

## CYANIDIUM CALDARIUM FERREDOXIN: A RED ALGAL TYPE?

T. HASE, S. WAKABAYASHI, K. WADA, H. MATSUBARA, F. JÜTTNER\*, K. K. RAO<sup>+</sup>, I. FRY<sup>+</sup>  
and D. O. HALL<sup>+</sup>

Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan, \*Institut für Chemische Pflanzenphysiologie, Universität Tübingen, 7400 Tübingen, FRG and <sup>+</sup>Department of Plant Sciences, University of London King's College, 68 Half Moon Lane, London SE24 9JF, England

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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 acidothermal 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. 1355/1 Allen) was from the Culture Collection of Algae and Protozoa,

Cambridge. The mass production of this alga was performed in a 110 l algal pilot plant [13]. A semi-continuous culturing was performed. Every week 95 l of the algal suspension were harvested. Then an equal volume of fresh and sterile medium was added to the 15 l 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°C was slowly added to the sonicate to final conc. 30%. The mixture was centrifuged at 23 000 × g for 1 h. DE23 (Whatman DEAE-cellulose), 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 20 l 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

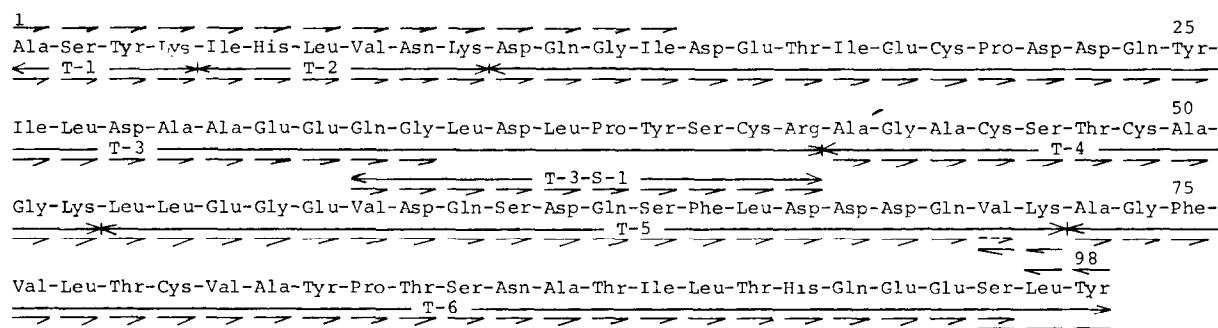
The amino acid composition of *C. caldarium* Cm-ferredoxin is shown in table 1. The total number of residues was 98. This composition agrees with that deduced from the sequence. Manual Edman degradation revealed the N-terminal sequence up to 14 residues without any ambiguity as shown in fig.1. Carboxypeptidase 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>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 valine and isoleucine were of 72 h hydrolysate

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



43

(a) 1 A A Y K V T L V T - P T G - N D V E I Q C P D D V Y I L D A A E E E G I D L P Y S C R A G S C S C A 50  
 (b) A T Y K V T L K T - P S G - N Q T E I E C P D D T Y I L D A A E E E A G L D L P Y S C R A G S C S C A  
 (c) A T F K V T L L I N E A E G - T D K H E I E V C P D D E Y I L D A A E E E G L D L P Y S C R A G S C S C A  
 (d) A S Y K I H L V N K D E Q G I D V T F D C S E D T Y I L D A A E E E Q G L D L P Y S C R A G S C S C A  
 (e) A D Y K I H L V S K D E G I D V T F D C S E D T Y I L D A A E E E G I E L

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51  
 (a) G K L K T G S L N Q D D Q S F L D D D Q I D E G W V L T C A A Y P V S D V T I E T H K E E E L T F 99  
 (b) G K V E A G T V D Q S D D Q S F L D D D Q I M D G G F V L T C V A Y P T S D C T I A T H K E E E D L T F  
 (c) G K L V S G T V D Q S D D Q S F L D D D Q I E A G F V L T C V A Y P T S D V V I Q T H K E E E D L Y  
 (d) G K L L E G T V D Q S D D Q S F L D D D Q I A G A G F V L T C V A Y P T S N A T I L T H Q E E S L Y