Electron-Transfer Reactions of Cytochrome *f* with Flavin Semiquinones and with Plastocyanin. Importance of Protein–Protein Electrostatic Interactions and of Donor–Acceptor Coupling†

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ABSTRACT: Reduction of turnip ferricytochrome *f* by flavin semiquinones and oxidation of this ferrocytochrome *f* by French bean cupriplastocyanin are studied by laser flash photolysis over a wide range of ionic strengths. Second-order rate constants (±15%) at extreme values of ionic strength, all at pH 7.0 and 22 °C, are as follows: with FMN semiquinone at 1.00 and 0.0040 M, 5.0×10^7 and 3.9×10^8 M^{-1} s^{-1}; with riboflavin semiquinone at 1.00 and 0.0040 M, 1.7×10^8 and 1.9×10^8 M^{-1} s^{-1}; with lumiflavin semiquinone at 1.00 and 0.0045 M, 1.8×10^8 and 4.5×10^8 M^{-1} s^{-1}; with cupriplastocyanin at 1.00 and 0.100 M, 1.4×10^6 and 2.0×10^8 M^{-1} s^{-1}. These reactions of cytochrome *f* are governed by the local positive charge of the interaction domain (the exposed heme edge), not by the overall negative charge of the protein. Lumiflavin semiquinone behaves as if it carried a small negative charge, probably because partial localization of the odd electron gives this electroneutral molecule some polarity; local charge seems to be more important than overall charge even for relatively small redox agents. The dependence of the rate constants on ionic strength was fitted to the equation of Watkins; this model recognizes the importance of local charges of the domains through which redox partners interact. There is kinetic evidence that a noncovalent complex between cytochrome *f* and plastocyanin exists at low ionic strength. The driving force for the intracomplex electron-transfer reaction in either direction is virtually nil, and the rate constant in either direction is 2800 ± 300 s^{-1}. For at least two reasons, cytochrome *f* reacts faster than cytochrome *c* with all four redox partners examined. First, the heme edge is exposed more in cytochrome *f* than in cytochrome *c*. Second, cytochrome *f* seems to attract the partners by a stronger electrostatic force than does cytochrome *c*. These differences should be taken into account when the latter protein is used as a model for the former. They are especially important in the reaction with plastocyanin, a physiological partner of cytochrome *f*.

Since most redox metalloproteins bear appreciable net charges and contain charged "patches" on the surface, the rates of their electron-transfer reactions depend on ionic strength. This dependence, and electrostatic effects in general, can be treated with varying degrees of rigor (Davis & McCammon, 1990; Marcus & Sutin, 1985; Harvey, 1989; Pettigrew & Moore, 1987; Sharp & Honig, 1990). Protein molecules can be considered as monopoles with uniform (spherical) distributions of charged groups or, more correctly, as dipoles with nonuniform (nonspherical) distribution of these groups. When only the protein composition or sequence is known, the former approach is suitable. When structures of both reactants are known, the latter approach is preferred for it takes into account not only the monopole–monopole interaction but also monopole–dipole and dipole–dipole interactions (Koppenol & Margoliash, 1982; van Leeuwen, 1981, 1983; Rush et al., 1987, 1988; Dixon et al., 1989). An intermediate approach recognizes the existence of charged patches and requires knowledge of the structure of only one of the reactants (Meyer et al., 1984; Tollin et al., 1984; Watkins, 1984; Senthilathipan & Tollin, 1989). Various rigorous treatments have been published (Allison et al., 1985; Northrup et al., 1986a,b, 1987, 1988, 1990).

Interprotein electron-transfer reactions have been studied mostly with cytochrome *c*, which bears a positive net charge, and metalloproteins that bear negative net charges [see Kostić (1991) and references cited therein; Pettigrew & Moore, 1987]. The heme protein cytochrome *f* (designated cyt)[1] and the blue copper protein plastocyanin (designated pc), whose charges are both negative, are nonetheless partners in electron-transport chains. They mediate the flow of electrons from photosystem II to photosystem I.

In this study, reactions (eq 1) of cytochrome *f* with three flavin semiquinones (FH) are examined for the first time, in order to determine electrostatic properties of the protein. The bimolecular reaction k_2 between ferrocytochrome *f* and cupriplastocyanin (eq 2) is examined over a wide range of ionic

$$\text{cyt(3+)} + \text{FH} \xrightarrow{k_1} \text{cyt(2+)} + \text{F} + \text{H}^+ \qquad (1)$$

$$\text{cyt(2+)} + \text{pc(2+)} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{cyt(3+)} + \text{pc(1+)} \qquad (2)$$

strengths and more thoroughly than in previous studies. (Parenthesized numerals in eq 1 and 2 are oxidation states of iron and copper.) The first kinetic evidence for noncovalent association between cytochrome *f* and plastocyanin is pres-

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[1] Abbreviations: cyt(3+), ferricytochrome (*f* or *c*); cyt(2+), ferrocytochrome (*f* or *c*); pc(2+), cupriplastocyanin; pc(1+), cuproplastocyanin; F, flavin; LF, lumiflavin; RF, riboflavin; DRF, 5-diazariboflavin; FMN, flavin mononucleotide; FH, flavin semiquinone; LFH, lumiflavin semiquinone; RFH, riboflavin semiquinone; DRFH, 5-deazariboflavin semiquinone; FMNH, flavin mononucleotide semiquinone; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; NHE, normal hydrogen electrode; *A*, absorbance; ϵ, molar absorptivity; M_r, molecular mass; *I*, ionic strength; p*I*, isoelectric point.

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ented. Unimolecular reactions within the diprotein complex (later designated k_{10} and k_{-10} in eq 10), which are analogous to the bimolecular reactions k_2 and k_{-2}, are examined for the first time. Cytochrome *f* and cytochrome *c* differ in their reactivity toward plastocyanin, and pitfalls in the use of the latter as a model for the former will be pointed out. Detailed three-dimensional structure is known for poplar plastocyanin (Guss & Freeman, 1983; Guss et al., 1986; Sykes, 1991a,b), and amino acid sequences are known for cytochrome *f* from five plants (Hauska et al., 1988). However, since the three-dimensional structure of cytochrome *f* is unknown, electrostatic factors in electron-transfer reactions are analyzed by the aforementioned "intermediate" method.

Materials and Methods

Chemicals. Cytochrome *f* from turnip was obtained from Sigma Chemical Co. Its absorbance quotient, A_{554}/A_{280}, was 0.80, and its purity was greater than 90% according to electrophoresis on an SDS–polyacrylamide gel. The protein was treated before each kinetic experiment with a 10-fold molar excess of dissolved $K_3[Fe(CN)_6]$, and this oxidant was removed by gel filtration on a P2 column 3 cm long. The concentration of ferricytochrome *f* was determined from the total concentration and the concentration of ferrocytochrome *f* (ϵ_{554} = 27 700 M^{-1} cm^{-1}) with an IBM 9430 UV–vis spectrophotometer; ferricytochrome *f* constituted ca. 75% of the total protein. Plastocyanin from French bean was isolated and purified by standard procedures (Milne & Wells, 1970) until its absorbance quotient A_{597}/A_{280} became 1.20 or less. Lumiflavin (LF), riboflavin (RF), and flavin mononucleotide (FMN) and all the chromatographic materials were obtained from Sigma Chemical Co. Pure 5-deazariboflavin (DRF) was obtained from Professor Gordon Tollin. Other chemicals were of reagent grade. Distilled water was demineralized further by a Barnstead Nanopure apparatus. Isoelectric focusing was done with a PhastSystem from Pharmacia.

Laser Flash Photolysis. The proteins and flavins were dissolved in a 1.0 mM sodium phosphate buffer at pH 7.00 that was made 0.50 mM in EDTA and whose ionic strength was adjusted with NaCl. Autoreduction of ferricytochrome *f* during kinetic experiments was negligible. The solutions were occasionally stirred during deaeration by wet argon for ca. 1 h. A Phase-R DL 1100 laser with the dye LTD 425 was perpendicular to an optical bench, and a data station was equipped with kinetic software by OLIS, Inc. The temperature was 22 °C. Reactions were started by a 0.4-μs laser pulse and followed by monitoring the transient absorbance of ferrocytochrome *f* at 554 nm. Solutions were shaken after each pulse. The proteins were equimolar, typically 15–20 μM each, and flavin semiquinone concentration was ca. 1.0 μM. Ferricytochrome *f* was present in excess over flavin semiquinones, and cupriplastocyanin was present in excess over ferrocytochrome *f*, so that first-order conditions were maintained. The observed rate constants depend linearly on protein concentration. Each rate constant is a mean value from at least four experiments. Individual values deviated from the mean by 15% or less.

Stopped-Flow Spectrophotometry. An apparatus by Kinetic Instruments, Inc., was interfaced to the aforementioned data station. The experiments were done at ambient temperature. The concentration of ferrocytochrome *f* was 0.25 μM, and the concentration of cupriplastocyanin was 2.5 μM. Oxidation of the former was monitored at 422 nm.

Electrostatic Calculations. The dependence of the bimolecular rate constant k on the ionic strength I was fitted to eq 3 (Meyer et al., 1984; Tollin et al., 1984; Watkins, 1984; Senthilathipan & Tollin, 1989). These are the symbols in eq 3–7: k_∞ is the rate constant at infinite ionic strength; V_{ii} is

$$\log k = \log k_\infty - V_{ii}X(I) \quad (3)$$

$$V_{ii} = \alpha\rho^{-2}D^{-1}Z_1Z_2r_{12} \quad (4)$$

$$\alpha = e^2/2\pi k_b T = 128.47 \text{ kcal mol}^{-1} \text{ at } 295 \text{ K} \quad (5)$$

$$X(I) = (1 + \kappa\rho)^{-1} \exp(-\kappa\rho) \quad (6)$$

$$\kappa = 0.3295I^{1/2} \quad (7)$$

the interaction energy between the parallel disklike domains bearing charges Z_1 (cytochrome *f*) and Z_2 (the other reactant); ρ is the radius of the interaction domains, and it depends on the smaller partner; r_{12} is the distance between the reactants, set at 3.5 Å; and D is the effective dielectric constant at the interface. This model was successfully applied to reduction of cytochrome *c* and plastocyanin by flavin semiquinones and flavodoxin semiquinone (Meyer et al., 1984; Tollin et al., 1984, 1986a,b). The disk radius of 4.0 Å for flavin semiquinones was taken from molecular dimensions (Meyer et al., 1984). For plastocyanin, the value of 7.0 Å was estimated from the effective radius of the protein (Rush et al., 1988) and also from the distance (15 Å) between the far edges of the two clusters of acidic residues that constitute the acidic patch. The dielectric constant (D) was set at 50 in the case of flavin semiquinones and at 10 in the case of plastocyanin. These values reflect partial exclusion of water molecules upon formation of the reactive complex for electron transfer (Matthew et al., 1983) and were used successfully in previous studies (Cheddar et al., 1989; Meyer et al., 1984; Tollin et al., 1984). Test applications of the electrostatic model to reactions between proteins of known structure gave dielectric constants in the range 10–15 (Watkins, 1984). Standard deviations in least-squares fitting were 10–15% for V_{ii} and 10–25% for k_∞.

Results and Discussion

Previous Studies. Although cytochrome *c* is not a physiological partner of plastocyanin, their reaction has been studied (Armstrong et al., 1986; Augustin et al., 1983; Bagby et al., 1990a,b; Chapman et al., 1984; King et al., 1985; Pan et al., 1990; Peerey & Kostič, 1989; Roberts et al., 1991; Senthilathipan & Tollin, 1989; Zhou & Kostič, 1991a,b, 1992; Zhou et al., 1992) as much as the reaction between the true partners cytochrome *f* and plastocyanin (Anderson et al., 1987; Beoku-Betts et al., 1983, 1985; Morand et al., 1989; Niwa et al., 1980; Takabe et al., 1980, 1984, 1989; Tanaka et al., 1981; Takenaka et al., 1980; Takabe & Ishikawa, 1989; Wherland & Pecht, 1978; Wood, 1974). Unavoidable autoreduction (Garewal et al., 1974; Garewal & Wasserman, 1972; Tanaka et al., 1978) and aggregation (Gray, 1978) complicate work with cytochrome *f*. Fortunately, the protein from turnip, used in this study, autoreduces very slowly and does not aggregate.

The dependence on ionic strength of the reaction between cytochrome *f* and plastocyanin has already been studied by others. The net charge of cytochrome *f* calculated by simple and complete versions of the Debye–Hückel theory was 0.1 and −5.9, respectively, and the charge calculated by the Wherland–Gray method was +4.0 (Takabe et al., 1980; Takenaka & Takabe, 1984). Trusting the Wherland–Gray method, these authors at first concluded that the net charge is positive. They later concluded that the net charge of cytochrome *f* is negative (Takabe & Ishikawa, 1989). Since both the Debye–Hückel theory and the Wherland–Gray method consider a protein as a sphere with uniformly distributed charges, they are unsuitable for treating electrostatic inter-

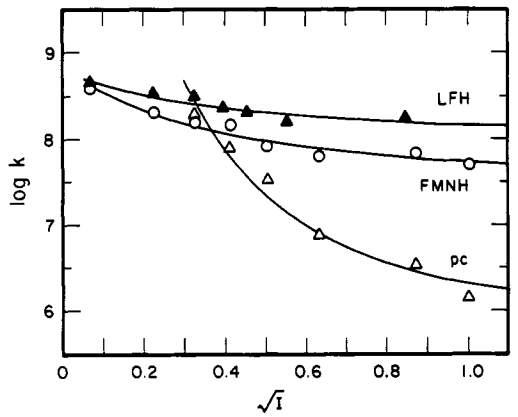

FIGURE 1: Dependence on ionic strength of bimolecular rate constants for reduction of ferricytochrome *f* by lumiflavin semiquinone (LFH) and FMN semiquinone ($FMNH^{2-}$) and for oxidation of ferrocytochrome *f* by cupriplastocyanin (pc). Solid lines are fittings to eq 3.

actions that are dominated by local charges.

Although cytochrome *f* has a negative net charge, it probably resembles cytochrome *c* in having an exposed heme edge surrounded by positively charged lysine residues (Simpkin et al., 1989; Wiley et al., 1984). Indeed, cross-linking of turnip cytochrome *f* and spinach plastocyanin by a carbodiimide indicated that lysine residues in the former protein interact with acidic residues in the negative patch in the latter (Morand et al., 1989). The only evidence for noncovalent association of cytochrome *f* and plastocyanin in solution comes from affinity chromatography (Molnar et al., 1987) and from cross-linking (Morand et al., 1989). Their association in electron-transfer reactions has not been studied before this work.

Cytochrome f and Flavin Semiquinones. Flavins (F) in a triplet state react with EDTA to form flavin semiquinones (FH), which disproportionate in the absence of oxidants (Ahmad et al., 1982; Simondsen & Tollin, 1983). In the presence of ferricytochrome *f*, two processes are detected because both the partially reduced and the fully reduced forms of the flavin (FH and FH^-) can reduce the protein. The former process accounts for ca. 90% of the cytochrome *f* absorbance change and occurs within 2 ms. The latter accounts for ca. 10% of the signal and lasts for more than 20 ms. These two processes were found also with cytochrome *c* (Hazzard et al., 1987; Meyer et al., 1983, 1984; Tollin et al., 1986a,b). Only the first process will be discussed.

The bimolecular rate constant k_1 (eq 1) depends on ionic strength as shown in Table I and Figure 1. In the case of FMN semiquinone, k_1 increases markedly as the ionic strength decreases from 1.00 M to 4.0 mM. Since this anion ($FMNH^{2-}$) sees cytochrome *f* as a cation, the reactivity of the protein is governed by the local positive charge near the exposed heme edge and not by the overall negative charge. The rate constants for the reactions of cytochrome *f* with $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ also increase as the ionic strength decreases (Takabe et al., 1980).

Although both riboflavin semiquinone and lumiflavin semiquinone are electroneutral, the reaction of ferricytochrome *f* with the former does not, but the reaction with the latter does, depend on ionic strength. This small but significant dependence cannot, for two reasons, be attributed to some structural change in cytochrome *f*. First, riboflavin semiquinone does not show the dependence. Second, lumiflavin semiquinone shows it with both cytochrome *c* and plastocyanin—in opposite directions, as expected from opposite charges of these two

Table I: Kinetics of Reduction of Ferricytochrome *f* by Flavin Semiquinones and of Oxidation of Ferrocytochrome *f* by Cupriplastocyanin at pH 7.0 and Different Ionic Strengths

	$k \times 10^{-7}$ (M^{-1} s^{-1})[a]			
I (M)	$FMNH^{2-}$	RFH	LFH	pc(2+)
1.00	5.0	17	18	0.14
0.700	7.0		17	0.34
0.500		18		
0.400	6.4			0.75
0.300			16	
0.250	8.4			3.3
0.200			21	
0.170	15			8.0
0.150			23	
0.100	16	21	32	20
0.050	21		34	
0.0045			45	
0.0040	39	19		

[a] Standard deviation ±15%.

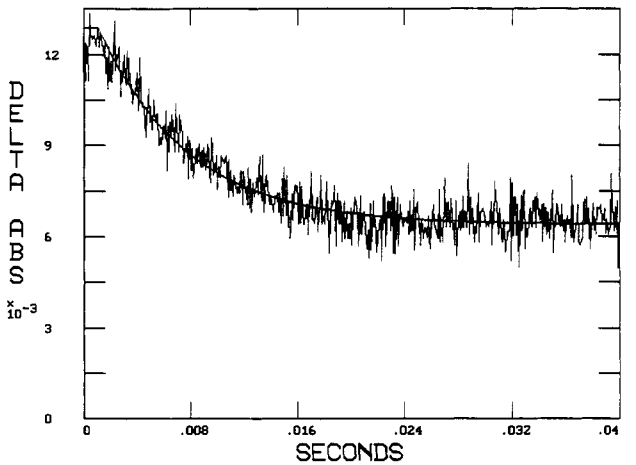

FIGURE 2: Change in the absorbance at 554 nm of ferrocytochrome *f* in its bimolecular reaction with cupriplastocyanin in phosphate buffer at pH 7.00 and an ionic strength of 0.400 M.

proteins (Peerey et al., 1991)—even though the conformations of these two proteins do not appreciably change with ionic strength. Electron spin resonance spectra show that the spin density is less delocalized in the latter than in the former semiquinone (Beinert, 1972). This partial localization perhaps gives lumiflavin semiquinone some dipolar character and behavior as if it carried a small positive charge. Similar dependence on ionic strength was found recently in a study of energy transfer (Northrup et al., 1990). Unsymmetric charge distribution is important for small agents as well as for proteins.

At the ionic strength of 1.00 M, when electrostatic interactions are negligible, the rate constants k_1 for riboflavin and lumiflavin semiquinones are identical within the error bounds and greater than the rate constant for FMN semiquinone even though all three reductants have reduction potentials of 0.23–0.24 V (Draper & Ingraham, 1968, 1970). The difference in reactivity is perhaps caused by a steric factor: the side chain is bulkier in FMN than in lumiflavin and riboflavin. A similar difference between these two types of flavin semiquinone in their reactions with cytochrome *c* was explained in the same way (Meyer et al., 1984). In general, cytochrome *f* reacts about 4 times faster than cytochrome *c* with a given flavin semiquinone under similar conditions.

Bimolecular Reactions between Cytochrome f and Plastocyanin. The reaction in eq 2 is induced by reduction of cytochrome *f* (eq 1) in the presence of cupriplastocyanin. The reductant of choice was riboflavin semiquinone because it reacts about 10 times faster with ferricytochrome *f* (Table I)

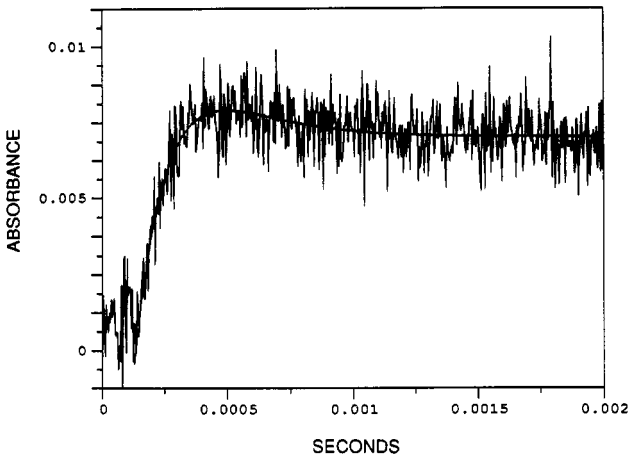

FIGURE 3: Change in the absorbance at 554 nm of ferrocytochrome *f* in its unimolecular reaction with cupriplastocyanin within the noncovalent diprotein complex in phosphate buffer at pH 7.00 and an ionic strength of 4.0 mM. The solid line is the best fit to eq 12.

than with cupriplastocyanin (Tollin et al., 1986a). A typical kinetic trace in Figure 2 shows that the concentration of ferrocytochrome *f*, whose formation is evident at the beginning of the trace, decreases and levels off at half the initial value. This kinetic profile and similar ones observed at other ionic strengths are characteristic of the reactions that satisfy eq 8

$$k_2 = k_{-2} = (1/2)k_{obs} \quad (8)$$

where k_{obs} is the observed second-order rate constant. Indeed, reduction potentials of cytochrome *f* and plastocyanin are equal (Morand et al., 1989), so that the equilibrium constant for the reaction in eq 2 is approximately equal to 1.

As Table I and Figure 1 show, the rate constant increases 143-fold as the ionic strength decreases 10-fold. The rate constants determined in this study are similar to those reported before for the same two proteins from other higher plants. The positively charged patch around the heme edge in cytochrome *f* and the negatively charged patch in plastocyanin seem to be conserved (Morand et al., 1989; Sykes, 1991a,b).

Cytochrome *f* (from turnip) differs from cytochrome *c*. The former is larger ($M_r \approx 30$ kDa), negatively charged (pI = 5.7), and a weaker reductant ($E°$ = 0.36 V vs NHE), whereas the latter is smaller (M_r = 12.4 kDa), positively charged in neutral solution (pI = 9.0), and a stronger reductant ($E°$ = 0.26 V vs NHE). Nonetheless, the rate constant for reactions (k_f) with cupriplastocyanin, which is negatively charged in neutral solution (pI = 3.8), is greater in the case of cytochrome *f* than in the case of cytochrome *c* over a wide range of ionic strengths. This difference in reactivity is explained in the next section.

Unimolecular Reactions between Cytochrome f and Plastocyanin. At low ionic strength, the two proteins form a noncovalent complexes. Since 5-deazariboflavin semiquinone reacts faster with ferricytochrome *f* than with cupriplastocyanin, the reaction in eq 9 is followed by intracomplex electron-transfer reactions in eq 10. The protein complex is

$$\text{cyt(3+)/pc(2+)} + \text{DRFH} \xrightarrow{k_9} \text{cyt(2+)/pc(2+)} + \text{DRF} + \text{H}^+ \quad (9)$$

$$\text{cyt(2+)/pc(2+)} \underset{k_{-10}}{\overset{k_{10}}{\rightleftharpoons}} \text{cyt(3+)/pc(1+)} \quad (10)$$

present in pseudo-first-order excess over the semiquinone. A typical kinetic trace in Figure 3 shows the initial reduction, k_9, but seemingly does not show the subsequent unimolecular oxidation, k_{10}. Nonetheless, this reaction does occur. The UV–vis spectrum of ferrocytochrome *f* at low ionic strength clearly shows oxidation of this protein by cupriplastocyanin. The spectrophotometric experiments were done with amounts and concentrations of the two proteins that are identical to those used in flash photolysis experiments. When the two proteins are equimolar, only ca. 50% of ferrocytochrome *f* is oxidized. This fact is consistent with the equilibrium in eq 10 for which $k_{10} \approx k_{-10}$ and with the fact that the redox potentials of the two proteins are equal. Stopped-flow spectrophotometric experiments with ferrocytochrome *f* and cupriplastocyanin at low ionic strength confirmed that the reaction in eq 10 occurs during the mixing time of ca. 2.0 ms. Since the rate constants k_{10} and k_{-10} are greater than ca. 500 s^{-1}, we returned to laser flash photolysis.

The kinetic trace in Figure 3 was fully explained in terms of the mechanism in eq 9 and 10 and the rate law in eq 11 and 12. The symbols mean the following: k_9 and k'_9 are the

$$k'_9 = k_9[\text{cyt(3+)/pc(2+)}]_0 \quad (11)$$

$$[\text{cyt(2+)/pc(2+)}] = \frac{1}{2}[\text{DRFH}]_0\left\{1 - \frac{2(k'_9 - k_{10})}{k'_9 - 2k_{10}}\exp(-k'_9 t) + \frac{k'_9}{k'_9 - 2k_{10}}\exp(-k_{10}t)\right\} \quad (12)$$

second-order and the pseudo-first-order rate constants for the reaction in eq 9; brackets indicate concentration; subscript 0 indicates the initial concentration. A nonlinear least-squares fitting of the experimental results to eq 12 (the solid line in Figure 3) yielded $k_{10} = k_{-10} = 2800 \pm 300$ s^{-1}. The other two parameters are $[\text{DRFH}]_0 = 0.60$ μM, consistent with pseudo first order, and $k_9 = 3 \times 10^8$ M^{-1} s^{-1}, comparable to the values in Table I. The rate constants k_{10} and k_{-10} are independent of the protein concentration (9.0 and 20.0 μM), as expected of intracomplex reactions. The plateau in Figure 3 does not mean the absence of the intracomplex reaction; it is a consequence of reversibility.

Plastocyanin in unimolecular (intracomplex) reactions reacts faster with its physiological partner, cytochrome *f* (2800 ± 300 s^{-1}), than with cytochrome *c* (1300 ± 200 s^{-1}) (Peerey & Kostić, 1989; Peerey et al., 1991) even though the driving force with the former is virtually nil and with the latter is ca. 0.10 eV. This difference in the rate constants and the similar difference in the bimolecular reactions probably are attributable to the same causes. The heme edge seems to be more exposed in cytochrome *f* (34%) than in cytochrome *c* (20%) (Morand et al., 1989); these semiquantitative percentages are adequate for the following analysis. The greater electronic coupling with plastocyanin may be decisive even though, because of the different sizes of the two protein molecules, the exposed heme edge probably constitutes a somewhat smaller fraction of the total surface in cytochrome *f* (0.4%) than in cytochrome *c* (0.6%) (Koppenol & Margoliash, 1982). The smaller fraction would be a disadvantage if the proteins collided randomly with each other. However, the reaction rate is increased by electrostatic attraction, and collisions can be made more efficient by preorientation of the proteins and by surface diffusion in the complex (Pettigrew & Moore, 1987; Zhou & Kostić, 1992).

Electrostatic Interactions. Our value of the isoelectric point (5.7) for cytochrome *f* from turnip agrees with the values of 5.5 for that from charloc (Gray, 1978) and of 5.2 for that from *Brassica komatsuna* (Takabe et al., 1984). Amino acid sequences also show a negative net charge for all of these cytochromes *f*, which are more than 80% homologous with one

Table II: Fitting to an Electrostatic Model[a] of the Dependence of the Bimolecular Rate Constant on the Ionic Strength

cyto-chrome	redox partner	ρ (Å)	V_{ii} (kcal/mol)[b]	Z_1	Z_2	k_∞ (M^{-1} s^{-1})[c]
cyt *f*	$FMNH^{2-}$	4.0	−2.8	2.5	−1.9	4.0×10^7
	LFH	4.0	−1.6			1.2×10^8
	pc	7.0	−20	5.0	−4.0	1.2×10^6
cyt *c*	$FMNH^{2-}$[d]	4.0	−3.0	3.1	−1.9	1.3×10^7
	pc[e]	7.0	−8.3	2.4	−4.0	3.9×10^5

[a] Watkins (1984). [b] Standard deviation 10–15%. [c] Standard deviation 10–25%. [d] Meyer et al. (1984). [e] Kinetic data are from Rush et al. (1988).

another (Hauska et al., 1988).

The reaction of cytochrome *f* with riboflavin semiquinone does not depend on ionic strength. The reactions with other redox partners were analyzed, and results are given in Table II.

Monopole–dipole and dipole–dipole interactions probably are unimportant for the reactions of flavin semiquinones because the dipole moment of FMN semiquinone is only 17 D (Meyer et al., 1984) and that of lumiflavin semiquinone must be even smaller. However, neglect of these interactions limits the accuracy of the theoretical treatment in the case of plastocyanin.

The variables are k_∞ and the product Z_1Z_2. The values of k_∞ in Table II are consistent with the rate constants in Table I. The effective charges Z_2 at pH 7.0 in Table II for FMN semiquinone (Meyer et al., 1984) and for plastocyanin (Beoku-Betts et al., 1983; Roberts et al., 1991) are known more or less accurately. The calculated effective charges Z_1 of cytochrome *f* for the reaction with FMN semiquinone (+2.5) and with plastocyanin (+5.0) are realistic, and the latter one agrees with the finding of at least two positively charged residues in the interaction domain of cytochrome *f* (Morand et al., 1989).

Chemical properties of cytochrome *f* and cytochrome *c* can be compared on the basis of Table II. The two cytochromes do not significantly differ in their reactions with FMN semiquinone ($-2.8 \approx -3.0$ and $2.5 \approx 3.1$), but do differ in the reactions with plastocyanin ($-20 < -8.3$ and $5.0 > 2.4$). The small molecule probably fits better than the macromolecule into the reactive domain in either cytochrome. The calculated values of Z_1 depend on the dielectric constant, whose choice is somewhat arbitrary although consistent with the choice made in previous studies. An effective dielectric constant depends on the accessibility of the interface to water molecules, which in turn depends on the complementarity of the cytochrome and plastocyanin. If this complementarity is lesser with cytochrome *c* than with cytochrome *f*, a likely proposition, the dielectric constant may be higher with the former than with the latter. Indeed, when the dielectric constant is changed from 10 to 15, the charge Z_1 of cytochrome *c* in Table II changes from 2.4 to the more realistic value of 3.6.

Any fitting of experimental results to an empirical model with several parameters is more or less arbitrary, but the large difference between the V_{ii} values calculated with identical parameters for cytochrome *f* and cytochrome *c* probably reflects a real difference in their electrostatic interactions with plastocyanin. The physiological partner, cytochrome *f*, seems to interact more specifically because of greater local positive charge or because of a better fit, or for both reasons.

Conclusions

Electrostatic interactions of cytochromes with small molecules and with other proteins seem to be governed by local charges of the interaction domains, not by overall charges. This study corraborates previous findings (Tollin et al., 1984; Takenaka & Takabe, 1984; Morand et al., 1989). Although the driving force for electron transfer between cytochrome *f* and plastocyanin is virtually nil and both proteins bear negative net charges, protein–protein electrostatic interactions and heme–copper coupling are favorable for the reaction. Cytochrome *f* and cytochrome *c* are alike in several important respects, but they differ in their capacity for electrostatic interactions with partners such as plastocyanin and in the rates of electron-transfer reactions within binary complexes that they form with those partners. This and other differences must be taken into account if the latter is used as a model or as a replacement for the former.

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