Flash-induced absorption changes in photosystem I at low temperature: evidence that the electron acceptor A_1 is vitamin K_1

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Low temperature flash absorption spectroscopy has been applied to elucidate the chemical nature of the secondary electron acceptor A_1 of photosystem I (PS-I). The flash-induced absorption changes measured in digitonin-fractionated spinach PS-I particles at 10 K between 240 and 525 nm are shown to comprise a major decay phase with $t_{1/2} \simeq 150$ μs which has been attributed to the recombination reaction $P\text{-}700^+...A_1^- \rightarrow P\text{-}700...A_1$ [(1984) Biochim. Biophys. Acta 767, 404–414]. We present the absorption difference spectrum of this reaction and demonstrate that it contains contributions in the ultraviolet due to A_1, which are characteristic of vitamin K_1 (phylloquinone).

Photosystem I   Electron transfer   Vitamin K_1 Absorption difference spectrum   Low temperature Photosynthesis

1. INTRODUCTION

The membrane-bound electron carriers of photosystem I (PS I) comprise the primary donor (named P-700 from the bleaching at 700 nm accompanying its oxidation) and a chain of electron acceptors named A_0, A_1, F_X, F_B and F_A (reviews [1–3]). Until recently, a single acceptor molecule was considered to precede the three iron-sulfur centers F_X, F_B and F_A. This primary acceptor was called A_1 and was claimed to be a chlorophyll (Chl) molecule (review [4]). Later studies showed that two electron acceptors A_0 and A_1 are in fact preceding the iron-sulfur centers [5,6]. The primary acceptor, therefore renamed A_0, is still thought to be a Chl molecule. In the new scheme (two acceptors A_0 and A_1), A_1 is a secondary acceptor whose anion has a g-value around 2.005 [5,6] with some g-anisotropy [7]. It was also argued that a reaction observed by flash-absorption spectroscopy and having a halftime of about 120 μs at 10 K [8] represents a recombina-

tion reaction between P-700$^+$ and A_1^- [9]. This reaction was tentatively associated [9] with polarized (CIDEP) EPR transient signals observed at low temperature which probably exhibit contributions from both P-700$^+$ and A_1^- [6,7,10]. From the absorption difference spectrum of the 120 μs phase in the red region [9] and from the lack of absorption changes in the visible region accompanying the formation of an EPR signal at g ~2.005 [11], it was concluded that A_1 is not a chlorophyll-like molecule, in accordance with the high g-value of A_1^-.

From both these EPR and absorption data, the possibility that A_1 is a quinone molecule has to be considered [7,9,11]. As a support for this hypothesis, vitamin K_1 (phylloquinone) has been found as an integral constituent of PS I [12–14]. We report here the absorption difference spectrum between 240 and 525 nm for the recombination reaction $P\text{-}700^+...A_1^- \longrightarrow P\text{-}700...A_1$ at 10 K and demonstrate that it contains contributions in the UV region due to A_1 which are characteristic of vitamin K_1.

2. MATERIALS AND METHODS

Digitonin-fractionated PS I particles (D144 particles) were prepared from spinach leaves [15]. They exhibit a Chl:P-700 ratio ~150.

For flash-induced absorption experiments, the PS I particles were contained either in a 1 mm thick plexiglass cuvette for measurements between 275 and 820 nm or a 0.5 mm thick quartz cuvette for measurements down to 240 nm. The cuvette was inserted in a cryostat (SCM, France) cooled with helium gas and positioned at 45° to the mutually perpendicular exciting and measuring beams. Excitation was provided by a (Nd-YAG)-pumped dye laser (broadband emission, ~600 nm; pulse energy, 5 mJ, checked repeatedly during the experiment; duration, 20 ns; repetition rate, 3 Hz). The laser beam passed through a OG 570 filter (Schott) and a piece of opal glass placed just before the cryostat to homogenize the excitation. The laser intensity was thus about two thirds saturating the reaction center photochemistry of the PS I particles (as estimated from absorption change measurements at differing laser intensities). The measuring light from either a deuterium lamp (240–280 nm) or a 800 W tungsten-halogen lamp passed through a Bausch and Lomb grating monochromator (bandwidth: 10 nm for measurements between 240 and 285 nm using the quartz cuvette, and 5 nm for all other measurements) before entering the cuvette. As detector, we used either (240–525 nm) a photomultiplier (9656 QR from EMI) which was protected against reflected laser light and fluorescence by suitable filter combinations, or (700 and 820 nm) a silicium photodiode protected by interference filters of the appropriate wavelength (bandwidth 3 nm). The output signals were filtered (DC, 10 kHz), amplified and finally recorded (Biomation 2805 coupled to a Tracor TN 1710). Depending on the wavelength studied, 512 to 4196 traces from 2 ms before to 8 ms after the laser pulse were added and an equivalent amount of fluorescence artefacts (without measuring light) were subtracted.

PS I samples were suspended in a buffer at pH 10 (0.2 M glycine NaOH) and glycerol was added (65% of final volume). Reduced samples were obtained in the presence of 10 mM sodium borohydride, which does not absorb in the wavelength region of interest, illuminated for 1 min with a flashlight and kept in darkness for 3 min before cooling in the dark. Oxidized samples were prepared with 2 mM potassium ferricyanide and 40 μM methylviologen and illuminated for 10 s with a flashlight before cooling under room light. The sample was not changed throughout a complete experiment either from 275 to 820 nm (plexiglass cuvette) or from 240 to 820 nm (quartz cuvette). The stability of the samples was verified by repeated control measurements at 430 nm. When changing from a reduced to an oxidized sample, much care was taken to reproduce the experimental conditions (Chl concentration and geometrical arrangements).

3. RESULTS AND DISCUSSION

When all the bound PS I electron acceptors are initially oxidized, or when F_A and F_B are prereduced with the other acceptors oxidized, it was shown previously that a 120 μs phase is the predominant relaxation phase of the flash-induced absorption changes at 820 nm (wavelength at which P-700 exhibits an absorption maximum) in PS I particles at 10 K and that this phase is about twice as large in the second case under steady state flash excitation [9]. Therefore D144 particles were treated with the strong reductant $NaBH_4$ to take advantage of the bigger signal when F_A and F_B are prereduced.

Under these reducing conditions, the flash-induced absorption changes in PS I particles at 10 K exhibit at most wavelengths 2 prominent decay phases (for examples, see fig.1): (i) A phase of $t_{1/2} \simeq 150$ μs which we identify essentially with the 120 μs phase ascribed to charge recombination in the pair P-700$^+$...A$_1^-$ that was observed previously [8,9]. The slightly larger apparent halftime in the present study may arise from the narrow bandwidth (DC, 10 kHz) that was used to improve the signal to noise ratio. (ii) A phase with $t_{1/2} \simeq 1$ ms, the absorption difference spectrum of this phase (not shown) resembles the triplet minus singlet ground state difference spectrum of Chl a [16] in the 270–525 nm range. At most wavelengths (e.g. 430 nm, see fig.1), a large portion of this phase remains even under oxidizing conditions (P-700 oxidized before excitation); at 700 nm, however, a signal of similar kinetics,
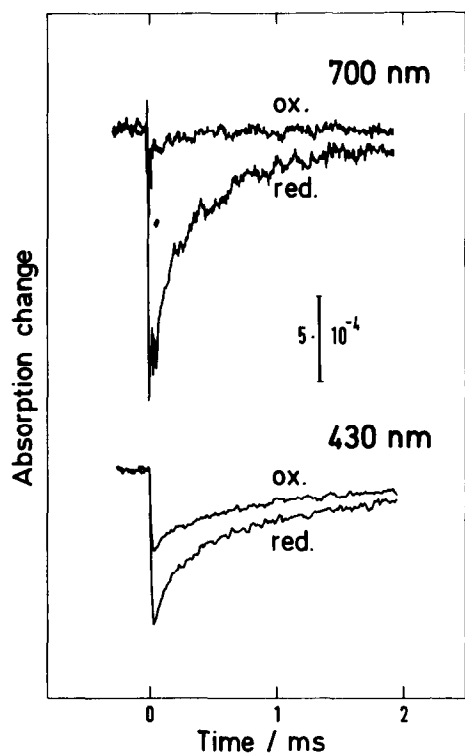
Fig.1. Flash-induced absorption changes at 700 nm and 430 nm in a reduced and an oxidized sample of D144 particles at 10 K; A_{676nm} (45°) = 0.7; see section 2 for further details.

which is present under reducing conditions, is largely suppressed under oxidizing conditions (fig.1). We assume that the triplet state of Chl a in the antennae and also the triplet state of P-700 formed in a minor fraction of reaction centers contribute to the 1 ms decay phase.

A small slower phase ($t_{1/2} > 5$ ms) which could be best resolved around 430 nm is probably due to charge recombination in the pair (P-700$^+$...F$_{\bar{x}}$) in a small fraction of reaction centers [9]. Around 520 nm, a strong positive component is observed which decays with $t_{1/2} \lesssim 20\,\mu$s (limit of the apparatus time resolution). This signal is on the whole insensitive to the oxidation of P-700 and is likely to arise from the triplet state of carotenoid molecules.

Under oxidizing conditions, we observed, in addition to the decay phases already mentioned ($t_{1/2} \lesssim 20\,\mu$s around 520 nm and $t_{1/2} \approx 1$ ms), some decay in the 100 μs range (see for example the

signal at 430 nm in fig.1). The nature of this component has yet to be clarified. However, it probably arises from antenna processes and not from incompletely oxidized P-700, as the small signal remaining at 700 nm under oxidizing conditions ($\approx 10\%$ of the signal detected under reducing conditions; see fig.1) shows that P-700 is largely oxidized under these experimental conditions.

In order to resolve the undisturbed spectrum of the 150 μs phase attributed to the recombination reaction between P-700$^+$ and A$_1^-$ under reducing conditions, it was necessary to subtract for each wavelength the signal which remains under oxidiz-
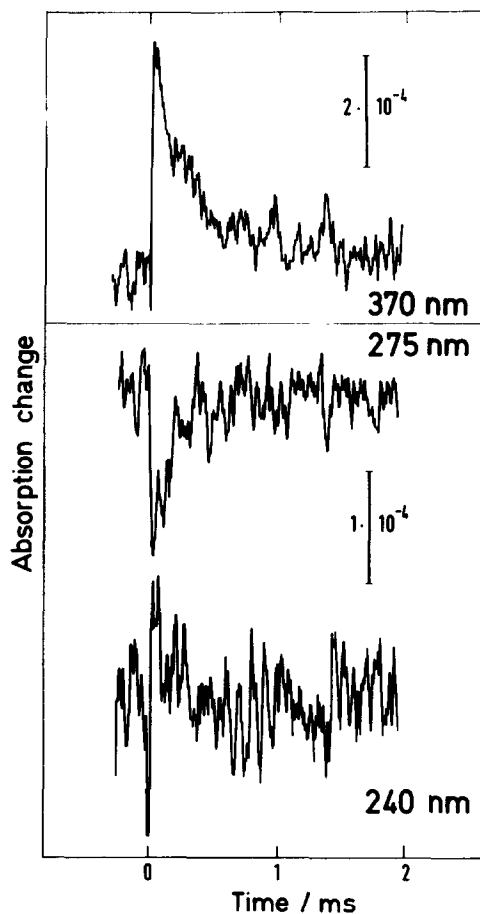

Fig.2. Flash-induced absorption changes at 370, 275 and 240 nm in reduced samples of D144 particles, corrected for absorption changes which are not related to reaction center photochemistry (see text); upper trace: A_{676nm} (45°) = 1.4; two lower traces: A_{676nm} (45°) = 0.7; see section 2 for further details.

ing conditions and is therefore not due to reaction center photochemistry. Fig.3 (lower part, symbols and continuous line) shows the spectrum of the amplitude of the 150 μs phase from 240 to 525 nm extracted from kinetic traces obtained after this correction procedure (see fig.2 for examples at 3 characteristic wavelengths). The $\Delta\epsilon$ scale of fig.3 was calculated by reference to the 150 μs phase obtained at 820 nm, assuming that P-700$^+$ alone contributes to that phase at 820 nm with $\epsilon = 6$ mM$^{-1}\cdot$cm^{-1} (room temperature value from [17]).

Ideally, the (P-700$^+$ − P-700) difference spectrum at low temperature should be subtracted from the spectrum of the 150 μs phase in order to determine the contribution of (A$_1^-$ − A$_1$). Since the
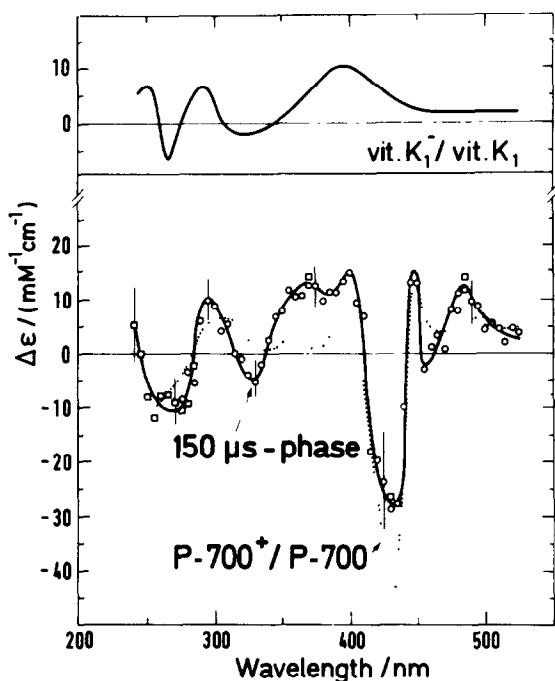

Fig.3. Absorption difference spectra for the 150 μs phase of D144 particles at 10 K (lower panel, symbols and continuous line), for the oxidation of P-700 at room temperature (lower panel, dotted line; redrawn from [17]) and for the reduction of vit.K$_1$ to its semiquinone anion in methanol (upper panel; as measured by Dr E.J. Land, redrawn from [18]); the amplitudes of the 150 μs phase were extracted from signals as those depicted in fig.2 (see text for the calibration of the $\Delta\epsilon$ scale); squares and circles refer to series of measurements with the quartz and plexiglass cuvettes, respectively.

former spectrum is not known in the UV region, we have to rely on the (P-700$^+$ − P-700) difference spectrum at room temperature that was previously measured by Ke [17] down to 260 nm with digitonin-fractionated spinach PS I particles (fig.3, dotted line).

Both the spectrum of the 150 μs phase and the (P-700$^+$ − P-700) spectrum exhibit a major bleaching around 430 nm which is, however, less pronounced for the 150 μs phase. This difference may arise either from an absorption increase due to the reduction of A$_1$ or from a temperature dependence of $\Delta\epsilon$ for the oxidation of P-700. In any case, the reduction of A$_1$ appears to be related to an absorption increase rather than to a bleaching at 430 nm, thus confirming that the partner of P-700$^+$ in the 150 μs recombination reaction is not the iron-sulfur center F$_X$ [9]. Outside the 430 nm band, the difference between the spectrum of the 150 μs phase and the (P-700$^+$ − P-700) is positive around 485 nm, from 340 to 410 nm, around 295 nm and probably also positive around 245 nm considering the slope of the (P-700$^+$ − P-700) spectrum above 250 nm. It is negative around 455, 325 and 270 nm. These differences correspond fairly well to the absorption changes due to the reduction of vitamin K$_1$ to its semiquinone anion in vitro (fig.3, upper panel, as measured by Dr E.J. Land, redrawn from [18]), except for the 450–500 nm region where electrochromic bandshifts may be superimposed. Concerning the most salient differences between the 150 μs spectrum and the (P-700$^+$ − P-700) spectrum in the UV region, namely around 380, 325 and 245 nm, it should be emphasized that the correspondence to the (vit.K$_1^-$ − vit.K$_1$) spectrum is not much dependent on a precise calibration of the $\Delta\epsilon$ scale of the 150 μs phase (our calibration is based on the assumption that the absorption of P-700$^+$ at 820 nm is identical at 10 K and at room temperature). These results show that the acceptor A$_1$, as defined by the 150 μs recombination reaction P-700$^+$...A$_1^-$ $\longrightarrow$ P-700...A$_1$ at low temperature can very well be a vitamin K$_1$.

From this report together with previous results [5–14], it can be concluded that vitamin K$_1$ takes an important part in the electron transfer steps in PS I at low temperature. At least two points are of major interest concerning the functional properties of this acceptor A$_1$. Firstly its role at room

temperature still needs to be determined and can now be studied more easily by taking advantage of the spectral features determined in the present paper. Secondly, for A_1 to be able to reduce the iron-sulfur centers, its redox potential has to be rather low, probably below -0.7 V vs NHE. Such a low potential might be achieved by the protein environment of vitamin K_1, providing possibly electron repulsive groups and aprotic conditions.

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