THE DETECTION OF A BOUND FERREDOXIN IN THE PHOTOSYNTHETIC LAMELLAE OF BLUE-GREEN ALGAE AND OTHER OXYGEN EVOLVING PHOTOSYNTHETIC ORGANISMS.

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Summary

The presence of an electron transport component with an EPR spectrum similar to that of a ferredoxin has been demonstrated in the blue-green alga Anabaena cylindrica, the green alga Euglena gracilis, and in chloroplasts from sorghum (Sorghum bicolour) and beans (Phaseolus vulgaris). The component is photoreduced at 77°K and is very similar to that previously reported in spinach. It seems likely that this component is a primary electron acceptor in photosynthesis in all of these organisms.

It has been shown that a chloroplast component with an electron paramagnetic resonance (EPR) spectrum similar to that of ferredoxin is photoreduced at 77°K or lower temperatures in spinach chloroplasts (1). This ferredoxin is associated with photosystem I in subchloroplast particles prepared with digitonin or using the french press (2)(3). It has been proposed that this component is the primary electron acceptor of photosystem I. We have now investigated the occurrence of this component in blue-green and green algae and higher plants other than spinach.

Materials and Methods

Lamellar particles were prepared from the blue-green alga Anabaena cylindrica as described by Smith, Noy & Evans (4). Chloroplasts were prepared from Euglena gracilis essentially as described by Katoh and San Pietro (5), from 14 day old greenhouse grown beans (Phaseolus vulgaris) by the procedure of Gregory and Bradbeer (6) and from greenhouse grown sorghum (Sorghum bicolour) by the procedure of Hall et al (7).

Samples for EPR spectroscopy were prepared and the spectra obtained using a Varian E4 spectrometer essentially as described previously (3).

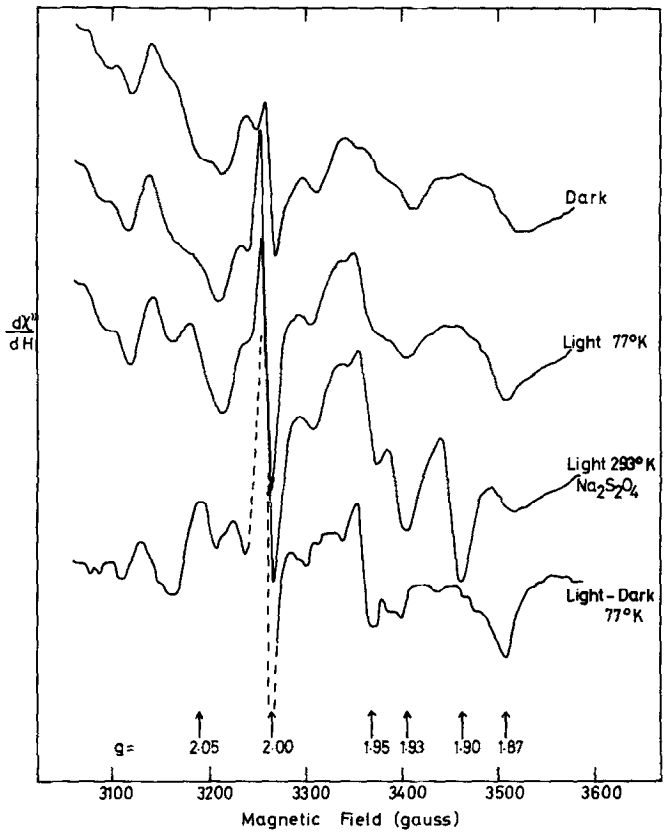

Figure 1
Low temperature EPR spectra of A. cylindrica lamellae.
(1) In the dark.
(2) Prepared and frozen in the dark and illuminated for 3 min. at 77°K.
(3) Prepared in the presence of 0.02M $Na_2S_2O_4$ (pH 7.5), illuminated for 3 min. at room temperature and frozen in the light.
(4) Difference spectrum of lamellae illuminated at 77°K minus dark. The lamellae preparation contained 2 mg. of chlorophyll per ml. EPR spectra were recorded at 20°K with the following instrument settings. Frequency 9.170 GHz; power 10 mW; modulation amplitude 6.3 gauss; scan rate 1000 gauss/min; gain 1.25×10^3.

Results and Discussion

Fig. 1 shows the EPR spectrum in the $g = 2.00$ region of a lamellar particle preparation of the blue-green alga Anabaena cylindrica. In the dark the spectrum shows a small signal at $g = 2.00$ due to chlorophyll and signals due to manganese. On illumination at 77°K a new signal appears with components at $g = 1.95$ and 1.87, this can be seen most clearly in the

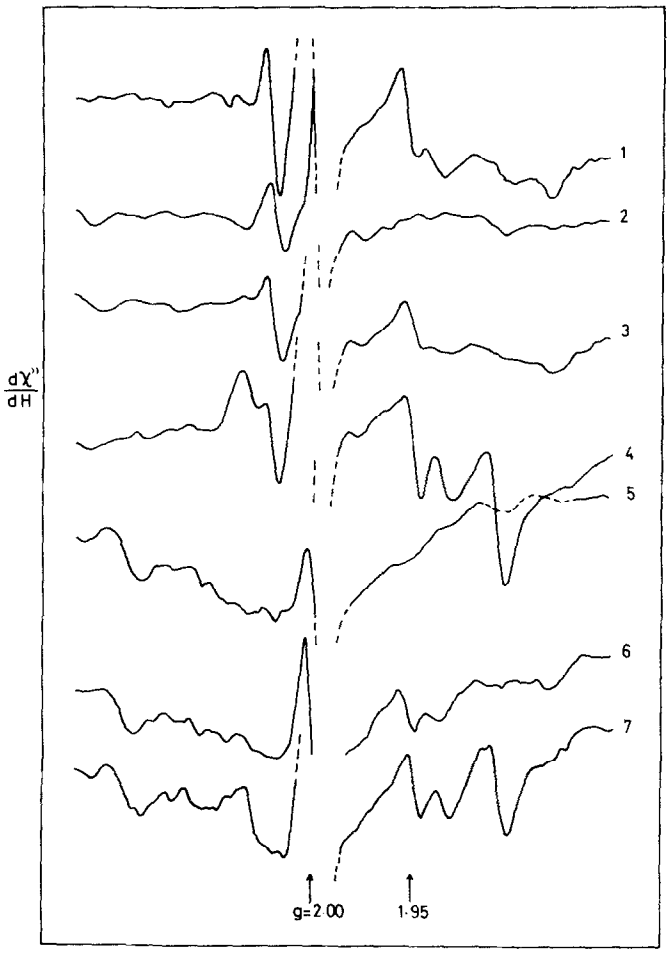

Figure 2
Low temperature EPR signals in
(1) Spinach chloroplasts illuminated at 77°K.
(2) Bean chloroplasts in the dark.
(3) Bean chloroplasts frozen in the dark and illuminated at 77°K for 3 min.
(4) Bean chloroplasts prepared in the presence of 0.02M $Na_2S_2O_4$ pH 7.5, illuminated at room temperature and frozen in the light.
(5) Sorghum chloroplasts in the dark.
(6) Sorghum chloroplasts frozen in the dark and illuminated for 3 mins. at 77°K.
(7) Sorghum chloroplasts prepared in the presence of 0.02M $Na_2S_2O_4$ and illuminated at room temperature and frozen in the light. All the preparations contained 2 mg. of chlorophyll per ml. Spectra were recorded as in Fig. 1, except that the gain was 1×10^3 for the bean chloroplasts and 2.5×10^3 for the sorghum chloroplasts.

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light minus dark difference spectrum. If the particles are illuminated at room temperature in the presence of sodium dithionite a second signal with components at g = 1.93 and 1.90 is seen. These changes are essentially

the same as those observed previously in spinach chloroplasts. The signals can only be seen at temperatures below 30°K.

Figure 2 shows similar spectra obtained with chloroplasts prepared from Phaseolus vulgaris and sorghum, and for comparison spinach chloroplasts on illumination at 77°K. In all of these preparations illumination at 77°K results in the appearance of a signal at g = 1.95 and 1.87 corresponding to the reduction of a ferredoxin, on illumination at room temperature in the presence of sodium dithionite the signals at g = 1.93 and 1.90 are also seen.

We have observed similar signals in chloroplasts from Euglena gracilis and whole cells of Chlorella vulgaris, although the signals are very small and difficult to observe in these organisms.

The presence of a bound ferredoxin in the photosynthetic lamellae of such a diverse group of organisms, and the demonstration that in all these organisms the ferredoxin can be photoreduced at 77°K, is strong supporting evidence for the proposal that this ferredoxin is the primary acceptor of electrons in the photosynthetic electron transport system of all oxygen evolving organisms.

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