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Purification and some properties of ferredoxin derived from the blue-green alga, *Anacystis nidulans*

We recently obtained ferredoxin in a highly purified state from the blue-green alga, *Anacystis nidulans*, and determined some properties of the protein. In the present paper, we briefly describe the purification procedure and some properties of the protein.

The strain of *Anacystis nidulans* Richt. (IAM M-6) used in the present study was kindly supplied by the Institute of Applied Microbiology, University of Tokyo. The microorganism was cultivated as described in the previous paper[1]. Acetone-dried cells (about 50 g) were suspended in 750 ml of 10 mM phosphate buffer (pH 7.4). After standing overnight, the suspension was centrifuged, the resulting supernatant was fractionated with $(NH_4)_2SO_4$, and the precipitate appearing between 40 and 90 % saturation was collected by centrifugation at 10000 × *g* for 15 min. The precipitate was dissolved in 10 mM Tris–HCl buffer at pH 8.0, and the solution thus obtained was dialysed against the Tris–HCl buffer. The dialysed solution was charged on the DEAE-cellulose column which had been equilibrated with the Tris–HCl buffer. Ferredoxin was adsorbed on the column together with cytochrome *c*-554, cytochrome *c*-549 and phycocyanin. When the column was washed with 10 mM Tris–HCl buffer containing 0.2 M NaCl, cytochrome *c*-554, cytochrome *c*-549 and phycocyanin were eluted, while ferredoxin remained on the column. Ferredoxin was then eluted from the column with 1 M $NH_4H_2PO_4$. The pH of the eluate was immediately adjusted to about 6 by the addition of 1 M $(NH_4)_2HPO_4$. The eluate thus obtained was fractionated by $(NH_4)_2SO_4$ and the precipitate appearing between 70 and 90 % saturation was collected by centrifugation at 10000 × *g* for 15 min. The precipitate obtained after the centrifugation was dissolved in 0.2 M phosphate buffer, and the resulting solution was used as the Anacystis ferredoxin preparation.

As shown in Fig. 1, the absorption spectrum of Anacystis ferredoxin possessed peaks at 276, 330, 420 and 463 mμ in the oxidized form, and on reduction by $Na_2S_2O_4$ the absorbances at 420 and 463 mμ decreased considerably.

The millimolar extinction coefficient at 420 mμ was calculated to be 6.5 assuming the molecular weight of the protein to be 11000 (based on the amino acid

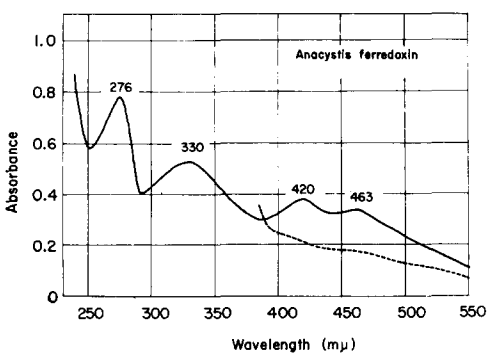

Fig. 1. Absorption spectra of Anacystis ferredoxin. The concentration of the protein was 58 μM. ———, oxidized; -------, reduced with $Na_2S_2O_4$.

composition). The ratio of $A_{330\ m\mu}/A_{276\ m\mu}$ was 0.68 and was considerably lower than that of Scenedesmus ferredoxin[2]. However, this seemed not to be caused by impurities, because the Anacystis ferredoxin obtained here was almost ultracentrifugally homogeneous. Thus, the ratio was comparable to those found with *Anabaena variabilis* (0.63 (ref. 3)) and *Nostoc muscorum* (0.70 (ref. 4)) ferredoxins.

The molecular weight of Anacystis ferredoxin was determined to be about 10000 by the gel filtration method[5] using Sephadex G-75. Bovine cytochrome *c* (mol. wt. 12300)[6], Pseudomonas blue protein (mol. wt. 17000)[7] and Chlorobium cytochrome *c*-551 (mol. wt. 60000)[8] were used as the standards. Nonhaem iron and labile sulphur were determined according to the methods of DUS *et al.*[9] and LOVENBERG *et al.*[10], respectively. 6400 g and 13700 g of the protein contained 1 atom of iron and 1 atom of labile sulphur, respectively. Therefore, it was concluded that one molecule of Anacystis ferredoxin contained 2 atoms of nonhaem iron and 1 atom of labile sulphur, since the molecular weight was determined to be 11000 from the amino acid composition (see below). Although this ratio of nonhaem iron to labile sulphur is anomalous for chloroplast-type ferredoxins[2,11–13], Anacystis ferredoxin greatly stimulated the photoreduction of $NADP^+$ with the illuminated spinach chloroplasts when assayed according to the method of SAN PIETRO[14]. Addition of Na_2S to the protein solution did not alter the absorbance spectrum. Analysis of the ferredoxin solution, which

TABLE I

AMINO ACID COMPOSITION OF ANACYSTIS FERREDOXIN

Values expressed as moles/mole protein.

Amino acid	*Values obtained after hydrolysis for*		*Average value*	*Integral number of residues*
	24 h	*48 h*		
Lys	2.87	2.92	2.90	3
His	0.82	0.79	0.81	1
Arg	1.17	1.16	1.17	1
(NH_3)	5.74	7.48	4.05*	4
Asp	15.27	14.19	14.73	15
Thr	10.49	9.29	11.67	12
Ser	5.73	4.91	6.53	7
Glu	10.43	10.43	10.70	11
Pro	2.49	2.83	2.66	3
Gly	6.30	5.81	6.06	6
Ala	12.20	11.83	12.02	12
Cys	2.90 (5.76)**	1.47		6**
Val	8.58	8.44	8.51	9
Met	0	0	0	0***
Ile	5.18	5.07	5.13	5
Leu	7.20	6.82	7.01	7
Tyr	4.57	4.59	4.58	5
Phe	2.11	2.17	2.14	2
Trp				0§
Total				105

* Extrapolated to zero hydrolysis time.
** As cysteic acid.
*** As methionine sulphone.
§ Based on spectrophotometric analysis[15].

had been dialysed against a buffer containing Na_2S, showed the same labile sulphur content as the original preparation.

In Table I is shown the amino acid composition of Anacystis ferredoxin. Amino acid analysis was performed with a Beckman amino acid analyser, model 120B.The protein differed both from higher plant[11–13] and Scenedesmus[2] ferredoxins in that it lacked both methionine and tryptophan. However, the amino acid composition of Anacystis protein as a whole resembled that of chloroplast-type ferredoxins[2,11–13]. It seems quite interesting that the ratio of nonhaem iron to labile sulphur in Anacystis ferredoxin is 2, while the content of half cystine is the same as that found for Scenedesmus ferredoxin[2]. The content of labile sulphur distinguishes Anacystis ferredoxin from the other chloroplast-type ferredoxins in which 2 atoms of sulphur exist in one molecule as far as is known. Further study should clarify whether these features observed with Anacystis ferredoxin are common to ferredoxins derived from blue-green algae.

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