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Electron spin polarization (CIDEP) of a primary electron acceptor in Photosystem II

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Photosystem II particles prepared according to Berthold et al. (Berthold, D.A., Babcock, G.T. and Yocum. C.F. (1981) FEBS Lett. 134, 231–234) and to Ganago and Klimov (Ganago, I.B. and Klimov, V.V. (1985) Biofizika, in the press) were subjected to an iron extraction procedure and cooled in the light under reducing conditions. The samples showed a 0.9 mT wide EPR line at $g = 2.0044$ attributed to the reduced primary acceptor Q_A^-. Further prolonged illumination at 15 K generated a wide, somewhat asymmetric EPR signal at $g = 2.0034–2.0038$ that showed strong, reversible polarization upon continuous illumination at 15 K and below. The signal is ascribed to an acceptor that becomes spin-polarized through exchange-mediated transfer of polarization as described previously for photosynthetic bacteria (Gast, P. and Hoff, A.J. (1979) Biochim. Biophys. Acta 548, 502–535). Arguments are given that the aceptor may be intermediate between the pheophytin transient acceptor and Q_A.

In recent years, electron spin polarization (CIDEP for Chemically Induced Dynamic Polarization) has been observed in photosystem (PS) I of plants and in the bacterial photosystem (see for a review Ref. 1). The study of electron spin polarization is of interest, because in principle it provides detailed information on the magnetic environment and on the magnetic interactions of the primary photosynthetic reactants, hence on reaction center structure [1]. A special and striking case of electron spin polarization is the polarization of the prereduced primary quinone acceptor (Q) in bacterial photosynthesis [2–5]. When the iron ion that is normally coupled to this acceptor is removed or uncoupled, the near-Gaussian absorptive EPR line of the prereduced semiquinone turns emissive under continuous illumination at cryogenic temperatures. This observation could be well explained by the concept of polarization induced in the radical pair $P\text{-}860^+ BPh^-$, where P-860 is the primary electron donor and BPh (bacteriopheophytin) a transient eletron acceptor, and subsequent transfer of the polarization of BPh^- to Q^- through the electron spin exchange interaction between BPh^- and Q^-. The observations allowed the determination of the exchange couplings between $P\text{-}860^+$ and BPh^- and between BPh^- and Q^- [2,3,5].

Abbreviations: PS, Photosystem; CIDEP, chemically induced dynamic electron polarization; BBY particles, particles prepared according to Berthold, Babcock and Yocum (Ref. 10); GK particles, particles prepared according to Ganago and Klimov (Ref. 11); BPh, bacteriopheophytin; Chl, chlorophyll.

Up to now, electron spin polarization of PS II has eluded observation. As it is clearly of importance to probe the structure of the PS II reaction center by means of this method, we have searched for the phenomenon in PS II, guided by the generally received notion that the acceptor side of PS II bears a close resemblance to that of the photosystem of purple bacteria [6]. Here too, the first stable acceptor is a (plasto)quinone, Q_A, and a pheophytin acts as intermediary electron acceptor (for a review on the primary photochemistry of PS II see Ref. 7). Q_A is normally coupled to an iron ion [8,9] but treatment with chaotropic agents and detergents decouples the iron from the Q_A and upon chemical or photoreduction a 0.8 mT wide, near-Gaussian EPR line at $g = 2.0044 \pm 0.003$ results [9]. We have searched for conditions for which this signal shows electron spin polarization.

In this communication we report the first observation of electron spin polarization in PS II. Prolonged illumination at 15 K of an iron-depleted PS II particle preparation that was frozen in the light in the presence of ascorbate produces an EPR signal that becomes strongly polarized upon continuous illumination at cryogenic temperatures. We interpret the phenomenon as in Ref. 2 as being due to transfer of emissive polarization of pheophytin$^-$ to an electron acceptor that is either Q_A or an accepter intermediate between pheophytin and Q_A.

PS II particles were prepared from spinach chloroplasts according to Ref. 10 (BBY particles) or to Ref. 11 (GK particles, prepared with deriphat 160, 30–40 Chl's per reaction center, no oxygen evolution), and stored in liquid nitrogen until further use. Iron extraction was carried out with $LiClO_4$ according to Ref. 9, which procedure reportedly results in a final Fe per reaction center concentration of 0.6. For the BBY particles 0.8 M $LiClO_4$ was used, for the GK particles 0.55 M plus 1.5 mM orthophenanthroline. The treated particles were dialyzed in 10 mM Tris-HCl (pH 8). EPR experiments were carried out in Pushchino on a superheterodyne spectrometer of local design operating at 9 GHz and equipped with a home-built helium gas flow cryostat. Samples contained 5–7 mg Chl/ml. g-values were measured using an NMR gauss meter, and are accurate ± 0.0002; linewidths (peak-to-peak of the derivative EPR spectrum) are accurate to ± 0.05 mT. To reduce Q_A the samples were slowly frozen to 220 K in the presence of 10 mM ascorbate under illumination with light from a 1000 W xenon arc. As controls, samples not treated with $LiClO_4$ with and without reductant were handled the same way.

Untreated BBY (PS II) particles without reductant showed at room temperature in the dark a weak broad signal similar to the so-called Signal II. Illumination at room temperature increased the amplitude of this signal 2-fold. No Signal I of the primary donor of PS I was induced, indicating that the particles were substantially free from PS I.

When treated BBY particles to which 10 mM ascorbate was added were slowly frozen to 220 K under illumination and then quickly cooled in the light to 120 K, a somewhat asymmetric EPR line appeared at $g = 2.0040$–2.0044 of width 0.95–1.05 mT. Following Klimov et al. [9] we attribute this signal to Q_A^-.

In some experiments treated BBY particles were frozen in the light at 220 K in the presence of 50 mM dithionite, cooled to 120 K in the light, and subsequently cooled to 60 K in the dark, where they showed a 1.2 mT wide line at $g = 2.0032$. We attribute this signal to the reduced intermediate acceptor pheophytin$^-$ [9]. Surprisingly, a similar line was observed at 60K when these particles were frozen in the dark at 220 K in the presence of dithionite and cooled to 60 K in the dark.

For the sample in which the Q_A^- signal was trapped at 120 K, we attempted to observe spin polarization during illumination at 15 K. At such low temperatures spin polarization is easier to see because then spin-lattice relaxation is considerably slowed. However, in none of the samples an emissive line or one with diminished absorption was produced, such as observed in the bacterial photosystem where the dark Q^- signal turns emissive directly upon illumination at cryogenic temperatures [2].

In further contrast to the bacterial system, the redox state of samples in which Q_A^- was trapped proved not to be stable under illumination at 15 K. Prolonged illumination caused an irreversible slow increase in the intensity of the EPR line and, in addition, a small g shift, until after 20 min irradiation a steady state was reached with a distinctly asymmetric EPR line of 1.0 to 1.1 mT

width at g between 2.0038 and 2.0034. Switching off the light now produced a considerable instantaneous increase in signal intensity (Fig. 1). This increase was fully reversible and approached a factor of two when measured at 6 K with low microwave power (Fig. 1). An increase in signal intensity due to sample heating by illumination, as sometimes observed for EPR lines measured at low temperature under saturating conditions, can be ruled out because (i) the effect increases when lowering the microwave power, (ii) the effect is absent when the sample is not pre-illuminated at 15 K, (iii) microwave saturation properties of the signal before and after preillumination at 15 K are similar. We conclude from the data shown in Fig. 1 that in PS II a redox state can be generated having an EPR signal that shows a strong spin polarization analogous to the one observed in bacterial reaction centers with prereduced quinone acceptor. The amplitude of the polarization in PS II is less than that found for the bacterial case. This is probably partly due to the high optical density of the preparation, as it was earlier found that reducing the absorbance of bacterial reaction centers enhanced the relative polarization effect [4]. In addition, the dark signal after preillumination at 15 K may be partly composed of a non-polarizable donor EPR line (see below).

The irreversible increase in EPR signal intensity upon illumination of a prereduced sample at 15 K must be the result of low temperature electron donation to P-680$^+$. Several low-temperature

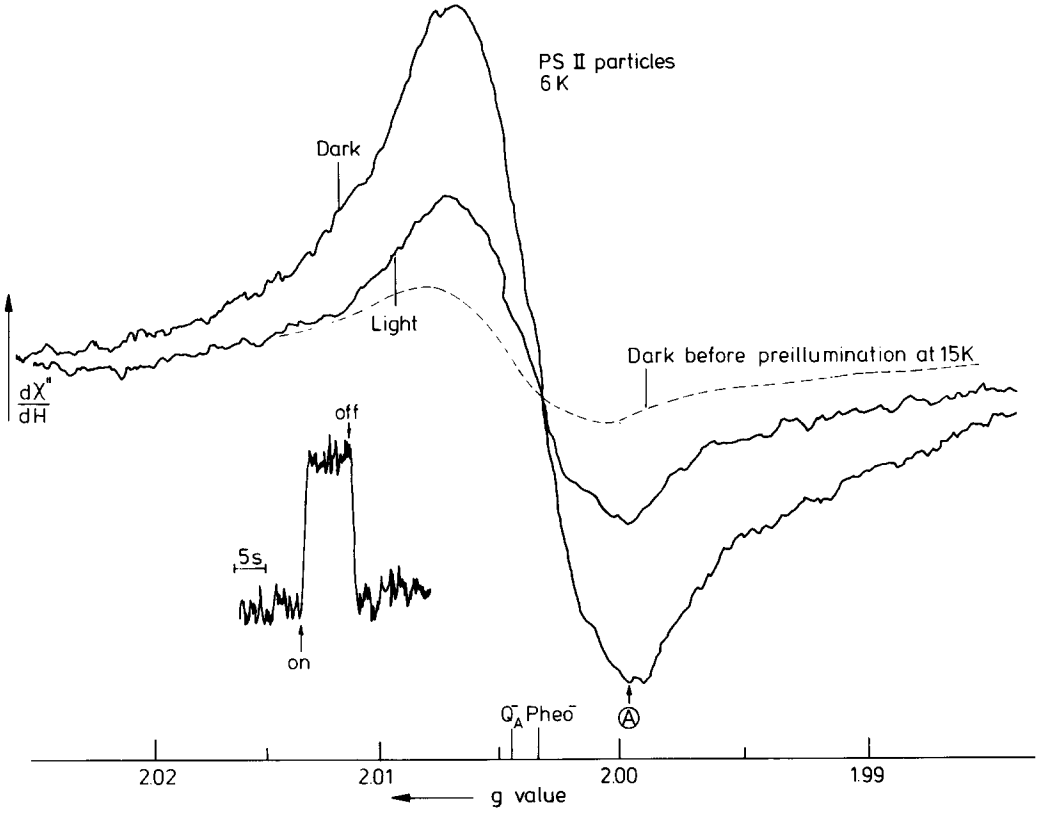

Fig. 1. EPR spectrum of GK Photosystem II particles at 6 K in the dark and under illumination with white light. Dashed trace: the signal produced by freezing at 220 K under illumination in the presence of 10 mM ascorbate (pH 8) measured at 15 K in the dark. Dark full trace: the signal produced by subsequent 20 min. illumination at 15 K. The g values of the EPR signals of Q_A^- and pheophytin (Pheo)a^- [9] are indicated. Conditions: microwave frequency, 9.1924 GHz; power, 5 μW; modulation amplitude, 5 G; time constant, 1 s. Inset: response to switching the light on (left arrow) and off (right arrow) at the position indicated by A. Time constant 100 ms, other conditions same as above.

donors have been described, amongst which are a carotenoid [12], a cyt *b*-559 [13], a species giving rise to a so-called Signal II type EPR spectrum [14] and one showing a narrow 1.0–1.1 mT wide EPR line at $g = 2.0033$ [14,15] that is possibly due to Car^+ [12]. From our lineshape we can exclude the Signal II species. If cyt *b*-559 is the donor and Q_A the acceptor, then one would not observe a g shift, contrary to observation. If Car is the donor and Q_A the acceptor, then one would expect to observe an EPR signal composed of about equal amounts of Car^+ ($g = 2.0033$) and Q_A^- ($g = 2.0044$) that is superimposed on the original dark signal of Q_A^-. Depending on the amount of $Car^+Q_A^-$ induced, the resulting EPR signal would show a g factor between 2.0044 and 2.0038. Our lowest g value of 2.0034 is somewhat lower than that of the pair $Car^+Q_A^-$, but this may be due to the inaccuracy in the reported g values of Car^+ and Q_A^-, which is about ± 0.0003.

A more severe argument against the induction of a composite EPR line by preillumination is the fact that the polarized signal has a lineshape that is similar to that of the dark signal, suggesting that we have to do with a single species, and not with two separate donor-acceptor radicals. This is because in the latter case we would expect (based on present knowledge of possible donors and acceptors) that the two purported radicals have a somewhat different g value and/or linewidth. By analogy to the bacterial case [2] we expect that only the acceptor radical becomes (emissively) polarized. Polarization of only one component of the composite EPR line would of course result in an appreciable change of the lineshape, contrary to experiment. Polarization of both the secondary donor and acceptor radicals seems to be ruled out, because the two radicals then must acquire polarizations that are opposite in sign and equal in magnitude (see, e.g., Ref. 1), and the polarization effects on Q_A^- and the secondary donor would largely cancel. Only when the two purported radicals have appreciably different spin-lattice relaxation times, would the cancellation be partial. However, no microwave power induced change in lineshape of the dark signal obtained after preillumination at 15 K has been observed. We feel therefore, that we can safely rule out the possibility that the signal induced by preillumination at 15 K is due to a donor-acceptor pair.

Alternatively, we may postulate a new acceptor, X, intermediate between Pheo and Q_A. In some PS II reaction center preparations the triplet state ^{3}P-680 could only be observed at redox potentials well below that of Q_A^- [16], indicating the presence of a low potential acceptor between Pheo and Q_A. In reaction centers in which Q_A^- is trapped, further low temperature electron donation may then accumulate X^-. If this radical has a g value of about 2.003 this would explain the g shift produced by preillumation at 15 K. As this preillumination enhances signal intensity up to 5-fold, the resulting signal would be almost pure X^- if the low-temperature donor is cyt *b*-559. Experiments to check this are now in progress.

The increase in integrated EPR intensity by illumination at 15 K is appreciably more than a factor of 2, indicating that our procedure of sample preparation leaves a fairly large amount of Q_A unreduced. This is perhaps not surprising, as P-680^+ is well-shielded and reduction of P-680^+ by ascorbate in the absence of redox mediators (which were left out to avoid contamination of the $g = 2$ region) has to complete with the back reaction of P-$680^+Q_A^-$. At 15 K further reduction of Q_A does not seem to occur, and instead X is reduced.

The identity of X is as yet unclear. An interesting possibility, pointed out by one of the reviewers of this paper, is that X is the second pheophytin present in PS II reaction centers that is not normally reduced. Slow electron donation to this pheophytin might generate the stable irreversible pheophytin$_{II}^-$ radical, which then might become polarized by interaction with transient pheophytin$_I^-$. This requires a fairly small distance of the order of 1.0 nm between pheophytin$_{II}$ and pheophytin$_I$, the normal transient acceptor. If we pursue the analogy with the bacterial reaction center, however, this does not seem to be likely [17].

Summarizing, we have demonstrated that in iron-depleted PS II reaction centers it is possible to accumulate a redox state of PS II that shows a strongly polarized EPR signal under continuous illumination at cryogenic temperatures. It is probable that this state is composed of cyt *b*-559^+ P-680 pheophytin X^-, where X is an acceptor earlier than Q_A. Illumination produces the

polarized radical pair P-680$^+$ pheophytin$^-$, pheophytin$^-$ is able to transfer emissive polarization to X^-, whose EPR signal intensity consequently diminishes.

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