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PURIFICATION AND MOLECULAR PROPERTIES OF A SOLUBLE FERREDOXIN FROM *RHODOPSEUDOMONAS CAPSULATA*

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A soluble ferredoxin was purified from the photosynthetic bacterium *Rhodopseudomonas capsulata* and characterized. Unlike *Rhodospirillum rubrum*, where two soluble ferredoxins have been found, only a single species was found in *Rps. capsulata*. The amino acid composition, ultraviolet-visible spectral properties, molecular weight (12000) and biological activity were determined. The ultraviolet-visible spectrum is similar to that of other bacterial ferredoxins, with a maximum when oxidized at 380 nm ($\epsilon = 26.1 \cdot 10^3\ M^{-1} \cdot cm^{-1}$). The possible roles of this ferredoxin in the cellular metabolism are discussed.

Introduction

Ferredoxins have previously been isolated from three photosynthetic bacteria: *Chlorobium limicola* [1,2], *Chromatium vinosum* [3] and *Rhodospirillum rubrum* [4,5]. Ferredoxin is believed to play a vital role as reductant in many reactions of photosynthetic bacteria; H_2 metabolism, N_2 fixation, pyridine nucleotide reduction and CO_2 fixation, but the mode of its reduction is in most cases entirely unknown [6]. *Rhodopseudomonas capsulata* exhibits a diversified metabolism, and is able to sustain growth with the intensive energy demands of nitrogen fixation under at least five different growth conditions: photoheterotrophic, photoautotrophic, microaerobic organotrophic, microaerobic chemoheterotrophic and fermentative (with an added organic oxidant). The mechanism of generation of reducing power (i.e., the mode of ferredoxin reduction) is largely unknown. Studies on the provision of reducing power to nitrogenase require the purification and characterization of soluble low-potential electron carrier(s) as well as auxiliary enzymes (ferredoxin reductases).

Chlorobium has been found to contain two ferredoxins, both containing two [4Fe-4S]clusters [1,2]. Only one ferredoxin has been described for *Chromatium* [3], and this ferredoxin will function in the energy-independent reduction of NAD by hydrogen and hydrogenase [7] as well as linking the reducing power of chloroplasts to nitrogenase [8]. Four ferredoxins have been described for *R. rubrum*. Two of these, ferredoxin III and ferredoxin IV, are membrane bound [5]; the other two, ferredoxin I and ferredoxin II, are soluble [9] and synthesized when cultures are grown photoheterotrophically; only ferredoxin II is synthesized under aerobic, dark conditions [4]. The two soluble ferredoxins also differ in molecular weight and iron-sulfur content. Both ferredoxins are reduced by illuminated chloroplasts; however, ferredoxin I is 2–3-times more effective than ferrodoxin II in reducing nitrogenase [10] or NADP oxidoreductase [5]. Here, we describe the isolation and some molecular properties of a ferredoxin from the photosynthetic bacterium, *Rps. capsulata*. The possible roles of this ferredoxin in the metabolism of the cell are discussed.

Materials and Methods

Bacterial culture. Rps. capsulata, strain B10, was a generous gift from the Photosynthetic Bacteria Group, Department of Microbiology, Indiana University, Bloomington, IN 47401, U.S.A. Cells were grown in a mineral salt medium [11] supplemented with DL-lactate (30 mM) and a limiting amount of ammonium sulfate (2 mM), in 10-l batches illuminated by an array of 16 100-W reflector light bulbs. The culture temperature was maintained at 32°C by circulating tap water through an internal glass cooling coil. After 24 h growth, cultures were harvested with a Sharples supercentrifuge and stored in liquid nitrogen until needed.

Isolation procedure. Purification was conveniently carried out on 50 g of cells. All operations, unless indicated otherwise, were anaerobic in the presence of 2 mM dithionite. Frozen cell paste was thawed in 100 ml of 0.125 M Tris-HCl (pH 8.0) and sonicated for a total of 3 min (with a 30 s break each minute for cooling) with a Branson Sonifier set at maximum output. The extract was loaded, with an argon-preflushed syringe, into 20-ml screw-cap centrifuge tubes, and centrifuged at $250000 \times g$ for 90 min. The supernatant from this centrifugation was applied to a 2.8×6.5 cm DEAE-cellulose column previously equilibrated with 50 mM Tris-HCl, pH 7.4 (used throughout the following procedure as the buffering component). The column was washed with several bed volumes of the buffer, and then with 5 bed volumes of 0.16 M NaCl in buffer. Finally, the ferredoxin was eluted with about 150 ml of buffered 0.25 M NaCl. The eluate was diluted 2-fold with anaearobic buffer, concentrated on a small (1.5×4.5 cm) DEAE-cellulose column, and eluted in a final volume of about 5 ml with 0.4 M NaCl. This fraction was subsequently chromatographed on a 2.1×45 cm column of Sephadex G-50 equilibrated with buffered 0.1 M NaCl, and developed at a flow rate of 0.4 ml/min. The fraction containing the ferredoxin was concentrated on-line by passing the effluent of the Sephadex G-50 column through a small (1.5×4.5 cm) DEAE-cellulose column and subsequently eluting with buffered 0.4 M NaCl. Final purification was achieved by further chromatography on a 1.6×77 cm column of Ultrogel ACA 202 (LKB), equilibrated and developed with anaerobic (but with dithionite omitted) buffer. This step eliminated the dithionite, allowing the determination of the spectrum and acid-labile sulfur. In addition, this step eliminated a contaminating cytochrome *c'* that was sometimes present.

Analytical procedures. The isoelectric point of the ferredoxin was determined by using the first dimension of the two-dimensional electrophoresis system of O'Farrell [12], adapted to a slab gel system. The established pH gradient was determined by measuring the pH of the eluates of successive gel slices equilibrated in distilled water. The proteins were visualized either by Coomassie blue staining, or by a brief immersion in highly effective iron stain (10% thioglycolic acid, 1 mM bathophenanthrolinedisulfonate).

Iron was determined by reaction with bathophenanthrolinedisulfonate [13], acid-labile sulfur through the formation of methylene blue [14]. Purified ($A_{390}/A_{285} = 0.75$) ferredoxin from *Clostridium pasteurianum* was used as the standard for both procedures. For amino acid composition determinations, protein samples were hydrolyzed with 6 M HCl containing 0.2% thioglycolic acid, which prevents the destruction of tryptophan [15]. For the determination of cysteine (as cysteic acid) and methionine (as methionine sulfone) the samples were first subjected to performic acid oxidation.

The C-terminal sequence was determined by carboxypeptidase Y digestion [16]. Cluster extrusion was carried out with freshly distilled dimethyl sulfoxide and thiophenol as described in Ref. 17. All spectral measurements were made with a Cary 219 spectrophotometer using anaerobic cuvettes. Protein concentration was determined by the micro-biuret method [18].

The molecular weight of the ferredoxin was determined by gel filtration on a 50×1.5 cm column of Ultrogel ACA 202 (LKB) equilibrated with 50 mM Tris, 0.25 M NaCl. The column was calibrated in a separate experiment by chromatographing mixtures of blue dextran, myoglobin, cytochrome *c*, aprotinin, insulin and glucagon, and determining the relationship between the logarithm of the molecular weight of the protein species, and its K_d value ($K_d = \frac{V_e - V_o}{V_t - V_o}$) [19].

Biological activity measurements. NADP reduction was carried out essentially as described in Ref. 20. Rates of NADP reduction were calculated from measurements of the increase in absorbance at 340 nm using anaerobic quartz cuvettes containing in a final volume of 2.6 ml the following (in μmol): Tris-HCl (pH 7.8), 100; $MgCl_2$, 60; NaCl, 75; sodium ascorbate, 20; 2,6-dichlorophenolindophenol, 0.2; dichlorophenyldimethylurea, 0.03; NADP, 1; and chloroplast fragments equivalent to 60 μg chlorophyll *a*. Quantities of ferredoxin are given in the text.

C_2H_2 reduction was carried out essentially as described in Ref. 21. Either ascorbate and illuminated chloroplasts or H_2 and hydrogenase were the electron donors to nitrogenase via ferredoxin. (a) When chloroplast fragments were used, the reaction mixtures contained in a final volume of 1 ml: chloroplast fragments equivalent to 35 μg chlorophyll *a*, an ATP-generating system, and the following (in μmol): sodium ascorbate, 8; 2,6-dichlorophenolindophenol, 0.05; dichlorophenyldimethylurea, 0.015; and ferredoxin as indicated in the text. (b) When H_2 (and hydrogenase) was used as source of electrons the reaction mixtures contained in 1 ml: purified hydrogenase from *C. pasteurianum* (0.5 unit per assay) an ATP-generating system and ferredoxin as indicated in the text (1 unit of hydrogenase = 1 μmol H_2 produced per min in the presence of dithionite-reduced methyl viologen). Incubation was carried out in 8-ml anaerobic flasks with a gas phase containing argon (or H_2 in test b) and acetylene at a 9:1 ratio. The reaction was started by addition of 30 μl of a partially purified ferredoxin-free nitrogenase from *Rps. capsulata* (unpublished data*) which produced 1215 nmol C_2H_2/min per ml with dithionite as reductant. Controls run without either chloroplast fragments or ferredoxin showed no significant reducing activity. Rates of acetylene reduction were linearly related to the concentration of ferredoxin added.

Measurement of metronidazole reduction was carried out as described in Ref. 22 using purified hydrogenase from *C. pasteurianum*.

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The purification of nitrogenase from *Rps. capsulata* has been recently described by Hallenbeck et al. [36].

Results

Purification

Using the purification procedure outlined in Materials and Methods, the ferredoxin from *Rps. capsulata* was isolated in a total of four steps with a final yield of approx. 3.2 mg ferredoxin per 50 g cells. This is considerably higher than the yield reported for the isolation of ferredoxins from either light- or dark-grown cells of *R. rubrum* [4]. An anaerobic technique was necessary for optimal recovery of ferredoxin. Early preparations, isolated in the presence of air, had low purity indices (see below) due to the loss of absorbance at 380 nm. Kinetic measurements showed that oxygen reacted with ferredoxin, causing the ferredoxin to lose active-site iron with apparent first-order kinetics. Measurement of the rate of iron loss, either by the decrease in absorbance at 380 nm or by the increased complexation of iron by bathophenanthrolinedisulfonate gave $K = 5.3 \times 10^{-5}\,s^{-1}$. These measurements were carried out in 50 mM Tris-HCl, pH 7.4, it is not known at present if this ferredoxin would be more oxygen stable at higher ionic strengths as has been found for a ferredoxin from *Desulfovibrio africanus* [23].

Molecular properties

Some of the molecular properties of *Rps. capsulata* ferredoxin are summarized in Table I. Gel chromatography gave a molecular weight of 12000 for the ferredoxin; similar values have been obtained for the molecular weights of *R. rubrum*

TABLE I

PROPERTIES OF THE FERREDOXIN FROM *RPS. CAPSULATA*

Molecular weight from gel filtration	12000
Isoelectric point	5.95
Extinction coefficient ($mM^{-1}\cdot cm^{-1}$) at 380 nm	26.1
Iron, sulfur content (mol/mol protein)	
Iron	6.0 ± 1.0 [a] 6.8 ± 0.05 [b]
Acid-labile sulfur	6.4 ± 0.3 [a]

[a] Chemical analysis.
[b] Determined by cluster extrusion.

ferredoxin II and ferredoxin IV, *Mycobacterium flavum* ferredoxin I and *Azotobacter vinelandii* ferredoxin I [6]. On the basis of this molecular weight, chemical determination gave an iron and acid-labile sulfur content of 6.0 ± 1.0 and 6.4 ± 0.3 mol/mol protein, respectively. Isoelectric focusing of the purified ferredoxin gave a p*I* of 5.95 when the ferredoxin was located by iron stain (see Materials and Methods); no protein contaminants were detected by Coomassie blue staining.

The amino acid composition of *R. capsulata* ferredoxin is given in Table II. In common with other ferredoxins, there were a large proportion of acidic residues relative to basic residues, consistent with the ferredoxin's acidic isoelectric point (5.95) (Table I). The compositional relatedness of the *Rps. capsulata* ferredoxin with some ferredoxins from other organisms was assessed by computing the compositional difference $S\Delta Q$ [24]. Briefly, to compute the $S\Delta Q$ values, the amino acid composition of each protein was expressed as residues per 100 residues. The amino acid composition of each protein was then compared pairwise with the amino acid composition of the protein from *Rps. capsulata*, and the squares of the differences were summed. The median value for totally unrelated proteins in this type of comparison has been found to be 300. The median values within families of proteins were found to be 80, 30 and 20 for hemoglobins, immunoglobulins and cytochromes, respectively. Furthermore, a significant correlation was found between $S\Delta Q$ value and differences in amino acid sequence [24]. As shown in Table III, *Rps. capsulata* ferredoxin appears completely unrelated to that of the nitrogen-fixing anaerobe *C. pasteurianum*. On the basis of $S\Delta Q$ values, *A. vinelandii* ferredoxin I and *Rps. capsulata* ferredoxin are somewhat different. The closest homology was observed with ferredoxin II from *M. flavum* and *R. rubrum* which were both found to contain a single [4Fe-4S] cluster. Using carboxypeptidase Y digestion, the C-terminal sequence of *R. capsulata* ferredoxin was determined to be: (Glu,Val)-Thr-Ala.

TABLE II

AMINO ACID COMPOSITION OF *RPS. CAPSULATA* FERRODOXIN

The values, calculated on the basis of a molecular weight of 12000, are to the nearest whole number of the average of four determinations (two samples after 24 and 72 h of hydrolysis). –, not detected.

Amino acid	mol/mol protein
Aspartate + asparagine	18
Threonine	7
Serine	4
Glutamate + glutamine	11
Proline	7
Glycine	5
Alanine	12
Cysteic acid	8
Valine	7
Methionine	2
Isoleucine	7
Leucine	5
Tyrosine	1
Phenylalanine	3
Histidine	2
Lysine	7
Arginine	1
Tryptophan	–
Total number of residues	107

TABLE III

COMPOSITIONAL RELATEDNESS BETWEEN *RPS. CAPSULATA* FERRODOXIN AND THOSE FROM SOME NITROGEN-FIXING BACTERIA AND HIGHER PLANTS (SPINACH)

Fd, ferredoxin.

	Molecular weight	$S\Delta Q$	Ref.
Rps. capsulata Fd	12000	–	–
R. rubrum Fd I	8800	81 [a]	4
R. rubrum Fd II	14500	54 [a]	4
A. vinelandii Fd I	14100	120	25
M. flavum Fd I	11230	87	26
M. flavum Fd II	13478	47	26
C. pasteurianum Fd	5600	214	27
Spinach Fd	10482	95	28

[a] From amino acid composition determined by T.E. Meyer (personal communication).

Ultraviolet-visible spectra

The ultraviolet-visible absorption spectra of a sample of oxidized ferredoxin and a sample of ferredoxin reduced with an excess of $Na_2S_2O_4$ are shown in Fig. 1. The spectrum of the oxidized sample is similar to that of eight-iron clostridial

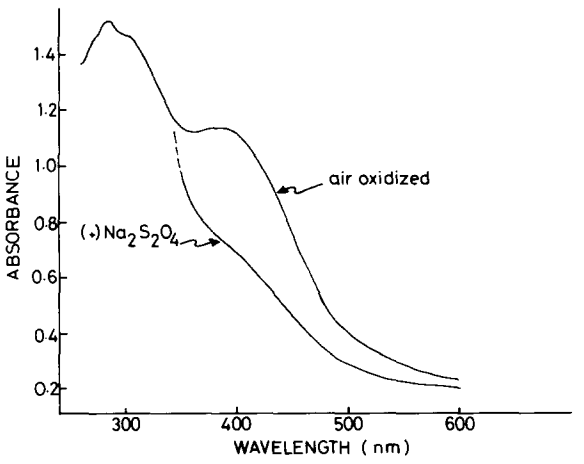

Fig. 1. Oxidized and $Na_2S_2O_4$-reduced optical absorption spectra of purified *Rps. capsulata* ferredoxin. The cuvette was fitted with a serum stopper and contained 39.5 μM ferredoxin in 50 mM Tris-HCl, pH 7.4, under an atmosphere of argon. The ferredoxin solution was reduced by addition of 20 μl of a 50 mM solution of $Na_2S_2O_4$ after recording the oxidized spectrum.

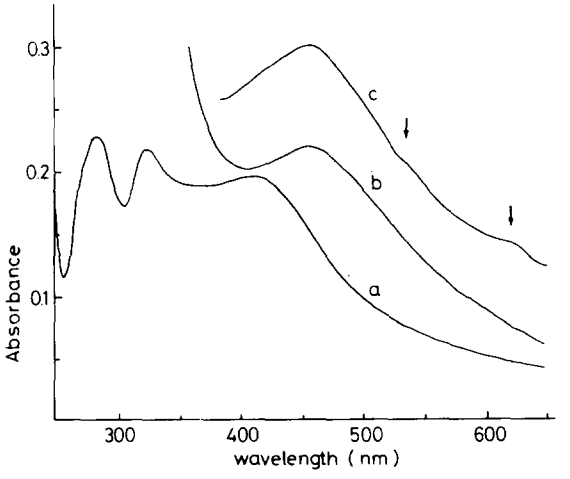

Fig. 2. Extrusion of the iron-sulfur clusters of *Rps. capsulata* ferredoxin. All spectra were recorded with anaerobic solutions made up in an anaerobic quartz cuvette of 0.20 cm path length. Dimethyl sulfoxide and thiophenol were freshly distilled and where indicated were at final concentrations of 80% and 78 mM, respectively. (a) *Rps. capsulata* ferredoxin (35 μM) in 80% dimethyl sulfoxide, 10 mM Tris-HCl, pH 9.0. (b) The same sample as in a after the addition of thiophenol. (c) The spectrum of the extrusion product(s) formed immediately after addition of thiophenol to a sample of ferredoxin (35 μM) in 80% dimethyl sulfoxide, 10 mM Tris-HCl, pH 7.4. The arrows indicate unusual long-wavelength features at 515 and 625 nm.

ferredoxin, but optical spectra alone are not diagnostic of cluster type. The purity index, the ratio of absorbance at 380 nm to that at 280 nm, was 0.74; four different preparations gave a purity index of 0.73 ± 0.02. The ratio of absorbance of the oxidized protein at 380 nm to the absorbance at 380 nm of the reduced protein was 0.68. Reduction by $Na_2S_2O_4$ was relatively slow; approx. 4–5 min were required before the decrease in absorbance at 380 nm reached a stable value. Attempted further oxidation of the oxidized protein with an excess of ferricyanide resulted in a loss of absorbance at 380 nm, probably due to oxidative damage [26].

The results of cluster extrusion experiments are shown in Fig. 2. The optical features normally present with maximal absorption at 315 nm were red shifted by 8 nm when the protein was dissolved in 80% dimethyl sulfoxide (Fig. 2a). An absorption maximum near 280 nm due to aromatic amino acid absorption was not red shifted, but the apparent chromophore-to-protein ratio increased ($A_{412}/A_{280} = 0.86$ in 80% dimethyl sulfoxide vs. $A_{390}/A_{280} = 0.74$ in aqueous solution) due to the effective subtraction of the chromophore contribution to the absorption at 280 nm. Upon extrusion of the cluster with thiophenol at pH 9.0, the chromophore long-wavelength absorption maximum was further red shifted by about 42 nm to 455 nm, giving a spectrum qualitatively similar to that obtained with ferredoxins known to contain [4Fe-4S] clusters [17].

Recently, *A. vinelandii* ferredoxin I, previously thought to contain only [4Fe-4S] clusters, has been shown to contain a novel [3Fe-3S] cluster [29,30]. Cluster extrusion experiments with *A. vinelandii* ferredoxin I have been ambiguous, since the spectra obtained, although qualitatively similar to those of [4Fe-4S] ferredoxins, contained additional long-wavelength features that disappeared with time. When the cluster(s) were extruded from *Rps. capsulata* ferredoxin at pH 7.4, additional long-wavelength features were observed (Fig. 2c) which disappeared after 10 min giving a spectrum identical to that found at pH 9.0 (Fig. 2b).

On the basis of an extinction coefficient per iron at 440 nm of 4.5 $mM^{-1} \cdot cm^{-1}$ [17], a molar ratio of iron to protein of 6.80 ± 0.05 was calculated, in fair agreement with the results of the

chemical analysis. According to one report [17], for the thiophenol/80% dimethyl sulfoxide system, the ratio of the absorbance at 450 nm to that at 440 nm can be used to determine the composition of solutions containing mixtures of [4Fe-4S] and [2Fe-2S] (the ratio for 100% [4Fe-4S] was reported to be 0.53). Applying this analysis to the spectrum at pH 9.0 shown in Fig. 2b gives 1.28 [4Fe-4S] and 0.85 [2Fe-2S]. However, similar experiments with *A. vinelandii* ferredoxin I have given 1.35 [4Fe-4S] and 1.87 [2Fe-2S] [17]. This fact and the short-lived presence of unusual long-wavelength features only at pH 7.4 (with *A. vinelandii* ferredoxin I unusual long-lived long-wavelenght features are observed at pH 9.0) suggest the cluster(s) in *Rps. capsulata* ferredoxin are arranged differently from those in *A. vinelandii*.

The biological activity of the ferredoxin was checked in three systems: (a) substitution for spinach ferredoxin in the light-induced reduction of NADP by spinach chloroplasts; (b) linking the *Rps. capculata* nitrogenase (ferredoxin free) to reducing power generated either photochemically by spinach chloroplasts or by H_2 via hydrogenase; and (c) substitution for clostridial ferredoxin in the hydrogenase-mediated reduction of metronidazole by molecular hydrogen. The results were compared with the activity of the ferredoxin from *C. pasteurianum* in the three assay systems (Table IV). The ferredoxin from *Rps. capsulata* gave no significant activity in the reduction of NADP (the rate shown in Table IV was essentially that of the control with no added ferredoxin), whereas the ferredoxin from *C. pasteurianum* was slightly active, giving about 30% of the rate found with spinach ferredoxin.

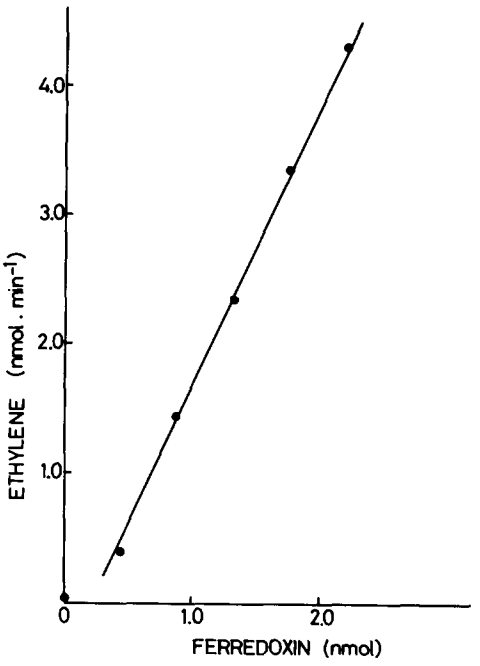

Fig. 3. Linear relationship between the rate of ethylene production and the ferredoxin concentration. Ascorbate-reduced chloroplasts were used as electron donors as described in Materials and Methods.

Rps. capsulata ferredoxin was capable of reducing nitrogenase with about 50% of the efficiency of *C. pasteurianum* ferredoxin on a molar basis. A lower range of concentrations of ferredoxin was tested with *Rps. capsulata* nitrogenase, but with both ferredoxins, nitrogenase activity was linearly related to ferredoxin concentrations (Fig. 3), thus

TABLE IV

BIOLOGICAL ACTIVITY OF *RPS. CAPSULATA* FERREDOXIN

The numbers in parentheses indicate the concentration, or range of concentrations, in μM of ferredoxin used. (a) Ferredoxin-mediated electron transfer between ascorbate-reduced chloroplasts and *Rps. capsulata* nitrogenase (b) Ferredoxin-mediated electron transfer between H_2-reduced *C. pasteurianum* hydrogenase and *Rps. capsulata* nitrogenase (see Materials and Methods). n.d., not detected.

Ferredoxin source	NADP reduction (nmol/min per nmol ferredoxin)	C_2H_2 reduction (nmol/min per nmol ferredoxin))		Metronidazole reduction (ΔA_{360}/min per nmol ferredoxin)
		a	b	
Rps. capsulata	0.09 (2.7)	2.12 (0.44–2.4)	0.85 (9.1)	n.d. (0.46)
C. pasteurianum	1.4 (3.6)	4.38 (12.5)	2.5 (12.5))	0.57 (0.17)
Spinach	5.0 (0.52)	1.32 (7.1)	0.065 (7.1)	-

indicating that this difference is really due to an inherent difference in reactivity. Since *Rps. capsulata* ferredoxin was capable of mediating electron flow from illuminated chloroplast to nitrogenase, lack of reactivity in NADP reduction suggests a lack of interaction with spinach ferredoxin-NADP oxidoreductase.

Rps. capsulata ferredoxin, at concentrations up to 0.5 μM, gave essentially no reaction with the hydrogenase from *C. pasteurianum* in the recently described hydrogenase-metronidazole test for ferredoxin [22] (Table IV). This lack of reactivity is probably due to a lack of interaction, at least at the concentrations used, with the hydrogenase from *Clostridium*, since metronidazole is an effective inhibitor of nitrogenase activity and photosynthetic growth by *Rps. capsulata* [31]. When the concentration of *Rps. capsulata* ferredoxin was increased about 20-fold to 9 μM, a weak electron transfer was catalyzed by ferredoxin between H_2-reduced hydrogenase (from *C. pasteurianum*) and nitrogenase (from *Rps. capsulata*) (Table IV). On the other hand, no reactivity of *Rps. capsulata* ferredoxin was observed with partially purified hydrogenase of *Rps. capsulata* when tested either by metronidazole reduction or mediating electron flow from dithionite to hydrogenase.

Discussion

When purified anaerobically, the ferredoxin from *Rps. capsulata* was rather stable at room temperature and was obtained in a relatively high yield. The molecular properties of this ferredoxin were rather similar to those of ferredoxins isolated from some other aerobic nitrogen-fixing bacteria; a relatively high molecular weight (12000), and EPR signal in the oxidized state (g 2.034) (unpublished results) and poor reactivity in some ferredoxin-requiring reactions. An EPR signal in the oxidized state has been found for *A. vinelandii* ferredoxin I which has been shown to have a high-potential [4Fe-4S] cluster and a novel low-potential [3Fe-3S] cluster [29,30] and it has been suggested that other ferredoxins isolated from other aerobic nitrogen-fixing bacteria may also contain this novel cluster type [32]. Active-site cluster extrusion experiments, and the quantity of iron and acid-labile sulfur (6.0 ± 1.0 and 6.4 ± 0.3) suggest that it may contain either a [3Fe-3S] cluster and a [4Fe-4S] cluster or two [4Fe-4S] clusters. However, the inaccuracy of measurement of iron and acid-labile sulfur along with the possibility of losses of variable amounts of iron and sulfur during purification make it difficult to distinguish between an iron-sulfur content of seven or eight each per molecule of ferredoxin. Furthermore, interpretation of the cluster extrusion experiments is at least uncertain for a ferredoxin of unknown stability and behavior in organic solvents. A final designation of cluster types in the *Rps. capsulata* ferredoxin will require quantitative EPR measurements and Mössbauer spectroscopy [32].

Rps. capsulata ferredoxin ressembles the *R. rubrum* ferredoxin I since, as suggested by the molar ratio of iron and labile sulfur, both might contain two iron-sulfur clusters. The cluster type of either ferredoxin has not been established. Yoch et al. [9] found that *R. rubrum* ferredoxin exhibits small EPR signals at g 1.94 in the reduced form and at g 2.01 in the oxidixed form which is consistent with the presence of two [4Fe-4S] clusters. The molecular weight and amino acid composition of *Rps. capsulata* ferredoxin are more similar to those of *R. rubrum* ferredoxin II (Table III) but the latter certainly contains only one [4Fe-4S] cluster as suggested by its iron and sulfur content (3.8 and 4.4, respectively) [9].

The low reactivity of *Rps. capsulata* ferredoxin in several biochemical ferredoxin-requiring systems is consistent with the results obtained with some other ferredoxins. *Rps. capsulata* ferredoxin gave a low reactivity in two systems, reduction by *C. pasteurianum* hydrogenase and reaction with spinach ferredoxin-NADP oxidoreductase; on the other hand, relatively good reactivity was obtained in mediating electron transfer from illuminated chloroplasts to nitrogenase. *A. vinelandii* ferredoxin I is also very unreactive with *C. pasteurianum* hydrogenase, but it is about equally effective as ferredoxin II as an electron carrier in nitrogenase and NADP reduction [25]. *M. flavum* ferredoxin I is not effective in NADP reduction, on the other hand, it is only one-fifth as effective as *M. flavum* ferredoxin II in electron transfer to nitrogenase [26]. *Chromatium* ferredoxin has been reported to be fairly active in both nitrogenase reduction [8] and NADP reduction [3]. Both soluble ferredoxins

of *R. rubrum* have been found to be reduced at approximately the same rate by illuminated chloroplasts [10]; however, ferredoxin I is 2–3-times more effective than ferredoxin II in reducing nitrogenase [10] or ferredoxin-NADP oxidoreductase [5]. Thus, although the ferredoxins discussed above give a varied pattern of biological reactivity, *Rps. capsulata* ferredoxin appears unusual in reacting only with nitrogenase (of the three systems tested) and appears similar to *A. vinelandii* ferrodoxin I in not reacting with *C. pasteurianum* hydrogenase.

The regulation of the synthesis of ferredoxin is poorly understood at present. Surprisingly, with *Rps. capsulata* only one soluble ferredoxin was found; the rather closely related bacterium *R. rubrum* has been found to contain two different ferredoxins in roughly equal amounts when grown photoheterotrophically [4]. On the other hand, the photosynthetic bacteria differ widely as to their content of cytochromes [33]; perhaps the variation in ferredoxin constitution is as great as that found for cytochromes. Previously, synthesis of one of the ferredoxins (ferredoxin I) of *R. rubrum* was reported to be light dependent [4]. Synthesis of ferredoxin I is, however, independent of nitrogen source [10]. Since *Rps. capsulata* is capable of growth under five different metabolic modes, the study of ferredoxin synthesis under different conditions of growth would be of great interest. The present report indicates that under diazotrophic, photoheterotrophic growth conditions, only one soluble ferredoxin appears to be present in significant amounts. Recent studies with the electron-transfer inhibitor metronidazole, thought to be a specific inhibitor of ferredoxin-catalyzed reactions, have shown that a ferredoxin may be required for photosynthetic growth independent of nitrogen source, as well as being required for diazotrophic growth independent of photosynthesis [31]. This corroborates earlier reports of a possible requirement of ferredoxin for cyclic photophosphorylation [34] or for noncyclic electron flow leading to NAD reduction [35]. The function of this ferredoxin in photosynthesis and nitrogen fixation obviously requires further study.

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