The Structure of an Electron Transfer Complex Containing a Cytochrome c and a Peroxidase*

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§ The abbreviations used are: CCP, cytochrome c peroxidase; Mes, 4-morpholineethanesulfonic acid.

Efficient biological electron transfer may require a fluid association of redox partners. Two noncrystallographic methods (a new molecular docking program and 1H NMR spectroscopy) have been used to study the electron transfer complex formed between the cytochrome c peroxidase (CCP) of Paracoccus denitrificans and cytochromes c. For the natural redox partner, cytochrome c<sub>550</sub>, the results are consistent with a complex in which the heme of a single cytochrome lies above the exposed electron-transfer heme of the peroxidase. In contrast, two molecules of the nonphysiological but kinetically competent horse cytochrome bind between the two hemes of the peroxidase. These dramatically different patterns are consistent with a redox active surface on the peroxidase that may accommodate more than one cytochrome and allow lateral mobility.

Our knowledge of the factors influencing electron transfer between biological redox partners remains sketchy. Important insights have come from the co-crystallization of redox partners (1, 2), but the nagging doubt remains that such crystalline complexes at best represent only one of a number of productive orientations or, at worst, a nonproductive association. Co-crystallography of biological molecules has at its heart the assumption that a single true native complex exists in a deep energy minimum that is selected for under crystallization conditions. However, a picture is now emerging of a biological strategy for electron transfer that involves rate enhancement by a “reduction of dimensions” to form an encounter complex that is fluid enough to allow lateral mobility and search for a productive electron transfer orientation. According to this scenario, although global electrostatic forces may enhance the initial collision, specific complementary interactions that are too strong may compromise the fluidity required to allow lateral search and also may hinder the dissociation of the oxidized product (3, 4).

The best studied biological electron transfer complex is that between yeast CCP and cytochrome c. Reaction with hydrogen peroxide yields a peroxidase with two oxidizing equivalents in the form of a Trp-191 radical (X+, in Fig. 1A) and a ferryl oxene heme (5). There has been a long running (and still not fully resolved) debate as to whether re-reduction of these sites occurs by a single pathway from a single binding site at which successive cytochromes c bind (6–8) or by separate pathways from distinct sites that simultaneously bind cytochrome c molecules (3, 4, 9–11). The balance of evidence seems to favor the former (which is shown in Fig. 1A), according to which each electron tunnels from the exposed edge of a bound cytochrome c heme along the backbone of Ala-194, Ala-193, and Gly-192 to Trp-191 as deduced from the co-crystallization study of Pelleter and Kraut (1).

The focus of our work has been a CCP from Paracoccus denitrificans. Previous work on this diheme enzyme (12) and its relative from Pseudomonas aeruginosa (13, 14) showed that one of the two hemes (E heme, in Fig. 1B) acts in an electron transferring role, whereas the other (P heme) is the peroxidatic center. Thus the electron-transferring heme functions like Trp-191 in the monoheme yeast enzyme but an important difference is that it is exposed at the molecular surface rather than buried. Re-reduction of the oxidized enzyme requires two electrons delivered from the monoheme cytochrome c<sub>550</sub> (15). Although this cytochrome is present in a monomer:dimer equilibrium in solution, we have shown that it is the monomer that binds to the peroxidase (16). Cytochrome c<sub>550</sub> is a close relative of mitochondrial cytochrome c and has a pronounced charge asymmetry with a positive front surface surrounding an exposed heme edge at which its proposed attachment to a negative surface of the peroxidase occurs (16, 17). The model of Fig. 1B indicates that there are a number of ways in which re-reduction of the peroxidase might be achieved which involve different binding sites for the cytochrome and different electron transfer routes. Here, we use simulation of molecular docking and 1H NMR spectroscopy to evaluate these possibilities.

EXPERIMENTAL PROCEDURES

Protein Structures—The protein coordinates of P. aeruginosa cytochrome c<sub>550</sub> (18), horse cytochrome c (19), and P. denitrificans strain LMD 22.21 cytochrome c<sub>550</sub> (20) were obtained from the Brookhaven Protein Data Bank as files 451pdb, 1hrcpdb, and 155cpdb, respectively. Higher resolution coordinates for the cytochrome c<sub>550</sub> (1cot.pdb) were kindly provided by H. Holden (University of Wisconsin) (21) prior to deposition. The coordinates of P. aeruginosa CCP were kindly provided by V. Fulop (University of Warwick, England) (22) prior to deposition. The 1cot.pdb coordinates for the cytochrome c<sub>550</sub> lack the N-terminal 2 residues and the C-terminal 13 residues. Those missing coordinates were grafted on to the 1cot.pdb file from the strain LMD 22.21. This sequence was mutated in Sybyl (Tripos Associates) to correspond to the cytochrome c<sub>550</sub> from strain LMD 52.44 (21 differences, see Ref. 16). The CCP from P. aeruginosa was mutated in Sybyl to correspond to the protein from P. denitrificans strain 52.44 (61% identity).
Molecular Docking—The molecular interaction simulations were performed with the docking program BIGGER.$^2$ This algorithm performs a complete and systematic search of the rotational space of one protein relative to the other, generating a large number of candidate docking geometries based solely on the complementarity of the molecular surfaces. This initial pre-filter of solutions was required to make the computation of a more complete set of energies of interaction manageable. The 1000 best solutions thus generated were finally evaluated and ranked according to a combination of additional interaction criteria that included electrostatic energy of interaction, relative solvation energy and the relative propensity of adjacent side chains to interact. This program has been shown to successfully predict the structure of a wide range of known protein:protein complexes (such as the homodimer of HIV-2 protease and the association of proteases with peptide protease inhibitors)$^2$.

Pathlength for Electron Transfer—We have implemented a routine for computation of the minimum pathlength between any two atoms of two interacting proteins based on the algorithm of Betts et al. (23) and the tunneling pathway model of Beratan et al. (24). The effective path-length is the sum of the effective distances each defined as a $2 \log$ (decay factor) for a particular segment of the path (covalent bond, hydrogen bond, through-space). In our implementation, the effective distance of the path segment at the intermolecular gap is set to zero to avoid undue influence of slight conformational adjustments at the interface that cannot be accommodated by the rigid body docking method used. We should note however that the size of this gap and its control by subtle movements of amino acid side chains in the complex are likely to be crucial influences on electron transfer rates.

$^1$H NMR Spectra—High resolution $^1$H NMR spectra were recorded in the Fourier transform mode on a Bruker ARX-400 spectrometer (400 MHz). Data were recorded at 299 K, and 2000 transients were acquired from each spectrum. To improve signal-to-noise ratio, an exponential multiplication by 10 Hz line broadening of the free induction decay was applied prior to Fourier transform. All chemical shifts are quoted in parts per million (ppm) from internal 3-trimethylsilyl [2,2,3,3 $^2$H$_4$]-propionate; positive values refer to low field shifts. Protein samples were exchanged several times with 10 mM Mes, pH 6.0, in D$_2$O by centrifugation in a Centricon microconcentrator (Amicon).

**FIG. 1.** Re-reduction of cytochrome c peroxidases. The reaction of hydrogen peroxide with the CCP of yeast (A) and Paracoccus (B) yields a compound 1 with two oxidizing equivalents, one as a ferryl heme and the other as a Trp radical (A) or a ferric heme (B). In principle, re-reduction of the Paracoccus enzyme could proceed via two separate electron transfer routes (1), a single electron transfer route that diverges within the protein (2) or a successive pair of electron transfers at the E heme with the first of the electrons passing to the P heme (3).
The docking results described in Fig. 2 are viewed from the side of the cytochrome c peroxidase looking down at the exposed edge of the electron-transferring heme group. In all three experiments, the 500 most favorable solutions are shown as individual unfilled circles marking the center of mass of the probe cytochrome. The top ten docking solutions are shown as black filled circles. The electron-transferring heme group (E) and the peroxidatic heme group (P) are shown as space-filled and shaded gray. For clarity, only the nearest two hemes of the four heme CCP dimer are shown. The protein itself is not shown and would be contained within the oval perimeter of clustered solutions that marks the dimer interface. Because the CCP is a symmetrical dimer, related solutions will be found at both “sides,” and for simplicity, these have been combined and they all appear on the single surface facing us.

![Fig. 3. The side view of docking of cytochromes to Paracoccus CCP. The docking results described in Fig. 2 are viewed from the side of the cytochrome c peroxidase looking down at the exposed edge of the electron-transferring heme group. In all three experiments, the 500 most favorable solutions are shown as individual unfilled circles marking the center of mass of the probe cytochrome. The top ten docking solutions are shown as black filled circles. The electron-transferring heme group (E) and the peroxidatic heme group (P) are shown as space-filled and shaded gray. For clarity, only the nearest two hemes of the four heme CCP dimer are shown. The protein itself is not shown and would be contained within the oval perimeter of clustered solutions that marks the dimer interface. Because the CCP is a symmetrical dimer, related solutions will be found at both “sides,” and for simplicity, these have been combined and they all appear on the single surface facing us.](image)

![Fig. 4. The top ranking docking solutions for cytochrome c<sub>550</sub> and horse cytochrome c<sub>551</sub>. The α-carbon backbone of a single monomer of the CCP enclosing the space-filled E and P hemes is shown in each case. A, the α-carbon backbone of the highest ranked docking orientation of cytochrome c<sub>550</sub> is shown in gray and encloses the space-filled heme group. B, the corresponding result for horse cytochrome c is shown. The distance between the iron of the c<sub>550</sub> heme and that of the E heme is 16 Å; the distance between the iron of the horse cytochrome c heme and that of the E heme is 23 Å; and the corresponding distance to the P heme is 22 Å.](image)

**RESULTS**

The Docking Simulation—Figs. 2 and 3 represent the results of docking simulations between *Paracoccus* CCP and *Paracoccus* cytochrome c<sub>550</sub> (panels A), horse cytochrome c (panels B), and *Pseudomonas* cytochrome c<sub>551</sub> (panels C). Each monoheme cytochrome was rotated and translated against the molecular surface of the peroxidase, and at each stage the docked complex was evaluated for favorable interactions. The small acidic cytochrome c<sub>551</sub> is not oxidized by the *Paracoccus* CCP and was used as a negative control.

The most favored docking solutions are represented by their heme groups in the front view of Fig. 2 and by black filled circles in the side view of Fig. 3. In the case of the cytochrome c<sub>550</sub>-CCP binding simulation (A), six of the top ten ranking solutions (including the top solution) belong to a tight cluster in which the cytochrome c<sub>550</sub> lies close to the E heme of the peroxidase. In this position, the distance between the iron of the cytochrome c and the iron atom of the CCP heme E is 15–16 Å. The top ranking solution is shown in Fig. 4A. Although there is a second cluster of solutions with cytochrome c<sub>550</sub> lying between the E and P hemes of the peroxidase, none of them scores within the high ranking group (the highest rank in that position is 17th). The remaining four of the top ten ranking solutions are situated at the “back” of the molecule and are not visible in Fig. 2 but are partly visible in the ring of solutions in Fig. 3A, which lie in the dimer interface. As discussed below these are electronically poorly coupled, and we regard them as artifacts of the docking procedure (see “Discussion”).

In contrast, in the case of the horse cytochrome c-CCP binding simulation, six of the top ten ranking solutions (including the top three themselves) cluster the cytochrome c between the E and P hemes of the peroxidase (Figs. 2B and 3B). In this position, the distance between the iron of cytochrome c and the iron atoms of either of the CCP hemes is 21–26 Å. The top ranking solution is shown in Fig. 4B. In this simulation, the horse cytochrome c shows little tendency to dock onto the peroxidase above the E heme (there is a single solution at that position, and it was ranked 12th). Again there are four of the top ten ranking solutions situated at the dimer interface and these are partly visible as filled black circles in Fig. 3B.

In the control docking simulation, cytochrome c<sub>551</sub> showed almost no tendency to bind on the side surface of the CCP (Figs. 2C and 3C); the highest rank in that position was 53rd and, overall, the docking scores were significantly poorer than those obtained for the other two docking cases (see “Discussion”). In this case, the top ten ranked solutions appear in the dimer interface but at the front rather than the back of the dimer (Fig. 3C) in a dip in the molecular surface large enough to accommodate the smaller cytochrome c<sub>551</sub>.

Electronic Coupling in the Docked Geometries—Electronic coupling can be assessed by calculation of the minimum “path-length” for electron transfer within a docked geometry. In Fig. 5, we show the correlation between the top ten docking solutions (unfilled symbols) and the best electronic coupling between the probe iron of the different cytochromes and the E heme of the peroxidase (filled symbols with no outline). In the case of cytochrome c<sub>550</sub> (circles), five of the six solutions that have the most favorable docking above the E heme were also the most strongly coupled electronically (broken line box). The four docking solutions in the top ten that bound at the dimer interface (unfilled circles) were very poorly coupled electronically, a result which supports the conclusion that the docking at the dimer interface is an artifact of the docking simulation (see “Discussion”).

Both docking and electron coupling are weaker for horse cytochrome c (Fig. 5, squares). In addition, there is poorer correlation between docking strength and electron coupling. Although the best coupled geometries do involve “between-hemes” binding (with the exception of the single E heme binding solution), only one of the top ten best coupled solutions...
Correlation of docking geometry with electronic coupling. Paracoccus cytochrome c\textsubscript{550} (circles), horse cytochrome c (squares), and Pseudomonas cytochrome c\textsubscript{551} (triangles). The electronic pathlength and the docking strength are calculated as described under “Experimental Procedures.” When a highly ranked docking solution corresponds to an electronically well coupled solution, the symbol is shown outlined and filled. Such solutions for cytochrome c\textsubscript{550} are shown boxed.

corresponds to a most favorable docking solution (single outlined and filled square in Fig. 5). Finally, for cytochrome c\textsubscript{551}, none of the top ten docking solutions (unfilled triangles) correspond to a top ten coupling solution (filled triangles with no outline).

\textsuperscript{1}H NMR Spectroscopy—The complex formation between Paracoccus peroxidase and the three different cytochromes c was independently studied by \textsuperscript{1}H NMR spectroscopy. The heme methyls of the c-type cytochromes are strongly downfield-shifted because of the influence of the unpaired electron in a d-orbital of the iron atom (25, 26). Their chemical shift and linewidth are sensitive to the presence of bound redox partners. When cytochrome c\textsubscript{550} is titrated with Paracoccus CCP, the two most downfield-shifted heme methyls shift and broaden dramatically (Fig. 6A), effects that are reversed by addition of NaCl. A further indication of the strength of the effect is the shift of the methyl of the coordinating methionine of cytochrome c\textsubscript{550} which moves from -17.4 to -16.6 ppm (Table I). The stoichiometry of the c\textsubscript{550}-CCP complex is 1:1 (Fig. 6C), and its complete dissociation requires greater than 300 mM NaCl (Fig. 6A). In contrast, the perturbation by addition of CCP of the equivalent resonances of horse cytochrome c (assigned as heme methyls 8 and 3) is much less pronounced (Fig. 7A), and the upfield methionine methyl resonance is not shifted (Table I). The main effect is a broadening of the heme resonances rather than a shift, and the data are consistent with a stoichiometry of two cytochrome c per CCP (Fig. 7C). The complex is completely dissociated by 300 mM NaCl (not shown).

The cytochrome c\textsubscript{551} appears to show binding, judging from the broadening of the methyl resonances, but these resonances experience no shift (Fig. 8A).

The \textsuperscript{1}H NMR spectrum of the diheme Paracoccus CCP reflects the presence of two distinct sets of heme c methyls (12). Those four most strongly downfield-shifted (50–60 ppm) are associated with the electron-transferring heme, whereas only two of the four methyls of the peroxidatic heme are visible in the spectra of Figs. 6B, 7B, and 8B (at 23.9 and 34.4 ppm). Additions of cytochrome c\textsubscript{550} or horse cytochrome c to a CCP solution have no effect on the latter resonances and have different effects on those of the electron-transferring heme. Binding of the cytochrome c\textsubscript{550} causes a movement of +2.1 ppm in the resonance at 51.6 ppm and +0.8 ppm in the resonance at 58.4 ppm (Table I). The corresponding changes associated with the binding of horse cytochrome c are +1.4 and +2.9 ppm, respectively. The binding of cytochrome c\textsubscript{551} does not cause any resonance shift (Fig. 8B).

\textbf{Fig. 5. Correlation of docking geometry with electronic coupling.} Paracoccus cytochrome c\textsubscript{550} (circles), horse cytochrome c (squares), and Pseudomonas cytochrome c\textsubscript{551} (triangles). The electronic pathlength and the docking strength are calculated as described under “Experimental Procedures.” When a highly ranked docking solution corresponds to an electronically well coupled solution, the symbol is shown outlined and filled. Such solutions for cytochrome c\textsubscript{550} are shown boxed.

\textbf{Fig. 6. \textsuperscript{1}H NMR spectroscopy of the complex formed between cytochrome c\textsubscript{550} and CCP.} High resolution \textsuperscript{1}H NMR spectra were collected for titrations performed in both directions. A, a solution (0.75 mM) of cytochrome c\textsubscript{550} in 10 mM Mes, pH 6.0, D\textsubscript{2}O was titrated with a solution of CCP (1.4 mM) in the same buffer. B, a solution of CCP (1 mM) in 10 mM Mes, pH 6.0, D\textsubscript{2}O was titrated with solutions (1.5–1.7 mM) of cytochrome c\textsubscript{550} in the same buffer. The molar proportions of titrant (cytochrome c\textsubscript{550}) to CCP are indicated at the right side of the individual spectra (Part 1). Broken lines indicate the free (f) and bound (b) positions of selected heme methyls of the cytochrome c. At a CCP/cytochrome ratio of 1:2, reversal of the cytochrome c\textsubscript{550} complex was achieved by additions of a solution (2 x) of NaCl in the same buffer to the final concentrations shown (Part 2). B, a solution of CCP (1 mM) in 10 mM Mes, pH 6.0, D\textsubscript{2}O was titrated with solutions (1.5–1.7 mM) of cytochrome c\textsubscript{550} in the same buffer. The molar proportions of titrant (cytochrome c\textsubscript{550}) to CCP are indicated at the right side of the individual spectra. The broken lines indicate the positions of the heme methyls of the CCP that are discussed in the text. Those at 23.9 and 34.4 ppm are unaltered during the titration. Those at 51.6 and 58.4 ppm are shifted to new positions (indicated by broken lines) in the bound state in the presence of cytochrome c\textsubscript{550}. C, the shift in the heme methyl resonance at 51.6 ppm with increasing cytochrome c\textsubscript{550} is plotted. Open circles are for the titration of CCP with cytochrome c\textsubscript{550}, and filled circles are for the titration of cytochrome c\textsubscript{550} with CCP. The upper curved line is theoretical for a K\textsubscript{d} of 5 mM and the lower for a K\textsubscript{d} of 20 mM.
Table I
The effect of complex formation on the chemical shifts of selected methyl resonances

| Compound | Free | Δ | Free + Horse c | Δ |
|----------|------|---|----------------|---|
| Paracoccus CCP heme methyls, effect of addition of cytochrome c | Methyl (E heme) | 51.6 | +2.1 | 51.3 | +1.4 |
| | Methyl (P heme) | 34.4 | 34.4 | 34.4 | 34.4 |
| | Methyl (P heme) | 23.9 | 23.9 | 23.9 | 23.9 |
| Cytochrome c heme methyls, effect of addition of Paracoccus CCP | c550 heme methyl 8 | 29.1 | -1.0 |
| | c550 heme methyl 3 | -28.7 | +2.5 |
| | c550 Met methyl | -17.4 | +0.8 |
| Horse c heme methyl 8 | 35.5 | -0.5 |
| Horse c heme methyl 3 | 32.3 | +0.9 |
| Horse c Met methyl | -24.9 | -24.8 |
| Yeast iso-1 c heme methyl | 34.7 | +0.2 |
| Yeast iso-1 c heme methyl | 31.4 | +1.9 |
| Yeast iso-1 c Met methyl | -23.4 | +0.6 |
| Horse c heme methyl 8 | 35.1 | -0.3 |
| Horse c heme methyl 3 | 32.2 | +0.7 |
| Horse c Met methyl | -24.4 | -24.4 |

DISCUSSION

The Docking Simulation and Electronic Coupling—The bulk of the solutions, each represented by an unfilled gray circle positioned at the center of mass of the probe cytochrome (Figs. 2 and 3), cluster in a perimeter at the dimer interface. We regard this as an artifact of the method used because a similar clustering is observed with a small protein that does not interact with the enzyme (Figs. 2C and 3). The extent of surface complementarity between the interacting molecules is the first criterion used to filter the billions of binding modes, blindly generated at the first stage. Most of these solutions are retained in the top ranked 1000 because of the increased possible surface contact for a small globular protein in a surface depression at the dimer interface on the peroxidase molecule. It is this effect that gives rise to the clustering of apparent docking solutions as a ring around the dimer in Figs. 2 and 3. Such solutions can appear highly ranked in the docking analysis but show poor electronic coupling.

The docking program combines the individual energy components of the association into a “global score.” Although this cannot be expressed in absolute energy units, it can be used to compare different docking profiles. Thus the global scores of the top ten solutions for the cytochrome c550 docking are in the range -71 to -80, those for the horse cytochrome c are in the range -63 to -71, and those for the negative control cytochrome c551 are in the range -52 to -59 (see the horizontal axis in Fig. 5). These numbers indicate the relative strengths of the docked complexes and show that the most favored horse cytochrome c complex ranks below the top cytochrome c550 complexes, and the most favored cytochrome c551 “complex” ranks below the top ten horse cytochrome c complexes.

The docking algorithm is not intended to point to one unique best solution but rather to present a reduced and manageable set of alternative binding modes which have a good probability of including a working model of the actual complex. Any independent information may be useful in validating or refuting individual docking models. All the high ranked solutions for both horse cytochrome c and cytochrome c550 binding to the sides of the peroxidase dimer have the heme group of the probe facing inwards in the complex, as would be expected for a productive encounter complex. Because no constraints were placed on the docking method to achieve this, we believe that this observation supports the conclusion that the docking simulation is producing results relevant to the actual complex.

For cytochrome c550, this conclusion is also supported by the strong correlation between the best docking solutions and strong electron coupling. It is interesting that there is a weaker correlation in the case of horse cytochrome c. Although best docking and coupling is achieved by between-hemes orientation of the horse cytochrome c, the best docking solutions and best coupled solutions do not exactly correspond in structure except in one case. These results may suggest a less specific and more fluid interaction of the Paracoccus peroxidase with horse cytochrome c, a conclusion also suggested by the less tightly kni...
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resonances associated with the CCP heme methyls at 23.9, 34.4, 51.6, and 57.9 ppm are shifted to new positions in the bound state in the presence of Pseudomonas cytochrome c$_{551}$. 

cluster of “between-hemes” solutions (Figs. 2B and 3B).

$^1$H NMR Spectroscopy—The methyl resonances previously assigned to the electron transferring heme (12) are shifted in the presence of cytochrome c$_{550}$ whereas the methyl resonances of the peroxidatic heme are unaffected. This is consistent with a model in which electron entry into the peroxidase is at the electron-transferring heme. Also, for cytochrome c$_{550}$, the strong reciprocal perturbations observed in the electron-transferring heme of the peroxidase and the heme of the cyto-

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The differences we see in the binding site and stoichiometry for the two kinetically competent electron donors is an intriguing result. This suggests that more than one pathway of electron transfer may have evolved to communicate with the protein surface of the peroxidase and that these pathways provide an opportunity for synchronous delivery of the two electrons required for restoration of the active enzyme (Fig. 1).

We should note a potential complication in our interpretation. We have studied the binding of the product ferricyto-

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The references are consistent with the physical chemistry of cytochrome c$_{551}$ as an electron donor and the lack of favorable docking to the side surface of the CCP.

There are remarkable similarities here with studies on the yeast CCP. The resonance of heme methyl 3 is strongly down-field-shifted in yeast cytochrome c when it binds to the yeast peroxidase, whereas that of heme methyl 8 experiences a small upfield shift (27, 28). Perturbations of the heme methyl resonances of horse cytochrome c are much smaller (29) and indeed can be mimicked by binding of polyglutamate (30). We found no change in the heme methyls on addition of polyglutamate to cytochrome c$_{550}$. However, polyglutamate also produced no inhibition of ferrocytochrome c$_{550}$ oxidation by peroxidase (in contrast to its strong inhibition of horse cytochrome c oxidation), and therefore polyglutamate is probably unable to bind the cytochrome c$_{550}$.

We can conclude that the small perturbations observed on binding horse cytochrome c to either the yeast or the bacterial peroxidase are because of the effect of changes in surface electrostatic charge on the electron distribution in the iron d-orbital. The larger perturbations observed in the physiological redox partners on binding their respective peroxidase could, in principle, be because of the proximity of the paramagnetic iron of the peroxidase heme group. However, this seems unlikely as the iron-iron separation is 26 Å for the yeast system. More likely, therefore, is that the strong perturbations in both the Paracoccus and the yeast systems arise from specific movement of a charged side chain in the complex close to the heme methyl 3 group.

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