Electron Transfer between Cytochrome P450cin and Its FMN-containing Redox Partner, Cindoxin*

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Cytochrome P450 reductase, which delivers electrons from NADPH to microsomal P450s, consists of a single polypeptide that contains both FAD and FMN. The bacterial P450cin utilizes a similar electron transport system except the FAD/FMN reductase consists of two separate polypeptides where the FMN protein, cindoxin, shuttles electrons between the FAD-containing cindoxin reductase and P450cin. Here we characterize the kinetics and specificity of electron transfer between cindoxin and P450cin as well as discuss the influence of possible binding surface interactions using homology models.

Cytochromes P450 catalyze the monoxygenation of a vast array of organic compounds in the following reaction, R–H + NAD(P)H + H+ + O2 → R−OH + NAD(P) + H2O.

Two electrons are required for O2 activation by the P450 heme iron that are delivered from NAD(P)H by P450 reductases. Until recently, P450s were divided into two classes based on the types of reductases used. Class I is a three-component system that uses a NAD(P)H-dependent FAD-containing protein, cindoxin, to shuttle electrons between the FAD-containing cindoxin reductase and P450cin. Class II consists of a FAD/FMN P450 reductase that mediates electron transfer from NADPH to P450. Not too long ago, mitochondria and prokaryotes were neatly grouped into class I while microsomal P450s were placed in class II. With an increasing genomic data base and a corresponding increase in the characterization of new P450s, it now is clear that a simple two-class categorization is too limited. Indeed, a recent review defines 10 different P450 electron transfer classes (1). For example, P450BM3 is a prokaryotic fatty acid hydroxylase that uses a microsomal-like FAD/FMN reductase with the important exception that the reductase is fused to the C-terminal end of the P450. The two-component class II consists of a FAD/FMN P450 reductase that transfers electrons to a FMN protein (2, 3). The two-component class I prokaryotic system except its flavin-containing components are class II-like. The P450cin monoxygenase system consists of a FAD reductase that transfers electrons to a FMN protein that then delivers electrons to P450cin. Sequence homologies clearly show that the FMN and FAD proteins are closely related to the FMN and FAD modules of P450 reductase (6). Indeed, the FMN protein cindoxin (Cdx) exhibits 37% sequence identity to the FMN domain of human microsomal P450 reductase (CPRFMN), whereas the FMN module of P450BM3 shares only 28% sequence identity with the human FMN domain.

We recently solved the crystal structure of P450cin (7) and had anticipated that the presumed electron transfer site on P450cin would resemble that of P450BM3 and other P450s that utilize an FMN module as the reductase partner. Instead, we found that the architecture of the proximal docking site is more similar to P450cam, which utilizes a Fe2S2 protein, putidaredoxin (Pdx). To further probe the interaction between Cdx and P450cin we have initiated a series of studies to characterize the electron transfer reaction. Here we report the kinetics and specificity of electron transfer between Cdx and P450cin.

MATERIALS AND METHODS

Protein Purification—Plasmids containing P450cin and Cdx were provided by Dr. James DeVoss. P450cin was expressed and purified as described previously (7). Adrenodoxin (Adx) and CPR expression plasmids were provided by Drs. Y. Sagara and C. Kasper, respectively. Expression and purification of Adx and Pdx were carried out as described elsewhere (8, 9). The FMN domain of human CPR containing residues 1–181 was cloned into EcoRI and KpnI sites of the pProEX expression vector (Invitrogen) with a cleavable N-terminal hexahistidine tag and purified using Ni2+ affinity and gel filtration chromatography. ε280 of 9.8 mM−1 cm−1 was employed to calculate concentration of oxidized CPRFMN.

Cdx was purified as follows. Overnight cultures of Escherichia coli DH5α harboring the Cdx expression plasmid were used to inoculate 1 liter of terrific broth (TB) medium in a 2.8-liter flask. Cells were grown at 37 °C and 220 rpm to a 600-nm absorbance of 1–1.5 followed by addition of 1 mM isopropyl β-D-1-thiogalactopyranoside. Cell growth was continued

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3 The abbreviations used are: Cdx, cindoxin; CPRFMN, FMN module of human cytochrome P450 reductase; Adx, adrenodoxin; Pdx, putidaredoxin; Sq, semiquinone; Hq, hydroquinone; Ox, oxidized.
at 27 °C and 70 rpm; 48 h after induction cells were harvested by centrifuging at 6000 rpm and kept frozen at −20 °C. All lysis and purification steps were carried out at 4 °C in 50 mM potassium phosphate, pH 7.4, and 0.5 mM dithiothreitol (buffer A).

Cell pellets were resuspended in buffer A containing 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin and pepstatin A and stirred for 1 h with 0.1 mg/ml lysozyme. Lysis was completed by sonication with a Branson Sonifier 450 using four to six alternations of 20-s pulses and 40-s incubations on ice. Cell debris was removed by centrifugation at 17,000 rpm for 1 h. The supernatant was diluted 3- to 5-fold with buffer A and loaded on a DEAE-Sepharose column equilibrated with buffer A. The column was washed with three column volumes of buffer A, two volumes of buffer A containing 100 mM KCl, and finally with four volumes of buffer A containing 180 mM KCl. Cdx was eluted with a gradient of 180–300 mM KCl in buffer A. Fractions were concentrated to 2 ml using an Amicon YM10 concentrator. The resulting sample was loaded onto a S-200-Sepharose gel filtration column and eluted with buffer A. Fractions were checked for purity by SDS-PAGE, and those containing a 16-kDa band were combined and concentrated to 2 ml using an Amicon YM10 concentrator. The resulting sample was loaded onto a S-200-Sepharose gel filtration column and eluted with buffer A. Fractions were checked for purity by SDS-PAGE, and those containing a 16-kDa band were combined and concentrated to 2 ml using an Amicon YM10 concentrator.

Yellow-colored fractions were checked for purity by SDS-PAGE, and those containing a 16-kDa band were combined and concentrated to 2 ml using an Amicon YM10 concentrator. The resulting sample was loaded onto a S-200-Sepharose gel filtration column and eluted with buffer A. Fractions were checked for purity by SDS-PAGE, and those containing a 16-kDa band were combined and concentrated to 2 ml using an Amicon YM10 concentrator.

Redox titration of substrate-free and 1,8-cineole-bound P450cin (A) and Cdx (B). The insets correspond to the Nernst plots used to calculate the redox potential of each species.

FIGURE 1. Redox titration of substrate-free and 1,8-cineole-bound P450cin (A) and Cdx (B). The insets correspond to the Nernst plots used to calculate the redox potential of each species.

where Cdx(hq) and Cdx(sq) are the hydroquinone (two-electron-reduced) and semiquinone (one-electron-reduced) forms of Cdx, respectively. The dissociation constant, $K_d$, and maximum rate of electron transfer, $k$, were estimated from double reciprocal plots of $1/k_{obs}$ rate versus $1/[Cdx]$ concentration.

Homology Model—The amino acid sequence of Cdx was submitted to the online program 3D JigSaw, which returned a PDB coordinate file with the sequence of Cdx threaded on to the x-ray crystal structure of the FMN binding domain of human cytochrome P450 reductase (PDB code 1B1C). FMN was manually added to the model, and the model was energy-minimized. Flavodoxins and P450 reductase FMN modules have small, compact, and highly homologous structures. As a result, there is a high level of confidence that the Cdx homology model is a good approximation of the correct structure.

Stopped-flow—All solutions were made anaerobic by alternating evacuating and flushing with pure argon. Concentration of the sodium dithionite solutions was determined by titrating cytochrome c solutions and measuring the amount of cytochrome c reduced upon addition of small aliquots of the reductant. Solutions were loaded into gas-tight syringes in an anaerobic glove box because the stopped flow instrument was in an aerobic environment, an oxygen-scrubbing system of 1 mM glucose, 1 unit/ml glucose oxidase, and 1 unit/ml catalase was included in the wash buffers and experimental solutions. Absorbance changes were monitored at 450 nm to follow formation of the ferrous-CO complex formation. Kinetic data were analyzed using IgorPro software (WaveMetrics, Inc.). Data were analyzed according to Reaction 1

$$P450\text{cin}(\text{Fe}^{2+}) + \text{Cdx}(\text{hq}) \rightleftharpoons P450\text{cin}(\text{Fe}^{2+}) - \text{Cdx}(\text{hq})$$

$$P450\text{cin}(\text{Fe}^{2+}) - \text{Cdx}(\text{hq}) \rightarrow P450\text{cin}(\text{Fe}^{2+}) - \text{Cdx}(\text{sq})$$

REACTION 1

$K_d$ and $k$ were estimated from double reciprocal plots of $1/k_{obs}$ rate versus $1/[Cdx]$ concentration.
pure argon while stirring. The final experimental volume was 1.3 ml containing 10–15 μM protein. The following typical redox mediators were used: methyl viologen (−430 mV), benzyl viologen (−374 mV), 2-hydroxy-1,4-naphthoquinone (−145 mV), and anthraquinone 2-sulfonate (−230 mV) to a final concentration of 2 μM each. All the potentials here are reported against standard hydrogen electrode (SHE).

The protein and mediator mixtures were deoxygenated under the flow of argon gas for several minutes. The protein was reduced by addition of a small excess of anaerobically prepared sodium dithionite solution (concentration was determined using a molar absorption coefficient 315 μM cm−1). The reduced protein spectrum was recorded to confirm complete reduction. After each addition of a small aliquot of oxidant/reductant, the solution was stirred until equilibration (stabilization of the potential) was reached (15–20 min), and then the spectrum (350–800 nm) was recorded. The titration continued until the sample solution was maximally oxidized by ferrocyanide (10).

The electrochemical potential was monitored using an Orion pH/mV meter (Model SA 720) coupled to a gold electrode and an Ag/AgCl reference electrode from Bioanalytical Systems, Inc. The gold electrode was modified using 4,4'-dithiodipyridine. The electrode system was calibrated using the ferrous-ferric ammonium sulfate couple (+675 mV) (11). The observed potential was obtained relative to the Ag/AgCl reference electrode. Hence, they were corrected (using the calibration data for the ferrous-ferric ammonium sulfate solution) to values relative to the standard hydrogen electrode by addition of 197 mV to the data obtained using the Ag/AgCl electrode.

RESULTS

Redox Potential of Cytochrome P450cin—The UV-visible spectrum of the oxidized substrate-free low spin protein has maxima at 416, 535, and 569 nm. The UV-visible spectrum of the oxidized substrate-bound protein has maxima at 391, 510, and 540 nm, which is typical of high spin P450s. The reduced protein has maxima at 408 and 540 nm. Any of these spectral differences could be used to monitor the change in population from the oxidized (Fe3+) to the reduced (Fe2+) state. Because the Soret absorbance in the 390–416-nm range overlapped with the absorbance of the mediators, the Q-bands from 500–570 nm were used to monitor the titrations. The percentage of oxidized/reduced protein was calculated from the spectral data and was plotted against the observed potential. The data were analyzed and titration curves were fitted to the Nernst equation

$$\Delta E = \Delta E^\circ + 2.303(RT/nF) \log_{10}(C_{\text{ox}}/C_{\text{RED}})$$

(Eq. 1)

where $\Delta E$ is the observed potential and is plotted against the logarithm of the ratio of the concentration of oxidized ($C_{\text{ox}}$) and the reduced species ($C_{\text{RED}}$). The intercept of the plot gives $\Delta E^\circ$, the redox potential of the protein, and the slope divided by 59 mV gives $n$, the number of electrons involved in the reaction.

| Reduction Potentials (mV) for Cytochrome P450 reductase (CPR) (17) and P450BM3 (BM3) (14) compared to Cdx |
|----------------------------------|
| BM3 | CPR | Cdx |
| FMN (ox/aq) | −216 | −66 | −93 ± 17 |
| FMN (aq/hq) | −177 | −269 | −226 ± 5 |
| FMN (ox/hq) | −196 | −168 | −167 ± 2 |

FIGURE 2. Cdx-to-P450cin electron transfer. A, kinetic traces of P450cin reduction by 0.2–9.6 μM Cdx. Increasing concentration is shown by the arrow. The reactions were run in 50 mM potassium phosphate buffer, 50 mM KCl, pH 7.4. B, plot of the electron transfer rate versus Cdx concentration from the data in panel A. C, Cdx-to-P450cin electron transfer rate as a function of salt concentration. The insets for both panels B and C are double reciprocal plots of 1/k_{obs} versus 1/[Cdx].
at 25 °C. The redox potential of substrate-free P450cin was determined to be $-330 \pm 5 \text{ mV}$ versus standard hydrogen electrode, while that for the P450cin substrate bound was determined to be $-202 \pm 15 \text{ mV}$, giving a net shift in the redox potential on binding the substrate 1,8-cineole of $+128 \text{ mV}$ (Fig. 1A). This finding is in contrast to previously reported results (12) where P450cin was bound to an electrode and showed no redox shift upon substrate binding. An increase in redox potential upon substrate binding is observed in many other P450s and is consistent with known structural changes that result from substrate binding. The shift in spin state from low to high is due in part to the displacement by the substrate of the axial water ligand from the heme iron, thus shifting the heme iron from low spin hexacoordinate to high spin pentacoordinate. In addition, substrate binding dehydrates the active site, which further destabilizes the lower redox potential. The change in redox potential also provides control of electron transfer. In some P450s electron transfer to the low spin substrate-free form is thermodynamically unfavorable whereas the increase in redox potential upon substrate binding removes the thermodynamic barrier to electron transfer. This prevents the futile consumption of electrons by the substrate-free enzyme (13–15).

**Redox Potentials of Cdx**—During the purification of Cdx, blue and green bands were observed during the loading of the cell-free extract onto the DEAE column, indicating the presence of partially reduced Cdx. Moreover, upon reduction of purified Cdx with small amounts of dithionite, the blue, neutral semiquinone form was readily observed with an absorption maximum of 590 nm. When the reduced Cdx is exposed to an aerobic environment, the blue semiquinone is reoxidized to the yellow quinone. The sensitivity of the Cdx semiquinone to oxygen is not shared by full-length CPR or the FMN and FAD domains (16). Despite the difference in the stability of the semiquinone, these initial spectral observations suggested that, like P450 reductase, the redox potential of the Sq/Hq couple should be lower than the Ox/Sq couple.

The wavelengths used for determining the redox potential of Cdx were 474 and 583 nm, which are close to the absorption maximum of the oxidized flavin and the absorption maximum for the blue semiquinone, respectively. These absorbances were used to calculate the percentage of oxidized FMN that was plotted against the observed potential (Fig. 1B). Data were fit using the Nernst equation where the two one-electron steps in reduction were analyzed separately because only oxidized and

![Figure 1](image1.png)

**TABLE 2**

**Comparison of electron transfer rates in various P450s and various electron donors**

| Source            | Electron donor | P450     | k      | Temperature | Reference |
|-------------------|---------------|----------|--------|-------------|-----------|
| *Pseudomonas putida* | PDX          | cam      | 30–33  | 25.1        | 19        |
| Cow               | ADX          | sec      | 0.67–1 | 15          | 20        |
| Human             | CPR          | 2E1      | 32     | 37          | 21        |
| Rabbit            | CPR          | 1A2      | 13.3   | 37          | 21        |
| Human             | CPR          | 2C9      | 2.67–4.17 | 37         | 21        |
| Arabidopsis thaliana | CPR (ATR1)  | 2B4      | 34–48  | 22          | 22        |
| Human             | CPR (ATR2)   | 2B4      | 29–39  | 22          | 22        |
| Saccharomyces cerevisiae | CPR (SCR1)    | 2B4      | 4.5–8  | 22          | 22        |
| Citrobacter braakii | Cdx         | cin      | 15     | 21          | This work |
| P. putida         | PDX          | cin      | 0.55   | 21          | This work |
| Cow               | ADX          | cin      | 0.027  | 21          | This work |
| Human             | CPR<sub>FMN</sub> | cin      | 0.6–0.7 | 21        | This work |

![Figure 3](image2.png)

**FIGURE 3.** Representative stopped-flow traces of 0.8 μM P450cin reduction with 40 μM Pdx (A), 28 μM CPR<sub>FMN</sub> (B), and 12 μM Adx (C). Insets are double reciprocal plots from which the maximum rate, k, and K<sub>D</sub> were calculated.
semiquinone species were present during most of the first step and only semiquinone and hydroquinone forms were present during most of the second step. Data points in the region of maximal semiquinone accumulation were not included in the Nernst plot as in this region all three redox species could be present (14, 17, 18). The values of the midpoint potentials obtained are: (a) \( E_{\text{m}}^{\text{Ox/Sq}} = 93 \pm 17 \text{ mV} \) for the Ox/Sq couple and (b) \( E_{\text{m}}^{\text{Sq/Hq}} = 226 \pm 5 \text{ mV} \) for the Sq/Hq couple. The redox potential of the mixed couple Ox/Red is \( E_{\text{m}}^{\text{Ox/Red}} = -167 \pm 2 \text{ mV} \). These values are close to those of CPR_{FMN} (Table 1) and suggest that the two-electron-reduced FMN transfers electrons to P450cin because the hydroquinone is a better reducing agent than the semiquinone. Despite these similarities, the Ox/Sq potential for Cdx is lower than that for P450 reductase, which may in part account for the greater air instability of the semiquinone in Cdx.

**Kinetics**—The first electron transfer between Cdx and P450cin was monitored by mixing oxidized CO-saturated P450cin and Cdx, fully reduced by a stoichiometric amount of dithionite, in a stopped-flow spectrophotometer and recording absorbance changes at 450 nm over time (Fig. 2). P450cin shows saturation at \( \sim 12 \)-fold excess Cdx, with a \( K_D = 3 \text{ mM} \) and \( k = 15 \text{ s}^{-1} \) (Fig. 2B). The rate of electron transfer value is comparable with that calculated for other class I and II redox couples (Table 2) (19–22). Electron transfer from Cdx to P450cin was also found to depend on ionic strength. As the salt concentration increased, the rate of P450cin reduction dramatically decreased (Fig. 2C). A full kinetic analysis (Fig. 2C, inset) at 200 mM salt shows that the maximum rate of electron transfer is not salt-dependent but \( K_D \) increases from 3 to 30 \( \mu \text{M} \) in going from 5 to 200 mM salt. This suggests that complementary electrostatic interactions play an important role in the P450cin-Cdx complex formation.

To test the specificity of P450cin, we investigated how the hemoprotein reacts with class I ferredoxins Adx and Pdx (Fig. 3). Adx-to-P450cin electron transfer was very slow, with a secondary reaction (data not shown) that quenched absorbance from reduced P450cin at longer time scales and higher Adx concentrations. This made it difficult to make an accurate determination of \( k \) and \( K_D \) from double-reciprocal plots (Fig. 3C, inset). For Pdx \( k \) is 27-fold lower and \( K_D \) is 45-fold higher than for Cdx, whereas for CPR_{FMN} \( k \) is 23-fold slower and \( K_D \) 6-fold higher (Fig. 3, Table 2).

**Homology Model**—Cdx has 37% identity and 56% similarity with human CPR_{FMN}. The energy-minimized model of Cdx, generated using the program 3D Jigsaw, showed minor deviations from the crystal structure of CPR_{FMN}. CPR_{FMN} is longer and has an extra small helix at the N terminus. In addition, the fragment comprising the third helix in CPR_{FMN} is not well structured in the Cdx model. The P450cin-Cdx redox complex was modeled based on the known structure of the P450BM3 complexed with its FMN module (PDB code 1BVY) (23). P450cin was first superimposed on P450BM3 by aligning the heme groups. Next, the energy-minimized homology model of Cdx was superimposed on the FMN domain of P450BM3. Fig. 4A shows the hypothetical P450cin-Cdx complex with the superimposed CPR_{FMN}. Even without any energy optimization of the complex, the fit between P450cin and Cdx is good with few steric clashes and good ionic pairing and the FMN within electron transfer distance of 20 Å from the P450cin heme (Fig. 4A). The ionic strength dependence of the Cdx-to-P450cin electron transfer reaction (Fig. 2) indicates that electrostatic interactions are important, similar to other P450s. Not surpris-

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**FIGURE 4.** A, hypothetical model of the P450cin-Cdx complex. P450cin is dark blue, Cdx is cyan, and CPR_{FMN} is red. B, close-up view at the potential interaction site between Cdx Glu-10 and P450cin His-342. C and D, views along the predicted docking surface of Cdx and CPR_{FMN} respectively. The Ca positions of acidic residues predicted to be directly at the docking interface are represented as green spheres and the rest as red spheres.
ingly, the docking surfaces have complementary electrostatic potentials with P450cin serving as the positively charged partner and Cdx the negatively charged partner.

Because CPRFmN and Cdx are so similar in structure, a more detailed comparison between these two might provide some insight into why CPRFmN is a poor reductant of P450cin compared with Cdx. Although Cdx and CPRFmN both present electronegative surfaces to their respective redox partners, the distribution of acidic side chains is quite different, as shown in Fig. 4, C and D. The acidic residues that can directly interact with P450 are represented as green spheres. CPRFmN has a larger number of acidic residues on the docking surface. Thus, the optimal electrostatic interactions with P450 are likely to lead to different orientations in the P450cin-Cdx and P450cin-CPRFmN complexes. One particularly noteworthy difference at the docking interface involves Glu-10 in Cdx (Gln-27 in CPRFmN) predicted to interact with His-342 in P450cin (Fig. 4B). A second potentially important difference is Asp-87 in CPRFmN, which is Gly-66 in Cdx. Gly-66 approaches close to Gln-66 in P450cin. In addition, the sequence in the immediate vicinity of Gly-66 in Cdx is Ala-Gly-Gly-Gly-Gly whereas the corresponding sequence in CPRFmN is Thr-Asp-Gln-Asn-Ala. Thus, CPRFmN might experience steric clashes with P450cin in this region. It thus appears that even though the polypeptide topology is very likely conserved at the docking interface, the distribution of negative charges and specific predicted contact points are sufficiently different to give significantly different docked complexes, which translates into lower $k$ and higher $K_D$ for CPRFmN compared with Cdx.

**DISCUSSION**

The present study shows that the Cdx-P450cin electron transfer reaction is specific for Cdx and proceeds at a rate similar to other known P450 systems. The reaction is highly ionic strength-dependent, which also is similar to other P450s and consistent with the electrostatic complementarity of the docking surfaces. The redox potentials of Cdx suggest that the fully reduced hydroquinone is the electron-donating species because the redox potential of the $\text{SQ/SH}_{2}$ Cdx couple is lower than that of substrate-bound P450cin ($-226$ versus $-202\text{mV}$, respectively). Thus, the reduction of P450cin by the Cdx Hq is a thermodynamically favorable reaction. This again is similar to microsomal P450 reductase and further illustrates that P450cin is more similar to microsomal P450s with respect to electron transfer than its prokaryotic homologue, P450cam. Given such similarities with microsomal P450s, it was surprising to find that the detailed topography of the expected proximal docking site on P450cin is far more similar to P450cam than microsomal P450s or P450BM3. It thus appears that a similarly shaped docking site can exhibit large differences in specificity. Part of such selectivity is no doubt due to the obvious electrostatic difference but also due to more subtle differences in orientation and other possible allosteric effects and docking-induced structural changes. For example, the P450cam redox partner Pdx is known to have a significant effector role (24) where binding to the proximal side of the heme leads to perturbations in the distal substrate binding pocket that are thought to be coupled to conformational changes required for oxygen activation (25, 26). Whether or not Cdx plays a similar effector role remains to be seen. Finally, the expected high structural homology between CPRFmN and Cdx and yet very different electron transfer parameters provide some insights into which regions of the docking surfaces are critical for electron transfer. The hypothetical model for the P450cin-Cdx complex predicts Glu-10 and Gly-66 as two regions that differ substantially from CPRFmN, which will be tested experimentally in our future studies.

**REFERENCES**

1. Hannemann, F., Bichet, A., Ewen, K. M., and Bernhardt, R. (2007) Biochim. Biophys. Acta 1770, 330–344
2. Narhi, L. O., and Fulco, A. J. (1987) *J. Biol. Chem.* 262, 6683–6690
3. Narhi, L. O., Wen, L. P., and Fulco, A. J. (1988) *Mol. Cell. Biochem.* 79, 63–71
4. Roberts, G. A., Celik, A., Hunter, D. J., Ost, T. W., White, J. H., Chapman, S. K., Turner, N. J., and Flitsch, S. L. (2003) *J. Biol. Chem.* 278, 48914–48920
5. Puchkova, A. V., and Ortiz de Montellano, P. R. (2005) *Arch. Biochem. Biophys.* 434, 169–177
6. Hawkes, D. B., Adams, G. W., Burlingame, A. L., Ortiz de Montellano, P. R., and De Voss, J. J. (2002) *J. Biol. Chem.* 277, 27725–27732
7. Meharenna, Y. T., Li, H., Hawkes, D. B., Pearson, A. G., De Voss, J., and Poulos, T. L. (2004) *Biochemistry* 43, 9487–9494
8. Sugahara, Y., Harab, T., Aiyayusa, Y., Ando, F., Tokunaga, N., and Horie, T. (1992) *FEBS Lett.* 308, 208–212
9. Sevioukova, I. F., Garcia, C., Li, H., Bhaskar, B., and Poulos, T. L. (2003) *J. Biol. Chem.* 333, 577–592
10. Dutton, P. L. (1978) *Methods Enzymol.* 54, 411–435
11. Light, T. S. (1972) *Anal. Chem.* 44, 1038–1039
12. Aguey-Zinsou, K. F., Bernhardt, P. V., De Voss, J. J., and Slessor, K. E. (2003) *Chem. Commun. (Camb.)* 13, 418–419
13. Huang, Y. Y., Hara, T., Sligar, S. C., Coon, M. J., and Kimura, T. (1986) *Biochemistry* 25, 1390–1394
14. Daff, S. N., Chapman, S. K., Turner, K. L., Holt, R. A., Govindaraju, S., Poulos, T. L., and Munro, A. W. (1997) *Biochemistry* 36, 13816–13823
15. Sligar, S. G. (1976) *Biochemistry* 15, 5399–5406
16. Yasukochi, Y., Peterson, J. A., and Masters, B. S. (1979) *J. Biol. Chem.* 254, 7097–7104
17. Munro, A. W., Noble, M. A., Robledo, L., Daff, S. N., and Chapman, S. K. (2001) *Biochemistry* 40, 1956–1963
18. Vermilion, J. L., and Coon, M. J. (1978) *J. Biol. Chem.* 253, 8812–8819
19. Hintz, M. J., and Peterson, J. A. (1981) *J. Biol. Chem.* 256, 6721–6728
20. Schiffer, B., Zollner, A., and Bernhardt, R. (2004) *J. Biol. Chem.* 279, 34269–34276
21. Guengerich, F. P., and Johnson, W. W. (1997) *Biochemistry* 36, 14741–14750
22. Louerat-Oriou, B., Perret, A., and Pompon, D. (1998) *Eur. J. Biochem.* 258, 1040–1049
23. Sevioukova, I. F., Li, H., Zhang, H., Peterson, J. A., and Poulos, T. L. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 1863–1868
24. Lipscomb, J. D., Sligar, S. G., Namtveld, M. J., and Gunalsuh, I. C. (1976) *J. Biol. Chem.* 251, 1116–1124
25. Nagano, S., Tosa, T., Ishimori, K., Morishima, I., and Poulos, T. L. (2004) *J. Biol. Chem.* 279, 42844–42849
26. Tosa, T., Yoshioka, S., Ishimori, K., and Morishima, I. (2004) *J. Biol. Chem.* 279, 42842–42843

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