Volume 239, number 1, 93–98 FEB 06428 October 1988

# Electron transfer from $A_1^-$ to an iron-sulfur center with $t_{1/2} = 200$ ns at room temperature in photosystem I

## Characterization by flash absorption spectroscopy

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Received 22 August 1988

Forward electron transfer in intact photosystem I particles from *Synechococcus* sp. at room temperature has been studied by flash absorption spectroscopy with a time resolution of 5 ns. A kinetic phase with $t_{1/2} = 200$ ns was resolved and characterized by its absorption difference spectrum between 325 and 495 nm. This phase is attributed to electron transfer from the reduced redox center $A_1$ to an iron-sulfur center, probably $F_X$. The difference spectrum for the reduction of $A_1$ is evaluated and the previously proposed identification of $A_1$ with vitamin $K_1$ is discussed.

Photosystem I; Electron transfer; Vitamin $K_1$; Absorption difference spectrum; Photosynthesis; (*Synechococcus* sp.)

## 1. INTRODUCTION

The electron transport chain of PS I comprises the primary electron donor P700, probably a chlorophyll-*a* dimer, and five electron acceptors: the primary acceptor $A_0$, probably a monomeric chlorophyll-*a*, an intermediate acceptor $A_1$ and three iron-sulfur centers, $F_X$, $F_B$ and $F_A$ (reviews [1–3]). The primary charge separation between singlet excited P700 and $A_0$ takes approx. 10 ps [4] and the subsequent reoxidation of $A_0^-$ proceeds in tens of picoseconds [5]. The following electron transfer steps have not yet been resolved kinetically. An upper limit of 100 ns has been reported [6] for the reduction of the terminal acceptor, called P430 according to its bleaching around 430 nm (P430 is now assumed to be constituted by $F_B$ and $F_A$ [2]).

Evidence for the existence of intermediate $A_1$ came from EPR studies under highly reducing conditions [7,8] and from absorption changes at low temperature [9]: in these studies, a photo-reduced species was observed which could neither be a chlorophyll nor an iron-sulfur center. Based on the detection of two molecules of vit.$K_1$ (phylloquinone) per P700 in isolated PS I particles [10–12] and some *g* anisotropy in the EPR spectrum of $A_1^-$ similar to semiquinone-anions in bacterial reaction centers [13], it was proposed that $A_1$ might be vit.$K_1$ [11]. This assignment was supported by the UV-absorption difference spectrum of the recombination between $P700^+$ and $A_1^-$ with $t_{1/2} \approx 150\,\mu s$ at 10 K [14]. The role of $A_1$ and vit.$K_1$, respectively, under physiological conditions, however, remained to be established. Recent studies based on photoinactivation or extraction and reconstitution of vit.$K_1$ are controversial (e.g., [15] and [16] and references therein).

In the present work, intact PS I particles at

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*Abbreviations:* Chl, chlorophyll; d, optical path for the measuring light; DPIP, 2,6-dichlorophenolindophenol; (FeS), iron-sulfur center; FWHM, full width at half maximum; PS, photosystem; vit.$K_1$, vitamin $K_1$

*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/88/$3.50 

room temperature are studied by flash absorption spectroscopy with a time resolution of 5 ns in the UV and blue spectral regions in order to resolve directly a participation of $A_1$ in electron transfer from $A_0^-$ to the iron-sulfur centers, i.e. the reactions $P700^+A_0^-A_1(FeS) \longrightarrow P700^+A_0A_1^-(FeS) \longrightarrow P700^+A_0A_1(FeS)^-$

## 2. MATERIALS AND METHODS

PS I particles with approx. 80 Chl/P700 were prepared from thermophilic cyanobacteria *Synechococcus* sp. according to [17] (there called SG1). The particles were suspended in a buffer containing 20 mM Tricine (pH 7.8), 20 mM $MgCl_2$ and 0.04% (w/w) $\beta$-dodecyl-D-maltoside. Further additions are given in the figure legends. Absorption change measurements with a time resolution of 5 ns were performed with the set-up described in [18], using repetitive (1.7 Hz) excitation by 532 nm laser flashes of 3 ns duration, approx. 2 mJ/cm$^2$. Measurements with a time resolution of 100 $\mu$s were performed with a flash photometer using a saturating Xe-flash of 20 $\mu$s duration, filtered by 3 mm OG 570 (Schott) for excitation, a tungsten halogen lamp as measuring light source and a photomultiplier (EMI 9558 BQ) coupled to a transient recorder (BIOMATION 4500 from Gould) as detection system. The measuring light beam passed through a monochromator (10 nm bandwidth) placed between lamp and sample and a combination of interference filters and colored-glass filters placed in front of the photomultiplier. The same filter combinations were used in both set-ups in order to make measurements performed on either set-up as comparable as possible with respect to wavelength. The bandwidth (FWHM) of the transmission of the filter combinations was usually between 7 and 10 nm, except for measurements centered at 433.5, 450 and 487.5 nm where it was only 3 nm. All measurements were performed at room temperature.

## 3. RESULTS AND DISCUSSION

As a control for normal charge separation between P700 and P430, flash-induced absorption changes were measured with a time resolution of approx. 100 $\mu$s in the presence of reduced DPIP and methylviologen. The results, e.g. at 433.5 nm (inset of fig.1), demonstrate the formation of $P700^+ \ldots P430^-$ within less than 100 $\mu$s, the subsequent reoxidation of $P430^-$ by methylviologen ($t_{1/2} \approx 1$ ms) and the rereduction of $P700^+$ by reduced DPIP ($t_{1/2} \approx 30$ ms) (cf. [6]). From such measurements, the difference spectra for the oxidation of P700 and for the reduction of P430 were deduced separately according to Ke [6]. These spectra (fig.1) agree well with those reported in [6] for PS I particles from spinach, except for two deviations: the slight bleaching in the $P700^+/P700$ spectrum around 335 nm, where Ke [6] reported a slight absorption increase, and a 40% lower differential extinction coefficient around 430 nm for $P430^-/P430$ compared to [6]. The dotted line in fig.1 represents the difference spectrum for the reduction of vit.$K_1$ in methanol (redrawn from [19]). From this plot it is evident, that a participation of vit.$K_1$ in electron transfer should be most easily detectable in the 350 to 400 nm range where contributions of $P700^+/P700$ and $P430^-/P430$ to the flash-induced absorption changes will be comparatively small.

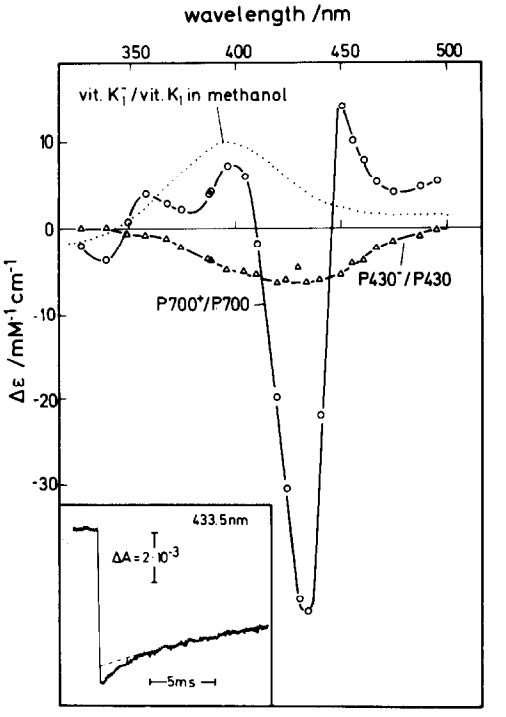


Fig.1. Absorption difference spectra for the oxidation of P700 (circles) and for the reduction of P430 (triangles), constructed according to [6] from time resolved measurements as the one at 433.5 nm depicted in the inset (74 $\mu$M Chl, 500 $\mu$M DPIP, 5 mM Na-ascorbate, 50 $\mu$M methylviologen; $d = 1.2$ mm; time resolution, 100 $\mu$s; 64 averages). To separate the faster phase ($t_{1/2} \approx 1$ ms; reoxidation of $P430^-$) from the slower phase ($t_{1/2} \approx 30$ ms; rereduction of $P700^+$), the latter was extrapolated to $t = 0$ according to the kinetics measured at 495 and 700 nm, where $\Delta\epsilon(P430^-/P430)$ is negligible [6] (dashed line in the inset). The $\Delta\epsilon$ scale is based on $\Delta\epsilon(P700^+/P700) = -45$ mM$^{-1}\cdot$cm$^{-1}$ at 433.5 nm [6]. (Dotted line) Absorption difference spectrum for the reduction of vit.$K_1$ to its semiquinone anion in methanol, as measured by Dr E.J. Land, redrawn from [19].

Fig.2A (upper trace) shows a measurement at 387 nm performed with a time resolution of 5 ns. A rapid instrument limited absorption increase is followed by a decay with $t_{1/2} \approx 200$ ns. This time course would be in line with a fast (<5 ns) reduction of vit.$K_1$ and subsequent electron transfer to an iron-sulfur center in 200 ns. This signal is completely suppressed in the presence of ferricyanide (fig.2A, lower trace), which blocks the photochemistry of PS I by chemical oxidation of P700. Two other typical examples are shown in fig.2B and C. At 430 nm, the strong initial bleaching, mainly due to the photooxidation of P700, is followed by a further bleaching with $t_{1/2} \approx 200$ ns, while at 455 nm an absorption increase with $t_{1/2} \approx 200$ ns is observed. The amplitude of the 200 ns phase is depicted as a function of wavelength in fig.3. The positive amplitudes between 340 and 430 nm would be roughly in line with a superposition of absorption changes due to the reoxidation of vit.$K_1^-$ and the reduction of an iron-sulfur center. In addition, the spectrum of the 200 ns phase shows a feature in the 440 to 500 nm range with a shape reminiscent of a red-shift of an absorption band centered at about 470 nm.

Further information can be gained from an inspection of the states at the beginning and at the end of the 200 ns reaction. Fig.4 shows the extrapolated initial absorption changes of measurements as in fig.2 (triangles, corresponding to the state at $t \approx 5$ ns) and the absorption changes after completion of the 200 ns reaction (circles, taken at $t = 1.6\,\mu$s). These spectra are compared with the $P700^+/P700$ spectrum (dotted line), because $P700^+$ is present in addition to the reduced electron acceptor under study. Inspection of fig.4 reveals that the reduced acceptor in the 'final' state ($t = 1.6\,\mu$s) is related to a bleaching between 370 and 470 nm. This is typical for the reduction of an iron-sulfur center, either $F_X$ or P430 (e.g., [21]). The 'initial' spectrum ($t \approx 5$ ns) matches well with the difference spectrum of the recombination between $P700^+$ and $A_1^-$ at 10 K [14] (inset of fig.4), except for the less pronounced bleaching around 430 nm at 10 K, which is probably due to a temperature dependence of the $P700^+/P700$ difference spectrum in this region [20]. The state at $t \approx 5$ ns is hence attributed to the radical pair $P700^+A_1^-$.

Summarizing, the 200 ns phase reflects the electron transfer reaction $P700^+A_0A_1^-(FeS) \longrightarrow P700^+A_0A_1(FeS)^-$, where (FeS) is an iron-sulfur center, probably $F_X$, because $F_X$ appears to precede $F_B$ and $F_A$ in the electron transfer chain [3]. This is in contrast to the upper limit of 100 ns for the reduction of P430 as estimated from a previous measurement at 430 nm with PS I particles from *Anabaena variabilis* [6]. Possibly, the relatively small 200 ns phase at 430 nm (fig.2B) was missed in [6] due to an insufficient signal to noise ratio.

With respect to the chemical nature of $A_1$, the difference spectrum for the reduction of $A_1$ alone ($A_1^-/A_1$) is of interest. Fig.5 shows the result of two different approaches to evaluate this spectrum.

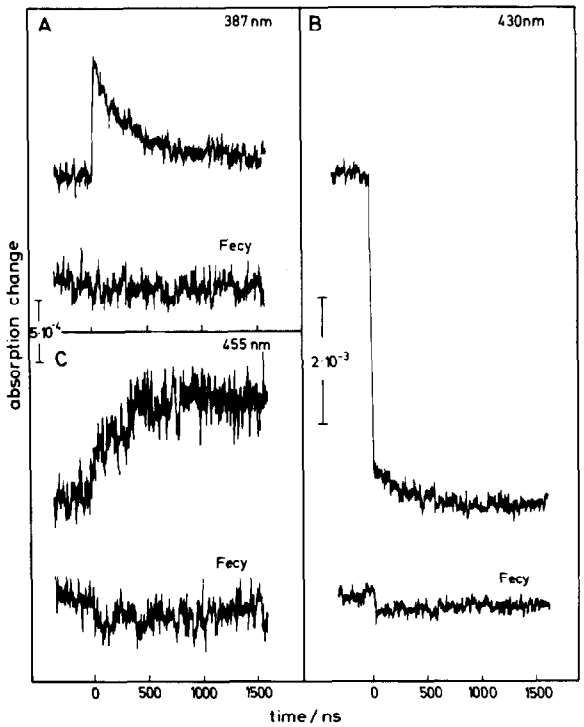


Fig.2. Flash-induced absorption changes at the indicated wavelengths, attributed to normal forward electron transfer (upper traces) and control measurements under conditions of chemically oxidized P700 (lower traces); 115 $\mu$M Chl; $d$ = 1.0 mm; time resolution, 5 ns. (A) Upper trace: presence of 500 $\mu$M DPIP, 5 mM Na-ascorbate, 100 $\mu$M methylviologen; 1024 averages. Lower trace: presence of 2 mM $K_3Fe(CN)_6$; 512 averages. (B) Same as A, but the upper trace represents the difference between the signal under normal conditions (640 averages) and the control with $K_3Fe(CN)_6$ (lower trace). (C) Same as B, but 256 averages under normal conditions.

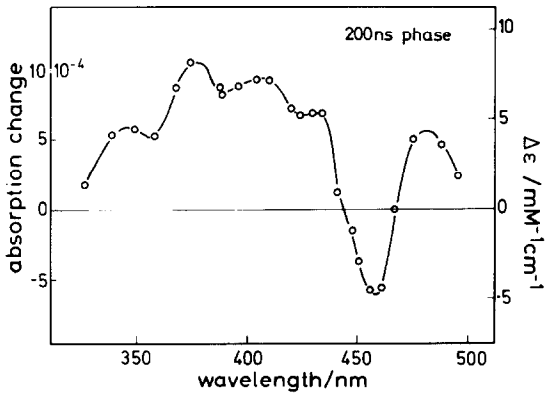


Fig.3. Spectrum of the amplitude of the 200 ns phase, extracted from measurements as those depicted in fig.2, upper traces. The $\Delta\epsilon$ scale was calibrated with the assumption, that the absorption change at 433.5 nm after 1.6 $\mu$s is identical with the extrapolated initial amplitude of the measurement performed with 100 $\mu$s resolution (fig.1, inset).

A (circles): Subtraction of the $P700^+/P700$ spectrum (fig.1, circles) from the initial ($t \approx 5$ ns) spectrum (fig.4, triangles), representing $P700^+A_1^-/P700A_1$. In this approach, the data points between 410 and 440 nm are not reliable because they represent differences between two very large signals measured with different set-ups.

B (triangles): Addition of the $P430^-/P430$ spectrum (fig.1, triangles) to the spectrum of the 200 ns phase (fig.3), which represents $A_1^-(FeS)/A_1(FeS)^-$ with (FeS) probably being $F_X$. The addition of the $P430^-/P430$ spectrum instead of the $F_X^-/F_X$ spectrum (which was not determined in this study), is an approximation, based on the published similarity of both difference spectra (e.g. [21]).

As a check for the previously proposed identification of $A_1$ with vit.$K_1$ (see section 1), the $A_1^-/A_1$ spectrum (fig.5) should be compared with the in vitro difference spectrum for the reduction of vit.$K_1$ (fig.1, dotted line). This comparison reveals a very significant deviation, namely the 440 to 500 nm feature of the $A_1^-/A_1$ spectrum. This feature even involves a bleaching at around 455 nm where neutral vit.$K_1$ does not absorb. Additionally, the positive absorption changes in the near UV in the $A_1^-/A_1$ spectrum appear at shorter wavelengths than for vit.$K_1^-$/vit.$K_1$ in methanol. The trough at 358 nm in the $A_1^-/A_1$ spectrum is in the order of the accuracy of the measurements, so that its significance is uncertain. These deviations

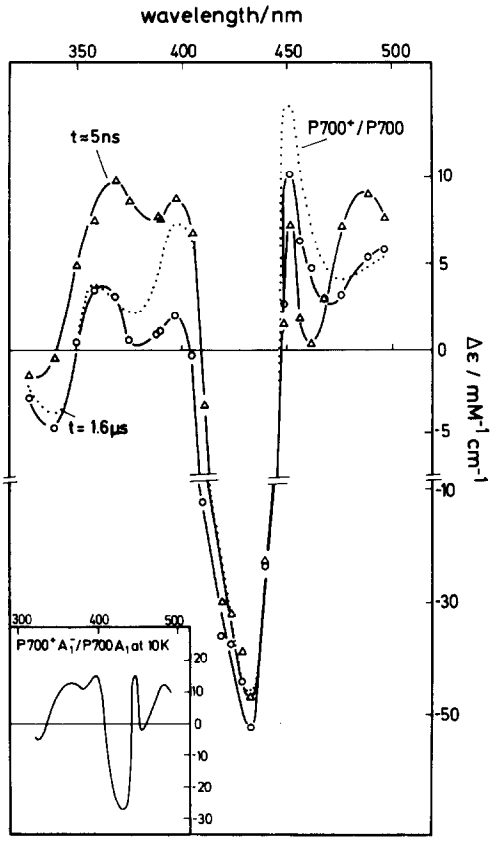


Fig.4. Spectra of the absorption changes at approx. 5 ns (triangles) and 1.6 $\mu$s (circles) after excitation, taken from measurements as those depicted in fig.2, upper traces. The $\Delta\epsilon$ scale was calibrated as in fig.3. (Dotted line) Difference spectrum for the oxidation of P700, redrawn from fig.1. (Inset) Difference spectrum of the 150 $\mu$s phase at 10 K in PS I particles from spinach, attributed to the recombination $P700^+A_1^- \longrightarrow P700A_1$, redrawn from [14].

can be explained in two ways: (i) $A_1$ is not vit.$K_1$. With respect to alternative candidates for $A_1$, one might think of other quinones which could also fit the EPR results (see section 1). Some other naphthoquinones and some anthraquinones have absorption bands in the blue spectral region [22], so that they may be bleached at around 455 nm upon reduction. However, vitamin $K_1$ is the only quinone so far detected in stoichiometric amount with P700 in isolated PS I [11,12]. Chl-*a* and $\beta$-carotene, which both absorb in the blue, can be excluded as candidates for $A_1$ because in either case the 200 ns phase should be detected at 820 nm [23,24] which has been checked and found not to be the case (not shown).

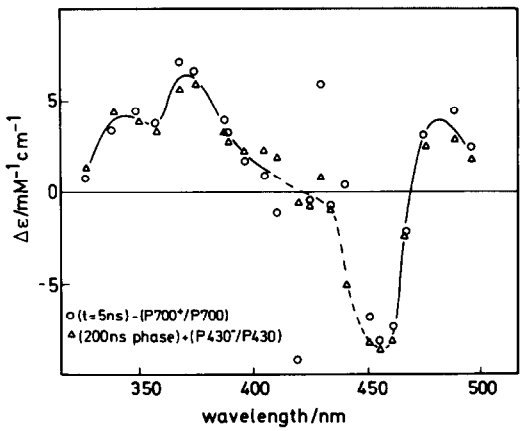


Fig.5. Absorption difference spectrum for the reduction of $A_1$ as evaluated by approach A (circles) and approach B (triangles) as explained in the text. All circles and triangles result from subtraction or addition of data points at equal wavelengths in figs 1,3 and 4. Between 410 and 440 nm (broken line), approach A is not reliable (see text).

(ii) $A_1$ is vit.$K_1$, but its difference spectrum upon reduction deviates from that in methanol due to the local environment of $A_1$ in PS I. This environment would have to provide at least two effects: (i) a blue-shift of the 390 nm band of vitamin $K_1^-$ by approx. 20 nm; (ii) an electrochromic red-shift of an absorption band of a neighbouring pigment, centered at about 470 nm, which would be induced by the electric field due to the negative charge of vit.$K_1^-$. A possible candidate for this pigment is $\beta$-carotene. Similar spectral effects of the local environment are known for other electron carriers in photosynthesis, e.g. the plastoquinone $Q_A$ in PS II [25]. Hence, the observed spectral deviations do not contradict an identification of $A_1$ with vit.$K_1$. An extension of the present study to shorter wavelengths might provide further evidence with respect to the chemical nature of $A_1$.

Electron transfer from $A_1^-$ to an iron-sulfur center might show up also in the spin-polarized EPR signals of PS I. The half-time of 200 ns determined for this reaction in the present work suggests that the striking change in the polarized EPR spectrum of PS I at room temperature during the first few hundred nanoseconds after excitation (review [26]), reflects the same reaction. This would be consistent with the interpretation that the spin-polarized signals at early times arise from $P700^+$ and a quinone-type $A_1^-$ [13,27,28].

After submission of the paper, I received a preprint of a similar study by Mathis and Sétif [29] performed with PS I from spinach. From measurements around 370 nm they conclude that electron transfer from $A_1^-$ to $F_X$ proceeds with $t_{1/2} = 15 \pm 5$ ns. The reason for the much faster kinetics compared to the present study remains to be clarified.

*Acknowledgements:* I wish to thank Ms D. DiFiore and Ms I. Geisenheimer for preparing the PS I particles and Dr E. Schlodder and Professor H.T. Witt for critically reading the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 312, Teilprojekt A3.

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