HALOBACTERIUM HALOBIUM FERREDOXIN

A homologous protein to choroplast-type ferredoxins

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1. Introduction

A ferredoxin from *Halobacterium halobium,* an extreme halophile, has a typical 2Fe–2S chromophore centre and shares several other properties with chloroplast-type ferredoxins isolated from algae and plants; in particular the optical, ORD-, CD- and EPR-spectra of the *Halobacterium* ferredoxin are very similar to the chloroplast-type ferredoxins [1,2]. However, it has a less negative redox potential, –350 mV, and a higher molecular weight (about 15 000) than those of chloroplast-type ferredoxins. More interestingly, it does not function in the NADP–photoreduction system of chloroplasts, though halobacterial cell-free extracts catalyse the equilibrium between ferredoxin and NADH at high salt concentrations [2].

The unique character of this ferredoxin from a bacterium led us to study its amino acid sequence and to compare it with those of other ferredoxins. We have found that the *H. halobium* ferredoxin molecule consists of 128 amino acid residues, but has only four cysteine residues whose relative positions in the sequence are the same as those of the four cysteines (found in all chloroplast-type ferredoxins) which are involved in the binding of the 2Fe–2S centre.

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2. Materials and methods

Ferredoxin was isolated from *Halobacterium halobium* NRL R_1 strain M 1 as previously described [2] and purified further by chromatography on hydroxylapatite. The amino acid sequence studies were essentially the same as described on several other occasions [3–5]. Carboxymethyl (Cm)–ferredoxin was separately digested with trypsin and *Staphylococcus* protease, the peptides were separated on a BioGel P-4 column and further purified by paper electrophoresis or chromatography. Thermolysin was also used to degrade the tryptic peptide T-4. The amino(N)- and carboxyl(C)-terminal sequences of the protein and peptides were determined by a manual Edman degradation method and a carboxypeptidase method, respectively.

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3. Results and discussions

The amino acid composition of Cm-ferredoxin was Lys (including an unidentified residue Lys*; see below) 4.93(5); His, 0.87(1); Arg, 2.78(3); Cm–Cys, 4.15(4); Asp, 20.8(21); Thr, 3.86(4); Ser, 3.87(4); Glu, 21.4(21); Pro, 3.18(3); Gly, 9.49(9); Ala, 13.9(14); Val, 5.59(8); Met, 3.72(4); Ile, 5.38(7); Leu, 8.29(9); Tyr, 6.52(7); Phe, 1.98(2). Tryptophan was not determined. The numbers in parentheses are deduced from the sequence study. Valine and isoleucine showed fairly low values after 24 h-hydrolysis because of the presence of these residues consecutively in order. One of the five lysine residues, tentatively designated Lys*, is probably an unknown amino acid which emerged at nearly identical position with lysine on the short column (5 cm) of the amino acid analyzer but which, during Edman degradation showed a different chromatographic behaviour, from lysine, for its PTH-derivative on thin layers. The nature of this amino acid is under investigation.

Figure 1 summarizes the sequence studies of *Halobacterium* ferredoxin. The N-terminal sequence was established by a manual Edman degradation up to 17 steps with ambiguous residue. Carboxypeptidase A released only isoleucine and valine in equal amounts, which were later sequenced to be –Val–Ile. Trypsin produced nine peptides, but Peptide T-1 was only partially sequenced because of the difficulty in Edman degradation at the cluster of aspartic acid residues. However, the studies of chymotryptic peptides, C-1 and C-2, supplemented the unsequenced portions of T-1. Tryptophan residues were ambiguously identified by Edman degradation, but the original analysis showed the presence of only two residues and Peptide T-1 and T-3 gave an Ehrlich-positive reaction. Carboxypeptidase A confirmed the C-terminal tryptophan of Peptide C-1. Therefore, the positions of two tryptophan residues are probably correct. Residues 74–76 were determined on a thermolysin peptide of T-4 to be Ile–Val–Lys. *Staphylococcus* protease produced 14 peptides and

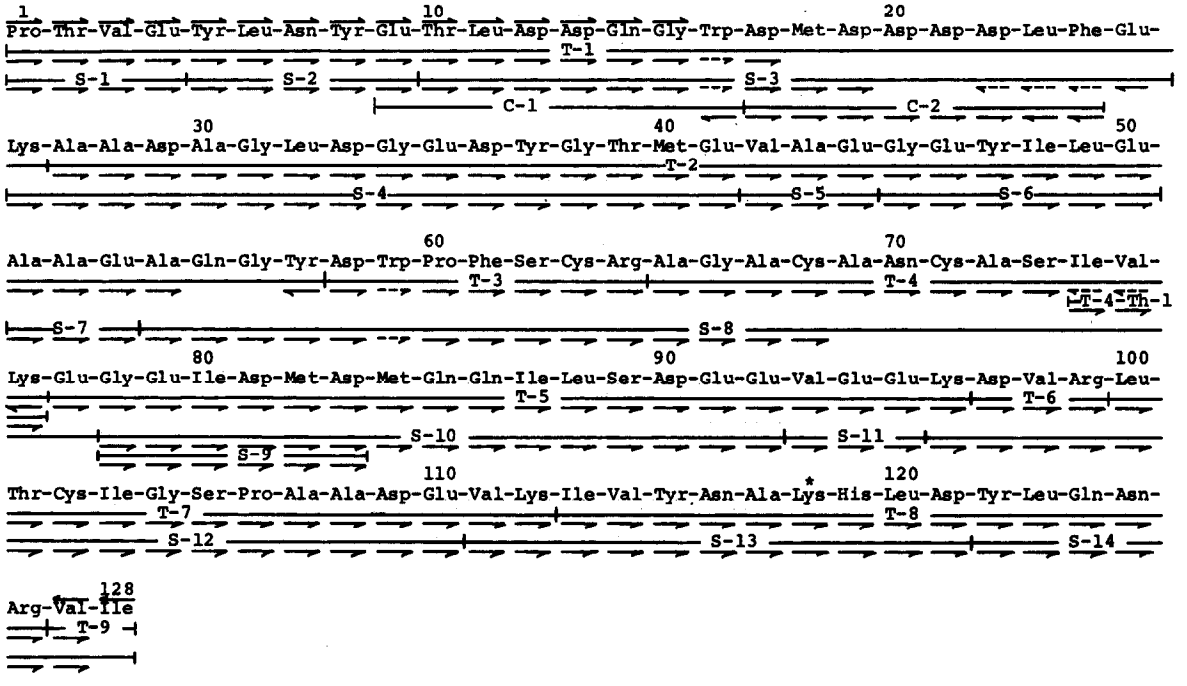

Fig.1. Summary of the sequence studies of *Halobacterium halobium* ferredoxin. The arrows (——→) and (←——) above the sequence indicate a manual Edman degradation and carboxypeptidase A digestion, respectively, on Cm-ferrodoxin. T-, S- and C- represent the peptides obtained after trypsin, *Staphylococcus* protease and chymotryptic digestion, respectively, of Cm–ferredoxin. Th- refers to a thermolysin peptide derived from Peptide T-4. Arrows (——↛) and (↚——) below the peptide sequences indicate Edman degradation and carboxypeptidases digestion, respectively. Dotted arrow indicates an ambiguous identification. Lys* is an unknown amino acid residue (see text). Cysteine was identified as Cm–cysteine.

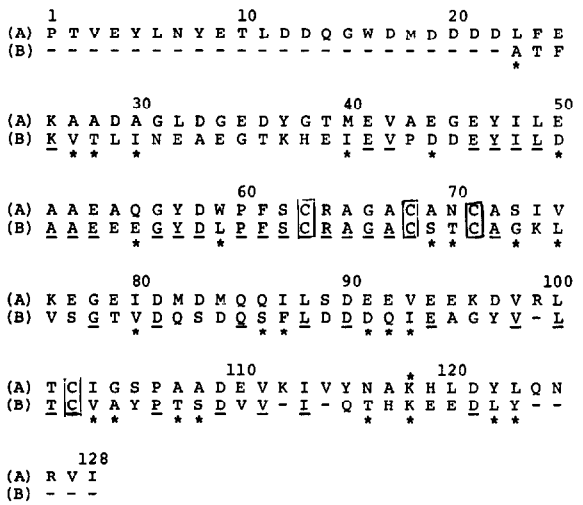

Fig.2. Sequence comparison of ferredoxins from *Halobacterium* (A) and *Nostoc muscorum*(B) [4]. The position with a bar below the sequences indicates the common residue in both the ferredoxins and that with a star the conservative residue. Hyphens represent a gap inserted in *Nostoc* ferredoxin to produce a higher homology between the two. K is an unknown amino acid expressed as a one letter notation for Lys.

several of them overlapped trypsin peptides. The unidentified residue, Lys, mentioned earlier, was present at position 118.

The total number of residues of *Halobacterium* ferredoxin is 128 which makes it the largest 2Fe–2S ferredoxin so far sequenced. However the protein has four, and only four, cysteine residues which fulfilled the minimum requirement for chelating two iron atoms, as has been discussed previously [3,4,6,7]. Figure 2 compares the sequences of *Halobacterium* ferredoxin and the ferredoxin from the blue–green alga *Nostoc muscorum*. It is striking to see that there is a very high similarity between these two except for the long extra N-terminal region of *Halobacterium* ferredoxin. Particularly the region around the cysteine residues is very similar to that in chloroplast-type ferredoxins. With this alignment there are 39 identical residues in 96 sites compared and 27 additional conservative residues. If compared with all other chloroplast-type ferredoxins so far sequences [8] a higher degree of similarity is obtained. Therefore, halobacterial ferredoxin is clearly homologous and should have a common ancestor with chloroplast-type ferredoxins. From the number of different amino acid residues it is postulated that *Halobacterium* (obligate aerobe) ferredoxin and chloroplast-type ferredoxins could have evolved independently from a common blue–green algal ferredoxin. The long extra N-terminal region contains 41% of acidic residues and no basic residue and may be caused by the adaptation to life in concentrated salt solutions since a large excess of acidic residues is common feature of all halophilic proteins [9].

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