extracted PS I particles in which $F_A$ and $F_B$ were initially prereduced. Two different parameters were derived from kinetic traces similar to traces a of Figs. 1 and 4: the halftime of the fast submicrosecond decay and the ratio of the absorption change measured 600 ns after the flash to the initial absorption change. They are given in Fig. 6 as a function of temperature. As a curve-fitting analysis has not been attempted at the intermediate temperatures due to the poor signal-to-noise ratio, the halftime of the fast decay has been determined by measuring $t_{1/2} = t_{max} - t_1$, where $t_{max}$ is the signal corresponding to $\Delta A_{max}$ (4–6 ns after the flash) and $t_1$ is the time at which $\Delta A = (\Delta A_{max} + \Delta A_{600ns})/2$. It increases regularly from 47 to 82 ns when the temperature decreases from 278 down to 10 K. Its value is slightly larger than the value than can be deduced from the curve fitting analysis of the fast decay with two exponential phases at 278 K (47 ns vs. 40 ns) as well as at 10 K (82 ns vs. 73 ns).

The ratio $\Delta A_{600ns}/\Delta A_{max}$ is fairly constant between 200 K and 278 K (22–25%) and is increasing continuously from 25 to 44% when the temperature decreases from 200 to 10 K (Fig. 6). The variation of this ratio presumably reflects the variations in the yield of the P-700 triplet state that is formed through the recombination reaction between P-700$^+$ and $A_0^-$. The relative triplet yields can be estimated more accurately at 10 K and 278

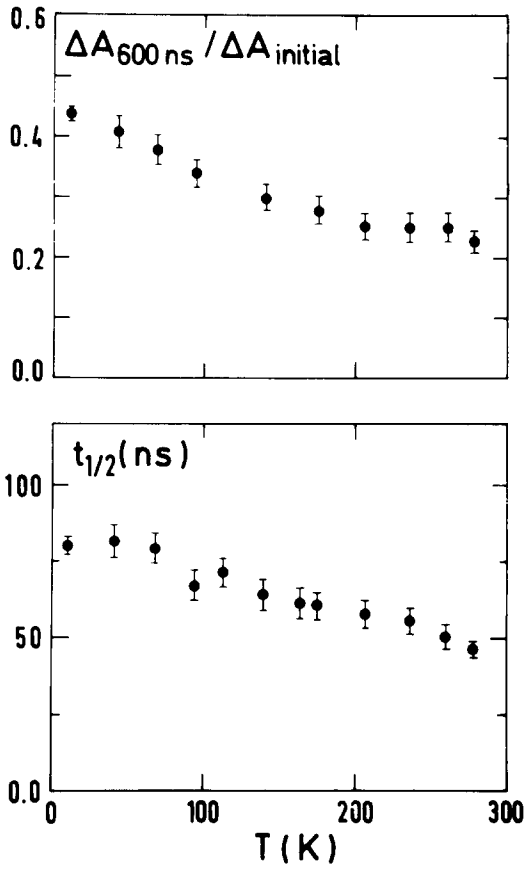


Fig. 6. Temperature dependence of flash-induced absorption changes at 820 nm in extracted PS I particles prepared as in Fig. 4. Upper part: ratio between the signal measured 600 ns after the flash and the initial signal versus temperature. Lower part: decay halftime of the fast phase versus temperature. Excitation: 30 ps pulses at 532 nm (10 mJ). Electrical bandwidth: 500 Hz–500 MHz.

K, as a more detailed kinetic analysis has been performed at these temperatures. At 820 nm, the signals that can be ascribed to the triplet state of P-700 represent about 15% and 37% of the initial signal at respectively 278 and 10 K. Assuming that the absorption of ($P\text{-}700^+ - A_0^-$) (with $A_0$ probably being chlorophyll *a*) is roughly twice that of $^3$P-700 at 820 nm, and that there is no very fast decay of the primary radical pair ($t_{1/2} < 1$ ns), these amounts correspond to yields of formation of $^3$P-700 from the primary radical pair of about 30% and 75% at respectively 278 and 10 K.

## Discussion

The absorption changes which are induced at 820 nm by laser flashes in extracted PS I particles present common features at 278 K and at 10 K. The initial absorption, which is not resolved by our experimental setup, is followed by a fast submicrosecond decay which probably reflects a recombination reaction between $P\text{-}700^+$ and $A_0^-$ [18,24]. This kinetic behavior is completely different from the kinetic behavior of control (unextracted) particles. As a matter of fact, control particles, under similar conditions, exhibit essentially a very slow decay phase at room temperature ($t_{1/2} \approx 30$ ms) ascribed to a recombination reaction between $P\text{-}700^+$ and an iron-sulfur acceptor $P430^-$ [23] and a 120 $\mu$s decay phase at 10 K which was attributed to a recombination reaction between $P\text{-}700^+$ and $A_1^-$ [29]. Such recombination reactions are observed because, after the initial charge separation, the electron on $A_0^-$ is passed onto secondary acceptors which then recombine with $P\text{-}700^+$. The kinetic behavior observed in the present study indicates that, in the diethyl-ether-extracted particles, the electron transfer is blocked at the level of $A_0$. This blocking can be correlated with the absence of vitamin K-1 in this material [12] and this interpretation is strongly supported by some recent reconstitution experiments in extracted reaction centers of the cyanobacterium *Synechocystis 6803* (Biggins, J. and Mathis, P., personal communication). However, a recent study concluded that electron transfer at room temperature remains unperturbed when vitamin K-1 is completely destroyed by ultraviolet irradiation [36]. Two opposite explanations can be proposed to resolve this discrepancy. The first could be that the product of ultraviolet irradiation of vitamin K-1 is still functioning as an efficient electron intermediate. In the second interpretation, the diethyl ether treatment not only extracts vitamin K-1, but also has other perturbing effects which by themselves preclude a normal electron transfer.

Assuming a single exponential phase for the fast submicrosecond decay results in a poor fit at 278 K as well as at 10 K. By contrast, the decay of the primary radical pair in bacterial reaction centers has been theoretically calculated to be essentially monoexponential [37], as has been

found experimentally. Theoretical calculations of the kinetics of the radical pair ($P\text{-}700^+ - A_0^-$) of PS I might help in understanding the origins of this discrepancy.

The addition of exogenous vitamin K-1 induces a decrease in the amplitude of the fast decay together with an increase in a very slow phase. These data indicate that vitamin K-1 can act as a very good electron acceptor from $A_0^-$, as its reduction appears to compete efficiently with the recombination reaction between $P\text{-}700^+$ and $A_0^-$. However these experiments do not point to the reconstitution of a functional acceptor, $A_1$, contrary to the results obtained with extracted reaction centers of *Synechocystis 6803* (Biggins, J. and Mathis, P., personal communication). First, the effect due to the addition of vitamin K-1 is reversed by the concomitant addition of sodium dithionite at pH 8, which is not expected to reduce the acceptor $A_1$. Second, addition of vitamin K-1 induces the appearance of a very slow phase ($t_{1/2} > 500$ ms), but does not restore the reduction of iron-sulfur centers, which would lead to faster recombination reactions between $P\text{-}700^+$ and the reduced iron-sulfur center. Therefore, these data can be best interpreted if, in the reconstituted reaction centers, vitamin K-1 accepts an electron from $A_0^-$ but cannot serve as an intermediate for the reduction of the terminal iron-sulfur acceptors.

In extracted PS I particles, the fast decay phase is followed by a slower decay that can be attributed to the triplet state of P-700 on the basis of its spectral features. This assignment is reinforced by the observation at 278 K of a magnetic field effect on the size of this decay phase. These two prominent components are most probably present at any temperature between 10 K and 278 K. A study of their temperature dependence shows that both the halftime of the recombination reaction between $P\text{-}700^+$ and $A_0^-$ and the yield of $^3P\text{-}700$ formation increase by a factor of about 2 when the temperature decreases from 278 K to 10 K (Fig. 6).

It has also been recently reported that the three iron-sulfur centers, $F_X$, $F_B$ and $F_A$, can be photoreduced at low temperature in similar PS I particles lacking vitamin K-1 [13]. A direct electron transfer from $A_0$ to these iron-sulfur centers has been tentatively proposed to explain these observations. The third minor component that we observe at 10 K ($t_{1/2} \approx 200$ ms) thus probably corresponds to a recombination reaction between $P\text{-}700^+$ and $F_X^-$ that has been previously observed by EPR when $F_A$ and $F_B$ are prereduced [13]. The very slow component that we observe at 278 K (7–8% of the total signal at 820 nm; $t_{1/2} \approx 10$ ms), might also reflect a low yield bypass from $A_0^-$ to iron-sulfur centers. The spectrum of this slow phase presents a negative maximum around 696 nm, indicating that $P\text{-}700^+$ is a partner in this reaction, but a more detailed examination of this spectrum in the blue and red regions is required to confirm such an hypothesis.

The decay kinetics of the P-700 triplet state at low temperature, with a halftime of 1 ms, are similar to the kinetics that we have measured in CP1-SDS particles [27,38] whereas these kinetics are completely different at room temperature. In CP1-SDS particles, the major decay phase of the $^3P\text{-}700$ state has a $t_{1/2}$ of about 6 $\mu$s [27,39]. No significant effect of $O_2$ removal could be found in these particles [27]. In diethyl-ether-extracted particles, the decay of the $^3P\text{-}700$ is slower (50 $\mu$s under air) and is slowed down upon removal of $O_2$ from the solution. These differences may be due to the removal of carotenoid molecules by the extraction procedure. The fast decay kinetics of the $^3P\text{-}700$ state in CP1 particles can be attributed to a quenching by carotenoids with a $t_{1/2} \approx 6$ $\mu$s. If the quenching rate of $^3P\text{-}700$ by molecular oxygen is identical in CP1 and ether-extracted particles (corresponding to a $t_{1/2} \approx 50$ $\mu$s), it is therefore expected that the removal of oxygen will have only a minor effect on the $^3P\text{-}700$ decay kinetics in CP1 particles if $\beta$-carotene is present and is the major quenching species. In the absence of $O_2$, the halftime of $^3P\text{-}700$ decay is about 560 $\mu$s at 278 K and 0.8–1 ms at 10 K and is therefore practically independent of temperature. This behavior is opposite to the strong temperature dependence of $^3P\text{-}700$ decay that has been measured in CP1 particles (activation energy of 0.94 kcal/mol, i.e., 0.04 eV) [39]. In the latter case, the temperature dependence of $^3P\text{-}700$ is very similar to the temperature dependence of $^3P\text{-}870$ in bacterial reaction centers devoid of carotenoid [40]. In such bacterial reaction centers, the magnetic field dependence of the $^3P\text{-}870$ decay kinetics led to the

proposal that the $^3$P-870 state decays through a thermally activated repopulation of the primary biradical state [40]. The nearly temperature-independent decay of the $^3$P-700 state in the PS I particles studied here indicates that this activated pathway is probably not effective in ether-extracted PS I particles and may be also ineffective in CP1-SDS particles. In the latter particles, the temperature dependence of the $^3$P-700 decay could be explained by a triplet energy transfer from an activated state $^3$D (for example, a triplet state of a neighbor antenna chlorophyll) to a carotenoid molecule, as has been previously proposed [41].

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