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Nanosecond electron transfer kinetics in photosystem I following substitution of quinones for vitamin K_1 as studied by time resolved EPR

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Room temperature transient EPR spectra of photosystem I (PS I) particles from *Synechocystis* 6803 are presented. Native PS I samples and preparations depleted in the A_1-acceptor site by solvent extraction and then reconstituted with the quinones (Q) vitamin K_1 (VK_1), duroquinone (DQ and DQd_{12}) and naphthoquinone (NQ) have been studied. Sequential electron transfer to $P_{700}^+A_1^-$ (FeS) and $P_{700}^+A_1$ $(FeS)^-$ is recovered only with VK_1. With DQ and NQ electron transfer is restored to form the radical pair $P_{700}^+Q^-$ as specified by a characteristic electron spin polarization (ESP)-pattern, but further electron transfer is either slowed down or blocked. A qualitative analysis of the K-band spectrum suggests that the orientation of reconstituted NQ in PS I is different from the native acceptor $A_1 = VK_1$.

Photosystem I; Vitamin K_1; Electron transfer; Time resolved EPR; A_1 extraction/reconstitution; *Synechocystis* 6803

1. INTRODUCTION

The photochemical charge separation in PS I proceeds from the donor P_{700} via a chain of acceptors termed A_0, A_1, F_X, F_A and F_B. The electron donor P_{700} is believed to be a chlorophyll dimer. It is generally accepted that A_0 is a chlorophyll a, and that F_X, F_A and F_B are iron-sulfur centers [1,2]. No agreement has been reached yet concerning the nature of A_1 but there is increasing evidence that it is a quinone molecule, probably VK_1 [3,4]. The quinone nature of acceptor A_1 is based on optical and EPR studies on intact [5–8] and prereduced [9–11] PS I particles. This has been confirmed by optical [12,13] and EPR [26] studies on samples in which VK_1 has been extracted and reconstituted. Here we wish to examine similar samples by fast transient EPR spectroscopy.

Kinetic data [3,14] for the primary electron transfer steps following by photoexcitation of the donor P_{700} are:

$$P_{700}^*A_0A_1(FeS) \xrightarrow{<10\ ps} P_{700}^+A_0^-A_1(FeS) \xrightarrow{30\ ps[15]} P_{700}^+A_0A_1^-(FeS) \quad (1)$$

For the subsequent electron transfer step rate constants (given as $t_{1/e}$ time) seem to converge in recent optical [7] and EPR [8,16] experiments:

$$P_{700}^+A_0A_1^-(FeS) \xrightarrow[260\ ns[8],\ 170\ ns[16]]{290\ ns[7]} P_{700}^+A_0A_1(FeS)^- \quad (2)$$

(FeS) represents the first iron-sulfur center in the electron transfer chain (probably F_X, a 4Fe-4S cluster [17]). Processes faster than 10 ns are either beyond the instrumental and physical limits of transient EPR at standard frequencies [18], thus the electron transfer step from A_1^- to $(FeS)^-$(2) is the first that can be detected by this method. Room temperature transient EPR measurements have yielded both kinetic [8,9] as well as structural [19] information for this electron transfer step.

In this paper we compare the respective transient EPR spectra of PS I particles from *Synechocystis* 6803 in the following samples: (i) intact, (ii) VK_1 depleted and (iii) reconstituted with VK_1, DQ, DQd_{12} and NQ. The results support the assignment of A_1 to VK_1. In addition, they open the way for further insights into structural and kinetic aspects of the A_1 acceptor site in PS I.

2. MATERIALS AND METHODS

Lyophilized PS I particles were prepared from cyanobacteria *Synechocystis* 6803 according to reference [20]. VK_1 was extracted with 0.3 vol% methanol in hexane [20]. Immediately before measurement both the intact and the VK_1 depleted PS I particles were rehydrated in 50 mM Tricine buffer (pH = 7.6) with 0.2% Triton X-100 to a concentration of 5 mg Chl/ml. In order to assure a fast optical excitation cycle suitable for averaging, 500 μM phenazine-metasulfate, 100 μM sodium ascorbate and 1 μM methylviologen were

Abbreviations: A, absorption; DQ, duroquinone; DQd_{12}, perdeuterated duroquinone; E, emission; ESP, electron spin polarization; (FeS), iron sulfur center; hfs, hyperfine structure; MW, microwave; NQ, naphthoquinone; PS I, photosystem I; Q, quinone; VK_1, vitamin K_1

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added as artifical donors and acceptors. For reconstitution the VK_1 depleted samples were incubated at 5°C for up to 15 h with 1.0 mM of the chosen quinone substitute. NQ and DQ were obtained from Merck, and NQ was sublimed (150°C, 10^{-2} Torr) prior to use. Perdeuterated DQ (DQd_{12}) was a gift of Prof. Dr. W. Lubitz (Universität Stuttgart, Physikalisches Institut). Ethanol was used as solvent for the stock solutions.

The transient EPR spectroscopy in the direct detection mode with a modified X-Band Varian spectrometer and K-Band spectrometer together with other experimental details are described elsewhere [8,18]. All measurements were done at room temperature using a flow system. Transient spin polarized EPR spectra were taken as complete time/field data sets s(t,B) after laser flash excitation (Nd-YAG-Laser, 532 nm) and were processed as described previously [8].

3. RESULTS

EPR data from intact PS I particles of cyanobacteria *Synechocystis* 6803 have been obtained by the same experimental procedures reported previously for *Synechococcus* sp. [8]. The two consecutive spin polarized spectra shown in Fig. 1a have been extracted from the full data set using the same kinetic model used in [8] according to the kinetic equation

$$s(t,B_0) = \alpha(B_0)\exp(-t/\tau_1) + \beta(B_0)(1-\exp(-t/\tau_1))\exp(-t/\tau_2) \quad (3)$$

Spectrum α (broken line) has been assigned to the radical pair $P_{700}^{+}A_1^{-}$ and spectrum β (dotted line) is due to $P_{700}^{+}FeS^{-}$. The electron transfer time from $P_{700}^{+}A_1^{-}$ to $P_{700}^{+}(FeS)^{-}$ is found to be 270 ns, and the decay time of spectrum β is 1500 ns (MW-power: 10 mW). This is in agreement with the corresponding times found for PS I particles from cyanobacteria and spinach chloroplasts [8,9] (see Table I).

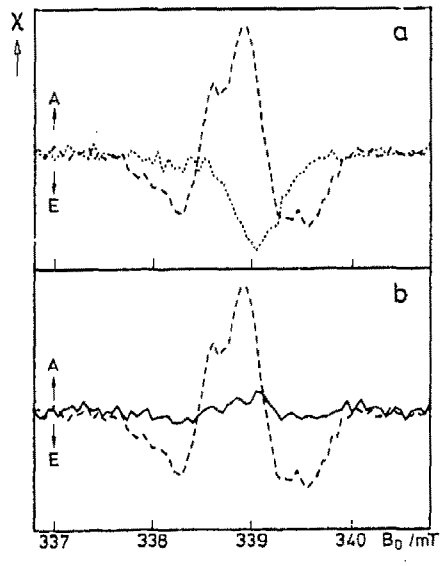

Fig. 1. Kinetically separated, spin polarized spectra of PS I particles from *Synechocystis* 6803, observed in the direct detection mode and derived from the original time/field data set $s(t,B_0)$ according to eq.(3). Room temperature, MW-frequency: 9.507 GHz, MW-power: 10 mW, laser repetition rate: 10 Hz. (a) Intact samples, early spectrum $\alpha(B_0)$ (broken line) and subsequent spectrum $\beta(B_0)$ (dotted line), E: emission, A: absorption. (b) VK_1 depleted samples, remaining spectrum $\alpha(B_0)$ (solid line), less than 5% intensity compared to intact sample (broken line, as in (a)).

Table I

Kinetic data observed for the electron transfer step in eq. 2 for different PS I particles as obtained from transient EPR

PS I sample	τ_1(1/e) (ns)	τ_2(1/e) (ns)	
Spinach chloroplasts	263	680*	[8]
Synechococcus sp.	260	1270	[8]
Synechocystis 6803	270	1500	this work
Synechocystis 6803 reconstituted with VK_1	280	1500	this work
Synechocystis 6803 reconstituted with DQ or DQd_{12}	675	-	this work
Synechocystis 6803 reconstituted with NQ	635	-	this work

MW-power: 10 mW, τ_1 and τ_2 are the results of the fit using eq. 3, S.D.: ±20 ns; * MW-power: 40 mW, -: amplitude is zero.

Fig. 1b shows the weak signal obtained from the VK_1 depleted sample (solid line). It is reduced by more than one order of magnitude compared with the amplitude of the respective signal in the intact PS I sample (broken line). The spin polarized spectra of samples reconstituted with DQ and DQd_{12} are shown in Fig. 3 (solid lines) as compared with spectrum α in intact PS I (broken lines, as taken from Fig. 1). In contrast to intact samples no subsequent spectrum β is observed after reconstitution with DQ. The observed spectrum appears within the instrumental rise time (~50 ns) and decays exponentially with $\tau_1 = 675$ ns (MW-power: 10 mW, see Fig. 2b and Table I). Reconstitution using DQ

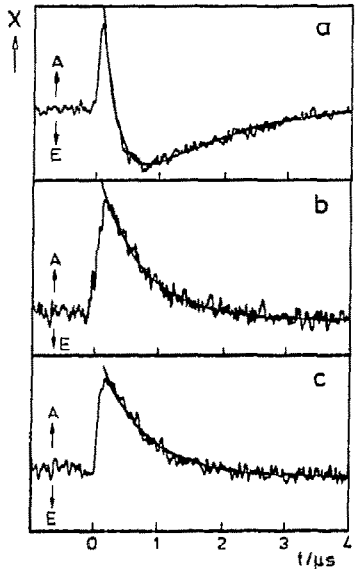

Fig. 2. Transients averaged in the field region of 338.80–339.05 mT. The smooth curves are the result of a fit to eq. (3), (a) intact PS I, $\tau_1 = 270$ ns, $\tau_2 = 1500$ ns, (b) with DQ or DQd_{12} reconstituted PS I, $\tau_1 = 675$ ns ($\beta = 0$), (c) with NQ reconstituted PSI $\tau_1 = 635$ ns ($\beta = 0$), S.D. in time constants: ±20 ns. Experimental parameters as in Fig. 1.

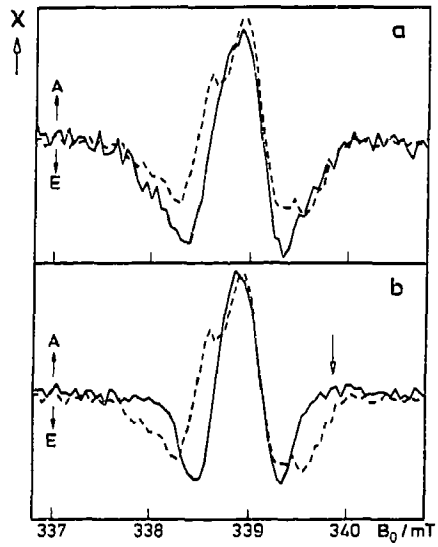

Fig. 3. Spin polarized spectra (solid lines) following reconstitution, with DQ (a) and DQd_{12} (b) compared with spectrum α (broken line) of intact PS I (taken from Fig. 1a). Experimental parameters as in Fig. 1.

yields a spectrum (Fig. 3a) comparable in overall width to that of intact samples, however, the emissive parts on the high- and low-field side appear with increased relative intensity. In the case of reconstitution with DQd_{12} the ESP-pattern is clearly narrowed (Fig. 3b) as expected from the reduced hyperfine coupling. This indicates also that DQ^- is one of the radical ions of the radical pair $P_{700}^{+}Q^-$ to which the ESP-pattern is assign-

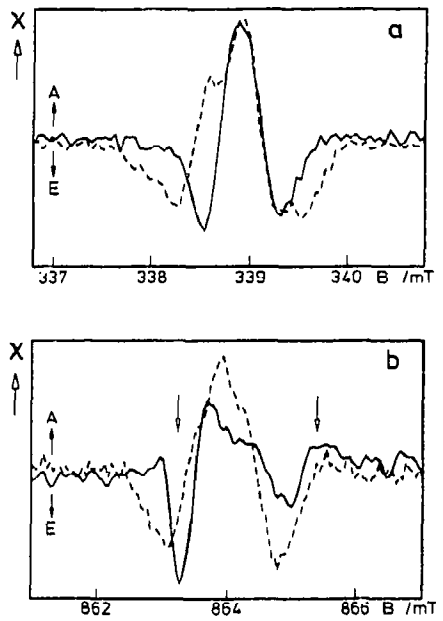

Fig. 4. Spin polarized spectra (solid line) following reconstitution with NQ compared with spectrum α of intact PS I (broken line); (a) X-band, experimental parameters as in Fig. 1, (b) K-band, experimental parameters as in Fig. 1, except MW-frequency: 24.211 GHz, MW-power: 6 mW.

Table II

Variation of τ_1 with MW-power for PS I sample reconstituted with NQ

Microwave power (mW)	τ_1 (1/e) (ns)
100	185
50	280
20	484
10	635
5	876
2	1067
1	1174

ed. A high-field absorption appears in addition to the prominent E/A/E pattern (see arrow in Fig. 3b), therefore DQ^- contributes also in the high-field region.

Reconstitution with NQ yields the ESP-pattern shown in Fig. 4. The observed spectrum decays with $\tau_1 = 635$ ns (MW-power; 10 mW, Fig. 2c and Table I). This decay time depends on the MW power (see Table II), hence it must be dominated by relaxation processes. The X-band (9 GHz) ESP-pattern (Fig. 4a) is markedly narrowed, even more than in samples using DQd_{12} (Fig. 3b). Improved resolution is obtained at K-band (24 GHz) (Fig.4b) and leads to several qualitative observations: (1) The overall spectral width which is largely determined by the anisotropy of the quinone g-tensor is not significantly affected by the replacement of VK_1 with NQ. This suggests that the narrowing of the spectral features in the NQ spectrum does not result from motional averaging. (2) The narrowing is not uniform over the whole spectrum, it is most pronounced near the low-field emissive peak (left arrow in Fig. 4b). (3) A high-field absorption (right arrow in Fig. 4b) appears as noted for the sample reconstituted with DQd_{12} (arrow in Fig. 3b).

Following reconstitution with VK_1 both successive spin polarized ESP-patterns were recovered. The electron transfer time $\tau_1 = 280$ ns and $\tau_2 = 1500$ ns (MW: 10 mW) agree well with the corresponding times in native PS I.

4. DISCUSSION

The usual, unpolarized P_{700}^{+} signal, which can be observed using standard EPR-detection with 100 kHz field modulation, provides a suitable reference signal. It remains essentially unchanged for all samples investigated here. In contrast, the first spin polarized spectrum seen in intact samples, with its characteristic E/A/E/(A) ESP-pattern, reduces to near zero intensity in VK_1 depleted samples (Fig. 1) and recovers to nearly equal overall intensity and general characteristics in samples reconstituted with Q. Electron transfer from the donor P_{700} to the acceptor A_1 is restored by reconstitution with different quinones and occurs on a time scale faster than 50 ns.

Earlier work on intact PS I samples (see [19] and

references therein) showed that the observed early ESP-pattern is due to the radical spin pair $P_{700}^{+}A_1^{-}$. This pattern depends strongly on the orientation of the acceptor A_1 relative to the dipolar axis z_d fixed in the reaction center. A comparable ESP-pattern was observed in all samples reconstituted with Q. Therefore we can assign it to the pair $P_{700}^{+}Q^{-}$, i.e. Q occupies the A_1-site. The VK_1 extraction procedure removes other pigments as well, especially some of the numerous antenna chlorophylls. During reconstitution Q could enter one of these non A_1-sites or could act otherwise as an exogenous acceptor. However, both the same fast rise time and the characteristic ESP-pattern found in the reconstituted samples argue against these possibilities. At least, the ESP-patterns are associated with structural constraints such as distance and molecular orientation which are characteristic for the A_1-site of PS I.

In contrast to intact samples, preparations reconstituted with DQ or NQ show only a single ESP-pattern whose decay is dominated by spin relaxation. As a consequence, further electron transfer past A_1 is slowed down by at least one order of magnitude ($\geq 5\ \mu s$). The redox potential of NQ^{-}/NQ (-0.58 V, [21]) and DQ^{-}/DQ (-0.74 V[12]) is more positive than that of A_1^{-}/A_1 (-1.04 V [22]) which may explain that electron transfer past Q^{-} is slowed down.

The most relevant experimental observation is a specific spectral narrowing when NQ is used for reconstitution. A higher mobility of NQ in the A_1-site can be excluded because no effect is observed on the overall width of the spectral pattern which is mainly due to the quinone *g*-tensor anisotropy. In addition, the smaller and potentially more mobile DQ molecule does not yield a spectral narrowing upon reconstitution.

The main difference between the three quinones used lies in the hyperfine interaction of the protons. Recent high-field EPR-spectra (95 GHz, W-band [24]) of NQ^{-} and similar quinone anions in solid solution show resolved hyperfine structure on the outer *g*-tensor components g_{xx} and g_{zz}, while the middle component g_{yy} appears narrow without detectable hfs. This is in contrast to VK_1 and DQ which show unresolved hfs on all *g*-tensor components. In the K-band spectrum of the NQ reconstituted sample (Fig. 4b) a narrow E/A feature is prominent in the low-field region, in addition the patterns begins with a weak A-peak from the low-field side. Thus, the narrow E/A feature is likely to be associated with the g_{yy} component and requires a NQ orientation with respect to z_d which is closer to $z_d \| g_{yy}$. This is in contrast to results obtained for perdeuterated PS I [19,25]. From simulations of these spectra it was concluded that the orientation of $A_1 = VK_1$ is close to $z_d \| g_{xx}$. As a consequence of this the lowest field *g*-tensor component g_{xx} appears in E/A polarization and g_{yy} in A/E. From this qualitative analysis we conclude that NQ in reconstituted PS I is oriented differently than the native quinone acceptor $A_1 = VK_1$ in intact PS I.

The main conclusions of a recent similar A_1 extraction/reconstitution study [26] on PS I particles from spinach are confirmed by the present paper. They are extended by the following complementary results:

(i) PS I particles of species as different as spinach [26] and cyanobacteria here lead to the same conclusions.

(ii) The study [26] uses light modulation and low temperature by which the $P_{700}^{+}A_1^{-}$ spectrum can only be studied on a slower time scale. Our results at room temperature establish compatible kinetics on the nanosecond time scale for both the intact as well as reconstituted samples. In both cases the spectrum of $P_{700}^{+}A_1^{-}$ appears within the instrumentally limited rise time (≤ 50 ns). This provides direct confirmation that the reconstituted Q acts as acceptor A_1 within the normal chain of chromophores and not as exogenous acceptor, as demonstrated also by recent optical studies [13].

(iii) We have demonstrated that reconstitution with suitable quinones leads to considerable spectral narrowing. This in turn results in better spectral resolution and the possibility of studying the influence of specific hyperfine interactions with selective deuteration experiments. Eventually, more specific structural information concerning the charge separation state can be extracted.

In summary, assignment and interpretation of the $P_{700}^{+}A_1^{-}$ ESP-pattern with A_1^{-} being a reduced VK_1 have been advanced considerably with these results. The potential for further structural and functional information on the charge separated state under physiological conditions has become clearer.

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