Electron Transfer between Flavodoxin Semiquinone and *c*-Type Cytochromes: Correlations between Electrostatically Corrected Rate Constants, Redox Potentials, and Surface Topologies†

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ABSTRACT: We have measured the ionic strength dependence of the rate constants for electron transfer from the semiquinone of *Clostridium pasteurianum* flavodoxin to 12 *c*-type cytochromes and several inorganic oxidants using stopped-flow methodology. The experimental data were fit quite well by an electrostatic model that represents the interaction domains as parallel disks with a point charge equal to the charge within this region of the protein. The analysis provides an evaluation of the electrostatic interaction energy and the rate constant at infinite ionic strength (k_∞). The electrostatic charge on the oxidant within the interaction site can be obtained from the electrostatic energy, and for most of those reactants for which structures are available, the results are in good agreement with expectation. The k_∞ values were found to correlate with redox potential differences, as expected from the theory of adiabatic (or nonadiabatic) outer-sphere electron-transfer reactions. Deviations from the theoretical curves are interpreted in terms of the influence of surface topology on reaction rate constants. In general, we find that electrostatic effects, steric influences, and redox potential all exert a much larger effect on reaction rate constants for the flavodoxin–cytochrome system than has been previously observed for free flavin–cytochrome interactions. The implications of this for determining biological specificity are discussed.

In a previous publication from this laboratory (Meyer et al., 1983), we demonstrated that the rate constants for reduction of a wide variety of heme-, iron-sulfur-, copper-, and flavin-containing proteins by reduced free flavins could be systematized using semiempirical free energy equations (Marcus, 1968; Rehm & Weller, 1970) which relate kinetic rate constants and thermodynamic redox potential differences. This allowed the establishment of relative intrinsic reactivities that could be qualitatively correlated with the extent of solvent exposure of the redox centers within a particular structural class of electron-transfer protein. We have recently (Meyer et al., 1984) extended this to a systematic investigation of reactions of riboflavin and FMN semiquinones with *c*-type cytochromes. This has demonstrated that steric and electrostatic interactions between small molecules and redox proteins can also be quantitated within this same framework. In the present study, we have attempted to apply this approach to redox reactions involving two proteins by replacing free flavin semiquinone as the reductant with the semiquinone form of *Clostridium pasteurianum* flavodoxin (which has FMN as a prosthetic group).

Such an extension to protein–protein interactions is complicated by two factors: (1) electrostatic interactions between two highly charged redox proteins can have much larger effects on electron-transfer rate constants [cf., for example, Simondsen et al. (1982) and Simondsen & Tollin (1983)] than is the case for small molecules such as FMN; (2) detailed three-dimensional structures are not available for all of the proteins which we have studied. As we will demonstrate below, electrostatically corrected rate constants obtained for 12 *c*-type cytochromes can generally be correlated with redox potential differences according to the above-mentioned free energy equations, as well as the Hopfield equation for vibronically assisted tunneling (Hopfield, 1974). The results are similar to what was found when FMN semiquinone was used as the reductant (Meyer et al., 1984). Reactivity differences, however, are much larger for the flavodoxin reactions than was the case with FMN. In general, we find that redox potentials, electrostatic effects, and steric interactions all exert a much greater influence on the rate constants of the flavodoxin–cytochrome reactions than for the free flavin–cytochrome system. This provides a quantitative basis for understanding the biological specificity of electron transfer between redox proteins.

Materials and Methods

Rate constants for electron transfer between *Clostridium pasteurianum* flavodoxin semiquinone and a variety of oxidized homologous cytochromes were measured by using stopped-flow spectrophotometry as previously described (Jung & Tollin, 1981; Simondson et al., 1982; Simondsen & Tollin, 1983). The sources of the various cytochromes used were as specified in an earlier publication (Meyer et al., 1983). Ionic strength was varied by addition of appropriate amounts of NaCl.

As was found for horse cytochrome *c* (Simondsen et al., 1982), the reactions of flavodoxin semiquinone with the other cytochromes used here were biphasic. All of the kinetic data were corrected for this biphasicity as in the previous work, and thus the rate constants reported below refer only to the fast phase of the reaction, which typically represents 70–80% of the total.

Over the range of ionic strengths (10–500 mM) and cytochrome concentrations (typically from 5 to 30 μM) used, all of the reactions studied gave linear plots of k_{obsd} vs. cytochrome concentration (i.e., no saturation effects were observed). The apparent second-order rate constants were calculated from the slopes of these plots. The estimated error in these values is ±15%. Although a nonlinear k_{obsd} vs. concentration plot would be expected at low ionic strengths for both *Candida* and tuna cytochromes *c* (Simondsen et al., 1982), it should be more difficult to observe with these proteins than with horse cytochrome *c*. This is because the smaller apparent second-order rate constants at low ionic strengths for these proteins (cf. Figure 1) would require that measurements be made at a higher cytochrome concentration range. Because of limitation of materials, and because this phenomenon was not being

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Table I: Electrostatic Analysis of Oxidant–Flavodoxin Kinetics

cytochrome source or reactant	$E_{m,7}$ (mV)	net charge	V_{ii}[a] (kcal/mol)	ρ (Å)	Z_1[b]	$k_\infty \times 10^{-4}$ (M^{-1} s^{-1})
$Fe(CN)_6^{3-}$	430	3–	+2.9	4.50	−2.6 (−3)	217
Rsp. tenue 3761	400	4+	−5.6	7.25	+1.6	214
Euglena sp.	370	8–	+7.0	7.25	−2.0	314
$Co(phen)_3^{3+}$	370	3+	−6.1	7.00	+3.0 (+3)	0.8
Rm. vanniellii	354	2+	−10.3	7.25	+3.0	17.6
Rps. capsulata	350	1+	−11.8	7.25	+3.4	33.6
Rsp. rubrum	324	0	−13.3	7.25	+3.9 (+4)	20.8
Ps. aeruginosa	270	2–	+3.2	7.25	−0.9 (+1)	11.5
Candida krusei	260	5+	+16.2	7.25	+4.7	1.5
tuna	260	7+	−16.0	7.25	+4.7 (+4)	0.9
horse	260	7+	−23.5	7.25	+6.8 (+4)	0.2
P. denitrificans	250	7–	−12.3	7.25	+3.6 (+4)	5.9
Chl. thiosulfatophilum	150	6+	−9.5	7.25	+2.8 (+2)	0.3
$Fe(EDTA)^{1-}$	120	1–	+1.1	5.00	−1.0 (−1)	0.003
E. halophila	58	10–	+6.5	7.25	−1.9	0.2

[a] V_{ii} and k_∞ were obtained by fitting the experimental data with eq 1, where $X(I) = (1 + \kappa\rho)^{-1} \exp(-\kappa\rho)$ with ρ defined in the text and taken as 7.25 Å for the cytochromes (see text) and $\kappa = 0.3295I^2$. [b] Z_1 was calculated from Z_1Z_2 obtained from eq 2 by using the fitted values of V_{ii}, the indicated value of ρ, $r_{12} = 3.5$ Å, $D_e = 10$, $\alpha = 128.47$, and $Z_2 = -4$. Values in parentheses are those expected from the structure (see text).

investigated in the present series of experiments, we did not make an effort to look for such effects.

All other procedures and materials were as described earlier (Simondsen et al., 1982).

Results and Discussion

The ionic strength dependencies of the calculated second-order rate constants for the electron-transfer reactions of flavodoxin semiquinone with various oxidized cytochromes are plotted in Figure 1. As described previously (Simondsen et al., 1982), flavodoxin behaves as a negatively charged molecule in redox reactions with horse cytochrome *c*, ferricyanide, and ferric EDTA, and Matthew et al. (1983) have shown that a large negative electrostatic potential exists on the surface of the protein in the region of the FMN prosthetic group. For all of the cytochromes described in Figure 1, the rate constant for reduction by flavodoxin semiquinone showed a much larger ionic strength dependence than was observed with FMN semiquinone (Meyer et al., 1984), although the direction of the effect was the same. For example, over the same ionic strength range (58–500 mM), the rate constant for the reaction of tuna cytochrome *c* decreased by a factor of 3 with FMN and by a factor of 100 with flavodoxin. This is consistent with the larger electrostatic charge on flavodoxin. With the exception of the *Euglena*, *Pseudomonas*, and *Ectothiorhodospira* proteins, all of the other cytochromes gave ionic strength dependencies that are indicative of an attractive (plus–minus) electrostatic interaction. Of particular interest among this latter group are *Rhodospirillum rubrum* c_2 and *Paracoccus denitrificans*[1] c_2, both of which show relatively strong ionic strength dependencies consistent with positively charged interaction domains, despite the fact that the former protein has a net zero charge and the latter has a net charge of 7–. A similar situation was found previously for the FMN semiquinone reaction with these cytochromes (Meyer et al., 1984). This result suggests that the interaction between flavodoxin and these cytochromes is being dominated by a local electrostatic potential near the site of electron transfer to the heme (Simondsen et al., 1982; Matthew et al., 1983). We will return to this below.

The solid lines in Figure 1 represent theoretical curves obtained by using eq 1 which describes the interaction domains of *c*-type cytochromes and flavodoxin as parallel disks (of

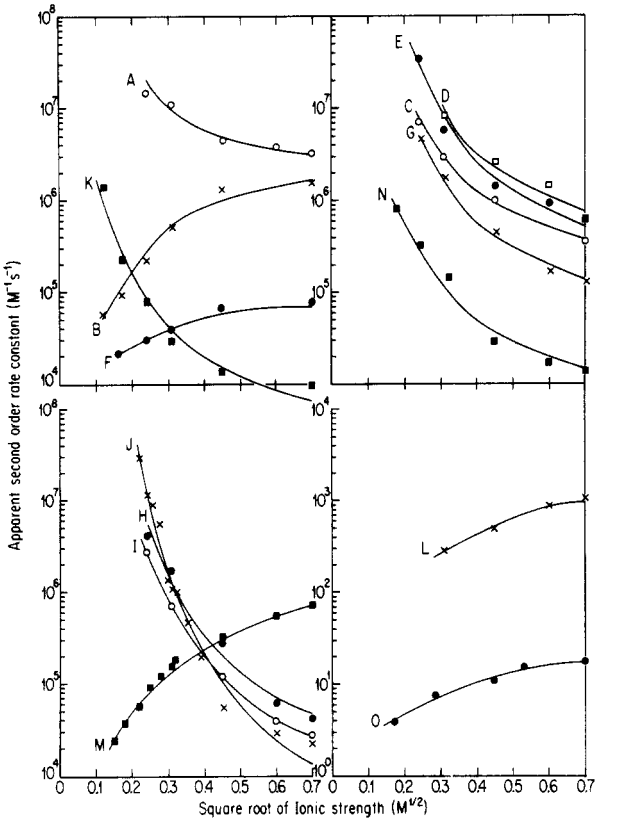

FIGURE 1: Ionic strength dependence of apparent second-order rate constants for electron transfer from flavodoxin semiquinone to oxidized *c*-type cytochromes and inorganic oxidants. Solid lines are theoretical fits to data points (see text for details). (A) *Rhodospirillum tenue* 3761 *c*-553; (B) *Euglena sp. c*-552; (C) *Rhodomicrobium vannielli* c_2; (D) *Rhodopseudomonas capsulata* c_2; (E) *Rhodospirillum rubrum* c_2; (F) *Pseudomonas aeruginosa c*-551; (G) *Paracoccus denitrificans* c_2; (H) *Candida krusei c*; (I) tuna *c*; (J) horse *c*; (K) *Chlorobium thiosulfatophilum c*-555; (L) *Ectothiorhodospira halophila c*-551; (M) ferricyanide; (N) cobalt phenanthroline; (O) ferric EDTA.

radius ρ), with discrete point charges (Z_1, Z_2), set equal to the total charge within this region, providing an electrostatic interaction energy, V_{ii} (Watkins, 1984). As is evident, the fits to the data are quite good. Table I summarizes the results of this analysis of the electrostatic interaction between the various cytochromes and inorganic oxidants and flavodoxin.

The equation used in the analysis is as follows:

$$\ln k(I) = \ln k_\infty - V_{ii}X(I) \qquad (1)$$

where $k(I)$ is the observed rate constant at any ionic strength

[1] This protein, previously referred to by the provisional designation cytochrome *c*-550, has recently been classified as a cytochrome c_2 on the basis of sequence homology and three-dimensional structure (Meyer & Kamen, 1982).

I, k_∞ is the rate constant at infinite ionic strength, V_{ii} is as defined above, and $X(I)$ is an ionic strength dependent term (see footnote *a* to Table I). This equation was derived by using a many-center multipole expansion of the charge distribution (J. A. Watkins and M. A. Cusanovich, unpublished results). Equation 1 does not include ion–dipole or dipole–dipole interactions. As we have shown previously (Meyer et al., 1984), however, this equation can adequately fit rate constant vs. ionic strength data for the interaction of FMN semiquinone and *c*-type cytochromes. Although other conceptual approaches [e.g., see Wherland & Gray (1976)] could be used, they yield generally similar results with regard to k_∞, which is the primary parameter to be determined (see below). Since we have seen that net protein charge and active site charge do not necessarily have the same sign, the approach used here is in fact more useful than the Wherland–Gray treatment which is based upon the use of the net protein charge.

For the purposes of the present analysis, we were interested in obtaining two parameters: the product of charges (Z_1Z_2; see eq 2) and the rate constant at infinite ionic strength. The value of ρ was chosen as described below. To obtain these parameters, we fit the experimental data given in Figure 1 (solid lines are the calculated fits) using a steepest descent approach and eq 1, yielding values for V_{ii} and k_∞. V_{ii} is related to the product of charges (Z_1Z_2) by eq 2 where α is a constant

$$V_{ii} = \alpha\rho^{-2}D_e^{-1}Z_1Z_2r_{12} \qquad (2)$$

(128.47), D_e is the effective dielectric constant within the interaction domain and r_{12} is the distance between the electron-transfer groups within the reactive complex (taken as the van der Waals radius 3.5 Å). Table I presents the midpoint potential and net charge (from the amino acid sequence or composition) for each oxidant studied for reference purposes and the values of V_{ii} and k_∞ obtained from the fits to eq 1. The values of ρ (given in Table I) used for the inorganic compounds were obtained from their crystal structures. A constant value of 7.25 Å for the radius of the cytochrome–flavodoxin interaction domain was used. Although ρ could have been varied and somewhat better fits to the data obtained, the value of 7.25 Å, which was derived from a structural analysis of a putative cytochrome *c*–flavodoxin complex (Simondsen et al., 1982), was used as a compromise so as to reduce the number of variables to be fitted.

Z_1Z_2 was calculated from eq 2 by using the fitted value of V_{ii}, as given in Table I, and a value of 10 for the D_e of the interaction domain of the cytochromes. This value for D_e was chosen since it appears to be a reasonable estimate for both the horse cytochrome *c*–flavodoxin and horse cytochrome *c*–cytochrome b_5 interactions, based on structural models (Matthew et al., 1983; Simondsen et al., 1982; Salemme, 1976); i.e., it reflects the suggestion that water is excluded from the interaction domain during electron transfer, and it provides generally good agreement between calculated and expected interaction domain charges (Table I). For the inorganic reactants, the values used for D_e were 80, 65, and 16 for ferricyanide, ferric ethylenediaminetetraacetate (ferric EDTA), and cobalt phenanthroline, respectively. Ferricyanide is hydrophilic and should not displace water when interacting with flavodoxin. Hence, the dielectric constant of water was used for this reactant. For ferric EDTA and cobalt phenanthroline, the D_e values were chosen so that the calculated and expected values for Z_1 were identical. Dielectric constants of 65 and 16 are not unreasonable for these systems in view of the structural properties of ferric EDTA (hydrophilic, but more hydrophobic than ferricyanide) and cobalt phenanthroline (hydrophobic at the site of interaction).

To calculate Z_1, we assumed a value of 4– for the interaction domain charge of flavodoxin (Z_2) (Simondsen et al., 1982). The value in parentheses in Table I following the value of Z_1 is that expected for the particular reactant on the basis of the charge distribution around the exposed heme edge, by analogy to the flavodoxin–cytochrome *c* complex (Simondsen et al., 1982), or from the known charge for the inorganic oxidants. In general, the values of V_{ii} that best fit the data had a range ±20%. Thus, the error limits on Z_1 are also ±20%. However, it is important to point out that over the range of acceptable V_{ii} values, k_∞ varied by only ±10%. It is interesting to note that the Z_1 values obtained previously with FMN (Meyer et al., 1984) are generally similar to those shown in Table I for flavodoxin, even though the V_{ii} values for FMN are much smaller. This is consistent with these two reductants interacting at the same electron-transfer site on the cytochromes, i.e., in the vicinity of the heme edge.

Examination of the entries in Table I for some of the cytochromes for which the three-dimensional structure is known indicates generally good agreement between the expected and calculated interaction domain charge (Z_1). Specifically, the tuna, *P. denitrificans*, *Chl. thiosulfatophilum*, and *Rsp. rubrum* cytochromes all give reasonable estimates of this parameter. *Ps. aeruginosa* cytochrome *c*-551 yields a calculated interaction domain charge which is opposite in sign from that expected from the structure about the heme edge (0.9– vs. 1+). This apparent anomaly warrants further investigation. The calculated charge for horse cytochrome *c* is appreciably larger than expected from its structure. The reason for this is unclear but may be related to our neglect of dipole effects. It is striking that *P. denitrificans* cytochrome c_2, even though it has a net charge of 7–, still behaves as a cation in its interaction with flavodoxin. Similar effects were obtained previously with FMN (Meyer et al., 1984).

By use of the theoretical fits to the rate constant vs. ionic strength data, it is possible to obtain an extrapolated value for the rate constant at infinite ionic strength (k_∞) (Table I). Presumably, such a value represents an intrinsic rate constant under conditions in which all of the electrostatic interactions are eliminated by screening effects of the salt ions. We have previously shown (Meyer et al., 1984) that it is possible to correlate k_∞ values for the FMN semiquinone reduction of cytochromes with redox potential differences and surface topologies (steric effects) using semiempirical free energy equations that relate log k_∞ and the redox potential difference, $\Delta E_{m,7}$. Such a plot for the data of Table I is shown in Figure 2. As was the case with FMN, it is apparent that a correlation between these two parameters indeed exists, as expected on the basis of theoretical treatments of outer-sphere reactions (cf. Meyer et al., 1983). In Figure 2 is shown a plot of the Marcus exponential equation (Marcus, 1968). A similar theoretical curve can also be obtained by using the Hopfield treatment of vibronically assisted tunneling (Hopfield, 1974). The values of the parameters used in constructing these theoretical curves are indicated in the figure legend of Figure 2. As is evident, the Hopfield equation gives approximately the same fit to the kinetic data as does the Marcus equation. We have no experimental evidence to discriminate between a nonadiabatic or tunneling (which requires only a weak electronic interaction between the redox centers) and an adiabatic (involving a strong interaction) mechanism for the electron-transfer process. Both viewpoints would yield identical conclusions regarding the influence of redox potential, electrostatics, and steric effects on reactivity. Since structural

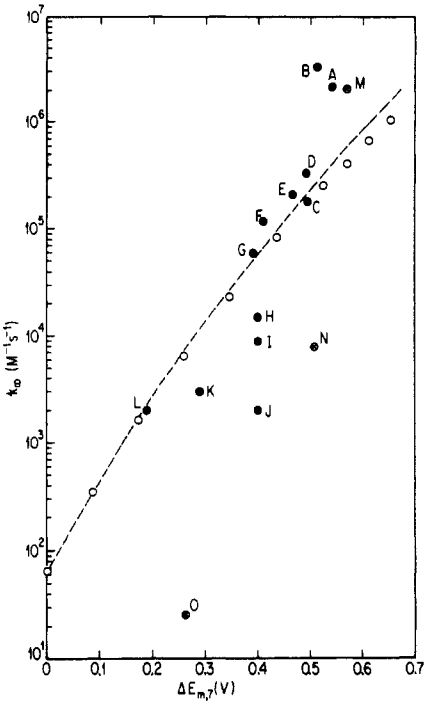

FIGURE 2: Semilog plot of second-order rate constants extrapolated to infinite ionic strength (k_∞) vs. difference in redox potential between reactants ($\Delta E_{m,7}$) for oxidation of flavodoxin semiquinone. Identification of reactants as in Figure 1. (O) Plot of Marcus exponential equation with $\nu_{ET} = 5 \times 10^9$ M^{-1} s^{-1} and $\Delta G^*(0) = 10.7$ kcal/mol. (---) Plot of Hopfield tunneling equation with $R = 15.9$ Å, $d = 3.5$ Å, and $\Delta = 1.75$ eV (R = protein radius; d = distance between reactants; Δ = vibronic coupling parameter).

modeling of an intermediate complex between flavodoxin and cytochrome *c* (Simondsen et al., 1982) supports the idea that the heme and the flavin are relatively close together (6–8 Å), and both prosthetic groups are partially exposed at the protein surface, a strong interaction mechanism is certainly feasible.

Although not all of the cytochromes lie on the theoretical lines, the general trend of the data is adequately represented. We will return to this point below. It is important to note that the steepness of the log k_∞ vs. redox potential plot for flavodoxin is much greater than was found for the FMN reactions (e.g., for a $\Delta E_{m,7}$ range of 0.18–0.5 V, approximately a factor of 5 was observed in the rate constants for the latter reactions as compared to 100 for the flavodoxin reactions). This is reflected in the much larger values for both of the Marcus equation parameters, ν_{ET} (5×10^9 M^{-1} s^{-1}) and $\Delta G^*(0)$ (10.7 kcal/mol) (for FMN, $\nu_{ET} = 2.7 \times 10^7$ M^{-1} s^{-1} and $\Delta G^*(0) = 2.75$ kcal/mol; Meyer et al., 1984). The larger ν_{ET} value (which corresponds to the limiting rate constant as ΔE approaches infinity) would suggest that the intrinsic reactivity of the flavin moiety in flavodoxin is greater than that of free FMN, i.e., that the protein environment acts to increase the ability of the flavin to transfer an electron to an acceptor such as a *c*-type cytochrome. The larger $\Delta G^*(0)$ (which represents the energy required to reach the transition state when $\Delta E = 0$) implies that more structural rearrangement (including solvent molecules) is required to attain the transition state in the flavodoxin system than with free FMN. This is reasonable in view of the greater structural complexity of the flavodoxin molecule. The relative effect of structural variations in the donor (see below for discussion) can also be assessed by, for example, the ratio of the *Euglena* *c*-552 rate constant to that of horse cytochrome *c*, which is 4 for FMN and 1600 for flavodoxin. In general, we can conclude that the effects of both redox potential and structure are considerably greater for the protein–protein system than for the protein–small molecule system (this was also the case for the electrostatic effects as discussed above). This is to be expected if these parameters are utilized physiologically to establish biochemical specificity.

Some interesting parallels, as well as differences, are found between the log k_∞ vs. ΔE plots observed here and with FMN. As in the FMN system, the mitochondrial cytochromes *c* (*Candida*, tuna, and horse) are all less reactive with flavodoxin as compared with the other cytochromes at the same redox potential difference (i.e., *Pseudomonas* *c*-551 and *Paracoccus* c_2); i.e., they fall well below the theoretical curve (cf. Figure 2). Even more striking is that among these, the same relative ordering is found as with FMN (i.e., *Candida* > tuna > horse). We have previously (Meyer et al., 1984) correlated these differences in intrinsic reactivity with the degree of steric hindrance around the heme edge in the mitochondrial cytochromes and *Pseudomonas* *c*-551, *Paracoccus* c_2, and *R. rubrum* c_2, based on the three-dimensional structures. We presume that this same factor is operative in the present situation, except that the resultant decrease in intrinsic reactivity is considerably greater (e.g., a factor of 30 below the theoretical curve for flavodoxin–horse cytochrome *c* vs. a factor of 1.5 for FMN). As was also true for FMN, *Euglena* *c*-552 falls above the theoretical curve (again by a significantly larger factor, 12 vs. 2). Although the three-dimensional structure of this cytochrome is unknown, this result would predict that the heme is more exposed or less sterically restricted than for those cytochromes which lie on or below the theoretical curve. This is subject to experimental test. The larger reactivity effects found with flavodoxin as compared to FMN can be thought of as being a consequence of the larger interaction domains for the protein–protein system and the restrictions on the orientational freedom of the flavin ring system, which result from its being bound within a protein matrix. Both of these factors would lead to more constraints on the attainment of a productive alignment of the heme and flavin prosthetic groups during a collisional encounter.

Three cytochromes appear to behave differently with flavodoxin than with FMN. *R. tenue* *c*-553, which lies above the flavodoxin–cytochrome curve (a factor of 5.5), was found to be on the FMN–cytochrome curve. *Pseudomonas* *c*-551, which is only slightly above the curve with flavodoxin, was appreciably above the curve with FMN (a factor of 1.5). *Ectothiorhodospira* *c*-551, which lies on the curve with flavodoxin, also was above the curve with FMN (a factor of 2). Of these, the *R. tenue* *c*-553 difference is probably within the experimental error of the FMN rate constant values (i.e., on the basis of the other differences noted above, its rate constant with FMN should only be slightly above the theoretical line). In the other two cases, the proteins are somewhat less reactive with flavodoxin (relative to the other cytochromes) than they are with FMN. This is again most probably a consequence of the factors mentioned above; i.e., within the interaction domain, flavodoxin samples a larger region of the cytochrome surface than does FMN, and thus, the possibility of unfavorable contacts is increased; the heme–flavin distance and degree of orbital overlap are determined by the precise nature of the protein–protein interaction which is more constrained than is the protein-free FMN interaction.

We have also studied several simple inorganic oxidants so as to provide a comparison with the more complex cytochromes. The k_∞ values for these reactants are also given in Table I and are plotted in Figure 2. It is noteworthy that the order of reactivity of the inorganic oxidants with flavodoxin

semiquinone (based on the absolute values of k_∞) is the same as was found for their reaction with horse cytochrome *c* by Wherland & Gray (1976), i.e., ferricyanide > cobalt phenanthroline > ferric EDTA. In terms of the free energy correlations, we can carry this analysis a step further and argue that ferric EDTA and cobalt phenanthroline appear to have approximately equal intrinsic reactivities (i.e., a curve parallel to the one for the cytochromes can be drawn through the k_∞ values for these two oxidants in Figure 2), whereas ferricyanide has a considerably larger intrinsic reactivity (about a factor of 100). Wherland & Gray (1976) argued that the relative reactivity of these reagents with horse cytochrome *c* was a consequence of steric and structural factors involving the hydrophobic heme edge region and the nature of the substrate. Thus, ferric EDTA is highly asymmetric, having both hydrophilic and hydrophobic surfaces, with π orbitals, which could overlap with the heme orbitals, concentrated mainly in the *hydrophilic* region and thus unfavorably located. Although cobalt phenanthroline is symmetric, it is highly hydrophobic and has considerable π character, but the aromatic ligands are quite rigid, requiring precise alignment to achieve optimal orbital overlap. Such an aligment would not necessarily be the same as that for the most favorable nonpolar interactions. On the other hand, ferricyanide has monodentate ligands with cylindrically symmetrical π orbitals and as a whole is spherically symmetric, so that the problems of orbital overlap and alignment are the least critical for this compound than for any of the other reactants. It would seem that these considerations could apply equally well to flavodoxin, in which the exposed π orbital of the nonpolar dimethylbenzene ring of the FMN cofactor is the most likely site for electron transfer (Simondsen et al., 1982; Simondsen & Tollin, 1983). Thus, a hydrophobic region not too different from that proposed for cytochrome *c* (Wherland & Gray, 1976) appears to be involved in the redox interaction with flavodoxin.

Conclusions

The results presented here demonstrate that is is possible to obtain information concerning the interactions between two redox proteins during electron transfer by applying a simple electrostatic model and correlations between thermodynamic free energy and electrostatically corrected rate constants to experimental measurements of the ionic strength dependence of reaction rate constants. This approach primarily focuses on the nature of the interactions that occur within the region of intermolecular contact between the two proteins. This is especially valuable in those cases for which three-dimensional structures are available, inasmuch as computer modeling techniques can be applied to construct hypothetical complexes which are then amenable to further experimental testing [e.g., see Salemme (1976) and Simondsen et al. (1982)]. We have shown that relatively small differences in the surface topology of proteins can lead to large differences in reactivity. Thus, for example, the relative intrinsic reactivities (i.e., k_∞ values) toward flavodoxin semiquinone of horse *c*, tuna *c*, *Candida c*, and *Pseudomonas c*-551, all structurally homologous cytochromes that have approximately the same redox potential, are 1:4.5:7.5:58. Reactivity differences can be even greater when electrostatic and redox potential effects are included. Thus, at 50 mM ionic strength, the relative reactivities of horse *c* and *Pseudomonas c*-551 are 1000:1, and at 100 mM ionic strength the ratio for *Ectothiorhodospira c*-551 and *R. rubrum* c_2 is $1:2 \times 10^4$. Such large differential reactivities could easily account for biological specificity. It should be noted, however, that we have thus far examined only a rather narrow range of redox protein types, and therefore whether or not this can be completely generalized remains to be established. To this end, we are currently studying a number of other systems, including iron-sulfur proteins, copper proteins, other flavodoxins, and other structural classes of cytochromes.

Registry No. Ferric EDTA, 17099-81-9; cobalt phenanthroline, 18581-79-8; ferricyanide, 13408-62-3; cytochrome *c*-553, 12624-01-0; cytochrome *c*-552, 9048-78-6; cytochrome c_2, 9035-43-2; cytochrome *c*-551, 9048-77-5; cytochrome *c*, 9007-43-6; cytochrome *c*-555, 9048-79-7.

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