EPR PROPERTIES OF PHOTOSYSTEM I IRON–SULFUR CENTERS IN THE HALOPHILIC ALGA, *DUNALIELLA PARVA*

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1. Introduction

The photosystem I complex in blue-green algae, green algae, and in plants has been shown to contain iron–sulfur electron-transferring biomolecules on the basis of low-temperature electron paramagnetic resonance (EPR) spectroscopy [1–3]. The presence of two distinct membrane-bound iron–sulfur centers in photosystem I has been confirmed by the determination of the midpoint oxidation–reduction potentials of these centers. In spinach preparations, reduction, either by illumination at cryogenic temperature or by the addition of a chemical reductant in the dark at 300 K, elicits an EPR spectrum with components $g_z = 2.05$, $g_y = 1.94$, and $g_x = 1.86$. This center has been designated 'A'. E_m-values of −530 mV and −553 mV have been reported in [4] and [5], respectively. Under more strongly reducing conditions, obtained by the addition of sodium dithionite to a sample or by the illumination of a sample while freezing, the signal at $g_x = 1.86$ is lost or shifted in value and new signals appear at $g = 1.92$ and 1.89 with additional signal intensity at $g = 2.05$. E_m-values for these additional components have been reported as −580 mV and −594 mV in [4] and [5], respectively. The apparent loss of, or shift in, the $g_x = 1.86$ component is argued to result from the magnetic interaction of center A with the second iron–sulfur center 'B' [5]. Since it is impossible to obtain a set of g-values for center B in the absence of center A, a specific set of g-values characteristic of this center only cannot be accurately assigned based on this data. Since a maximum of 3 g-values can be obtained from an $S = 1/2$ transition-metal ion but 5 g-values are observed (two contributions at $g = 2.05$), the fully reduced spectrum, therefore, must represent a total $S > 1/2$. Excluding the possibility of hyperfine interactions, this implies the presence of either > 1 $S = 1/2$ center or 1 center with an effective $S > 1/2$.

For comprehensive reviews of the properties of membrane-bound iron–sulfur centers in photosynthetic systems see [6,7].

In a study [8] on the thermophilic blue–green alga *Phormidium laminosum*, a g-value of 1.92 was suggested as a consequence of an exchange interaction between spins. Additional information concerning the assignment of various g-values in spinach preparations has been obtained by studying the angular dependence of the EPR signal intensity in oriented preparations [9,10].

Studies with the halophilic alga, *Dunaliella parva*, have proven useful in the further examination of these bound iron–sulfur centers. In membrane fragments from *D. parva*, it is possible to photoreduce 2 iron–sulfur centers at cryogenic temperatures in chemically unpoised samples. The ability to observe the photoreduction of 2 iron–sulfur centers (centers A,B) in *D. parva* may be related to structural differences between these centers relative to those in spinach photosystem I since an examination of the oxidation–reduction potentials of the centers in *D. parva* reveal significant differences compared with those of spinach. Unusual temperature dependence of the EPR signal intensity in *D. parva* also supports the idea of differences existing between the two systems. These results are discussed in terms of the role of the

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2 membrane-bound iron–sulfur centers in the photosystem I electron-acceptor complex.

2. Materials and methods

A culture of *D. parva* obtained from Professor M. Avron was grown as in [11] but under anaerobic, sterile conditions. Cells were harvested by centrifugation during middle log-phase and fragments were prepared by osmotically shocking in a 1:20 dilution of a wash solution containing 40 mM Hepes buffer (pH 7.6), 15 mM NaCl, 5 mM $NaHCO_3$ and 4 mM EDTA. Fragments obtained by this procedure were washed a second time and concentrated by centrifugation at 2000 × *g* for 5 min.

Redox titrations were performed on fragments prepared from 2 l stock medium after washing with the addition of the following agents: 2.0 ml 0.5 M glycine buffer (pH 10.5), 0.2 ml 5.0 mM benzyl viologen, 0.2 ml 5.0 mM methyl viologen, 0.2 ml 5.0 mM triquat, 0.1 ml 100 mM EDTA (pH 7.6) and 7.3 ml distilled water to make the final reaction mixture 10 ml. The titration was done by adding μl amounts of an anaerobic solution containing 100 mM sodium dithionite in 0.2 N KOH following [12].

X-band EPR spectra were obtained at cryogenic temperatures employing a modified JEOL ME-IX spectrometer with a TE_{011} mode cylindrical reflection cavity. First-derivative spectra were recorded employing 100 kHz magnetic field modulation and a phase-sensitive detection system. Field modulation amplitudes were typically 1.0×10^{-3} Tesla. Spectrometer modifications include the replacement of the conventional klystron microwave source by a low-noise solid-state Gunn oscillator (Central Microwave) to enhance sensitivity and by adding a double-balanced mixer (RHG, DM 8-12B) as a homodyne phase detector.

3. Results and discussion

Illumination of *D. parva* membrane fragments with red light at cryogenic temperatures in chemically unpoised samples results in the photoreduction of 2 distinct iron–sulfur centers. Six *g*-values are discernable from the light – dark difference spectra when observed at 10 K (fig.1): $g = 2.072$, 2.053, 1.940, 1.925, 1.876 and 1.844. The $g = 1.940$ and 1.925

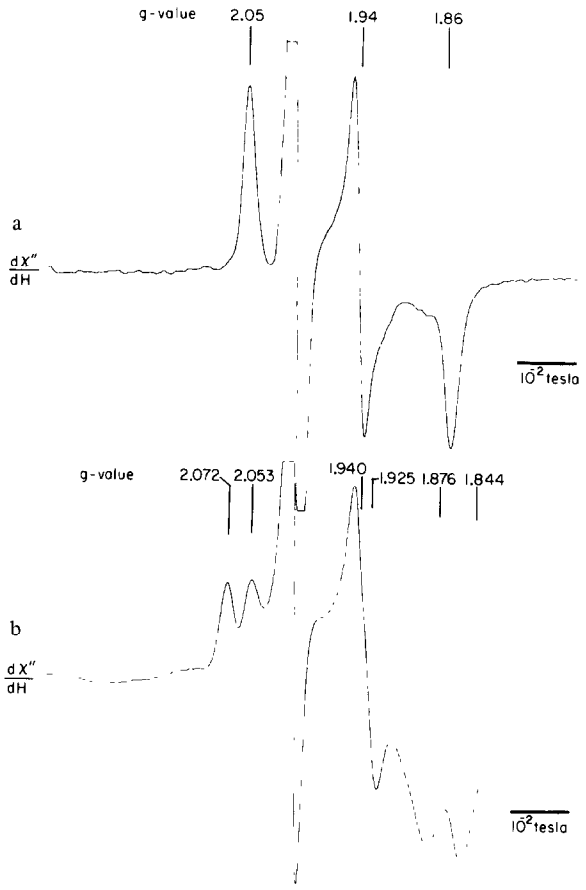

Fig.1. Red light illumination at 10 K by continuous light source. Spectra were recorded at different gains and light–dark difference spectra are shown for (a) spinach chloroplasts and (b) *D. parva* membrane fragments. EPR conditions: $T = 10$ K; power = 10 mW; $H_m = 10^{-3}$ T; $\Delta H = 10^{-1}$ T; $Q_I = 5000$; $t = 0.1$ s; $\nu = 9.2$ GHz.

components are difficult to resolve but an inflection point can frequently be observed between these signals. Further reduction, either by warming to 300 K and re-freezing in the presence of white light illumination or by the addition of sodium dithionite to the sample, results in the disappearance of the $g = 1.844$ component.

In contrast, photoreduction of spinach preparations at cryogenic temperatures produces a spectrum consisting of only 3 *g*-values (fig.1). Further reduction produces 2 additional *g*-values and a disappearance of the $g = 1.86$ signal. This component is not generally observed in the presence of a second reduced center. Sometimes, however, under strongly reducing conditions, the $g = 1.86$ component is not entirely eliminated from the spectra.

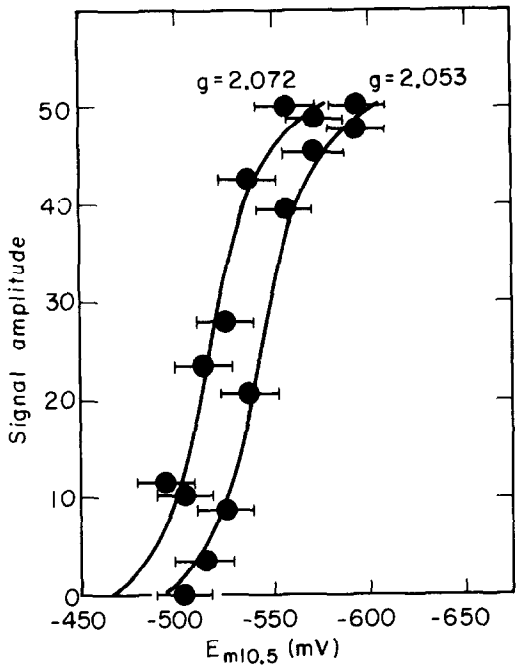

Fig.2. Oxidation–reduction Nernst plot of the $g = 2.072$ (center B) and $g = 2.053$ (center A) components of *D. parva* membrane fragments. Data are best fit with $n = 2$ titration curves.

The oxidation–reduction titration data illustrated in fig.2 represents the signal amplitude of the $g = 2.072$ and 2.053 components produced by chemical reduction in the dark at 300 K of *D. parva* membrane fragments. These g-values were selected as representative of the redox state of the 2 iron–sulfur centers since they are well-resolved and free from g-value shifts and lineshape changes throughout the potential range indicated. The $g = 2.072$ signal remains stationary and constant in width irrespective of the absence of the $g = 2.053$ component. Both titration curves are best fit with $n = 2$ Nernst curves as presented. The significance of this is not understood at present. Values of $n = 2$ for centers A,B have been reported in

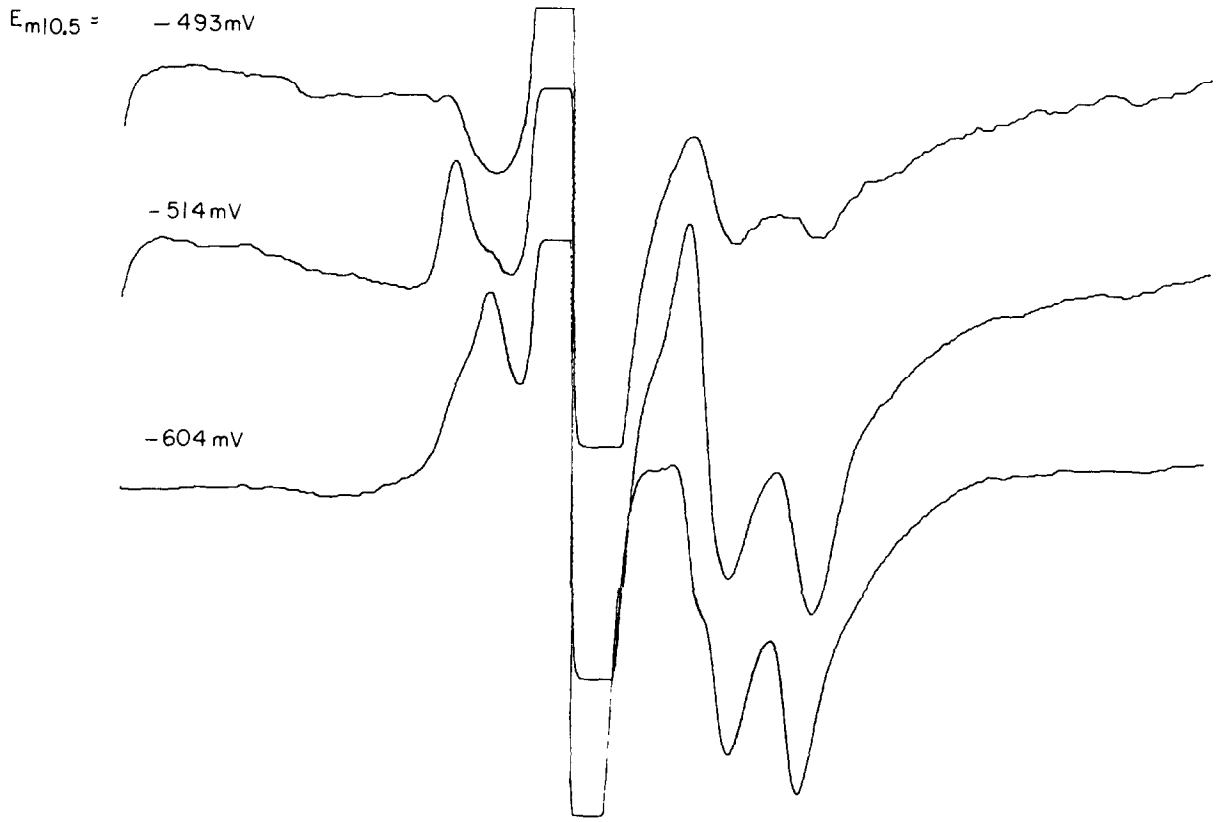

Fig.3. Spectra obtained from oxidation–reduction titration in which each spectrum was recorded at the chemically poised potential indicated. Spectra obtained at different gains. EPR conditions were as in fig.1

spinach [4]. Midpoint potentials determined from these plots are $E_{m10.5}$ = −518 mV and −543 mV for the g = 2.072 and 2.053 components, respectively. As indicated by the titration curves, it is possible to obtain one set of 3 g-values by chemically poising at ~−500 mV. Fig.3 reproduces EPR spectra taken at several different oxidation–reduction potentials illustrating both the partial and the complete chemical reduction of the 2 iron–sulfur centers. The g-values obtained after only partial reduction are g_z = 2.072, g_y = 1.932 and g_x = 1.876 and designated as center B. Complete chemical reduction at potentials <−543 mV or photoreduction of a sample containing chemically reduced center B results in a spectrum containing additional signals at g = 2.053, 1.940 and 1.925 with a loss of, or shift in, the g = 1.932 component. This is in contrast to photoreduction of an unpoised, dark-adapted sample at cryogenic temperature in which the above g-values are present as well as an additional signal at g = 1.844. The more complicated spectrum is considered as a consequence of the reduction of a second iron–sulfur center, center A. The assignment of these centers as A or B is based on the similarity of EPR properties between these centers in *D. parva* with those of spinach. The disappearence of the g = 1.844 signal under strongly reducing conditions is similar to that observed for the g = 1.86 component in spinach preparations. Although the g = 2.053 signal can be assigned to center A, either one or both of the g = 1.940 and 1.925 components, observed only in the composite spectrum, may result from magnetic interaction between the centers and, therefore, cannot be assigned unambiguously to a specific center. Since we can chemically reduce center B (reducing electron from the reductant) and then photoreduce the second center, center A (reducing electron from P700) it is clear that iron–sulfur centers A,B can be reduced in the same reaction center. Therefore, the loss of the g = 1.932 and the production of new g-values at g = 1.940 and 1.925 might argue for the presence of magnetic interaction between these centers. Since center B can be observed in the absence of reduced center A, the assignment of the g-values at 2.072, 1.932, and 1.876 can be made explicitly.

The amount of photoreducible g = 1.844 component of center A in *D. parva* appears to correlate with the extent of chemical reduction of center B. Although the g = 1.844 signal cannot be produced by chemical reduction at even the most negative potentials, it can be photoreduced unless center B had been reduced by sodium dithionite. Indeed, a plot of the amount of photoreducible g = 1.844 *vs* oxidation–reduction potential gives a titration curve with a similar midpoint potential to that of center B. Apparently, chemically reducing center B eliminates the ability to photoreduce the g = 1.844 component of center A.

Another contrast between *D. parva* and spinach is evident when observing the temperature dependence of the EPR signal intensity of the reduced centers. In spinach preparations, the relative intensities between centers A and B remain approximately constant as a function of temperature over 5–30 K. In *D. parva* preparations, however, the 2 centers show unusual temperature dependences as illustrated in fig.4.

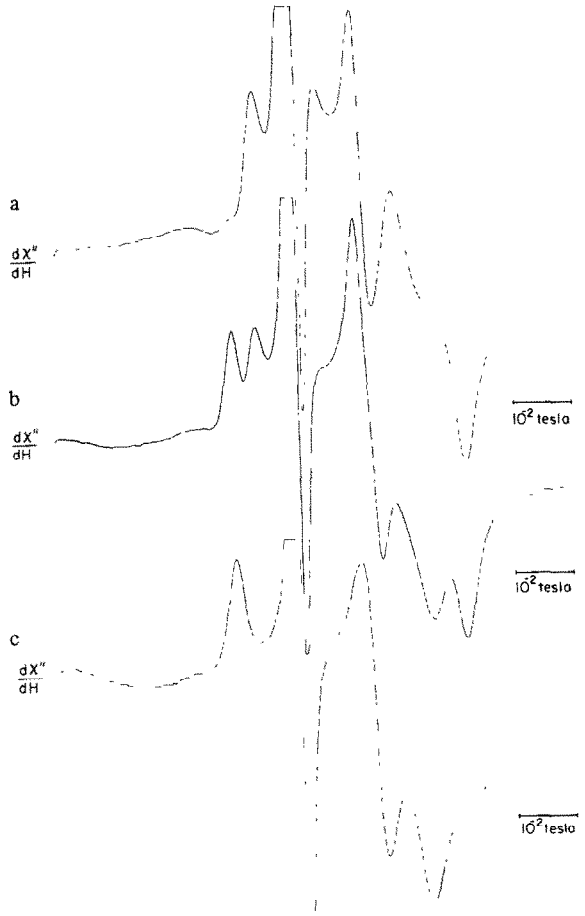

Fig.4. Red light illumination at 10 K by continuous light source. Spectra were recorded at different gains. Light–dark difference spectra are shown for the following temperatures: (a) 5 K; (b) 10 K; (c) 25 K. EPR conditions were as in fig.1.

One set of 3 g-values are observed at 5 K (g_z = 2.053, g_y = 1.940, g_x = 1.844) while at 25 K a second, non-equal set of 3 g-values are observed (g_z = 2.072, g_y = 1.925, g_x = 1.876). At intermediate temperatures, all 6 g-values are observed with amplitudes proportional to the temperature. The optimal temperature for observing the maximal signal intensity of each component also varies as a function of the microwave power incident to the cavity. It is tempting to assign g-values to each center based on this temperature dependence of EPR signal intensity. However, spin-lattice relaxation times may not be isotropic among the principal values of each g-tensor depending upon the relaxation mechanism.

4. Conclusions

Several hypotheses concerning the function of bound iron–sulfur centers in photosystem I have been advanced. They consider various relationships between the bound centers A and B with each other and with respect to electron flow through the photosystem I complex. One model suggests that electron transfer first occurs through component X then to either center A or B [13]. A more recent proposal considers electron flow to be linear between center B and center A [14]. These models imply a close physical association between these centers. A third proposal suggests a different function for each center in which center A is involved in non-cyclic electron transport to NADP while center B is involved in cyclic electron transfer around photosystem I [15]. This arrangement suggests that the 2 centers exist in separate proteins located at different sites in the chloroplast membrane although both in close proximity to component X. Whether one envisions a series or parallel relationship between these iron–sulfur centers with respect to electron flow, our results indicate that both centers A and B can act as a trap for electrons at cryogenic temperatures and, therefore, both are involved in the primary charge separation.

The close association between the centers in spinach is given additional support by the g-value shift of the g = 1.86 component during reductive titration. The disappearence of the g = 1.86 signal of center A during the reductive titration of center B was proposed [5] to result from spin–spin interactions between the reduced centers. Although the loss of or shift in the g = 1.932 signal of center B in *D. parva* upon either chemical or photoreduction of center A also supports this close association, the loss of the g = 1.844 component in *D. parva* from reductive titration of center B may not result from spin–spin interactions between these centers since photoreduction of a dark-adapted sample results in all 6 g-values being produced, simultaneously. Since in this case the g = 1.844 is observed in the presence of the other reduced center, we suggest that its loss under certain conditions does not result from an interaction with another g-value.

The unusual temperature dependence of the iron–sulfur centers A and B in *D. parva* may indicate different relaxation (perhaps even structural) properties and further illustrates the importance of temperature on the EPR observability. One must be cautious when considering the extent of reduction of a particular electron carrier when based on the observed signal amplitude at a single temperature and power.

Acknowledgements

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