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Efficiency of ferredoxins and flavodoxins as mediators in systems for hydrogen evolution

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1. The efficiencies of ferredoxins and flavodoxins from a range of sources as mediators in systems for hydrogen evolution were assessed. 2. In supporting electron transfer from dithionite to hydrogenase of the bacterium *Clostridium pasteurianum*, highest activity was shown by the ferredoxin from the cyanobacterium *Chlorogloeopsis fritschii* and flavodoxin from the bacterium *Megasphaera elsdenii*. The latter was some twenty times as active as comparable concentrations of Methyl Viologen. Ferredoxins from the cyanobacterium *Anacystis nidulans* and the red alga *Porphyra umbilicalis* also showed high activity. 3. In mediating electron transfer from chloroplast membranes to *Clostridium pasteurianum* hydrogenase the flavodoxin from *Anacystis nidulans* proved the most active with *Nostoc* strain MAC flavodoxin and *Porphyra umbilicalis* ferredoxin also being appreciably more active than other cyanobacterial and higher plant ferredoxins. 4. In both hydrogenase systems the ferredoxin and flavodoxin from the red alga *Chondrus crispus* and the ferredoxin from another red alga *Gigartina stellata* showed very low activity. 5. There appeared to be no apparent correlation of efficiency in supporting hydrogenase activity with midpoint redox potential (E_m) of the mediators, though some correlation of E_m with the efficiency of the mediators in supporting $NADP^+$ photoreduction by chloroplasts, or pyruvate oxidation by a *Clostridium pasteurianum* system, was evident. 6. Activity of the mediators in the hydrogenase systems therefore primarily reflects differences in tertiary structure conferring differing affinities for the other components of the systems.

One of the reasons for the continuing interest in purification and characterization of hydrogenases [H_2-ferredoxin oxidoreductase, EC 1.12.7.1] from different sources is the possibility of using them in a chloroplast–ferredoxin–hydrogenase (Benemann *et al.*, 1973) or other suitable system *in vitro* to produce molecular hydrogen, a potential fuel source. The continuous production of H_2 by reconstituted systems composed of higher-plant chloroplasts, ferredoxin and bacterial hydrogenase has been successfully demonstrated (Rao *et al.*, 1976; Fry *et al.*, 1977). The rate of H_2 evolution was dependent on chlorophyll and ferredoxin concentrations and was enhanced by the presence of oxygen (glucose, glucose oxidase) and peroxide (ethanol, catalase) traps and bovine serum albumin (Fry *et al.*, 1977; Rao *et al.*, 1978*a*).

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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Hydrogenase from the bacterium *Clostridium pasteurianum* proved more effective than those from the bacteria *Desulfovibrio desulfuricans* (strains Norway and 9974), *Thiocapsa roseopersicina*, *Chromatium viridis*, *Rhodospirillum rubrum*, *Alcaligenes eutrophus* and cyanobacteria, both in the chloroplast system and in an alternative assay using dithionite as electron donor (Rao *et al.*, 1978*a*,*b*). This is due to the fact that, of all these hydrogenases and that of the bacterium *Escherichia coli* (Adams & Hall, 1978), only that of *Clostridium pasteurianum* reacted readily with the ferredoxin from the cyanobacterium *Spirulina maxima* used as mediator (Rao *et al.*, 1978*a*), whereas the oxygen-insensitive hydrogenases from photosynthetic and other bacteria including *Alcaligenes eutrophus* and cyanobacteria proved ineffective (Bothe *et al.*, 1978; Llama *et al.*, 1979). The latter group of hydrogenases accepted electrons more readily from reduced Methyl Viologen.

Though the ferredoxin from *Spirulina maxima* has been routinely used as electron mediator in

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these systems, flavodoxin from the bacterium *Megasphaera elsdenii* (formerly *Peptostreptococcus elsdenii*) also appeared to be active (Rao *et al.*, 1978*b*). This observation prompted a survey of the efficiency of a range of ferredoxins and flavodoxins in promoting H_2 evolution by *Clostridium pasteurianum* hydrogenase.

Experimental

Chloroplasts were prepared from leaves of *Chenopodium album* (goose-foot) or *Spinacia oleracea* (spinach) in a sorbitol medium (Reeves & Hall, 1973), and would support the evolution of approx. 400 μmol of O_2/h per mg of chlorophyll measured as electron transport from water to potassium ferricyanide in the presence of NH_4Cl at a light intensity of 400 W/m^2 (Rao *et al.*, 1978*b*). Hydrogenase was purified from *Clostridium pasteurianum* (Rao *et al.*, 1978*a*). Ferredoxins and flavodoxins were prepared by standard procedures in our laboratories (Hall & Rao, 1977; Hutber *et al.*, 1977; Hutson *et al.*, 1978).

H_2 evolution with dithionite as electron donor

The 2 ml reaction mixture, in 15 ml vials, contained routinely 80 units of *Clostridium pasteurianum* hydrogenase and 20 μmol of sodium dithionite in 20 mM-potassium phosphate buffer, pH 7.0, under an N_2 atmosphere at 30°C in a shaking water-bath. Experiments were also carried out with other amounts of hydrogenase and in Tris/HCl or Hepes buffers as shown in individual experiments. The ferredoxin or flavodoxin as required was added by syringe to start the reaction. Gas in the head-space was withdrawn at 5, 10 and 15 min, and H_2 was determined as described by Rao *et al.* (1976). Controls were run in the absence of hydrogenase or mediator. In one vial, 5 μmol of Methyl Viologen was used as mediator of electron flow from dithionite to hydrogenase in place of ferredoxin or flavodoxin. Under these conditions one unit of hydrogenase catalysed the evolution of 1 μmol of H_2/h.

H_2 evolution from the chloroplast–hydrogenase system

The 2 ml reaction mixture, in 15 ml vials, contained *Chenopodium album* or *Spinacia* chloroplasts equivalent to 100 μg of chlorophyll, 100 μmol of glucose, 20 units of glucose oxidase [EC 1.1.3.4], 170 or 340 nmol of ethanol respectively, 1000 units of catalase [EC 1.11.1.6], 10 mg of bovine serum albumin (fat-free, fraction V) in 50 mM-Hepes buffer, pH 7.5, under an N_2 atmosphere at 25°C in a shaking water-bath. The system was illuminated at a light intensity of 10000 lx. Ferredoxin or flavodoxin as indicated was injected and the reaction was started by the further addition of 75 or 120 units of *Clostridium pasteurianum* hydrogenase as indicated. H_2 was determined at 30 min intervals for at least 2 h, as described by Rao *et al.* (1976). Controls were run in the absence of mediator.

Results

Activities in the dithionite assay

In this assay (Fig. 1) dithionite, or rather the dissociation product SO_2^- (midpoint redox potential, E_m, −0.66 V) (Mayhew, 1978), is the source of electrons, with ferredoxin or flavodoxin as mediator of electron flow to hydrogenase. The capacity of the hydrogenase for H_2 evolution was assessed alternatively by using excess Methyl Viologen (E_m −0.446 V) as mediator.

We tested the efficiency in this system of a range of two-iron ferredoxins from cyanobacteria, red algae and higher plants, and flavodoxins from two cyanobacteria and a red alga. The two-cluster four-iron ferredoxin of *Clostridium pasteurianum* and the flavodoxin from *Megasphaera elsdenii*, both obligate anaerobes, were also included in the survey (Table 1).

In the concentration range used, H_2 evolution for a selected hydrogenase activity should be stoichiometric with mediator concentration, as seen when Methyl Viologen concentration is considered (Fig. 2). As far as was possible, therefore, the data in individual series of experiments were obtained at comparable concentrations of the mediators. A number of proteins were very effective mediators to *Clostridium pasteurianum* hydrogenase, in particular ferredoxin from the cyanobacterium *Chlorogloeopsis fritschii* and *Megasphaera elsdenii* flavodoxin, the latter at 40 μM catalysing an H_2 evolution of 95 μmol/h. Ferredoxins from the other cyanobacteria and from the red alga *Porphyra umbilicalis*

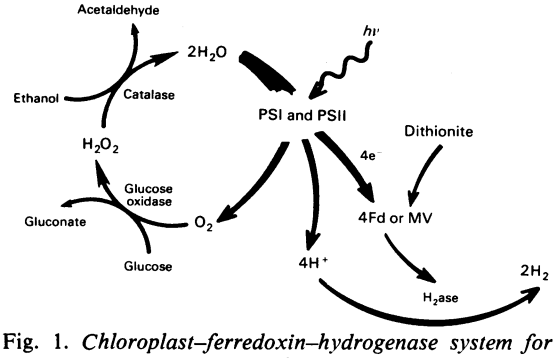

Fig. 1. *Chloroplast–ferredoxin–hydrogenase system for H_2 evolution*

Abbreviations used: PSI and PSII, chloroplast Photosystems I and II; Fd, ferredoxin; MV, Methyl Viologen; H_2ase, hydrogenase.

Table 1. *Efficiency of ferredoxins and flavodoxins as electron donors to hydrogenase in the H_2-evolution assay with dithionite as electron donor*

Reaction mixtures contained *Clostridium pasteurianum* hydrogenase, sodium dithionite and the mediator shown, and were incubated under anaerobic conditions. Controls without mediator or hydrogenase showed negligible activity. (*a*) Ferredoxins (45 μM) in 50 mM-Tris/HCl, pH 7.4, with 50 units of *Clostridium pasteurianum* hydrogenase. (*b*) Ferredoxins and flavodoxins (12 μM, except*, 20 μM) in 20 mM-phosphate, pH 7.0, with 80 units of *Clostridium pasteurianum* hydrogenase. Results in italics were obtained on a separate occasion. †, Oxygen scavengers omitted.

	Activity (μmol of H_2/h)	
Mediator	(*a*)	(*b*)
Methyl Viologen 2.5 mM	45.8	75.2
25 μM	2.6	4.4
Ferredoxins		
Bacterial		
Clostridium pasteurianum	14.8	16.8
Cyanobacterial		
Anacystis nidulans	19.4	25.0
Chlorogloeopsis fritschii	33.4	
Nostoc MAC I	18.0	17.6
Nostoc MAC II	15.0	14.7
Spirulina maxima	20.8	*8.0*
Algal		
Chondrus crispus	0.2	*0.6*
Gigartina stellata	2.4	5.4
Porphyra umbilicalis		24.0
Higher-plant		
Pisum sativum I		11.2
Pisum sativum II		8.6†
Spinacia oleracea		20.0
Flavodoxins		
Bacterial		
Megasphaera elsdenii		*54.6**
Cyanobacterial		
Anacystis nidulans		8.8
Nostoc MAC		9.0
Algal		
Chondrus crispus		6.6
		6.0

were also equally as active as, or more active than, *Clostridium pasteurianum* ferredoxin when compared in parallel experiments.

In general, most of the ferredoxins were much better electron donors to hydrogenase in this system than the same molar concentrations of Methyl Viologen, but notable exceptions were the ferredoxins from the related red algae *Chondrus crispus* and *Gigartina stellata*. Flavodoxins from the cyanobacteria and *Chondrus crispus* were less efficient as mediators, in notable contrast with the very high activity of the flavodoxin from an obligate anaerobe.

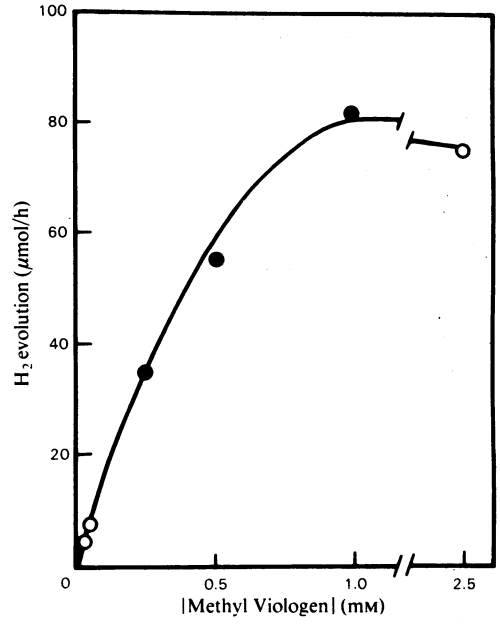

Fig. 2. *Dependence of H_2 evolution on Methyl Viologen concentration*

Reaction mixtures contained 80 units of *Clostridium pasteurianum* hydrogenase, 10 mM-sodium dithionite and the indicated concentration of Methyl Viologen, and were incubated under anaerobic conditions. Data are for two experiments (○, ●) on separate occasions, corresponding to Table 1, column (*b*).

The basis of the chloroplast–ferredoxin–hydrogenase system (Fig. 1) is the photolysis of water by Photosystem II to produce molecular oxygen, protons and electrons. These latter pass via Photosystem I to reduce ferredoxin (or flavodoxin), which acts as electron mediator to facilitate the reduction of protons by hydrogenase. The oxygen formed will progressively inactivate hydrogenase and must be removed by supplementing the reaction system with glucose and glucose oxidase. The H_2O_2 resulting from this reaction and from the autoxidation of ferredoxin is scavenged by inclusion of catalase and ethanol. The additional supplementation with bovine serum albumin stabilizes the chloroplast membranes, probably by formation of a complex with non-esterified fatty acids. Such a reconstituted system will function for several hours (Rao *et al.*, 1976) and can use up to 70% of the potential electrons for H_2 production, depending on ferredoxin concentration (Packer & Cullingford, 1978). In this system the mediating ferredoxin or flavodoxin has to couple with the electron donor on the reducing side of Photosystem I and to the electron acceptor, *Clostridium pasteurianum* hydrogenase.

Table 2. *Efficiency of ferredoxins and flavodoxins as electron donors to hydrogenase in the chloroplast–hydrogenase system*

Reaction mixtures contained *Clostridium pasteurianum* hydrogenase, higher-plant chloroplasts and the mediator shown, and were incubated under anaerobic conditions, in the light. Ferredoxins and flavodoxins were at 22.5 μM final concentration except: *, 12.5 μM and †, 27 μM; ‡, ferredoxin not saturating; §, activity ceased after 90 min incubation (cf. Fig. 3). (*a*) *Chenopodium album* chloroplasts, with 120 units of *Clostridium pasteurianum* hydrogenase. (*b*) *Spinacia oleracea* chloroplasts, with 75 units of hydrogenase. Results in italics were obtained on a separate occasion.

Mediator	Activity (μmol of H_2/h per mg of chlorophyll) (*a*)	(*b*)
Ferredoxins		
Cyanobacterial		
Anacystis nidulans	16.7	17.4
Chlorogloeopsis fritschii	14.5	
Nostoc MAC I	14.0	16.0‡
Nostoc MAC II	16.5	18.9
Spirulina maxima	14.5†	*15.4*
Algal		
Chondrus crispus	4.2	*0.5*
Gigartina stellata	14.0§	1.5
Porphyra umbilicalis		20.5
Higher-plant		
Pisum sativum I		13.2
Pisum sativum II		15.3
Spinacia oleracea		15.2
Flavodoxins		
Bacterial		
Megasphaera elsdenii		9.2*
Cyanobacterial		
Anacystis nidulans		27.2
Nostoc MAC	22.3†	21.0
Algal		
Chondrus crispus	1.5†	0.9
		1.6

The activities of the ferredoxins and flavodoxins in this system are shown in Table 2. Only a fraction of the electron-transport capacity of the chloroplasts is utilized for the hydrogen-evolution reaction, which saturates at about 20 μM-ferredoxin (Rao *et al.*, 1978*a*; Packer & Cullingford, 1978).

The time course of H_2 evolution for experiments with *Chenopodium album* chloroplasts, *Clostridium pasteurianum* hydrogenase and representative mediators is shown in Fig. 3. After a slight lag in some cases, as observed by Adams & Hall (1979) and Fry *et al.* (1977), and interpreted as the need to reduce the hydrogenase itself, the hydrogen-evolution rate was linear for about 2 h before declining.

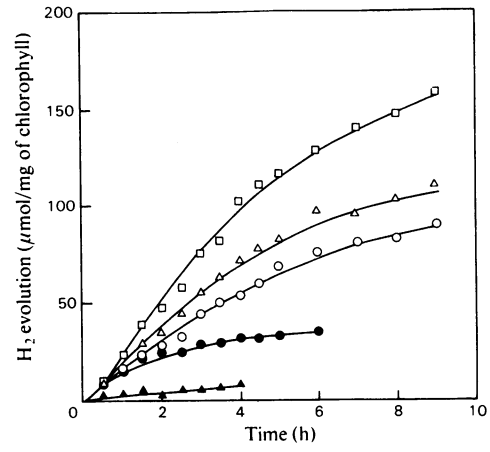

Fig. 3. *Relative activities of some ferredoxins and flavodoxins in supporting hydrogen evolution by a chloroplast and hydrogenase system in vitro*

Reaction mixtures contained 120 units of *Clostridium pasteurianum* hydrogenase, *Chenopodium album* chloroplasts and the mediator shown, and were incubated under anaerobic conditions, in the light. Ferredoxins (22.5 μM) and flavodoxins (25 μM) were: ○, *Chlorogloeopsis fritschii* ferredoxin; ●, *Gigartina stellata* ferredoxin; △, *Nostoc* strain MAC ferredoxin II; ▲, *Chondrus crispus* ferredoxin; □, *Nostoc* strain MAC flavodoxin.

Nevertheless, in most cases, appreciable H_2 evolution was still occurring when the reactions were terminated after 9 h incubation. In the case of *Chondrus crispus* and *Gigartina stellata* ferredoxins, however, activity ceased after only 90 min. The relative activities of the range of ferredoxins and flavodoxins tested are given in Table 2. The flavodoxin from the cyanobacterium *Anacystis nidulans* proved the most active, with that from the cyanobacterium *Nostoc* (strain MAC) also showing high activity. With the former, a total output of 160 μmol of H_2/mg of chlorophyll was obtained over a 9 h period, with a maximum rate of 25 μmol/h per mg of chlorophyll maintained for 4 h. The most active of the ferredoxins was that from the red alga *Porphyra umbilicalis*. However, the ferredoxins from the other red algae were poor mediators; though the data from one experiment suggest that *Gigartina stellata* ferredoxin shows good activity, this mediator was inactive within 90 min of initiating the reaction (Fig. 3). The higher-plant and cyanobacterial ferredoxins all showed similar activity to that of *Spirulina maxima* ferredoxin; this is the ferredoxin routinely used in studies of reconstituted hydrogenase systems (Fry *et al.*, 1977; Rao *et al.*, 1978*a*). The optimal activity of *Nostoc* strain MAC ferredoxin I

may be underestimated, since of those tested this was the only ferredoxin not saturating at the concentration used; at 40 μM, Nostoc strain MAC ferredoxin I supported an H_2 evolution of 19 μmol/h per mg of chlorophyll. Accompanying experiments showed that, in this system, *Clostridium pasteurianum* ferredoxin has similar activity to the two-iron ferredoxins.

Flavodoxin from *Megasphaera elsdenii* was only tested at 12.5 μM; it is likely that, at the higher concentration used for the other mediators, it would show activity comparable with that of the cyanobacterial and higher-plant ferredoxins. The remaining flavodoxin, that from *Chondrus crispus*, showed negligible activity.

Discussion

In mediating electron flow from dithionite to *Clostridium pasteurianum* hydrogenase it is the interaction with the latter that will dictate the observed activity of the mediating ferredoxin or flavodoxin. However, a number of two-iron ferredoxins proved equally or more effective than the native ferredoxin; in particular, *Chlorogloeopsis fritschii* ferredoxin at equivalent concentration supported twice the rate of H_2 evolution. In addition to other cyanobacterial ferredoxins, the ferredoxins from the red alga *Porphyra umbilicalis*, and a higher plant, *Spinacia oleracea*, also showed high activity. Clearly the relative activities of the mediators do not therefore correlate with possession of an hydrogenase by the various organisms. Higher plants do not possess the enzyme, and the status of red algae in this respect is uncertain (Frenkel & Rieger, 1951); even the nature of the hydrogenases in cyanobacteria is still a subject of debate (Bishop & Jones, 1978; Bothe *et al.*, 1978). It should also be borne in mind in subsequent discussion that even in organisms where hydrogenases occur, neither ferredoxin nor flavodoxin may be the immediate donor *in vivo*, but, as in the bacterium *Desulfovibrio gigas*, this may be a cytochrome (Bell *et al.*, 1978). Flavodoxins from two cyanobacteria and a red alga were not as effective in this system as the native bacterial ferredoxin; however, flavodoxin from *Megasphaera elsdenii* proved to be extremely effective, and, at 40 μM, activity with this mediator was limited only by the activity of hydrogenase. At comparable concentrations, *Megasphaera* flavodoxin was twice as active as *Clostridium pasteurianum* ferredoxin and some 15–20 times as active as the artificial electron-transfer mediator Methyl Viologen. *Megasphaera elsdenii* produces a flavodoxin only when grown under iron-limiting conditions. Like *Clostridium pasteurianum*, it is an obligate anaerobe and one of its natural habitats is the highly reducing environment of the rumen. The activity in hydrogenase systems of *Megasphaera* ferredoxin and *Clostridium* flavodoxin would be of interest.

When the mediators couple the reducing potential of the chloroplast membranes to the *Clostridium pasteurianum* hydrogenase, there is the additional interaction with the physiological electron donor to consider. Under these circumstances the higher-plant and cyanobacterial ferredoxins, including *Chlorogloeopsis fritschii* ferredoxin, show comparable activity, but the ferredoxin from *Porphyra umbilicalis* is still somewhat more active than the others. The ferredoxins from two other red algae showed low activity in both hydrogenase assays. In the case of *Chondrus crispus* the organism produces a constitutive flavodoxin and only negligible amounts of ferredoxin (Fitzgerald *et al.*, 1978). This has so far prevented structural and functional characterization of the latter, but, in view of the very low quantities present, the ferredoxin may have only a limited, or very specific, biochemical role *in vivo*. *Gigartina stellata* also contains similarly low quantities of extractable ferredoxin, but in this case no compensating flavodoxin. The low activity of *Gigartina* ferredoxin is not due to an unusual E_m; at −395 mV (M. P. Fitzgerald, L. J. Rogers & R. Cammack, unpublished work) this is similar to that of *Porphyra umbilicalis* ferredoxin, which has high activity. The flavodoxins from the cyanobacteria show the highest activity in this system, though taking into account its lower concentration in the assay, *Megasphaera* flavodoxin also shows good activity. Nevertheless, though some of these mediators show higher activity than *Spirulina maxima* ferredoxin, which is routinely used in studying the potentiality of solar-energy-conservation systems (Fry *et al.*, 1977; Rao *et al.*, 1978*a*,*b*), their usefulness is limited by lack of availability. With the exception of *Porphyra umbilicalis* ferredoxin, which can be obtained in high yield from an abundant naturally occurring alga (Andrew *et al.*, 1976), the organisms concerned have to be grown in large-scale laboratory culture, and, for flavodoxin production, under carefully defined iron-limiting conditions. These and other considerations have led to the exploration of use of synthetic analogues of the biochemical components, and it has been demonstrated that a synthetic Mo–Fe–S centre will act as a mediator in the chloroplast–ferredoxin–hydrogenase system (Rao *et al.*, 1978*b*; Adams *et al.*, 1979*a*), whereas PtO_2 (Adams' catalyst) will replace hydrogenase itself (Adams *et al.*, 1979*b*).

In mediating electron transfer from dithionite to *Rhodospirillum rubrum* hydrogenase, Adams & Hall (1979) demonstrated a correlation between H_2 evolution and the redox potentials of a range of mediator dyes. Methyl Viologen (E_m −446 mV)

proved more active than Benzyl Viologen (E_m −359 mV). FMN (E_m −219 mV) showed negligible activity, whereas electron carriers with E_m values greater than +50 mV were quite inactive. The E_m values for the ferredoxins used in the present survey cover the range −340 to −455 mV (Cammack *et al.*, 1977); E_m values for the semiquinone/hydroquinone couples of the *Anacystis* and *Megasphaera* flavodoxins are about −450 mV (Mayhew & Ludwig, 1975), and the other flavodoxins are probably similar. A comparison of the activities of these mediators in the two hydrogen-evolution systems with their E_m shows little correlation. For example, in interaction with *Clostridium* hydrogenase, as assessed in the dithionite assay, *Chlorogloeopsis fritschii* ferredoxin and *Nostoc* strain MAC ferredoxin I show quite different activities, but have a very similar E_m value; a similar conclusion is reached from consideration of the *Spinacia oleracea* and *Pisum sativum* (pea) proteins. In the chloroplast assay the cyanobacterial and higher-plant ferredoxins have similar activity, irrespective of their E_m. It may be that the E_m values for all these ferredoxins are sufficiently negative for rapid electron transfer to *Clostridium pasteurianum* hydrogenase, so that this step is not rate-limiting. Though their redox potentials are probably similar, the flavodoxins showed widely differing activities.

It is also worth comparing the activity of the mediators in other biological systems. Comparable data for activities in supporting $NADP^+$ photoreduction by chloroplasts and pyruvate oxidation in bacterial extracts are available for seven of the ferredoxins and two of the flavodoxins included in the present survey (Table 3). Discussion has been limited to those proteins studied in our laboratories under conditions where direct comparison was possible.

In $NADP^+$ photoreduction, the mediator couples to the electron donor from photosystem I, as in the chloroplast–ferredoxin–hydrogenase system, and to the chloroplast ferredoxin–$NADP^+$ reductase. It is surprising, therefore, that *Clostridium pasteurianum* ferredoxin mediates electron flow more

Table 3. *Comparative efficiencies of ferredoxins and flavodoxins*

Values for E_m are from Cammack *et al.* (1977), except *Clostridium pasteurianum* (Lode *et al.*, 1976*b*). Comparative activities in the H_2-evolution assays are based on the values in Tables 1 and 2. For assays with dithionite as electron donor, activities are compared with that of *Clostridium pasteurianum* ferredoxin, and for the chloroplast–hydrogenase assay, activities are compared with ferredoxin II of *Pisum sativum*. *In separate experiments to those shown in Table 2, *Clostridium pasteurianum* ferredoxin had similar activity to the two-iron ferredoxins. The assays of NADP photoreduction were with *Hordeum vulgare* (barley) chloroplasts; activities are compared with that of *Pisum sativum* ferredoxin II. Activities in the phosphoroclastic reaction were determined from formation of acetyl phosphate from pyruvate by *Clostridium pasteurianum* extracts, and are compared with that of *Clostridium pasteurianum* ferredoxin. Comparative efficiencies for NADP photoreduction and the phosphoroclastic reaction are from Hutson *et al.* (1978, 1980), Fitzgerald *et al.* (1978), Hutber *et al.* (1978) and Dutton *et al.* (1980). Abbreviation used: n.d. not determined.

		H_2-evolution assays			
Electron donor	E_m (mV)	Chloroplast–hydrogenase system (%)	Dithionite as electron donor (%)	NADP photoreduction (%)	Phosphoroclastic reaction (%)
Ferredoxins					
Bacterial					
Clostridium pasteurianum	−405	100*	100	75	100
Cyanobacterial					
Chlorogloeopsis fritschii	−340	100	200	55	85
Nostoc MAC I	−350	100	105	55	85
Nostoc MAC II	−455	120	90	100	65
Algal					
Porphyra umbilicalis	−380	140	145	85	80
Higher-plant					
Pisum sativum I	−425	90	70	120	35
Pisum sativum II	−410	100	50	100	40
Flavodoxins					
Cyanobacterial					
Nostoc MAC	n.d.	140	55	105	30
Algal					
Chondrus crispus	n.d.	10	40	75	45

successfully in this system than some of the cyanobacterial ferredoxins. A possible explanation for this may be its smaller size (mol.wt. 6000) compared with the other ferredoxins, under the assay conditions used facilitating coupling to membrane-associated ferredoxin–$NADP^+$ reductase. With the other ferredoxins there is some correlation of activity in supporting $NADP^+$ photoreduction with redox potential, the ferredoxins with lowest E_m values being the most active. In this system the flavodoxin from *Nostoc* strain MAC shows good activity and that from *Chondrus crispus* is now appreciably more active than in the chloroplast–hydrogenase system.

In the phosphoroclastic system of *Clostridium pasteurianum*, the mediator acts as electron acceptor and the assay therefore differs in this respect from the others. In this system all the ferredoxins and flavodoxins are less active than the native ferredoxin, though three ferredoxins, those of *Chlorogloeopsis fritschii*, *Nostoc* strain MAC (ferredoxin I) and *Porphyra umbilicalis*, approach its effectivenesss. For the cyanobacterial and algal ferredoxins there is correlation of activity with redox potential, with ferredoxins of highest E_m being most active, though differences are not marked. The flavodoxins and higher-plant ferredoxins show less than half the activity of *Clostridium pasteurianum* ferredoxin. Relatively small changes in structure might be responsible for these differences in biological activity and E_m. Thus *Clostridium acidi-urici* ferredoxin with a tryptophan instead of a tyrosine residue adjacent to the *N*-terminal alanine residue has only 80% the activity of the natural ferredoxin in the phosphoroclastic reaction and in a cytochrome *c*-reduction assay (Lode *et al.*, 1976*a*). The change in E_m consequent upon the substitution of tryptophan is some 20mV more negative (Lode *et al.*, 1976*b*).

Although, therefore, there is some correlation between redox potential and activities in NADP photoreduction and the phosphoroclastic reaction, this breaks down when activity in the hydrogenase systems is also considered. In the latter assays, activity of the ferredoxins and flavodoxins may primarily reflect differences in tertiary structure conferring differing affinities for the other components of the system. Even in the apparently closely related flavodoxins from *Clostridium* MP and *Desulfovibrio vulgaris*, X-ray-crystallographic studies reveal that there are significant differences in the binding of flavin mononucleotide to the apoprotein, which should be reflected in relative efficiencies of transfer of electrons from the two flavodoxins to a given acceptor (Mayhew & Ludwig, 1975). Greater knowledge of the structure of ferredoxins and flavodoxins than is presently available is therefore needed to clarify the factors affecting their interactions with biochemical systems.

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