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MIDPOINT POTENTIAL OF SIGNAL II (SLOW) IN TRIS-WASHED PHOTOSYSTEM-II PARTICLES

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In Tris-washed Photosystem-II particles we are able to induce an EPR signal in the dark by addition of an iridium salt (K_2IrCl_6). This signal is attributed to signal II_s (slow) (D^+) and the redox titration gives an E_m value of 760 mV for the couple D^+/D. On the basis of our previous studies on the equilibrium between D^+Z and DZ^+ ($K = 10^4$) (Boussac, A. and Etienne, A.L. (1982) Biochem. Biophys. Res. Commun. 109, 1200–1205), we therefore attribute a value of 1 V for the E_m of the Z^+/Z couple. A second effect of K_2IrCl_6 is to modify the spectral characteristics of signal II. We conclude that K_2IrCl_6 is able to change the environment of the species from which signal II_s and signal II_f originate.

Introduction

Since the discovery by Commoner et al. [1] of EPR signals related to the photosynthetic electron-transport chain, several studies have shown that at room temperature the observed signals correspond to the oxidized form of the chlorophyll center of Photosystem I (P^+-700), called signal I [2], and to the oxidized form of secondary electron donors to Photosystem II (signal II) [3]. A signal corresponding to P^+-680, the chlorophyll center of Photosystem II, has been described [4], but under most experimental conditions it is not detected at room temperature because its reduction kinetics are very fast [5]. Signal II corresponds to two distinct species on the donor side of Photosystem II (PS II). The electron donor to P^+_{680}, Z/Z^+, is reduced with kinetics which depend upon the integrity of the oxygen-evolving system. In fully active PS-II centers reduction of Z^+ is achieved in less than 1 ms (signal II_{vf}, i.e., 'very fast') [6]. After treatments like Tris or hydroxylamine washing which inhibit the oxygen-evolving complex, the reduction of Z^+ is slowed down (and the corresponding EPR signal becomes signal II_f [7]). A slowly decaying component of signal II, signal II_s, corresponds to a lateral donor D which can be slowly oxidized by the S_2 and S_3 states of the oxygen evolving complex in intact system-II centers [8] or more rapidly by Z^+ in Tris-washed chloroplasts [9,10]. The optical spectrum of Z^+ and the hyperfine structure of the EPR spectra of signal II_f and II_s [11,12,13] show that Z and D are likely to be plastoquinone cation radicals, tightly bound to a protein of the thylakoid membrane.

In PS-II particles devoid of PS-I centers the spectral characteristics of signal II remain identical to those of chloroplasts [14]. Tris washing causes the release of manganese and of the 17, 23 and 34 kDa protein [15]. Oxygen evolution is totally inhibited, but EPR signals II_f and II_s are still detected [14].

Abbreviations: Z and D, species from which originate signal II_f (fast) and II_s (slow), respectively, in electron paramagnetic resonance spectroscopy (EPR); PS I and II, Photosystem I and II; χ'' magnetic susceptibility; H, magnetic field; DPPH; 1,1-diphenyl-2-picrylhydrazyl.

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Our aim, in the present report, is to determine the redox midpoint potential of the D^+/D couple and, by deduction, of the Z^+/Z couple by the use of a mixture of the reduced (K_3IrCl_6) and oxidized (K_2IrCl_6) forms of an iridium salt known to be a strong oxidant.

Materials and Methods

Photosystem-II particles were prepared from fresh pea leaves (grown 15 days), according to the method of Berthold, Babcock and Yocum [14], with a single Triton X-100 treatment. The chloroplasts were previously washed with Tris buffer (0.8 M, pH 8.5) for 15 min at 4 °C in day light.

The Tris-washed PS-II particles were resuspended in a buffer containing 0.4 M sorbitol/10 mM NaCl/5 mM $MgCl_2$/20 mM Tris (pH 8.5), with 0.2% (w/v) bovine serum albumin at a concentration of 2 mg total chlorophyll/ml and were stored at −30 °C until use. The same medium was used in all experiments.

EPR measurements were performed with a Bruker B-ER 420 spectrometer at room temperature with a slotted cavity to allow illumination. Continuous white light illumination was provided by a 900 W xenon lamp. The first derivative spectra were recorded on a signal averager (Tracor NS-575A) and stored on a HP 45 microcomputer. Areas of the absorption spectra were subsequently computed by double integration. The potentiometric titration curve shown in Fig. 3 was obtained by adding a constant volume of a mixture of K_2IrCl_6 and K_3IrCl_6 freshly dissolved in water (at a concentration of 25 mM) to a constant volume of PS II particle suspension.

The redox midpoint potential of the $IrCl_6^{2-}/IrCl_6^{3-}$ was measured in the presence of PS-II particles in the resuspension medium with a Pt ‖ Ag | AgCl ‖ KCl combined electrode. A value of 720 mV was obtained. All others experimental conditions are specified in the figure captions. The *g* values were determined in separate experiments with the use of DPPH (1,1-diphenyl-2-pikrylhydrazyl) as *g* factor standard to calibrate the magnetic field.

Results

Fig. 1 shows the spectra recorded for dark-adapted particles (a), during continuous illumination (b) and in the dark after illumination (c).

The a area is small and for some preparations is close to zero (Table I). This fits with our previous studies on Tris-washed chloroplasts which showed

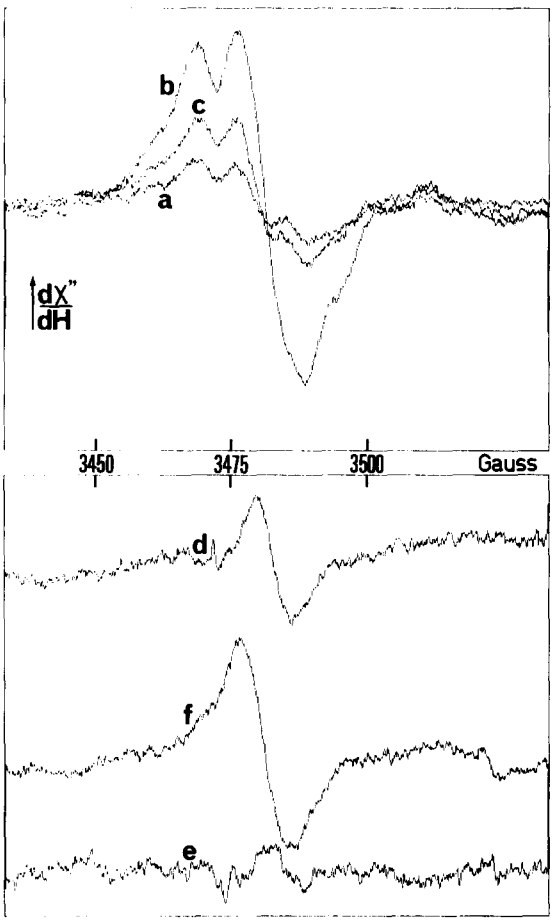

Fig. 1. First derivative of EPR spectra of PS-II particles. The instrument settings were as follows: Microwave power, 25 mW; sweep time, 100 Gauss/3 mn; temperature, 20 °C; chlorophyll concentration, 1.5 mg/ml; frequency, 9.77 GHz. Each trace is the result of 12 accumulations. Trace a: dark-adapted sample. Trace b: signal induced by continuous illumination. Trace c: signal in the dark after continuous illumination. Trace d: trace b minus twice trace c. Trace e: difference between a spectrum recorded in the dark in the presence of 5 mM FeCN and the spectrum recorded in the dark (trace a). Trace f: dark adapted sample +10 mM K_2IrCl_6, the vertical scale is multiplied by two.

that, at pH 8.5, D is mostly reduced in dark-adapted samples [9,10].

The difference between the dark spectra in the presence and absence of 5 mM FeCN (spectrum e in Fig. 1) shows that there is no detectable amount of P-700 in these particles and that FeCN is unable to oxidize D in the dark.

The area during continuous illumination (b) is a little larger than twice that remaining in the dark after illumination (c); the additional signal is represented by the spectrum (d). It is a 6 gauss wide, gaussian-shaped signal in the $g = 2$ region, with a g value equal to 2.0026 ± 0.0007, therefore smaller than that of signal II that we found equal to 2.0044 ± 0.0005 in these particles. By analogy with the light-induced EPR signal (gaussian-shaped spectrum, 7 gauss wide) in the presence of ferricyanide for chloroplasts at pH 4 [16] we attribute this signal to P^+-680. An EPR signal with 7 gauss wide and attributed to P^+-680 was also observed in PS-II particles in the presence of a high concentration of hydroxylamine [17]. The centers eliciting this signal (approx. 30%, Table I) are in a D^+Z^+ P^+-680 state. The data in Table I show that the stoichiometry of D to Z is 1 : 1 in agreement with previous findings [14]. The corresponding spectra, with a width of 19 gauss, have identical shapes and hyperfine structure.

Fig. 2 shows the spectrum of dark-adapted PS II particles in the presence of 10 mM K_2IrCl_6 (also shown in Fig. 1, spectrum f). Its area is equal to that of signal II_s (Fig. 1, spectrum c). It has no hyperfine structure and its bandwidth is 10 gauss with a g value equal to 2.0044 ± 0.0005, therefore

TABLE I

The areas are normalized to the value of the area signal in dark after illumination with no addition. average of 2–4 independent preparations of PS-II particles.

Conditions	No addition	With 10 mM K_2IrCl_6
Area of the signal of the dark-adapted sample	0.25 ± 0.16	1.1 ± 0.1
Area of the light-induced signal	2.4 ± 0.3	2.0 ± 0.1
Area of the signal in dark after illumination	1	1.1 ± 0.1
Area of the light-induced signal minus twice the dark signal after illumination	0.30 ± 0.15	0

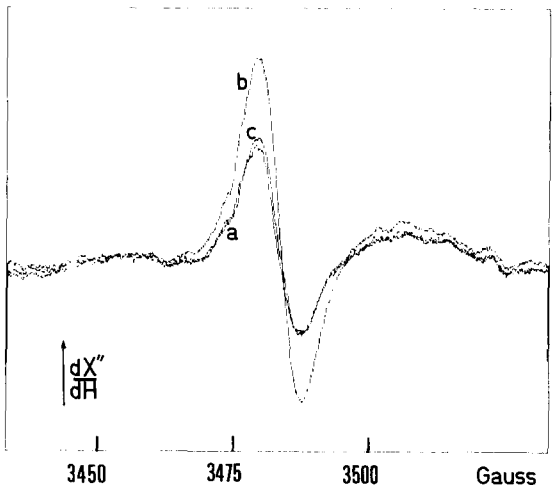

Fig. 2. First derivative EPR spectra of PS-II particles EPR spectra in the presence of 10 mM K_2IrCl_6. The settings are the same as in Fig. 1. Each trace is the result of six accumulations. Trace a: dark-adapted sample. Trace b: signal induced by continuous illumination. Trace c: dark signal after the continuous illumination.

the same as for Signal II. Under continuous illumination an increase in the amplitude of the signal is observed and its area is twice that of the dark signal (Fig. 2 and Table I). The light-induced signal (spectrum b) has the same characteristics as spectrum a. The signal in the dark after illumination (spectrum c) is identical to the signal recorded before the illumination. In Fig. 3, the area of the signal in the dark is plotted vs. the potential imposed by mixtures of $IrCl_6^{2-}/IrCl_6^{3-}$. A midpoint potential of 760 mV is found. This oxido-reduction reaction involves one electron as shown by the good fit between the experimental points and the theoretical curve computed for $E_m = 760$ mV and $n = 1$.

The signal observed in the presence of K_2IrCl_6 cannot be attributed to P^+-680; the E_m value is too low, its g value, exactly identical to that of signal II, does not correspond a chlorophyll radical and the doubling of the area during illumination would need a special interpretation. Because of the similarities of the areas of signal II and this signal in light and dark conditions and because no other signal with different lineshapes is formed during illumination, we postulate that the signal produced by the addition of K_2IrCl_6 is signal II_s.

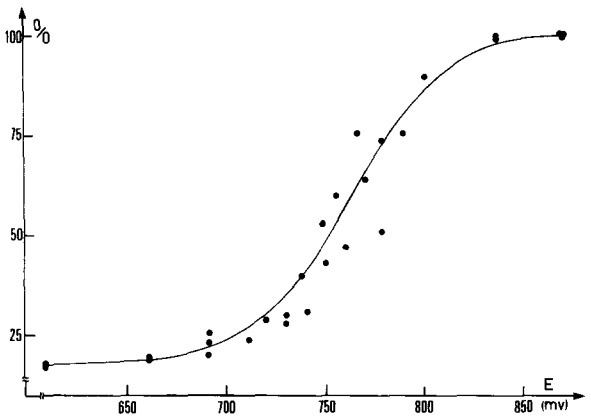

Fig. 3. Area of the dark signal (normalized in percentage of the area of total signal II_s) vs. the redox potential in mV (E) imposed by a mixture of the oxidised and reduced form (K_2IrCl_6, K_3IrCl_6). For each point the value is the mean of four accumulations and total $K_xIrCl_6 = 6.25$ mM. This figure is the result of experiences on 3 independent PS-II particles preparations.

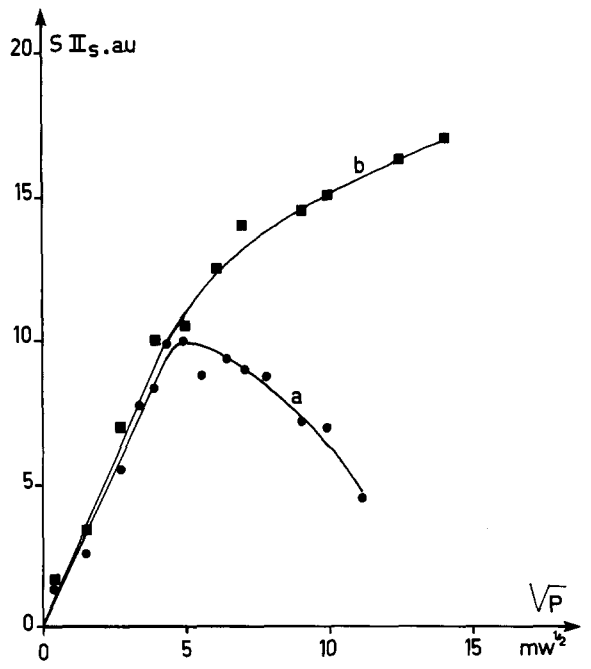

Fig. 4. Power saturation curve. The amplitude (in arbitrary unit) of the first derivative EPR spectra of signal II_s after an illumination (curve a) or in dark-adapted sample (curve b) is plotted versus the square root of the microwave power. Mean of three independent PS-II particle preparations. Curve a: no addition. Curve b: +10 mM K_2IrCl_6.

Therefore, the E_m of 760 mV corresponds to the D^+/D redox couple. The E_m of the Z^+/Z couple is too high (see Discussion) to be oxidised by K_2IrCl_6, but the shape of the spectrum of Z^+ is modified in the same way as that of D^+.

The modification in shape of signal II is associated with a substantial change in its power saturation behavior as shown in Fig. 4. While signal II_s of Tris-washed particles in the absence of K_2IrCl_6 saturates at low-power levels (about 25 mW) signal II_s with 10 mM K_2IrCl_6 present is still not saturated at the maximum microwave power (225 mW). Babcock et al. observed a similar alteration in the power saturation behavior for signal II_s in the presence of NH_4Cl, but the spectrum was unchanged [18].

Discussion

The Tris-washed PS-II particles we used have no detectable amount of P-700, and a stoichiometry of 1 : 1 for signal II_f and II_s. Under continuous illumination, an additional signal with a 6 gauss bandwidth was attributed to the formation of a small amount of P^+-680. At the pH used (pH = 8.5), D was mainly reduced in the dark, as was the case for Tris-washed chloroplasts in our previous studies [9,10].

In the presence of an iridium salt K_2IrCl_6, known to be a strong oxidant, a new signal was observed in the dark with no hyperfine structure, but with a g value and area identical to those of signal II_s. In the presence of K_2IrCl_6, the light-induced signal shape was identical to the dark signal with a doubling of the area. In the dark following illumination, the signal decayed back to its original amplitude.

We attribute this novel signal to D^+ and interpret our results by two distinct effects of K_2IrCl_6:
- the capability to oxidize D in the dark (FeCN is not a sufficiently strong oxidant to achieve this oxidation);
- a large modification of the bonding between the cation plastoquinone radicals, D^+ and Z^+, and their associated protein(s), although K_2IrCl_6 is unable to directly oxidize Z. If the hyperfine structure of signal II is due to the stabilization of the antisymmetric orbital by the membrane interaction [19], then the structureless shape observed in

the presence of K_2IrCl_6 might be due to a stabilization of the symmetric orbital.

The redox titration curve of Fig. 3 gives a value of 760 mV for the redox midpoint potential of the D^+/D couple. This signal is not related to the signal observed by Bearden and Malkin [20] at 77 K, in light, in presence of $IrCl_6^{2-}$. The radical formed in these conditions is P^+-680 and the species titrated is the same as that titrated in Refs. 21 and 22 with an E_m in the range 425–475 mV, and designated as A_H in Refs. 23 and 24. Under certain experimental conditions, carotenoids were oxidized by Photosystem II [25,26]. In the present case it seems very unlikely that the signal described corresponds to the cation radical of carotenoids. The stoichiometry would then be of two carotenoids per PS-II center and K_2IrCl_6 would only oxidize half of it in the dark. According to Ref. 26, only one carotenoid is tightly associated with the donor side of PS II. If the midpoint potential of the D^+/D couple is equal to 760 mV, then our previous findings of an equilibrium between Z^+D and ZD^+ in Tris-washed chloroplasts with an equilibrium constant, $K = ZD^+/Z^+D$, of 10^4 [10], lead to the conclusion that the E_m of the Z^+/Z couple is around 1 V, a value close to the predictions of Bouges-Bocquet [24], and to the value found in vitro for the cationic quinone radicals [19]. This value of 1 V may be underestimated if we take into account for the hypothesis formulated in Ref. 27.

In the presence of K_2IrCl_6, no P^+-680 is formed in the light, this might be due to a reoxidation of Q^- by $IrCl_6^{2-}$ with a concomitant reduction of Z^+ by the $IrCl_6^{3-}$ formed as suggested in Ref. 28 with ferricyanide.

In conclusion, we have determined the redox midpoint potential values for the D^+/D couple and estimated that of the Z^+/Z couple. Experiments are under progress to know if the modification induced in the shape of the signal-II spectrum by K_2IrCl_6 might give new insights on the structure and environment of the D^+ and Z^+ radicals.

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