CHARACTERIZATION OF A NEW PHOTO-ESR SIGNAL ASSOCIATED WITH PHOTOSYNTHESIS*

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SUMMARY

A new photo-active electron spin resonance signal has been observed in a blue-green alga. By using fully deuterated organisms and manipulating the iron content of the growth medium, we can identify the neutral semiquinone form of the flavoprotein flavodoxin as the source of this signal. This ESR signal is associated with photosystem I activity. Kinetic data indicate that this flavodoxin is probably not the primary electron acceptor of photosystem I.

INTRODUCTION

Isotopically substituted photosynthetic organisms have been used for some time in studies directed toward the characterization of the species responsible for ESR[1/] Signals I and II (1,2). Spectral perturbations produced by isotopic substitution greatly facilitate characterization of these signals in vivo. We describe here a new photo-active signal whose detection and characterization has been made possible by the use of fully-deuterated organisms (3).

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1/ Abbreviations: ESR, electron spin resonance, EDTA, ethylenediaminetetraacetic acid; CAT, computer of average transients; Fld, oxidized flavodoxin; $\dot{\text{F}}\text{ldH}$, flavodoxin semiquinone; FldH_2, reduced flavodoxin; RdH_2, reduced ferredoxin NADP^+ reductase.

MATERIALS AND METHODS

The thermophilic blue-green alga Synechococcus lividus was cultured at 45° in $^{1}H_2O$ and $^{2}H_2O$ according to methods previously described (4-6). The availability of iron to the algae was controlled by varying the total iron content of the growth medium and by using or omitting EDTA (7,8). Scanlon S-808 aqueous sample cells were used for all ESR measurements. Flavodoxin, from S. lividus (9), was dissolved in 0.01 M sodium phosphate, 0.05 M EDTA $^{1}H_2O$ or $^{2}H_2O$ buffers of pH 7.5 (^{1}) (pH 7.9 (^{2})) for generation of the semiquinone radical (3). Spectra were recorded as described earlier (3) or as described in the text. In the ESR experiments, slurried whole cells were irradiated with light from a Xenon arc lamp passed through Baird-Atomic B3 interference filters (20 nm band pass). Repetitive scans with programmed light and dark cycling was accomplished with a Fabritek (Nicolet) 1072 computer in conjunction with automatic routing and shutter control (10).

RESULTS AND DISCUSSION

As indicated in our previous report (3), ESR signals from ^{2}H-flavin radicals are much more amenable to identification and quantitative study than are ^{1}H-flavin radicals because of the characteristic, resolved hyperfine structure of the ^{2}H-flavin system. These advantages useful in the study of possible flavoprotein ESR signals from photosynthetic organisms, particularly in the presence of the ESR photosignals designated Signal I and Signal II (1,2). Comparing ^{1}H and ^{2}H-organisms, Signal I collapses from a 7 gauss line width in ^{1}H systems to a 3 gauss line, while Signal II changes from a structured 20 gauss line in ^{1}H systems to an unstructured 6 gauss line in ^{2}H-systems. The ^{1}H-flavoprotein

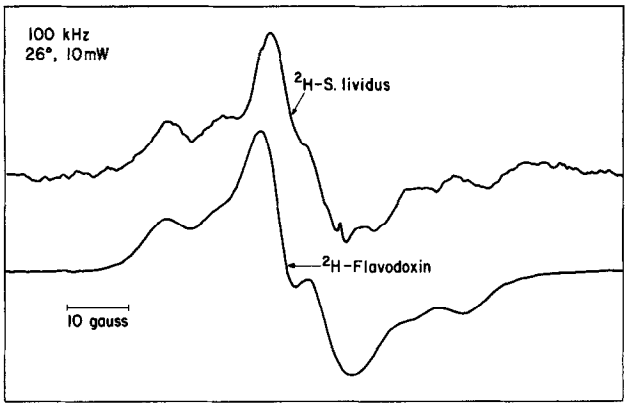

Figure 1 Top: The ESR spectrum of ^{2}H-S. lividus cells (32 CAT scans). Bottom: The ESR spectrum of the flavodoxin neutral semiquinone radical in ^2H_2O buffer. The similarity of the two spectra is striking. Small differences are due to the fact that the in vivo radical flavodoxin is tumbling in solution of greater viscosity than ^2H_2O buffer. (^{2}H-flavin radicals constitute a new class of highly useful intrinsic spin labels (12).)

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neutral free radical signal is an essentially featureless 20 gauss line (11) whereas the ^{2}H-flavoprotein yields a structured 13 gauss line (3), easily identified and monitored for photoactivity, even in the presence of Signals I and II.

Figure 1 shows ESR spectra obtained from fresh, fully deuterated S. lividus cells and from the radical form of the fully deuterated flavoprotein flavodoxin in ^2H_2O buffer. The similarity of the two spectra is striking and leaves little doubt that the in vivo signal is due to a flavin radical. The in vivo signal of Fig. 1 is a dark signal recorded from cells previously heated to 65° to destroy Signal II, so there are no spectral contributions from Signals I or II. In the light the intensity of this signal drops and, as explained above and illustrated in Fig. 2, the photo-

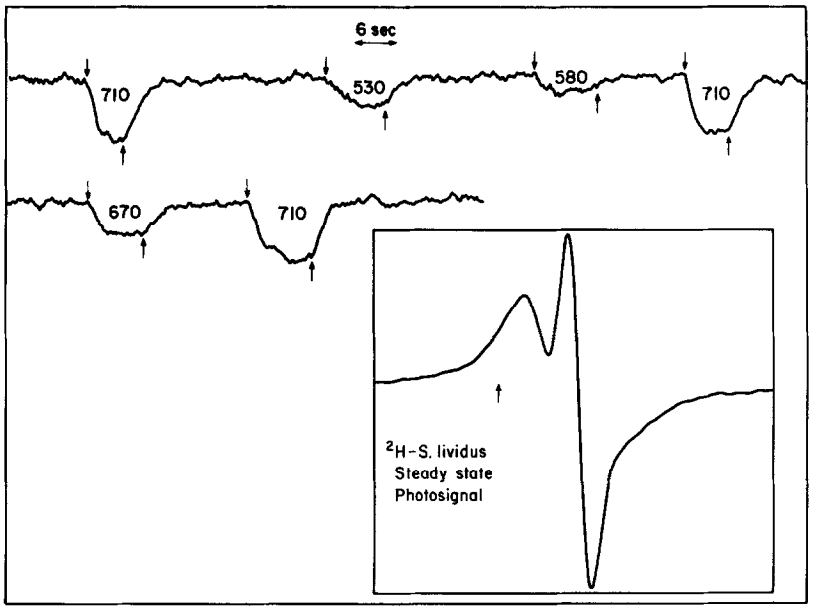

Figure 2 The kinetic behavior of ^{2}H-S. lividus cells after heating to destroy Signal II. The arrow on the inset spectrum indicates the point of observation. (The sharp line in this spectrum is Signal I.) Wavelengths from 530 to 710 are effective in reducing the flavodoxin signal. The down arrows indicate light on, the up arrows, light off.

response can be monitored easily in fully deuterated cells. The kinetics of the drop in signal intensity depends on wavelength, light intensity, and the nature of the previous treatment. We have not yet investigated these parameters in detail. If the cells are kept in continuous light, the semiquinone signal slowly builds to about its previous dark level, concomitant with the approach of Signal I to a steady state level. The response time of the flavodoxin signal is about 200 milliseconds, while that of Signal I is about 100 milliseconds. In frozen cells, the semiquinone signal is unchanged in the light as Signal I grows. We

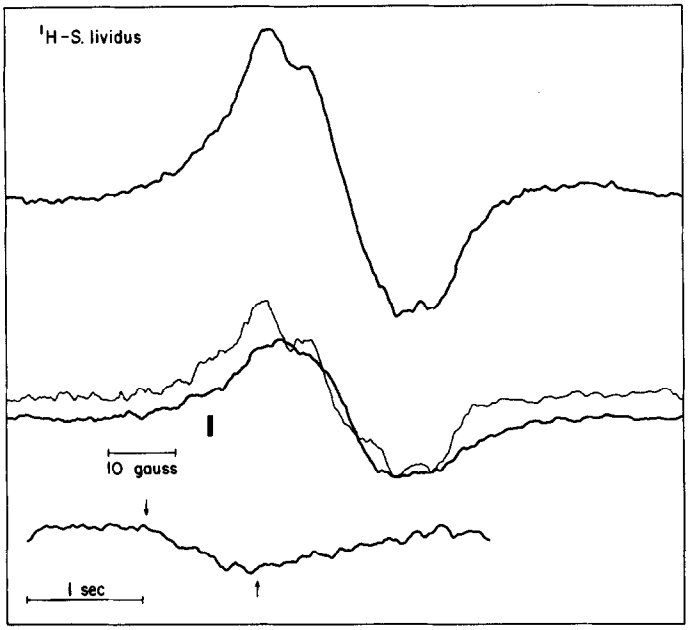

Figure 3 Top: The dark signal from ^{1}H-S. lividus cells, 25°. This signal is a mixture from flavoprotein free radical and Signal II, as Signal II decays very slowly in the dark. Middle: Heavy line, the dark signal at 25° after heating to 65° for 15 minutes. This signal is characteristic of ^{1}H-flavodoxin. Fine line, the flavodoxin spectrum subtracted from the top spectrum. The net signal is Signal II. Note that at the upfield extreme, Signal II drops directly to the baseline while the flavodoxin signal characteristically tails. All these spectra are CAT accumulations of 16 0.5 minute scans. The modulation amplitude was 2 gauss, modulation frequency, 100 kHz, microwave power, 2mW. Bottom: The kinetic behavior of the flavodoxin signal in the light (at 25° with algae heated to destroy Signal II). The signal was monitored with a time constant of 0.03 sec., modulation amplitude 5 gauss, modulation frequency 100 kHz, and microwave power 2 mW. The down arrow indicates light on, the up arrow, light off. The bar indicates the field positions at which the signal was monitored.

have confirmed this kinetic behavior at room temperature and at 45° with unheated cells by monitoring the wings of the flavodoxin spectrum at high ESR microwave power levels. At high power levels any "tail" of Signal II will be saturated while the flavodoxin signal is emphasized (12).

Figure 3 shows the flavin radical signal obtained with ^{1}H-*S. lividus* cells. In the dark, both Signal II and flavodoxin semiquinone signals are evident. Because these two signals have similar g values and are both about 19-20 gauss broad, Signal II obscures the rather featureless ^{1}H-flavodoxin radical. However, by heating the cells to 65° for 15 minutes, Signal II is destroyed, while photosystem I remains competent. Spectrum C of Fig. 1 was obtained in this way. The data of Fig. 3 are presented mainly to show the qualitative similarity between the ordinary and fully deuterated organisms, i.e., the deuterated *in vivo* system is a valid mimic of the ^{1}H system. It should be noted that the ESR parameters of the signal described here are quite different from those of "Signal III" described recently by Weaver (13).

If *S. lividus* is cultured in the presence of high levels of EDTA-iron complex, no *in vivo* flavin signal is observed. This observation, and the observation that the flavin signal is associated with photosystem I activity (14) leads us to conclude that this signal must be due to the flavoprotein flavodoxin. Flavodoxins are known to replace ferredoxin in many microorganisms grown under conditions of iron deficiency (15), hence, our data provides direct evidence that in the cell flavodoxin shuttles between the semiquinone radical and fully reduced state during electron transport associated with photosystem I, as suggested by others (16).

Flavodoxin is probably involved in both $NADP^+$ reduction and cyclic phosphorylation, which could lead to complex kinetics. It appears plausible that $NADP^+$ reduction may result from the following series of reactions:

(1) $2FldH^{\cdot} + 2e^- + 2H^+ \longrightarrow 2FldH_2$

(2) $RdH_2 + FldH_2 + NADP^+ \rightleftharpoons RdH_2 + Fld + NADPH + H^+$

(3) $FldH_2 + Fld \rightleftharpoons 2FldH^{\cdot}$

These reactions result in a net reduction of $NADP^+$ and the maintenance of a high steady state level of $FldH^{\cdot}$.

Reaction 2 indicates a pure catalytic function for the fully reduced form of ferredoxin $NADP^+$ reductase, as RdH_2 appears on both sides of the equation. Two electrons are transferred from fully reduced flavodoxin to $NADP^+$, and reaction 2 is drawn to completion by the reaction of fully oxidized flavodoxin via the highly favored reaction 3 (16). We tend to favor a mechanism of $NADP^+$ reduction that does not involve the semiquinone (free radical) form of ferredoxin $NADP^+$ reductase,[17] as we have searched for a flavin semiquinone signal (light and dark) from deuterated C. vulgaris and deuterated S. lividus grown in EDTA-iron (no flavodoxin) without success.

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