MEASUREMENT OF THE MIDPOINT POTENTIAL OF THE PHEOPHYTIN ACCEPTOR OF PHOTOSYSTEM II

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1. Introduction

Primary photochemistry in photosystem II (PSII) involves at least three components: (1) P680, the primary electron donor [1], which has been reported to be a chlorophyll dimer [2,3] or trimer [3] although recent evidence favors its identification as a monomeric chlorophyll species [4,5]; (2) Q, a special plastoquinone [6], which functions as a relatively stable acceptor (200–600 μs at room temperature when secondary electron transport is not blocked [7]); (3) Ph, a pheophytin molecule which functions as an intermediate electron carrier between P680 and Q.

The involvement of pheophytin as an electron acceptor in PSII was initially demonstrated in [8–10]. Reversible photo-reduction of pheophytin in PSII was observed when Q was chemically reduced. At the same time an unsplit EPR radical signal attributed to Ph^- was reported [10]. Later it was shown that a split EPR signal could be photo-induced under some circumstances and this was attributed to the reduced Ph which was involved in an interaction with the semiquinone–iron form of Q [11] by analogy to the situation in photosynthetic bacteria [12]. Similar EPR [5] and optical spectra at 200 K [13] were observed in a different PSII reaction center preparation. These spectra were also attributed to a pheophytin anion. Recent ENDOR measurements are consistent with the identification of the reduced intermediate acceptor in PSII as a pheophytin anion [14]. Also a picosecond time-resolved optical spectrum of Ph^- has been reported in PSII particles in PSII particles in which Q is chemically reduced [15].

Strong evidence for the presence of an intermediate acceptor between P680 and Q has also come from the discovery of a reaction center triplet state in PSII particles [5]. This triplet state was detected by low-temperature EPR in PSII particles in which Q was reduced. The spin polarization of this triplet state results from its formation by the recombination of a radical pair (for a discussion of this kind of spin polarization pattern see [16]). This indicates that, even when Q is reduced, light driven radical pair formation can occur [5]:

$$\text{P680 Ph Q}^- \xrightarrow{h\nu} \underbrace{\text{P680}^+ \text{Ph}^- \text{Q}^-}_{\text{radical pair}} \rightarrow \underbrace{\text{P680}^t \text{Ph Q}^-}_{\text{triplet}} \qquad (1)$$

We have shown that reaction center triplet formation does not occur when the pheophytin acceptor is trapped in its reduced form (by illumination while freezing or at 200 K) [5]. Here, we have used the EPR signal of the reaction center triplet as a probe of the redox state of the Ph/Ph^- couple and the E_m of this couple has been determined in a potentiometric titration.

2. Materials and methods

PSII particles were prepared from pea chloroplasts as in [17]. Some biophysical characteristics of these particles are described in [13]. PSII particles (120 chl/P680) were used at 1 mg chl/ml in all experiments. Redox potentiometry was carried out essentially as in [18] at pH 11.0 (100 mM glycine/KOH buffer, 100 mM KCl). The following mediators were used at 100 μM; benzyl viologen, neutral red, methyl viologen, triquat (1,1-trimethylene-2,2-dipyridilium dibromide) and tetraquat (1,1-trimethylene-5,5′-dimethyl-2,2-dipyridilium dibromide). The pH was

monitored and adjusted as necessary throughout the titration. Samples were taken anaerobically and were frozen in the dark in 3 mm i.d. quartz EPR tubes, which had been pre-calibrated by measuring the extent of the EPR signal in a (0.5 mM) $CuSO_4$, (5 mM) EDTA solution.

Adjustments of the redox potential were made by adding small amounts of sodium dithionite solution (2% (w/v) in 0.1 M Tris–HCl (pH 9.0)) or potassium ferricyanide solution (0.2 mM in H_2O). Titrations were carried out in the reducing and oxidizing directions.

EPR measurements were made at ~5 K using a Varian E9 X-band EPR spectrometer with an Oxford Instruments cryostat and temperature control system. Illumination in the EPR cavity was provided by a 500 W projector which provided ~0.35 J . cm^{-2} . s^{-1} of white light at the cavity window. A Nernst curve for a one electron accepting component was computer fitted using a Control Data Corp. CYBER 175 computer.

3. Results and discussion

Fig.1a shows the EPR spectrum of the PSII reaction center triplet observed in PSII particles while under steady state illumination at 5 K. The sample in fig.1a was poised at –439 mV in a redox titration experiment. Fig.1b shows the decrease in the extent of the triplet at very low potential (–640 mV). After titrations in the reducing direction, several samples were taken after the reaction mixture was reoxidized. Fig.1c shows such a sample posed at –480 mV. It can be seen that the effect of poising at low potentials is ~100% reversible. The decrease in the triplet signal at low potential is therefore attributed to a reduction, rather than a destruction process.

Fig.2 shows the results of redox titrations carried out at pH 11. An $n = 1$ curve with E_m ~–604 mV has been computer fitted to the points. Since the reaction center triplet is formed by radical pair recombination (see eq. (1)), reduction of Ph, photochemically or chemically, before illumination would result in the blocking of the formation of the radical pair and consequently the ability to photo-induce the triplet would be lost. We have demonstrated the dependence of the formation of the PSII reaction center triplet signal on the redox state of Ph in photoreduction experiments [5]. Identical relationships between the reaction center triplet states of photosynthetic bacteria [19,20] and PSI [21] with their respective intermediate acceptors have also been established. In bacteria the reaction center triplet has been used as a redox probe of the bacteriopheophytin intermediate acceptor in redox titrations [12]. Similarly the attenuation of the PSII reaction center triplet as the potential is lowered (fig.1,2) is a reflection of the reduction state of Ph. Thus the Ph/Ph^- couple is measured here to have E_m ~–604 mV.

This value is in good agreement with the reported

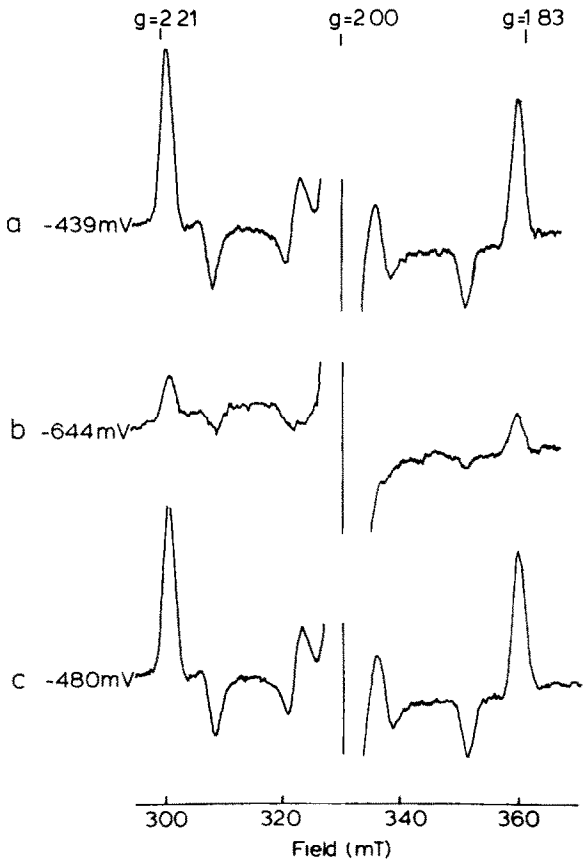

Fig.1. The effect upon the extent of reaction center triplet signal of poising PSII particles at low potentials. PSII particles (1 mg chl/ml) were poised at the potential shown for 10–15 min and frozen anaerobically in the dark: (a) poised at –439 mV; (b) poised at 640 mV, (c) poised at –480 mV taken from the titration mixture which had undergone reduction at –640 mV followed by reoxidation. The spectra shown are recorded under illumination at 5 K. Dark spectra were featureless except for a large $g \simeq 2$ signal due to mediator radicals. EPR conditions were as follows: microwave power, 0.05 mW; frequency, 9.26 GHz; modulation amplitude, 1.0 mT (10 G); instrument gain 10 000, response time 3 s; scan rate 30 min/2000 G.

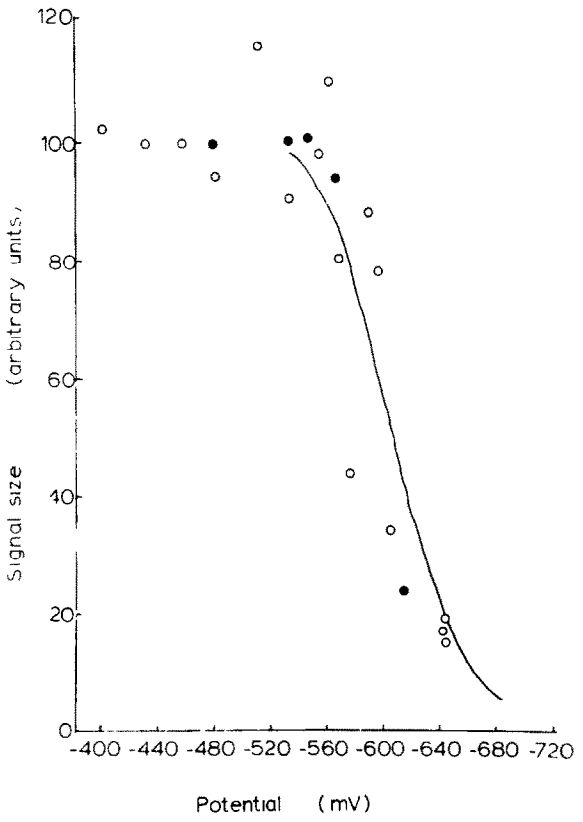

Fig.2. The redox potential dependence of the extent of the light-induced spin-polarized reaction center triplet EPR signal in PSII particles. The open circles are points obtained in titrations carried out in the reducing direction while the solid circles are points obtained in titrations carried out in the oxidizing direction. The curve is a computer fitted $n = 1$ Nernst curve with an E_m at −604 mV. EPR conditions were as described in the legend of fig.1.

value of −610 mV for Ph/Ph^- obtained by monitoring optically the disappearance of the light-induced change at 685 nm [9].

4. Conclusion

The extent of EPR signal of the light-induced reaction center triplet in PSII particles decreases as the potential is lowered. This effect is attributed to the one electron reduction of Ph. An E_m of ~−604 mV for the Ph/Ph^- couple has been measured by monitoring the triplet EPR of PSII as a function of potential.

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