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Midpoint Redox Potentials of Plant and Algal Ferredoxins

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Midpoint potentials of plant-type ferredoxins from a range of sources were measured by redox titrations combined with electron-paramagnetic-resonance spectroscopy. For ferredoxins from higher plants, green algae and most red algae, the midpoint potentials (at pH 8.0) were between −390 and −425 mV. Values for the major ferredoxin fractions from blue–green algae were less negative (between −325 and −390 mV). In addition, *Spirulina maxima* and *Nostoc* strain MAC contain second minor ferredoxin components with a different potential, −305 mV (the highest so far measured for a plant-algal ferredoxin) for *Spirulina* ferredoxin II, and −455 mV (the lowest so far measured for a plant-algal ferredoxin) for *Nostoc* strain MAC ferredoxin II. However, two ferredoxins extracted from a variety of the higher plant *Pisum sativum* (pea) had midpoint potentials that were only slightly different from each other. These values are discussed in terms of possible roles for the ferredoxins in addition to their involvement in photosynthetic electron transport.

Two-iron ferredoxins were first isolated from the leaves of higher plants (Davenport *et al.*, 1952). Subsequently they have been isolated from many species of plants and algae (see Hall *et al.*, 1975*a*,*b*). In chloroplasts, ferredoxin has a well-defined role as a carrier of electrons from Photosystem I to $NADP^+$ (San Pietro & Lang, 1958) via the flavoprotein ferredoxin–$NADP^+$ reductase.

The ferredoxin from spinach chloroplasts was shown by Tagawa & Arnon (1968) to have a midpoint potential (E_m) at pH 7 of −420 mV. Similar values have been obtained by others workers [−423 mV (Ke *et al.*, 1974); −428 mV (Stombaugh *et al.*, 1976)].

The ferredoxins from green, red and blue–green algae are very similar to those of higher plants in their spectroscopic properties (Rao *et al.*, 1972; Andrew *et al.*, 1976), and the amino acid sequences of the green- and blue–green-algal ferredoxins show a considerable degree of homology (Matsubara *et al.*, 1976). Ferredoxin from a *Nostoc* species had an E_m value of −406 mV according to Mitsui & Arnon (1971).

There have been reports of the isolation of two ferredoxins from the blue–green algae *Aphanothece sacrum* (Hase *et al.*, 1975) and *Nostoc* strain MAC (Hutson & Rogers, 1975). In the latter case the ferredoxins were isolated from a monophyletic culture, indicating that they are not due to two subspecies growing together. The two ferredoxins can be readily separated on DEAE-cellulose chromatography, and though of essentially the same molecular weight they have different amino acid sequences. Significantly the two proteins from *Nostoc* strain MAC also differ in their activity in supporting the phosphoroclastic cleavage of pyruvate by *Clostridium pasteurianum* extracts or catalysing $NADP^+$ photoreduction by chloroplasts from higher plant, implying that they may fulfil different roles in the organism (K. G. Hutson, L. J. Rogers, B. G. Haslett, D. Boulter & R. Cammack, unpublished work). In the present paper we also describe the separation of a second, less acidic, ferredoxin from *Spirulina maxima*, designated ferredoxin II. The major fraction that was previously isolated (Hall *et al.*, 1972) is called ferredoxin I.

In higher-plant ferredoxins there is evidence for genetic variation in the amino acid sequences. Benson & Yasunobu (1969) found differences in amino acid sequence in ferredoxins from different individual trees of the species *Lucaena glauca* (koa). Glickson *et al.* (1971) detected differences in ^1H nuclear-magnetic-resonance spectra of ferredoxins from different varieties of *Glycine max* (soya bean). Probably these were due to hybridization, since Kwanyuen & Wildman (1975) noted two variants of ferredoxin (corresponding to the parent strains) in a hybrid of *Nicotiana* (tobacco). In the present

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investigation we have examined two ferredoxins extracted from a strain of pea (*Pisum sativum*) similar to that described by Mukhin *et al.* (1975).

We have measured the redox potentials of ferredoxins from a wide range of algal and higher-plant species by the method of Dutton (1971). A protein was poised, in the presence of mediator dyes, at various potentials measured by a platinum electrode. The degree of reduction of the ferredoxin at each potential was determined by removing samples anaerobically and freezing them for e.p.r.* spectroscopic measurements. Although somewhat cumbersome, this method is sensitive, requiring only about 1 mg of protein for each determination, and is applicable over a wide range of redox potentials. The results showed that the E_m values of ferredoxins from algae and higher plants vary over a much wider range than had previously been supposed.

Experimental

Preparation of ferredoxins

The isolations of the ferredoxins from *Chlorogloeopsis fritschii* and *Aphanocapsa* 6714 were based on that outlined for *Nostoc* strain MAC (Hutson & Rogers, 1975). *Nostoc* strain MAC, *Chlorogloeopsis fritschii* and *Aphanocapsa* 6714 are some of the few blue-green algae capable of growth both autotrophically in light and heterotrophically in the dark. The ferredoxins isolated from photo-autotrophically grown cells were used in this study, but there appears to be no doubt that the same ferredoxins are produced under heterotrophic conditions.

The isolation of the ferredoxin from *Porphyra umbilicalis* is described by Andrew *et al.* (1976). The preparation of ferredoxin from *Rhodymenia palmata* was similar, though homogenization of the fronds proved ineffective and thus freeze-drying and milling to a fine powder were necessary before extraction of ferredoxin. Compared with *Porphyra*, two further purification steps, chromatography on Sephadex G-100 and preparative polyacrylamide (15%)-gel electrophoresis, were also necessary to purify the ferredoxin. The preparation of ferredoxins from the unicellular red algae *Porphyridium cruentum* and *Porphyridium aerugineum* were based on the procedures used for the blue-green algae (Hutson & Rogers, 1975). The purification of the two ferredoxins from *P. sativum* (var. Onward) was based on steps 1–3 of the method of Rao (1969). Their separation exploited the observation that the Type-II ferredoxin binds slightly less tightly to DEAE-cellulose columns.

S. maxima ferredoxins I and II were prepared from dry algal powder, obtained from Sosa Texcoco, Mexico, by the procedure of Hall *et al.* (1972), except that in the final DEAE-cellulose (DE23; Whatman) chromatography the ferredoxins were eluted with 0.28M-NaCl instead of 0.35M-NaCl, when a minor red band separated from the major Type-I ferredoxin. This Type-II ferredoxin was eluted first and constituted less than 5% of the total ferredoxin.

Mastigocladus laminosus cells were cultured in the water of a hot spring near Reykjavik, Iceland, and processed for the extraction of allophycocyanin II by Gysi & Zuber (1974). The supernatant obtained after 50%-satd.-$(NH_4)_2SO_4$ fractionation was kindly supplied by Professor H. Zuber and was used for the purification of ferredoxin by the method of Rao *et al.* (1971).

The sources of other ferredoxins were as described by Tel-Or *et al.* (1977). Homogeneity of the ferredoxins was confirmed by polyacrylamide-gel electrophoresis (see e.g. Hutson & Rogers, 1975).

Redox titrations and e.p.r. measurements

Titrations were carried out as previously described (Cammack *et al.*, 1976). The ferredoxin concentration was 20–50μM. The mediators used were 3,7-diamino-5-phenylphenazinium chloride (phenosafranine), 1,1′-dibenzyl-4,4′-bipyridylium dichloride (Benzyl Viologen); 1,1′-dimethyl-4,4′-bipyridylium dichloride (Methyl Viologen) (from BDH Chemicals, Poole, Dorset, U.K.), 6,7-dihydrodipyrido[1,2-*a*:2′,1′-*c*]pyrazinediium dibromide (diquat) and 7,8-dihydro-6*H*-dipyrido[1,2-*a*:2′,1′-*c*]-diazepinediium dibromide (triquat) (kindly provided by Dr. B. White, I.C.I. Plant Protection, Bracknell, Berks., U.K.), all at concentrations of 10μM. The potential was adjusted with small additions of 0.1M-$Na_2S_2O_4$ in 0.1M-Tris/HCl, pH9.2, or of 0.2M-$K_3Fe(CN)_6$. After adjustment to a particular potential, a sample was withdrawn after 1 min and frozen in liquid N_2. The equilibration time was considered to be adequate, as similar results were obtained whether titrations were carried out by adjusting to progressively lower potential, or by adjusting to higher potentials. Samples, in quartz e.p.r. tubes (diam. approx. 3mm), were stored at 77K before reading the intensity of the ferredoxin e.p.r. signals.

E.p.r. spectra were recorded on a Varian E4 spectrometer (Varian Associates, Palo Alto, CA, U.S.A.) by using a flow of cold helium gas to cool the sample. Spectra were recorded at 25K, with a microwave power of 1mW and frequency of 9.2GHz. The size of the signal corresponding to reduced ferredoxin was measured from the overall amplitude of the derivative-type feature (g_y) at $g = 1.96$.

The fitting of the intensity data to curves derived from the Nernst equation was carried out as described by Cammack *et al.* (1976).

* Abbreviation: e.p.r., electron paramagnetic resonance.

Results

Comparison of potentials of ferredoxins

All the ferredoxins exhibited the rhombic e.p.r. signal in the reduced state characteristic of two-iron ferredoxins from plants or algae. The e.p.r. spectra of four of the blue–green-algal ferredoxins, including ferredoxins I and II from *Nostoc* strain MAC, are shown in Fig. 1. The e.p.r. spectra of the ferredoxins from *Porphyra umbilicalis*, *S. maxima* and spinach (*Spinacia oleracea*) have been presented elsewhere (Andrew *et al.*, 1976; Cammack, 1975; Hall *et al.*, 1975*a*; respectively). E_m values of the ferredoxins from various plant and algal sources were compared in 0.15M-Tris/HCl, pH8.0 (Table 1). This pH value was chosen because of the difficulty in obtaining sufficiently low potentials with dithionite at pH7. Results for signal intensity were plotted both directly or according to the Nernst equation as a function of redox potential. The plots indicated one-electron-accepting species in all cases. Reproducibility of results for the titrations was within ±10mV.

The E_m value of spinach ferredoxin (average of five determinations) was −415mV (S.D. ±9mV), in good agreement with previous (Tagawa & Arnon, 1968; Ke *et al.*, 1974; Stombaugh *et al.*, 1976) measurements. This indicates that the presence of Viologen dyes is not affecting the results; Stombaugh *et al.* (1976) found that Methyl Viologen at high concentration (0.64mM) affected the potential of *Cl. pasteurianum* ferredoxin.

Table 1. *E_m values of ferredoxins*

Samples were in 0.15M-Tris/HCl, pH8.0 at 25°C. E.p.r. spectra were at 25K. Potentials are expressed relative to the standard hydrogen electrode.

Source	Potential (mV)
Higher plants	
S. oleracea	−415
P. sativum, I	−425
P. sativum, II	−410
M. sativa	−415
Z. mays	−390
Equisetum telemateia (horsetail)	−405
Green algae	
Scenedesmus obliquus	−385
Red algae	
Porphyra umbilicalis	−380
Porphyridium cruentum	−405
Porphyridium aerugineum	−394
R. palmata	−402
Cy. caldarium	−340
Blue–green algae	
S. maxima, I	−390
S. maxima, II	−310
Spirulina platensis	−381
Nostoc MAC, I	−350
Nostoc MAC, II	−455
Ch. fritschii	−340
Anabaena variabilis	−355
Aphanocapsu 6714	−375
M. laminosus	−325
Oscillatoria limnetica	−346

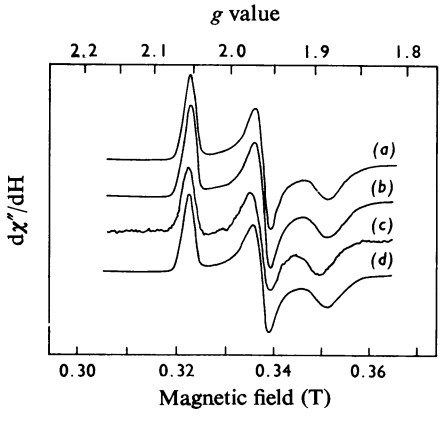

Fig. 1. *E.p.r. spectra of reduced blue–green-algal ferredoxins*

The ferredoxins are those from (*a*) *Aphanocapsa* 6714, (*b*) and (*c*) *Nostoc* strain MAC, ferredoxins I and II respectively and (*d*) *Ch. fritschii*. Samples (approx. 0.5mM) in tubes (diam. 0.3cm) were reduced with 2.5mM-$Na_2S_2O_4$ under argon at 20°C for 2min before being frozen. Instrument settings: modulation amplitude, 1.0mT; modulation frequency, 100KHz; microwave power, 1mW; microwave frequency, 9.26GHz; temperature 22K.

Ferredoxins from the other dicotyledonous plants, alfalfa (*Medicago sativa*) and pea, gave E_m values in the same range as spinach, though a small difference was observed between the two ferredoxins from pea. This difference is probably too small to represent a significant functional difference. Ferredoxin from the monocotyledonous plant, maize (*Zea mays*), consistently gave a slightly higher E_m value (−390mV). Ferredoxin from the primitive plant, *Equisetum*, gave a value of −405mV, which is probably not significantly different from that of spinach.

Ferredoxins from the eukaryotic green and red algae all have potentials around −390mV with the exception of *Cyanidium caldarium* at −340mV. It is noteworthy that this organism resembles the red and blue–green algae in aspects of its pigment composition (Bisalputra, 1974). In the prokaryotic blue–green algae the differences are more striking. Their potentials all lie within the range −310 to −390mV, apart from ferredoxin II from *Nostoc* strain MAC. The minority species II in *Nostoc* strain MAC has a lower E_m (−455mV), in contrast with the minority

species II in *Spirulina*, which has the highest E_m value observed (−310mV).

Discussion

The redox potentials reported for the plant and algal ferredoxins vary from −310 to −455mV, a much wider range than had previously been supposed. However, it might be noted that the two-iron ferredoxins from some bacteria and from adrenal mitochondria have less-negative redox potentials. That from adrenal ferredoxin has an E_m value of −270mV, whereas the ferredoxins from *Pseudomonas putida* and *Agrobacterium tumefaciens* have E_m values of approx. −230mV (see Van Beeumen *et al.*, 1975). The first two, and possibly also the *A. tumefaciens* ferredoxin, are involved in hydroxylation reactions.

All the ferredoxins listed in Table 1 have a suitable potential for transferring electrons from Photosystem I at about −550mV (Evans *et al.*, 1974) to $NADP^+$, with an E_m value of −320mV. However, the difference in potential from those in higher plants suggests that the ferredoxins in the lower organisms are adapted to additional roles. This is supported by the existence of two proteins with different E_m values in *Nostoc* strain MAC and *S. maxima*.

In higher plants and algae, ferredoxin can act as electron donor to nitrite reductase (Ramirez *et al.*, 1965) and sulphite reductase (Asada *et al.*, 1971) and as an electron donor for glutamate synthase (Lea & Miflin, 1974). In blue-green algae, ferredoxin can also act as an electron acceptor in the phosphoroclastic cleavage of pyruvate (Leach & Carr, 1971; Bothe *et al.*, 1974) and as electron donor to nitrogenase (Smith *et al.*, 1971). It is noteworthy that, of the blue-green algae used, *S. maxima* and *Spirulina platensis* and *Aphanocapsa* 6714 do not fix N_2, whereas *Ch. fritschii* and *M. laminosus* do. The position with the other species is less certain, as some strains have been shown to fix N_2, but others are recognized as non-nitrogen fixers. At present there is insufficient information to determine whether this activity can be correlated with the E_m value of the ferredoxin. However, preliminary studies have shown that the Type-II ferredoxin from *Nostoc* strain MAC is less active in the phosphoroclastic system of *Cl. pasteurianum*, but more active in catalysing $NADP^+$ photoreduction by chloroplasts, than the Type-I ferredoxin. Such comparative results are generally lacking for the two-iron ferredoxins, though it has been reported that the two *Aphanothece sacrum* ferredoxins (Hase *et al.*, 1975) also differ in their ability to support $NADP^+$ photoreduction. The redox potentials of the *A. sacrum* ferredoxins have not yet been reported. It would be useful to investigate further the relationship between ferredoxin E_m values and various metabolic activities of the algae.

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