Putidaredoxin Competitively Inhibits Cytochrome b_5–Cytochrome P-450$_{cam}$ Association: A Proposed Molecular Model for a Cytochrome P-450$_{cam}$ Electron-Transfer Complex†

Patrick S. Stayton,‡ Thomas L. Poulos,§ and Stephen G. Sligar*,‡

Departments of Biochemistry and Chemistry, University of Illinois, Urbana, Illinois 61801, and The Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, Maryland 20850

Received January 30, 1989; Revised Manuscript Received May 11, 1989

ABSTRACT: Cytochrome b_5 has been genetically engineered to afford a fluorescent derivative capable of monitoring its association with cytochrome P-450$_{cam}$ from *Pseudomonas putida* [Stayton, P. S., Fisher, M. T., & Sligar, S. G. (1988) *J. Biol. Chem.* 263, 13544–13548]. In the mutant cytochrome b_5, threonine is replaced by a cysteine at position 65 (T65C) and has been labeled with the environmentally sensitive fluorophore acrylodan. In this paper, the physiological P-450$_{cam}$ reductant putidaredoxin, an Fe_2S_2·Cys_4 iron–sulfur protein, is shown to competitively inhibit the cytochrome b_5 association, suggesting that cytochrome b_5 and putidaredoxin bind to a similar site on the cytochrome P-450$_{cam}$ surface. Since the crystal structures for both cytochrome b_5 and cytochrome P-450$_{cam}$ have been solved to high resolution, the complex has been computer modeled, and a good fit was found on the proximal surface of nearest approach to the P-450$_{cam}$ heme prosthetic group. The proposed model includes electrostatic contacts between conserved cytochrome b_5 carboxylates Glu-44, Glu-48, Asp-60, and the exposed heme propionate with cytochrome P-450$_{cam}$ basic residues Lys-344, Arg-72, Arg-112, and Arg-364, respectively. Putidaredoxin has similarly been shown to contain a carboxylate-based binding surface, and the current results suggest that if the model is correct, then it also interacts at the proposed site, probably utilizing similar P-450$_{cam}$ electrostatic contacts.

The cytochrome P-450$_{cam}$ reaction cycle (Figure 1) requires the input of two reducing equivalents to yield product, 5-*exo*-hydroxycamphor, and water from molecular dioxygen and the camphor substrate. These reducing equivalents are ultimately derived from NADH, being transferred to cytochrome P-450$_{cam}$ via two redox-linked proteins, a flavin adenine dinucleotide (FAD)[1]-containing reductase and putidaredoxin, an Fe_2S_2·Cys_4 iron–sulfur protein. Putidaredoxin serves as a one-electron shuttle between the flavoprotein and cytochrome P-450$_{cam}$.

The electron-transfer reactions of putidaredoxin with cytochrome P-450$_{cam}$ are interesting in several respects. This redox couple represents a well-characterized example of conformational gating of electron transfer via binding of substrate, protein, and protons (Sligar & Gunsalus, 1976, 1979; Sligar, 1976). The interaction is also interesting in regard to control of the reaction cycle. Cytochrome P-450$_{cam}$ is unusual in utilizing an electron-transfer step as the rate-limiting control point, rather than the usual enzymatic control at the release of product (Pederson et al., 1977; Brewer & Peterson, 1988). Finally, the P-450$_{cam}$ reaction cycle serves as a functional model for mammalian P-450 activities, particularly the adrenal methylene hydroxylase system, which utilizes a similar Fe_2S_2·Cys_4 iron–sulfur protein, adrenodoxin.

It is clear than an understanding of putidaredoxin–cytochrome P-450$_{cam}$ interactions is crucial to an understanding of the P-450 reaction cycle. Unfortunately, the lack of a putidaredoxin crystal structure has stymied efforts to relate the determined chemical and kinetic data to the level of molecular structure. Putidaredoxin and adrenodoxin do contain analogous primary clusters of carboxylate residues that have been implicated in establishing the basis for complex formation with the flavoprotein reductases (Geren et al., 1984, 1986) and, for adrenodoxin, with cytochrome P-450$_{scc}$ (Lambeth et al., 1984; Tuls et al., 1984). Recent characterization of a cytochrome b_5–cytochrome P-450$_{cam}$ association reaction (Stayton et al., 1988) established the involvement of the conserved cytochrome b_5 anionic binding surface, similarly composed of a carboxylate-derived array of negative charge. We thus report the results of a binding competition study that suggests cytochrome b_5 and putidaredoxin bind at a similar cytochrome P-450 molecular surface. On the basis of the cytochrome b_5 and cytochrome P-450$_{cam}$ crystal structures and the implicated cytochrome b_5 binding surface, a computer-modeled b_5–P-450$_{cam}$ complex structure is proposed that is rooted in good electrostatic and van der Waals complementarity. Besides representing the first proposed molecular model of a biologically relevant cytochrome P-450 electron-transfer complex, the model also suggests that a similar site is utilized by the physiological redox partner putidaredoxin.

Materials and Methods

Site-Directed Mutagenesis. The synthesis, cloning, and expression of the rat liver cytochrome b_5 gene in *Escherichia coli* have been previously described (Beck von Bodman et al.,

† This research was supported by National Institutes of Health Grants GM 33775, GM 31756, and GM 33688.

* To whom correspondence should be addressed at the Department of Chemistry, University of Illinois.

‡ University of Illinois.

§ The Center for Advanced Research in Biotechnology.

[1] Abbreviations: FAD, flavin adenine dinucleotide; b_5, cytochrome b_5; P-450$_{cam}$, cytochrome P-450$_{cam}$; T65C, mutant cytochrome b_5 where the first letter is the one-letter amino acid code for the native protein at the position specified by the following number and the final letter is similarly the one-letter amino acid code for the residue introduced by site-specific mutagenesis; therefore, T65C refers to the mutant protein where threonine-65 has been changed to a cysteine; T8C, mutant b_5 produced by changing threonine-8 to cysteine; acrylodan, 6-acryloyl-2-(dimethylamino)naphthalene; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; D, aspartic acid; E, glutamic acid; K, lysine; R, arginine; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

0006-2960/89/0428-8201$01.50/0 © 1989 American Chemical Society

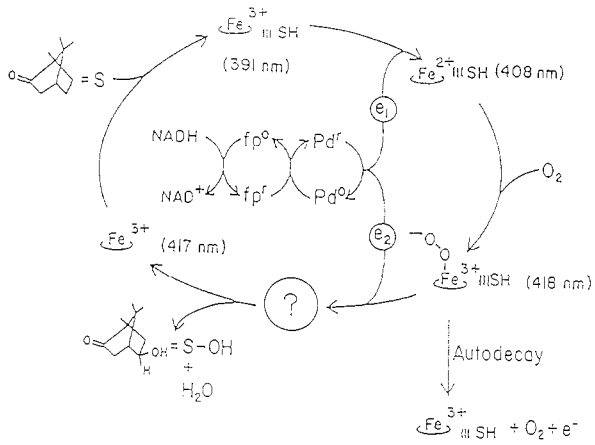

FIGURE 1: Generalized cytochrome P-450$_{cam}$ reaction cycle showing the spectrally observable intermediates and the associated redox chain where m^{os} is the oxidized camphor-bound P-450, m^{rs} is one-electron-reduced, camphor-bound P-450, m^{o} is oxidized camphor-free P-450, fp is putidaredoxin reductase, and Pd is putidaredoxin.

1986). The T65C and T8C mutant proteins were produced by standard cassette mutagenesis (Stayton et al., 1988) with the additional design feature of silent codon changes to facilitate screening by colony hybridization. Sequence analysis confirmed positive hybridization clones as the desired mutants.

Protein Purification. The T65C and T8C mutant cytochromes b_5 were purified by published procedures (Stayton et al., 1988; Beck von Bodman, 1986). Cytochrome P-450$_{cam}$ and putidaredoxin were purified from camphor-induced *Pseudomonas putida* cells as previously described (Gunsalus & Wagner, 1978).

Fluorescent Labeling of the Mutant Cytochrome b_5. Labeling of the T65C and T8C mutant cytochrome b_5 molecules with acrylodan has been previously described (Stayton et al., 1988). The T65C mutant spontaneously dimerizes in the purification procedure, and the resulting disulfide must be reduced prior to labeling. Typically, 500 μL of 200 μM T65C in 50 mM HEPES, pH 7.3, is reduced with 20 mM dithiothreitol and passed over a nitrogen-purged Whatman P-4 column equilibrated with 50 mM HEPES at pH 7.3. The protein band is collected in a nitrogen-purged Erlenmeyer flask containing a 4–5-fold excess of acrylodan (Molecular Probes, Eugene, OR) and reacted for 6–8 h. The labeling mixture is then passed over the P-4 column equilibrated with 1 mM phosphate buffer, pH 7.1. The T8C protein does not dimerize and is reacted directly with acrylodan under similar conditions.

Binding Competition Studies. Static fluorescence titrations were executed on a commercial photon counting ISS Greg PC (Urbana, IL) instrument. The excitation wavelength was 360 nm, and emission spectra were recorded from 440 to 600 nm. Titrations were executed in 1 mM phosphate buffer, pH 7.1, with 200 μM camphor at 20 °C. The resulting spectra were evaluated for integrated area under the curve. For the competitive binding studies, 0.2 μM acrylodan-labeled T65C cytochrome b_5 was titrated with cytochrome P-450$_{cam}$ in the presence of varying levels of putidaredoxin. Control experiments demonstrated that the T65C acrylodan fluorescence was unperturbed by the presence of putidaredoxin.

Computer Modeling. Computer models of the cytochrome b_5–cytochrome P-450$_{cam}$ association were generated on a Silicon Graphics IRIS 2400 using the University of California at San Diego Molecular Modeling System and an Evans and Sutherland system utilizing FRODO. The cytochrome b_5 conserved carboxylates at residues Glu-43, Glu-44, Glu-48, Asp-60, and the heme propionate were then used as fixed points to search the P-450$_{cam}$ surface for complementary basic amino acid side chains where salt bridge contacts and van der Waals complementarity could be modeled.

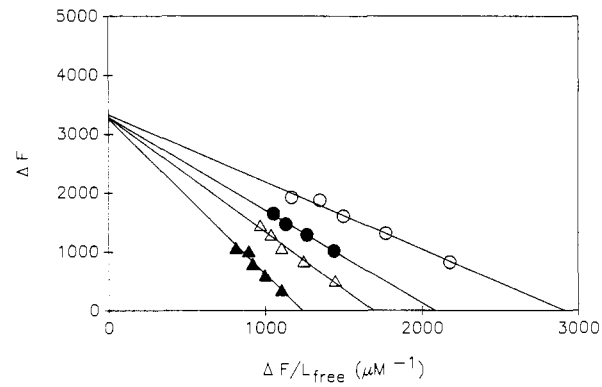

FIGURE 2: Cytochrome b_5/putidaredoxin binding competition for cytochrome P-450$_{cam}$ association: (○) 0.0 μM Pd; (●) 0.6 μM Pd; (△) 0.9 μM Pd; (▲) 1.2 μM Pd. The reaction conditions are given under Materials and Methods.

RESULTS

Cytochrome b_5 contains conserved surface carboxylates near the solvent-accessible prosthetic heme group at amino acid residues E43, E44, E48, and D60 and the exposed heme propionate. The involvement of this surface in specific protein–protein association reactions with a variety of redox partners is well supported experimentally (Stayton et al., 1988; Dailey & Strittmatter, 1979; Rodgers et al., 1988; Tamburini et al., 1985). Putidaredoxin, the Fe_2S_2·Cys_4 iron–sulfur protein responsible for the physiological one-electron reduction of cytochrome P-450$_{cam}$, also contains a cluster of carboxylic amino acid residues in its primary sequence (Tanaka et al., 1974). A binding competition study was conducted to investigate the possibility that a similar cationic site on cytochrome P-450$_{cam}$ was involved in the association of both proteins.

Static fluorescent titrations utilizing a cytochrome b_5 sulfhydryl mutant labeled with acrylodan at amino acid position 65, located on the outer edge of the proposed binding region, suggest that cytochrome P-450$_{cam}$ association occurs at the conserved cytochrome b_5 anionic surface surrounding the exposed heme cleft (Stayton et al., 1988). A control mutant, T8C cytochrome b_5, containing the surface cysteine on the opposite face of the molecule, does not exhibit a fluorescence enhancement upon titration of cytochrome P-450$_{cam}$, further supporting this observation. The reaction is ionic strength dependent, indicating an electrostatic contribution.

The results of the binding competition study are plotted in Eadie–Hoffstee formalism in Figure 2. This treatment assumes a 1:1 interaction, and the linearity of the data using this transformation suggests that the stoichiometry of cytochrome b_5–cytochrome P-450$_{cam}$ association is 1:1. The convergence at the y intercept representing ΔF_{max} suggests that the binding is indeed competitive. The secondary plot yields an inhibitor constant, K_I, corresponding in this case to the dissociation constant, K_d, for putidaredoxin and cytochrome P-450$_{cam}$, of 0.8 μM. This preparation of acrylodan-labeled T65C cytochrome b_5 gave a slightly higher value for the cytochrome b_5–cytochrome P-450$_{cam}$ dissociation constant than previously reported, 1.1 vs 0.7 μM.

The cytochrome b_5 and cytochrome P-450$_{cam}$ structures derived from X-ray crystallography studies were visually searched on the graphics systems for complementary electrostatic docking orientations assuming the involvement of the

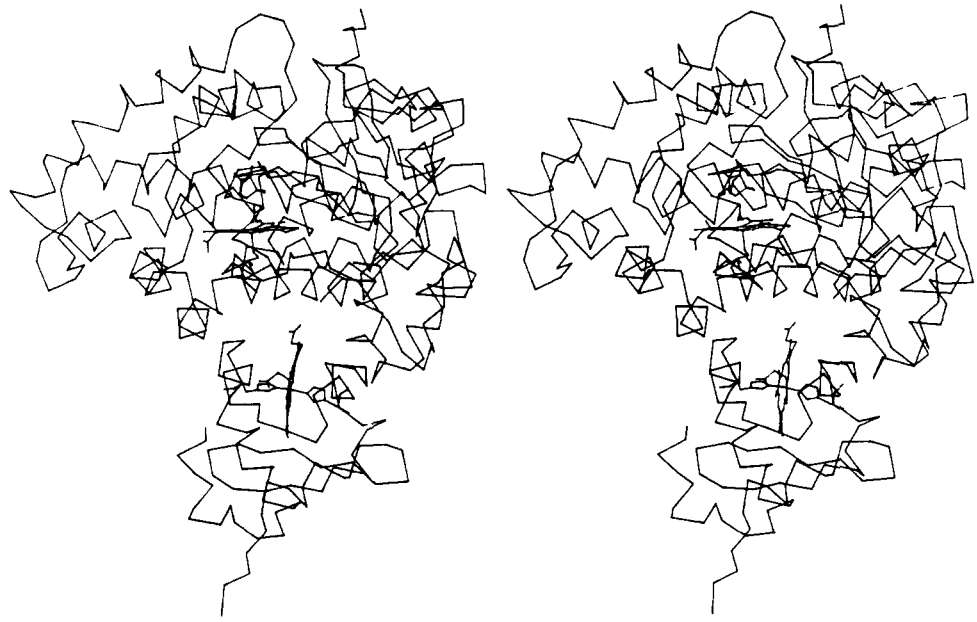

FIGURE 3: Stereoview of the proposed cytochrome b_5–cytochrome P-450$_{cam}$ complex.

conserved cytochrome b_5 carboxylates. The P-450$_{cam}$ surface does not contain an obvious asymmetry of total charge distribution such as that found with cytochrome b_5, but patches on the surface represent possible cationic binding domains. A good electrostatic fit based on charged pair interactions was found at the proximal surface of nearest approach to the buried cytochrome P-450$_{cam}$ prosthetic heme group. In addition to containing appropriately positioned positive charges, the P-450$_{cam}$ proximal surface defines a broad concave depression that would provide a good macromolecular docking site. This surface also is the closest approach of the P-450$_{cam}$ heme to the molecular surface, and, therefore, docking at this site would provide the shortest distance between donor and acceptor redox centers.

On the basis of simple manipulation of the two proteins in a computer graphics system, we found two reasonable orientations of cytochrome b_5 relative to P-450$_{cam}$. In the first, Glu-44, Glu-48, Asp-60, and the exposed heme propionate of cytochrome b_5 interact with Lys-344, Arg-72, Arg-112, and Arg-364 of P-450$_{cam}$. The second orientation is related to the first approximately as follows: a 180° rotation about a vector connecting the b_5 heme α and β meso carbon atoms followed by a 60° rotation about the heme normal passing through the iron atom and an 11-Å translation. This second model pairs the b_5 carboxylates at Glu-44, Glu-48, Asp-60, and the propionate with Arg-112, Arg-364, Lys-344, and Arg-72, respectively, of cytochrome P-450$_{cam}$. In both orientations, the b_5 and P-450$_{cam}$ hemes are nearly perpendicular with the shortest distance between aromatic heme atoms about 12–14 Å. The preferred complex where there appear to be more favorable nonbonded contacts and intramolecular ion pairs is the first orientation, and it is shown in Figure 3. The salt bridge contacts and metrical information of this model are given in Table I. Although this model has not been more rigorously examined using electrostatic calculations or energy minimization, it will serve as a useful starting model for the design of site-directed variants of both b_5 and P-450$_{cam}$.

Table I: Summary of Intermolecular Geometry in the Preferred Cytochrome b_5–Cytochrome P-450$_{cam}$ Computer-Docked Complex

cytochrome b_5 residue	cytochrome P-450 residue	intermolecular distance (Å)
b_5 propionate	Arg-364	2.90
Glu-48	Arg-72	3.08
Glu-44	Lys-344	2.80
Asp-60	Arg-112	2.97
Fe	Fe	18.46
C2D (heme)	Phe-350	8.68
Glu-43	Gln-343	4.56

DISCUSSION

Despite the vast library of research documenting the advanced physical–chemical characterization of the cytochromes P-450 and their associated redox systems, there remains a striking paucity of structural information at the molecular level. The major advancement occurred with the high-resolution of the bacterial cytochrome P-450$_{cam}$ crystal structure (Poulos et al., 1985). The availability of the X-ray structure for substrate-free, substrate-bound, and inhibitor-bound P-450$_{cam}$ has provided the first three-dimensional view of enzyme structure–function relationships (Poulos et al., 1985, 1986; Poulos & Howard, 1986). It also provides the basis for a systematic dissection of structure–function relationships via the technique of site-directed mutagenesis. The first such active-site study has been recently reported (Atkins & Sligar, 1988).

The extension of structure–function studies to P-450 protein–protein interactions is made difficult by the lack of structural information for both the partner molecules and the mammalian cytochromes P-450 themselves. The cytochromes P-450 interact with three classes of proteins, depending on their source and cellular location. The *Pseudomonas* and adrenal cytochromes P-450 are reduced by an Fe_2S_2·Cys_4 iron–sulfur protein, while the liver microsomal cytochromes P-450 utilize a flavin-linked reductase (Lu & Coon, 1968; French et al., 1980). In addition, certain mammalian cytochromes P-450 are known to interact with the membrane form of cytochrome b_5 (Hildebrandt & Estabrook, 1974; Bonfils et al., 1981; Pompon & Coon, 1984). The only crystal structure available for this set of proteins is that of the soluble, trypsin-released form of cytochrome b_5 (Mathews et al., 1972).

The goal of the present work and a recent related study (Stayton et al., 1988) was 2-fold: to establish the validity of a soluble cytochrome b_5/cytochrome P-450 complex as a model for the mammalian system and to relate the structural findings to the physiological cytochrome P-450$_{cam}$ system itself. The findings that cytochrome P-450$_{cam}$ associates with a soluble

form of cytochrome b_5 is promising in the first regard. The synthetic rat cytochrome b_5 is also a functional electron-transfer partner with cytochrome P-450$_{cam}$ (P. S. Stayton, M. Fisher, and S. G. Sligar, unpublished experiments; Lipscomb et al., 1976).

Since the crystal structures are available for both the soluble cytochrome b_5 and cytochrome P-450$_{cam}$, the P-450$_{cam}$ surface was searched for a likely binding site, i.e., primarily for a region containing basic residues complementary to the b_5 carboxylates. A good fit was found near the proximal surface of nearest approach to the heme prosthetic group (Figure 3).

The complementary P-450$_{cam}$ surface was found to accommodate two major cytochrome b_5 docking geometries. Within each of these models, numerous orientations can be assumed where certain contacts are optimized and others worsened, leading to slight differences in heme–heme orientation and distance. Such modeling observations probably reflect the dynamic and ensemblic nature of protein–protein association processes, as attested to by a growing number of theoretical and experimental reports [e.g., see Northrup et al. (1988), Wendoloski et al. (1987), and Hazzard et al. (1988)]. The static models of diprotein complexes have been very useful in describing general structure–function relationships (e.g., a structural basis for the rapid electron transfer between the heme edges of cytochromes) and in suggesting potential roles for specific amino acids via their location within the modeled complex (Liang et al., 1987).

However, the static models are unlikely to uniquely define the solution complex. A dynamic modeling of the cytochrome b_5–cytochrome c complex with energy minimization resulted in an even better docking geometry, i.e., shorter heme–heme separation distance, than that realized with static modeling (Wendoloski et al., 1987). Dynamic simulations incoporating electrostatic energy calculations have emphasized the ensemblic sampling of geometries (Northrup et al., 1988). The best synthesis with available evidence pictures complex formation occurring first by longer range electrostatic steering to the general locus of interaction (Koppenol & Margoliash, 1982; Matthew et al., 1983; Northrup et al., 1988) with subsequent sampling and optimization of geometries in a reduced dimension situation (Northrup et al., 1988), with deemphasis of rigid, specific salt bridge formation. Electron transfer can occur from a number of orientations.

With these caveats, it is still useful to model electrostatically based complex formation using specific salt bridge contacts to guide cytochrome b_5 to potential P-450$_{cam}$ electrostatic binding surfaces. As previously discussed, a number of realistic complex geometries centered on the same P-450$_{cam}$ surface can be modeled with cytochrome b_5. Metrical information for the orientation containing the best salt bridge contacts and minimal van der Waals overlap is given in Table I, and a stereoview of this complex is shown in Figure 3.

This model has several interesting and potentially physiologically relevant features. The prosthetic heme groups are oriented in a nearly perpendicular fashion. In addition, Phe-350 of cytochrome P-450$_{cam}$ is located directly on the path between the two hemes. This is a completely conserved residue in both bacterial and eukaryotic cytochromes P-450 (Nelson & Strobel, 1988), and it is possible that the aromatic side chain participates in electron transfer to the solvent-inaccessible P-450$_{cam}$ heme, in similar fashion to that demonstrated with Phe-87 of cytochrome c (Liang et al., 1987). The side chain of Arg-364 is situated on the surface directly above Phe-350, in position to orient a redox partner relative to that residue. In the model shown in Figure 3, the exposed b_5 propionate forms a salt bridge contact with this residue. Relevant distance information concerning these residues is also listed in Table I. Distances from the nearest conjugated porphyrin carbon have been included as suggested in recent electron-transfer studies with [Fe,Zn] hemoglobin hybrids (Gingrich et al., 1987). In the alignment of 33 vertebrate cytochrome P-450 primary sequences with P-450$_{cam}$ proposed by Strobel, the basic charge at Arg-72 of P-450$_{cam}$ is 82% conserved, at Arg-112 is 100% conserved, and at Arg-364 is 85% conserved, and Lys-344 is a uniquely aligned basic side chain (Nelson & Strobel, 1988).

The physiological P-450$_{cam}$ electron-transfer partner putidaredoxin is reduced by a flavin-linked protein, putidaredoxin reductase. Modification studies have implicated a cluster of acidic amino acids located between positions 55 and 83 in the primary putidaredoxin structure as being important in establishing the putidaredoxin–putidaredoxin reductase complex (Geren et al., 1986). The analogous mammalian iron–sulfur protein, adrenodoxin, contains a similar set of carboxylates that have been shown to constitute the binding surface for both the adrenodoxin reductase and the relevant cytochrome P-450, P-450$_{scc}$ (Geren et al., 1984; Lambeth et al., 1984; Tuls et al., 1987), consistent with the proposed role of adrenodoxin as a soluble redox mediator.

The demonstration that putidaredoxin competitively inhibits the binding of mammalian cytochrome b_5 to cytochrome P-450$_{cam}$ suggests that an anionic binding site may similarly be present on the putidaredoxin surface. Thus, the same cluster of carboxylates responsible for binding putidaredoxin reductase may also contribute in establishing the P-450$_{cam}$ association. If the modeled cytochrome b_5–cytochrome P-450$_{cam}$ complex is correct, then putidaredoxin may interact at a similar position on the proximal surface of nearest approach to the P-450$_{cam}$ heme prosthetic group. Electrostatic interactions between the set or a subset of putidaredoxin carboxylates at amino acid positions 58, 65, 67, and 72 and the basic P-450$_{cam}$ residues modeled for cytochrome b_5 may contribute to the binding free energy. It should be noted that the competition experiment does not directly demonstrate an electrostatic basis for putidaredoxin binding. Indeed, a hydrophobic contribution toward cytochrome P-450$_{cam}$ binding free energy has been suggested on the basis of kinetic measurements (Hintz & Peterson, 1981; Geren et al., 1986). A putidaredoxin-induced conformational change, at a site distant from the location of complexation, affecting cytochrome b_5 association also cannot be ruled out.

In summary, we have demonstrated that putidaredoxin competitively inhibits the cytochrome b_5–cytochrome P-450$_{cam}$ association reaction, presumably by utilizing similar cationic P-450$_{cam}$ electrostatic contacts. The cytochrome b_5 and P-450$_{cam}$ crystal structures and known electrostatic dependencies have been used to model for the first time a cytochrome P-450 electron-transfer complex. This complex is itself of physiological relevance since cytochrome b_5 is known to function in mammalian P-450 activities. It is also of particular relevance to the bacterial P-450$_{cam}$ system. Since putidaredoxin is known to contain a set of carboxylates important in protein–protein association, analogous to the cytochrome b_5 binding surface, and competitively inhibits b_5–P-450$_{cam}$ association, we suggest that putidaredoxin binds at the proximal surface of nearest approach to the P-450 heme prosthetic group, probably utilizing some or all of the same basic amino acid residues modeled for b_5. It is interesting that this structure provides a natural path of electorn transfer from the iron–sulfur center of putidaredoxin through the conserved Phe-350 of cytochrome P-450$_{cam}$ to the heme iron. The C-terminal tryptophan of

putidaredoxin may also be involved in complex recognition and electron transfer (Sligar et al., 1974; M. D. Davies, J. L. Beck, and S. G. Sligar, unpublished experiments). We are currently using site-directed mutants of cytochrome b_5, cytochrome P-450$_{cam}$, and putidaredoxin to experimentally probe the modeled function of all of these amino acids.

ACKNOWLEDGMENTS

Some of the experiments and analyses of the fluorescence data produced were conducted at the Laboratory for Fluorescence Dynamics (LFD) at the University of Illinois at Urbana—Champaign (UIUC). The LFD is supported jointly by the Division of Research Resources of the National Institutes of Health (RR03155-01) and the UIUC. Howard Robinson provided invaluable assistance with the graphics system at the University of Illinois. We also acknowledge Dr. Mark Fisher for initial work done with this system.

REFERENCES

Atkins, W. M., & Sligar, S. G. (1988) *J. Biol. Chem. 263*, 18842–18849.

Beck von Bodman, S., Schuler, M. A., Jollie, D. R., & Sligar, S. G. (1986) *Proc. Natl. Acad. Sci. U.S.A. 53*, 9443–9447.

Bonfils, C., Balny, C., & Maurel, P. (1981) *J. Biol. Chem. 256*, 9457–9465.

Brewer, C. B., & Peterson, J. A. (1988) *J. Biol. Chem. 263*, 791–798.

Dailey, H. A., & Strittmatter, P. (1979) *J. Biol. Chem. 254*, 5388–5396.

French, J. S., Guengerich, F. P., & Coon, M. J. (1980) *J. Biol. Chem. 255*, 4112–4119.

Geren, L. M., O'Brien, P., Stonehuerner, J., & Millett F. (1984) *J. Biol. Chem. 259*, 2155–2160.

Geren, L., Tuls, J., O'Brien, P., Millett, F., & Peterson, J. A. (1986) *J. Biol. Chem. 261*, 15491–15495.

Gingrich, D. J., Nocek, J. M., Natan, M. J., & Hoffman, B. M. (1987) *J. Am. Chem. Soc. 109*, 7533–7534.

Gunsalus, I. C., & Wagner, G. C. (1978) *Methods Enzymol. 52*, 166–188.

Hazzard, J. T., McLendon, G., Cusanovich, M. A., Das, G., Sherman, F., & Tollin, G. (1988) *Biochemistry 27*, 4445–4451.

Hildebrandt, A., & Estabrook, R. W. (1971) *Arch. Biochem. Biophys. 143*, 66–79.

Hintz, M. J., & Peterson, J. A. (1981) *J. Biol. Chem. 256*, 6721–6728.

Koppenol, W. H., & Margoliash, E. (1982) *J. Biol. Chem. 257*, 4426–4437.

Lambeth, J. D., & Kriengsiri, S. (1985) *J. Biol. Chem. 260*, 8810–8816.

Lambeth, J. D., Green, L. M., & Millet, F. (1984) *J. Biol. Chem. 259*, 10025–10029.

Liang, N., Mauk, A. G., Pielak, G. J., Johnson, J. A., Smith, M., & Hoffman, B. M. (1988) *Science 240*, 311–313.

Lipscomb, J. D., Sligar, S. G., Namtvedt, M. J., & Gunsalus, I. C. (1976) *J. Biol. Chem. 251*, 1116–1124.

Lu, A. Y. H., & Coon, M. J. (1968) *J. Biol. Chem. 243*, 1331–1332.

Mathews, F. S., Levine, M., & Argos, P. (1972) *J. Mol. Biol. 64*, 449–464.

Matthew, J. B., Weber, P. C., Salemme, F. R., & Richards, F. M. (1983) *Nature (London) 301*, 169–171.

Nelson, D. R., & Strobel, H. W. (1988) *J. Biol. Chem. 263*, 6038–6050.

Northrup, S. H., Boles, J. O., & Reynolds, J. C. L. (1988) *Science 241*, 67–70.

Pederson, T. C., Austin, R. H., & Gunsalus, I. C. (1977) in *Microsomes and Drug Oxidations* (Ulrich, V., Roots, I., Hildebrandt, A., Estabrook, R. W., & Conney, A. H., Eds.) pp 275–284, Pergamon Press, New York.

Pompon, D., & Coon, M. J. (1984) *J. Biol. Chem. 259*, 15377–15385.

Poulos, T. L., & Howard, A. J. (1987) *Biochemistry 26*, 8165–8174.

Poulos, T. L., Finzel, B. C., Gunsalus, I. C., Wagner, G. C., & Kraut, J. (1985) *J. Biol. Chem. 260*, 16122–16130.

Poulos, T. L., Finzel, B. C., & Howard, A. J. (1986) *Biochemistry 25*, 5314–5322.

Rodgers, K. K., Pochapsky, T. C., & Sligar, S. G. (1988) *Science 240*, 1657–1659.

Sligar, S. G. (1976) *Biochemistry 15*, 5399–5406.

Sligar, S. G., & Gunsalus, I. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1078–1082.

Sligar, S. G., & Gunsalus, I. C. (1979) *Biochemistry 18*, 2290–2295.

Sligar, S. G., Debrunner, P. G., Lipscomb, J. D., Namtvedt, M. J., & Gunsalus, I. C. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3906–3910.

Stayton, P. S., Fisher, M. T., & Sligar, S. G. (1988) *J. Biol. Chem. 263*, 13544–13548.

Tamburini, P. P., White, R. E., & Schenkman, J. B. (1985) *J. Biol. Chem. 260*, 4007–4015.

Tanaka, M., Haniu, M., Yasunobu, K. T., Dus, K., & Gunsalus, I. C. (1974) *J. Biol. Chem. 249*, 3689–3701.

Tuls, J., Geren, L., Lambeth, J. D., & Millett, F. (1987) *J. Biol. Chem. 262*, 10020–10025.