PROPERTIES OF IRON-SULFUR PROTEIN ISOLATED FROM PSEUDOMONAS OVALIS

Daijiro Ohmori

Department of Chemistry, School of Medicine,
Juntendo University, Chiba, 275, JAPAN

Received June 18, 1976

SUMMARY

An iron-sulfur protein containing eight iron atoms and eight acid-labile sulfur atoms per one molecule of 19,000 daltons was found in Pseudomonas ovalis which had been grown on medium supplemented with glucose and ammonium sulfate. This protein molecule was composed of two subunits of identical size. The absorption spectrum exhibited a peak at 283 nm with shoulders at 320 nm and 400 nm. The purified iron-sulfur protein catalyzed the photoreduction of NADP by spinach chloroplast.

Iron-sulfur proteins have been isolated from various kinds of origin. They act mainly as electron carriers in various biological functions. Two iron-sulfur proteins have been isolated from Pseudomonas: rubredoxin from P. oleovolans(1) containing one or two iron atoms and no acid-labile sulfur atom, and putidaredoxin from P. putida(2) containing two iron atoms and two acid-labile sulfur atoms.

In an earlier investigation of iron-sulfur protein in Pseudomonas, 4Fe-4S iron-sulfur protein (molecular weight, 14,000 daltons) was isolated from a Pseudomonas species(3). Recent study on iron-sulfur protein in Pseudomonas ovalis have shown that 8Fe-8S dimeric iron-sulfur protein is a major iron-sulfur protein component of Pseudomonas ovalis. The present communication reports the purification and properties of 8Fe-8S dimeric iron-sulfur protein from Pseudomonas ovalis. Evidence will be presented that two different iron-sulfur proteins appeared to be presented in the cell.

Abbreviation Used: SDS, sodium dodecyl sulfate

MATERIALS AND METHODS

Polyacrylamide disc electrophoresis was performed according to the method of Ornstein(4) and Davis(5) at pH 9.5 using 20% gels. Polyacrylamide gel electrophoresis in SDS was done by the method of Weber and Osborn(6) using 15% gels. Nonheme iron was determined with bathophenanthroline(7). Inorganic sulfide was determined by the method of Kimura and Suzuki(7). Protein was determined by the method of Itzhaki and Gill(8) using bovine serum albumin as a standard. The molecular weight of each protein was estimated by polyacrylamide gel electrophoresis in SDS, Sephadex G-75 gel filtration(9), and sedimentation equilibrium ultracentrifugation(10).

Spinach chloroplasts, as used for studying the electron transfer capability of protein in the NADP reduction by ascorbate(11), were prepared according to Whatley and Arnon(12). NADPH: ferredoxin oxidoreductase, as used for assay of cytochrome c reduction by NADPH(13), was partially purified (about 45%) from spinach leaves.

Pseudomonas ovalis Chester was aerobically grown at 30°C for 24 hrs in the medium containing, per liter: $Na_2HPO_4 \cdot 12H_2O$, 16.4g; KH_2PO_4, 1.5g; $MgSO_4 \cdot 7H_2O$, 0.01g; $CaCl_2 \cdot 2H_2O$, 0.002g; $FeSO_4 \cdot 7H_2O$, 0.01g; glucose, 4g; and $(NH_4)_2SO_4$, 4g. The cells were stored at -20°C.

The cells (1,700g wet weight) were suspended in ice cold 0.01M sodium phosphate, pH 7.4 (500 ml of buffer per 100g cell past), and were sonicated at 9 kHz for 10 min at 4°C with a sonic disintegrater (Kubota 200M). The suspension was centrifuged at 10,000 X g for 1 hr, at a temperature ranging from 0 to 4°C. (This same temperature was used in all the following steps.) The supernatant was filtered through a DEAE-cellulose column (10 X 13 cm) equilibrated with 0.01M sodium phosphate, pH 7.4. (This buffer was used throughout the purification process.) The column was washed with 2,400 ml of the buffer and subsequently with the buffer to which had been added 0.2M NaCl until A_{280} was 0.1. The iron-sulfur protein was eluted from the column with the buffer containing 0.7M NaCl. After dialysis against 0.005M sodium Phosphate, pH 7.4, the eluate was applied to a DEAE-cellulose column (Whatman DE-32, 6.0 X 40 cm). The column was washed with 4 liters of the buffer containing 0.25M NaCl. The brown iron-sulfur protain was eluted with the buffer containing 0.3M NaCl. The iron-sulfur protein fraction was concentrated to 7 ml on a small DEAE-cellulose column, and applied to a Sephadex G-75 column (3.4 X 87cm). The fractions with absorbance ratio A_{410}/A_{280} above 0.55 were combined and were applied to a DEAE-Sephadex A-50 column (1.5 X 20 cm), which had been previously equilibrated with the buffer containing 0.2M NaCl. The column was washed with the buffer containing 0.35M NaCl, and subsequently the iron-sulfur protein was eluted with the buffer containing 0.38M NaCl. The fractions with absorbance ratio A_{410}/A_{280} above 0.56 were combined and concentrated to 2 ml on a small DEAE-cellulose column, and were applied to a Sephadex G-75 column (superfine, 3.4 X 89 cm), Fig. 1a. The fractions containing a minor component (P-2, in Fig. 1a) were rechromatographed on the same column (Fig. 1b).

RESULTS

The fractions containing the iron-sulfur proteir from the DEAE-Sephadex column were applied to a Sephadex G-75 column. As shown in Fig. 1a, an unsymmetrical protein peak was obtained, suggesting that two or more components are contained in the peak. The contents of tubes 90 through 97 were pooled (P-1 in Fig. 1a). The contents of tubes 98

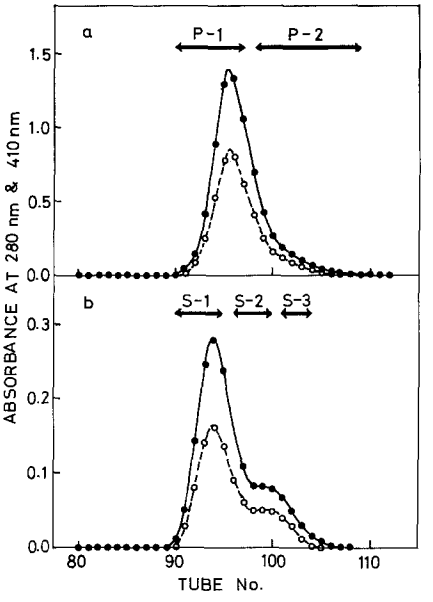

Fig. 1. Gel filtration of P. ovalis iron-sulfur protein on Sephadex G-75. (a) Two ml of iron-sulfur protein fraction from DEAE-Sephadex column were applied to a Sephadex G-75 column (3.4 X 89 cm) equilibrated with 0.01M sodium phosphate buffer containing 0.1M NaCl, pH 7.4. Fractions of 5 ml were collected at a flow rate of 26 ml/hr. —●— , absorbance at 280 nm and ---○--- , absorbance at 410 nm.
(b) P-2 fraction in (a) was concentrated to 2 ml and applied to a Sephadex G-75 column. The conditions were the same as in (a) except that the flow rate was 15 ml/hr.

through 109 were also pooled (P-2 in Fig. 1a), and then chromatographed on a Sephadex G-75 column. The elution pattern is shown in Fig. 1b. This pattern shows a minor protein peak just behind the major protein peak. The eluates were separated into three fractions: S-1 (tube No's 90-95), S-2 (tube No's 96-100), and S-3 (tube No's 101-104). The pattern of polyacrylamide disc electrophoresis of each of these fractions is shown in Fig. 2. Gels were not stained, since the iron-sulfur protein could be identified by their brown color. The S-1 fraction migrated as a single brown band, and its mobility was the same as that of the P-1 fraction. The P-1 and S-1 fractions were combined (ISP-I). The S-3 fraction contained mostly a component (ISP-II) which migrated as a brown

TABLE I Summary of Purification

Step	Volume (ml)	Protein (mg)	A_{260}	A_{280}	A_{410}	A_{280}/A_{260}	A_{410}/A_{280}
Cell extract	9,070	171,423	–	–	–	–	–
DEAE-cellulose	2,430	1,652	36.6	18.7	0.36	0.51	0.02
DEAE-cellulose column	40.5	48.6	4.32	4.64	0.24	1.07	0.52
First gel filtration	11.0	32.1	10.6	12.8	7.3	1.21	0.57
DEAE-Sephadex column	6.5	26.7	9.2	11.0	6.3	1.19	0.57
Second gel filtration	5.8	23.9	9.1	11.3	6.4	1.24	0.57

band slightly slower than the major component. The gels were stained for protein with Amide Black, but no other protein bands were observed except the protein band corresponding to the brown band. (TABLE I)

The molecular weight of ISP-I was estimated by Sephadex G-75 gel filtration to be 18,800. Bovine serum albumin, ovalbumin, chymotrypsinogen A, myoglobine (whale), and cytochrome c (horse heart) were used as markers. Sedimentation equilibrium analysis yielded a molecular weight of 19,100. The sedimentation coefficient ($S_{20,w}$) was calculated to be 1.8 S. Polyacrylamide gel electrophoresis in SDS of ISP-I was carried out, and the patterns are shown in Fig. 3. The molecular weight was estimated to be about 9,500 using the same marker proteins as in gel filtration. From these results, it is concluded that ISP-I is composed of two subunits of

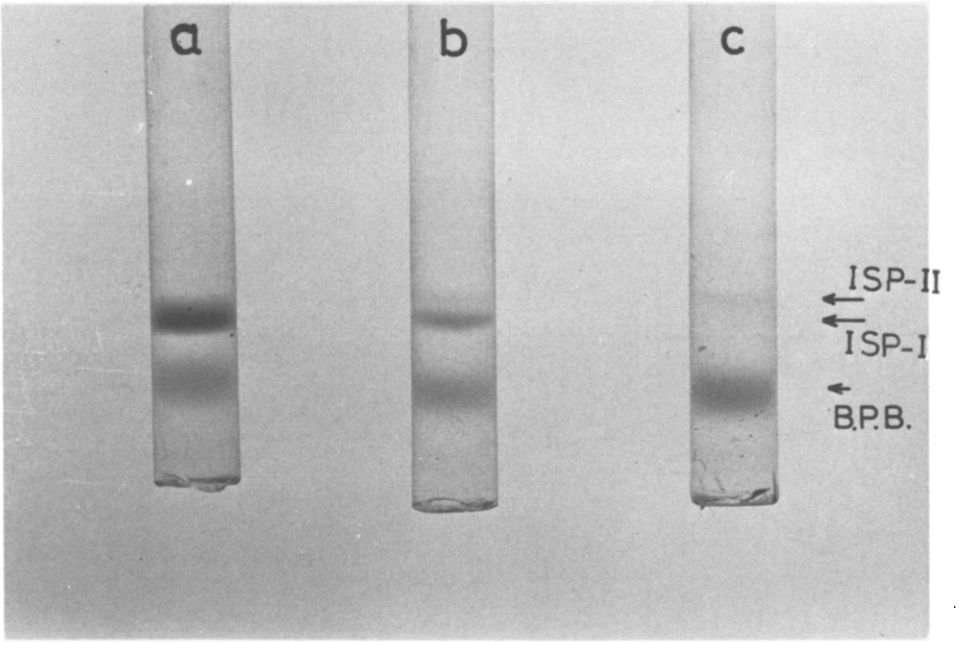

Fig. 2 Polyacrylamide disc electrophoresis patterns.
(a) S-1 fraction (about 0.066 mg protein), (b) S-2 fraction (about 0.028 mg protein), (c) S-3 fraction (about 0.014 mg protein) in Fig. 1b. Gels were not stained since the iron-sulfur protein could be identified by their brown color.

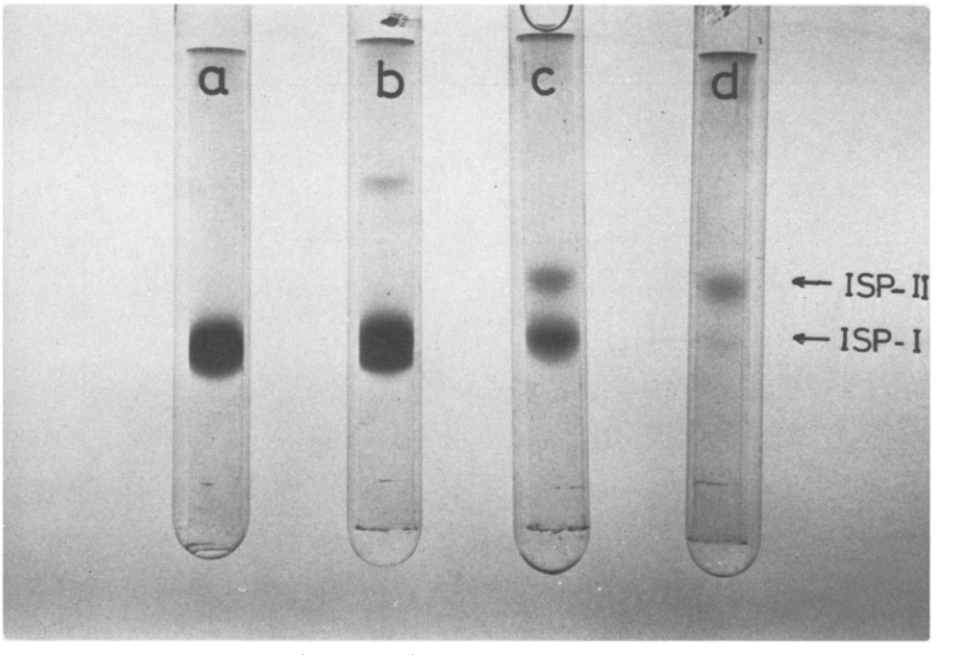

Fig. 3. SDS polyacrylamide gel electrophoresis patterns.
(a) S-1 fraction (about 0.035 mg protein), (b) S-1 fraction in the absence of 2-mercaptoethanol (about 0.035 mg protein), (c) S-2 fraction (about 0.007 mg protein), (d) S-3 fraction (about 0.005 mg protein).

TABLE II

Some Properties of P. ovalis Iron-Sulfur Protein(ISP-I)

Parameter	
Molecular weight (g/mole)	
a. Sedimentation equilibrium	19,100
b. Gel filtration	18,800
c. Polyacrylamide gel electrophoresis	9,500
Sedimentation coefficient ($S_{20,w}$)	1.8 S
Chemical analysis (mole/mole protein)	
Iron	8.3 [2]
S	7.9 [2]
Molar extinction coefficient[2] (cm^{-1}, mM^{-1})	
E_{283}	58.7
E_{320}	43.9
E_{400}	34.8

1. Solvent was 0.01M sodium phosphate buffer containing 0.1M NaCl. The value of $\bar{v}$ was assumed to be 0.72.
2. Based on a molecular weight of 19,000 g per mole.

identical size. Molecular weight of ISP-II was estimated to be about 14,000 by Sephadex G-75 gel filtration and by polyacrylamide gel electrophoresis in SDS.

ISP-I appears to contain eight nonheme iron atoms and eight acid-labile sulfur stoms per mole, based on a molecular weight of 19,000 (TABLE II). ISP-II appears to contain 6-8 atoms of nonheme iron and 6-8 atoms of acid-labile sulfur per mole, based on a molecular weight of 14,000.

The absorption spectrum of purified iron-sulfur protein is shown in Fig. 4; it exhibits a peak at 283 nm and shoulders at 320 nm and 400 nm.

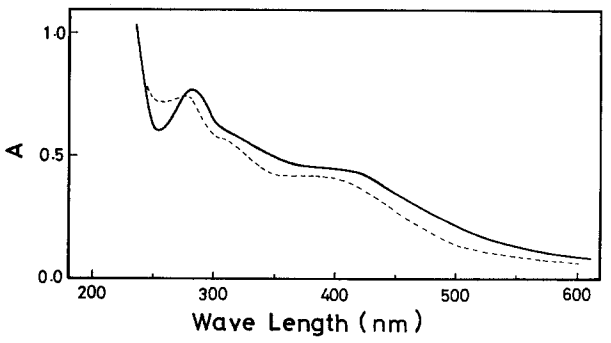

Fig. 4. Absorption spectra of P. ovalis iron-sulfur proteins.
Solid line: Absorption spectrum of ISP-I. ISP-I is in 0.01M sodium phosphate buffer, pH 7.4, at a concentration 0.26 mg/ml.
Dashed line: Absoption spectrum of ISP-II (S-3 fraction). ISP-II is in the same buffer, at a concentration 0.27 mg/ml.

The absorbance ratio A_{400}/A_{283} was 0.59. The absorption spectrum of the minor component was slightly different from that of ISP-I (Fig. 4).

In the photoreduction of NADP, ISP-I could replace the spinach ferredoxin. This protein could also substitute for spinach ferredoxin in the cytochrome c reduction by spinach NADPH: Fd oxidoreductase and NADPH. ISP-II had a similar biological activity.

DISCUSSION

Pseudomonas ovalis iron-sulfur protein (ISP-I) has quite different properties from the previously described iron-sulfur proteins of Pseudomonas: rubredoxin from P. oleovolans and putidaredoxin from P. putida. ISP-I contained eight iron atoms and eight acid-labile sulfur atoms per mole with a molecular weight of 19,000. This protein was composed of two subunits of identical size, therefore one subunit (molecular weight, 9,500) probably contains four iron atoms and four acid-labile fulfur atoms. From this point of view, ISP-I resembles 4Fe-4S ferredoxin from Bacillus polymyxa(14, 15) and Bacillus stearothermophilus (16), and the high potential iron-sulfur protein(17). The iron center of bacterial 8Fe-8S ferredoxin (Micrococcus aerogenes ferredoxin) consists

of two tetranuclear iron complexes with four bridging sulfur atoms(18). Chromatium high potential iron-sulfur protein has a tetranuclear iron complex center also(19). ISP-I appears to consist of two identical subunits which contain single tetranuclear iron center.

Some iron-sulfur proteins have been isolated in the dimeric form: nitrogenase Fe-protein(20, 21), and hydrogenase(22). However, these protein were high molecular weight molecules. The dimerization of Clostridium ferredoxin has been described; it takes place in the presence of oxygen with an intermolecular disulfide bond(23). From the results of polyacrylamide gel electrophoresis in SDS (Fig. 3a, b), however, the two subunits of ISP-I are held together probably by ionic or hydrophobic force and not by disulfide bond.

There are several reports on the presence of two different molecular species of ferredoxin in one organism; Rhodospirillum rubrum(24, 25), Azotobacter vinelandii(26, 27), Bacillus polymyxa(14, 15), and Aphanothece sacrum(28, 29). From the results, it is suggested that there are two types of iron-sulfur protein (ISP-I and ISP-II) in P. ovalis. These iron-sulfur proteins differ in molecular weight and absorption spectra. The amount of the minor component (ISP-II) was estimated as about 1/20 of the amount of the major component (ISP-I) by densitmetry of polyacrylamide disc electrophoresis at the DEAE-Sephadex column step. Further characterization of the minor component protein and relationships of this protein to the major component are now under way in this laboratory.

ACKNOWLEDGMENT

The auther is grateful to Professor K. Suzuki, Professor J. Tobari, Mr. F. Yamakura, and Mr. T. Matsumoto, for their helpful discussions.

REFERENCES

1. Lode, E. T. and Coon, M. J. (1971) J. Biol. Chem., 246, 791-802.
2. Cushman, D. W., Tsai, R. L. and Gunsalus , I. C. (1967) Biochem. Biophys. Res. Commu., 26, 577-583.

3. Matsumoto, T., Tobari, J., Suzuki, K., Kimura, T. and Tchen, T. T. (1976) J. Biochem., 79, 937-943.
4. Ornstein, L. (1964) Ann. N. Y. Acad. Sci., 121, 321-349.
5. Davis, B. J. (1964) Ann. N. Y. Acad. Sci., 121, 404-427.
6. Weber, K. and Osborn, M. (1969) J. Biol. Chem., 244, 4406-4412.
7. Kimura, T. and Suzuki, K. (1967) J. Biol. Chem., 242, 485-491.
8. Itzhaki, R. F. and Gill, D. M. (1964) Anal. Biochem., 9, 401-410.
9. Andrews, P. (1965) Biochem. J., 96, 595-606.
10. Yphantis, D. A. (1964) Biochemistry, 3, 297-317.
11. Mcswain, B. D. and Arnon, D. I. (1968) Proc. Natl. Acad. Sci. U. S. A., 61, 989-996.
12. Whatley, F. R. and Arnon, D. I. (1963) Methods Enzymol., 6, 308-318.
13. Forti, G. and Sturani, E. (1963) Eur. J. Biochem., 3, 461-472.
14. Yoch, D. C. (1973) Arch. Biochem. Biophys., 158, 633-640.
15. Stombaugh, N. A., Burris, R. H. and Orme-Johnson, W. H. (1973) J. Biol. Chem., 248, 7951-7956.
16. Mullinger, R. N., Cammack, R., Rao, K. K. and Hall, D. O. (1975) Biochem. J., 151, 75-83.
17. Dus, K., Deklerk, H., Sletten, K. and Bartsch, R. G. (1967) Biochim. Biophys. Acta, 140, 291-311.
18. Adman, E. T., Sieker, L. C. and Jensen, L. H. (1973) J. Biol. Chem., 248, 3987-3996.
19. Carter, C. W. Jr., Freer, S. T., Xuong, Ng. H., Alden, R. A. and Kraut, J. (1972) Cold Spring Harbor Symp. Quant. Biol., 36, 381-385.
20. Nakos, G. and Mortenson, L. E. (1971) Biochemistry, 10, 455-458.
21. Eady, R. R., Smith, B. E., Cook, K. A. and Postgate, J. R. (1972) Biochem. J., 128, 655-675.
22. Nakos, G. and Mortenson, L. E. (1971) Biochemistry, 10, 2442-2449.
23. Gersonde, K., Tritielvitz, E., Schlaak, H. E. and Stabel, H. H. (1971) Eur. J. Biochem., 22, 57-65.
24. Shanmugam, K. T., Buchanan, B. B., and Arnon, D. J. (1972) Biochim. Biophys. Acta, 256, 477-486.
25. Yoch, D. C. and Arnon, D. I. (1975) J. Biol. Chem., 250, 8330-8336.
26. Yoch, D. C., Beneman, J. R., Valentine, R. C., and Arnon D. I. (1969) Proc. Natl. Acad. Sci. U. S. A., 64, 1404-1410.
27. Yoch, D. C. and Arnon, D. I. (1972) J. Biol. Chem., 247, 4514-4520.
28. Wada, K., Kagamiyama, H., Shin, M. and Matsubara, H. (1974) J. Biochem., 76, 1217-1225.
29. Hase, T., Wada, K. and Matsubara, H. (1975) J. Biochem., 78, 605-610.