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SHORT REPORTS

FERREDOXIN FROM THE CYANOBACTERIUM *OSCILLATORIA AGARDHII*

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Key Word Index—*Oscillatoria agardhii* Cyanobacteria, nitrogen fixation, ferredoxin

Abstract—*Oscillatoria agardhii* contained a single ferredoxin It was a [2Fe-2S] protein of MW 11 075, with a mid-point redox potential (−380 mV) characteristic of ferredoxins from non-nitrogen-fixing cyanobacteria and different from that of the nitrogen-fixing *Oscillatoria limnetica*

INTRODUCTION

Ferredoxins serve as low potential electron donors to several physiological processes in cyanobacteria [1] Some degree of functional specialization of ferredoxins may have evolved Some species appear to contain multiple ferredoxins differing in their biochemical properties *in vitro* [2–4] Cammack *et al* [5] speculated that the mid-point redox potentials (E_m) of cyanobacterial ferredoxins may be correlated with the ability to fix atmospheric nitrogen We have purified the ferredoxin from *Oscillatoria agardhii* and, by comparison of its properties with those of *O limnetica* [5], suggest that this relationship may hold within a single genus

RESULTS AND DISCUSSION

Table 1 summarises the properties of the ferredoxin purified from *O agardhii* They are typical of the plant-type [2Fe-2S] ferredoxins

Cammack *et al* [5] showed that the known mid-point redox potentials of the major ferredoxins (Fd I in those cases where multiple ferredoxins were found) of cyanobacteria were within the range −310 to −390 mV Inspection of their data suggests that two subdivisions occurred Ferredoxins with E_m values in the range −310 to −355 mV were all from cyanobacteria capable of fixing atmospheric nitrogen (sometimes only under anaerobic conditions), while those in the range −375 to −390 mV were all from non-nitrogen-fixing species Ferredoxins from two species of *Oscillatoria* have now been characterized The ferredoxin from *O limnetica* [5] had an E_m (−346 mV) within the range typical of nitrogen-fixing species and this species has subsequently been shown to fix nitrogen anaerobically [6] Attempts to demonstrate anaerobic nitrogen fixation by *O agardhii*, however, have failed [7] and our value for the E_m of its ferredoxin (−380 mV) falls correspondingly within the range of those from non-nitrogen-fixing species A comparison of ferredoxins from other closely related nitrogen-fixing and non-fixing species would be of great interest

EXPERIMENTAL

Isolation and purification of ferredoxin Oscillatoria agardhii Gom, Cambridge Culture Collection of Algae and Protozoa no 1459/11, was grown in a large scale batch culture facilty Cells

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Table 1 Properties of *O agardhii* ferredoxin

λ_{max} (nm) of oxidized form	274, 282 (sh), 330, 442, 464
A_{max}/A_{274nm}	1 0, —, 0 68, 0 52, 0 46
MW	11 075 ± 9% ($p < 0 05$)
ε (422 nm)	10026 M^{-1} cm^{-1}
Mol ratio	non-haem iron = 2 08 labile sulphide = 1 90
pI	3 55
E_m	E_m = −380 (±9 9) mV at pH 8 0 ($p < 0 05$)
NADP reduction	3 9 mmol NADP/mg Chl μmol Fd hr

Table 2 Purification of ferredoxin

Purification step	Vol (ml)	Ferredoxin (μmol)	$\frac{A420}{A275}$	$\frac{A260}{A275}$
$(NH_4)_2SO_4$ supernatant	520	2 65	0 01	nd
1st DEAE column	52	2 58	0 05	1 41
2nd DEAE column	120	1 98	0 13	1 35
3rd DEAE column	105	1 61	0 19	1 26
Sephadex G-75 column	22	0 61	0 50	0 95
Peak G-75 fraction	4 5	nd	0 52	0 92

Ferredoxin was obtained from 200 g wet wt of cells The $A420/A275$ ratio was used to indicate the purity of the preparation and the $A260/A275$ ratio reflected the removal of contaminating nucleic acids nd = Not determined

were resuspended in 100 mM Tris, pH 7 7, disrupted and the cell debris was removed by centrifugation The supernatant was brought to 50% saturation with $(NH_4)_2SO_4$ and the precipitated proteins were discarded after being assayed for ferredoxin activity All subsequent solns used in the purification were made up in 50 mM Tris, pH 7 7 The supernatant was applied to a DEAE cellulose column equilibrated with 50% satd $(NH_4)_2SO_4$ [8] and eluted with 0 6 M NaCl After application to a second DEAE cellulose column, ferredoxin was eluted with a linear gradient of 0 1–0 3 M NaCl The ferredoxin was recovered as a single, well defined peak with no shoulders The most active fractions were purified further by a slow elution from a third DEAE cellulose column with 0 27 M NaCl After concn on a small DEAE cellulose column, the ferredoxin was applied to a Sephadex G-75 column which was equilibrated and developed in an upward direction with 0 4 M NaCl in Tris buffer Fractions with $A420/A275$ ratios of 0 49 or greater were retained as purified ferredoxin [9] Table 2 summarizes the purification process

Characterization of ferredoxin Ferredoxin activity was assayed by light-dependent NADP reduction using pea chloroplasts [9] Non-haem iron was determined by the method of Lovenberg *et al* [10], acid-labile sulphide by that of Suhara *et al* [11] and mid-point redox potential by that of Dutton [12] MW was estimated by gel filtration [13] through a Sephadex G-75 column, and the pI by determining the precipitation ($A750$ nm) of ferredoxin (2 5 μM in 50 mM potassium hydrogen phthalate) between pH 3 0 and 4 0 at intervals of 0 05 pH units Soluble protein was determined by a modified Lowry method [14] more suitable for proteins low in tryptophan

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REFERENCES

1 Stewart, W D P (1980) *Annu Rev Microbiol* **34**, 497
2 Shin, M, Sukenobu, M, Oshino, R and Kitazume, Y (1977) *Biochim Biophys Acta* **460**, 85
3 Hutson, K G, Rogers, L J, Haslett, B G and Boulter, D (1980) *FEBS Micro Letters* **7**, 279
4 Wada, K, Matsubara, H, Chain, R K and Arnon, D I (1981) *Plant Cell Physiol* **22**, 275
5 Cammack, R, Rao, K K, Bargeron, C P, Hutson, K G, Andrew, P and Rogers, L J (1977) *Biochem J* **168**, 205
6 Belkin, S, Arieli, B and Padan, E (1982) *Isr J Botany* **31**, 199
7 Kalininskaya, T A, Pankratova, E M and Khokhlova, V F (1981) *Mikrobiologiya* (Eng tr) **50**, 401
8 Mayhew, S G (1971) *Analyt Biochem* **42**, 191
9 Buchanan, B B and Arnon, D I (1971) *Methods Enzymol* **23**, 413
10 Lovenberg, W, Buchanan, B B and Rabinowitz, J C (1963) *J Biol Chem* **238**, 3899
11 Suhara, K, Takemori, S, Katagiri, M, Wada, K, Kobayashi, H and Matsubara, H (1975) *Analyt Biochem* **68**, 632
12 Dutton, P L (1978) *Methods Enzymol* **54**, 411
13 Andrews, P (1970) in *Methods of Biochemical Analysis* (Glick, D, ed) Vol 18, p 1 Interscience, New York
14 Herbert, D, Phipps, P J and Strange, R E (1977) in *Methods in Microbiology* (Norris, J R and Ribbons, D W, eds) Vol 5B, p 209 Academic Press, London