## CYANIDIUM CALDARIUM FERREDOXIN: A RED AŁGAL TYPE?

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## 1. Introduction

Chloroplast-type ferredoxin is a 2 Fe-2 S iron—sulfur protein found in blue-green algae and photosynthetic eukaryotes [1]. A similar ferredoxin was also present in *Halobacterium* [2]. Recent studies on immunological cross reaction of ferredoxins showed the interrelations between blue-green and green algae or blue-green and red algae [3]. The amino acid sequences of ferredoxins from blue-green algae [4-9] and a green alga [10] have been determined, but no other algal ferredoxin sequence is available, although a partial sequence of a red alga, *Porphyra umbilicalis* is known [11]. The algal ferredoxins are evolutionarily a very diverse group judging from sequence comparisons [5,11].

We have purified a ferredoxin from an acido-thermal alga, Cyanidium caldarium which is a unicellular eukaryotic organism of uncertain classification. This alga may represent the evolutionary transition from the prokaryotic blue-green algae to the simplest eukaryotic red alga [12]. We describe here the amino acid sequence of Cyanidium caldarium ferredoxin and compare it with other chloroplast-type ferredoxins to assess a taxonomic and evolutionary status of Cyanidium.

### 2. Materials and methods

## 2.1. Mass production of C. caldarium

C. caldarium Geitler em. Hirose (no. 1355/I Allen) was from the Culture Collection of Algae and Protozoa,

Cambridge. The mass production of this alga was performed in a 1101 algal pilot plant [13]. A semicontinuous culturing was performed. Every week 951 of the algal suspension were harvested. Then an equal volume of fresh and sterile medium was added to the 151 algal suspension remaining in the plant. Details of sterilization, inoculation and harvest were similar to those in [14]. On average, 750 g Cyanidium were harvested every 7 days.

### 2.2. Purification of ferredoxin

Wet cells, 1 kg, were homogenised with 20 mM Tris-HCl buffer (pH 7.5). The homogenate was sonicated for 30 s intervals for total of 10 min in an ice bath. Acetone at  $-15^{\circ}$ C was slowly added to the sonicate to final conc. 30%. The mixture was centrifuged at 23 000  $\times$  g for 1 h. DE23 (Whatman DEAEcellulose), 40 g, was stirred into the supernatant and the mixture let stand for 1 h. The DE23 was transferred to a small column (3.2 × 30 cm) and washed successively with 0.1 M and 0.2 M NaCl in buffer. The ferredoxin was eluted by 0.8 M NaCl in buffer. After adding ammonium sulphate to the eluate to 50%, the mixture was centrifuged and the supernatant containing the ferredoxin was dialysed against 201 buffer overnight. Further purification of ferredoxin was carried out by standard procedures, viz. chromatography on DEAE-cellulose, Sephadex G-75 and hydroxylapatite [1].

## 2.3. Sequence analyses

About 1.3 µmol carboxymethyl (Cm)-ferredoxin

[6] were hydrolyzed with 0.3 mg trypsin for 2 h at 40°C in 1 ml 0.1 M Tris-HCl (pH 8.0). The digest was chromatographed on a Bio-Gel P-6 column (2 × 197 cm) by 0.2 M ammonium bicarbonate. Some fractions were further purified by paper electrophoresis, at pH 6.5. Staphylococcal protease (a gift of Dr R. Ambler, Edinburgh Univ.) was used to hydrolyze a large tryptic peptide T-3. The amino (N)- and carboxyl (C)-terminal sequences of Cm-ferredoxin and various peptides were determined by a manual Edman degradation [15] and a carboxypeptidase method [16,17], respectively. Phenylthiohydantoin derivatives of amino acids were identified by the thin-layer chromatography [18] or by paper electrophoresis, at pH 6.5 [19]. Amino acid compositions of CM-ferredoxin and peptides were determined by an amino acid analyzer (Beckman Model 120B) as usual [20] after 6 N HCl hydrolysis for 24 h or 72 h. Detailed procedures of sequence studies were essentially as in [2,6-8].

#### 3. Results and discussion

Purified *Cyanidium* ferredoxin (5 mg/kg cells) showed similar optical absorption and electron paramagnetic spectra to those of other chloroplast-type ferredoxins. The absorbance ratio,  $R=A_{420}/A_{280}$ , was 0.52.

# 3.1. Amino acid compositions and N- and C-terminal sequences of Cm-ferredoxin

The amino acid composition of *C. caldarium* Cm-ferredoxin is shown in table 1. The total number of residues was 98. This composition agrees with that deduced from the sequence. Manual Edman degradation revealed the N-terminal sequence up to 14 residues without any ambiguity as shown in fig.1. Carboxypeptidase A released only leucine (1.00) and tyrosine (1.02) from Cm-ferredoxin after 15 min and no other residue was released after further digestion.

Table 1

Amino acid compositions of Cm-ferredoxin<sup>a</sup> and its tryptic peptides<sup>b</sup>

Cm- ferredoxin	T-1	T-2	T-3	T-4	T-5	T-6	Total
Lys 4.22(4)	1.09(1)	1.18(1)		1.05(1)	1.03(1)		4
His 1.71(2)		0.88(1)				1.12(1)	2
Arg 0.94(1)			0.88(1)				1
Cmc 5.31(5)			1.97(2)	1.89(2)		1.02(1)	5
Asp 12.7 (13)		1.06(1)	5.84(6)		5 00(5)	1.07(1)	13
Thr 5.54(6)			1.02(1)	1.01(1)		3.83(4)	6
Ser 6.92(7)	0.96(1)		1 09(1)	0.93(1)	1.81(2)	1.97(2)	7
Glu 15.2 (15)			6.88(7)		5.04(5)	3.08(3)	15
Pro 3.31(3)			1.99(2)			1.14(1)	3
Gly 6.29(6)			2.05(2)	1.88(2)	1.00(1)	1.03(1)	6
Ala 9.05( 9)	0.98(1)		2.06(2)	2.90(3)		2.91(3)	9
Val 5.13(5)		1.02(1)			2.02(2)	1.97(2)	5
Ile 4.94( 5)		0.82(1)	3.08(3)			0.98(1)	5
Leu 9.55(10)		1.05(1)	2.97(3)		2.84(3)	2.72(3)	10
Tyr 4.83(5)	0.96(1)		1.81(2)			1.96(2)	5
Phe 2.05(2)					1.01(1)	0.87(1)	2
Total 98	4	6	32	10	20	26	98
Yield (%)	80	45	96	95	94	63	

<sup>&</sup>lt;sup>a</sup> Acid hydrolyses were carried out for 24 h and 72 h. The values of threonine and serine were obtained by extrapolation to 0 time hydrolysis. The values of value and isoleucine were of 72 h hydrolysate

b The values were of 24 h hydrolysates without any corrections for incomplete hydrolysis or destruction

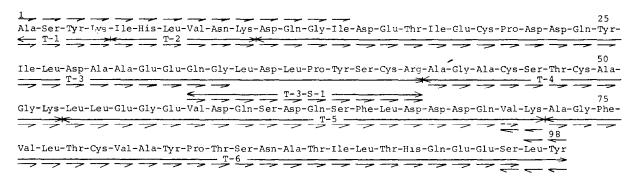


Fig.1. Summary of the sequence studies of Cyanidium ferredoxin. T- and S- refer, respectively, to peptides derived by tryptic digestion of Cm-ferredoxin and staphylococcal protease digestion of peptide T-3. Arrows (——) above the sequence and below the sequences of peptides show, respectively, Edman degradations for Cm-ferredoxin and peptides. Arrows (——) above and below the sequence show, respectively, carboxypeptidase A digestion on Cm-ferredoxin and peptides. A dotted arrow indicates an ambiguous identification.

## 3.2. Tryptic peptides and reconstruction of the ferredoxin sequence

Six peptides, T-1 to T-6, were obtained from the tryptic digest in good recovery (45-95%). The compositions and properties of the tryptic peptides are summarized in table 1. The sequence studies of these peptides are summarized in fig.1. Peptides T-1, T-2 and T-4 were completely sequenced by Edman degradation (T-1,4 steps; T-2, 6 steps; T-4, 10 steps). Large peptides T-3, T-5 and T-6 were partially sequenced by Edman degradations. Peptide T-3 was further digested with staphylococcal protease to give peptide T-3-S-1, C-terminal region of peptide T-3. Its amino acid composition was Arg, 0.92(1); Cm-cys, 0.89(1), Asp, 1.03(1); Ser, 0.86(1); Glu, 0.98(1), Pro, 0.92(1); Gly, 0.98(1); Leu, 1.80(2); Tyr, 0.85(1); and its sequence was determined by Edman degradation and gave the supplement of the unsequenced portion of peptide T-3. Carboxypeptidase digestions were performed on peptides T-5 and T-6 to obtain C-terminal sequences. Carboxypeptidase B digestion released lysine (1.09) from peptide T-5 after 1 h and further digestion with carboxypeptidase A released valine (1.05) after 1 h. Carboxypeptidase Y digestion of peptide T-6 released leucine (0.40) and tyrosine (0.73) after 5 min and leucine (0.84), tyrosine (0.87)and serine (0.47) after 1 h. Thus, C-terminal sequences of peptides T-5 and T-6 were determined as -Val-Lys and -Ser-Leu-Tyr, respectively.

N-terminal sequence of Cm-ferredoxin gave the overlaps of peptides T-1, T-2 and T-3. No effort to

overlap peptides T-4 and T-5 was made and they were aligned on the basis of homology to all other chloroplast-type ferredoxins. Peptide T-6 was C-terminal peptide. These results gave the complete sequence of *C. caldarium* ferredoxin (fig.1) and mol. wt 10 695 excluding iron and sulfur atoms.

## 3.3. Comparison of sequences of Cyanidium and other ferredoxins

Sequence of Cyanidium ferredoxin is compared with those of spinach, Scenedesmus quadricauda (a green alga), Nostoc muscorum (a blue-green alga), and Porphyra umbilicalis (a red alga) (fig.2). There are 37, 27, and 28 amino acid differences between Cyanidium and spinach, Scenedesmus, and Nostoc ferredoxins, respectively. From the number of amino acid differences, Cyanidium ferredoxin shows closer similarity to algal ferredoxins than to spinach ferredoxin, Cyanidium ferredoxin has two extra residues, lysine and isoleucine at positions 10 and 14, respectively. From the data available at present time all blue-green algal ferredoxins except for Aphanothece sacrum ferredoxin I [6] has two insertions at these positions, although the amino acid residues are different. Recently a partial sequence of Porphyra ferredoxin was reported and this ferredoxin had also such insertions [11]. In terms of the presence of these insertions Cyanidium ferredoxin seems to be more closely related to blue-green and red algal ferredoxins. Cyanidium caldarium is a unicellular eukaryotic alga of uncertain classification. In the

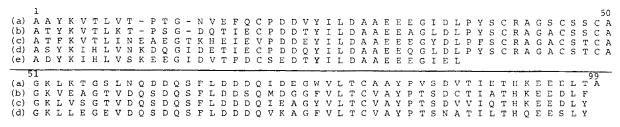


Fig. 2. Comparison of several representative chloroplast-type ferredoxins: (a) spinach [1], (b) Scenedesmus quadricauda [10]; (c) Nostoc muscorum [7], (d) Cyanidium caldarium, here: (e) Porphyra umbilicalis [11].

sequence comparison of 19 ferredoxins unique amino acid residues occupy several positions in *Cyanidium* and in the partial sequence of *Porphyra* ferredoxins, such as, Ile-5, His-6 and Lys-10. This suggests *C. caldarium* may be a member of red algae. However, more complete and extensive sequence study of red algal ferredoxins is required to confirm that *Cyanidium* forms an evolutionary link between the blue-green and red algae.

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### Addendum

During the preparation of this manuscript we were notified by Dr L. J. Rogers that *P. umbilicalis* ferredoxin sequence has been completed.

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