EPR PROPERTIES OF AN INTERMEDIARY ELECTRON ACCEPTOR (PHEOPHYTIN) IN PHOTOSYSTEM-II REACTION CENTERS AT CRYOGENIC TEMPERATURES

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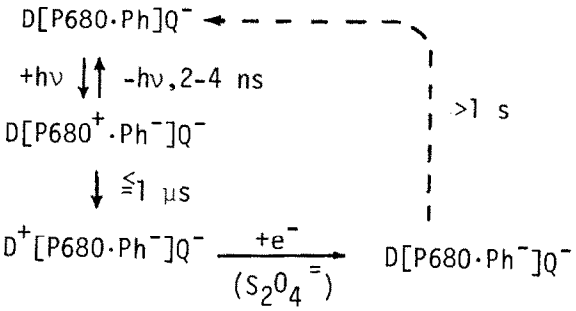
1. Introduction

The primary photochemical act in photosystem II (PS II) reaction centers consists of an electron transfer from the primary donor, P680 (probably a chlorophyll (chl) dimer), to the primary acceptor, Q (a special form of plastoquinone) (reviewed [1,2]). When Q is chemically reduced beforehand, reversible photoreduction of pheophytin (Ph) is observed in PS II subchloroplast preparations [3–8] as well as in chloroplasts [7–8]. This photoreaction, which can occur at temperatures as low as 100 K [3], is inactivated by heat treatment or Tris washing but can be reactivated by the addition of Mn^{2+} [8]. It has been proposed [3–8] that pheophytin acts as an intermediary electron carrier in PS II, analogous to the role played by bacteriopheophytin (BPh) in photosynthetic bacteria [9–11] and by chlorophyll in PS I [12].

It has been suggested [3–5] that the 'variable' fluorescence of PS II, which appears upon reduction of Q [13] but disappears upon photoreduction of Ph [3–8], may indeed be recombination luminescence arising from $[P680^+ \cdot Ph^-]$. Investigation of this luminescence has shown that the return of the electron from Ph^- to the excited level of P680 has an activation energy of 0.06–0.08 eV, and the lifetime of $[P680^+ \cdot Ph^-]$ is 2–4 ns at 295 K [5]. The redox potential of the Ph/Ph^- couple in PS II has been measured to be –610 mV [7].

The quantum yield for photoaccumulation of the long-lived state $[P680^+ \cdot Ph^-]Q^-$ after prior reduction of Q ($\leq$ –200 mV vs SHE) has been estimated to be 0.002–0.005 [3,4,8] at 295 K (cf. scheme 1). This value is consistent with the ratio of the recombination time for $[P680^+ \cdot Ph^-]$ and the time for reduction of $P680^+$ by a secondary electron donor, D ($\leq$1 μs [14–16]):



Trapping of Ph^- in PS II at 295 K is accompanied by the appearance of an EPR signal with $g = 2.0035$ and $\Delta H = 12.5$ G [6], similar to that of the anion radical of pheophytin in vitro [17]. The analogous EPR signal of BPh^- in bacterial reaction centers is characterized by an additional split signal below 15 K, attributed to interaction between BPh^- and the singly-reduced electron acceptor, a menaquinone–iron complex ($MQ^- \cdot Fe^{2+}$) [11,18–20]. We report here the observation of a similar, low-temperature EPR doublet in addition to the singlet after phototrapping Ph^- in PS II reaction centers.

2. Experimental

Subchloroplast fragments (TSF-IIa) highly enriched in PS II reaction-center components (one in 30–40 chl molecules [21–24]) and free of P700 [22,23]

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were fractionated from spinach chloroplasts following Triton treatment [21,22]. The fragments were suspended in 0.1 M tricine–NaOH (pH 8.0) which contained additional 50% glycerol for low-temperature samples.

Changes in absorbance and fluorescence yield, induced by continuous actinic light from a 1000 W incandescent lamp filtered by 5 cm $CuSO_4$ solution, were measured in a phosphoroscopic photometer similar to that in [25], using a measuring beam modulated at 25 kHz and a Dynatrac-3 lock-in amplifier (Ithaco). EPR measurements were performed with a Varian V-4500 spectrometer at 9.15 GHz with 100 kHz field modulation. Sample temperature was maintained by an LTD-3-110 liquid-helium transfer line-cryostat (Air Products). Recording and manipulation of the EPR signals were made in a Tracor-Northern model 1710 signal averager.

3. Results

Illumination of TSF-IIa at ~300 mV induced a 1.5–2-fold increase in chlorophyll fluorescence yield, a result of Q becoming photoreduced [13]. At ~–450 mV, actinic light caused a reversible decrease in fluorescence yield as a result of photoreduction of Ph in PS II. The accumulation of Ph^- under these same conditions is shown by the light-minus-dark difference absorption spectrum in fig.1, which is similar to that in [3–8]. A similar spectrum was also observed upon photoreduction of Ph at 220 K (not shown). Using the value of 32 $mM^{-1} \cdot cm^{-1}$ for the difference extinction coefficient at 685 nm (taken from the long-wavelength band of Ph in vitro [17]),

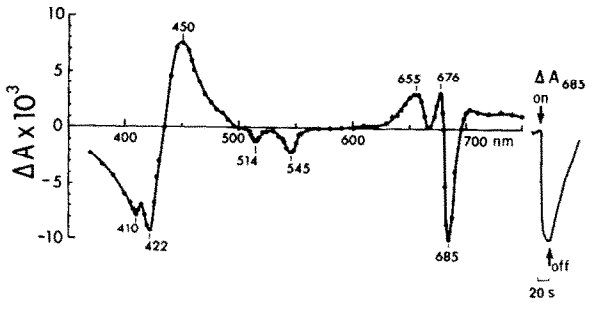

Fig.1. Light-minus-dark difference spectrum of TSF-IIa fragments at ~–450 mV and 295 K (10 μg/ml chl; 1 cm pathlength). The $\Delta A685$ transient (right) is shown on the same absorbance scale.

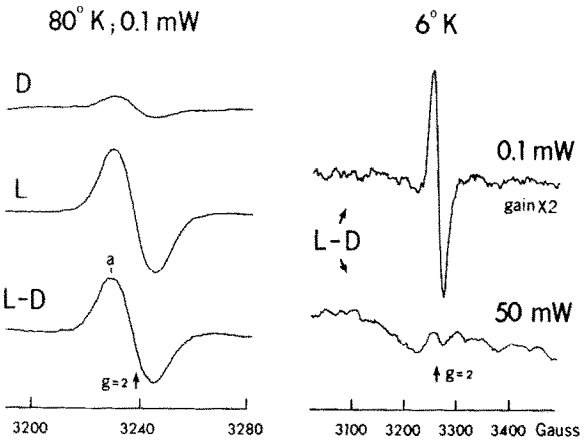

Fig.2. EPR spectra of TSF-IIa fragments before (D) and after (L) 30 s illumination at 295 K (at ~–450 mV) followed immediately by freezing in liquid nitrogen. Two identical EPR samples were prepared in 3 mm i.d. tubes containing dithionite, one of which was illuminated before freezing. Microwave power and temperature are indicated in the figure. Amplitude modulation, 7 G; 300 μg chl/ml.

our TSF-IIa sample has 1 photoactive Ph/30–40 chl molecules. This is in good agreement with the concentration of P680 estimated [24] and other reaction-center components [22,23] and consistent with the idea that Ph is an early electron acceptor in PS II.

Photoreduction of Ph in TSF-IIa at 295 K (as monitored by light-induced decrease of fluorescence yield in the EPR tubes) produced an EPR signal with $g = 2.0033 \pm 0.0003$ and $\Delta H = 12.6 \pm 0.3$ G at 80 K (fig.2) which is characteristic of Ph^- [6]. At 6 K, this signal was also seen at low microwave power (0.1 mW), but almost unobservable at 50 mW due to power saturation (fig.2).

Fig.3 shows EPR signals obtained after trapping Ph^- in the TSF-IIa at 220 K (trapping being monitored at point 'a' on the spectrum in fig.2). At 6 K, in addition to the narrow singlet of Ph^- with $g = 2.003$ and $\Delta H = 13$ G (cf. fig.2), a doublet centered at $g = 2.00$ with a splitting of 52–55 G appeared. This EPR doublet, in contrast to the singlet, was highly temperature sensitive (not detectable above 15 K) and not easily power saturated. Thus, at 6 K and 50 mW only the EPR doublet was observed, while at 25 K only the singlet was seen either at 0.1 or 50 mW (fig.3).

Preliminary investigation showed that the EPR doublet was also detected in lyophilized TSF-IIa

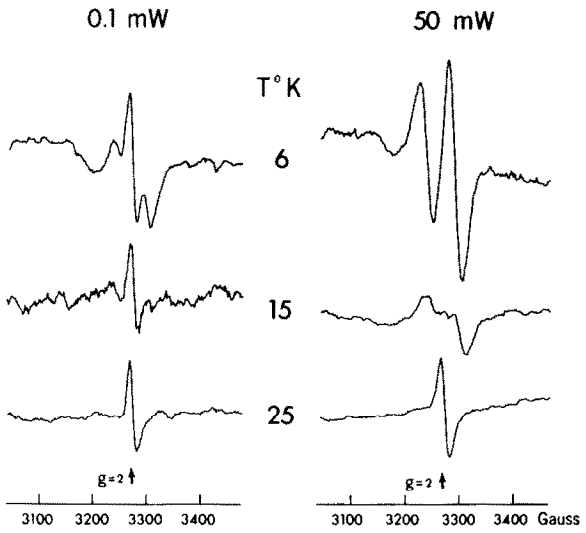

Fig.3. EPR spectra of TSF-IIa fragments after 90 s illumination at 220 K (at ~−450 mV) followed immediately by freezing at 77 K. The EPR spectra shown here are the difference between signals of separate illuminated and non-illuminated samples prepared from the same material. Microwave power and temperature are as shown. Amplitude modulation, 7 G. The spectrum at 6 K and 0.1 mW was recorded with a 2-fold gain; 300 μg chl/ml.

resuspended in buffer. Extraction of Q from the lyophilyzed TSF-IIa at room temperature with hexane containing 0.15% methanol [26] eliminated the light-induced increase in fluorescence yield, indicating that Q was indeed removed by extraction. However, although photoreduction of Ph (at ~−450 mV) at 220 K was observed, only the EPR singlet of Ph^- could be detected at 6 K.

4. Discussion

Reaction centers of PS II and photosynthetic bacteria have many similarities. In both photosystems a quinone is the primary electron acceptor [1,2,11, 18–20,26–30]. Photoreduction of the quinone is accompanied by a shift of the absorption bands of BPh in bacteria [30] and of Ph in PS II [3,4,29] toward shorter wavelength. When the quinones are reduced beforehand, illumination traps BPh^- in bacteria [10,11,18–20] as well as Ph^- in PS II [3–8], thus supporting the contention that these pheophytins act as intermediary electron carriers. Reduction of the quinones is accompanied by the appearance of ns recombination luminescence, which is abolished upon trapping of BPh^- [10,31,32] or Ph^- [3–8]. We now note that the EPR doublet detected after trapping Ph^- in PS II and that observed after trapping BPh^- in photosynthetic bacteria [11,18–20] also have similar characteristics: trapping conditions, *g*-values, magnitude of the splitting, dependence of the signal amplitude on temperature and microwave power. These similarities indicate that the splitting of the EPR signal in PS II probably has the same origin, namely, an interaction between Ph^- and the singly reduced primary acceptor, Q^-, which probably also includes iron[+] or some other transition-metal ion. This suggestion is further supported by the lack of an EPR doublet after extraction of Q from TSF-IIa by hexane.

Results from the extraction experiments also suggest that the split signal is not related to an oxidized secondary electron donor in PS II. Although photoaccumulation of Ph^- occurs in extracted TSF-IIa (indicating donation of electron from D to $P680^+$), no EPR doublet was observed.

In *Rhodopseudomonas viridis*, which contains MQ in the reaction center, a greater proportion of doublet in the EPR signal at 8 K was seen after trapping BPh^- at 200 than at 300 K [19]. In *Rps. spheroides* reaction centers, only the EPR singlet was observed after trapping BPh^- at 300 K [20]. Its absence was explained by very fast electron transfer (10 ms at 300 K) from BPh^- to reduced ubiquinone, UQ^-, leading to the photoaccumulation of the state $BPh^-{\cdot}UQ^{2-}{\cdot}Fe^{2+}$, which is characterized by a narrow singlet EPR signal of BPh^- [20]. When UQ in the bacterial reaction center was replaced by MQ, the EPR doublet could be seen after trapping BPh^- at 300 K, since the reduction of MQ^- by BPh^- required ~4 s [20]. The structure of plastoquinone is much more like that of UQ than MQ (cf. [28]) and thus the absence of an EPR doublet in TSF-IIa after trapping Ph^- at 295 K might also be attributed to a rapid reduction of Q^- by Ph^-, leading to photoaccumulation of the state $Ph^-{\cdot}Q^{2-}$. At 220 K, trapping of the state $Ph^-{\cdot}Q^-$ is apparently more favorable due to slower electron transfer from Ph^- to Q^-*. Further investigations of the reaction kinetics are planned to clarify this point.

[+] The presence of nonheme iron in PS II preparations not in the form of iron–sulfur proteins has been reported [26].

* The activation energy for electron transfer from BPh^- to UQ^- has been estimated to be 0.42 eV [20]

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