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Claire Chatelet · Jacques Meyer

The [2Fe-2S] protein I (Shetna protein I) from *Azotobacter vinelandii* is homologous to the [2Fe-2S] ferredoxin from *Clostridium pasteurianum*

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Abstract The [2Fe-2S] protein from *Azotobacter vinelandii* that was previously known as iron-sulfur protein I, or Shethna protein I, has been shown to be encoded by a gene belonging to the major *nif* gene cluster. Overexpression of this gene in *Escherichia coli* yielded a dimeric protein of which each subunit comprises 106 residues and contains one [2Fe-2S] cluster. The sequence of this protein is very similar to that of the [2Fe-2S] ferredoxin from *Clostridium pasteurianum* (2Fe*Cp*Fd), and the four cysteine ligands of the [2Fe-2S] cluster occur in the same positions. The *A. vinelandii* protein differs from the *C. pasteurianum* one by the absence of the N-terminal methionine, the presence of a five-residue C-terminal extension, and a lesser number of acidic and polar residues. The UV-visible absorption and EPR spectra, as well as the redox potentials of the two proteins, are nearly identical. These data show that the *A. vinelandii* FeS protein I, which is therefore proposed to be designated 2Fe*Av*FdI, is the counterpart of the [2Fe-2S] ferredoxin from *C. pasteurianum*. The occurrence of the 2Fe*Av*FdI-encoding gene in the *nif* gene cluster, together with the previous demonstration of a specific interaction between the 2Fe*Cp*Fd and the nitrogenase MoFe protein, suggest that both proteins might be involved in nitrogen fixation, with possibly similar roles.

Key words Iron-sulfur · Nitrogen fixation · Evolution

Abbreviations *Cp: Clostridium pasteurianum* · *EPR:* Electron paramagnetic resonance · *ESI-MS:* Electrospray ionization mass spectrometry · *Fd:* Ferredoxin · *2FeCpFd:* [2Fe-2S] *Clostridium pasteurianum* ferredoxin · *HPLC:* High performance liquid chromatography · *ORF:* Open reading frame · *NHE:* Normal hydrogen electrode · *PCR:* Polymerase chain reaction · *SDS-PAGE:* Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

C. Chatelet · J. Meyer (✉)
DBMS-Métalloprotéines, CEA-Grenoble, F-38054 Grenoble France
e-mail: jacques.meyer@cea.fr, Fax: +33-4-76885872

Introduction

Numerous proteins assuming a variety of important functions contain [2Fe-2S] active sites [1, 2, 3]. Among these, the [2Fe-2S] ferredoxin (2Fe*Cp*Fd) from the nitrogen-fixing saccharolytic anaerobe *Clostridium pasteurianum* possesses a number of remarkable properties. It displays unique interactions between the metal site and the polypeptide chain [4, 5, 6], as well as unprecedented magnetic properties of the [2Fe-2S] cluster in some of its variants [7, 8, 9]. The primary structure of this protein [10, 11] has long remained unique [12], but is now known to have counterparts in diverse enzymes and complexes, including hydrogenases [13, 14], complex I (NADH-ubiquinone oxidoreductase) of respiratory chains [15], and possibly other proteins [6, 16].

The function of the 2Fe*Cp*Fd has remained unknown, although a possible involvement in nitrogen fixation was suggested by its presence in larger amounts in N_2-fixing than in ammonia-grown cells ([17]; Meyer and Moulis, unpublished data). These inferences have recently been substantiated by the demonstration of a specific interaction between the 2Fe*Cp*Fd and the nitrogenase MoFe protein [18]. The biochemical significance of this unprecedented phenomenon has remained unknown. Genetic tools afford potentially powerful means of elucidating this question, for instance by analyzing the effects of 2Fe*Cp*Fd gene deletions on nitrogenase and nitrogen fixation. Up to now, however, *C. pasteurianum* has remained refractory to genetic manipulation, and it would therefore be desirable to carry out similar investigations in a genetically tractable organism.

Azotobacter vinelandii has for many years been a reference microorganism for the study of most aspects

of nitrogen fixation [19, 20]. It contains a number of small iron-sulfur proteins, several of which have been biochemically and genetically characterized (reviewed in [21]). Among these, a [2Fe-2S] protein displaying similarities with the 2Fe*Cp*Fd had been isolated 35 years ago [22, 23, 24], characterized biochemically and spectroscopically [22, 23, 24, 25, 26, 27], but neither sequenced nor studied functionally or genetically. The known similarities with the 2Fe*Cp*Fd were therefore restricted to UV-visible absorption and EPR spectra only. We report here the overproduction in *Escherichia coli* of this protein, formerly known as *A. vinelandii* FeS protein I, or Shethna protein I, and demonstrate that it has a primary structure and other properties very similar to those of the 2Fe*Cp*Fd.

Materials and methods

All common DNA manipulations were as described [28]. Enzymes were purchased from Boehringer Mannheim. Oligonucleotides were from Life Technologies (Cergy-Pontoise, France). Polymerase chain reactions (PCRs) were run on a Perkin Elmer 2400 machine. Plasmid DNA was purified with the EasyPrep kit (Pharmacia). DNA sequencing was performed either in the laboratory using the USB (Amersham) Sequenase 2.0 kit, or by Genome Express (Grenoble, France).

The gene encoding the FeSI protein was lifted from *A. vinelandii* (strain DJ) genomic DNA by PCR using the following primers: 5′-gcttgaggaggaaga*CATat*ggccaaacccg-3' hybridized to the non-coding strand near the 5′-terminus of the gene and included mismatches (capital letters) for the introduction of a *Nde*I (italics) site; 5′-gtagccgacatggc*GgatCC*gctgctgttcgcc-3' hybridized to the coding strand near the 3′-terminus of the gene and included mismatches (capital letters) for the introduction of a *Bam*HI (italics) site. Genomic DNA (1.6 μg) was denatured for 5 min at 94 °C in the presence of the primers (0.5 μM each). DNA polymerase (*Taq* or *Pwo,* 2 units) and deoxynucleotides (0.2 mM each) were added. Twenty five reaction cycles (30 s at 94 °C, 30 s at 50 °C, 2 min at 72 °C) were then performed, followed by a 7 min elongation at 72 °C. The PCR product was extracted with neutral phenol, precipitated with ethanol, digested by *Nde*I and *Bam*HI, electrophoresed through a 2% low-melting agarose gel (NuSieve, FMC), and ligated into the pT7-7 vector [29] cleaved with the same enzymes and dephosphorylated with calf intestine phosphatase. The ligation mixture was used to transform *E. coli* DH5α cells. Two clones containing the FeSI protein-encoding gene and derived from two idependent PCR reactions were sequenced and both inserts were found to match perfectly the sequence encompassing bases 12251 to 12574 of the main *nif* gene cluster (GenBank entry M20568 [30]).

Expression of the FeSI protein-encoding gene was performed with the pT7-7 and pGP1-2 double plasmid system in *E. coli* K38 cells [29], as described previously for 2Fe*Cp*Fd [31]. Cells from 10-L cultures were harvested by centrifugation, resuspended in Tris-HCl (20 mM, pH 8), and stored at –80 °C. They were thawed, disrupted by sonication, and centrifuged for 30 min at 35,000 *g* and 10 °C. The supernatant was brought to 30% saturation in ammonium sulfate over a period of 2 h at 0 °C, centrifuged 30 min at 35,000 *g* to remove the precipitate, dialyzed overnight against Tris-HCl (20 mM, pH 8), and loaded on a 6 × 3 cm DE-52 (Whatman) column equilibrated with the same buffer. The FeSI protein was visible as a diffuse red-brown band in the upper half of the column. The column was washed with Tris-HCl (20 mM, pH 8) containing successively increasing concentrations of NaCl (0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 M). The red fraction eluted from the column at 0.2–0.25 M NaCl and was concentrated in an Amicon cell fitted with a PM10 membrane. Precipitated material was removed by centrifugation, and the fraction was loaded on a 100 × 2 cm Superdex-75 (Pharmacia) column equilibrated with Tris-HCl (20 mM, pH 8) and NaCl (0.2 M). The last purification step was an anion-exchange HPLC chromatography on a PL-SAX (Polymer Labs) column developed with a 0–0.4 M NaCl gradient in Tris-HCl (0.01 M, pH 8) over 50 min. The protein eluted at 0.22 M NaCl, was concentrated on a PM10 membrane, and stored in liquid nitrogen.

Transfer of proteins from SDS-PAGE gels onto nylon membranes was as described [18]. Proteins were sequenced on a 477A (Applied Biosystems) automated sequencer equipped with an on-line phenylthiohydantoin analyzer 120A (Applied Biosystems) and run with the sequencing program recommended by the manufacturer.

UV-visible spectra were recorded on Hewlett-Packard 8452 or 8453 diode array spectrophotometers.

X-band EPR spectra were recorded on a Bruker Instruments ESP 300D spectrometer equipped with an Oxford Instruments ESR 900 flow cryostat (4.2–300 K).

Electrospray ionization mass spectra (ESI-MS) were recorded in the positive ion detection mode as described [32, 33]. The infusion solvent was 1% acetic acid-20% methanol in water. For the detection of the dimeric protein, 20% methanol was implemented. The negative ion detection mode at neutral pH, which has been successfully used for the detection of acidic holoferredoxins [32, 33], has not yielded any data for *A. vinelandii* FeSI, most likely because of the nearly neutral isoelectric point of this protein (6.5, see below).

Redox titrations were performed in an anaerobic glovebox (< 2 ppm O_2). The reaction mixture (2.3 mL) contained Tris-HCl (20 mM, pH 7.4), NaCl (80 mM), [2Fe-2S] protein (0.1 mM), and the following mediators: indigo disulfonic acid, safranine T, and benzyl viologen, each at a concentration of 2.5 μM. The potential was measured between the platinum electrode and the Ag/AgCl reference electrode of a combined electrode. Proteins were titrated in both the reductive and the oxidative directions, with stepwise additions of dithionite or ferricyanide, respectively. UV-visible absorption spectra in the 300–800 nm range were recorded at each step, and the absorbance at 420 nm or 550 nm, where the contribution of the mediators is negligible, were used for the calculations [34].

Electrochemical experiments were performed in a closed three-electrode cell as described previously [35]. The three-electrode design included a saturated Ag/AgCl reference micro-electrode MI-401F from Microelectrodes, a platinum wire counter electrode from EG&G Instruments, and a glassy carbon working electrode from Radiometer. The three electrodes were connected to a model 263 potentiostat controlled with Model 270/250 (EG&G Instruments) software. The carbon disc tip of the working electrode was immersed in 65% HNO_3 at room temperature for several hours, thoroughly washed with water, gently polished with Al_2O_3, rinsed with water, and dried. The deaerated sample (15–20 μL) was placed on the working electrode, and the other electrodes were positioned to contact the drop.

Results and discussion

Identification of the gene encoding the *A. vinelandii* FeS protein I

The FeS protein I has been shown (see below) to be homologous to the 2Fe*Cp*Fd. Accordingly, it will hereafter be designated 2Fe*Av*FdI. The roman number I is conserved to avoid any confusion with the FeS protein II from the same bacterium [24, 25, 36, 37, 38]. The 2Fe*Av*FdI-encoding gene has been identified in two independent ways. In one, sequence databanks were searched on the http://www.ncbi.nlm.nih site using the

TBLASTN software [39] with the 2Fe*Cp*Fd sequence [10, 11] as a query. One of the best matches was a putative open reading frame (ORF) encompassing bases 12251 to 12574 of the major *nif* gene cluster of *A. vinelandii* (GenBank entry M20568 [30]). This ORF is preceded by a ribosome binding sequence, and encodes a putative protein of which the sequence is very similar to that of the 2Fe*Cp*Fd (Fig. 1). Another approach was implemented to ensure that the cloned gene would encode the previously isolated FeS protein I [22, 23, 24, 25, 26, 27]. It involved the acquisition of N-terminal sequence data as follows. Previous reports indicated that the target protein was present in low amounts in *A. vinelandii* cells (5–10 mg/kg wet weight) and membrane-bound to some extent at least [23, 24, 25]. Extractions and purifications were therefore run as described [25] on *A. vinelandii* (strain DJ) cell pellets obtained by osmotic shock or sonication. Following butanol extraction, ammonium sulfate precipitation, and anion-exchange chromatography, fractions enriched in 2Fe*Av*FdI were identified by the characteristic EPR spectrum of this protein ([25]; see also Fig. 4 below), and were electrophoresed through SDS-polyacrylamide gels. These were blotted onto nylon membranes and bands of interest were analyzed by automated Edman degradation (see Materials and methods). A band corresponding to a size (ca. 14 kDa) near the expected one yielded a sequence, Ala-Lys-Pro-Glu-Phe-His-Ile-Phe-Ile-(–)-Ala-Gln-Asn-Arg-Pro-Ala-, that was reminiscent of the N-terminal sequence of 2Fe*Cp*Fd. Two degenerate primers were derived from this sequence and used to amplify the corresponding part of the gene (ca. 50 bp), which was cloned in the pBluescript II SK^+ plasmid (Stratagene) as described [40]. The sequence of this fragment was found to perfectly match the 5′-region of the ORF belonging to the main *nif* gene cluster and identified by searching databanks with the 2Fe*Cp*Fd sequence (see above). This ORF was thus confirmed to be the 2Fe*Av*FdI-encoding gene, and it has therefore been cloned and expressed in *E. coli* as described (Materials and methods).

Purification of the 2Fe*Av*FdI

Purification of 2Fe*Av*FdI (see Materials and methods) was in most ways similar to that of 2Fe*Cp*Fd [4, 31]. The main difference arose from the significantly higher isoelectric point of 2Fe*Av*FdI (6.5 versus 4.5 for the 2FeCpFd, Table 1) which resulted in a lesser affinity for anion-exchange phases. It was noted that the two proteins had identical elution volumes on gel filtration columns, from which it was inferred that the size of 2Fe*Av*FdI is similar to that of 2Fe*Cp*Fd (25 kDa [41]). The progress of the purification was monitored by SDS-PAGE electrophoresis (Fig. 2), which showed that the band corresponding to 2Fe*Av*FdI was visible in the crude extract, and that the protein was pure after the anion-exchange HPLC step. The purity of these chromophoric proteins can also be assessed by their A_{464}/A_{280} ratio, which reflects the relative contributions of the Fe-S chromophore and of the polypeptide chain to the UV-visible absorption spectrum. Pure 2Fe*Cp*Fd has an A_{464}/A_{280} ratio of 0.50 [31, 41]. This ratio is 0.37 (0.32 in a previous report [24]) for 2Fe*Av*FdI, a lower value which can be rationalized by the higher content of UV-absorbing aromatic residues (2 Trp and 4 Tyr versus 1 Trp and 3 Tyr in 2Fe*Cp*Fd; Fig. 1, Table 1).

Polypeptide chain of 2Fe*Av*FdI

The protein sequence translated from the gene was checked by N-terminal sequencing, which showed that the N-terminal methionine was removed in recombinant 2Fe*Av*FdI as well as in the native protein from *A. vinelandii* (see above). The ESI-MS spectra were dominated by two peaks at $M = 11265$ and $M = 11439$ (not shown). The former is attributable to the polypeptide chain as encoded by the gene, with the N-terminal methionine removed, in keeping with the N-terminal sequence data (calculated mass 11264). The $M = 11439$ peak is attributable to the holoprotein. The similarity of the sequences of 2Fe*Cp*Fd and 2Fe*Av*FdI is high, with 32 identities and 24 similarities (Fig. 1). Of particular interest is the presence in 2Fe*Av*FdI of only four cysteine residues in positions matching those of the demonstrated ligands of the [2Fe-2S] cluster in 2Fe*Cp*Fd [4]. Cysteine 14 of 2Fe*Cp*Fd, which had been shown not to be a ligand of the chromophore [4], has no counterpart in 2Fe*Av*FdI. Significant differences between the two proteins are observed concerning their charge and hydrophobicity (Table 1). The two proteins contain the same numbers of basic residues but differ by their con-

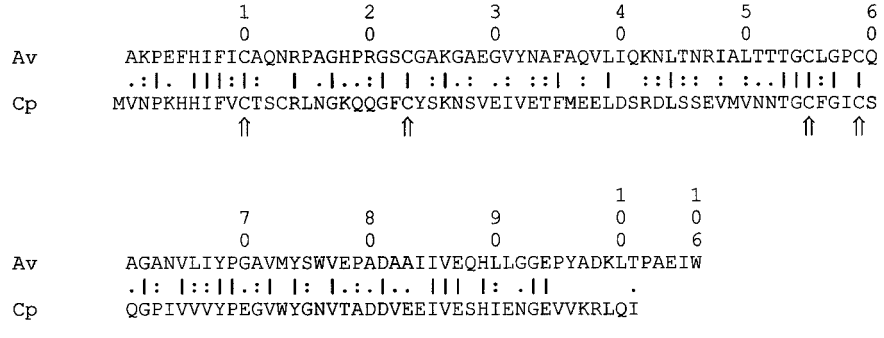

Fig. 1 Alignment of the sequences of the [2Fe-2S] ferredoxin from *C. pasteurianum* and of the [2Fe-2S] protein I from *A. vinelandii*. *Vertical bars* are for identities, *colons* for high similarities, and *dots* for low similarities. Residue numbering is for the *A. vinelandii* protein. *Arrows* indicate the cysteine ligands of the [2Fe-2S] clusters

Table 1 Comparison of significant properties of the [2Fe-2S] proteins from *C. pasteurianum* and *A. vinelandii*

	2Fe*Cp*Fd	2Fe*Av*FdI
Amino acid residues	102	106
F	4	3
Y	3	4
W	1	2
Basic (K,R)	7	7
Acidic (D,E)	15	8
Hydrophobic	32	43
Polar	31	25
Subunit molecular mass	11600[a]	11439[a]
(including [2Fe-2S])	11600[b]	11436[b]
Dimer molecular mass	23200[a]	22876[a]
	23200[b]	22872[b]
Isoelectric point	4.5[c]	6.5[d]
UV-vis absorption bands of the chromophore (nm)	336, 420, 464, 550	336, 420, 462, 546
EPR *g* values	2.004, 1.950, 1.922	2.009, 1.944, 1.918
Redox potential (vs. NHE)		
Spectrophotometric titration	–280 mV	–300 mV
Electrochemistry	–355 mV	–370 mV

[a] Determined by electrospray ionization mass spectrometry
[b] Calculated
[c] Calculated and measured by isoelectrofocusing [10]
[d] Calculated

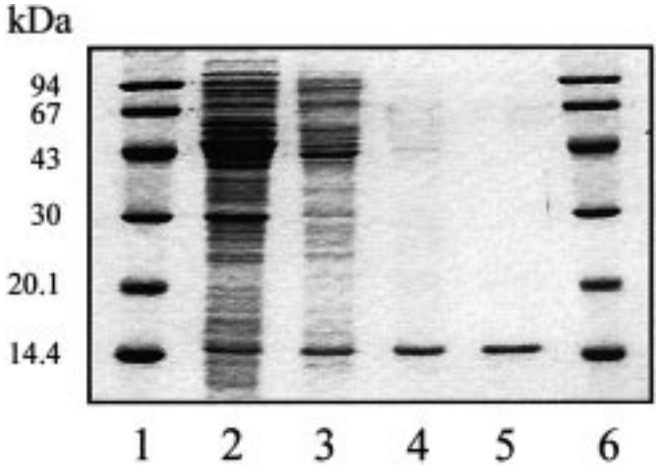

Fig. 2 Purification of the recombinant [2Fe-2S] protein I from *A. vinelandii*. Fractions at various purification steps (see Materials and methods) were analyzed by 5–15% SDS-PAGE. *Lanes 1 and 6* Molecular mass markers. *Lane 2* Soluble cell extracts. *Lane 3* DE-52 anion exchange. *Lane 4* Superdex 75 gel filtration. *Lane 5* PL-SAX anion exchange

tent in acidic residues (Table 1), and consequently have different isoelectric points (4.5 for the *C. pasteurianum* protein, 6.5 for the *A. vinelandii* protein). Also, 2Fe*Av*FdI is more hydrophobic than 2Fe*Cp*Fd (Table 1).

[2Fe-2S] chromophore

The UV-visible absorption spectrum of the 2Fe*Av*FdI, observable early in the purification process, displays the characteristic absorption bands, at 330, 420, 460, and 550 nm, of $[2Fe\text{-}2S]^{2+}$ clusters [1, 2]. Furthermore, the unusual inversion of the relative intensities of the 420 and 460 nm bands, which is an indiosyncrasy of 2Fe*Cp*Fd [17, 41] and native *A. vinelandii* protein [24], is confirmed here with the recombinant 2Fe*Av*FdI. The nearly identical spectra of 2Fe*Cp*Fd and 2Fe*Av*FdI (Fig. 3) are in keeping with their sequence homology and suggest very similar structures of the [2Fe-2S] chro-

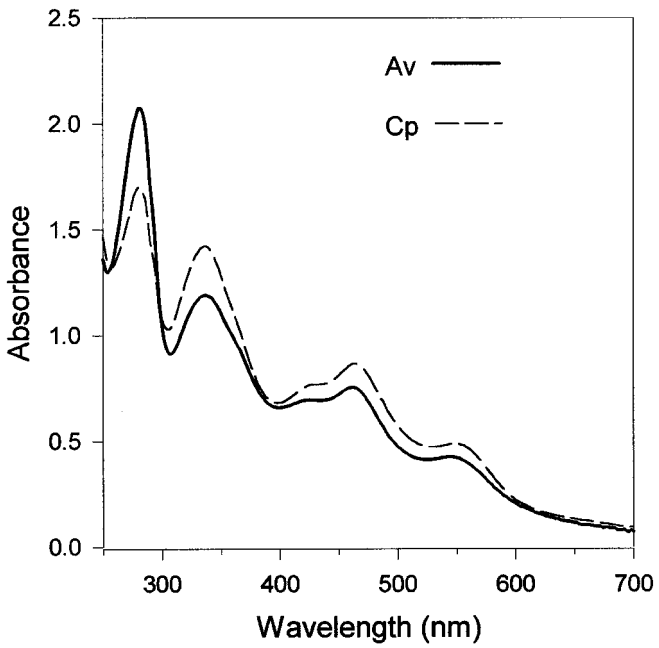

Fig. 3 UV-visible absorption spectra of [2Fe-2S] proteins from *C. pasteurianum* (*thin broken line*) and *A. vinelandii* (*thick solid line*). Protein concentrations were 0.5 mM in [2Fe-2S] chromophore. The solvent was NaCl (0.2 M)/Tris-HCl (10 mM, pH 8)

mophores and of their polypeptidic environment. The structural similarity of the chromophores is maintained in the reduced level $[2Fe\text{-}2S]^{+}$, as witnessed by the UV-visible absorption (not shown) and EPR spectra (Fig. 4). The latter show only marginal differences in *g* values (Table 1) and have very similar relaxation properties (not shown). The *g* values of 2Fe*Av*FdI are nearly identical with those previously reported [25] for the FeS protein I.

The UV-visible and EPR spectra discussed above afford compelling evidence that 2Fe*Av*FdI contains a [2Fe-2S] cluster. This was further substantiated by the ESI-MS results which included, in addition to the $M = 11265$ peak arising from the apoprotein, an $M = 11439$ peak corresponding to the mass of the apoprotein plus two iron and two sulfur atoms (calculated $M = 11435.7$).

Spectrophotometric titration of 2Fe*Av*FdI yielded a redox potential of –300 mV (Table 1), slightly more negative than values previously obtained (–280 mV) for 2Fe*Cp*Fd using the same technique [5]. More negative values were obtained by electrochemistry, –370 mV and –355 mV for 2Fe*Av*FdI and 2Fe*Cp*Fd, respectively (Fig. 5, Table 1). The two techniques yielded different redox potential values, but in both cases 2Fe*Av*Fd was found to have a slightly more negative potential (by ca. 20 mV) than 2Fe*Cp*Fd.

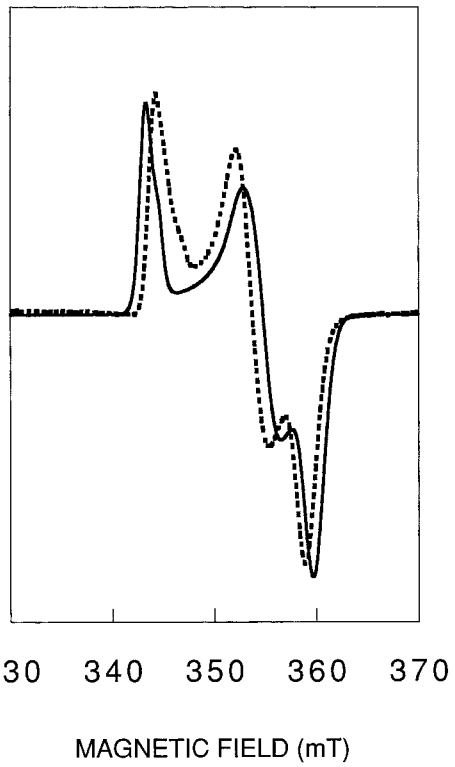

Fig. 4 EPR spectra of dithionite-reduced [2Fe-2S] proteins from *C. pasteurianum* (*broken line*) and *A. vinelandii* (*solid line*). Temperature 10 K, microwave frequency 9.655 GHz, microwave power 0.01 mW, modulation frequency 100 kHz, modulation amplitude 10 G. The protein concentrations were 0.5 mM and the solvent was NaCl (0.2 M)/Tris-HCl (10 mM, pH 8.0)

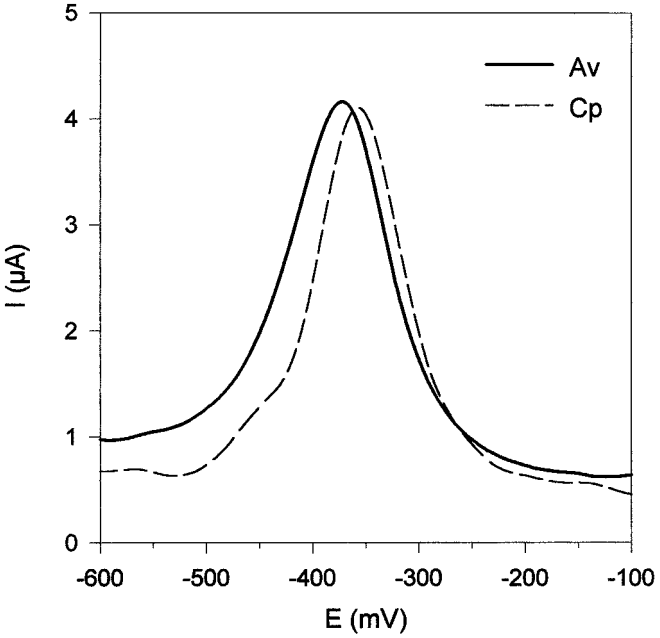

Fig. 5 Square-wave voltammograms of 2Fe*Av*FdI (*thick solid line*) and 2Fe*Cp*Fd (*thin broken line*). Data recorded at the planar glassy carbon electrode. The protein concentrations were 0.5 mM and the solvent was NaCl (0.2 M)/Tris-HCl (10 mM, pH 8.0). Pulse frequency was 5 Hz, scan increment 2 mV, and pulse height amplitude 25 mV. Potentials are versus the normal hydrogen electrode

Quaternary structure

In a similar fashion to 2Fe*Cp*Fd [17], 2Fe*Av*FdI was first reported to be a monomer of 21 kDa [24]. The former protein was later demonstrated to be a dimer [10, 33, 41]. Our gel filtration data confirm that 2Fe*Av*Fd has a size similar to that of the 2Fe*Cp*Fd, ca. 25 kDa, in agreement with previous reports [23, 24, 25]. However, the sequence and ESI-MS data indicate a mass of 11435 Da for the monomeric holoprotein (Table 1), and therefore imply a dimeric structure for 2Fe*Av*FdI. Indeed, ESI-MS results recorded with 20% MeOH as an infusion solvent revealed the presence of a peak at $M = 22876$ (not shown) arising from the dimeric protein containing one [2Fe-2S] cluster in each of its subunits. These observations are very similar to those previously reported for 2Fe*Cp*Fd [33] and indicate a dimeric structure for both proteins, in keeping with their other structural similarities.

Interaction with nitrogenase

The 2Fe*Cp*Fd has been shown, by affinity chromatography and covalent cross-linking, to specifically interact with the nitrogenase MoFe protein from *C. pasteurianum* [18]. Preliminary cross-linking experiments between 2Fe*Av*FdI and the MoFe proteins from either *C. pasteurianum* or *A. vinelandii* have been unsuccessful.

However, since the 2Fe*Av*FdI misses the acidic residues in positions 31, 34, 38, and 39 that have been shown to be essential contributors to the interaction in the case of *C. pasteurianum* ([18]; C. Chatelet and J. Meyer, unpublished), hasty conclusions should not be drawn from these results. Further experiments aimed at detecting interactions between 2Fe*Av*FdI and nitrogenase are under way. In any event, the occurrence of the 2Fe*Av*FdI-encoding gene within the *nif* gene cluster, together with indications that 2Fe*Cp*Fd might be involved in nitrogen fixation ([18]; J. Meyer and J.-M. Moulis, unpublished), suggest that both of these homologous proteins might have roles related to this important metabolic process.

Conclusion

Among the numerous small Fe-S proteins that are found in *A. vinelandii*, two [2Fe-2S] proteins, FeSI and FeSII, had been isolated early in the development of this field [22, 23, 24, 25], but have long escaped full biochemical and genetic characterization. FeSII has subsequently been shown to protect nitrogenase against oxygen damage [36, 37, 42], and its gene has been overexpressed in *E. coli* [38]. We now report the identification and overexpression in *E. coli* of the gene encoding FeSI (or 2Fe*Av*FdI). Most of the formerly known properties of this protein have been confirmed, with the notable exception of its quaternary structure, which is dimeric.

The most significant result is the similarity of 2Fe*Av*FdI with the [2Fe-2S] ferredoxin from *C. pasteurianum*. The two proteins have closely related sequences and Fe-S chromophores, and both are dimers. The previous demonstration of a specific interaction of 2Fe*Cp*Fd with nitrogenase and the occurrence of the 2Fe*Av*FdI-encoding gene within the *nif* gene cluster [30] suggest that both proteins are involved in nitrogen fixation. This, together with their structural similarity, would indicate that they have closely related roles. The genetic tractability of *A. vinelandii* [19, 20] now provides powerful tools for the elucidation of the function of both proteins.

The present confirmation that structurally and probably functionally similar [2Fe-2S] proteins are present in *C. pasteurianum* and *A. vinelandii* affords an opportunity of pointing out that additional homologues of these proteins occur in multimeric hydrogenases [14], in Complex I of aerobic respiratory chains [15], or are known merely as putative proteins [6, 16]. Such a wide distribution almost certainly implies different functions and a long history of structural and functional evolution. This growing family of [2Fe-2S] proteins may therefore turn out to be as ubiquitous as the better known one comprising the [2Fe-2S] plant- and mammalian-type Fds [1].

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