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Extraction of electron acceptor A_1 from pea photosystem I

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Photosystem I particles were washed twice in either 0, 50 or 100% water-saturated diethyl ether. It was found that the characteristic electron spin resonance signal associated with electron acceptor A_1 was progressively lost with increasing percentage saturation of the ether. Light-induced electron flow to the terminal iron-sulphur acceptors was inhibited although these acceptors were still present and could be chemically reduced. The kinetics of optical measurements of $P700^+$ re-reduction at 820 nm following ether washing were consistent with removal of electron acceptor A_1.

Photosystem I; Electron acceptor; Ether extraction; *(Pisum sativum)*

1. INTRODUCTION

Following light-induced charge separation in PS I, the displaced electron is transferred through intermediary carriers to a complex of membrane bound iron-sulphur centres and then to soluble ferredoxin [1]. The intermediary carriers have been shown to give both optical and ESR signals upon reduction [2-8]. These signals have been resolved to show the presence of two distinct components known as A_0 and A_1. Neither component has been reduced by chemical means. Their mid-point potentials are presumed to be between that of the lowest potential iron-sulphur acceptor, X (-0.7 V [1]), and the excited donor, $P700^+$ (-1.4 V [1]). Illumination at low temperature in the presence of sodium dithionite causes the irreversible transfer of electrons to A_1 (at 205 K), and to A_0 (at 225-230 K) [4,5]. The characteristics of optical and ESR difference spectra recorded upon reduction of A_0 suggest that it is a chlorophyll *a* monomer [2-4] although there are conflicting reports as to the position of the red absorption minimum for this component [4,7,8].

There is a consensus of opinion that the component responsible for the A_1 signal is a quinone. This is suggested by the shape and position of its ESR [2,5] and electron spin polarisation [9,10] spectrum. Moreover, two recent reports using complementary techniques have produced very similar optical difference spectra which suggest that the component is vitamin K_1, otherwise known as phylloquinone [5,6]. Two molecules of vitamin K_1 are found in each PS I reaction centre [11,12] although only one appears to be essential for forward electron flow [13,14]. Extraction of PS I with hexane removes the non-functional quinone [14] and was shown to alter but not eliminate the ESR spectrum from A_1 [15]. In this report we have used a more rigorous extraction method that has been shown to extract a large proportion of the reaction centre pigments and quinones [16,17]. Our results from ESR and optical measurements indicate that this treatment effectively removes electron acceptor A_1.

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Abbreviations: Chl, chlorophyll; ESR, electron spin resonance; PS, photosystem

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2. MATERIALS AND METHODS

PS I particles were prepared from pea (*Pisum sativum* var. Feltham First) leaves using the procedure described in [4,5]. The P700:Chl ratio obtained using this method was 1:32. Samples were diluted by a factor of four in distilled water and concentrated above an Amicon XM-100 filter in order to decrease salt and detergent concentration before freeze-drying overnight. Freeze-dried material was then washed twice with either anhydrous (0%), 50% or 100% water-saturated diethyl ether. In each case 10 mg of dried material was treated with 2×20 ml diethyl ether solution on ice in the dark for 1 min before recovery of the sample by centrifugation ($16000 \times g$ for 20 min). Supernatants were concentrated by rotary evaporation and stored at 77 K. Pellets were dried in the dark on ice under a stream of nitrogen before resuspension in a medium containing 0.25% Triton X-100 and 20 mM glycine, pH 10.0. ESR measurements were carried out as in [4,5] under the conditions detailed in the figure legends. Flash-induced absorbance changes at 820 nm were measured with a single-beam spectrophotometer essentially as described in [18], except that the measuring beam was detected using an end-on, 2 inch diameter, quartz window photomultiplier with S20 response. Actinic flashes (658 nm, pulse duration 20 ns, energy output approx. 10 mJ at 10 Hz) were produced by a J.K. 2000 YAG pumped dye-laser. The dye used was LC 6500 DCM (from Lambda Physik) dissolved in methanol. Samples were prepared in the above Triton X-100/glycine medium at 30 μg Chl/ml with 10 mM sodium ascorbate added.

3. RESULTS AND DISCUSSION

In fig.1, we show the effect of extraction with diethyl ether solutions saturated to various extents with water on the ESR signals from PS I electron acceptors A_0 and A_1. The 0% water-saturated (i.e. anhydrous) ether solution had no effect on the linewidth, shape or amplitude of either signal (fig.1a). The A_1^- signal was photoaccumulated at 205 K and that for A_0^- at 225 K. This was identical to the transition seen in untreated and freeze-dried PS I, only the ether-washed sample is shown here therefore and this may be compared with subsequent samples. The 50% water-saturated ether-extracted sample showed a significant modification of the signal recorded after photoaccumulation at 205 K for 1 min (fig.1b). The g value of the signal was closer to that expected of A_0 and the high field trough was broad, suggesting the presence of minima due to both A_1 and A_0. After illumination for a further 5 min at 205 K a signal with g value and linewidth characteristic of A_0 was formed. This signal did not alter further, except for an increase in amplitude, upon photoaccumulation at 225 K. It seemed likely that the

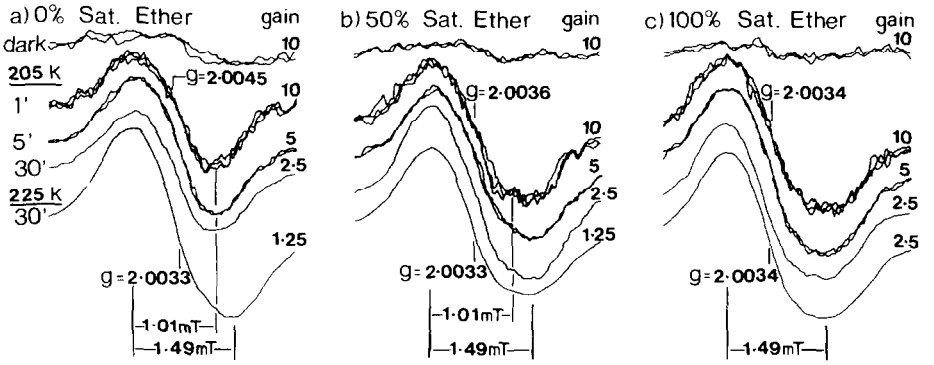

Fig.1. ESR spectra from PS I samples washed in diethyl ether solutions saturated to various extents with water. Anaerobic samples, at 1 mg Chl/ml, were pre-illuminated for 2 min at room temperature in the presence of 1% sodium dithionite before freezing at 77 K in the dark. Samples were then illuminated in the ESR cryostat at 205 K and subsequently 225 K for the time increments shown and the spectra recorded in the dark. Instrument settings were as follows: microwave power, 100 μW; frequency, 9.05 GHz; modulation, 0.2 mT; response, 1 s; gain, as shown in the figure $\times 10^3$; assay temperature, 205 K.

amplitude of the signal measured with 1.01 mT width following 1 min of illumination at 205 K was indicative of the full extent of A_1 photoaccumulation in this sample since a significant amount of A_0 was also present. When compared with the amplitude of fully reduced A_1 in control samples the difference represents extraction of about 60% of A_1. The increase in amplitude due to A_0 photoaccumulation in this sample was the same as that in control samples indicating that no A_0 had been removed. The 100% water-saturated ether-washed sample showed no signal that might be related to A_1 (fig.1c). The g value and linewidth of the signal following illumination for 1 min at 205 K were indicative of A_0 reduction only. This signal increased in amplitude but did not alter the shape upon further illumination, the extent of the increase indicated that only A_0 was photoreducible and that none of the latter had been extracted. We suggest that the A_0^- signal accumulates in these samples because the subsequent acceptor, A_1, had been removed.

Extraction of an intermediate electron carrier would be expected to prevent electron flow to components further along the chain. In fig.2a we demonstrate that electrons are prevented from reaching the terminal membrane bound iron-sulphur acceptors A and B. On illumination for 30 s at 18 K, iron-sulphur centre A became fully reduced in the freeze-dried unextracted control sample. In the various ether-extracted samples some reduction was observed but it was only 5% of that seen in the control. The control is plotted at a quarter of the gain used for the extracted samples. The decrease in amplitude of the signal due to centre A in the extracted samples therefore represents a loss of electron transport ability of about 95%. However, this occurred in 0% water-saturated as well as 100% water-satured ether-extracted samples although electron acceptor A_1 was still present in 0% water-saturated ether-extracted material (fig.1a). Fig.2b shows that the ESR signals characteristic of iron-sulphur centres A and B may be induced by chemical reduction.

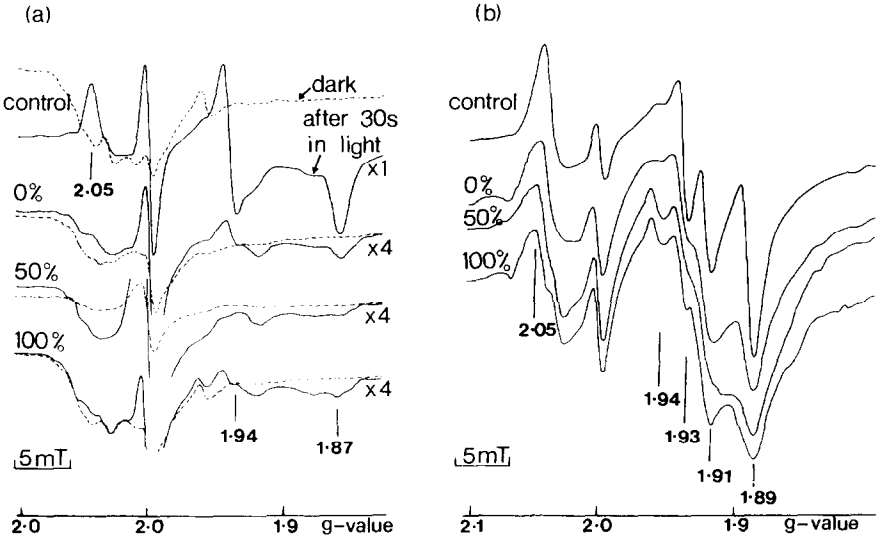

Fig.2. (a) Light-induced reduction of iron-sulphur centre A in various ether-extracted PS I samples. The freeze-dried control, 0%, 50% and 100% water-saturated ether-washed samples at 1 mg Chl/ml were dark adapted for 1 h and frozen in the presence of 10 mM sodium ascorbate in the dark. ESR spectra of the dark-adapted samples were then recorded at 18 K and again in the dark following a 30 s illumination period at that temperature. Instrument settings were as follows: microwave power, 5 mW: frequency, 9.05 GHz; modulation, 1 mT; response, 0.3 s; gain, control = 250; 0%, 50% and 100% water-saturated ether-washed = 1000. (b) Chemical reduction of iron-sulphur centres A and B in various ether-extracted PS I samples. The samples used in (a) were thawed, made anaerobic under nitrogen and sodium dithionite was added to give 1% final concentration. They were then illuminated at room temperature for 2 min before freezing in liquid nitrogen in the dark. Samples were assayed in the dark at 18 K with the instrument settings as in (a) except the gain was 250 for each.

The amplitudes of the signals were similar to those in the control. This indicates that although light-induced electron flow to A and B had been blocked the centres were still present in the samples. The spectra recorded in the treated samples were modified in shape which suggests that ether washing alters the spatial relationship of the iron-sulphur centres, possibly by modification or extraction of lipids surrounding the proteins.

The results shown in fig.2 are somewhat ambiguous since electron flow to the iron-sulphur centres was blocked in 0% water-saturated ether-washed samples which retained the intermediate electron carriers. We therefore carried out an optical kinetic study to determine at which point blockage had occurred in each type of extracted sample. In fig.3a, we show the rise and decay at 820 nm of absorbance changes due to P700 in a freeze-dried, unextracted sample. The half-time of the decay was 30 ms which, at room temperature, is characteristic of the back reaction between the iron-sulphur centres A and B and $P700^+$ [1]. In the 0% water-saturated ether-washed sample (fig.2b) a transient with a half-time of about 500 μs forms the dominent part of the decay. This probably represents back reaction from either X^- or A_1^- [1]. The residual slow phase represents about 10% of the signal, which is consistent with the amount of light-induced reduction of centres A and B observed in fig.2. In the 50% and 100% water-saturated ether-washed samples (fig.3c and d, respectively) a phase with a half-time of about 25 μs became prominant. The fast (25 μs) phase is indicative of the back reaction from the P700 triplet to the ground state. The P700 triplet is formed by the back reaction from reduced A_0 (see [1]). This suggests that in these samples electron transport to A_1 in the majority of reaction centres had been blocked. The recombination reaction with $P700^+$ was therefore from A_0. Since more A_1 was extracted in the 100% than the 50% water-saturated ether-washed sample (fig.1), we had expected a proportionately larger amount of the 25 μs phase in the former. However, the 100% water-saturated ether-washed sample was somewhat particulate and some of the fast phase was probably obscured by scattered light.

In summary then, we have shown that washing of freeze-dried PS I particles with ether solutions of increasing water saturation progressively eliminates the ESR signal associated with component A_1. This treatment blocks electron flow to the terminal iron-sulphur acceptors, although this may be the result of modification of the lipid or protein environment of the components involved. It is possible that inclusion of chloroplast lipids in the ether solution during extraction would prevent the loss of electron transport to the iron sulphur centres in the samples which retain component A_1. However, light-induced charge separation in centres lacking the A_1 ESR signal is dissipated primarily by the back reaction from A_0. It has been reported [17,19] that ether extraction removes all vitamin K_1 which is currently thought to be the molecular component responsible for the A_1^- ESR signal [5,6]. Our results show that ether extraction does indeed remove centre A_1. We have been unable to reconstitute electron flow to the terminal acceptors (as measured by ESR) by addition of

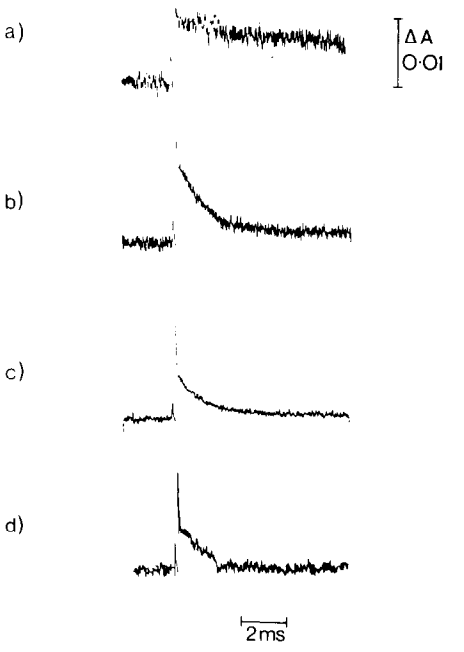

Fig.3. Flash-induced absorbance changes at 820 nm in PS I following ether washing. (a) Freeze-dried control, (b) 0% water-saturated ether-extracted, (c) 50% water-saturated ether-extracted, (d) 100% water-saturated ether-extracted. Samples were flashed at 5 Hz and the following number of measurements were averaged: (a) 512; (b,c,d) 1024.

either purified vitamin K_1 or the ether-extracted supernatant to washed particles. This may be due to the secondary disruption of the membrane noted earlier. Recently however, workers using similar techniques have been able to regain the optical kinetic properties of reaction centres containing A_1 by addition of vitamin K_1 to ether-extracted PS I [19]. The most likely explanation of the results presented here and in [19] is that A_1 is ether-extractable vitamin K_1.

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