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Nanosecond electron transfer kinetics in photosystem I as obtained from transient EPR at room temperature

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Transient EPR spectra of photosystem I (PS I) in spinach chloroplasts and PS I particles prepared from *Synechococcus* are presented. Two consecutive spectra are observed after the laser pulse. The decay time of the first spectrum is equal to the rise time of the second spectrum. The two spectra represent sequential charge-separated states in the electron transfer chain and the time constant of the electron transfer step between them is found to be $t_{1/e} = 260 \pm 20$ ns for the *Synechococcus* PS I particles as well as for the spinach chloroplasts. The first spectrum is assigned to the $P_{700}^+ A_1^-$ pair, where A_1^- is the second electron acceptor, probably a quinone-like molecule. The second spectrum covers the region of the P_{700}^+ signal and may be part of the spectrum due to the coupled radical pair P_{700}^+ (FeS), where $(FeS)^-$ is the first iron-sulfur center along the electron transfer chain.

Photosystem I; Electron spin polarization; P700; (*Synechococcus*)

1. INTRODUCTION

The primary photochemistry of photosystem I as reviewed recently [1,2] involves the primary donor P_{700} and a series of membrane-bound acceptors, A_0, A_1, F_X, F_B and F_A. It is widely accepted that A_0 is a chlorophyll *a*, and that F_X, F_B and F_A are iron-sulfur centers. The identity of A_1 has not yet been firmly established. However, there is ample evidence that it is a quinone-type molecule, probably vitamin K_1 [3–6], but see also [7,8]. Existing information points to the following chain of electron transfer steps, initiated by photoexcitation of the donor P_{700} [1]:

$$P_{700}^* A_0 A_1(FeS) \xrightarrow{<10\ ps} P_{700}^+ A_0^- A_1(FeS) \xrightarrow{200\ ps}$$

$$P_{700}^+ A_0 A_1^-(FeS) \xrightarrow{(?)} P_{700}^+ A_0 A_1(FeS)^-.$$

(FeS) denotes the first iron-sulfur center acceptor, usually identified with F_X [1,2].

Conflicting data exist for the electron transfer time from A_1^- to $(FeS)^-$. Recently, two quite different sets of kinetics have been published: Mathis and Setif [9] state a time constant as fast as $t_{1/2} =$ 15 ns, while Brettel [10] measured an A_1^- reoxidation time of $t_{1/2} = 200$ ns. Since both reduced acceptor states of the electron transfer chain are paramagnetic, EPR should be able to solve this problem.

An important feature of the early EPR signals of PS I is their electron spin polarization (ESP), which provides a crucial increase in signal strength. Although the first observations of polarized transient EPR spectra of PS I date back to 1975 [11]

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Abbreviations: A, absorption; Chl, chlorophyll; CIDEP, chemically induced dynamic electron polarization; CIDNP, chemically induced dynamic nuclear polarization; E, emission; ESP, electron spin polarization; FeS, iron-sulfur center; MV, methylviologen; PMS, phenazine methosulfate; PS I, photosystem I; T_1, longitudinal spin relaxation time; T_2, transverse spin relaxation time

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and 1978 [12], their interpretation on the basis of chemically induced dynamic electron polarization (CIDEP) concepts (for a review, see [13]) remained inconclusive. More recent work [14,15] (also compare [16]) strongly suggests that the commonly known E|A|E transient ESP pattern is due to the EPR transitions in a correlated, magnetically coupled spin pair, a mechanism demonstrated earlier in hydrogenation reactions [17] and photoinduced radical pair reactions [18,19]. In PS I, the first EPR-detectable state is the radical ion pair P_{700}^{+} A_1^{-} originating from the singlet precursor excited state P_{700}^{*} via the pair P_{700}^{+} A_0^{-}, which is too short-lived to be observed by EPR and probably also too short-lived to generate any polarization that could be transferred to the state P_{700}^{+} A_1^{-}. The ESP pattern reflects the magnetic spin interactions in the P_{700}^{+} A_1^{-} radical pair and is nicely consistent with the assumption of a semiquinone-like g tensor of A_1^{-} [3,15].

Intact PS I in whole cells of algae *Synechococcus lividus* under physiological conditions have shown various transient EPR spectra with different decay kinetics and characteristic ESP patterns [20,21]. The fast-decaying ESP pattern is consistent with that found for the P_{700}^{+} A_1^{-} pair at higher spectral and time resolution [3,15]. A second spectrum characterized by a slower decay rate could not be assigned conclusively.

It is the purpose of this paper to restudy the transient EPR spectra of PS I from various species with a higher time resolution (50 ns). We will show that the kinetics observed are consistent with sequential electron transfer along the acceptor chain from A_1 to the first iron-sulfur center (FeS). A consistent interpretation of the observed ESP patterns will be proposed, which is in agreement with the kinetic assignment.

2. MATERIALS AND METHODS

PS I particles with approximately 80 Chl/P_{700} were prepared from thermophilic cyanobacteria *Synechococcus* sp. according to [22] (therein referred to as SG1). The material was stored in the dark at −30°C. The particles were suspended in a buffer containing 20 mM Tricine (pH 7.8) and 20 mM $MgCl_2$ to a final concentration of 5 mg Chl/ml. An artificial donor (sodium ascorbate/PMS) and an artificial acceptor (methylviologen (MV)) were added immediately before the EPR measurements were made. Final concentrations: Na ascorbate, 100 mM; PMS, 500 μM; MV, 1 mM.

Chloroplasts from market spinach were made by a standard procedure [23] with a chlorophyll concentration of 4 mg/ml. These samples contained 100 μM NADP and 20 μg/ml ferredoxin as an acceptor system.

The EPR experiments were performed on a Varian E-line X-band spectrometer in direct detection mode without field modulation. To gain the best possible time resolution, the signal was tapped directly after the back diode detector (Omni Spectra) and amplified by an Avantek GDP 461-462-463 amplifier cascade yielding a total time response of 50 ns. 1.4 ml of the sample was circulated through a 0.3 mm flat cell inside a modified E-231 (TE 102) resonator. The beam of a frequency-doubled Nd-YAG laser (Quanta Ray DCR-2, λ = 532 nm, pulse energy 12 mJ, pulse width 6 ns, repetition rate 10 Hz) homogeneously illuminated a flat cell area of 3 cm^2. The transient EPR signal produced by each laser pulse was digitized by a Gould 4500 transient recorder at a rate of 10 ns/sample and immediately transferred to a PDP 11/73 computer for storage and averaging. In order to obtain the complete spectral information, transients were taken at 161 field positions spaced 0.025 mT apart. Flash artifacts were eliminated by subtracting an average of off-resonance transients and baseline deviations at different field positions were corrected for by subtracting the baseline after the transients decayed. Data acquisition, field sweep and laser triggers were under computer control. Evaluation of information from the time/field data set is described below.

3. RESULTS

Fig.1 shows the complete time/field data set of the spin-polarized EPR signals around g = 2 of PS I particles from *Synechococcus* sp. Two consecutive spectra can be observed: the first spectrum (spectrum A) rises with the response time of the spectrometer. The second spectrum (spectrum B) rises during the time in which spectrum A decays. If the two spectra A and B result from sequential charge-separated states in the charge transfer chain, then the rise time of spectrum B should be equal to the decay time of spectrum A and the observed signal as a function of time at any given field position may be expressed by:

$$s(t,B_0) = \alpha(B_0)\exp(-t/\tau_1) + \beta(B_0)(1-\exp(-t/\tau_1))\exp(-t/\tau_2), \quad (1)$$

where $\alpha(B_0)$ and $\beta(B_0)$ are the EPR signal amplitudes of the two charge-separated states giving rise to spectra A and B, respectively. τ_1 is the decay time of the early signal as well as the rise time of the second spectrum B. Here, for simplicity, we have ignored spin relaxation in the charge-separated state, which produces spectrum A. This assumption seems reasonable, because from the

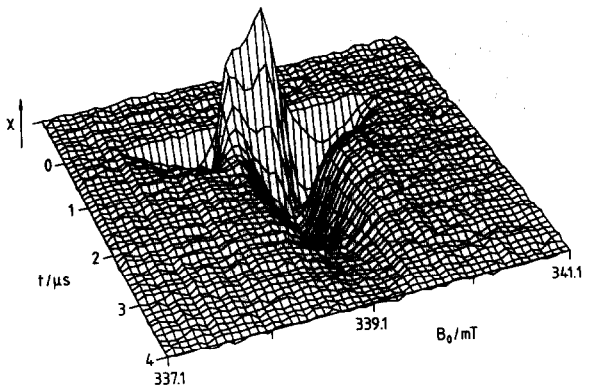

Fig.1. Direct detection mode EPR signal of PS I particles from *Synechococcus* sp. is plotted against time and magnetic field. Ambient temperature, microwave frequency 9.511 GHz (X band), microwave power 10 mW. The raw data set contains 161 transients spaced 0.025 mT apart with 500 points. Each transient represents the average of 256 shots. In this presentation, the original number of 161 × 500 data points is reduced to 51 × 51 net points. Positive signals represent absorption, negative signals emission.

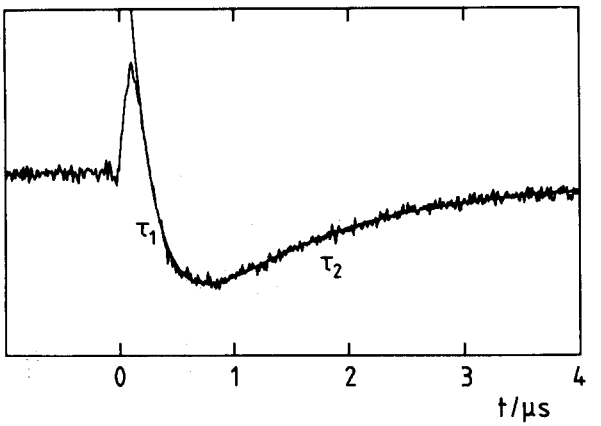

Fig.2. Transient of the experiment with PS I particles from *Synechoccocus* sp. in the region of 338.975–339.200 mT. Average of 2560 events. The smooth curve is the result of a fit to eqn 1. τ_1 = 240 ns, τ_2 = 1277 ns.

decay of spectrum B it is clear that the spin relaxation in this charge-separated state is much longer than τ_1. However, the relaxation time constants for the two charge-separated states are not necessarily the same, and it is difficult to distinguish clearly between contributions to τ_1 from relaxation and electron transfer. Because no other spectrum is observed after spectrum B, it is likely that τ_2 is dominated by the relaxation of the spin system.

Using a linear least-squares method, α, β, τ_1 and τ_2 have been fitted with eqn to the average of several transients around 339.1 mT extracted from the data set shown in fig.1. The result of this fit is shown in fig.2.

To evaluate spectra A and B, eqn 1 was fitted to all individual transients of the data set. In these fits, τ_1 and τ_2 were held constant at the values obtained in the fit shown in fig.2. The coefficients α and β are plotted against the magnetic field in fig.3 (bottom).

The same experiment has been performed on chloroplasts from spinach. Fig.4 shows the two spectra for this sample. The spectra of both samples are clearly very similar. However, spectrum B of the *Synechococcus* sample shows a somewhat weaker high field absorption than the spinach chloroplast sample.

Finally, we have obtained a value for τ_1 at each field position by fitting eqn 1 to all transients of the *Synechococcus* sample. In these fits, τ_2 was held constant at the value obtained from the fit shown in fig.2, while α, β and τ_1 were varied. The values of τ_1 as a function of the field are plotted in fig.3 (top) [24]. Within experimental accuracy, they are constant over the entire spectral range, regardless of the relative polarizations and

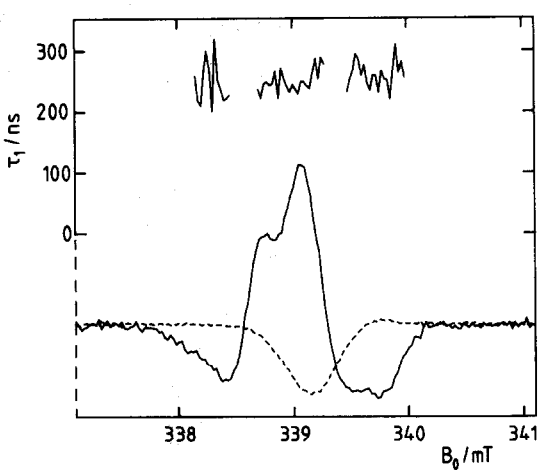

Fig.3. (Bottom) Spectra as derived from the original data set of fig.1 by a fit to eqn 1. Solid line: early spectrum A, i.e., coefficients α of eqn 1; broken line: late spectrum B, i.e., coefficients β of eqn 1. In this fit, α and β were varied. τ_1 = 240 ns, τ_2 = 1277 ns for all field positions. (Top) Plot of the transition time τ_1 representing the decay of spectrum A and the rise of spectrum B. In this fit α, β and τ_1 were varied, τ_2 = 1277 ns for all field positions.

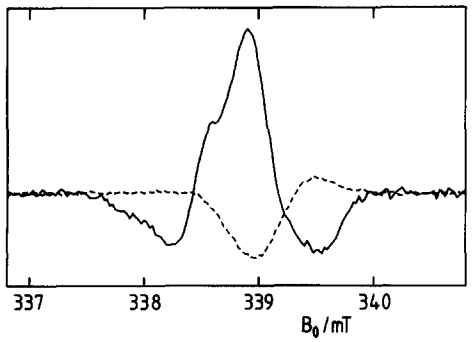

Fig.4. Spectra as derived from the data set of the experiment on chloroplasts from spinach. Parameters as in fig.1 except for microwave power 40 mW, microwave frequency 9.507 GHz, average of 768 events at each field position. (Solid line) Early spectrum A, i.e., coefficients α of eqn 1; (broken line) late spectrum B, i.e., coefficients β of eqn 1. τ_1 = 263 ns, τ_2 = 679 ns.

amplitudes of the two spectra A and B. Consequently, the time constant with which spectrum A decays is the same as the rise time of spectrum B.

Values of τ_1, obtained for the two samples under various conditions, are nearly constant (see table 1), whereas τ_2 varies considerably depending on the microwave power. From these values, it is clear that our assumption $\tau_1 \ll T_1, T_2$ is well justified. Moreover, the fact that τ_1 is almost independent of sample, microwave power and frequency provides convincing evidence that τ_1 is indeed dominated by the kinetics of the electron transport. Due to possible relaxation effects, the real electron transfer time may be somewhat longer than indicated by τ_1. The strong microwave power dependence of τ_2 shows that this time constant is largely determined by the relaxation of the spins and suggests that it reflects the relaxation times T_1 and T_2 at low and high power levels, respectively.

Table 1

Variation of τ_1 or τ_2 with microwave power

Sample	Microwave power (mW)	τ_1 (1/e) (ns)	τ_2 (1/e) (ns)
Spinach chloroplasts' X-band	40	263	679
PS I particles' X-band	100	261	393
	50	270	582
	20	278	955
	10	254	1274
	10[a]	240[a]	1277[a]
	5	258	1557
	2	230	1972
	1	243	1933
PS I particles' K-band (26.2 GHz)	6	270	580

[a] The data from this experiment were used for figs 1–3

4. DISCUSSION

Two polarized transient EPR spectra appearing consecutively after the laser flash are observed within the time range of our experiment. Their characteristic ESP patterns are well known [11–13,21]. The first spectrum, A, displays an E|A|E pattern with an indication of proton hyperfine splittings [13,21]. It has to be associated with the correlated radical pair $P_{700}^+ A_1^-$ [14,15]. The second spectrum B exhibits an E|A pattern in the g factor region of P_{700}^+ [11,21].

This work establishes a clear sequential time development from spectrum A to spectrum B over the whole spectral range with the time constant of τ_1 = 260 ns. Because the early spectrum A is assigned to the charge-separated state $P_{700}^+ A_1^-$, the observed decay and rise kinetics must be assigned to the electron transfer step past A_1 along the chain of acceptors

$$P_{700}^+ A_1^- (FeS) \xrightarrow{260\ ns} P_{700}^+ A_1 (FeS)^-.$$

This conclusion is strongly supported by a parallel investigation with transient absorption difference spectroscopy [10] with the same PS I particles used in this work, yielding a compatible time constant of $t_{1/2}$ = 200 ns. Within experimental accuracy, identical kinetics have been demonstrated in this work for chloroplasts from spinach.

An analogous time constant was first observed by Thurnauer and Norris [16] as the kinetics of an 'out-of-phase' electron spin echo in phase with the microwave field B, indicating a change in spin interaction due to an electron transfer. The authors stated a decay time of $t_{1/e}$ = 170 ns [16,25]; however, the decay curve in [16] yields a decay time of $t_{1/e}$ = 235 ns, and is thus compatible with our results.

Varying the redox potential, Thurnauer et al.

[25] found that the out-of-phase echo disappears when the iron-sulfur centers $F_{A,B}$ are prereduced and, therefore, assigned their 170 ns phase to the electron transfer step $F_X^- F_{A,B} \longrightarrow F_X F_{A,B}^-$. This would imply that our spectrum A is due to the $P_{700}^+ F_X^-$ pair. However, the width of our spectrum readily excludes this possibility. The spectral contributions of iron-sulfur center radicals like F_X are unobservable at room temperature due to their large g factor anisotropy and our spectrum covers too large a spectral range to be assigned to P_{700}^+ alone. In order to satisfy both the redox potential studies as well as our assignment of spectrum A to the radical pair $P_{700}^+ A_1^-$, one can either conclude that F_X is not normally involved in the electron transfer chain or that electron transfer from A_1^- to F_X is blocked or slowed down under conditions of prereduced $F_{A,B}$. Recent flash absorption data show that the radical pair $P_{700}^+ A_1^-$ has a halflife of approximately 250 μs under such conditions (Brettel, K., to be published).

In contrast, another optical study on spinach subchloroplasts enriched in PS I [9] showed a considerably faster kinetic phase ($t_{1/2} = 15 \pm 5$ ns) attributed to the reoxidation of A_1^- and electron transfer to F_X. This result cannot be confirmed by our data.

Additional information on the charge-separated states may be derived from the patterns of spectra A and B. A pattern similar to that of spectrum A has been observed in PS I ESP spectra of fully deuterated whole algae *Synechococcus lividus* [3,15,20]. Recent work suggests the use of the correlated radical pair mechanism [14,18,19] to explain this ESP spectrum. It is interesting to note that the basis of such a concept was first introduced by Thurnauer and Norris [16]. With this concept, the spectra obtained from PS I in freeze-dried deuterated whole algae can be simulated satisfactorily, yielding valuable information on g tensors and their relative orientations in the reaction center structure [15]. Although our spectrum A shows evidence for an additional influence of a partly resolved large hyperfine splitting, all other features of the spectrum are compatible with the parameters obtained from such simulations [15].

Spectrum B has already been observed since the initial reports of PS I ESP at lower time resolution [11] or as a slower or later secondary signal [12,13,21,26]. All authors agree that the observed transient E|A pattern coincides with the spectral range of P_{700}^+ and, therefore, represents a polarized P_{700}^+ signal. Early interpretations based on the established mechanism of chemically induced electron spin polarization (CIDEP) [13] assumed an uncoupled P_{700}^+ to which polarization was transferred from the last coupled spin pair.

In the first microsecond after the laser pulse, the electron moves along the acceptor chain A_0, A_1, FeS with sequential generation of three spin pairs. Spectrum A for the spin pair $P_{700}^+ A_1^-$ establishes this state as a coupled spin pair, with no detectable polarization transferred from the preceding pair $P_{700}^+ A_0^-$ as expected from the short lifetime of the A_0^- state (200 ps). Two limiting cases have to be discussed for polarization transfer from $P_{700}^+ A_1^-$ to $P_{700}^+ (FeS)^-$: (i) electron transfer past A_1 separates the unpaired electron spins so far that the dipole–dipole interaction between the spins may be neglected and P_{700}^+ may be considered as a free radical; (ii) the radical ions P_{700}^+ and $(FeS)^-$ are still close enough together that the dipole–dipole interaction is not negligible and $P_{700}^+ (FeS)^-$ must be considered as a coupled radical pair.

From the recently proposed geometry for the PS I reaction centre [2], it is possible that the distance between P_{700}^+ and F_X^- is small enough for case ii to apply to this pair. However, without a definite assignment of spectrum B to a specific iron sulfur center and without precise geometric information, it is not clear which of the above two cases applies.

In both cases, the $(FeS)^-$ radical does not contribute to the observable ESP pattern due to its large g anisotropy [1,2]. In case i, transferred polarization can result only from $P_{700}^+ A_1^-$. The observed E|A pattern could be rationalized with established CIDEP concepts [13,15] assuming a small g anisotropy for P_{700}^+ [27]. For case ii, S T_0 mixing in the coupled pair yields as much emission as absorption for each partner when the precursor is a pure singlet state. For g factor differences very much larger than the dipolar or exchange interaction, this is also true for a mixed S T_0 initial population. However, large g anisotropy of $(FeS)^-$ also leads to S T_+ mixing, which would result in a predominantly emissive contribution, as seen in spectrum B.

A more detailed explanation requires rigorous treatment of the coupling between all spin states in

the successive spin pairs including the role of hyperfine couplings.

A final note concerns the observation of a similar E|A pattern (P^+ region) in bacterial reaction centers with substituted quinones in the Q_A site [28]. Here, EPR detection clearly occurs in the coupled spin pair state P_{860}^+ $(Q_AFe)^-$ and the observed E|A pattern for P_{860}^+ has to be explained within the above-mentioned case ii.

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