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Double-reduction of A_1 abolishes the EPR signal attributed to A_1^-: Evidence for C_2 symmetry in the Photosystem I reaction centre

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Illumination of Photosystem I preparations at room temperature at pH 10.0 in the presence of dithionite has been shown to double-reduce the bound phylloquinone electron acceptor A_1 (Setif, P. and Bottin, H. (1989) Biochemistry 28, 2689–2697). The asymmetric EPR signal ($g = 2.0048$, ΔH ptp = 0.95 mT) photo-accumulated by illumination of Photosystem I at 205 K is removed by such treatment, confirming the attribution of this EPR signal to A_1^-. Photoaccumulation of A_0^- and A_1^- EPR signals at 230 K in Photosystem I frozen at pH 10.0 approaches a maximum of 4 spins per $P700^+$, suggesting that Photosystem I exhibits the C_2 symmetry of other types of photosynthetic reaction centre.

Introduction

The reaction centre of Photosystem I (PS I) in oxygen-evolving organisms is believed to contain five electron acceptors [1,2]. The secondary electron acceptors F_X, F_B and F_A are iron-sulphur centres and reduce ferredoxin. The presence of two intermediary electron acceptors A_0 and A_1 were first suggested by multiple back reaction times for re-reduction of $P700^+$ following single turnover flashes at room temperature. These two intermediary acceptors were resolved as distinct chemical entities by detection of electron paramagnetic resonance (EPR) spectra at cryogenic temperatures [3,4]. Photosystem I particles prepared using the non-ionic detergent Triton X-100 were frozen under illumination [4] or illuminated at 230 K [3] in the presence of sodium dithionite to photoaccumulate the reduced acceptors A_1^- and A_0^- in addition to the reduced iron-sulphur centres. On the basis of their EPR spectra A_1^- ($g = 2.0051$, ΔH ptp = 1.05 mT [3] or $g = 2.0054$, ΔH ptp = 1.08 mT [4]) were attributed to a semi-quinone, and A_0^- ($g = 2.0017$, ΔH ptp = 1.15 mT [4] and $g = 2.0024$ [3]) to a chlorophyll monomer anion. A_1^- was photoaccumulated before A_0^- and so it was suggested that A_0 was the initial electron acceptor from P700. Studies of the P700 triplet indicated that it increased in amplitude when A_1 was reduced, and decreased back to the base level when A_0 was subsequently reduced [3]. By analogy with the reaction centres of purple photosynthetic bacteria, where the P870 triplet arises from recombination between the $P870^+$ BPh^- pair, these results seem to confirm that A_0 is the initial electron acceptor. The EPR signal of A_0^- plus A_1^- was compared with the EPR signal of $P700^+$ in the same samples [3,4]. These results indicated that there were approx. 2 spins in $A_0^- + A_1^-$ per P700, suggesting that A_0^- and A_1^- were present in stoichiometric quantities.

Although it is now generally accepted that A_0 is a chlorophyll monomer and acts as the first intermediary electron acceptor, there is still considerable controversy over the chemical identity and function of A_1 [1,2]. Two molecules of vitamin K-1 (phylloquinone) are present per P700 in Photosystem I reaction centres [5], and have been identified with A_1 by extraction and reconstitution experiments. $NADP^+$ photoreduction and forward electron transfer from A_0 at room temperature are correlated with the presence of at least one of the phylloquinones [6]. Extraction of both of the phylloquinones removed the EPR signal attributed to A_1^- [7,8]; subsequently, Mansfield and Evans [9] suggested that the EPR signal of A_1 had a narrower linewidth than that previously reported ($g =$ 2.0058,

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ΔH ptp = 0.95 mT). Photo-accumulation of the EPR signal of A_1^- was correlated with the appearance of an optical signal in the ultraviolet that would seem to be from a quinone/semi-quinone couple rather than a chlorophyll [10] and point-by-point spectra of an acceptor thought to be A_1 were consistent with a quinone [11].

However, experiments involving photo-destruction of phylloquinone by ultraviolet irradiation indicate that A_1 is not a quinone, and that phylloquinone does not have a role in electron transfer. Palace et al. [12] found that PS I reaction centres are still photoactive after UV inactivation of phylloquinone and Ziegler et al. [13] found that the EPR signal of A_1^- was still present after such treatment. The assignment of the EPR signal of A_1^- to phylloquinone was also challenged by experiments in which cyanobacteria were grown under conditions that should result in the deuteration of phylloquinone [14]. The linewidth of the EPR signal attributed to A_1^- was not narrowed as might be expected when proton hyperfine interactions are lost. This has led to the suggestion that the photoaccumulated EPR signal does not represent phylloquinone, but rather a reporter species that is influenced by the redox state of the true A_1.

Another monitor of A_1 is the electron spin polarised (ESP) signal thought to arise from the radical pair $P700^+$ A_1^- [15]. Rustandi et al. [16] have recently shown that extraction of phylloquinone abolishes the ESP signal, and that replacement with a deuterated phylloquinone causes narrowing of the ESP signal. Recently, Setif and Bottin [17] have shown that illumination of digitonin PS I particles at pH 10.0 in the presence of dithionite leads to loss of a back-reaction to $P700^+$ following single-turnover flashes with a $t_{1/2}$ of 750 ns. This phase is attributed to the back-reaction between $P700^+$ and A_1^-, whereas back-reaction between $P700^+$ and A_0^- has a $t_{1/2}$ of 25–30 ns. This change in kinetic behaviour was conserved in the dark, even though EPR spectra showed that F_x^- and to some extent F_B^- had re-oxidised during this dark adaption suggesting an E_m for recovery of the 750 ns back reaction of ~ 550 mV [17]. Since the dark samples showed no $g = 2.00$ EPR signal arising from A_1^-, the authors concluded that a process of double-reduction of the quinone was occurring that supported the identification of A_1 with the phylloquinone. Subsequent experiments showed that A_1 could be double-reduced in the dark at pH 10.0 by dithionite [18], although the process was rather slow unless mediators (i.e., methyl viologen) were added. Recently it was reported [19] that preparation of PS I particles with the quinone double-reduced reversibly abolished the ESP signal, confirming that the ESP signal arose from the $P700^+$ A_1^- radical pair and supporting the conclusions of Setif and Bottin [17,18].

These results have shown that:
(a) the component A_1 identified by a back-reaction to $P700^+$ following a single-turnover flash of $t_{1/2}$ 750 ns is probably a quinone, since it can be doubly reduced [17,18].
(b) that the ESP signal attributed to $P700^+$ A_1^- radical pair arises from a phylloquinone [16] that can be double-reduced [19].

However, they have not resolved the controversy over whether the photoaccumulated EPR signal attributed to A_1^- [3,4] arises from the phylloquinone or a reporter species. A further complication (see discussion [17]) is that the conditions for double-reduction of A_1 [17,18] are essentially those used in the initial experiments that first observed the EPR signal attributed to A_1^- [3,4]. In order to determine whether this EPR signal does arise from A_1^-, we have re-investigated the photoaccumulation of the EPR signals of A_1^- and A_0^- at 205 K and 230 K, taking care to control the extent to which A_1 might be double-reduced prior to photoaccumulation.

Materials and Methods

Chloroplasts were prepared from market spinach [20] and Photosystem I was prepared using either digitonin (high-purity digitonin, Calbiochem) [21] or Triton X-100 [22].

EPR samples were prepared in 3 mm internal diameter tubes. All samples were prepared at a chlorophyll concentration of 0.5 mg/ml. Samples with P700 and all of the acceptors oxidised were prepared by illuminating samples in 50 mM Tris-HCl (pH 8.0) for 30 s and freezing in liquid nitrogen under illumination. Reduced samples were prepared in either 50 mM Tris-HCl (pH 8.0) or 200 mM glycine-KOH (pH 10.0), the samples were made anaerobic under a stream of oxygen free nitrogen and sodium dithionite (0.2% w/v; final concentration, approx. 11.5 mM) added. They were then incubated for 30 min in the dark before freezing in liquid nitrogen. In order to prepare samples with A_1 double-reduced samples reduced with sodium dithionite at pH 10.0 were illuminated at 4° in an unsilvered dewar for appropriate periods, placed in the dark for 60 s and then frozen in liquid nitrogen. Samples were stored frozen in liquid nitrogen. Samples were illuminated at 205 K or 230 K in an unsilvered dewar containing a solid CO_2/ethanol mixture. EPR spectra of samples illuminated at this temperature were recorded immediately after illumination. All illumination procedures were done using quartz halogen lamps with an incident photon flux density of 1500 μmol/m^2 per s. The light was filtered through water, or for prolonged illumination to double reduce A_1 copper sulphate, filters to reduce infrared heating.

EPR spectra were recorded with a Jeol RE-1X spectrometer fitted with an Oxford Instruments Liquid Helium Cryostat. Spectra were stored on a Dell microcomputer using software written in this laboratory. The spin intensity of signals was determined using double-integration to obtain the area of the spectra.

All g-values and linewidths were calculated with reference to a powdered manganese oxide standard sample.

Results

Initially Photosystem I prepared using digitonin was used, since it is known that Triton X-100 perturbs the structure of the Photosystem I reaction centre [9], and the orientation of redox components in these preparations is similar to that in the native membrane [23]. The experiments reporting double-reduction of A_1 used similar preparations [17,18]. In order to observe the EPR signal of A_1^-, samples are usually frozen in the presence of dithionite, which then acts as an electron donor in photoaccumulation experiments. Such samples are also adjusted to pH 10.0 [4,10], in order to lower the redox potential of the samples and facilitate the dark reduction of centres F_A and F_B. However, it has now been reported that A_1 is double-reduced to the quinol under such conditions [18], although these authors also added redox mediators. In order to avoid double-reduction of A_1, samples were incubated in the dark with dithionite for 30 min at pH 8.0 in the absence of redox mediators – conditions which do not lead to double-reduction of this quinone [18] – and compared with identical samples prepared at pH 10.0. Fig. 1 shows the EPR signals at $g = 2.00$ generated in these samples by a brief period of illumination at 205 K, in order to preferentially reduce A_1 [10,24]. In the pH 10.0 sample, the signal was that previously attributed to A_1^- [9] with $g = 2.0048$ and ΔH ptp = 0.95

Fig. 1. The EPR spectra of A_1^- obtained by 205 K illumination of digitonin Photosystem I particles frozen in the dark following incubation with sodium dithionite at pH 8.0 or pH 10.0. Samples prepared as described in Materials and Methods were illuminated for 2 min at 205 K. Spectra are the average of two recordings. EPR conditions: Microwave power, 5 μW. Modulation width, 0.2 mT. Recording temperature, 75 K.

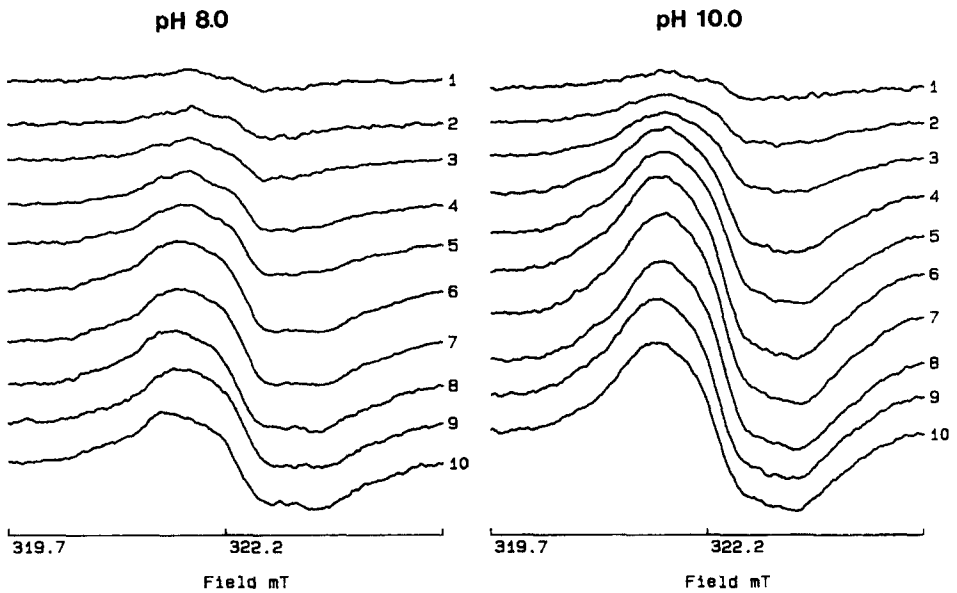

Fig. 2. The EPR spectra of A_1^- and A_0^- produced by progressively longer periods of illumination at 230 K of digitonin Photosystem I particles frozen in the dark following incubation with sodium dithionite at pH 8.0 or pH 10.0. Samples prepared as described in Materials and Methods were illuminated at 230 K for (1) 0.5 min, (2) 1 min, (3) 2 min, (4) 5 min, (5) 10 min, (6) 30 min, (7) 60 min, (8) 90 min, (9) 120 min, (10) 180 min. At intervals during the illumination the samples were removed to the EPR spectrometer and the spectra recorded. Spectra are the average of two recordings. EPR conditions: Microwave power, 5 μW. Modulation width, 0.1 mT. Recording temperature, 75 K.

TABLE I

Spin intensities of the g = 2.00 signals photo-accumulated in digitonin PS I particles at pH 8.0 and pH 10.0 following illumination at 230 K

The spin intensities were estimated by double integration of the spectra presented in Fig. 2.

Cumulative period of illumination at 230 K (min)	Spin intensity (arbitrary units)	
	pH 8.0	pH 10.0
0.5	209	420
1	325	838
2	515	1427
5	899	2232
10	1180	2769
30	1681	3338
60	1773	3489
90	1906	3432
120	1867	3312
180	1844	3064

mT. Although the signal in the pH 8.0 sample had approximately the same linewidth and g-value, it was more symmetrical, lacking the shoulder on the high-field side of the peak. The pH 10.0 signal size was approx. 80% larger than that at pH 8.0 after the same illumination period

Similar samples were illuminated for progressively longer periods at 230 K in order to reduce both A_1 and A_0, and after 90 min of illumination the EPR signals at $g = 2.00$ in both pH 8.0 and pH 10.0 samples have reached a maximum (Fig. 2). It was immediately apparent that the EPR signals at $g = 2.00$ in the pH 10.0 sample were considerably larger. Having confirmed that the microwave power used (5 μW) was non-saturating at 75 K, the double-integrated intensity of the two sets of spectra during the time course of 230 K illumination was determined (Table I). It can be seen that the pH 10.0 $g = 2.00$ signals at maximum (60 min illumination) contained approximately twice (1.83) as many spin equivalents as the maximum (90 min illumination) $g = 2.00$ signals in the pH 8.0 sample.

However, this did not seem to be due to the appearance of different EPR signals at pH 10.0 in addition to those seen at pH 8.0, since both samples contained only the EPR signals attributed to A_1^- and A_0^-.

In order to investigate this phenomenon, a number of samples from different digitonin Photosystem I preparations were compared, and the maximum EPR signals at $g = 2.00$ photo-accumulated at 230 K in the presence of dithionite at pH 8.0 and 10.0 compared with the signal arising from $P700^+$ in samples frozen under illumination in the absence of a reductant (Table II). The samples frozen under illumination in the absence of a reductant contained $P700^+$ in 100% of the Photosystem I reaction centres, since illumination at 15 K did not generate any stable charge separation

TABLE II

The number of spins relative to $P700^+$ in the maximum g = 2.00 signals accumulated following illumination at 230 K in different digitonin PS I preparations at pH 8.0 and pH 10.0

Samples prepared as described in Materials and Methods were illuminated at 230 K until the maximum $g = 2.00$ signal had been attained. The spin intensity was estimated by double integration of the spectra and divided by the spin intensity in $P700^+$ in matching samples.

	Spin intensity relative to $P700^+$	
	pH 8.0	pH 10.0
Preparation 1	2.21	3.90
Preparation 2	1.98	3.73
Preparation 3	2.08	4.09

either as an increase in the size of $P700^+$ or the appearance of a signal due to centre F_A^-. The EPR signal of $P700^+$ with $g = 2.0025$ and ΔH ptp = 7.5–7.75 g indicated that no spurious radical signal was being generated by this treatment. In all preparations at pH 10.0 the signals photo-accumulated at 230 K contained considerably more than 2 spin equivalents per $P700^+$ and approximated 4 (Table III). In contrast, at pH 8.0 the maximum $g = 2.00$ signals photo-accumulated at 230 K contained approx. 2 spin equivalents per $P700^+$. This result strongly suggests that, whereas at pH 8.0 only one A_0^- and A_1^- are reduced by photo-accumulation, at pH 10.0 two A_0^- and two A_1^- can be reduced. At pH 10.0 (Table I) two of the spins were photo-accumulated in the first 2–5 min (one A_0^- and one A_1^-), whilst the next two spins photo-accumulated more slowly over the next 55 min.

This result contrasts with the observations in Refs. 3,4, which report that A_0^- and A_1^- contained approx. two spin equivalents per P_{700}^+. In one case [3], samples were illuminated at room temperature at pH 9.0 in the

TABLE III

The number of spins relative to $P700^+$ in the g = 2.00 signals accumulated in Triton X-100 PS I preparations at pH 8.0 and pH 10.0 following 230 K illumination

The spin intensity of the photo-accumulated spectra were estimated by double integration of the spectra presented in Fig. 3, and divided by the spin intensity estimated by double integration of the spectrum of $P700^+$ in a matching sample.

Cumulative period of illumination at 230 K (min)	Spin intensity relative to $P700^+$	
	pH 8.0	pH 10.0
0.5	0.633	0.959
1	0.935	1.256
2	1.279	1.840
5	1.943	2.902
10	2.004	3.300
30	1.602	3.681
60	1.208	3.239

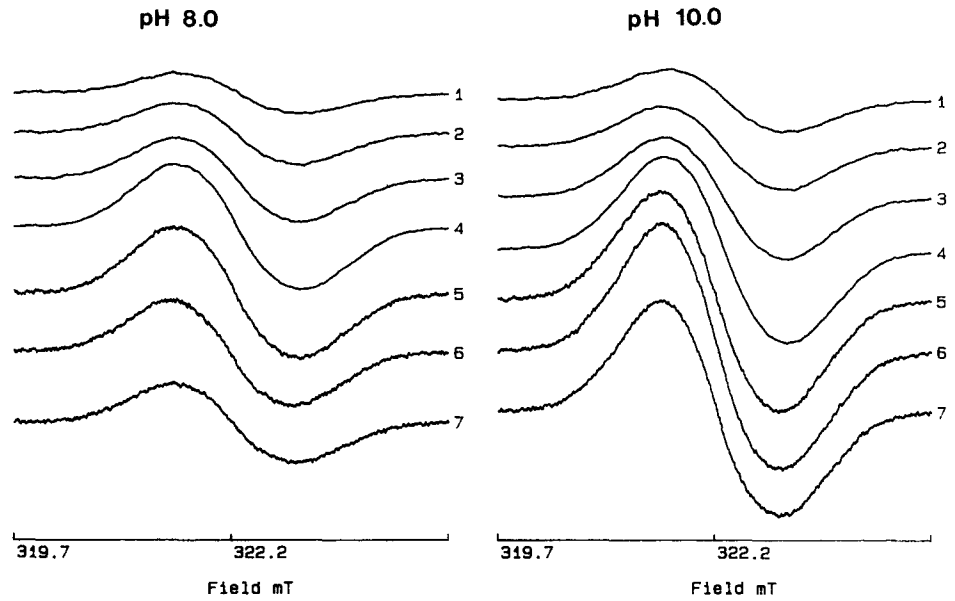

Fig. 3. The EPR spectra of A_1^- and A_0^- produced by progressively longer periods of illumination at 230 K of Triton X-100 Photosystem I particles frozen in the dark following incubation with sodium dithionite at pH 8.0 or pH 10.0. The experimental procedures were as for Fig. 2. Samples were illuminated at 230 K for (1) 0.5 min, (2) 1 min, (3) 2 min, (4) 5 min, (5) 10 min, (6) 30 min, (7) 60 min.

presence of dithionite, dark-adapted to allow reoxidation of $A_0^- + A_1^-$, frozen and $A_0^- + A_1^-$ photo-accumulated at 230 K. The other group [4] photo-accumulated $A_0^- + A_1^-$ by room temperature illumination at pH 10.0 in the presence of dithionite. Both these groups were using Photosystem I prepared with Triton X-100. We have now reinvestigated the photo-accumulation of $g = 2.00$ signals at 230 K at pH 8.0 and pH 10.0 using a similar preparation.

We did not photo-accumulate the $g = 2.00$ signals at room temperature in the presence of dithionite [3,4], since it is known that this treatment could lead to the double-reduction of A_1^- [17,18]. The results (Fig. 3) show that in Triton X-100 Photosystem I the maximum

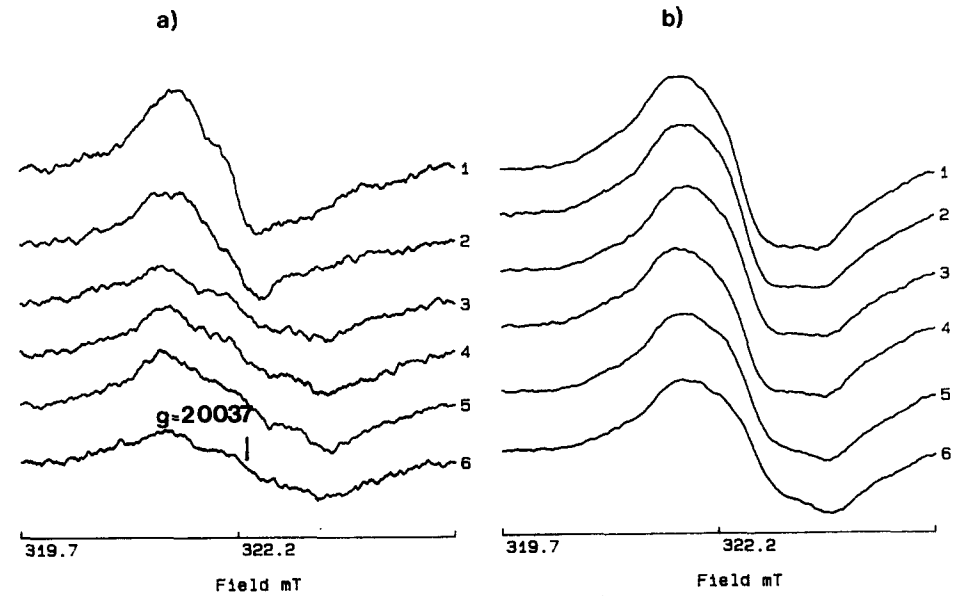

Fig. 4. The effect on the EPR spectra of A_1^- and of A_1^- plus A_0^- in digitonin Photosystem I particles of progressively longer periods of illumination at 4° at pH 10.0 in the presence of sodium dithionite. Samples prepared as described in Materials and Methods were illuminated at 4° for (1) Dark. (2) 2 min, (3) 15 min, (4) 30 min, (5) 60 min, (6) 90 min. After freezing the samples were subsequently illuminated for 2 min at 205 K (Spectra a) and then at 230 K until no further increase in signal size was observed (Spectra b). EPR conditions (a) Microwave power, 5 μW. Modulation width, 0.2 mT. Recording temperature, 75 K. (b) as (a) except modulation width 0.1 mT.

$g = 2.00$ signals photo-accumulated at 230 K at pH 10.0 in the presence of dithionite are much larger than those seen in pH 8.0 samples. Comparison of the double-integration of these $g = 2.00$ signals with that of $P700^+$ suggests that at pH 10.0 approx. 4 $A_0^- + A_1^-$ can be reduced per $P700^+$ (Table III), whereas at pH 8.0 only 2.2 $A_0^- + A_1^-$ can be reduced per $P700^+$. It is noticeable (Fig. 3), as previously reported [9], that the signal arising from A_0^- in Triton X-100 Photosystem I is not as broad as that seen in digitonin PS I preparations, and therefore not as easily distinguished from the EPR signal of A_1^-.

We then proceeded to establish whether the EPR signal attributed to A_1^- did in fact arise from the quinone, by monitoring this EPR signal as A_1 was double-reduced. At first we attempted to double-reduce A_1 by illumination of digitonin Photosystem I at pH 8.0 in the presence of dithionite at 4°, but found that incomplete double-reduction of A_1 was achieved. This is unfortunate, since the results above suggest that at pH 8.0 only one A_0^- and A_1^- can be photo-accumulated, simplifying the situation. Previous reports [18] indicated that the presence of a mediator such as methyl viologen may assist in double-reduction of A_1 at pH 8.0, but we wished to avoid the use of such mediators, since they contribute EPR signals at $g = 2.00$. Instead, digitonin PS I was illuminated at pH 10.0 in the presence of dithionite at 4° for progressively longer periods, in order to double-reduce A_1 [17]. The illumination was turned off for a brief period (see Materials and Methods) and the samples were frozen in the dark in liquid nitrogen. The redox state of the samples after this treatment was P700, A_0 A_1 X^- B^- A^-, and the iron-sulphur centre EPR signals remained at the same intensity, even at the longest periods of illumination, suggesting that these treatments were not damaging the Photosystem I. The samples had no $g = 2.00$ signals at this stage. The samples were then illuminated for 2 min at 205 K in order to see if the EPR signal attributed to A_1^- could be observed (Fig. 4a). After short periods of illumination at 4° subsequent illumination at 205 K produced the signal attributed to A_1^- [9]. However, after longer periods of illumination at 4° 205 K illumination produced a much broader EPR signal. After 90 min of illumination at 4°C, the EPR signal photo-accumulated at 205 K (Fig. 4a) had a g-value of 2.0037 and a ΔH ptp of 1.75 mT. This is similar to the EPR signal previously attributed to A_0^- in digitonin PS I [9], and its appearance indicates that A_1^- has been double-reduced and that the EPR signal attributed to A_1^- is not seen. Previous reports have suggested that prolonged periods of ultraviolet illumination can photo-inactivate phylloquinone [12,13], although it was suggested that such treatment did not remove the EPR signal of A_1^- [13]. In order to confirm that the disappearance of the EPR signal of A_1^- is due to double-reduction and not photo-inactivation, samples with A_1 double reduced were dialysed against pH 8.0 buffer in the dark at 4°C in order to re-oxidise the quinol to the quinone.

TABLE IV

Spin intensity of the maximum $g = 2.00$ signals accumulated following 230 K illumination of digitonin PS I particles at pH 10.0 previously illuminated at 4°C in order to double reduce A_1

The spin intensity of the maximum $g = 2.00$ signal induced by 230 K illumination was estimated by double integration of the spectra shown in Fig. 4b and divided by the spin intensity of $P700^+$ in matching samples.

Period of illumination at 4°C (min)	Spin intensity relative to $P700^+$
0	3.900
2	3.642
15	3.412
30	3.352
60	3.315
90	3.057

Such samples were then frozen in the dark at pH 8.0 in the presence of dithionite, and illuminated for 2 min at 205 K to see whether the EPR signal of A_1^- appeared. Dialysis for 24 hours usually restored the EPR signal attributed to A_1^-, although in some digitonin PS I preparations more prolonged periods of dialysis were required.

A_1 in Triton X-100 Photosystem I could also be double-reduced by illumination at pH 10.0 in the presence of dithionite, abolishing the EPR signal attributed to A_1^-, although 60 min illumination was required to achieve complete double-reduction. As with digitonin PS I, the EPR signal of A_1^- could be restored by dialysis for 24 h at 4°C.

Since illumination at 230 K of pH 10.0 digitonin PS I has suggested that two A_1^- could be photo-accumulated, samples from the time-course of A_1 double-reduction were illuminated for 90 min at 230 K in order to generate the maximum $g = 2.00$ signals (Fig. 4b). These signals were double-integrated and their spin intensity compared to that of $P700^+$ in the same preparation (Table IV). As the EPR signal of A_1^- photo-accumulated by brief illumination at 205 K is replaced by that of A_0^-, the spin intensity of the maximum $g = 2.00$ signals declined from 3.9 spins per $P700^+$ to 3.0 spins per $P700^+$. The spectra show that the component that is decreasing in intensity is in the A_1 region of the spectrum, although the signal is not completely abolished (Fig. 4b). This result indicates that, whereas one of the A_1^- species can be fully double-reduced by prolonged illumination at 4°C at pH 10.0 in the presence of dithionite, the other A_1 is more difficult to double-reduce. The result also indicates that the A_1^- EPR signal seen after brief illumination at

205 K is arising from the quinone that is more easily double-reduced, and that the other quinone is not reduced by brief periods of illumination at 205 K.

Discussion

The results demonstrate that the conditions that lead to double-reduction of the optically detected A_1, illumination at room temperature at pH 10.0 in the presence of dithionite [17], lead to the disappearance of the photo-accumulated EPR signal attributed to A_1^-. This result indicates that all four assignments of the term A_1 are to the same redox component, the bound phylloquinone. These four assignments of the term A_1 are:

(a) the asymmetric EPR signal at $g = 2.00$ in photo-accumulation experiments [3,4];

(b) the 750 ns flash-induced transient at room temperature [17,18];

(c) the ESP signal attributed to the $P700^+$ A_1^- radical pair [15,16,19];

(d) the two chemically extractable molecules of phylloquinone [5].

The suggestion [17] that the photo-accumulated EPR signal attributed to A_1^- [3,4] was in fact arising from another component, because A_1 could have become doubly-reduced before photo-accumulation is shown not to be the case. However, it is true that the preparation of samples in the presence of dithionite at pH 10.0 is altering the EPR signal attributed to A_1^-. The signal of A_1^- at pH 10.0 (Fig. 1) contains an additional contribution on the high-field side of the peak in comparison with the spectrum of A_1^- at pH 8.0. We believe that this is due to a contribution from A_0^- (cf Fig. 4a) and indicates that some A_1 may be double reduced in the pH 10.0 samples. Mansfield and Evans [9] also concluded that both A_0 and A_1 were being reduced by illumination of pH 10.0 samples at 205 K, as the spectra of A_1^- contained a shoulder on the high field side at $g = 1.9985$. The spin intensity of the maximum $g = 2.00$ signal accumulated at 230 K in pH 10.0 samples is often less than 4 spins per P700, also suggesting that a small amount of A_1 has been double-reduced (Tables I–IV).

The results of Barry et al. [14] are frequently cited as indicating that the photo-accumulated EPR signal attributed to A_1^- is of a reporter species rather than A_1. However, it is important to note that they prepared samples by illumination at pH 10.0 in the presence of dithionite at room temperature prior to freezing and photo-accumulation [13] suggesting that some A_1 would have been doubly-reduced and a mixture of A_1^- and A_0^- observed. Some narrowing of the EPR signal upon 65% deuteration of the quinone and an effect on lineshape might still be expected. However, Mansfield et al. [25] showed that the line width is sensitive to the media protons. A combination of proton broadening and double reduction may have masked the expected effects of deuterating the methyl protons of the quinone.

In the course of these investigations it has also been shown that the routine preparation of Photosystem I samples at pH 10.0 is having an additional effect. It seems that Photosystem I prepared at pH 10.0 is able to photo-accumulate at 230 K two A_0^- and two A_1^- per $P700^+$, rather than the one A_0^- and one A_1^- previously reported [3,4]. This result is the first direct evidence that Photosystem I reaction centres exhibit the same C_2 symmetry as other photosynthetic reaction centres (see [26] for a discussion of this point). Although it is known that Photosystem I contains two phylloquinones [5], it had not been established that it contained two chlorin primary electron acceptors (A_0). It has been argued that it is not the primary acceptor A_0^- which is being photo-accumulated by illumination at 230 K. This photo-accumulated radical absorbs at 670 nm [24], whereas transient experiments [27] measure an absorption maximum for A_0^- at around 690 nm. However, it has proved difficult to repeat the observation of a picosecond transient around 690 nm attributable to A_0^- ([28] and unpublished observations in this laboratory), and we believe that the EPR signal at $g = 2.0037$ photo-accumulated by 230 K illumination is A_0^-. It should be stressed that in the experiments reported here once the maximum of 2 (pH 8.0) or 4 spins (pH 10.0) per $P700^+$ had been photo-accumulated, doubling of the period of illumination did not increase the amplitude of the photo-accumulated $g = 2.00$ EPR signals (Fig. 2, Table I). In fact the further periods of illumination slightly reduced the amplitude of these signals (Tables I and III), suggesting that A_1 can be double-reduced by illumination at 230 K, albeit rather slowly.

The photosynthetic reaction centres of purple photosynthetic bacteria exhibit a preference of electron transfer for one of two symmetrical electron transfer pathways (A branch), in that only the L polypeptide bacteriopheophytin (H_A) is reduced following an excitation flash. However, it is possible to phototrap the BPh_M (H_B) [29] at 219 K, although it is photo-trapped 274-times more slowly than BPh_L (H_A). These results show the same phenomenon in the Photosystem I reaction centre, in that the A_1 (phylloquinone) and A_0 (chlorophyll *a*) on one branch of electron transfer can be photo-trapped at 230 K, but more slowly than the corresponding electron transfer components in the other electron transfer pathway. This indicates that the second phylloquinone in the Photosystem I reaction centre accepts electrons from the A_0, which is photo-trapped more slowly and is presumably a dead-end, rather than the two phylliquinones functioning in series, as Q_A and Q_B do in purple bacteria.

This difference in efficiency of electron transfer in the two branches is also shown at room temperature, in that it is much more difficult to double-reduce the second A_1 by illumination in the presence of dithionite at pH 10.0.

However, for reasons that are not clear, such photo-trapping of both electron transfer pathways requires preparation of Photosystem I at an alkaline pH, a requirement not seen in the reaction centres of purple photosynthetic bacteria [29].

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