Optical difference spectrum of the electron acceptor A_0 in photosystem I

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Optical measurements were made during low temperature photoreduction of photosystem I acceptors, A_1 and A_0. In the presence of a significant amount of A_1 (detected by EPR), no absorbance changes occurred between 750–350 nm, indicating that this species is not a chlorophyll or pheophytin molecule. Spectral changes in this region that may be correlated with the appearance of A_0^-, suggest that this component is a chlorophyll *a* anion monomer. The species is present in reaction centres in a ratio of 0.94 A_0/ P700.

Photosystem I *Primary acceptor* *Optical difference spectrum* *Chlorophyll a monomer*

1. INTRODUCTION

Light absorption by photosystem I (PS I) leads, within 40 ps [1] to charge separation in the photosynthetic reaction centre. The primary donor, P700 (E_m = +0.4 V) becomes oxidised and an electron is passed along a chain of components that ultimately reduce soluble ferredoxin (E_m = −0.42 V). These components comprise the secondary acceptors, iron-sulphur centres X, A and B and an intermediary acceptor complex 'A_1' [2,3]. Centre X has a midpoint potential of ≃ −0.70 V [4], therefore 'A_1' must have a greater reducing potential in order to function in linear electron flow. Under conditions where the iron-sulphur centres were reduced or removed, optical and electron paramagnetic resonance (EPR) spectra due to charge separation between P700 and 'A_1' have been detected. These indicate that 'A_1' is a chlorophyll *a* (chl *a*) anion [5,6]. 'A_1' can be progressively accumulated by illumination of PS I particles at cryogenic temperatures. During such reduction two distinct EPR signals have been detected, indicating that 'A_1' consists of two components, A_1 and an earlier acceptor A_0 [7,8]. The multiple component nature of the intermediary acceptor complex has also been indicated by chemically-induced dynamic electron polarisation (CIDEP) EPR signals (reviewed in [9]). A 120 μs component of the low temperature decay kinetics of 820 nm absorption changes has been related to a back reaction between $P700^+$ and A_1^- [10]. The only absorption changes in the range 650–730 nm accompanying this change were those due to $P700^+$ relaxation and some minor electrochromic shifts. The absence of any spectral contribution by the other partner in the radical pair strongly suggests that A_1 is not a chlorophyll (chl) or pheophytin molecule. The asymmetric line shape of the A_1^- X-band EPR spectrum [7] and the anisotropic *g* values of its Q-band spectrum [11] resemble those of a reduced quinone.

Previous attempts to obtain a spectrum of the primary PS I acceptor have not clearly distinguished between the two components of the A_0/A_1 complex. A point-by-point spectrum of slow absorption changes ($t_{½}$ ≃ 2 s) in reduced PS I particles [5] and a steady state difference spectrum of the acceptors trapped in the reduced state by freezing under illumination [6] were consistent with a chl *a* monomer. A spectrum of absorbance changes with 1.3 ms lifetime recorded under reducing conditions at 5 K has been related to A_1 and resembles a chlorophyll dimer [12]. However, this kinetic

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signal has been shown to be due to decay of a P700 triplet state and not to the $P700^+/A_1^-$ radical pair [13]. The g value and linewidth of the A_0^--EPR signal, on the other hand, indicate that it is a chlorophyll monomer [7,8].

Here we confirm that 'A_1' consists of two components, A_0 and A_1. We also show that an optical difference spectrum indicative of a chl a anion monomer can be correlated with the reduction of A_0 as monitored by EPR. Moreover, the reduction of A_1, similarly detected, was not accompanied by any optical absorbance changes in the visible region.

2. MATERIALS AND METHODS

PS I particles were prepared from pea (*Pisum sativum* var. Feltham First) leaves. A partially enriched fraction was obtained by retaining the supernatant following Triton X-100 digestion of magnesium-stacked chloroplasts [14]. This was then subjected to chromatography on a hydroxyapatite column as in [15]. The final P700:chl ratio was 1:45.

The standard assay mixture for both EPR and optical measurements consisted of 60% glycerol, 20 mM glycine, pH 10, 0.1% sodium dithionite, 0.3% silicone DC antifoam and sample at 150 μg chl/ml. The mixture was bubbled with oxygen-free nitrogen for 1 h and then illuminated for 2 min with a 1250 W lamp through a copper sulphate solution to reduce fully electron acceptors A, B and X (as determined by EPR). No discolouration of the sample occurred during this treatment indicating that no gross chlorophyll oxidation had taken place.

Optical measurements were made on an Aminco-Chance DW2 spectrophotometer in the split beam mode with 3 nm slit width. The sample was placed in a cuvette (2 mm path length) with a metal tongue which was dipped into liquid nitrogen in a glass dewar. The sample was thereby maintained at the required temperature as determined by the probe of an Oxford Instruments carbon thermometer immersed within the cuvette. The sample was clear at 200 K, with no cracks. An anaerobic environment was maintained around the sample by the evaporating nitrogen.

EPR measurements were made using a Jeol FE-1X spectrometer with 100 KHz field modulation and were below the power saturation level of each signal. The carbon thermometer was also used to measure the sample temperature in EPR tubes within an Oxford Instruments cryostat during the assays. g values were measured using a powdered manganese oxide sample as a standard. The measuring temperature for both EPR and optical measurements was 200 K. Illumination at low temperature was carried out using a Barr and Stroud 150 W fibre-optic light source. Optical spectra were recorded on an Apple II microcomputer for computation of difference spectra.

3. RESULTS AND DISCUSSION

The results from the EPR and optical experiments are directly comparable since the same

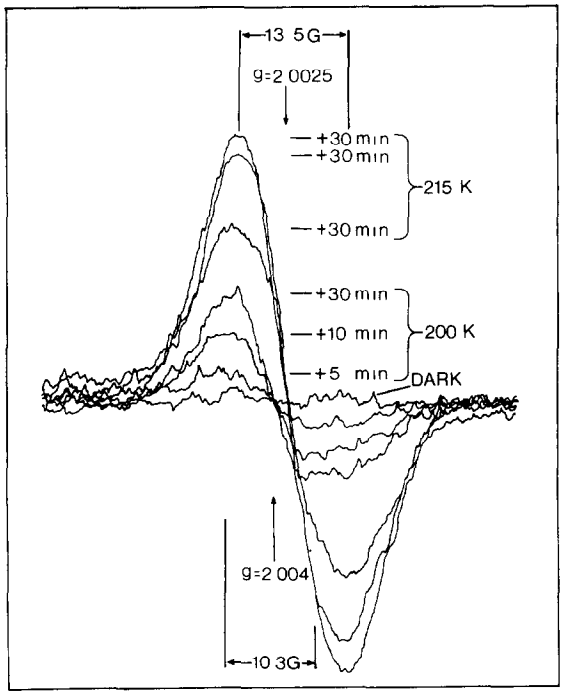

Fig.1. EPR spectra of A_1 and A_0 recorded after illumination (for stated time increments) of PS I particles at 200 and 215 K. The chlorophyll concentration was 150 μg/ml with 1 P700/45 chl. The iron-sulphur acceptors (A, B and X) were reduced prior to low temperature illumination. The spectra were recorded at 200 K with the following instrument settings: microwave power, 100 μW; frequency, 9.043 GHz; modulation amplitude, 2 G; instrument gain, 10^4.

chlorophyll concentration and illumination regime was used in each. Fig.1 shows the progressive appearance, upon illumination, of an EPR signal in the $g = 2.00$ region which is stable in the dark. The components responsible for this spectrum were trapped in the reduced state and were prevented from decaying by back reaction with $P700^+$ because the latter had been re-reduced by dithionite. The increase in the amount of stably reduced acceptor is dependent on competition between dithionite and the back reaction for reduction of $P700^+$. In the temperature range 200–230 K the back reaction is sufficiently slowed to increase the probability of dithionite reducing $P700^+$ and trapping an electron on the acceptor.

Spectra were recorded in the dark after illumination for the stated time increments. Some of the earlier traces are omitted from fig.2 for clarity but corresponding values are recorded in fig.3. Illumination at 200 K led to the appearance of a radical signal characteristic of A_1, having an asymmetric 10.3 G wide spectrum centred at $g = 2.0040$. This signal closely resembles that previously reported for A_1 [7]. Subsequently, the sample temperature was raised to 215 K during illumination and returned to 200 K for assay. The EPR signal then broadened to 13.5 G and was centred at $g = 2.0025$, characteristic of A_0 [7]. The appearance of the $g = 2.00$ signal has been shown to be temperature sensitive [16], being more rapid at 230 K than at 200 K. Illumination at 200 K for the duration used here trapped mainly A_1^- and warming to 215 K allowed trapping of A_0^-. 215 K was the temperature of choice since samples began to become opaque on warming above 220 K.

An identical temperature and illumination regime was used to follow optical changes in the range 350–750 nm (fig.2). The spectra were assayed in two parts, 750–550 nm and 550–350 nm. Each 200 nm section was recorded as 1000 data points, each point being the average of 5 readings. Under conditions in which A_1 became reduced, as detected by EPR, no optical changes were seen. The absence of any absorbance changes in the visible region due to the $A_1 \rightarrow A_1^-$ transition is not obscured by changes due to P700 oxidation since the donor is rapidly re-reduced by dithionite. Moreover, the traces are not complicated by transient electrochromic band shifts since the spectrum is recorded in the dark after such changes have

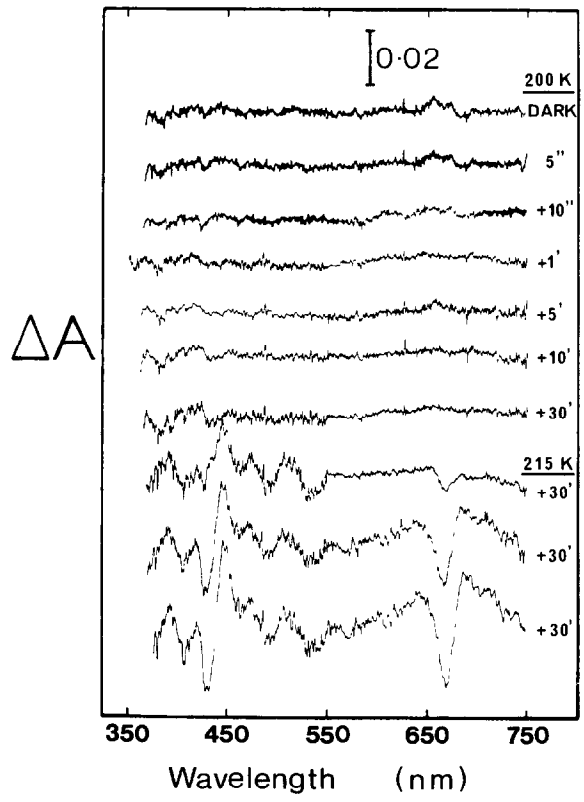

Fig.2. Optical difference spectra of A_1 and A_0 recorded after illumination (for stated time increments) of PS I particles at 200 and 215 K. The chlorophyll concentration was 150 μg/ml with 1 P700/45 chl, spectra were recorded at 200 K.

decayed. The lack of optical changes in the measured region upon reduction of A_1 strongly suggests that this component is not a chlorophyll or a pheophytin molecule.

Subsequent illumination at 215 K, which led to the appearance of the A_0^- EPR spectrum, also gave rise to distinct optical changes (fig.2). The difference spectrum consisted of bleachings at 670, 430 and 405 nm with absorbance increases at 450 and in a broad range around 690 nm. There were also some less distinct changes in the 480–550 nm region, the amplitudes of which did not bear any relationship to the duration of illumination. The absorbance changes in the blue and the red regions were linearly related to each other and to duration of illumination. Therefore, they were probably derived from a single component. The increase in amplitude of the 670 nm bleaching correlated well

with the increase in the $g = 2.00$ signal due to A_1 reduction (fig.3). Therefore, it is likely that the optical and EPR signals are derived from the same component.

The optical difference spectrum for A_0, presented here, closely resembles the chl a^-/chl difference spectrum generated electrolytically in dimethylformamide by Fujita et al. [17]. However, only a minor absorbance increase occurred at 640 nm. In this respect the spectrum is similar to those given in [6] and [10] which probably represent $A_0^- + A_1^-$. The spectrum has maxima and minima in similar positions to those in [6,10]. These characteristics lead us to suggest that the spectrum presented in fig.2 represents the reduction of a chl *a* monomer. Assuming an extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ the absorbance change at 670 nm in fig.2 represents 1 A_0:48 chl. This is equivalent to 0.94 A_0:1 P700. The component is therefore present in stoichiometric amounts in relation to the reaction centre concentration.

The changes seen in the 480–550 nm region resemble a difference spectrum of pheophytin a^-/pheophytin [17] but their rapid appearance upon 215 K illumination was not accompanied by large absorbance changes at 410 and 670 nm as would be expected for such a transition. Moreover, the signals did not increase upon further illumination and bore no relationship to the rate of formation of the $g = 2.00$ EPR signal due to A_0^-. It is therefore unlikely that they are part of a pheophytin spectrum or of a component of the acceptor complex.

We are able to confirm that 'A_1' has two distinct components. These are (i) A_1, which upon reduction has no absorbance changes in the visible region and is therefore not a chlorophyll or pheophytin molecule and (ii) A_0, which has optical and EPR spectra characteristic of a chl *a* anion monomer when reduced. It seems more probable that chl a^-, with in vitro reduction potential of about -0.9 V, will act as the reductant for the iron-sulphur centres (E_m of X > -700 mV) rather than pheophytin ($E_m = -0.64$ V). Upon excitation, P700* is energetically capable of reducing a chlorophyll acceptor [17]. The chemical nature of A_1 remains elusive. By analogy with bacterial reaction centres [18], from the shape and position of its EPR spectrum and by its lack of optical spectrum in the visible region it may be a quinone. To resolve the problem, experiments similar to those described above are being carried out to establish if an optical difference spectrum characteristic of a quinone can be found in the UV region.

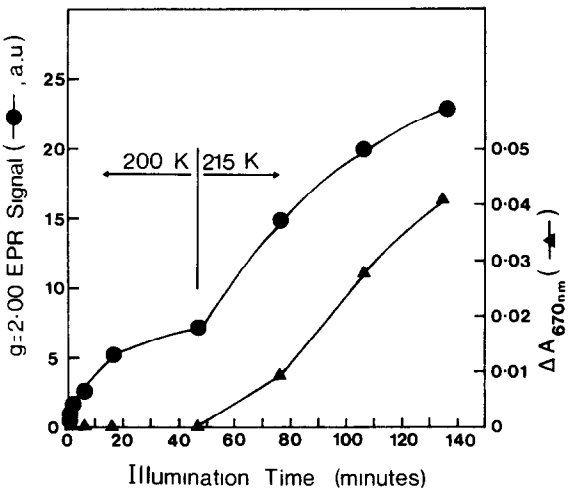

Fig.3. Comparison of the amplitude of EPR and optical spectral changes following low temperature illumination of PS I. The data are taken from figs 1 and 2.

ACKNOWLEDGEMENT

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