PSEUDOMONAS OVALIS FERREDOXIN: SIMILARITY TO *AZOTOBACTER* AND *CHROMATIUM* FERREDOXINS

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1. Introduction

An iron–sulfur protein isolated [1] from *Pseudomonas* sp. had amino acid composition and physical properties similar to those of *Azotobacter vinelandii* ferredoxin I [2]. Two iron–sulfur proteins isolated [3] from *Pseudomonas ovalis* (identical with *Ps. putida*) had ferredoxin I (iron–sulfur protein I) as the major component having similar characteristics to those of *Pseudomonas* sp. [1]. However, *Ps. ovalis* ferredoxin I appeared to consist of 2 identical subunits and the molecular weight of one subunit, 9500, was smaller than that of *Pseudomonas*, sp., 14 000. To clarify the still ambiguous nature of *Ps. ovalis* ferredoxin I, we studied the sequence of this ferredoxin. With the amino (N)-terminal sequence of *Az. vinelandii* ferredoxin I [4] the unusual nature of two 4 Fe–4 S chromophore centers was explained by the distribution of cysteine residues.

This paper describes the amino acid sequence of *Ps. ovalis* ferredoxin I, demonstrates the similarity to *Azotobacter* and *Chromatium* ferredoxins and discusses the structure and evolutionary relationships among these proteins.

2. Materials and methods

Pseudomonas ovalis ferredoxin I was prepared as in [3]. After precipitation of ferredoxin with trichloroacetic acid, the apoferredoxin was reduced with 2-mercaptoethanol and carboxymethylated with iodoacetic acid. The amino acid composition of carboxymethyl (Cm)-ferredoxin was determined as usual after 6 N HCl hydrolysis for 24 h. Tryptophan was analyzed after hydrolysis with 3 N mercaptoethanesulphonic acid [5].

The amino acid sequence studies were essentially the same as in [6,7]. Cm-ferredoxin was separately digested with trypsin and staphylococcal protease and the peptides produced were separated by gel filtration on a Bio-Gel P-4 or P-6 column developed by 0.2 M ammonium bicarbonate and further purified by paper electrophoresis, at pH 3.6 or pH 6.5, or by paper chromatography. The N-terminal and carboxyl (C)-terminal sequences of the Cm-ferredoxin and peptides were determined by a manual Edman degradation method and a carboxypeptidase method, respectively.

In order to obtain overlapping sequence in the region of the Cm-protein beyond the single methionine (residue 64), Cm-protein was acetylated with acetic anhydride, then cleaved with cyanogen bromide in 70% formic acid. After these treatments the product was directly sequenced by Edman degradation without any purification procedure.

3. Results and discussions

3.1. *Sequence studies of* Ps ovalis *ferredoxin I*

The amino acid composition of Cm-ferredoxin I was:

Lys, 5.16 (5); His, 0.94 (1); Arg, 2.06 (2); Cm-cys, 9.33 (9); Asp, 14.9 (15); Thr, 3.90 (4); Ser, 2.00 (2); Glu, 15.6 (16); Pro, 8.61 (9); Gly, 4.39 (4); Ala, 8.20 (8); Val, 6.27 (7); Met, 1.01 (1); Ile, 7.31 (8); Leu, 6.20 (6); Tyr, 2.04 (2); Phe, 5.23 (5); Trp, 1.83 (2).

The numbers in parentheses are deduced from the sequence study. Figure 1 shows the summary of the sequence studies of *Ps. ovalis* ferredoxin I. The N-terminal sequence was established by a manual Edman degradation up to 18 steps without any ambiguity. Carboxypeptidase B released arginine (0.86) from Cm-protein for 1 h and no other residue was released after further digestion with carboxypeptidase A. Ten peptides, T-1–T-10, and 12 peptides, S-1–S-12, were obtained from tryptic and staphylococcal protease digests. All staphylococcal protease peptides, except for peptides S-2, S-11, and S-12, were completely sequenced by Edman degradations and the C-terminal sequence of peptide S-4 was confirmed by a carboxypeptidase Y digestion. The C-terminal residue of peptide S-11 was arginine in accord with that of Cm-protein and therefore, peptide S-11 was considered to be C-terminal peptide of the original protein. Tryp-

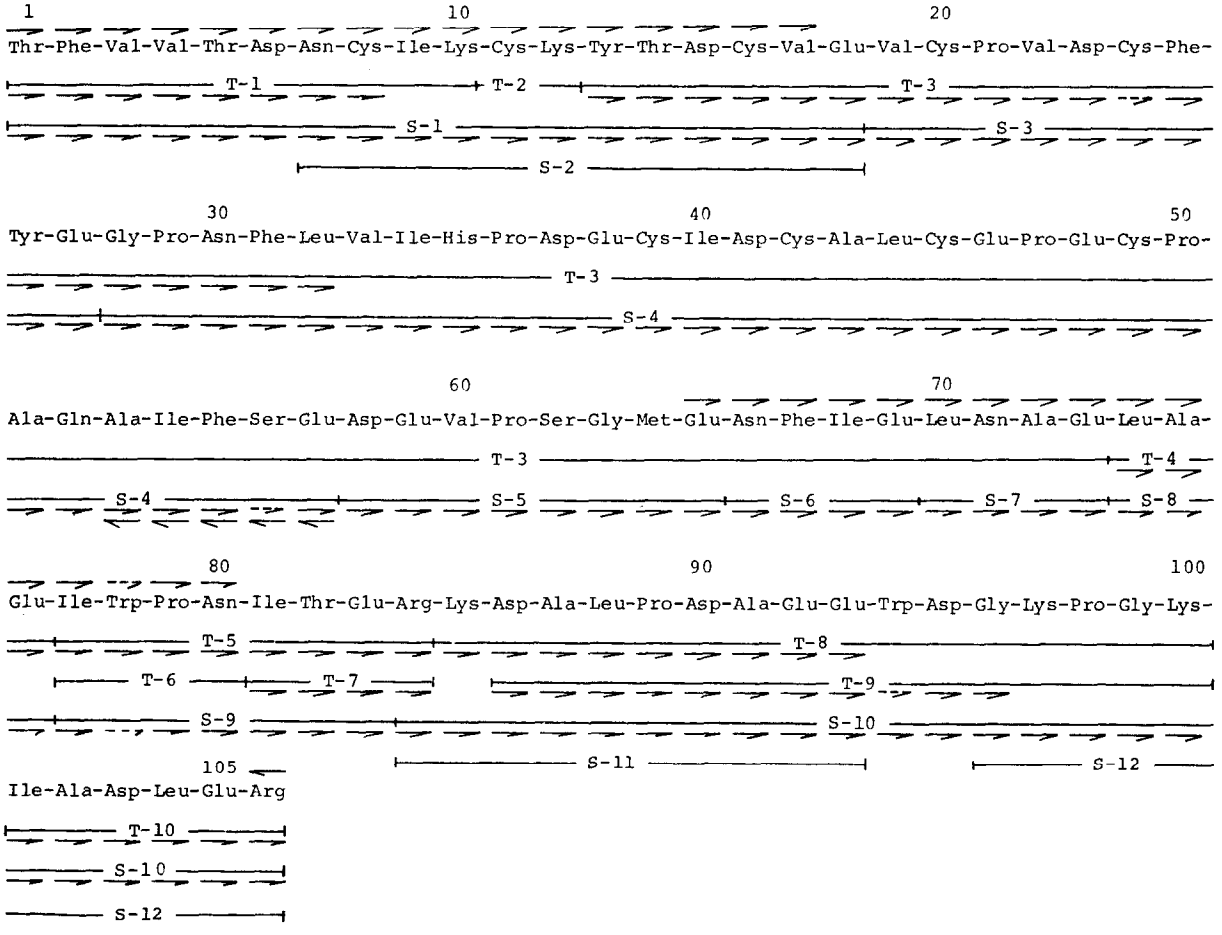

Fig.1. Summary of the sequence studies of *Ps. ovalis* ferredoxin I. The arrows (—→) and (←—) above the sequence indicate a manual Edman degradation and carboxypeptidase B digestion, respectively, on Cm-ferredoxin. T- and S- represent the peptides obtained by trypsin and staphylococcal protease digestion, respectively, of Cm-ferredoxin. Arrows, (—→) and (←—) below the peptide sequences indicate Edman degradation and carboxypeptidase Y digestion, respectively. Dotted arrows indicate ambiguous identifications.

tic peptides were also sequenced by Edman degradation. The sequences of some peptides were not completed, but unsequenced portions of tryptic peptides could be supplemented by the sequence studies of staphylococcal protease peptides and Cm-ferredoxin. Unexpected cleavages by trypsin were observed at Glu–Leu (73–74) and Glu-Ile (76–77) bonds and this might be due to contamination of staphylococcal protease into our trypsin solution by some reason, judging from the cleavage site.

N-terminal sequence of Cm-ferredoxin and the comparison of the sequence of tryptic and staphylococcal protease peptides gave the overlaps of several peptides. Edman degradation of the cleaved product of acetyl-Cm-ferredoxin with cyanogen bromide gave the sequence of residues 65–80 which overlapped peptides T-3–T-5 and also peptides S-5–S-9. These results aligned all peptides in order establishing the complete amino acid sequence of *Ps. ovalis* ferredoxin I as shown in fig.1.

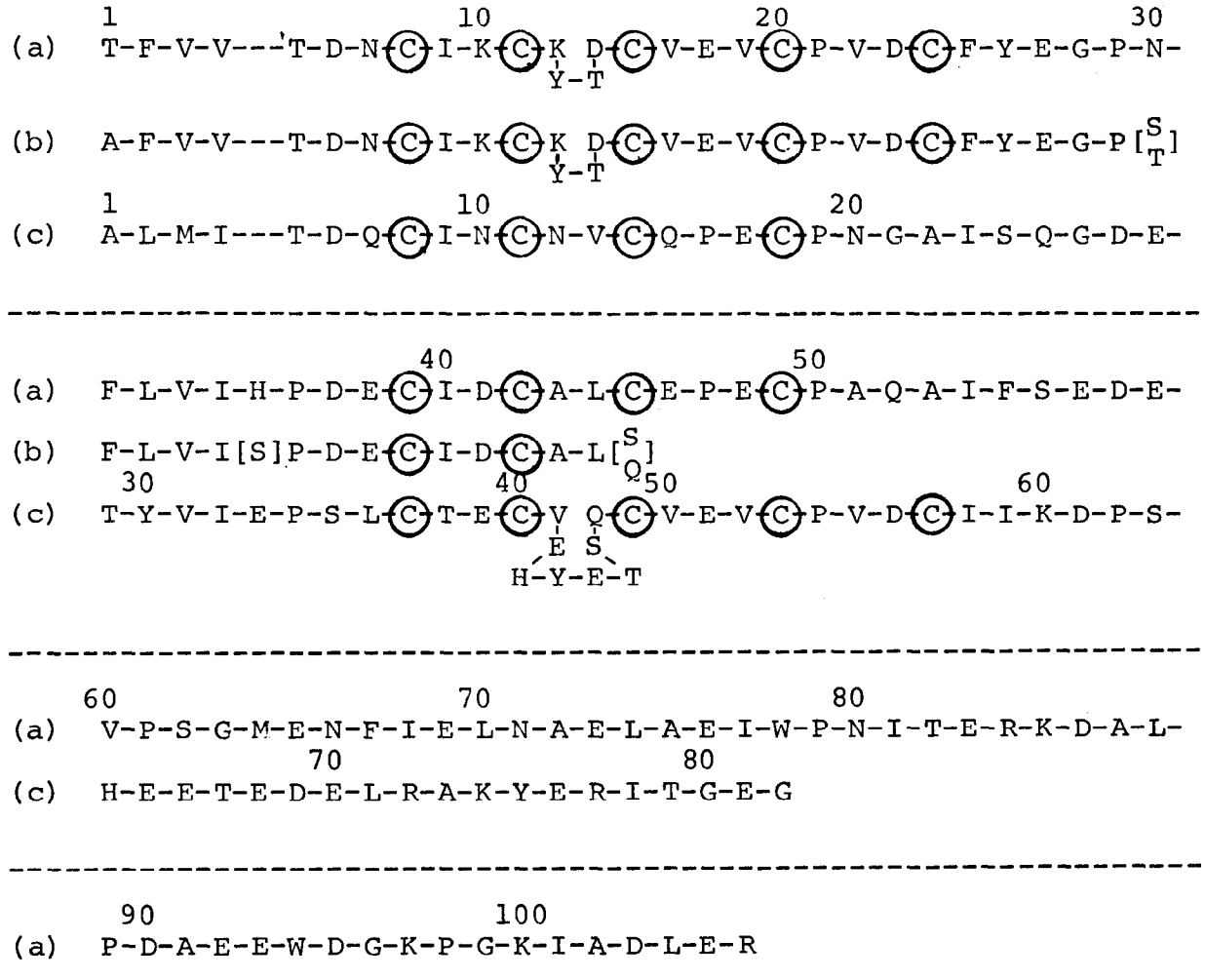

Fig.2. Sequence comparison of ferredoxins from (a) *Ps. ovalis*, (b) *Az. vinelandii* [4] and (c) *Chr. vinosum* [7,8]. [ ] in *Azotobacter* ferredoxin were not yet unambiguously established [4]. Gaps and insertions are placed into the sequences in order to produce a higher homology. Similarities between sequences (1–30) of (a) and (29–63) of (c) and between sequences (31–59) of (a) and (1–28) of (c) should be noted.

3.2. *Sequence homology of* Ps. ovalis *ferredoxin I with* Az. vinelandii *ferredoxin I and* Chr. vinosum *ferredoxin*

Ps. ovalis ferredoxin I was composed of 106 amino acid residues with 9 cysteine residues. The molecule was much larger than that of clostridial-type ferredoxins. The N-terminal sequence of *Ps. ovalis* ferredoxin up to about 40 residues was surprisingly similar to that of *Az. vinelandii* ferredoxin I [4] as shown in fig.2, although its complete sequence is not available yet. The comparison of *Chromatium* [9,10] and *Ps. ovalis* ferredoxins is also shown in Fig.2, in which the 2 ferredoxins are aligned from the N-termini. This alignment is based on the suggestion that the clostridial and photosynthetic bacterial ferredoxins were derived by a gene duplication of an ancestral ferredoxin followed by fusion and variation of molecular size with insertion or deletion [8]. However, cysteine cluster regions at the N- and C-terminal segments of *Ps. ovalis* ferredoxin I show a close similarity to those at the C- and N-terminal segments of *Chromatium* ferredoxin, respectively. Especially the sequence of residues 16–24 of *Ps. ovalis* ferredoxin I is completely identical with that of residues 49–57 of *Chromatium* ferredoxin. An insertion after residue 11 in *Ps. ovalis* ferredoxin I is also found in *Chromatium* ferredoxin after residue 41, although the length of insertion is different. In contrast to the large homology at cysteine segments, no similarity is found between the long C-terminal regions of the two sequences. This evidence suggests that after an ancestral form of *Chromatium* and *Ps. ovalis* ferredoxins diverged from the line of clostridial ferredoxins at an early evolutionary stage, an unequal crossing over must have occurred in the ferredoxin gene to deliver *Ps. ovalis* ferredoxin, although the detailed genetic mechanism is reserved at the present moment.

3.3. *Distribution of cysteine residues*

Recent study showed that *Ps. ovalis* ferredoxin I had two 4 Fe–4 S clusters in one single polypeptide chain and that it exhibited an EPR signal of g 2.01 in its oxidized form (D.O., unpublished results). These characteristics are similar to those of *Azotobacter* ferredoxin. *Azotobacter* ferredoxin contained two 4 Fe–4 S clusters, both of which appeared to function between the same pair of oxidation states as the single 4 Fe–4 S cluster in *Chromatium* high-potential-iron-protein [11], but the midpoint reduction potentials of two clusters was 760 mV different; one was with E_0 –420 mV and the other with E_0 +350 mV. Recently a ferredoxin with similar magnetic properties with that of *Azotobacter* ferredoxin was also isolated from *Rhodospirillum rubrum* [12]. This evidence suggests the strong influence of protein structure to reduction potentials of 2 clusters.

The chelate structures of two 4 Fe–4 S clusters of *Peptococcus aerogenes* ferredoxin is known [13]. The distribution of cysteine involved in iron chelation is summarized in fig.3. From the comparison of *Pp. aerogenes, Chromatium,* and *Ps. ovalis* ferredoxins, the iron chelation of cysteine residues in *Ps. ovalis* ferredoxin is proposed as follows: at one cluster, cysteine residues 8, 11, 16 and 49; at the other, cysteine residues 20, 39, 42 and 45. A cysteine residue at position 24 which corresponds to cysteine 57 of *Chromatium* ferredoxin will not be involved in iron chelation. The striking similarity of sequences of *Ps. ovalis* and *Azotobacter* ferredoxins indicates that Cys-24 of *Azotobacter* ferredoxin is unnecessary for iron chelation in spite of the suggestion in [4]. Although we do not find the substantial difference in cysteine distri-

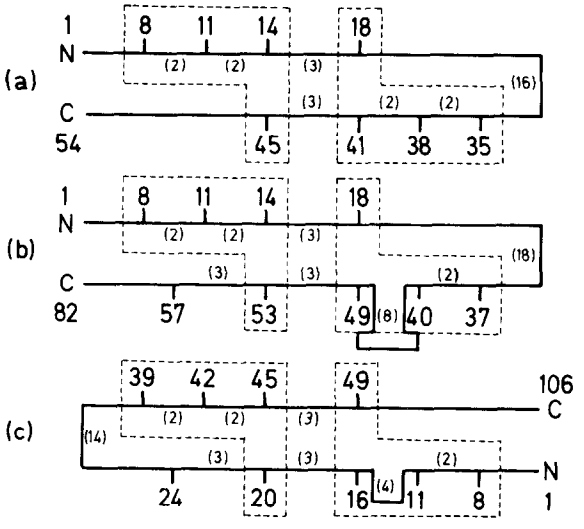

Fig.3. Distributions of cysteine residues in ferredoxins of (a) *Pp. aerogenes,* (b) *Chromatium* and (c) *Ps. ovalis*. Numbers in ( ) are those of residues present between cysteine residues. The relative lengths of peptide chains are valid only at around cysteine cluster regions.

bution of *Ps. ovalis* ferredoxin I from other bacterial ferredoxins, the cysteine cluster containing the loop in *Ps. ovalis* ferredoxin locates at the region near N- and C-termini of the molecule in contrast with the cluster in *Chromatium* ferredoxin which is in the middle portion of the molecule. This difference might influence one of the iron–sulfur cluster structures to derive the large difference in redox potentials of the 2 clusters [11].

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