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Iron-Sulfur Centers and Activities of the Photosynthetic Electron Transport Chain in Iron-Deficient Cultures of the Blue-Green Alga *Aphanocapsa*[1]

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GERHARD SANDMANN[2] AND RICHARD MALKIN
Division of Molecular Plant Biology, University of California, Berkeley, California 94720

ABSTRACT

Cultures of the blue-green alga, *Aphanocapsa*, were grown under iron-limiting conditions and changes in concentration of redox components of the photosynthetic electron transport chain, particularly iron-sulfur centers, were monitored by spectroscopic methods. A moderate iron depletion (1/10 of the normal concentration) had little effect on photosynthetic electron transport reactions and growth. Nevertheless, the amount of membrane-bound non-heme iron decreased sharply, and ferredoxin was nearly totally replaced by a flavin-containing protein, flavodoxin. Severe iron-deficiency (1/100 of the normal concentration) was accompanied by growth inhibition and decreased rates of photosynthetic electron flow. The Photosystem I reaction center was most affected by iron depletion as evidenced by a decrease in the amounts of iron-sulfur centers A, B, and X. However, formation of other redox proteins, even those that do not contain iron, was also inhibited by severe iron deficiency.

Various Fe-containing proteins are involved in electron transfer processes (17) in energy-transducing membranes. In the photosynthetic electron transport chain, heme-containing proteins (cytochromes) and non-heme Fe-containing proteins (Fd-like proteins) can be found. Except for the plastidal 'Rieske' Fe-S center, which is a component of the Cyt b_6-f complex (5), other Fe-S centers are located in the electron acceptor complex of PSI (9).

In contrast to mitochondria, where Fe deficiency has been reported to result in a decrease of respiratory Fe-S centers and respiratory function (8, 13), a detailed study of the effect of limited Fe supply on plastidal Fe-S centers as well as on photosynthetic electron transport has not been made. There are reports of decrease in P_{700}, the reaction center Chl of PSI and c-type Cyt in algae grown at low Fe concentrations (2, 12) but there are no reports of the effect of such growth conditions on membrane-bound Fe-S centers. In the present study, we have investigated the effect of Fe deficiency on the formation of Fe-S centers and other redox components of PSI and on the plastidal Cyt b_6-f complex in the blue-green alga *Aphanocapsa*. The effect of Fe deficiency on partial reactions of the photosynthetic electron transport chain was also considered.

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[2] On leave from Lehrstuhl für Physiologie und Biochemie der Pflanzen, University of Konstanz, Konstanz, Federal Republic of Germany.

MATERIALS AND METHODS

Cell Culture. *Aphanocapsa* 6714 was cultured in a liquid mineral medium as previously described (18). The standard Fe concentration for growth was 36 μM and this was decreased to 3 or 0.3 μM in Fe-deficient cultures. Fe-deficient cultures were inoculated with cells grown for several generations in 3 μM Fe-containing medium. Cell growth was measured as PVC[3] per ml suspension in calibrated microcentrifuge tubes of 80 μl capacity.

EPR Spectroscopy of P_{700} and Fe-S Centers. The harvested cells were washed twice in 5 mM K-phosphate (pH 6.0) containing 10 mM NaCl and 5 mM EDTA to remove extraneous paramagnetic metal ions. The cells were then resuspended in the same medium in the absence of EDTA and frozen at −20°C. Samples were thawed prior to refreezing at 77 K for EPR studies. EPR measurements were carried out in calibrated quartz tubes (3 mm i.d.) at 12 to 15 K in a modified JEOL X-band spectrometer operating with 100 kHz field modulation (15). A microwave power of 5 mw and a modulation amplitude of 10 g were routinely used for detection of iron sulfur centers while 0.02 mw microwave power and 2.5 g modulation were used for P_{700}.

Chemical Detection of Redox Proteins and Non-Heme Fe. *Aphanocapsa* cells, suspended in 0.2 M Tris-HCl buffer (pH 7.5), were broken in a Ribi cell fractionator at 40,000 p.s.i. Plastocyanin and Cyt f-556 were determined by difference optical spectroscopy after chemical oxidation and reduction of the samples as described for other blue-green algae (16). The concentration of P_{700} was measured according to the procedure of Almon and Böhme (1). Fd and flavodoxin were determined in the supernatant of a 70% $(NH_4)_2SO_4$ precipitation of the cell extract from the absorbance difference spectrum obtained after reduction of the reference sample with solid dithionite (~0.5 mg/ml). Differential extinction coefficients (oxidized minus reduced) of 4.4 mM^{-1} cm^{-1} at 420 mM and 9.5 mM^{-1} cm^{-1} were used for Fd and flavodoxin, respectively. Membrane-bound non-heme Fe was determined as described by Lovenberg *et al.* (6). All spectra were recorded in a Cary model 219 spectrophotometer at 22°C.

Electron Transport Reactions with Spheroplast Preparations. Spheroplasts of *Aphanocapsa* cells were prepared as previously described (18). They were osmotically shocked in a reaction mixture that contained 3 mM NaCl, 10 mM $MgCl_2$, and 10 mM K-phosphate (pH 7.5). Electron transfer reactions were monitored by following O_2 uptake or evolution in a Rank O_2 electrode. Samples were illuminated with light (170 w/m^2) that was passed through a 530-nm cut-off filter (Corning No. CS 3-68). For comparison of the different partial reactions, rates were expressed in μeq/h·mg Chl.

[3] Abbreviations: PVC, packed volume of cells; EPR, electron paramagnetic resonance.

Chl Determination. Chl was determined in 80% acetone according to MacKinney (7).

RESULTS

EPR Spectra of Fe-S Centers in *Aphanocapsa*. The first-derivative EPR spectra of Fe-S centers were recorded in cells of *Aphanocapsa* that were made permeable to exogenously added chemicals by a freeze-thaw cycle. All Fe-S centers present in the cells could be identified by their characteristic EPR g values in the reduced state. A discrimination between the EPR signals of six different Fe-S centers was possible by adjusting the redox potential in the cells with durohydroquinone or dithionite at different pH values or by photoreduction of the centers at cryogenic or room temperatures (Figs. 1 and 2).

At a redox potential of approximately +60 mv, only the high-potential Rieske Fe-S center was observed, as indicated by its characteristic $g = 1.89$ signal (Fig. 1C) (5). Lowering the redox potential to approximately −330 mv resulted in the appearance of an additional Fe-S signal at $g = 1.92$ (Fig. 1B). This signal has been associated with a membrane-bound NAD(P)H-quinone oxidoreductase present in blue-green algae. This signal is different from that of Fe-S center B, which also has a $g = 1.92$ component but appears at lower redox potentials. The redox potential of

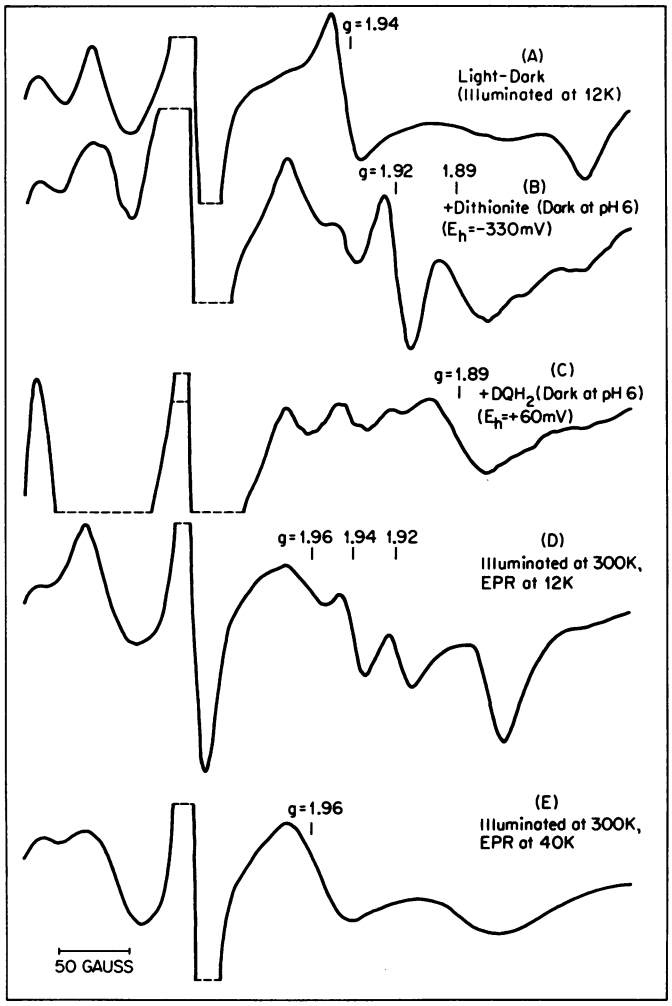

FIG. 1. Determination of Fe-S centers in frozen-thawed *Aphanocapsa* cells. Conditions for each sample are indicated in the respective trace. EPR spectra were recorded at 13 K, except in (E), which was recorded at 40 K. Relative amplifier gains were: (A), × 2.5; (B), × 10; (C), × 10; (D), × 1; (E), × 2.

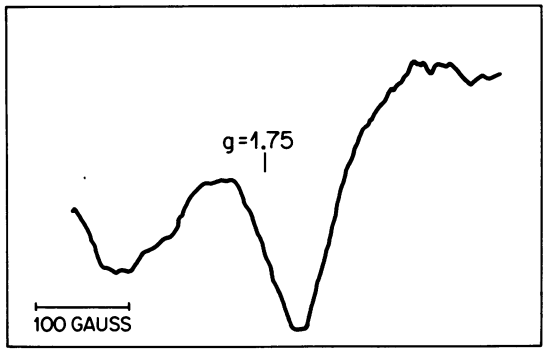

FIG. 2. Fe-S center X in frozen-thawed *Aphanocapsa* cells. Cells were suspended in 0.1 M glycine buffer (pH 10) and reduced with a small amount of sodium dithionite. The sample was frozen to 77 K during continuous illumination with white light. EPR spectra were recorded at 7 K.

sodium dithionite at pH 6.0 was not sufficiently low to reduce the Fe-S centers associated with the PSI primary electron acceptor complex (10). The reduction of Fe-S centers A and B as well as Fd could be achieved by photoreduction at room temperature (Fig. 1D). Illumination resulted in the appearance of the EPR signals of reduced center A ($g = 1.94$), center B ($g = 1.92$), and Fd ($g = 1.96$). When the EPR spectra were recorded at 40 K instead of 12 K, only the characteristic EPR g values of Fd ($g_x = 1.89$, $g_y = 1.96$, and $g_z = 2.05$) were observed (Fig. 1E) as well as signals from P_{700} and Signal II in the $g = 2.00$ region. The signals from centers A and B are broadened at this higher temperature and these components are no longer detectable. Following illumination of dark-adapted samples at 12 to 15 K, photoreduction of only center A was observed (Fig. 1A), and the g values of this center at $g_x = 1.86$, $g_y = 1.94$, and $g_z = 2.05$ are clearly identifiable under these conditions (10). In Figure 1D, the g_x value of center A has shifted to 1.89, and this has been shown to occur when both centers A and B are found in the reduced state (9, 10). Center X could be reduced by illumination at room temperature in the presence of dithionite at alkaline pH values; this center is characterized by a g value of 1.75 (Fig. 2). The amplitude of the EPR signals is directly proportional to the amount of the reduced Fe-S center and these signals have been used to study the effect of Fe deficiency on the respective paramagnetic centers. The signals described under the specific conditions of Figure 1A through 1E and Figure 2 were used to determine the relative concentrations of Fe-S centers as a function of iron depletion. In addition, the relative amount of P_{700} could be estimated from the difference spectra from EPR spectra as well as by the optical method of (1). The P_{700} EPR signal in the $g = 2.00$ region was corrected for signal II, which does not show any change in amplitude after low temperature illumination. Except for soluble Fd, which can be easily isolated, no absolute calibration of the EPR signals was possible and only relative amounts of the membrane-bound Fe-S centers could be determined.

Decrease of Redox Proteins in Fe-Depleted Cells. All membrane-bound Fe-S centers and P_{700} were found to decrease when cells were grown in a medium supplemented with low amounts of Fe (0.3 μM). The Fe-S centers of PSI (centers A, B, and X) were found at a level of 10 to 20% of that of control cultures grown in the presence of 36 μM Fe (Table I). The Rieske Fe-S center of the Cyt b_6-f complex showed a less pronounced decrease but was still diminished significantly as compared with its relative concentration in Fe-sufficient cultures. Furthermore, the relative amount of Cyt f-556, another Fe-containing redox protein of the Cyt complex, was decreased to the same extent as the Rieske center in Fe-deficient cultures.

In addition to these Fe-containing redox proteins, a decrease

Table I. *Relative Amount of Fe-S Proteins and P_{700} in Aphanocapsa Cells Grown with Different Fe Concentrations*

	Fe Concn. during Growth (μM)		
	36	3	0.3
	units/mg Chl[a]		
Component			
Center A	0.45 (100)	0.42 (93)	0.06 (13)
Center B	0.21 (100)	0.18 (86)	0.04 (19)
Center X	0.24 (100)	0.20 (83)	0.03 (13)
'Rieske' center	0.14 (100)	0.14 (100)	0.04 (29)
P_{700}	1.57 (100)	1.37 (87)	0.43 (27)

[a] Units are relative; per cent is expressed as the amount in cultures grown in the presence of 36 μM Fe.

Table II. *Fe-Dependent Concentrations of Various Redox Proteins and Membrane-Bound Non-Heme Iron in Aphanocapsa Cells*

	Fe Concn. during Growth (μM)		
	36	3	0.3
	nmol/μmol Chl		
Component			
P_{700}	2.5	2.6	0.6
Cyt *f*-556	1.9	1.7	0.5
Plastocyanin	2.7	3.2	0.9
Flavodoxin	ND[a]	4.9	5.3
Fd	9.4	0.5	ND
PHOTOREDUCIBLE	3.8	ND	ND
Membrane-bound non-heme Fe	181	72	13

[a] Not detected. Chl concentrations were 1.9, 1.8, and 1.5 μg/ml PVC in cultures grown with 36, 3, or 0.3 μM Fe^{3+}.

Table III. *Electron Transport Reactions of Thylakoids from Aphanocapsa Grown with High or Low Fe*

Reactions[a,b]	Fe Concn. during Growth (μM)	
	36	0.3
	μeq/h·mg Chl	
(a) $H_2O \rightarrow NADP$	83	33 (40)
(b) $H_2O \rightarrow MV$	183	56 (30)
(c) Asc/DAD→MV	1164	187 (17)
(d) $H_2O \rightarrow BQ$	409	221 (54)

[a] Rates are expressed as μeq/h·mg Chl or per cent of activity in the cultures grown with 36 μM Fe^{3+}.

[b] Additions: (a) 1 mM NADP; 10 μM Fd; (b) 0.5 mM methylviologen, 0.5 mM azide; (c) 10 μM DCMU, 1 mM ascorbate, 0.1 mM diaminodurene (DAD); (d) 0.2 mM benzoquinone (BQ).

of the relative concentration of plastocyanin and P_{700} to about 30% of control values was observed in Fe-deficient cultures (Tables I and II), although these two components do not contain Fe. The formation of Fd was extremely sensitive to Fe depletion, and at an Fe concentration of 3 μM in the growth medium, most of the Fd was absent and this carrier was replaced by the flavin mononucleotide-containing protein, flavodoxin. However, this replacement was not stoichiometric since, on a molar basis, only half as much flavodoxin was present as Fd. Nevertheless, this amount of flavodoxin exceeded the amount of Fd that was photoreducible at physiological temperatures with steady-state illumination (Table II). Decreasing the Fe concentration during growth from 36 to 3 μM had only a slight effect on other redox proteins (Tables I and II), and the major effect appeared to be related to the decrease in Fd concentration.

Blue-green algae contain large amounts of membrane-bound Fe-storage proteins (4). The presence of membrane-bound non-heme Fe could be demonstrated in *Aphanocapsa* (Table II). Moderate Fe depletion affected this concentration more than the concentration of the Fe-containing redox proteins of the photosynthetic apparatus. With 3 μM Fe in the growth medium, less than 50% of the membrane-bound non-heme Fe was found compared to cultures grown in 36 μM Fe. Further decrease of Fe to 0.3 μM during growth resulted in a sharper decrease in the concentration of membrane-bound non-heme Fe (Table II).

Effect of Fe Deficiency on Photosynthetic Electron Transport Reactions. The decrease of Fe-containing and other redox proteins during growth at low Fe concentrations (0.3 μM) was accompanied by an inhibition of various electron transport reactions in spheroplasts. Spheroplast preparations from cultures grown at an Fe concentration of 3 μM showed the same rates of electron transport from H_2O to NADP or methyl viologen as the control culture grown with 36 μM Fe. Table III compares electron transfer rates for various donors and acceptors. Electron transfer in all cases showed some inhibition, but the most pronounced effect was on the reaction that utilizes only PSI (Asc/DAD → MV) where 83% inhibition was observed in preparations from cultures grown at 0.3 μM Fe. The least sensitive reaction (H_2O → BQ) involved only PSII while reactions involving both photosystems as well as the Cyt *b-f* complex showed intermediate inhibition patterns.

Growth in the Presence of High and Low Fe Concentrations. Growth of cells was determined by PVC from cultures supplemented with 0.3, 3, or 36 μM Fe (Fig. 3). Decreasing the Fe concentration in the growth medium from 36 to 3 μM had no significant effect on the shape of the growth curve. In cultures grown with 0.3 μM Fe, this deficiency resulted in much slower growth. The growth rate estimated from the slope of the growth curve in the stationary phase was only one-third of the value obtained for the cultures grown in the presence of higher concentrations of Fe.

DISCUSSION

Cammack *et al.* (3) described the first systematic study of the bound Fe-S centers in cells of blue-green algae. In the present work using *Aphanocapsa*, it was possible to demonstrate the presence of the EPR signals of all PSI Fe-S centers, the Rieske Fe-S center, and Fd (Figs. 1 and 2). Furthermore, conditions were defined that allowed for obtaining the EPR spectra of individual Fe-S centers in *Aphanocapsa* cells with negligible interference by other centers. These included changes in redox potential and conditions of illumination as well as changes in the temperature of recording EPR spectra. Applying these conditions to a study of the effect of Fe deficiency, it has been possible to examine the relative amounts of various Fe-S centers in response to Fe deficiency. Other methods, such as differential absorbance spectroscopy, which may be convenient for the measurements of other electron carriers, such as Cyt, plastocyanin, and P_{700} (1, 16), cannot be used for the study of membrane-bound Fe-S centers because of the broad absorbance bands and low differential extinction coefficient of Fe-S centers (10).

At an Fe concentration of 3 μM during growth (1/10 of the standard Fe concentration), cell growth and non-cyclic electron transport were unaffected, but Fe limitation was indicated by a decreased pool of endogenous membrane-bound non-heme Fe. Another response to moderate Fe depletion was a substitution of the flavin-containing flavodoxin for the Fe-S protein Fd. This replacement has previously been described in procaryotic algae (19, 21). When Fe was further depleted by reducing the Fe concentration in the growth medium to 0.3 μM, photosynthetic O_2 evolution in *Aphanocapsa* cells was more affected than was respiration, as measured by O_2 uptake in the dark in the presence of reduced pyridine nucleotide. This limiting Fe condition af-

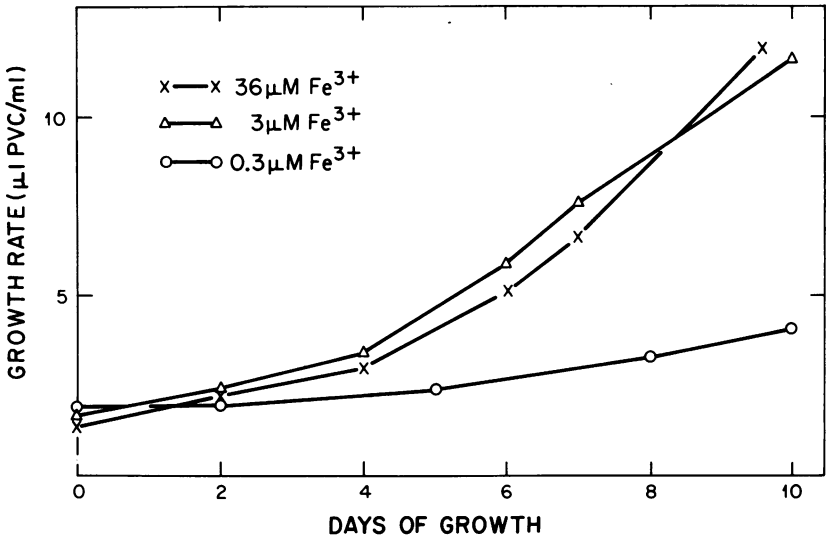

FIG. 3. Growth of *Aphanocapsa* cells in the presence of varying Fe concentrations. Cultures were grown with 36, 3, or 0.3 μM iron and monitored as described in "Materials and Methods."

fected all the photosynthetic electron carriers, even those not containing Fe, in a comparable manner, although components of the Cyt *b-c* complex (Cyt *f* and the Rieske Fe-S center) were slightly less affected than the Fe-S centers of the PSI electron acceptor complex. An inhibition of P_{700} synthesis in Fe-deficient *Anacystis* led to the conclusion that an absence of the PSI Fe-S centers affects the formation of both P_{700} (12) and other Chl proteins (14). This would indicate that all the components of an integral protein complex must be present for the assembly of a functional unit in the photosynthetic membrane. A similar situation would hold for the Cyt *b-c* complex where Fe deficiency affects both the Rieske Fe-S center and Cyt *f*-556, although both of these carriers are obviously Fe-containing proteins. This response of the redox proteins of *Aphanocapsa* towards Fe deficiency differs from observations with *Beta vulgaris* in which Fe deficiency caused a simultaneous decrease of the total photosynthetic unit without changing the ratios of the redox carriers or Chl relative to each other (20). Under conditions where Fe deficiency was most pronounced, we found the same degree of inhibition for PSI reactions as for the synthesis of PSI Fe-S enters while the corresponding effect on PSII was less. Inhibition of electron flow involving both PSI and PSII dependent on Fe concentration during growth also reflects an inhibition in the Cyt *b-c* complex, which contains the Rieske Fe-S center and Cyt *f*.

Inhibition of electron transport involving the complete noncyclic chain in Fe-deficient cells from *Aphanocapsa* was approximately the same as that observed with membrane preparations. However, the inhibition was definitely greater for the PSI reaction than for reactions involving both PSI and PSII. This suggests there is a surplus of PSI over the other integral protein complexes in Fe-sufficient *Aphanocapsa* cells. Melis and Brown (11) determined the PSI to PSII ratio in cells of another blue-green alga, *Anacystis*, and found a ratio of 2:1, indicating an excess of PSI. Using their results and the results of Table II, one can calculate a PSII:Cyt complex:PSI ratio of 1.3:2:2.5 (per 1000 Chl molecules) for *Aphanocapsa*. Assuming that the 50% decrease of PSII activity is indicative of 50% decrease in PSII content, the above ratio changes to 0.6:0.5:0.6 in cultures grown with 0.3 μM Fe. Subsequently, there is a decrease from 1.3 complete electron transport chains to 0.6/1000 Chl molecules. This is a concentration of only 38% of the relative concentration in cells grown without Fe limitation and is in good agreement with the inhibition of overall electron transport observed.

In summary, we conclude that *Aphanocapsa* cells that require Fe for the synthesis of various Fe-containing redox proteins exhibit a varied strategy to deal with Fe deficiency. Moderate Fe depletion is countered by exchanging the Fe-S containing Fd, which is present in excess amounts, by a non-Fe-containing flavoprotein, flavodoxin, and at the expense of internal Fe storage compounds. This adaptation can fully compensate for Fe deficiency, and rates of photosynthetic electron transport as well as rates of growth are unaffected. Greater Fe depletion, however, causes a decrease in Fe-containing redox proteins. In addition, other photosynthetic redox components are also adjusted to lower levels. This, of course, has negative consequences on rates of photosynthesis and cellular growth.

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