Crystal Structure of 2-Nitropropane Dioxygenase Complexed with FMN and Substrate

IDENTIFICATION OF THE CATALYTIC BASE\textsuperscript{a,b}

Received for publication, February 22, 2006, and in revised form, May 1, 2006. Published, JBC Papers in Press, May 8, 2006, DOI 10.1074/jbc.M601658200

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Nitroalkane compounds are widely used in chemical industry and are also produced by microorganisms and plants. Some nitroalkanes have been demonstrated to be carcinogenic, and enzymatic oxidation of nitroalkanes is of considerable interest. 2-Nitropropane dioxygenases from Neurospora crassa and Williopsis mrakii (Hansenula mrakii), members of one family of the nitroalkane-oxidizing enzymes, contain FMN and FAD, respectively. The enzymatic oxidation of nitroalkanes by 2-nitropropane dioxygenase operates by an oxidase-style catalytic mechanism, which was recently shown to involve the formation of an anionic flavin semiquinone. This represents a unique case in which an anionic flavin semiquinone has been experimentally observed in the catalytic pathway for oxidation catalyzed by a flavin-dependent enzyme. Here we report the first crystal structure of 2-nitropropane dioxygenase from Pseudomonas aeruginosa in two forms: a binary complex with FMN and a ternary complex with both FMN and 2-nitropropane. The structure identifies His\textsuperscript{152} as the proposed catalytic base, thus providing a structural framework for a better understanding of the catalytic mechanism.

Nitroalkanes are widely used in industry, because they are useful as intermediate compounds in chemical synthesis (1, 2). They are also synthesized by various organisms. Many antibiotics, e.g. chloramphenicol and azosycin, contain nitro groups, and many leguminous plants produce nitro toxins such as 3-nitro-1-propionic acid and 3-nitro-1-propanol (3). However, many nitroalkanes are expected to be toxic, and some have been shown to be carcinogenic (4–10). For example, 2-nitropropane causes the formation of both 8-hydroxy- and 8-aminoguanine (12) and a yeast (13). The atomic coordinates and structure factors (code 2GJL and 2GJN) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

The two enzymes have similar molecular masses of ~40 kDa, but their prosthetic groups are different. FMN and FAD are found in the N. crassa and W. mrakii (H. mrakii) enzymes, respectively (14, 15). The ncd-2 gene encoding for 2-nitropropane dioxygenase in N. crassa has been cloned and expressed in Escherichia coli (16). The heterologously expressed enzyme was found to be a homodimer containing 1 mol of non-covalently bound FMN per mol of subunit (16). A steady-state kinetic analysis showed that the preferred substrates for the enzyme are anionic nitronates as compared with neutral nitroalkanes and that the enzyme has broad substrate specificity that is independent of substrate size (16).

Despite many interesting features, no three-dimensional structure has yet been reported for any 2-nitropropane dioxygenase. Because all previous biochemical works have been done on the enzymes from N. crassa and W. mrakii (H. mrakii), the genomic DNAs of N. crassa and W. mrakii (H. mrakii) were not commercially available. Therefore, we chose 2-nitropropane dioxygenase from Pseudomonas aeruginosa as part of the laboratory-scale structural genomics project. P. aeruginosa 2-nitropropane dioxygenase is a 328-residue protein (M, 34,819), showing 23% sequence identity to 2-nitropropane dioxygenase from N. crassa. It also shows significant levels of sequence similarity to putative 2-nitropropane dioxygenases from various sources. In this study, we have overexpressed 2-nitropropane dioxygenase from P. aeruginosa in E. coli, crystallized it, and solved its structure, representing the first structure of any 2-nitropropane dioxygenase. We describe the structure in two forms: (i) a binary complex with FMN and (ii) a ternary complex with FMN and 2-nitropropane. This study provides detailed structural information on the binding mode of the cofactor and substrate. The structure also suggests that the highly conserved His\textsuperscript{152} is the likely candidate for the catalytic base, thus allowing a better understanding of the catalytic mechanism of 2-nitropropane dioxygenase family.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The PA1024 gene encoding 2-nitropropane dioxygenase from P. aeruginosa was amplified by PCR using the genomic DNA of P. aeruginosa strain PAO1 as template. The forward and reverse oligonucleotide primers were designed using the published genome sequence (17) as 5'-G GAA TTC CAT ATG GGC GTG TTC CGT ACC CGT TT-3' and 5'-CCG CCG CTC GAG GAC CCC GGC GAG CAT GCC C-3', respectively. The underlined bases

\textsuperscript{a} This work was supported by the Korea Ministry of Science and Technology (Grant NRL-2001). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{b} The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S7 and Tables S1 and S2.

\textsuperscript{1} Recipients of the BK21 fellowship from the Korean Ministry of Education.

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denote the NdeI and XhoI cleavage sites. The PCR product was digested with NdeI and XhoI and was then inserted into the NdeI/XhoI-digested expression vector pET-21a(+) (Novagen). This vector construction adds an eight-residue tag (LEHHHHHH) to the C terminus of the gene product to facilitate protein purification.

The recombinant enzyme was expressed in E. coli C41(DE3) cells (18) using Luria-Bertani broth. Protein expression was induced by 1 mM isopropyl 1-thio-D-galactopyranoside, and the cells were incubated for an additional 22 h at 20 °C following growth to mid-log phase at 37 °C. The cells were lysed by sonication in a lysis buffer (20 mM Tris-HCl at pH 7.9, 500 mM NaCl) containing 50 mM imidazole. The crude lysate was centrifuged at ~36,000 × g for 60 min.

The supernatant was applied to a Ni2+-chelated HiTrap chelating column (Amersham Biosciences). The protein was eluted with the lysis buffer containing 500 mM imidazole. Next, gel filtration was performed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham Biosciences) that was previously equilibrated with buffer A (50 mM Tris-HCl, pH 7.5) containing 100 mM sodium chloride. At the third chromatography step, the protein was loaded onto a mono Q HR5/5 ion-exchange column (Amersham Biosciences) that was previously equilibrated with buffer A, and the protein was eluted with a linear gradient of 0–1.0 M sodium chloride.

The selenomethionine (SeMet)3-substituted protein was expressed in E. coli B834(DE3) cells, using the M9 cell culture medium containing extra amino acids. The procedure for purifying the SeMet-substituted protein was the same except for the presence of 10 mM dithiothreitol in all buffers used during purification steps.

**Enzyme Assay and Mutagenesis—2-Nitropropane dioxygenase activity was assayed with 10 mM 2-nitropropane (anionic form) as a substrate. The reaction was carried out at 30 °C in a final volume of 1 ml of Britton-Robinson’s buffer (pH 6.5) (a mixture of 16 mM phosphoric acid, 16 mM acetic acid, and 16 mM boric acid, whose pH is adjusted with NaOH), and the amount of nitrite was determined with sulfanilamide and N-(1-naphthyl)ethylenediamine by measuring the absorbance at 540 nm (19). One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of nitrite per minute. Protein concentrations were measured with a Bio-Rad protein assay kit.

H152A and S288A mutants were prepared using the QuikChange site-directed mutagenesis kit (Stratagene). The mutations were confirmed by DNA sequencing.

**Crystallization—Crystals were grown by the hanging-drop vapor diffusion method at 24 °C by mixing equal volumes (2 μl each) of the protein solution (at 13 mg ml−1 concentration in a buffer consisting of 50 mM Tris-HCl at pH 7.5, 1.4 mM β-mercaptoethanol, and 100 mM sodium chloride) and the reservoir solution.

To grow native crystals, we used a reservoir solution consisting of 0.1 M HEPES at pH 7.0, 5.0% (w/v) tacsimate at pH 7.0, and 10% (w/v) polyethylene glycol monomethyl ether 5000. Crystals grew to approximate dimensions of 0.1 mm × 0.1 mm × 0.07 mm within a day. To grow crystals of the native protein complexed with 2-nitropropane (neutral form), 160 mM 2-nitropropane solution (dissolved in 50 mM Tris-HCl, pH 7.5, and 100 mM sodium chloride) was mixed with the protein solution in a 1:20 volume ratio, resulting in an ~24-fold molar excess of 2-nitropropane over the enzyme. The protein mixed with 2-nitropropane was incubated for 30 min at 4 °C before crystallization. Crystals of 2-nitropropane-bound form grew under identical reservoir conditions as the native crystals within a day.

The SeMet-substituted protein was crystallized under conditions identical to those for the native crystals except for the presence of 10 mM dithiothreitol in the protein solution. The crystals of the SeMet-substituted grew to approximate dimensions of 0.1 × 0.1 × 0.07 mm within a few days.

**X-ray Data Collection**—Table 1 summarizes the statistics of data collection. A crystal of the SeMet-substituted protein was dipped into a cryoprotectant solution for a few seconds and then frozen in the cold nitrogen gas stream at 100 K. The cryoprotectant solution consisted of 0.1 M HEPES at pH 7.0, 5% (w/v) tacsimate at pH 7.0, 10% (w/v) polyethylene glycol monomethyl ether 5000, and 20% (w/v) glycerol. X-ray diffraction data were collected at 100 K at three different wavelengths using an Area Detector System Corporation Quantum 210 charge-coupled device detector at the experimental station NW12A of Photon Factory, Tsukuba, Japan. The crystal was rotated through a total of 180° with a 1.0° oscillation range per frame. The raw data were processed and scaled using the program suite HKL2000 (20). The SeMet-substituted crystal belongs to the space group P3121, with unit cell parameters of a = b = 93.28 Å, c = 81.05 Å, α = β = 90°, γ = 120°. A monomeric molecule is present in the asymmetric unit, with the calculated crystal volume per protein mass (Vmol) of 2.84 Å3 Da−1 and the solvent content of 57%. X-ray diffraction data of the native protein as a ternary complex with both FMN and 2-nitropropane were collected similarly as above. X-ray diffraction data of the native protein as a binary complex with FMN were collected using a Bruker charge-coupled device detector at the beamline BL-6B of Pohang Light Source, Pohang, Korea. The raw data were processed and scaled using the program suite HKL2000 (20).

**Structure Determination and Refinement**—Phasing statistics are summarized in Table 1. Seven of the eight expected selenium atoms in each monomer of the recombinant enzyme, except the SeMet residue at the N terminus of the polypeptide chain, were located with the program SOLVE (21), and the selenium sites were used to calculate the phases with the program RESOLVE (22). The MAD-phased electron density map was of sufficient quality to allow automatic model building by RESOLVE (22), giving an initial model that accounted for ~36% of the backbone of the polypeptide chain with much of the sequence assigned. Subsequent manual model building was done using the program O (23).

The model was refined with the program CNS (24), including the bulk solvent correction. 10% of the data were randomly set aside as the test data for the calculation of Rfree (25). Several rounds of model building, simulated annealing, positional refinement, and individual B-factor refinement were performed. Subsequently, this model was used to refine structures of the native enzyme as a binary complex with FMN and as a ternary complex with both FMN and 2-nitropropane. Refinement statistics are summarized in Table 1. All of the models have excellent stereochemistry, as evaluated by the program PROCHECK (26).

**RESULTS AND DISCUSSION**

**Protein Expression and Enzymatic Assay**—When 2-nitropropane dioxygenase from P. aeruginosa was expressed in E. coli as a fusion with the C-terminal tag (LEHHHHHHH), ~20% of the expressed protein was present in the soluble fraction. The yield was typically ~1.2 mg of the purified enzyme per liter of culture. This low yield was due to a considerable loss of the enzyme that occurred during the concentration step before gel filtration. Without concentrating the protein solution and by repeated gel filtration steps, we could significantly improve the yield (supplementary Table S1). The recombinant enzyme has a native molecular mass of ~37 kDa (calculated monomer mass including the C-terminal tag = 35,884 Da), as estimated by our dynamic light scatter-
ing analysis. This result indicates that P. aeruginosa 2-nitropropane dioxygenase is monomeric in solution, unlike the homodimeric 2-nitropropane dioxygenase from N. crassa (16).

The UV-visible absorption spectrum of the recombinant enzyme in the wavelength range between 300 and 700 nm (Fig. S1A) resembles that of the glucose oxidase containing the oxidized flavin (27). Our structures reveal the presence of non-covalently bound FMN (Figs. 1, 2, and S3). We have also found that the recombinant enzyme exhibits the enzyme dose-dependent 2-nitropropane dioxygenase activities (Fig. S3). We have additionally determined the structure of the ternary complex of FMN and 2-nitropropane. The ternary complex model was refined again at 15.0–2.30 Å data to R_work and R_free values of 18.8% and 23.2%, respectively (Table 1). The model (PDB code 2GJN) accounts for 324 amino acid residues (3–326), 224 water molecules, 1 FMN molecule, and 1 2-nitropropane molecule. N-terminal two residues (Met1–Gly3) and C-terminal two residues (Gly327–Val328) of 2-nitropropane dioxygenase, as well as the C-terminal eight-residue tag, are missing from both models, because there is no electron density. The models of data collection, phasing, and refinement statistics

| Data collection and phasing |
|-----------------------------|
| **Figure of merit** |
| **R** free (%) | 19.1/22.7 | 18.8/23.2 |
| **r.m.s. deviation from ideal geometry** |
| Bond lengths/angles (Å, °) | 0.005/1.18 | 0.005/1.17 |

**Overall Structure**—We have determined the crystal structure of P. aeruginosa 2-nitropropane dioxygenase (Table 1). The model of the binary complex with FMN (Figs. 1 and 2) was refined against 8.0–2.00 Å data to R_work and R_free values of 19.1% and 22.7%, respectively (Table 1). The binary complex model (Protein Data Bank (PDB) code 2GJL) accounts for 324 residues (3–326), 288 water molecules, and 1 FMN molecule in the asymmetric unit. We have additionally determined the structure of the ternary complex with FMN and 2-nitropropane. The ternary complex model was refined against 15.0–2.30 Å data to R_work and R_free values of 18.8% and 23.2%, respectively (Table 1). The model (PDB code 2GJN) accounts for 324 amino acid residues (3–326), 224 water molecules, 1 FMN molecule, and 1 2-nitropropane molecule. N-terminal two residues (Met1–Gly3) and C-terminal two residues (Gly327–Val328) of 2-nitropropane dioxygenase, as well as the C-terminal eight-residue tag, are missing from both models, because there is no electron density. The models of binary and ternary complexes are highly similar to each other, with a root mean square (r.m.s.) deviation of 0.14 Å for 324 C

**TABLE 1**

| Data collection, phasing, and refinement statistics |
|---------------------------------------------|
| **Resolution range (Å)** |
| 8.0-2.00 | 15.0-2.00 |

**Data set**

| Data set | SeMet A1 (peak) | SeMet A2 (edge) | SeMet A3 (remote) |
|----------|----------------|----------------|----------------|
| Wavelength (Å) | 0.97915 | 0.97948 | 0.95000 |
| Resolution range (Å) | 50.2-2.8 | 50.2-2.9 | 50.2-3.0 |
| Unique reflections/redundancy | 17.978/4.3 | 17.638/4.3 | 13.157/4.3 |
| Completeness (%) | 99.7 (97.6) | 99.7 (97.7) | 99.3 (95.7) |
| R_work (%) | 6.5 (37.1) | 6.6 (37.7) | 7.1 (40.1) |
| R_free (%) | 7.4 (27.9) | 7.7 (23.8) | |

**Refinement**

| Data set | Binary complex | Ternary complex |
|----------|----------------|----------------|
| Resolution range (Å) | 8.0-2.00 | 15.0-2.30 |
| No. of reflections used | 26,759 | 17,697 |
| R_work/R_free (%) | 19.1/22.7 | 18.8/23.2 |
| No. of protein atoms (β-factor, Å²) | 2,418 (23.0) | 2,418 (25.7) |
| No. of waters (β-factor, Å²) | 288 (35.2) | 224 (37.1) |
| No. of ligand atoms (β-factor, Å²) | 31 (14.8) | 37 (24.0) |

**Ramachandran plot**

| Most favorable (%) | 92.0 |
| Allowed (%) | 7.6 |
| Generously allowed (%) | 0.0 |
| Disallowed (%) | 0.4 |
of the strands. The inserted domain resides between strand $\beta 8$ and helix $\alpha 15$, the eighth strand-helix pair of the $(\beta/\alpha)_8$-barrel (Fig. 1). The N-terminal portion of the C-terminal helix $\alpha 15$ is located between helices $\alpha 2$ and $\alpha 8$ of the $(\beta/\alpha)_8$-barrel, and its C-terminal portion protrudes out from the main body of the $(\beta/\alpha)_8$-barrel (Fig. 1C). The inserted domain consists of six $\alpha$-helices ($\alpha 9$–$\alpha 14$), two antiparallel $\beta$-strands ($\beta 9$–$\beta 10$), and the loops connecting these secondary structure elements. The strand $\beta 10$ is located at the interface between the two domains, making contacts with the long, winding loop between $\beta 6$ and $\alpha 7$ (Fig. 1, A and C). This loop covers the FMN binding pocket on the C-terminal side of the strands in the $(\beta/\alpha)_8$-barrel. A long loop before helix $\alpha 14$ of the inserted domain buries the bound FMN (Fig. 1, A and C).

Structural Similarities—A DALI structural similarity search (29) with the inserted domain did not reveal any similar structure. The highest Z-score is obtained with the mouse transport protein lin 7 homolog b fragment (PDB code 1Y74; an r.m.s. deviation of 2.8 Å for 37 equiv-
lent Cα positions, a Z-score of 1.6, and a sequence identity of 5%). This result indicates that the inserted domain has a novel fold.

The DALI search (29) with the main domain revealed many similar structures, as one can expect from its common (β/α)8-barrel fold. The highest Z-score is obtained with the inosine-5'-monophosphate dehydrogenase from *Trichomonas fetus* (PDB code 1AK5; an r.m.s. deviation of 2.3 Å for 213 equivalent Cα positions, a Z-score of 21.0, and a sequence identity of 21%) (Fig. S2A) (30). The second highest Z-score is obtained with human guanosine monophosphate reductase (unpublished deposition; PDB code 2BLE; an r.m.s. deviation of 2.8 Å for 216 equivalent Cα positions, a Z-score of 18.8, and a sequence identity of 16%) (Fig. S2B).

**Binding of FMN and Substrate**—Even though we have not included FMN in the crystallization medium, one molecule of FMN is non-covalently bound in the deep active site pocket of the recombinant enzyme (Figs. 2 and S3). FMN is located near the interface between the two domains, on the C-terminal side of the strands in the main (β/α)8-barrel domain (Fig. 1, A and C). The phosphate moiety of FMN, buried completely inside the pocket, is not solvent-accessible, whereas the edge of the isoalloxazine ring opposite to the ribityl chain is partially accessible from the protein surface (Fig. 1, A and C). The bulk of the FMN binding site is contributed by the main (β/α)8-barrel domain. The FMN-binding pocket is lined by the following ten residues of the main domain: Gly22, Gln24, Thr75, Lys124, Asp145, Ala150, Ser178, Gly180, Gly201, and Thr202.

The inserted domain makes relatively little contribution to the FMN binding site. A loop region around Ser288 of the inserted domain covers the dimethylbenzene part of the isoalloxazine ring and contributes to forming the binding site of the substrate 2-nitropropane (Figs. 1A, 1C, 2A, and 2B).

The phosphate moiety of FMN contacts the backbone amide atoms of Ala150, Gly180, Gly201, and Thr202, where the two strands β7 and β8 diverge from each other (Figs. 1A and 2C). The last three residues Gly180, Gly201, and Thr202 constitute the standard phosphate binding motif that is also utilized by other members of the “FMN-dependent oxidoreductase and phosphate-binding enzymes” family of (β/α)8-barrel proteins (31). In *P. aeruginosa* 2-nitropropane dioxygenase, the active site B6–α7 loop also contributes to phosphate binding. No positively charged residue interacts directly with the phosphate moiety to neutralize its negative charge. Arg203 is located close to the phosphate moiety but its side chain points away from the phosphate.

The isoalloxazine ring of the bound FMN displays no significant deviation from the planarity. The O2 atom of the isoalloxazine ring is hydrogen-bonded to the side chain of Lys178 (Figs. 2 and S3B). This is in...
agreement with the previous observation that the N1–C2/H11005/O2 locus of flavin is always in contact (<3.5 Å distance) with a positively charged entity (32). The hydroxyl group of Thr75 is hydrogen-bonded to the N3 and O2 atoms of FMN. In addition, the O4 atom of FMN is hydrogen-bonded to the backbone nitrogen of Gln24 and two water molecules. The hydroxyl O21 atom of the ribityl chain of FMN is hydrogen-bonded to the carbonyl oxygen of Gly22 and the side chain of Lys124. The hydroxyl O31 atom of the ribityl chain is hydrogen-bonded to the side chains of Lys124, Asp145, and Ser178.

We have also determined the structure of P. aeruginosa 2-nitropropane dioxygenase as a ternary complex with both FMN and the neutral form of the substrate 2-nitropropane. The enzyme is sufficiently inactivated under crystallization conditions, and thus the reaction with the neutral form of 2-nitropropane did not proceed in the crystal to an appreciable extent before the crystal was flash-cooled for x-ray data collection within a few days after setting up crystallization. The electron density of 2-nitropropane itself does not clearly distinguish the nitro group from the isopropyl group (Fig. S3C). In one orientation of the substrate, the side chain of Ser288 and one water molecule near the electron density of the substrate make hydrogen bonds with the nitro group (Fig. 2B), whereas Ser288 cannot make a hydrogen bond in the other orientation of the substrate. Therefore, we prefer the former orientation of 2-nitropropane.

The substrate is located underneath the protein surface on the si face of the isoalloxazine ring of FMN (Figs. 2B and S3D), and it is protected from the bulk solvent. This kind of substrate protection is also observed
Structure of 2-Nitropropane Dioxygenase

in other flavin-dependent dehydrogenases and NAD(H)-dependent dehydrogenases (32). In the binary complex with FMN only, a water molecule is present at the substrate position (Fig. 2A). The highly conserved loop between β1 and α2 (part of motif I) covers the re face of the flavin ring.

In both models of the binary and ternary complexes, a single tryptophan residue at position 25 in is in the disallowed region of the Ramachandran plot, although its main-chain conformation is clearly defined by the electron density (Fig. S4). This residue is part of a conserved sequence region and is next to the conserved Gln24 that interacts with the electron density (Fig. S4). This residue is part of a conserved flavin ring.

The inserted domain and is involved in interactions with the highly conserved residues Gly201, and Thr202) that form the standard phosphate-binding motif and stabilizing structural integrity of the active site (Fig. 1).

Gly201, and Thr202) that form the standard phosphate-binding motif and stabilizing structural integrity of the active site (Fig. 1).

Oxidation of the neutral nitroalkanes by 2-nitropropane dioxygenase from N. crassa requires a catalytic base with pK_a of ~7.5, which initiates oxidation of the neutral substrates by abstracting the proton from the substrate α-carbon (16). A similar requirement for a catalytic base that abstracts the α-proton from the nitroalkane substrate was previously established in nitroalkane oxidase from a fungus F. oxysporum, a member of the other family of the nitroalkane-oxidizing enzymes, where the catalytic base was identified as Asp^102 with mutagenesis studies (33). However, the identity of the catalytic base in 2-nitropropane dioxygenases has remained elusive, due to the lack of sequence similarity between 2-nitropropane dioxygenase and nitroalkane oxidase, and the lack of structural information on 2-nitropropane dioxygenase.

Our structures of P. aeruginosa 2-nitropropane dioxygenase reveal that His^152 and Ser^288 are located in proximity of 2-nitropropane on the si side of the isoalloxazine ring of FMN in the active site (Figs. 2A, 2B, and S3D). Between these two candidates for the catalytic base, we propose that His^152 is most likely to act as the essential catalytic base. It belongs to the highly conserved sequence motif IV and is strictly conserved among other bacterial homologs (Fig. S3) and 2-nitropropane dioxygenases from N. crassa and W. mrakii (H. mrakii) (Fig. S5). We suggest that the equivalent residues, His^156 in N. crassa and His^157 in W. mrakii (H. mrakii), play the role of the catalytic base. There are many examples in which histidine plays a similar role as a catalytic base. For example, His^274 (of (S)-mandelate dehydrogenase from Pseudomonas putida acts as the base that abstracts the α-proton to form a carbanion in the initial step of the reaction (Fig. S6) (34).

Ser^288 of P. aeruginosa 2-nitropropane dioxygenase belongs to a semi-conserved region of the inserted domain (Fig. 3). The side chain of Ser^288 recognizes the nitro group of 2-nitropropane via a hydrogen bond (Figs. 2B, 2C, and S3D). This hydrogen bond may increase the acidity of the adjacent carbon, facilitating its deprotonation by His^152. To further characterize the role of Ser^288, we substituted it with alanine. Unfortunately, the mutant enzyme formed inclusion bodies when we overexpressed it in E. coli.

To summarize, we have determined the first crystal structure of 2-nitropropane dioxygenase from P. aeruginosa. The cofactor FMN is bound non-covalently in the active site, and the structure suggests that strictly conserved His^152 likely functions as the catalytic base that initiates oxidation of neutral substrates by abstracting a proton from the α-carbon.
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