Osborne, G. A., Cheng, J. C., & Stephens, P. J. (1973) *Rev. Sci. Instrum. 44*, 10–15.
Palmer, G. (1979) in *The Porphyrins* (Dolphin, D., Ed.) Vol. 4, pp 313–354, Academic Press, New York.
Palmer, G. (1982) in *Iron Porphyrins* (Gray, H. B., & Lever, A. B. P., Eds.) Part II, pp 45–88, Addison-Wesley, Reading, MA.
Palmer, G. (1985) *Trans. Biochem. Soc. 13*, 548–560.
Rawlings, J., Stephens, P. J., Nafie, L. A., & Kamen, M. D. (1977) *Biochemistry 16*, 1725–1729.
Rigby, S. E. J., Moore, G. R., Gray, J. C., Gadsby, P. M. A., George, S. J., & Thomson, A. J. (1988) *Biochem. J. 256*, 571–577.
Sadler, I., Suda, K., Schatz, G., Kaudewitz, F., & Haid, A. (1984) *EMBO J. 3*, 2137–2143.
Salerno, J. C., McGill, J. W., & Gerstle, G. C. (1983) *FEBS Lett. 162*, 257–261.
Salerno, J. C., Yoshida, S., & King, T. E. (1986) *J. Biol. Chem. 261*, 5480–5486.
Salmeen, I., & Palmer, G. (1966) *J. Chem. Phys. 48*, 2049–2052.
Siedow, J. N., Power, S., de la Rosa, F. F., & Palmer, G. (1978) *J. Biol. Chem. 253*, 2392–2399.
Siedow, J. N., Vickery, L. E., & Palmer, G. (1980) *Arch. Biochem. Biophys. 203*, 101–107.
Sievers, G., Gadsby, P. M. A., Peterson, J., & Thomson, A. J. (1983a) *Biochim. Biophys. Acta 742*, 637–647.
Sievers, G., Gadsby, P. M. A., Petersen, J., & Thomson, A. J. (1983b) *Biochim. Biophys. Acta 742*, 659–668.
Stephens, P. J., & Clark, R. (1979) in *Optical Activity & Chiral Discrimination* (Mason, S. F., Ed.) pp 263–287, D. Reidel, Amsterdam.
Stephens, P. J., Sutherland, J. C., Cheng, J. C., & Eaton, W. A. (1976) in *Excited States of Biological Molecules* (Birks, J. W., Ed.) pp 434–442, Wiley, New York.
Sutherland, J., Greenwood, C., Peterson, J., & Thomson, A. J. (1986) *Biochem. J. 233*, 893–898.
Takano, T., Kallai, O. B., Swanson, R., & Dickerson, R. E. (1973) *J. Biol. Chem. 248*, 5234–5255.
Tanaka, K., Takahashi, M., & Asada, K. (1978) *J. Biol. Chem. 253*, 7397–7403.
T'sai, A., & Palmer, G. (1982) *Biochim. Biophys. Acta 681*, 484–495.
Wakabayashi, S., Matsubara, H., Kim, C. H., Kawai, K., & King, T. E. (1980) *Biochem. Biophys. Res. Commun. 97*, 1548–1554.
Widger, W. R., Cramer, W. A., Herrmann, R. G., & Trebst, A. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 674–678.

Plastocyanin Cytochrome *f* Interaction†

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ABSTRACT: Spinach plastocyanin and turnip cytochrome *f* have been covalently linked by using a water-soluble carbodiimide to yield an adduct of the two proteins. The redox potential of cytochrome *f* in the adduct was shifted by −20 mV relative to that of free cytochrome *f*, while the redox potential of plastocyanin in the adduct was the same as that of free plastocyanin. Solvent perturbation studies showed the degree of heme exposure in the adduct to be less than in free cytochrome *f*, indicating that plastocyanin was linked in such a way as to bury the exposed heme edge. Small changes were also observed when the resonance Raman spectrum of the adduct was compared to that of free cytochrome *f*. The adduct was incapable of interacting with or donating electrons to photosystem I. Peptide mapping and sequencing studies revealed two sites of linkage between the two proteins. In one site of linkage, Asp-44 of plastocyanin is covalently linked to Lys-187 of cytochrome *f*. This represents the first identification of a group on cytochrome *f* that is involved in the interaction with plastocyanin. The other site of linkage involves Glu-59 and/or Glu-60 of plastocyanin to as yet unidentified amino groups on cytochrome *f*. *Euglena* cytochrome *c*-552 could also be covalently linked to turnip cytochrome *f*, although with a lower efficiency than spinach plastocyanin. In contrast, a variety of cyanobacterial cytochrome *c*-553's and a cyanobacterial plastocyanin could not be covalently linked to turnip cytochrome *f*.

Cytochrome *f* (cyt *f*)[1] of the photosynthetic electron-transport chain functions between the two photosystems to reduce plastocyanin (PC) using electrons from the Rieske iron–sulfur protein of the cytochrome *b6/f* complex as a reductant (Bendall, 1982; Hauska et al., 1983). Cyt *f* and PC are functional analogues of cytochrome c_1 and cytochrome *c*, which function in the mitochondrial electron-transport chain. In some algae and cyanobacteria, a small *c*-type cytochrome, cyt *c*-553, replaces PC under certain growth conditions (Wood,

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[1] Abbreviations: Chl, chlorophyll; cyt *f*, cytochrome *f*; PC, plastocyanin; PC–cyt *f*, covalently linked adduct between plastocyanin and cytochrome *f*; cyt *c*-553, cytochrome *c*-553; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; SDS, sodium dodecyl sulfate; PS I, photosystem I; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; MV, methylviologen; CNBr, cyanogen bromide; TFA, trifluoroacetic acid.

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1977, 1978; Sandmann & Boger, 1980).

Cyt *f* has been purified from a variety of plant (Bendall, 1982), algal (Kamimura & Matsuzaki, 1978; Bohme et al., 1980a), and cyanobacterial (Ho et al., 1979; Ho & Krogmann, 1980; Bohme et al., 1980b) sources. From most higher plants, purification of cyt *f* results in the production of oligomeric or aggregated forms of the protein (Bendall, 1982). Monomeric forms of cyt *f* have been purified from members of the Cruciferae family (Takahashi & Asada, 1975; Matsuzaki et al., 1975; Tanaka et al., 1978; Gray, 1978). Cyt *f* is generally found to have an apparent molecular weight near 33 000 and a redox potential near +370 mV (Bendall, 1982). In contrast to other *c*-type cytochromes that have a methionine sulfur as the sixth iron ligand, recent resonance Raman studies (Davis et al., 1988) indicated that cyt *f* utilizes a lysine amino group as the sixth iron ligand. This observation is consistent with the suggestion of Siedow et al. (1980) and is supported by other recent physical measurements (Rigby et al., 1988).

Electron transfer from cyt *f* to PC is extremely rapid, with rate constants in excess of 10^7 M^{-1} s^{-1} having been reported (Wood, 1974; Niwa et al., 1980; Tanaka et al., 1981). The rate of electron transfer is sensitive to the ionic strength of the medium, suggesting that electrostatic interactions contribute to the interaction between the two proteins (Niwa et al., 1980; Takabe et al., 1986). As PC is extremely rich in negatively charged carboxyl groups, it seems likely that some of these groups on PC might interact with positively charged lysine or arginine residues on cyt *f*. Several amino acid sequences are now known for cyt *f* (Hauska et al., 1988; Kallas et al., 1988). Comparison of charge distribution in these sequences relative to that in the sequence of cyt c_1 has led to the suggestion that three regions of cyt *f* (residues 45–69, 150–166, and 180–190) might be potential candidates for the PC binding site (Davis, 1987). Consistent with the electrostatic nature of the interaction between the cyt *f* and PC is the observation that the two proteins can be covalently linked in the presence of the water-soluble carbodiimide EDC (Davis & Hough, 1983). In this paper, we describe further characterization of the PC–cyt *f* adduct obtained when the two proteins are incubated in the presence of EDC, including effects on the redox potential of the components, heme accessibility, and identification of groups involved in the covalent linkages between the two proteins. Characterization of a similar adduct has been recently described by Takabe and Ishikawa (1989).

Materials and Methods

Turnip cyt *f* was purchased from Sigma or purified according to the method of Gray (1978). PC was purified from spinach according to previously published procedures (Geren et al., 1983). Cyanobacterial cyt *c*-553's were purified as described by Ho et al. (1979), and *Euglena* cyt *c*-552 was purified by the method of Mitsui (1971).

PC was cross-linked to turnip cyt *f* by using EDC as previously described (Davis & Hough, 1983). The cross-linking reaction was terminated after 2 h by the addition of ammonium acetate to a final concentration of 200 mM. For peptide mapping studies, the entire reaction mixture was passed through a Sephadex G-50 column equilibrated with 100 mM ammonium bicarbonate. The collected adduct was concentrated to less than 5 mL with an Amicon ultrafiltration unit utilizing a YM-30 membrane. For other studies, the quenched reaction mixture was passed through a Sephadex G-10 column equilibrated with 10 mM Tris-HCl, pH 8.0, to remove excess reagents, and the PC–cyt *f* adduct was purified by ion-exchange chromatography on DEAE-cellulose (Whatman DE-52). The properties of the PC–cyt *f* adduct were identical regardless of which preparative method was used.

The ability of cyanobacterial and algal proteins to be cross-linked to turnip cyt *f* in the presence of EDC was examined under the conditions used for the initial characterization of the PC–cyt *f* adduct (Davis & Hough, 1983).

SDS polyacrylamide gel electrophoresis was performed by using a Bio-Rad minigel apparatus. Samples were denatured by the addition of SDS to a concentration of 1%. Electrophoresis was done by using 10% polyacrylamide gels with a Laemmli (1970) Tris–glycine running buffer. After the completion of the run, protein bands were visualized by staining with Coomassie Brilliant Blue. Molecular weight markers (Sigma MW-SDS-70L) were run simultaneously and used as the basis for molecular weight determinations.

Routine spectral data were collected with a Cary 210 spectrophotometer. Spectral data for determination of the redox potentials were collected by using an Aminco DW2a spectrophotometer with samples that had been equilibrated against ferri-/ferrocyanide couples of varying ratio. The sum of the ferri- and ferrocyanide concentrations was kept constant at 1 mM, and an $E°$ of +420 mV was assumed for the ferri-/ferrocyanide couple. The protein concentrations were at 10 μM. All measurements were made in 10 mM sodium phosphate, pH 7.0. At any wavelength, the absorbance was assumed to be the sum of the contributions from all absorbing species described by

$$A = e_{f,ox}[f]_{total} + e_{p,ox}[P]_{total} + \Delta e_{f,red\text{-}ox}[f]_{red} + \Delta e_{p,red\text{-}ox}[P]_{red} \quad (1)$$

where e is the extinction coefficient for the indicated component (f = cyt *f*, P = PC) in the indicated redox state. By measuring the absorbance at two wavelengths, it was possible to set up simultaneous equations to solve for $[f]_{red}$ and $[P]_{red}$. As the total concentration of each component was known, it was then possible to calculate $[f]_{ox}$ and $[P]_{ox}$. The log $([f]_{ox}/[f]_{red})$ and log $([P]_{ox}/[P]_{red})$ were then plotted against the half-cell potential (E_h) calculated from the ratio of ferri- and ferrocyanide in the solution against which the sample had been equilibrated. The two wavelengths used in the present study were 554 and 597 nm, which correspond to the α peak in the spectrum of reduced cyt *f* and the visible peak of oxidized PC, respectively. At 554 nm, the following millimolar extinction coefficients were used: cyt f_{ox}, 6.61; cyt f_{red}, 27.7; PC_{ox}, 3.10; PC_{red}, 0.00. At 597 nm, the following millimolar extinction coefficients were used: cyt f_{ox}, 1.07; cyt f_{red}, 0.00; PC_{ox}, 4.90; PC_{red}, 0.00.

Solvent perturbation studies to assess heme exposure were done on the cyt *f* and the PC–cyt *f* adduct in the reduced state by using 20% ethylene glycol as the perturbant as in Schlauder and Kassner (1979). The degree of heme exposure was calculated on the basis of their data by using the heme octapeptide of cyt *c* as a model for fully exposed heme (Schlauder & Kassner, 1979). Protein concentrations were 10 μM. Cyt *c* was also examined as a control.

Resonance Raman spectra of the PC–cyt *f* adduct were recorded with the instrumentation described by Davis et al. (1988). Excitation was with the 514-nm line from an Ar^+ ion laser. Laser power at the sample was 50 mW. Samples were illuminated for 25-s intervals with a total accumulation of 50 scans. Because of the large luminescence background, peak positions were determined by taking the second derivative of the acquired spectral data.

The ability of the PC–cyt *f* adduct to donate electrons to PS I was examined by using PS I particles prepared from spinach by the procedure of Mullet et al. (1980). The PC–cyt *f* adduct (15 μM) and PS I particles (25 μg of chl/mL) were

placed in a cuvette containing 50 mM Tris-HCl, pH 8.0, and the absorbance at 554 nm was followed by using an Aminco DW2a spectrophotometer equipped for side illumination. Illumination was provided through a 650-nm cut-off filter with the photomultiplier being protected by a complementary filter. Experiments were done in the presence and absence of 5 mM $MgCl_2$.

PS I dependent oxygen consumption was also measured by using the PS I particles of Mullet et al. (1980). The reaction mixture contained 50 mM Tricine, pH 8.0, 5 mM $MgCl_2$, 10 mM sodium ascorbate, 67 μM TMPD, 133 μM MV, and PS I particles at 16 μg of chl/mL. PC was at a concentration of 3 μM when added. The concentrations of cyt *f* and the PC–cyt *f* adduct were varied between 0.38 and 12.5 μM when added. Illumination was provided by a Kodak Ektagraph Model B2 slide projector, and oxygen consumption was measured with a Yellow Springs Instrument Co. Model 53 oxygen electrode.

CNBr digestion of the PC–cyt *f* adduct was performed according to the method of Gross (1967) after the adduct was lyophilized and resuspended with 75% (v/v) TFA (Pierce HPLC/spectro grade). A single clean white crystal of CNBr was added and the reaction allowed to proceed in the dark for 16 h. The mixture was dried under nitrogen. The dried cleavage products were dissolved in 10% (v/v) formic acid and subjected to reversed-phase HPLC. Under these conditions, quantitative cleavage at Met-57 of PC was obtained, but no detectable cleavage at Met-55 of cyt *f* was observed.

A proteolytic predigestion of the concentrated purified PC–cyt *f* adduct was performed after the addition of urea (BRL, ultrapure) to a concentration of 2 M. The adduct was predigested with endoprotease Arg-C (Boehringer-Mannheim) and passed through a Sephadex G-50 column (28 × 1.5 cm) equilibrated with 100 mM ammonium bicarbonate. The entire eluate between the void volume and the salt fraction was collected and concentrated with an Amicon ultrafiltration unit using a YM-10 membrane. After lyophilization, the dried predigest was dissolved in 500 μL of 8 M urea after which 2 mL of 100 mM ammonium bicarbonate was added. Further digestion was done with TPCK-treated trypsin (Pierce) or endoprotease Asp-N (Boehringer-Mannheim). The reactions were stopped by the addition of 350 μL of 88% formic acid. The acidified mixture was microfuged at maximum speed for 2 min and the supernatant subjected to reversed-phase HPLC. All protease digestions were done in the dark at 37 °C for 20 h. Aliquots of the protease were added at 0, 3, and 6 h with a final protease to substrate ratio of 1:40 (w/w).

All HPLC work was performed on a Varian Model 5060 instrument with a built-in UV–100 detector using a Synchropak RP-R C-18 column (250 × 1.4 mm). The column was developed by using a 0–60% (v/v) gradient of acetonitrile in 0.1% (v/v) TFA at a flow rate of 1 mL/min. Collected fractions from the chromatogram were lyophilized.

Lyophilized peptides obtained by HPLC were redissolved in 10% (v/v) formic acid and rechromatographed by reversed-phase HPLC. The resulting samples were lyophilized and then sequences as described by Cohn et al. (1989) by using an Applied Biosystems 470 protein sequencer.

Results

Stoichiometry of PC–Cyt f Adduct. When examined by SDS gel electrophoresis, spinach PC and turnip cyt *f* were found to have apparent molecular weights of 12 300 and 29 300, respectively. The PC–cyt *f* adduct was observed to have an apparent molecular weight of 43 000, indicative of a stoichiometry of 1:1 between the two proteins in the adduct.

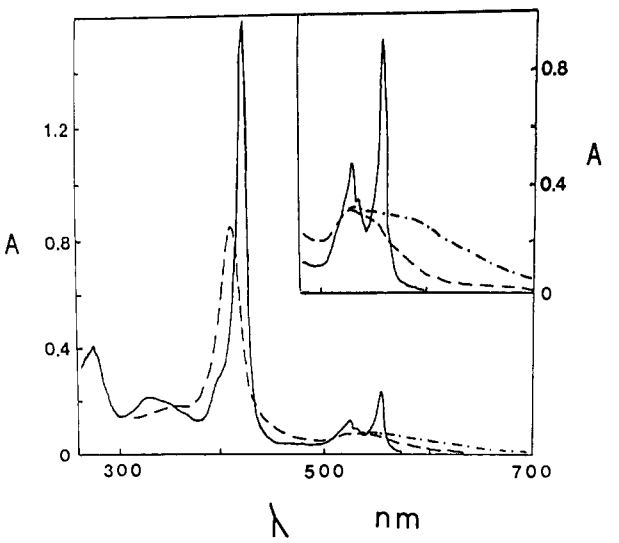


FIGURE 1: Spectral analysis of PC–cyt *f* adduct. The spectra of the PC–cyt *f* adduct in the reduced (—) and oxidized state (-·-) are shown. The spectrum of cyt *f* (at the same concentration) in the oxidized state (---) is also shown for comparison. All samples were in 10 mM sodium phosphate, pH 7.5. The main panel shows data collected with a sample having a PC–cyt *f* concentration of 8.5 μM. The inset shows data obtained with a sample having a concentration of 34 μM. The difference between the spectra of the oxidized adduct and oxidized cyt *f* in this region is due to the presence of PC in the adduct.

The UV–visible spectrum of the PC–cyt *f* adduct is shown in Figure 1. The spectrum of the reduced adduct in the visible region is essentially identical with that of reduced cyt *f* as reduced PC has no absorbance in the visible region. The UV region of the spectrum is higher than normal and exhibits some of the fine structure commonly associated with this spectral region in PC. The spectrum of the reduced PC–cyt *f* adduct was used to determine the amount of cyt *f* present in the adduct. The amount of PC present in the adduct was determined from the difference between the spectra of the oxidized PC–cyt *f* adduct and that of a sample of oxidized cyt *f* at the same concentration (Figure 1, inset). By use of this method, the stoichiometry of the adduct is routinely found to be between 0.8:1 and 1:1 PC per cyt *f*.

Redox Properties, Heme Accessibility, and Resonance Raman Spectrum of the PC–Cyt f Adduct. The redox potential for both components of the PC–cyt *f* adduct was determined from spectra of samples of the adduct that had been equilibrated against ferri-/ferrocyanide couples of predetermined potential. Figure 2 shows the results of such determinations for the individual proteins and for the components of the PC–cyt *f* adduct. The determined redox potentials for PC (+381 mV, n = 0.95) and the PC component of the PC–cyt *f* adduct (+383 mV, n = 0.90) are identical and consistent with values reported in the literature, indicating that the redox properties of PC have not been altered by its covalent linkage to cyt *f*. In contrast, the redox potential for cyt *f* in the adduct (+355 mV, n = 0.95) is 20 mV more negative than that reported for other cyt *f* preparations or for our preparation of turnip cyt *f* (+372 mV, n = 1.08), suggesting that the heme environment may be altered by the covalent linkage of cyt *f* to PC.

To examine this possibility, the heme exposure in the PC–cyt *f* adduct has been examined by solvent perturbation spectroscopy using 20% ethylene glycol as the perturbant. The results (Table I) indicate that the degree of cyt *f* heme exposure is decreased from 34% to 7% as a result of the covalent linkage of PC to cyt *f*. As a control, the degree of heme

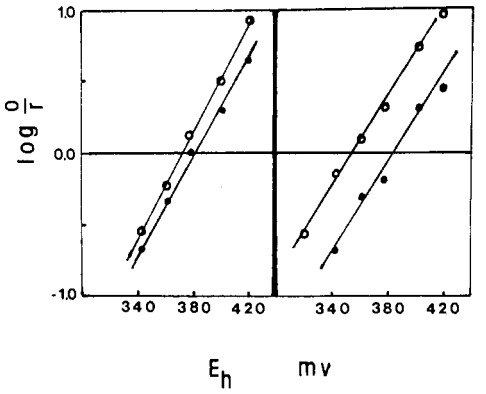


FIGURE 2: Redox potential determination for PC, cyt *f*, and the PC–cyt *f* adduct. The amounts of PC and cyt *f* in the oxidized and reduced states were determined as described in the text after equilibration against ferri-/ferrocyanide couples of known potential. Data were plotted according to the Nernst equation. All measurements were made in 10 mM sodium phosphate, pH 7.0. The concentrations of the individual proteins or the PC–cyt *f* adduct were 10 μM. The left panel shows data for the individual proteins and the right panel data for the PC–cyt *f* adduct. Solid circles are PC data and open circles cyt *f* data in each case.

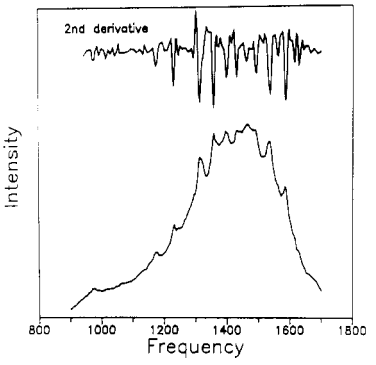


FIGURE 3: Resonance Raman spectrum of PC–cyt *f* adduct. The resonance Raman spectrum of the PC–cyt *f* adduct was recorded as described under Materials and Methods. The frequency scale is in cm^{-1}. Shown above the raw spectrum is the second derivative of the spectral data which was used in assigning peak positions. The second derivative of the spectrum was obtained by using the signal processing software of the Tracor TN-6500 diode array controller subsequent to a 15-point Savitsky–Golay polynomial smoothing of the experimental data.

Table I: Heme Exposure in Cyt *f* and PC–Cyt *f* Adduct Measured by Solvent Perturbation Spectroscopy with 20% Ethylene Glycol

	$\Delta A/A$	% heme exposure[a]	reference
cyt *f*	0.0309 ± 0.0042	31.5 ± 4.5	this work
PC–cyt *f* adduct	0.0067 ± 0.0024	7.3 ± 2.6	this work
cyt *c*	0.0197 ± 0.0014	21.6 ± 1.5	this work
cyt *c*	0.0186	20.4	Schlauder and Kassner (1979)

[a] Heme exposure was measured by solvent perturbation spectroscopy using 20% ethylene glycol as the perturbant as described by Schlauder and Kassner (1979). All values shown represent an average of three determinations.

exposure in cyt *c* was also determined and found to be near 20%, a value consistent with that reported in the literature (Schlauder & Kassner, 1979).

Figure 3 shows the resonance Raman spectrum of the PC–cyt *f* adduct in the reduced state. The spectrum shown is similar to that reported previously for cyt *f* (Davis et al., 1988). Analysis of the peak positions by taking the derivative of the spectrum shown did, however, reveal shifts of 1–4 cm^{-1} in the positions of some of the peaks relative to those observed with

Table II: Comparison of Resonance Raman Peak Positions at Cyt *f* and PC–Cyt *f* Adduct

peak position (cm^{-1})		
cyt *f* (Davis et al., 1988)	PC–cyt *f* adduct (this study)	difference (cm^{-1})
1581	1585	+4
1532	1535	+3
1490	1491	+1
nd[a]	1462	
nd	1431	
1398	1398	0
1361	1357	−4
1312	1313	+1
1226	1229	+3
1173	1174	+1

[a] nd, not detected. These peaks were detected with the PC–cyt *f* adduct but were not found in the resonance Raman spectrum of cyt *f* (Davis et al., 1988) that was run at a lower power level.

Table III: Examination of Ability of PC–Cyt *f* Adduct To Serve as an Electron Donor to Photosystem I

conditions	μmol of O_2 consumed (mg of chlorophyll)$^{-1}$ h^{-1}[a]
(1a) Asc + TMPD	68 ± 27
(1b) 1a + cyt *f*	68 ± 27
(1c) 1a + PC–cyt *f* adduct	68 ± 27
(2) 1a + PC	540 ± 30
(3) 1a + PC + cyt *f*	622 ± 78
(4) 1a + PC–cyt *f* adduct + PC	541 ± 21

[a] O_2 consumption was measured by using reaction mixtures and illumination conditions described under Materials and Methods.

cyt *f* (Table II), also suggesting some change in environment of the cyt *f* heme in the PC–cyt *f* adduct.

Inability To Act as Electron Donor to Photosystem I. The PC–cyt *f* adduct has also been examined to determine whether it is capable of serving as an electron donor to photosystem I. In an attempt to examine this question, two approaches were taken. In the first, the possibility that the PC–cyt *f* adduct could be photooxidized by PS I particles prepared as described by Mullet et al. (1980) was examined by spectral analysis of oxidation of the cyt *f* component of the adduct when illuminated in the presence of PS I particles. No photooxidation of the cyt *f* component of the adduct was observed in either the presence or absence of 5 mM $MgCl_2$ (data not shown).

In the second approach, the ability of the PC–cyt *f* adduct to donate electrons to PS I was measured by PS I reduction of O_2 via methylviologen (Table III). An ascorbate plus TMPD electron-donating system supported electron transport through PS I at a low rate that was increased nearly 8-fold in the presence of PC and 9-fold in the presence of both PC and cyt *f*. In contrast, no increase in the rate was observed when the PC–cyt *f* adduct was used with the ascorbate plus TMPD electron-donating system, indicating the PC–cyt *f* adduct to be incapable of interacting with PS I. Furthermore, the addition of free PC in the presence of the PC–cyt *f* adduct increased the electron-transfer rate to the same degree as did PC alone, indicating that the presence of the PC–cyt *f* adduct did not inhibit the interaction of free PC with PS I.

Identification of Groups Involved in Covalent Linkages in the PC–Cyt f Adduct. Four large-scale preparations of the PC–cyt *f* adduct each resulted in 9–10 mg of adduct, representing a 68–75% recovery of the cyt *f* present in the cross-linking reaction mixture. A portion of the concentrated adduct (about 2 mg) was lyophilized without prior chemical or enzymatic treatment for the purpose of determining N-terminal sequences. When analyzed by reversed-phase HPLC, cyt *f* and the PC–cyt *f* adduct had identical retention times, indi-

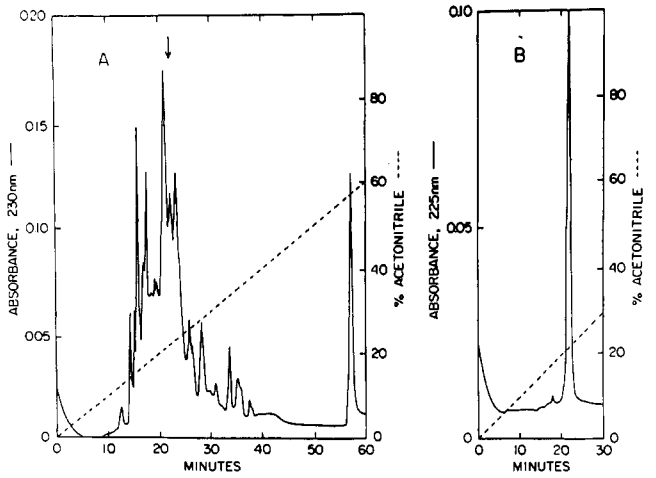


FIGURE 4: HPLC purification of PC–cyt *f* adduct before and after CNBr digestion. Prior to sequencing experiments, the PC–cyt *f* adduct (panel A) and the CNBr digest of the PC–cyt *f* adduct (panel B) were purified by HPLC. CNBr digestion and HPLC procedures were as described under Materials and Methods.

Table IV: N-Terminal Sequencing of PC–Cyt *f* Adduct

		interpretation	
cycle	raw data	cyt *f* sequence	PC sequence
1	V (183),[a] Y (112)	Y (112)	V (183)
2	E (131), P (86)	P (86)	E (112)
3	V (124), I (72)	I (72)	V (124)
4	L (227), F (109)	F (109)	L (227)
5	L (188), A (96)	A (96)	L (188)
6	G (45), Q (33)	Q (33)	G (45)
7	G (43), Q (58)	Q (58)	G (43)
8	G (84)	G (42)	G (42)
9	D (26), Y (49)	Y (49)	D (26)
10	G (84), E (81)	E (81)	G (84)
11	S (30), N (38)	N (38)	S (30)
12	L (35), P (36)	P (36)	L (35)
13	A (45)	nd[b]	A (45)
14	F (34)	nd	F (34)
15	L (50)	nd	L (50)
16	P (50)	nd	P (50)
17	G (53)	nd	G (53)
18	D (13)	nd	D (13)
	1	10	18
PC N-terminus	V E V L L G G G D G S L A F L P G D -		
cyt *f* N-terminus	Y P I F A Q Q G Y E N P -		

[a] Values in parentheses indicate the number of picomoles for each amino acid determined in that cycle. [b] nd, not determined.

cating that retention of the adduct by the column was due to interaction with the cyt *f* component of the adduct and not with the PC component. The chromatographic behavior of PC alone was irregular and not reproducible (data not shown). Figure 4A shows the chromatogram of the adduct. The peak fraction, labeled "adduct", was subjected to N-terminal sequencing. The results obtained (Table IV) were consistent with a dual sequence consisting of the 18 N-terminal residues of cyt *f* and the 12 N-terminal residues of PC. Overall, the sequences are of roughly equivalent picomole values, consistent with the 1:1 stoichiometry of the adduct. Exactly equivalent values were not expected as each sequence will have a difference in its sequencing efficiency. Cycle 8 has a glycine for both sequences. The raw value of 84 pmol was evenly distributed between the two sequences. The N-terminal sequence determined for PC was identical with that reported by Scawen et al. (1975) for spinach PC. The N-terminal sequence of turnip cyt *f* is identical with that of several other higher plant cyt *f* for which complete sequences are known (Hauska et al., 1988).

Table V: Sequencing of Cyanogen Bromide Treated PC–Cyt *f* Adduct

		interpretation		
cycle	raw data	cyt *f* (N-terminus)	PC (N-terminus)	PC (res 58–62)
1	V (72),[a] Y (46), S (41)	Y (46)	V (72)	S (41)
2	E (80), P (20)	P (20)	E (40)	E (40)
3	V (53), I (38)	I (38)	V (53)	(E missing)
4	L (34), F (41), D (40)	F (41)	L (34)	D (40)
5	L (60), A (38)	A (38)	L (30)	L (30)
6	G (25), Q (18)	Q (18)	G (25)	nd[b]
7	G (30), Q (20)	Q (20)	G (30)	nd
		1	7	
	PC N-terminus		V E V L L G G -	
	cyt *f* N-terminus		Y P I F A Q Q -	
			58	62
	PC (res 58–62)		S E D x D L	

[a] Values in parentheses indicate the number of picomoles of each amino acid determined in that cycle. [b] nd, not determined.

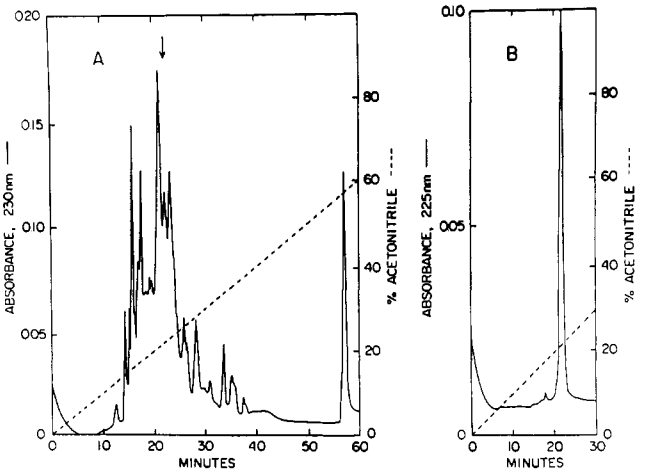


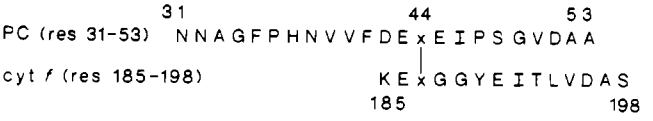
FIGURE 5: HPLC peptide mapping of trypsin digest of PC–cyt *f* adduct. Following a predigestion with endoprotease Arg-C, the PC–cyt *f* adduct was digested with trypsin, and the resulting fragments were separated by HPLC as described under Materials and Methods. The initial separation is shown in panel A with the peak for which sequence data were obtained indicated with an arrow. The results of rechromatography of this peak prior to sequencing are shown in panel B.

Figure 4B shows the chromatogram of CNBr digested adduct. The peak had an irregular shape, suggesting the possibility of mutiple fragments. Fractions from the front and back sides of the main peak were sequenced separately. Table V summarizes results from the back side of the peak. The seven N-terminal residues from both PC and cyt *f* were obtained in addition to an internal sequence of PC representing residues 58–62. In cycle 2, the glutamate was distributed between position 2 of the PC N-terminus and position 59 of the PC internal sequence. The leucine in cycle 5 was distributed in a similar manner. However, the glutamate at position 60 in the PC internal sequence (cycle 3) was absent, indicating that it is cross-linked to cyt *f*. Similar results (data not shown) were obtained from the front side of the peak except that in this case glutamate 59 of PC appeared to be cross-linked to cyt *f* rather than glutamate 60. Glutamate 59 was not totally absent from these sequences, but its sequencing yield was very poor relative to all other residues, suggesting that most but not all of the adducts in this fraction were linked via Glu-59. It has not yet been possible to identify the lysine(s) on cyt *f* to which these residues on PC are covalently linked.

Resuspension of the lyophilized adduct and CNBr cleaved products proved difficult. Heavy sample loss occurred, presumably due to irreversible aggregation of the denatured and dehydrated sample. Exploiting the fact that PC is devoid of arginine, the adduct was predigested with endoprotease Arg-C,

Table VI: Sequencing of Trypsin Digestion Fragment of PC–Cyt *f* Adduct

cycle	raw data	interpretation: PC (31–53)	cyt *f* (185–193)	cyt *f* (186–198)
1	N (250),[a] K (165), E (114)	N (250)	K (165)	E (114)
2	N (243), E (172)	N (243)	E (172)	(K missing)
3	A (318), G (185)	A (318)	(K missing)	G (185)
4	G (502)	G (167)	G (167)	G (168)
5	F (290), Y (138)	F (290)	(G missing)[b]	Y (138)
6	P (188), Y (214), E (180)	P (188)	Y (214)	E (180)
7	H (25), E (174), I (171)	H (25)	E (174)	I (171)
8	N (149), I (241), T (45)	N (149)	I (241)	T (45)
9	V (229), T (73), L (214)	V (229)	T (73)	L (214)
10	V (452)	V (226)	nd[c]	V (226)
11	F (145), D (56)	F (145)	nd	D (56)
12	D (137), A (161)	D (137)	nd	A (161)
13	E (77), S (15)	E (77)	nd	S (15)
14	no release	(D missing)	nd	nd
15	(E 120)	E (120)	nd	nd
16	I (102)	I (102)	nd	nd
17	P (67)	P (67)	nd	nd
18	S (11)	S (11)	nd	nd
19	G (82)	G (82)	nd	nd
20	V (82)	V (82)	nd	nd
21	D (33)	D (33)	nd	nd
22	A (61)	A (61)	nd	nd
23	A (53)	A (53)	nd	nd



[a] Values in parentheses indicate the number of picomoles of each amino acid determined in that cycle. [b] G was expected in this position but not observed. The reason for the apparent absence of G is not known. [c] nd, not determined.

Table VII: Sequencing of Endoprotease Asp-N Digestion Fragment of PC-Cyt *f* Adduct

cycle	raw data	interpretation: PC (res 61–70)
1	D (32)[a]	
2	L (37)	61 70
3	I (12)[b]	D L I N A P G E T Y
4	N (26)	
5	A (21)	
6	P (20)	
7	G (18)	
8	E (11)	
9	T[c]	
10	Y (12)	

[a] Values reported in parentheses are the number of picomoles for each amino acid determined in that cycle. [b] This assignment may be in error as the amino acid in this position is reported as L in the original report of the sequence of spinach PC (Scawen et al., 1975) and is conserved in all PCs for which sequences have been determined. [c] Picomole value not determined.

a treatment that greatly reduced sample loss. The predigested adduct was then digested with either trypsin or endoprotease Asp-N. Figure 5 shows the chromatographic profilie of the trypsin digest. Sequence data are presented in Table VI for the peak indicated by the arrow. Three internal sequences were obtained, one from PC and two from cyt *f*. The two from cyt *f* are staggered sequences of the same portion of cyt *f* which resulted from cleavages after Arg-184 or Lys-185. The PC sequence represented residues 31–53. Glycine in cycle 4 was distributed between all three sequences, while valine at cycle 10 was distributed between only two sequences. Asp-44 of PC (cycle 14) was absent as was Lys-187 of cyt *f* (cycle 3, residues 185–193; cycle 2, residues 186–198), indicating that Asp-44 of PC is linked to Lys-187 of cyt *f*. The inability of trypsin to cleave at Lys-187 is also consistent with its being "nonavailable" due to covalent linkage to PC.

Figure 6 shows the chromatographic profile for the endoprotease Asp-N digestion of the PC–cyt *f* adduct. Sequence

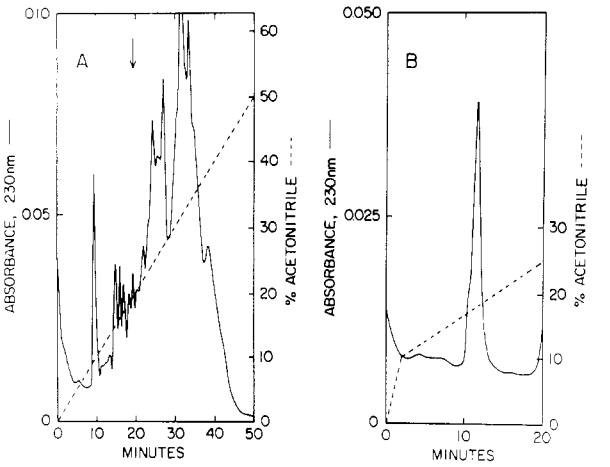


FIGURE 6: HPLC peptide mapping of endoprotease Asp-N digest of PC–cyt *f* adduct. Following predigestion with endoprotease Arg-C, the PC–cys *f* adduct was treated with endoprotease Asp-N, and the resulting peptides were separated by HPLC as described under Materials and Methods. Panel A shows the initial separation with the peak for which sequence data were obtained indicated with an arrow. The results of rechromatography of this peak prior to sequencing are shown in panel B.

data for the peak indicated with the arrow are summarized in Table VII. It was consistent with a single internal peptide of PC consisting of residues 61–70 containing Glu-68. The only carboxyl groups on PC not covered by the sequencing efforts were Glu-25, Glu-26, and Glu-76.

Additional Cross-Linking Studies. We have also covalently linked spinach PC to spinach cyt *f* with EDC using a modified procedure of Davis and Hough (1983). As purified spinach cyt *f* forms oligomers, a 16-h preincubation of the reaction mixtures in the presence of 0.1% (v/v) Triton X-100 at 4 °C was required. EDC was added and the reaction allowed to proceed at room temperature. The preincubation may result

Table VIII: Cross-Linking of Various Electron-Acceptor Proteins to Turnip Cyt *f*

protein	cross-linked to turnip cyt *f* in the presence of EDC
spinach PC	YES, strong formation (70–100%) of 1:1 adduct when performed in the presence of a 2-fold excess of PC
A. variabilis PC	NO, no adduct formed in the presence of a 2- or 4-fold excess of PC
Euglena cyt *c*-552	YES, weak (<20%) yield of 1:1 adduct when cyt *c*-552 is present in 4-fold excess; no adduct detected when cyt *c*-552 is present in 2-fold excess
S. maxima cyt *c*-553	NO, no adduct formed in the presence of a 2- or 4-fold excess of cyt *c*-552
A. flos-aquae cyt *c*-553	NO, no adduct formed in the presence of a 2- or 4-fold excess of cyt *c*-553
Microcystis aeruginosa cyt *c*-553	NO, no adduct formed in the presence of a 2-fold excess of cyt *c*-553
Anacystis nidulans cyt *c*-553	NO, no adduct formed in the presence of a 2-fold excess of cyt *c*-553

in the dissociation of the cyt *f* oligomer. If the preincubation was omitted and/or the reaction time was longer than 90 min, the cross-linked reaction product would remain on top of the 10% resolving gel during SDS gel electrophoresis. This occurred when cyt *f* was incubated with EDC in the presence or absence of PC, suggesting that EDC was forming cross-linkages within the cyt *f* oligomer.

The ability of algal and cyanobacterial proteins serving as electron acceptors for cyt *f* to be covalently linked to turnip cyt *f* in the presence of EDC has also been examined (Table VIII). While *Euglena* cyt *c*-552 could be covalently linked to turnip cyt *f* in the presence of EDC, the efficiency of cross-linking was substantially less than that observed with spinach PC. None of the cyanobacterial proteins tested could be cross-linked to turnip cyt *f* in the presence of EDC.

Discussion

The PC-cyt *f* adduct described in this paper has a molecular weight and spectral characteristics consistent with a 1:1 stoichiometry for the two components in the adduct. While the E° for the PC component of the adduct was unchanged from that of free PC, that of the cyt *f* component of the adduct was shifted by −20 mV relative to free cyt *f*. Takabe and Ishikawa (1989) have reported an E° for cyt *f* in their PC–cyt *f* adduct similar to that which we have measured. They did not, however, compare their value to that of free cyt *f*. A similar shift has been previously reported for the cyt *c* component of a PC–cyt *c* adduct prepared in a similar fashion (Geren et al., 1983). While it is not certain that the shift in E° for cyt *f* is physiologically significant, it is interesting that the shift is in the direction which would favor electron transfer from cyt *f* to PC.

Although Gross and co-workers (Burkey & Gross, 1981, 1982; Anderson et al., 1987) demonstrated that modification of PC with ethylenediamine in the presence of EDC alters the E° of PC, our data showed no significant difference between the E° of PC in the adduct and that of free PC. This difference may be due to the fact that the modification used by Gross and co-workers changes the charge at the site of modification from negative to positive, while the use of EDC in covalent attachment of PC to cyt *f* would only change the charge from negative to neutral. The recent observation (Jackman et al., 1987) that the positively charged PC from *Anabaena variabilis* has an E° of +340 mV in contrast to the +370–380 mV values typical of higher plant PC's suggests that the nature and distribution of charges on PC may affect its E°.

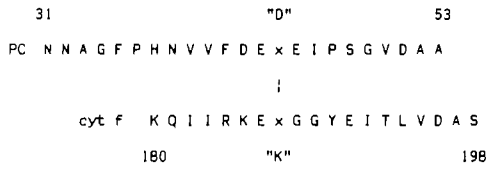
The possibility that the shift in redox potential of the cyt *f* component of the PC–cyt *f* adduct might be a reflection of a change in heme environment was examined by solvent perturbation spectroscopy. Siedow et al. (1980) have suggested that the high redox potential of cyt *f* might be due to the heme being buried within the protein. Our solvent perturbations studies would discount this explanation as we find the degree of exposure to be slightly greater than that observed with cyt *c*. The degree of heme exposure was found to be decreased in the PC–cyt *f* adduct relative to that observed in free cyt *f*. This observation suggests that PC is covalently attached to cyt *f* in such a manner that the exposed portion of the heme in cyt *f* is buried at the interface between the two proteins. It seems likely that the exposed portion of heme in cyt *f* may be important in electron-transfer reactions as has been observed in the case of cyt *c*.

Small shifts in the resonance Raman spectrum of the PC–cyt *f* adduct were observed relative to the peak positions reported previously for cyt *f*. These shifts may also be indicative of a change in heme environment. It is not possible from the present data to determine whether this change is due to alteration of the exposure of the heme to solvent or due to microenvironmental changes in cyt *f* as a result of the covalent attachment of PC.

The PC–cyt *f* adduct was demonstrated by two criteria to be incapable of donating electrons to PS I. This observation suggests that the same region (or at least some common feature) of PC may be involved in the interaction of PC with both cyt *f* and PS I such that when the PC component is covalently linked to cyt *f* it is no longer capable of interacting with PS I. Results of chemical modification studies of Anderson et al. (1987) did not rule out the idea of a ternary complex involving cyt *f*, PC, and PS I, although they consider such a complex to be unlikely. Our data would tend to more strongly refute the idea of a ternary complex as our results indicate that when PC is (covalently) bound to cyt *f*, it is incapable of interacting with PS I. PC may thus be similar to cyt *c*, which uses a common portion of its surface in its interaction with both its oxidase and reductase. The observation that the addition of free PC restored full activity would seem to indicate that the PC–cyt *f* adduct does not even bind to PS I. Otherwise, one might have expected a competition between free PC and the PC–cyt *f* adduct for the site. In contrast to our results, Takabe and Ishikawa (1989) found their PC–cyt *f* adduct to be partially active as an electron donor to PS I. However, the activity they reported was extremely low (about 1% that of native PC) with PS I particles comparable to those used in the present study. The inability to observe the photooxidation of cyt *f* in our PC–cyt *f* adduct might be interpreted as due to an absence of electron transport within the adduct as has been observed by Peerey and Kostic (1989) for a covalently linked adduct involving PC and cyt *c*. However, Takabe and Ishikawa (1989) have demonstrated that electron transfer from cyt *f* to PC can occur in a PC–cyt *f* adduct similar to that used in this study.

Cleavage of the PC–cyt *f* adduct, peptide mapping, and sequencing have allowed identification of two sites of covalent linkage between the two proteins. In one of these it has been possible to identify both partners in the covalent attachment

with Asp-44 of PC being covalently linked to Lys-187 of cyt *f*.

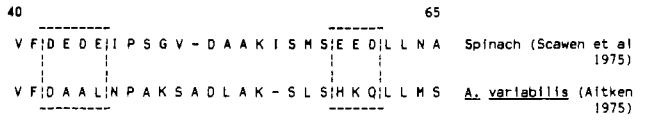


The lysine of cyt *f* involved in the covalent attachment to PC lies in one of the three regions of cyt *f* suggested previously to be candidates for contributing to the PC binding site (Davis, 1987). This paper is the first identification of a binding site on cyt *f* for PC. This region of the cyt *f* amino acid sequence is rich in basic residues as would be expected for interaction with a negatively charged protein such as PC and is highly conserved in all cyt *f* sequences known to date (Hauska et al., 1988; Kallas, 1988) except that of the cyanobacterium, *Nostoc* PCC 7906 (Kallas, 1988), where the region is rich in acidic residues. This change may be functionally important as *Nostoc*, being a filamentous cyanobacterium, would be expected to utilize a positively charged electron acceptor (PC or cyt *c*-553) in contrast to the negatively charged electron acceptors utilized by unicellular cyanobacteria, algae, and higher plants (Ho & Krogmann, 1984). The differences in this region of the cyt *f* amino sequence of *Nostoc* as compared to those of higher plants may be indicative of an evolutionary adaption to match the charge characteristics of the protein serving as the electron acceptor.

A second site of covalent linkage between PC and cyt *f* has also been identified as involving Glu-60 (and perhaps Glu-59) of PC. It has not yet been possible to determined the lysine residues on cyt *f* to which these groups are covalently attached. Our data have also allowed us to eliminate other carboxyl groups on PC at positions 2, 9, 18, 42, 43, 45, 51, 61, and 68 in PC as being involved in the covalent linkage between PC and cyt *f* in the adduct. No determination could be made regarding carboxyl groups in PC at positions 25, 26, and 76, although their positions in the structure of PC would make it unlikely that they would be involved in interaction with cyt *f*.

The locations of the residues on PC involved in covalent linkage to cyt *f* in the PC–cyt *f* adduct observed in the present work are supported by the chemical modification studies of Anderson et al. (1987) which indicated that both of the acidic patches on PC (residues 42–45 and 59–61) are involved in its interaction with cyt *f*. The involvement of the acidic patch including residues 42–45 on PC in its interaction with cyt *f* has also been suggested by Beoku-Betts et al. (1983, 1985). The involvement of the second acidic patch in covalent linkage of PC to cyt *f* in the adduct may explain the inability of the PC–cyt *f* adduct to serve as an electron donor to PS I. The chemical modification studies of Anderson et al. (1987) indicated that, in addition to alteration of interaction with cyt *f*, modification of the second acidic patch also altered the interaction of PC with PS I. If the second acidic patch is a feature shared in common by the binding sites for cyt *f* and PS I, the PC–cyt *f* adduct would not be expected to interact with PS I. The observation that modification of the first acidic patch (residues 42–45) affects the interaction of PC with cyt *f* but not with PS I would also suggest that although the two binding sites on PC are not identical, there must be a considerable overlap such that PC would be incapable of interacting with both cyt *f* and PS I simultaneously.

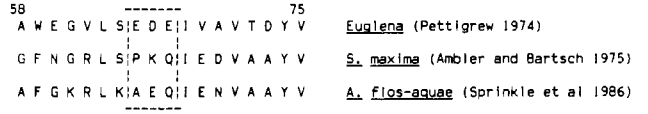
The inability of *A. variabilis* PC to be covalently linked to turnip cyt *f* in the presence of EDC is most likely due to sequence variation in the region of the two acidic clusters.



Comparison of these regions of the amino acid sequences of the two proteins indicates the negative clusters found in spinach (and most higher plant PC's) are not present in the *A. variabilis* sequence.

Changes in the surface charge distributions may also be responsible for the differences in the abilities of the various *c*-type cytochromes examined to be covalently linked to turnip cyt *f*. We observed that *Euglena* cyt *c*-552 could be covalently linked to cyt *f* but with a lower efficiency than observed for PC. Wood (1978) has noted a cluster of negative charges in the sequence of *Euglena* cyt *c*-552 that might be functionally equivalent to the acidic cluster near residue 60 in PC. As our cross-linking data on the PC–cyt *f* adduct (described above) and the chemical modification studies of Anderson et al. (1987) indicate that both acidic clusters of PC are involved in its interaction with cyt *f*, the *Euglena* cyt *c*-552, having only a single acidic cluster, might be expected to interact less strongly with cyt *f*, resulting in a lowered efficiency of cross-linking of cyt *c*-552 to cyt *f*.

No evidence was found for formation of a covalent linkage between cyt *f* and the cyanobacterial cyt *c*-553's examined. The positive charge of *Aphanizomenon flos-aquae* cyt *c*-553 (Sprinkle et al., 1986) is probably responsible for its inability to be covalently linked to turnip cyt *f* in the presence of EDC. As the cyt *c*-553 from *Spirulina maxima* is a negatively charged protein, we were initially surprised that it was not covalently linked to cyt *f* in the presence of EDC. However, kinetic studies have shown it to react with higher plant cyt *f* with a rate constant approximately 1 order of magnitude less than does PC (Ho & Krogmann, 1980), suggesting that the interaction between the two proteins might be less efficient. In addition, comparison of the amino acid sequences in the region suggested by Wood (1978) to be analogous to the negative cluster near residue 60 of PC shows the negative cluster present in *Euglena* cyt *c*-552 to be missing in the cyanobacterial cyt *c*-553's examined here.



It is probably the precise arrangement of negative charges on the cyt *c*-553's rather than just their presence that contributes to the specificity of the cross-linking reaction. The observation that there are significant sequence differences in the cyanobacterial cyt *f* (Kallas et al., 1988) relative to those of higher plant cyt *f* may also be indicative of differences in the binding site(s) for electron acceptors in procaryotic and eucaryotic systems.

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Registry No. Cyt *f*, 9035-46-5; Asp, 56-84-8; Lys, 56-87-1; Glu, 56-86-0; cyt *c*-552, 9048-78-6.

REFERENCES

Aitken, A. (1975) *Biochem. J., 149*, 675–683.

Ambler, R. P., & Bartsch, R. G. (1975) *Nature 253*, 285–288.
Anderson, G. P., Sanderson, D. G., Lee, C. H., Durell, S., Anderson, L. B., & Gross, E. L. (1987) *Biochim. Biophys. Acta 894*, 386–398.
Bendall, D. S. (1982) *Biochim. Biophys. Acta 683*, 119–151.
Beoku-Betts, D., Chapman, S. K., Knox, C. V., & Sykes, A. G. (1983) *J. Chem. Soc., Chem. Commun.*, 1150–1153.
Beoku-Betts, D., Chapman, S. K., Knox, C. V., & Sykes, A. G. (1985) *Inorg. Chem. 24*, 1677–1681.
Bohme, H., Brutsch, S., Weithmann, G., & Boger, P. (1980a) *Biochim. Biophys. Acta 590*, 248–260.
Bohme, H., Pelzer, B., & Boger, P. (1980b) *Biochim. Biophys. Acta 592*, 528–535.
Burkey, K. O., & Gross, E. L. (1981) *Biochemistry 20*, 5495–5499.
Burkey, K. O., & Gross, E. L. (1982) *Biochemistry 21*, 5886–5890.
Cohn, C. L., Hermondson, M. A., & Krogmann, D. W. (1989) *Arch. Biochem. Biophys.* (in press).
Davis, D. J. (1987) *Prog. Photosynth. Res., Proc. Int. Congr. Photosynth., 7th, 1986, 2*, 473–476.
Davis, D. J., & Hough, K. (1983) *Biochem. Biophys. Res. Commun. 116*, 1000–1006.
Davis, D. J., Frame, M. K., & Johnson, D. A. (1988) *Biochim. Biophys. Acta 936*, 61–66.
Geren, L. M., Stonehuerner, J., Davis, D. J., & Millett, F. (1983) *Biochim. Biophys. Acta 724*, 62–68.
Gray, J. C. (1978) *Eur. J. Biochem. 82*, 133–141.
Gross, E. (1967) *Methods Enzymol. 11*, 238–255.
Hauska, G., Hurt, E., Gabellini, N., & Lockau, W. (1983) *Biochim. Biophys. Acta 726*, 97–133.
Hauska, G., Nitschke, W., & Herrmann, R. G. (1988) *J. Biomembr. Bioenerg. 20*, 211–228.
Ho, K. H., & Krogmann, D. W. (1980) *J. Biol. Chem. 255*, 3855–3861.
Ho, K. H., & Krogmann, D. W. (1984) *Biochim. Biophys. Acta 766*, 310–316.
Ho, K. H., Ulrich, E. L., Krogmann, D. W., & Gomez-Lojero, C. (1979) *Biochim. Biophys. Acta 545*, 236–248.
Jackman, M. P., Sinclair-Day, J. D., Sisley, M. J., Sykes, A. G., Denys, L.A., & Wright, P. E. (1987) *J. Am. Chem. Soc. 109*, 6443–6449.
Kallas, T., Spiller, S., & Malkin, R. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 5794–5798.
Kamimura, Y., & Matsuzaki, E. (1978) *Plant Cell Physiol. 19*, 1175–1183.
Laemmli, U. K. (1970) *Nature 227*, 680–685.
Matsuzaki, E., Kamimura, Y., Yamasaki, T., & Yakushiji, E. (1975) *Plant Cell Physiol. 16*, 237–246.
Mitsui, A. (1971) *Methods Enzymol. 23*, 368–371.
Mullet, J. E., Burke, J. J., & Arntzen, C. J. (1980) *Plant Physiol. 65*, 814–822.
Niwa, S., Ishikawa, H., Nikai, S., & Takabe, T. (1980) *J. Biochem. 88*, 1177–1183.
Peerey, L. M., & Kostic, N. M. (1989) *Biochemistry 28*, 1861–1868.
Pettigrew, G. W. (1974) *Biochem. J. 139*, 449–459.
Rigby, S. E. J., Moore, G. R., Gray, J. C., Gadsby, P. M. A., George, S. J., & Thompson, A. J. (1988) *Biochem. J. 256*, 571–577.
Sandmann, G., & Boger, P. (1980) *Plant Sci. Lett. 17*, 417–424.
Scawen, M. D., Ramshaw, J. A. M., & Boulter, D. (1975) *Biochem. J. 147*, 343–349.
Schlauder, G. G., & Kassner, R. J. (1979) *J. Biol. Chem. 254*, 4110–4113.
Siedow, J. N., Vickery, L. E., & Palmer, G. (1980) *Arch. Biochem. Biophys. 203*, 101–107.
Sprinkle, J. R., Hermondson, M., & Krogmann, D. W. (1986) *Photosynth. Res. 10*, 62–73.
Takabe, T., & Ishikawa, H. (1989) *J. Biochem. 105*, 98–102.
Takabe, T., Takenaka, K., Kawamura, H., & Beppu, Y. (1986) *J. Biochem. 99*, 833–840.
Takahashi, M., & Asada, K. (1975) *Plant Cell Physiol. 16*, 191–194.
Tanaka, K., Takahashi, M., & Asada, K. (1978) *J. Biol. Chem. 253*, 7397–7403.
Tanaka, K., Takahashi, M., & Asada, K. (1981) *Plant Cell Physiol. 22*, 33–39.
Wood, P. M. (1974) *Biochim. Biophys. Acta 357*, 370–379.
Wood, P. M. (1977) *Eur. J. Biochem. 72*, 605–612.
Wood, P. M. (1978) *Eur. J. Biochem. 87*, 9–19.