Biochem. J. (1984) **217**, 845–850
Printed in Great Britain

Redox potentials of algal and cyanobacterial flavodoxins

Gerald A. SYKES and Lyndon J. ROGERS
Department of Biochemistry, University College of Wales, Aberystwyth, Dyfed SY23 3DD, Wales, U.K.

(*Received 26 August 1983/Accepted 14 October 1983*)

The redox potentials of flavodoxins from the cyanobacteria *Synechococcus* PCC 6301 (formerly *Anacystis nidulans*) and *Nostoc* strain MAC, and from the red alga *Chondrus crispus*, were determined by potentiometric titration. For the oxidized–semiquinone interconversion the potentials at pH 7.0 of the three flavodoxins were between −210 and −235 mV, and these were pH-dependent over the range pH 6.9–8.2. For the semiquinone–reduced interconversion the potentials of the cyanobacterial flavodoxins were close to −414 mV, and that for the algal flavodoxin, −370 mV, is the highest reported in this group of flavoproteins.

Flavodoxins are small-M_r electron-transfer proteins that have been shown to substitute for ferredoxin in a number of biological reactions (Mayhew & Ludwig, 1975). In previous work we have isolated and characterized the flavodoxins produced by the cyanobacterium *Nostoc* strain MAC (Hutber *et al.*, 1981) and the marine red alga *Chondrus crispus* (Fitzgerald *et al.*, 1978). Although the flavodoxin in *Nostoc* strain MAC, like most other flavodoxins, is produced in response to iron deficiency (Hutber *et al.*, 1977) and replaces ferredoxin, that in *Chondrus crispus* is constitutive. In this latter organism the flavodoxin is the predominantly formed low-potential electron carrier, an accompanying ferredoxin occurring in very small amounts. *Chondrus crispus* flavodoxin differed in another respect when compared with cyanobacterial flavodoxins, since dissociation of FMN resulted in extensive aggregation of the apoflavodoxin (Fitzgerald *et al.*, 1980*a*). In various biological assays (see Fitzgerald *et al.*, 1980*b*) *Chondrus crispus* flavodoxin differed in activity compared with flavodoxin from *Nostoc* strain MAC. In particular, *Chondrus crispus* flavodoxin was far less active in supporting H_2 evolution by a chloroplast/hydrogenase system than was the cyanobacterial flavodoxin or the ferredoxins from a range of cyanobacteria and algae. These earlier observations have prompted a comparative study of the redox potentials of this flavodoxin from a eukaryote with those from the cyanobacteria *Nostoc* strain MAC and *Synechococcus* PCC 6301. The last-mentioned flavodoxin was studied since it was included in earlier comparative studies of these flavodoxins (Fitzgerald *et al.*, 1980*a*,*b*) and because there was some discrepancy in previous reports (Entsch & Smillie, 1972; van Lin & Bothe, 1972) of its redox potentials.

Experimental

Growth of organisms

Synechococcus PCC 6301 (*Anacystis nidulans* 1405/1 Kratz/Allen) was obtained from the Culture Collection of Algae and Protozoa, Cambridge, U.K. It was grown as described by Smillie & Entsch (1971) except that iron in the growth medium was 0.25 μM. *Nostoc* strain MAC was grown autotrophically in the light on medium Cg 10 of van Baalen (1967) supplemented with 0.9 g of $KHCO_3$/l. Cultures at 30°C were gassed continuously with air/CO_2 (19:1) during illumination (5000 lux). Iron [$Fe(NO_3)_3$] in the medium was 0.5 μM. *Chondrus crispus* (L.) Stakh was collected from local beaches.

Preparation of flavodoxins

Flavodoxins were isolated by methods detailed elsewhere from acetone-dried powders of *Synechococcus* PCC 6301 and *Nostoc* strain MAC (Hutber *et al.*, 1981) or a freeze-dried powder of *Chondrus crispus* (Fitzgerald *et al.*, 1978).

All were purified to homogeneity as assessed by analytical polyacrylamide-gel electrophoresis at pH 8.2 on 15% (w/v) acrylamide gels in which 2.5% of the total acrylamide was *NN'*-methylenebisacrylamide, with Coomassie Brilliant Blue G-250 in 3.5% (w/v) $HClO_4$ being used for detection.

Redox titrations

Redox potentials were determined by potentiometric titration with $Na_2S_2O_4$ (reduction) and di-

chlorophenol-indophenol or $K_3[Fe(CN)_6]$ (oxidation) in a 1cm-light-path spectrophotometric cuvette, of similar design to that of Dutton (1978), whose contents could be maintained under anaerobic conditions by a flow of Ar gas purged of traces of O_2 by passage through Fieser's solution.

The cell oxidation–reduction potential at ambient temperature was measured with a platinum electrode by reference to a calomel electrode connected by a salt bridge to the cuvette contents. Reductant or oxidant, maintained under anaerobic conditions, were added by micro-syringe as necessary.

The degree of reduction of the flavodoxin (40–100 μM) was monitored spectrophotometrically with a Hitachi–Perkin–Elmer dual-wavelength spectrophotometer (model 356) by following absorbance change at 580nm (appearance and disappearance of semiquinone) or at 464 or 466nm as appropriate for a particular flavodoxin [absorbance decrease on successive reduction to semiquinone and quinol (hydroquinone)], relative to a reference wavelength of 750nm. Choice of monitoring wavelength for each flavodoxin was based on absorption spectra for oxidized, semiquinone and reduced forms (see, e.g., Fitzgerald *et al.*, 1978; Hutber *et al.*, 1981). Though there was in each case a well-defined isosbestic point at about 520nm for the oxidized–semiquinone interconversion at pH7.0, this was less satisfactory in titrations at higher pH values, and a suitable isosbestic point for the semiquinone–reduced interconversion was lacking.

The mediators used, with their $E_{m,7}$ values, were 5,5′-indigodisulphonate (−125mV), anthraquinone-1,5-disulphonate (−174mV), phenosafranine (−252mV), 1,1′-dibenzyl-4,4′-bipyridylium dichloride (−358mV) and 1,1′-dimethyl-4,4′-bipyridylium dichloride (−449mV), in various combinations. Mediators were supplied by Aldrich Chemical Co., Gillingham, Dorset, U.K. (the first two listed) or by BDH Chemicals, Poole, Dorset, U.K. At the concentrations used (1–10 μM) correction for absorbance of the mediators at each potential was insignificant. The potentials at equilibrium after each addition of reductant or oxidant were plotted against A_{580} or A_{466} and the data fitted to theoretical curves based on the Nernst equation, enabling E_m and stoichiometry of electron transfer to be determined. The same information was also obtained from plots of log(redox ratio) against cell oxidation–reduction potential.

Results and discussion

Flavodoxins contain FMN as the prosthetic group and consequently exhibit redox potentials that can be assigned to the oxidized–semiquinone (E_2) and semiquinone–reduced interconversions (E_1) respectively. In flavodoxins E_2 is more positive than E_1, since the binding of apoprotein causes a large negative shift in E_1 compared with FMN in free solution.

We have determined from potentiometric titrations the E_m values for both the oxidized–semiquinone and semiquinone–reduced interconversions for three flavodoxins. The flavodoxins from two cyanobacteria, *Synechococcus* PCC 6301 and *Nostoc* strain MAC, are produced in response to growth under iron-deficient conditions, whereas the flavodoxin from the marine red alga *Chondrus crispus* is constitutive and is the predominant low-potential electron-transfer protein present. *Synechococcus* PCC 6301 was included both to serve as a check on the experimental techniques used and to resolve the disagreement in the E_m values quoted previously for this flavoprotein (Entsch & Smillie, 1972; van Lin & Bothe, 1972).

Changes in oxidation state of the flavodoxins were followed by two methods. Absorbance changes at 580nm in response to changes in applied potential reflect changes in the amount of semiquinone present, since this is the only molecular species absorbing at this wavelength. Alternatively E_m values could also be found by monitoring in separate titrations changes in absorbance at about 465nm. Here the absorbance decreases as the flavoprotein progresses from the oxidized form through the semiquinone to the fully reduced form during a reductive titration. We found that data for either titration gave good fits to theoretical curves for successive one-electron transfers.

Representative data for *Synechococcus* PCC 6301 flavodoxin are given in Fig. 1. At pH7 (Fig. 1*a*) the value of E_2 from this experiment was −228mV and that of E_1 was −419mV. The potentials calculated from plots of log(redox ratio) versus cell oxidation–reduction potential were in agreement with those taken directly from the titration, and the slopes corresponded to one-electron processes. At pH8 the value of E_1 is within experimental error unchanged, but that for E_2 has become markedly more negative. The E_m values were confirmed by separate titrations in which reduction of the flavodoxin was followed from ΔA_{464}; data for a titration at pH7.6 are given in Fig. 1(*b*). Here there was more difficulty in fitting the data for E_1 to theoretical curves, because of the small absorbance change for the semiquinone–reduced interconversion. There was also difficulty in some titrations in obtaining total reduction of the flavodoxin, possibly through O_2 leakage into the cuvette.

The data for these E_m values derived from a number of experiments over the pH range 7.0–8.0 are given in Fig. 2; various amounts and different

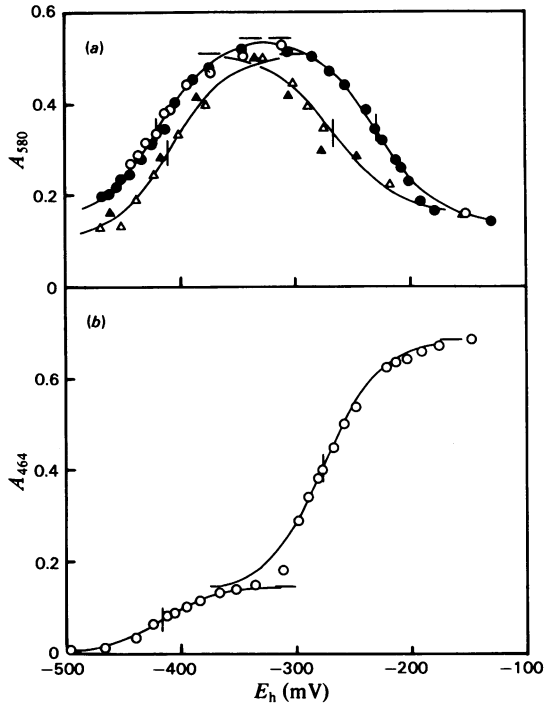

Fig. 1. *Mid-point redox potentials of Synechococcus PCC 6301 flavodoxin*

Experimental points are the A_{580} or A_{464} shown as a function of the redox potential at which the samples in 0.15M-Tris/HCl were poised. Data are superimposed on theoretically derived plots for successive one-electron transfers. (*a*) Potentiometric titration of flavodoxin at pH 7.0 (○ and ●) in the presence of Benzyl Viologen, Methyl Viologen, anthraquinone-1,5-disulphonate, indigo-5,5′-disulphonate and phenosafranine, all at 10 μM, and at pH 8.0 (△ and ▲) in the presence of Methyl Viologen and Isopropyl Viologen, both at 1 μM; flavodoxin was at 65 and 60 μM respectively. Open symbols are reductive titrations, and black symbols represent a successive oxidative titration with dichlorophenol-indophenol. (*b*) Reductive titration of flavodoxin (110 μM) at pH 7.6 in the presence of Benzyl Viologen, Methyl Viologen, anthraquinone-1,5-disulphonate, indigo-5,5′-disulphonate and phenosafranine, all at 8 μM.

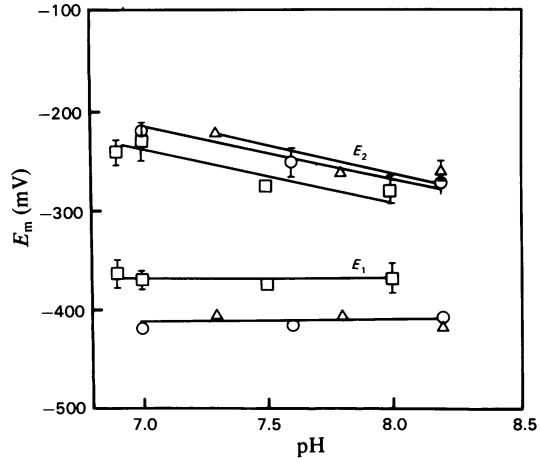

Fig. 2. *Dependence on pH of the mid-point potentials of flavodoxins*

E_2 is the potential of the quinone/semiquinone couple, and E_1 that of the semiquinone/quinol couple, for the flavodoxins from *Synechococcus* PCC 6301 (○), *Nostoc* strain MAC (△) and *Chondrus crispus* (□). Reaction mixtures were as described for Figs. 1, 3 and 4, except that combinations and amounts of mediators were varied. Some values are given as means ± S.D. for three experiments; others are the means for two determinations.

combinations of mediators were used in confirmatory titrations at each pH. These studies showed that the value of E_1 for *Synechococcus* PCC 6301 flavodoxin was independent of pH over this range, whereas E_m for the oxidized–semiquinine interconversion (E_2) became markedly more negative as pH was increased. This dependence was consistent with the change of −59 mV per pH unit, which would be in accord (Mayhew & Ludwig, 1975) with the reaction sequence:

$$Fld_{ox}.H \overset{e^-}{\underset{}{\rightleftharpoons}} \overset{+H^+}{\underset{}{\rightleftharpoons}} FldH_2 \overset{\cdot e^-}{\underset{}{\rightleftharpoons}} Fld_{red}.H_2^-$$

where Fld represents flavodoxin.

At pH 7.0 the values for E_1 and E_2 based on these titrations were −414 mV and −215 mV respectively.

The value for E_2 agrees with that reported by Entsch & Smillie (1972) and is some 40 mV more negative than the value quoted in van Lin & Bothe (1972). Our value for E_1 is also in closer agreement with the former group but is more positive by some 30 mV (cf. Table 1) than the value they obtained by equilibration with H_2 in the presence of hydrogenase.

The redox potentials of the flavodoxin from *Synechococcus lividus*, another cyanobacterium in section I of this group (Rippka *et al.*, 1979), have been determined by Crespi *et al.* (1973). The value of E_1 was in the same range as that for *Synechococcus* PCC 6301, but E_2, determined by equilibration with mediator dyes, was appreciably more positive (Table 1), and apart from an apparently anomalous value for *Azotobacter vinelandii* flavo-

Table 1. *Redox potentials of flavodoxins at pH7.0*

E_1 and E_2 are the redox potentials for the semiquinone–reduced and oxidized–semiquinone interconversions respectively. To facilitate comparison the data quoted are for pH7; where data in the original reference were for higher pH values, corrections have been based on a −59mV change per pH-unit increase for E_2 and no pH-dependence for E_1 over the range pH7–8.2. The method of determination of E_m and the mediators present in each case are also given: [a]equilibration with H_2 in the presence of hydrogenase; [b]equilibration with mediator dye; [c]equilibration with NADPH in the presence of ferredoxin–$NADP^+$ oxidoreductase; [d]potentiometric titration; [e]coulometric titration. Mediators that have been used (with their $E_{m,7}$ in mV) are: BV, Benzyl Viologen (−358); MV, Methyl Viologen (−449); IV, Isopropyl Viologen (not determined); AS, anthraquinone-2-sulphonate (−225); ADS, anthraquinone-1,5-disulphonate (−174); IDS, indigo-5,5′-disulphonate (−116); ITS, indigotrisulphonate (−81); DHNQ, 1,4-dihydroxynaphthoquinone (−195); MB, Methylene Blue (+11); PC, pyocyanine (−36); PS, phenosafranine (−252); S, safranine (−289); FNR–NADPH, ferredoxin–NADP oxidoreductase and NADPH (−324). Mediators present are listed though any role in the respective interconversions will depend on $E_{m,7}$.

	Oxidized–semiquinone		Semiquinone–reduced			
	E_2 (mV)	Mediator titrant[b]	E_1 (mV)	Mediator titrant[b]	Mediators present	Reference
Alga						
Chondrus crispus	−235[d]		−370[d]		BV, MV, ADS, IDS, PS	Present work
Cyanobacteria						
Nostoc strain MAC	−210[d]		−414[d]		BV, MV, ADS, IDS, PS	Present work
Synechococcus PCC 6301	−215[d]		−414[d]		BV, MV, ADS, IDS, PS	Present work
	−221[b]	AS	−447[a]		FNR–NADPH[b]	Entsch & Smillie (1972)
	−180[b]	IDS	−470[a]		FNR–NADPH[b]	Quoted in van Lin & Bothe (1972)
Synechococcus lividus	(−50)[b]	MB or PC	−450[b]	BV or MV		Crespi *et al.* (1973)
Bacteria						
Azotobacter vinelandii	(+121)[d]		−495[d]		MV	Barman & Tollin (1972)
	−229[b]	BV or S	−464[b]	MV		Yoch (1972)
	−245[e]		−515[e]		BV, MV, IV, PS	Watt (1979)
Clostridium MP	−92[b]	IDS	−399[ac]	FNR–NADPH[c]	FNR–NADPH[b]	Mayhew (1971)
Clostridium pasteurianum	−132[b]	IDS	−419[ac]	FNR–NADPH[c]	FNR–NADPH[b]	Mayhew (1971)
Desulfovibrio vulgaris	−102[d]		−438[a]			Dubourdieu *et al.* (1975)
Escherichia coli	−245[b]	BV or S	−455[b]	MV		Vetter & Knappe (1971)
Megasphaera elsdenii	−115[b]	IDS or ITS	−373[ac]	FNR–NADPH[c]	FNR–NADPH[b]	Mayhew *et al.* (1969)
	−116[d]		−375[d]		IDS, MV	Mayhew (1971)
	−114[d]		−373[d]		IDS or DHNQ (E_1); BV or MV (E_2)	Stankovich (1980)

doxin is the highest redox potential quoted for a flavodoxin.

The properties of *Nostoc* strain MAC flavodoxin have been reported previously (Hutber *et al.*, 1981), and the determination of its E_m values (Fig. 3) extends this knowledge. In potentiometric titrations its behaviour closely paralleled that of *Synechococcus* PCC 6301, and the E_m values found were very similar.

The remaining flavoprotein studied was the constitutive flavodoxin from *Chondrus crispus* (Fig. 4). Here the value for E_1 at −370mV was markedly more positive than for the cyanobacterial proteins, and with *Megasphaera elsdenii* flavodoxin is the highest recorded (Table 1).

The value for E_2 was somewhat more negative and is among the lowest values for flavodoxins. This flavodoxin provided more problems in potentiometric titrations. Firstly, the difference in E_1 and E_2 values are less than for the cyanobacterial flavodoxins, and, since at intermediate potentials three species may be present, the fits to the theoretical curves in this region are not so precise. Secondly, there was difficulty in fitting data from successive reductive and oxidative titrations to the same theoretical curve (Fig. 4*b*). The data shown in Fig. 4(*b*) are from a titration where this occurred, with E_1 and E_2 both being some 15mV more positive in the successive oxidative titration. The reason for this is unclear, though it is known that the apoflavodoxin shows unusual conformational changes and aggregates when the flavin is dissociated (Fitzgerald *et al.*, 1980*a*), and possibly this occurs to some extent when some FMN is removed from the flavodoxin under the strongly reducing conditions at completion of a reductive titration. The data used in Fig. 2 and summarized in Table 1 for *Chondrus crispus* flavodoxin are from reductive titrations, though, as comparison of Fig. 4(*a*) and Fig. 4(*b*) demonstrates, the values from

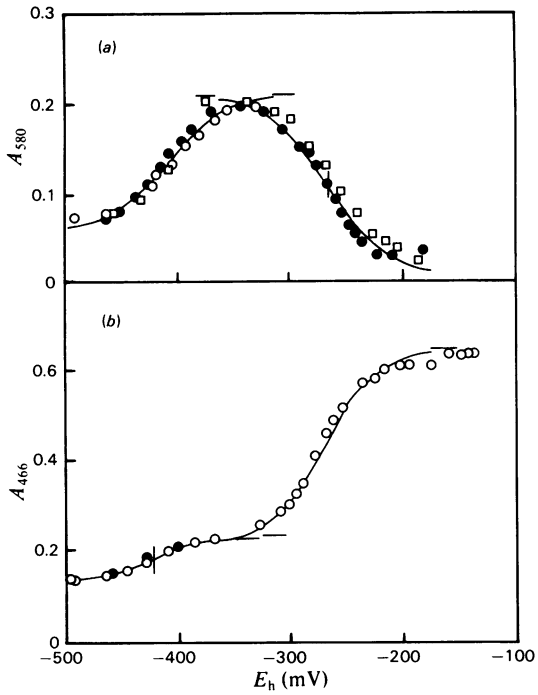

Fig. 3. *Mid-point redox potentials of Nostoc strain MAC flavodoxin*

Experimental points are the A_{580} or A_{466} shown as a function of the redox potential at which the samples in 0.15M-Tris/HCl were poised. Data are superimposed on theoretically derived plots for successive one-electron transfers. (*a*) Potentiometric titration of flavodoxin (45μM) at pH7.8 in the presence of Benzyl Viologen, Methyl Viologen, anthraquinone-1,5-disulphonate, indigo-5,5'-disulphonate and phenosafranine, all at 8μM. The data shown are for a reductive titration (○), followed by an oxidative titration with ferricyanide (●) and another reductive titration (□). (*b*) Potentiometric titration of flavodoxin (60μM) at pH8.2 in the presence of Benzyl Viologen, Methyl Viologen, anthraquinone-1,5-disulphonate, indigo-5,5'-disulphonate and phenosafranine, all at 6μM. Data are for a reductive titration (○) and a successive oxidative titration (●) with dichlorophenol-indophenol.

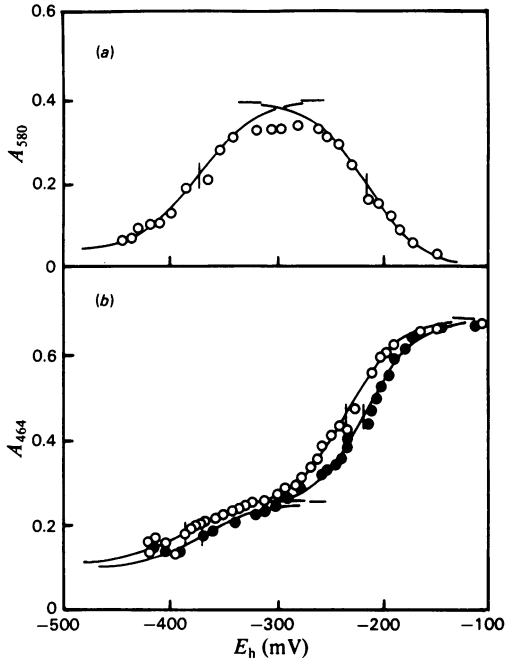

Fig. 4. *Mid-point redox potentials of Chondrus crispus flavodoxin*

Experimental points are the A_{580} or A_{464} shown as a function of the redox potential at which the samples in 0.15M-Tris/HCl were poised. Data are superimposed on theoretically derived plots for successive one-electron transfers. (*a*) Reductive titration of flavodoxin (110μM) at pH7.0 in the presence of Benzyl Viologen, Methyl Viologen, anthraquinone-1,5-disulphonate, indigo-5,5'-disulphonate and phenosafranine, all at 6μM. (*b*) Potentiometric titration of flavodoxin (70μM) at pH7.0 in the presence of Methyl Viologen, indigo-5,5'-disulphonate and phenosafranine, all at 3μM. Data are for a reductive titration (○) and a successive oxidative titration (●) with ferricyanide.

the oxidative titration were still in the same range as those obtained from reductive titrations in separate experiments.

A summary of the redox potentials reported for flavodoxins from algae and bacteria is given (Table 1), noting the method of determination. These data suggest that the organisms fall into two groups with values for E_2 of either approx. −230mV or approx. −110mV. In the first group are placed the flavodoxins from the red alga, the cyanobacteria except *Synechococcus lividus*, *Escherichia coli* and *Azotobacter vinelandii*, disregarding the value of +121mV quoted by one group for the latter. This grouping broadly coincides with the division of flavodoxins on the basis of molecular mass; all the above have M_r values of about 21000 except *E.* coli flavodoxin, whose M_r of 15000 places it in the second category of M_r values. The M_r for *E. coli* flavodoxin was based on amino acid analysis and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, but Sephadex G-100 gel chromatography and sucrose-gradient centrifugation suggested an M_r of approx. 20000. Calculations from sedimentation-equilibrium studies with use of the partial specific volume $\bar{v}$ of 0.73ml/g calculated for *Nostoc* flavodoxin (Hutber *et al.*, 1981) would have been consistent with the higher M_r value, and the authors needed to assume an unusually low value for $\bar{v}$ in order for their data to be consistent with the M_r value of 15000 now quoted

in the literature. This value should possibly be re-assessed. It might be noted that *E. coli* is unusual in also possessing a two-iron ferredoxin (Vetter & Knappe, 1971), a characteristic of cyanobacteria and photosynthetic eukaryotes, rather than a bacterial-type ferredoxin. The redox potential (E_2) (Crespi *et al.*, 1973) and the M_r of 17000 (Crespi *et al.*, 1972) given for *Synechococcus lividus* flavodoxin, if confirmed, would distinguish it from the other cyanobacterial and algal flavodoxins. The values for E_1 for the flavodoxins fall between −370mV and −490mV without correlation with the grouping of the organisms evident for E_2.

The consequence of the differing behaviour of the red-algal flavodoxin is that the redox potentials for E_1 and E_2 are appreciably closer (135mV at pH7) than for any other flavodoxin (Table 1); the next closest is *Synechococcus* PCC 6301, where the E_m values differ by 205mV. For comparison, the widest differences are 400mV for *Synechococcus lividus* and 327mV for *Desulfovibrio vulgaris*. At pH8.0, which is the pH of the chloroplast stroma under conditions of CO_2 assimilation (Buchanan, 1980), the values of E_1 and E_2 are only some 75mV apart. This situation could well have physiological implications. An accepted view is that flavodoxins can substitute for ferredoxins in a whole range of reactions in which the latter participates (see, e.g., Bothe *et al.*, 1971; Bothe & Falkenberg, 1972; Irie *et al.*, 1973; Moura *et al.*, 1980; Tollin & Edmondson, 1980), but most importantly in photosynthetic organisms as an electron-transfer protein on the reducing side of Photosystem 1. Here its role would be ideally suited by the redox potential of the semiquinone–quinol interconversion. It might be expected, therefore, that with its less-favourable redox potential for $NADP^+$ reduction *Chondrus crispus* flavodoxin would be less active in its photosynthetic role. A comparative survey of efficiencies of a range of ferredoxins and flavodoxins in $NADP^+$ photoreduction bears this out, *Chondrus crispus* flavodoxin being only some 75% as active as the flavodoxin from *Nostoc* strain MAC (Fitzgerald *et al.*, 1980*b*).

The red-algal flavodoxin also showed very low activity in a chloroplast/hydrogenase assay. Conversely, in a phosphoroclastic assay, where the mediator acts as an electron acceptor, the flavodoxin from *Chondrus crispus* was significantly more active than that from *Nostoc* strain MAC. For the other cyanobacterial flavodoxins the protein from *Synechococcus lividus* appeared to show very low activity in a ferredoxin–$NADP^+$ reductase assay compared with the physiological carrier, whereas for *Synechococcus* PCC 6301 (Bothe, 1969) the flavodoxin would effectively substitute for the ferredoxin in $NADP^+$ photoreduction, other photosynthetic assays and a phosphoroclastic assay. Clarification of the physiological roles of the constitutive flavodoxin in *Chondrus crispus*, which lacks a conventional ferredoxin, is therefore awaited with interest.

References

Barman, B. G. & Tollin, G. (1972) *Biochemistry* **11**, 4755–4759

Bothe, H. (1969) *Prog. Photosynth. Res. Proc. Int. Congr. 1st* **3**, 1483–1491

Bothe, H. & Falkenberg, B. (1972) *Z. Naturforsch. B Anorg. Chem. Org. Chem. Biochem. Biophys. Biol.* **27**, 1090–1094

Bothe, H., Hemmerich, P. & Sund, H. (1971) in *Flavins and Flavoproteins* (Kamin, H., ed.), pp. 211–226, University Park Press, Baltimore

Buchanan, B. B. (1980) *Annu. Rev. Plant Physiol.* **31**, 341–374

Crespi, H. L., Smith, U., Gajda, L., Tisue, T. & Ammeral, R. M. (1972) *Biochim. Biophys. Acta* **256**, 611–618

Crespi, H. L., Norris, J. R., Bays, J. P. & Katz, J. J. (1973) *Ann. N.Y. Acad. Sci.* **222**, 800–815

Dubourdieu, M., LeGall, J. & Favaudon, V. (1975) *Biochim. Biophys. Acta* **376**, 519–532

Dutton, P. L. (1978) *Methods Enzymol.* **54**, 411–435

Entsch, B. & Smillie, R. M. (1972) *Arch. Biochem. Biophys.* **151**, 378–386

Fitzgerald, M. P., Husain, A. & Rogers, L. J. (1978) *Biochem. Biophys. Res. Commun.* **81**, 630–635

Fitzgerald, M. P., Sykes, G. A. & Rogers, L. J. (1980*a*) *Biochim. Biophys. Acta* **625**, 127–132

Fitzgerald, M. P., Rogers, L. J., Rao, K. K. & Hall, D. O. (1980*b*) *Biochem. J.* **192**, 665–672

Hutber, G. N., Hutson, K. G. & Rogers, L. J. (1977) *FEMS Microbiol. Lett.* **1**, 193–196

Hutber, G. N., Smith, A. J. & Rogers, L. J. (1981) *Phytochemistry* **20**, 383–387

Irie, K., Kobayashi, K., Kobayashi, M. & Ishimoto, M. (1973) *J. Biochem. (Tokyo)* **73**, 353–366

Mayhew, S. G. (1971) *Biochim. Biophys. Acta* **235**, 276–288

Mayhew, S. G. & Ludwig, M. L. (1975) *Enzymes 3rd Ed.* **12**, 57–118

Mayhew, S. G., Foust, G. P. & Massey, V. (1969) *J. Biol. Chem.* **244**, 803–810

Moura, I., Moura, J. J. G., Bruschi, M. & LeGall, J. (1980) *Biochim. Biophys. Acta* **591**, 1–8

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. (1979) *J. Gen. Microbiol.* **111**, 1–61

Smillie, R. M. & Entsch, B. (1971) *Methods Enzymol.* **23**, 504–514

Stankovich, M. T. (1980) *Anal. Biochem.* **109**, 295–308

Tollin, G. & Edmondson, D. E. (1980) *Methods Enzymol.* **69**, 392–406

van Baalen, C. (1967) *J. Phycol.* **3**, 154–157

van Lin, B. & Bothe, H. (1972) *Arch. Mikrobiol.* **82**, 155–172

Vetter, H. & Knappe, J. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 433–436

Watt, G. D. (1979) *Anal. Biochem.* **99**, 399–407

Yoch, D. C. (1972) *Biochem. Biophys. Res. Commun.* **49**, 335–342