QUANTITATIVE STUDIES ON THE EPR SIGNALS OF PHOTOSYNTHETIC SYSTEM I AND FERREDOXIN

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SUMMARY

The relative sizes of the electron paramagnetic resonance absorptions of ferredoxin and photosynthetic System I were measured at 1.5°K after illuminating spinach chloroplasts at 77°K or at 1.5°K. The total amount of reduced ferredoxin produced by the irradiation was less than 10% of the induced Photosystem I free radical. The EPR signal of a second photosensitive nonheme iron center has also been observed. The reported role of ferredoxin as a primary electron acceptor is discussed.

INTRODUCTION

The identity of the primary electron acceptor in photosynthetic System I of the green plant has been studied for many years. Arnon (1) proposed that this role is played by ferredoxin, a nonheme iron protein with a midpoint reduction potential of -0.42 volt. However, several other workers were in favor of a more electronegative compound (2). Recently, Malkin and Bearden (3) have reported an EPR signal characteristic of the reduced ferredoxin in spinach chloroplasts after irradiating with strong light at 77°K. Assuming that the electron transfer by ordinary chemical reactions should be minimal at this low temperature, they suggested that some bound form of ferredoxin may serve as the primary acceptor of Photosystem I.

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The present study was undertaken to test the above proposal by measuring the relative quantities of the reduced ferredoxin EPR signal and that of the primary electron donor of Photosystem I. A 1:1 ratio between these two species should be expected if ferredoxin is the primary electron acceptor under conditions that electron transfer by ordinary chemical reactions is negligible.

MATERIALS AND METHODS

Chloroplasts were prepared by grinding fresh spinach leaves in a Waring Blender, followed by differential centrifugation as described by Avron (4). The blending solution contained 0.25 M sucrose, 0.05 M Tris.HCl, 0.01 NaCl and 2 mM sodium-EDTA. EPR measurements at 77°K were carried out with a Varian E-3 EPR spectrometer. The illumination source was a 500-W projector lamp with a red filter and a 1.5 inch thick cold water shield. For measurement of both the ferredoxin and the Photosystem I signals the chloroplast-containing EPR cavity was first immersed in liquid nitrogen and irradiated with a 500-W quartz-iodine lamp (Sylvania Sun-Gun) placed about 6 inches away from the sample. It was then measured at 1.5°K with an X-band EPR spectrometer previously described (5). The total amounts of the different paramagnetic centers were obtained by comparing the observed signals to similar standards of known concentration. In some experiments, the chloroplasts were also illuminated at liquid helium temperature in the sample cavity by means of a mirror device.

RESULTS

Two types of photo-induced EPR signals are apparent in chloroplasts (6), a Gaussian shape (c.f. Spectrum C in Fig. 1) with a g-value of 2.0023 and a peak-to-peak width ($\triangle$H) of 8.2 gauss, and a broader signal (c.f. Spectrum A in Fig. 1) with $\triangle$H of about 20 gauss and a g-value of 2.0042. These signals have been designated Signal I and Signal II, respectively (7). Signal I has generally been attributed to a chlorophyll radical on the reactive center of Photosystem I, i.e., P700 (8-10). Since very little or no Signal II is induced by light at 77°K, the contribution of Signal II in Spectrum C of Fig. 1 is minimal. Therefore the appearance and disappearance of Signal I can be followed by measuring the amplitude of the peak at 3288.7 gauss under these experimental conditions.

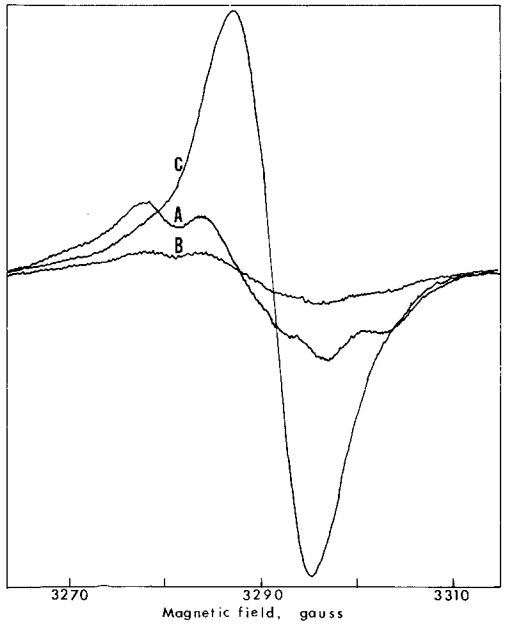

Fig. 1: Light induced EPR signals in chloroplasts. A, chloroplasts (chlorophyll, 3 mg/ml) frozen in the dark after brief illumination; B, after thawing the same sample in the dark and keeping at 0°C for 30 min; C, the same sample after illumination. First derivative EPR spectra were recorded at 77°K with the following instrument settings: microwave frequency 9.22 GHz: Power, 1.6 mW; modulation amplitude, 4G; gain 2.5×10^5; scan rate, 6G/min.

As shown in Fig. 2, Signal I increased rapidly at 77°C upon illumination. With the high concentration of chloroplast (3 mg chlorophyll per ml) used in the experiment a portion of the sample was photoactivated at a much slower rate which may account

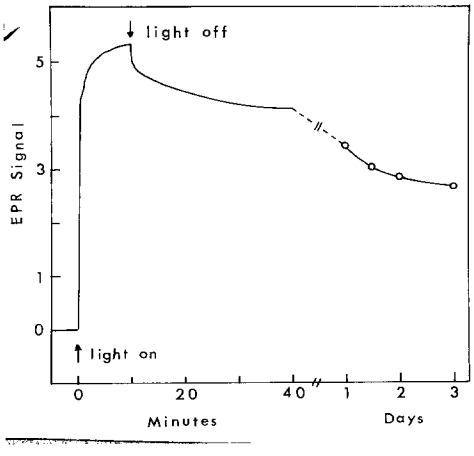

Fig. 2: The appearance and decay of Signal I. The field was set at 3288.7 gauss and other conditions were similar to Fig. 1.

for the gradual rise of the signal after the initial jump. In the dark, the signal showed complex decay kinetics at 77°K, with a fast initial phase followed by a slow phase with rates slower than a first order decay rate. The rate of disappearance of Signal I in the first 30 min. was much faster than the rate calculated from a half-life of 8 hrs. for ferredoxin at 77°K (3).

Figure 3 shows the EPR absorptions in the regions on either side of the free radical absorptions for two typical experiments. These intense signals obscure all other features between approximately g = 1.96 to g = 2.04. In the irradiated samples, spectral features arising from reduced ferredoxin can be seen at g = 1.94 and g = 1.87. In addition, there is another nonheme iron center which gives rise to absorptions at g = 1.90 and 1.81 and is present in small amounts in the dark control. Small absorptions are consistently observed in the region of g = 2.09 to g = 2.12 which are not part of the reduced ferredoxin spectrum and might be part of the spectrum of the second nonheme iron center. The signal from the second nonheme iron center is reminiscent of the nonheme iron center occurring in mitochondria in the cytochrome $\underline{b}$, $\underline{c}_1$ region (11). Table I shows the quantitative results of these two experiments. Both Photosignal I and reduced ferredoxin were unobservable in the dark control, which did, however, have the customary

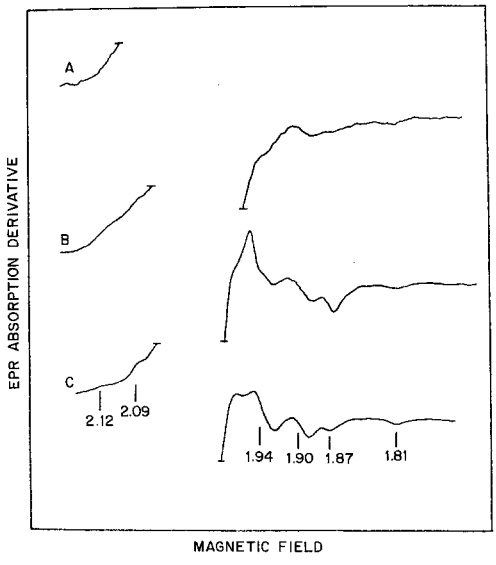

Fig. 3: EPR signal of chloroplasts (chlorophyll, 3 mg/ml) observed at 1.5°K after irradiation at 77°K. A, dark control; B, after 40 min.; C, another sample after 60 min. The intense free radical signal obscures the region near g = 2.

Table I. Amounts of Paramagnetic Centers Induced by Light

Irradiation	Photosignal I	Reduced Ferredoxin	Second Nonheme Iron
Dark Control	0	0	0.2
40 min.	10	0.6	0.3
60 min.	14	1.0	1.3

Irradiation was carried out at 77°K and EPR measurement at 1.5°K. The sample contained 3 mg chlorophyll per ml. The concentration of photo-induced centers is expressed as μ moles/g of chlorophyll.

resting Photosignal II and a small amount of the second nonheme iron signal. On irradiation, both Photosignal I and reduced ferredoxin increased approximately in proportion to the irradiation time but not in equal amounts. The ferredoxin absorption always was only 5-10% of Photosignal I. A similar proportion was observed when the irradiation was carried out at liquid helium temperature. The absorption due to the second nonheme iron center was of comparable magnitude to that of the reduce ferredoxin.

DISCUSSION

The present study leaves the role of ferredoxin as a primary electron acceptor in photosynthesis completely open. Vernon and co-workers (12) found that in small sub-chloroplast particles, there is a 1:1 ratio between the number of spin of Signal I and the change in P700 during illumination. In whole spinach chloroplasts, the spin can be twice as much as P700 (8). Taking this factor into consideration, the presently observed ferredoxin signal is still only about 10-20% that of Photosystem I cation radical. The result indicates that either ferredoxin is the primary electron acceptor and can pass electrons to secondary acceptors at liquid nitrogen and liquid helium temperatures, or the primary acceptor is not ferredoxin but can transfer electrons to it at these temperatures. Theoretically these two mechanisms may be distinguished by comparing the kinetics of the appearances of the ferredoxin and P700 signals upon illumination. However, such kinetic information was not obtained with the present experimental set-up. The observation of the P700 signal in P-D10 particles, a subchloroplast preparation that is essentially devoid of ferredoxin (9), seems to favor the second mechanism if the primary acceptc

is not altered by the treatment during preparation. The nature and function of the newly observed nonheme iron center (g = 1.90 and 1.81) remain to be investigated.

The primary step in photosynthesis involves a charge-separation at the photoactive center. The primary electron donor in Photosystem I is generally believed to be a chlorophyll molecule in the P700 unit (9, 10, 13), but no conclusion has been reached on the structur of the primary acceptor. Fuller and Nugent (14) have proposed pteridines as the primary acceptor for both green plants and bacteria. Recently Bobst (15) demonstrated the similarity between Signal II and a radical species of a pteridine derivative. This radical can be induced by red light in the presence of chlorphyll a. However, the lack of photoinduced Signal II (c.f. Fig. 1) at 77°K tends to counteract this evidence, unless freezing to 77°K changes the structure of the photoactive center and alters the primary acceptor. Based on model studies, the suggestion has also been made that flavin may serve as the primary electron acceptor of Photosystem I (16). "P430" which has recently been proposed as a possible primary electron acceptor (2), may well be a bound form of flavin or ferredoxin. The absorption peak at 450 mμ of flavin may be shifted to the 430 region when the flavin and chlorophyll form a molecular complex (17). A red shift of the ferredoxin 420 mμ peak may also be demonstrated (18). If Signal I is a chlorophyll cationic radical, the corresponding radical species of its acceptor remains to be detected. The interesting discovery of a photoinduced ferredoxin radical in chloroplast by Malkin and Bearden (3), however, does not fully permit the role of this nonheme iron protein to qualify as a primary electron acceptor in view of the present results. The suggestion that Signal I may be composed of both the signals of electron donor and acceptor is consistent with the present observations.

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