Structural insights from a P450 Carrier Protein complex reveal how specificity is achieved in the P450_{BioI} ACP complex

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Cytochrome P450_{BioI} (CYP107H1) from the biotin operon of *Bacillus subtilis* forms a seven-carbon diacid through a multistep oxidative cleavage of a fatty acid linked to acyl carrier protein (ACP). Crystal structures of P450_{BioI} in complex with three different length fatty acyl-ACP (*Escherichia coli*) ligands show that P450_{BioI} binds the fatty acid such as to force the carbon chain into a U-shape above the active site heme. This positions the C7 and C8 carbons for oxidation, with a large additional cavity extending beyond the heme to accommodate the methyl termini of fatty acids beyond the site of cleavage. The structures explain the experimentally observed lack of stereo- and regiospecificity in the hydroxylation and cleavage of free fatty acids. The P450_{BioI}-ACP complexes represent the only structurally characterized P450-carrier protein complexes to date, which has allowed the generation of a model of the interaction of the vancomycin biosynthetic P450 OxyB with its proposed carrier protein bound substrate.

acyl carrier protein | biotin biosynthesis | crystal structure | cytochrome P450 | protein-protein interaction

Cytochrome P450 enzymes (P450s) form a superfamily of oxidative hemoproteins, capable of catalyzing an extensive array of chemical transformations, with biological roles as varied as natural product biosynthesis and xenobiotic metabolism (1, 2). Within the P450 superfamily, ever increasing diversity is being identified in both the chemical reactions that these enzymes catalyze and also in the manner in which P450s interact with other proteins (3). One important and increasingly populated subsection of P450s includes those which, instead of binding free ligands, accept their substrates bound to a carrier protein (CP). Such P450s have, to date, mostly been identified in antibiotic biosynthesis pathways and have been found to catalyze several different types of oxidative reactions with substrates bound to peptidyl carrier proteins (PCPs). These reactions include the hydroxylation of aliphatic or aromatic amino acid C-H bonds, found in the biosynthesis of the antibiotics novobiocin (4, 5) and coumermycin A (6), the antifungal compound nikkomycin (7), and the oxidative phenolic coupling of heptapeptides, found in the biosynthesis of vancomycin-type antibiotics (8, 9). The crucial roles that such P450s play in the biosynthesis of these important medicinal compounds make it imperative that all aspects of their function are well understood, with the aim of developing new and effective antibiotic compounds.

Although structural biology has provided a wealth of valuable information for many different P450s covering a broad range of organisms and catalytic functions, the interaction of carrier proteins and P450s has so far eluded structural analysis. To redress this balance, a model of CP-P450 interactions was sought that would provide information about the nature of the protein contacts involved in CP-P450 interactions. To this end, P450_{BioI} (CYP107H1), a biosynthetic P450 isolated from the biotin operon of *Bacillus subtilis* (10), was chosen as an ideal candidate for investigating P450-CP interactions. P450_{BioI} is a perfect model system to study P450-CP interactions as it forms a tight protein-protein complex and, in addition, is also interesting in its

own right due to the nature of the oxidative transformations that it performs. P450_{BioI} catalyzes the formation of pimelic acid, a seven-carbon diacid that makes up the majority of the carbon skeleton of biotin, through the oxidative cleavage of a fatty acid carbon-carbon bond (Fig. 1) (11, 12). This type of reaction is performed by other P450s, many of which are involved in mammalian steroid metabolism, making it important to delineate the mechanism of this reaction (13).

P450_{BioI} forms a tight complex *in vivo* with fatty acids bound to *Escherichia coli* acyl carrier protein (ACP) (11), a small, acidic protein that plays a vital role in fatty acid transport and polyketide biosynthesis (14). The turnover of this complex affords pimeloyl-ACP, which not only supports the biological relevance of the CP-P450 interaction but also is in agreement with labeling studies showing that P450_{BioI} catalyzes the formation of pimelic acid from a fatty acid precursor formed via the standard fatty acid biosynthesis pathway (15). The *in vitro* oxidation of free fatty acids by P450_{BioI} produces, in addition to a small quantity of pimelic acid, a suite of fatty acids that are hydroxylated on the carbons adjacent to, but not including, the methyl terminus (11, 16). The mechanism of in-chain carbon-carbon bond cleavage has been investigated using the turnover of free fatty acids possessing the oxidation pattern of potential intermediates, which indicated a probable mechanism involving sequential oxidation via alcohol and vicinal *threo*-diol intermediates (Fig. 1) (12).

Here, we report the high resolution crystal structures of P450_{BioI} in complex with fatty acyl-ACPs from *E. coli*, acylated with tetradecanoic acid (C₁₄), hexadec-9Z-enoic acid (9-Z-C₁₆), and octadec-9Z-enoic acid (9-Z-C₁₈). These structures reveal the insertion of the phosphopantetheine linked fatty acid into the P450 active site upon binding, provide clear support for an in-chain C7-C8 carbon-carbon bond cleavage function of P450_{BioI}, and show the site and nature of the CP-P450 interaction interface. This knowledge has been applied to generate a model of the complex of a heptapeptide bound PCP with the P450 OxyB, responsible for the first phenolic coupling in the biosynthesis of vancomycin.

Results and Discussion

Overall P450_{BioI} Structure. The structure of the P450_{BioI}-ACP complex was determined by single anomalous diffraction of

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Data Deposition: Coordinates and structural factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 3EJB, 3EJD, and 3EJE).

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Fig. 1. Mechanism of oxidative cleavage of fatty acids by P450_{BioI} (observed for R: OH, $n \geq 1$; hypothesized for R: S-acyl ACP).

selenomethionine labeled crystals for three different fatty acid-acylated ACPs: C₁₄ (2.0 Å resolution), 9-Z-C₁₆ (2.1 Å resolution) and 9-Z-C₁₈ (2.1 Å resolution) [data collection and refinement statistics are available in [supporting information \(SI\) Table S1](#)]. P450_{BioI} (Fig. 2) has a similar overall structure to other P450s, although there are some notable differences in the structure of P450_{BioI} compared to the closest structures available in the Protein Database (StaP (17)—PDB code 2Z3U, 32% identity, 2.1 Å rmsd; PikC (18)—PDB code 2C6H, 36% identity, 2.2 Å rmsd; EryF (19, 20)—PDB code 1Z8Q, 34% identity, 2.0 Å rmsd, see [Table S2](#)). There are three regions of major difference in the substrate recognition sites (SRSs) (ref. 21; Fig. 2): the β -1 sheet, the loop between helices B and B₂ (SRS 1) and the region between the F- and G-helices (SRSs 2 and 3) (22). The differences in the β -1 sheet are caused by the interaction of P450_{BioI} with ACP and the phosphopantetheine linker; this region is closed in toward other structural elements in StaP and moved further away in the PikC/EryF structures due to the positioning of the substrates within the active site. In a similar manner, the loop between helices B and B₂ in P450_{BioI} moves due to interactions with the ACP bound substrate, whilst in the StaP structure, a second substrate molecule enforces a more open conformation with a longer loop. In PikC and EryF, this loop moves closer to the nearby β -sheet as there is no need to provide space and binding residues for ACP, as required with P450_{BioI}. The loop between the F- and G-helices adopts a more closed conformation in P450_{BioI} (similar to StaP) due to it forming part of the hydrophobic pocket for the fatty acid.

Comparison of the structures of P450_{BioI} and P450_{BM3} (PDB code 1FAG), a widely studied fatty acid hydroxylase from *Bacillus megaterium*, reveals major differences in the regions

involved in fatty acid binding (23, 24). In P450_{BM3}, the loop extension between the F- and G-helices is longer and would clash with the bound ACP in the P450_{BioI} complex. Additionally, in P450_{BM3} the loop between helices B and B₂ would clash with the phosphopantetheine linker and restrict the ligand binding pocket that in P450_{BioI} accommodates the methyl terminus of the fatty acid.

Comparison of the ACP-complexed P450_{BioI} structure with those of substrate free OxyB and OxyC (PDB codes 1LFK and 1UED), P450s involved in vancomycin biosynthesis, reveals that the entire region from the C terminus of the E-helix to the beginning of the I-helix is moved away from the active site in the Oxy structures, providing a larger binding pocket for their CP-bound peptide substrates (8, 9). Additionally, a loop region in the β -1 sheet and the loop between helices B and B₂, which provide the interface for ACP binding in P450_{BioI}, are not defined in the OxyB structure. This suggests that this region is highly flexible without carrier protein bound substrate (9). The loop between helices B and B₂ is also not defined in the OxyC structure, whilst the β -1 sheet adopts a very similar orientation to that seen with P450_{BioI}. A PEG fragment bound over the heme in the OxyC structure appears to mimic the heptapeptide and adopts a similar orientation to that seen with the methyl terminal region of the ACP-bound fatty acid, whilst the β -1 sheet is somewhat extended toward the probable position of the PCP (8).

Active Site Architecture. The active site of P450_{BioI} contains the conserved catalytic residues and typical structure found in P450s, which includes a kink in the I-helix where the highly conserved acid/alcohol residue pair (Glu-238/Thr-239 in P450_{BioI}) control the protonation of intermediate oxygen species during oxygen activation (25). The proximal heme thiolate ligand Cys-345 is present in the N-terminal loop before the L-helix. The fatty acid chain is accommodated in a hydrophobic active site and takes up a U-shaped conformation (Fig. 3). This conformation is caused by Leu-82, Phe-83, Ile-166, Ile-169, Ile-234, and Phe-384 that line the fatty acid binding pocket and provide an upper “lid” over the heme (Fig. 3B). The carbon atoms to be oxidized, C7 and C8 of the fatty acid, are located in the bend of the “U” and thus positioned above the heme iron. The hydrophobic binding pocket extends beyond the heme, providing space into which the methyl terminus of the fatty acid projects and which is principally

Fig. 2. Stereo diagram showing the overall structure of the P450_{BioI}-ACP complex with a C₁₄ ligand (ACP, fatty acid and phosphopantetheine linker shown in blue; P450_{BioI} is shown in gray except certain structural motifs that are colored; heme shown in red), with structural motifs labeled.

Fig. 3. Active site of the P450_{BioI}-ACP complex. (A) Critical P450 catalytic residues and those interacting with the phosphopantetheine linker via hydrogen bonds (distances averaged across the four molecules in the crystallographic asymmetric unit), with the three different length fatty acid chains overlaid (cyan, C₁₄; pink, 9-Z-C₁₆; orange, 9-Z-C₁₈; hydrogen bonds shown as dashed black lines). (B) The hydrophobic pocket of P450_{BioI} and residues that enforce a U-shaped conformation upon the fatty acid chain, with the C7 and C8 atoms of the fatty acid labeled. Phosphopantetheine linker and C₁₄ fatty acid are shown in orange.

composed of hydrophobic residues of the F-, G-, and I-helices (Fig. 3B). This pocket allows long chain fatty acids to project past the bend in the fatty acid over the heme and thus to accommodate the varying length of the residual chain beyond the C7-C8 cleavage site. The structure of the 9-Z-C₁₈ complex indicates that beyond C16 the fatty acid chain is mobile, as no electron density was observed beyond C16 (Fig. S1).

The ability of P450_{BioI} to oxidize two specific and adjacent carbons within the center of a fatty acid chain (C7 and C8) is in line with the positioning of the substrate within the active site. Although the location of the C7 and C8 carbons differs somewhat between the structures of the three P450_{BioI}-acyl ACP complexes with different bound fatty acids and within the four molecules in the crystallographic asymmetric unit, both the average carbon atom-heme iron distances for C7 (4.3 ± 0.3 Å) and for C8 (4.7 ± 0.5 Å) are within the range seen for other P450s, such as in EryF or P450_{cam} (4.7 Å and 4.2 Å) (20, 26). The similarity in the Fe-C7 and Fe-C8 distances indicates an apparent lack of regioselectivity for the initial site of oxidation. The *cis*-double bonds present in both the 9-Z-C₁₆ and 9-Z-C₁₈ fatty acids do not prevent binding of these fatty acids in the correct orientation for oxidation: the C7 and C8 carbons are still the closest to the heme.

The insertion of the fatty acid into the active site of P450_{BioI} occurs at the junction between several structural elements, notably sections of the β -1 sheet, the region between the F- and G-helices and the region C-terminal of the B-helix. In addition to these features, the binding site involves the interactions of various portions of the B₂-, F-, and I-helices and the loop C-terminal of the L-helix. The phosphopantetheine linker of ACP, bound to Ser-1057,[†] makes several contacts with P450_{BioI} (Fig. 3A). Specifically, the carbonyl oxygens of the phospho-

pantetheine linker form hydrogen bonds with Arg-60 (2.8 Å) and Arg-173 (2.8 Å) of P450_{BioI}, which are oriented via additional hydrogen bonds with other P450_{BioI} residues (Arg-173 with Pro-62 (2.9 Å) and Arg-60 with Gln-305 (2.7 Å)). The amide nitrogen atoms of the phosphopantetheine linker form hydrogen bonds with the phenol oxygen of Tyr-307 (3.2 Å) and the backbone carbonyl of Ile-169 (3.0 Å). The phosphate group of the phosphopantetheine linker interacts with several P450_{BioI} residues via water mediated hydrogen bonds, including Thr-61, Glu-65, Gln-305, and Ser-1057 (Fig. 4A). The hydroxyl group of the phosphopantetheine linker also interacts with protein residues via water mediated hydrogen bonds, specifically P450_{BioI} residues Lys-36, Pro-38, and Tyr-307 and Ser-1057 (Fig. 4B).

Previous biochemical investigations have shown that although P450_{BioI} is capable of oxidizing free C₁₄ and C₁₆ fatty acids in the center of the fatty acid chain (11, 16), the majority of oxidation occurs toward the methyl terminus. Arg-60 and Arg-173, present in the fatty acid entry tunnel of P450_{BioI}, represent plausible sites for adventitious interactions with the carboxyl groups of free fatty acids (distances of 12.4–13.5 Å and 16.0–16.9 Å from the carboxyl oxygen to C7/C8 carbons, respectively). Depending on the fatty acid chain length, such interactions would place the carbon atoms close to the methyl chain terminus of the fatty acids near the heme for oxidation in the case of C₁₄ (Arg-60) and C₁₆ fatty acids (Arg-173). In addition, docking studies of hexadecanoic acid with P450_{BioI} using Autodock (27) indicate that favorable docking conformations exist over the plane of the heme, with the central carbons of the fatty acid chain placed above the heme iron (Fig. S2). The lack of anchoring residues for such orientations would also explain the appearance of cleavage products other than pimelic acid (unpublished results, M.J.C. and J. De Voss), while the different docking orientations observed for the fatty acids explain the relatively low enantiospeci-

[†]ACP residues are numbered beginning at 1001 to avoid confusion with P450_{BioI} residues.

Fig. 4. Phosphopantetheine linker region of the P450_{BioI}-ACP complex (phosphopantetheine linker and C₁₄ fatty acid shown in cyan, waters shown in green, detergent—n-heptyl β -D-thiogluconopyranoside—shown in yellow, distances averaged across the four molecules in the crystallographic asymmetric unit, hydrogen bonds shown as dashed black lines). (A) Residues interacting with the phosphate group via water-mediated hydrogen bonds. (B) Residues interacting with the hydroxyl group of the phosphopantetheine linker via water-mediated hydrogen bonds ($\approx 90^\circ$ rotation of A).

Fig. 5. Two regions (A and B) of hydrogen bonding and salt bridge interactions between P450_{BioI} (shown in gray) and ACP (shown in blue) oriented along the α -2_{ACP} helix, with selected residues and hydrogen bond/salt bridge distances indicated as dashed black lines (averaged across the four molecules in the crystallographic asymmetric unit with the exception of Lys-69, see text).

ficity observed for the in-chain cleavage process (12). From the orientations of the fatty acid chain relative to the heme in the P450_{BioI}-ACP structure, both the C7 pro-*R* and pro-*S* hydrogen atoms of the fatty acid are presented favorably for oxidation in different molecules in the crystallographic asymmetric unit. This would lead to low stereochemical selectivity for the initial hydroxylation, which was observed in studies using free fatty acid intermediates (12).

P450_{BioI}-ACP Binding. ACP binding to P450_{BioI} occurs through interactions at the C terminus of helix α 1_{ACP}, the N terminus of helix α 3_{ACP} and through residues that are C-terminal of the phosphopantetheine bearing Ser-1057 on helix α 2_{ACP} (Fig. 5). Most interactions involve salt bridges between acidic residues on ACP and basic residues on P450_{BioI}, including Gln-1035, Asp-1059 and Glu-1062 to Lys-69 (3.2 Å, 2.8 Å and 2.6 Å respectively), Asp-1077 to Lys-36 (2.7 Å) and Glu-1069 to Arg-175 (2.9 Å). In addition, several non-polar residues from both ACP (Ile-1075 to Lys-36: 2.7 Å) and P450_{BioI} (Phe-34 and Leu-35 to Asp-1077: 3.4 Å and 3.5 Å) establish hydrogen bonds via their backbone amide groups. P450_{BioI} residue Lys-69 forms hydrogen bonds to ACP residues in several conformations that differ in the four crystallographically independent molecules in the asymmetric unit.

Overall ACP Structure. The structure of *E. coli* ACP bound to P450_{BioI} superimposes well onto both the apo- (28) and C₁₀-acylated forms of *E. coli* ACP (29) (PDB codes 1T8K and 2FAE, rmsd 1.1 Å and 0.9 Å respectively, Fig. 6). The major difference between C₁₀-acyl ACP and the P450_{BioI}-bound form of ACP is the orientation of the phosphopantetheine bearing Ser-1057 side chain, which points toward or away from the ACP core, respectively. This affects the orientation of the fatty acid, which is accommodated between helices α 2_{ACP} and α 4_{ACP} in the free C₁₀-acyl ACP, whereas it projects into P450_{BioI} in the P450-ACP

Fig. 6. Overlay of C_α traces of *E. coli* ACP and phosphopantetheine linked fatty acids from the P450_{BioI}-ACP C₁₄ complex (shown in blue), apo-ACP (shown in pink) and C₁₀ acyl-ACP (shown in orange); the P450_{BioI} protein surface is displayed in gray and the heme in red.

complex. Interestingly, in the P450_{BioI}-ACP complex a detergent molecule binds at the position corresponding to the entry point of the fatty acid in C₁₀-acyl ACP (Fig. 4). Insertion of the ACP bound fatty acid into P450_{BioI} most likely follows the initial binding of ACP to P450_{BioI}, mediated through protein-protein interactions. Generation of P450_{BioI} complexes with apo-ACP or ACP bearing the phosphopantetheine linker alone were not successful, which indicates that the presence of the fatty acid is necessary to afford a stable P450_{BioI}-ACP complex.

Relevance of the P450_{BioI}-ACP Complex in Vivo. As the ACP ligand in the complex described here originates from *E. coli* and is not the natural *B. subtilis* ACP, it is important to clarify the relevance of this complex. An overlay of the solution structure of *B. subtilis* ACP and the ACP chain in the complex (2.2 Å rmsd, Table S2) indicates that the interactions for *B. subtilis* ACP would be identical for four of the six amino acids observed with *E. coli* ACP, with only Arg-1035 changed to a glutamine and Asp-1069 changed to a glutamate in the *E. coli* ACP structure (30). The *B. subtilis* ACP-P450_{BioI} complex forms *in vitro* under the same conditions used for the *E. coli* ACP-P450_{BioI} complex, although crystallization of this complex has so far proven elusive. Sequence alignment of P450_{BioI} with homologous P450s from other *Bacillus* species also shows that all residues identified in binding or active site architecture are conserved or are replaced by conservative amino acids (Fig. S3), which supports similar acyl-ACP binding and turnover by these P450s (31–33).

Implications for Other CP Binding P450s. With the availability of the P450_{BioI}-ACP complex structure as a blueprint, a homology model of the complex of P450 OxyB and the PCP-7 unit of the vancomycin biosynthetic non-ribosomal peptide synthetase VpsC was created (34, 35). In this manually docked, low-resolution model, the heptapeptide substrate occupies the expected substrate binding cavity above the OxyB heme without serious clashes with the protein backbone. Furthermore, the prospective phenolic link catalyzed by P450 OxyB is within 4.5 Å of the heme iron. This model suggests that the protein-protein interface of the OxyB-PCP complex may resemble that of the P450_{BioI}-ACP complex, with comparable residues interacting with the phosphopantetheine linker.

Conclusions

We have determined crystal structures of three P450_{BioI} acyl-ACP complexes, which represent the first examples of P450-carrier protein complexes to be structurally characterized. The P450_{BioI} acyl-ACP complexes show the covalently bound ACP-phosphopantetheine linker and the attached fatty acid inserted into P450_{BioI} in a hydrophobic binding pocket above the heme, where the fatty acid chain adopts a highly kinked U-shaped conformation over the heme iron. In this conformation, the C7 and C8 carbons of the fatty acid chain are presented close to the heme iron for oxidation, independent of the fatty acid chain

length, thus enabling the formation of a C₇-diacid via a multistep in-chain cleavage reaction. The ability of P450_{Biol} to oxidize both saturated and unsaturated fatty acids of different lengths is demonstrated, as the structures of P450_{Biol}-ACP bearing C₁₄, 9-Z-C₁₆ and 9-Z-C₁₈ fatty acids all present the C7 and C8 carbons above the heme for oxidation. These complexes provide the first structures of a carbon-carbon bond cleaving P450. Moreover they offer valuable insight into the potential binding of related carrier proteins to other P450s, such as the vancomycin phenolic coupling biosynthetic P450s, as exemplified by the generation of a homology model of the complex between OxyB and a PCP domain linked with OxyB's heptapeptide substrate. This knowledge greatly improves the prospects for further structural characterization of the important Oxy biosynthetic proteins, which would in turn allow specific modifications to be introduced into the substrates in the vancomycin biosynthetic pathway.

Materials and Methods

Protein Expression and Purification. For full experimental details, see *SI Text*. Briefly, the coding region for ACP from *E. coli* was cloned into expression vector pET28a(+) (Novagen), with an N-terminal 6xHis tag and a thrombin cleavage site and overexpressed in *E. coli* strain C41(DE3). The apo-ACP was purified at 4 °C by Ni-affinity chromatography followed by precipitation at pH 3.9 and resuspension of the pellet in 1.0 M Tris-HCl (pH 7.4). The coding region for P450_{Biol} from *Bacillus subtilis* was cloned into expression vector pET24b(+) (Novagen), with a C-terminal Strep-II tag and overexpressed in *E. coli* strain BL21(DE3). Selenomethionine labeled P450_{Biol} was expressed using the same construct in *E. coli* strain BL21(DE3) grown in minimal media, supplemented with all amino acids except methionine, plus 50 mg/L selenomethionine.

ACP Acylation and Complex Formation. For full experimental details, see *SI Text*. Briefly, acyl-ACP was prepared in a two-step procedure, firstly converting apo-ACP in the presence of purified, recombinant acyl carrier protein synthase (AcpS) and CoA for 2 h at 37 °C to *holo*-ACP, which was in turn converted the appropriate acyl-ACP using purified, recombinant acyl-ACP synthetase (Aas), fatty acid and ATP for 12 h at 22 °C. The cleared lysate from P450_{Biol} overexpression was then incubated with the appropriate acyl-ACP for 30 min at 4 °C, before the P450_{Biol}-ACP complex was purified at 4 °C by nickel-affinity and streptactin-affinity chromatography. Before crystallization, the complex was concentrated to 8–10 mg/ml.

Crystallization, Data Collection, and Structure Determination. All P450_{Biol}/acyl-ACP complexes were crystallized using the hanging-drop vapor diffusion method by mixing 1 μl of protein—8 mg/ml protein in buffer (0.1 M Tris-HCl [pH 7.4] and 0.15 M NaCl)—with 1 μl of reservoir solution containing 0.1 M Na Hepes (pH 6.5), 0.15 M Li₂SO₄, 0.25 M NaCl, 19% PEG 4000, and 0.2% *n*-heptyl β-D-thioglucoopyranoside. Reddish crystals (rods ≈ 300 μm long) formed over a period of 5–10 days at 4 °C. For cryoprotection, crystals were passed through the reservoir solution supplemented with 20% glycerol and flash-cooled in liquid nitrogen. Diffraction data were collected at beamline X10SA at the SLS, with the crystal kept at 100 K and processed using the XDS program suite (36). All crystals are in space group P1 with four P450_{Biol}-ACP complexes per asymmetric unit. Selenomethionine labeled protein crystals were used for phasing by seleno single anomalous diffraction (2.3 Å resolution). Forty selenium positions were identified by the program SHARP (37) and, after density modification, clearly interpretable electron density maps were obtained. An initial protein model (80% of the combined complex sequence) was built automatically by the program ARP/wARP (38) and extended manually in the program COOT (39) following simulated annealing in the program CNS (40). During several cyclic rounds of refinement with the program REFMAC (41) and manual rebuilding, fatty acid plus phosphopantetheine linker, heme, and solvent molecules were included in the model. The native crystal structures were built manually using COOT, with cyclic rounds of refinement with REFMAC. Structure-based sequence alignments were carried out with SSM (42) as implemented in COOT, with comparisons to known structures performed using DaliLite (43). All structural figures were prepared using PyMol (44).

SI Text. See SI text for crystallographic data tables, structural alignment of P450_{Biol} and ACP with related structures; sequence alignment of homologous P450 proteins, initial electron density maps for the three different fatty acid chains, comparison of the P450_{Biol} crystal structure with a published P450_{Biol} homology model (45), docking of hexadecanoic acid and P450_{Biol}, protein expression and purification protocols, ACP-acylation protocol and P450_{Biol}-ACP complex formation/purification protocol, and details of OxyB-PCP model generation.

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