The nonoxidative decarboxylation of aromatic acids occurs in a range of microbes and is of interest for bioprocessing and metabolomic engineering. Although phenolic acid decarboxylases provide useful tools for bioindustrial applications, the molecular bases for how these enzymes function are only beginning to be examined. Here we present the 2.35-Å-resolution X-ray crystal structure of the ferulic acid decarboxylase (FDC1; UbiD) from *Saccharomyces cerevisiae*. FDC1 shares structural similarity with the UbiD family of enzymes that are involved in ubiquinone biosynthesis. The position of 4-vinylphenol, the product of *p*-coumaric acid decarboxylation, in the structure identifies a large hydrophobic cavity as the active site. Differences in the B2ε-o5 loop of chains in the crystal structure suggest that the conformational flexibility of this loop allows access to the active site. The structure also implicates Glu285 as the general base in the nonoxidative decarboxylation reaction catalyzed by FDC1. Biochemical analysis showed a loss of enzymatic activity in the E285A mutant. Modeling of 3-methoxy-4-hydroxy-5-decaprenylbenzoate, a partial structure of the physiological UbiD substrate, in the binding site suggests that an ~30-Å-long pocket adjacent to the catalytic site may accommodate the isoprenoid tail of the substrate needed for ubiquinone biosynthesis in yeast. The three-dimensional structure of yeast FDC1 provides a template for guiding protein engineering studies aimed at optimizing the efficiency of aromatic acid decarboxylation reactions in bioindustrial applications.
the metabolic relationship between these two proteins is unclear. In *S. cerevisiae*, both ferulic acid decarboxylase (FDC1) and phenylacrylic acid decarboxylase (PAD1) are required for *in vivo* decarboxylation of substituted cinnamic acids (21). In other microbes, the biochemical functions of UbiX and UbiD may be redundant (29, 32).

Given the interest in using aromatic acid decarboxylases in bioprocessing and metabolic engineering applications, we present the three-dimensional structure of FDC1 from *S. cerevisiae*. The overall structure establishes FDC1 as a UbiD-like enzyme. Crystallization with a decarboxylated reaction product identifies a large apolar cavity as the active site of the enzyme and suggests a catalytic mechanism for the nonoxidative decarboxylation reaction of aromatic substrates.

**MATERIALS AND METHODS**

**Isolation of yeast FDC1 clone and generation of bacterial expression vector.** Genomic DNA was prepared from *S. cerevisiae* YIM993 (33). Yeast cells (1.5 ml) were grown for ~24 h at 30°C in yeast extract-peptone-dextrose (YPD) medium. Cells were pelleted by centrifugation (20,000 × g; 5 min). Following addition of resuspension buffer (2% [vol/vol] Triton X-100, 1% [wt/vol] SDS, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA), the tube was immersed in a dry ice-ethanol bath (2 min) and then transferred to a 95°C water bath (1 min). Following chloroform extraction and centrifugation (20,000 × g; 5 min), the upper aqueous phase was transferred to a microcentrifuge tube containing ice-cold ethanol. After incubation at room temperature (5 min) and centrifugation (20,000 × g; 5 min), the supernatant was removed by vacuum aspiration, and the pellet was washed with 70% (vol/vol) ethanol. After a second wash, the final pellet was air dried and resuspended in TriS-EDTA (TE; pH 8.0) buffer.

For PCR amplification of FDC1 (YDR359W), which does not contain an intron, from the genomic DNA, the following primers were used: YFDC-F, 5'-ATGGAAGGAACATCCAGACCGTTA-3' and YFDC-R, 5'-TATATATATATGATTTTACTGGTTTAAA-3'. The resulting 1,512-bp PCR fragment was gel purified and subcloned into the PCR8/GWFF OPO vector (Invitrogen). The resulting plasmid was fully sequenced by Genewiz (South Plainfield, NJ), using standard M13 forward and M13 reverse primers. For expression of FDC1 in *E. coli*, the coding region was cloned into the Gateway vector pDEST7 (Invitrogen), in which expression of a hexahistidine-tagged protein is driven by a T7 promoter, using LR Clonenas (Life Technologies) for *in vitro* recombination of the entry clone and the destination vector to yield the pDEST7-FDC1 vector, according to the manufacturers’ protocols. Site-directed mutagenesis of Glu285 to alanine (E285A) in yeast FDC1 was performed by QuikChange PCR mutagenesis using the pDEST7-FDC1 vector as the template, with appropriate mutant oligonucleotides.

**Protein expression and purification.** The pDEST7-FDC1 plasmid was transformed into *E. coli* BL21(DE3). Cells were grown at 37°C in Terrific broth supplemented with ampicillin (100 μg ml<sup>−1</sup>) to an *A<sub>600</sub>* of ~0.8 to 1.0. Protein expression was induced by addition of isopropyl-β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.2 mM, and the cells were grown for 16 h at 16°C. Cells were pelleted by centrifugation (10,000 × g; 10 min; 4°C), washed with 1× phosphate-buffered saline (PBS), and then resuspended in buffer A [50 mM Tris-HCl, pH 8.0; 50 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>; 25 mM Tris (2-carboxyethyl-phosphine), 500 mM NaCl, 0.5 mM phenylmethanesulfonyl fluoride, 20 mM β-mercaptoethanol, 10% glycerol, and 10 mM imidazole] containing 100 mM MgCl<sub>2</sub>, 0.2% (vol/vol) Triton X-100, 2 μg ml<sup>−1</sup> DNase, 2 μg ml<sup>−1</sup> RNase, and 4 μg ml<sup>−1</sup> lysozyme. The cells were disrupted by sonication, and cell debris was removed by centrifugation (15,000 × g; 30 min; 4°C). The resultant supernatant was filtered through a 0.45-μm polyethersulfone filter and then applied to a Ni<sup>2+</sup>-nitrotriacetic acid-agarose affinity purification column (GE Healthcare) equilibrated with buffer A. The column was washed with buffer A and the protein eluted with buffer A containing 250 mM imidazole. Size-exclusion chromatography was performed using a Superdex-200 26/60 fast-performance liquid chromatography (FPLC) column equilibrated in buffer A. The final protein concentration was determined spectrophotometrically (*S<sub>280</sub> = 64,455 M<sup>−1</sup> cm<sup>−1</sup>).*

**Protein crystallization and structure determination.** Crystals of yeast FDC1 were obtained by the hanging-drop vapor diffusion method, using a 2-μl drop of protein (6.6 mg ml<sup>−1</sup>) and 5 mM 4-hydroxycinnamic acid (p-coumaric acid) mixed with a 2-μl drop of reservoir buffer (100 mM HEPES, pH 7.5; 10% [wt/vol] polyethylene glycol 6000 [PEG 6000]; and 0.2% [vol/vol] 2-methyl-2,4-pentanediol [MPD]) over a 0.5-ml crystallization reservoir. Single crystals grew within 7 days at 4°C. For X-ray data collection, a crystal was harvested from the mother liquor with a nylon loop, mounted on a goniometer, and directly frozen in a liquid N<sub>2</sub> vapor stream at 100 K. Diffraction data were collected using beamline 19ID at the Advanced Photon Source of the Argonne National Laboratory, with a charge-coupled device (CCD) detector (ADSC Quantum 315R) at a distance of 378.9 mm. Images (180° frames) were collected at 0.979 Å in 1.0° oscillations (a 180° hemisphere), indexed, and scaled using HKL3000 (34). Unit cell parameters and data reduction statistics are summarized in Table 1. The three-dimensional structure of yeast FDC1 was phased by molecular replacement implemented with PHASER (35). The search model for molecular replacement was a homology model of yeast FDC1 generated in I-TASSER (36), using the *P. aeruginosa* UbiD-like protein (PDB entry 4WVS) (30) as the template. Based on the Matthews coefficient, eight protein monomers were expected in the asymmetric unit. The solution from PHASER had a top log likelihood gain (LLG) of 498, a top translation function Z-score (TFZ) of 28.7, and an initial *R<sub>cryst</sub>* value of 55.9%. Refinement of the model was performed using PHENIX (37). Simulated annealing and B-factor refinement of the initial solution produced a model with an *R<sub>cryst</sub>* value of 36.3% and an *R<sub>free</sub>* value of 46.4%. At this stage, manual fitting was initiated using COOT (38). Iterative rounds of model building and refinement, which included TLS refinement, led to a final model with the statistics summarized in Table 1. The final model includes residues 3 to 165, 169 to 188, 208 to 228, and 236

**TABLE 1 Crystallographic statistics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value or description</th>
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<td><strong>Crystal statistics</strong></td>
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<td>Space group</td>
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<td>Cell dimensions (Å) (a, b, c)</td>
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<td><strong>Data collection statistics</strong></td>
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<tr>
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</tr>
<tr>
<td>&lt;I&gt;/&gt; or R&lt;sub&gt;cryst&lt;/sub&gt;</td>
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<tr>
<td>R&lt;sub&gt;cryst&lt;/sub&gt;=R&lt;sub&gt;free&lt;/sub&gt; (%)</td>
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<td>No. of water molecules</td>
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<tr>
<td>No. of ligand atoms</td>
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<td>RMSD for bond angles (°)</td>
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<tr>
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<tr>
<td>Stereochemistry (most favored, % allowed, % outliers)</td>
<td>96.5, 3.3, 0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> R<sub>cryst</sub> = <I>-<I>/>)/<I>/>, where <I>/> is the average intensity over symmetry.  
<sup>b</sup> R<sub>free</sub> is calculated with 5% of the data excluded from refinement.  
<sup>c</sup> R<sub>cryst</sub> was calculated with 5% of the data excluded from refinement.  
<sup>d</sup> Data in parentheses are for the highest shell.
to 503 of chain A, residues 3 to 187, 208 to 228, 235 to 293, and 297 to 503 of chain B, residues 3 to 503 of chain C, residues 3 to 188, 208 to 231, 234 to 293, and 297 to 503 of chain D, residues 3 to 228, 235 to 293, and 297 to 503 of chain E, residues 3 to 503 of chain F, residues 4 to 187, 208 to 229, 235 to 294, and 298 to 503 of chain G, residues 4 to 187 and 207 to 503 of chain H, and 1,381 waters. 4-Vinylphenol, the product of decarboxylation of p-coumaric acid, was modeled into the active sites of chains A, C, E, F, and G.

Docking. Molecular docking of 3-methoxy-4-hydroxy-5-decaprenylbenzoate, a partial substrate analog, into the yeast FDC1 active site was performed using AutoDock Vina (ver. 1.1.2) (39). The ligand was generated by editing the structure of amofruitin B [3-[(2E)-3,7-dimethylocta-2,6-dien-1-yl]-2-hydroxy-4-methoxy-6-(2-phenylethyl)benzoic acid], which was obtained from PDB entry 4A4W (40). The ligand was manually placed into the active site by using the position of 4-vinylphenol as a guide, with docking using a grid box of 30 by 30 by 30 Å and the level of exhaustiveness set to 8.

Assay of phenolic acid decarboxylase activity. Decarboxylation of ferulic acid and p-coumaric acid catalyzed by yeast FDC1 was assayed by high-pressure liquid chromatography (HPLC) (12). The standard reaction mixture (0.5 ml; 30°C) consisted of 25 mM potassium phosphate buffer (pH 6.5), 5 mM dithiothreitol, 0 to 3 mM substrate, and enzyme (0.1 mg). The reaction was started by the addition of the enzyme and quenched by the addition of glacial acetic acid. After quenching, 2-propanol was added in equal volume to the reaction mixture to solubilize the product. An Agilent 1100 series HPLC system using a SymmetryShield C18 column (150 × 3.9 mm; Waters) was used for separation of the substrate and product. Samples (30 μl) were injected for analyses at a flow rate of 1.0 ml min⁻¹. The mobile phase consisted of solvent A (0.1% trifluoroacetic acid) and solvent B (acetonitrile). The elution program was as follows: 0 to 5 min, 0% B; 5 to 35 min, a linear gradient from 0 to 90% B; 35 to 38 min, 90% B; and 38 to 40 min, 0% B. Conversion of substrate to product was determined using a standard curve of either ferulic acid or p-coumaric acid. Steady-state kinetic parameters were determined under standard assay conditions with varied substrate concentrations (0 to 3 mM) by fitting the untransformed data to the equation \( v = \frac{k_{cat}[S]}{K_m + [S]} \), using Kaleidagraph (Synergy Software).

Protein structure accession number. Coordinates and structure factors were deposited in the Protein Data Bank (PDB) under accession number 4S13.

RESULTS
Overall structure of yeast FDC1. For crystallographic analysis of yeast FDC1, the full-length protein (residues Met1 to Lys503) was expressed in E. coli as an N-terminally His₆-tagged protein (monomer molecular mass, ~56.1 kDa) and purified by Ni²⁺-affinity and size-exclusion chromatographies. Yeast FDC1 eluted from the gel filtration column as a dimeric species (molecular mass, ~100 kDa). The three-dimensional structure of yeast FDC1 was determined at a 2.35-Å resolution by molecular replacement with eight molecules (chains A to H) in the asymmetric unit (Table 1).

Yeast FDC1 folds into a three-domain structure (Fig. 1). Domains 1 and 2 form the N-terminal portion of the monomer and are linked to the C-terminal domain 3 by α8. The first domain consists of a central, four-stranded β-sheet (β1a to -d) flanked by two α-helices (α1 and α2). Three of the β-strands are from N-terminal residues (β1a to -c; residues 23 to 69), and one is from C-terminal residues (β1d; residues 312 to 317). Two α-helices (α3 and α4) connect the first domain to the second domain. Domain 2, the largest of the three domains, predominantly contains multiple β-structural features, including a six-stranded antiparallel β-sheet. One side of the β-sheet is capped by α7 and a two-stranded β-sheet (β3a and -b). The third domain also contains a core β-sheet (β4a to -e) that is capped by multiple α-helices (α8 to...
An extended flexible loop leads to the C-terminal helix (α14) and terminus. Dimerization of yeast FDC1 is mediated through domain 3 of each monomer. Domain 3 of one monomer packs against domain 3 and α8 of domain 2 of the adjacent monomer (Fig. 2). The overall shape of the yeast FDC1 dimer resembles a “U,” with domains 1 and 2 of each monomer extending from the domain 3 dimerization region. The C-terminal loop after α14, which extends to α15 of one monomer, exclusively interacts with the α1 and α2 helices of domain 1 in the second monomer.

Comparison of yeast FDC1 with structures in the Protein Data Bank by using the DALI server (41) reveals the highest structural similarity with the UbiD-like PA0254 protein from P. aeruginosa (39% amino acid sequence identity; Z = 52.3; 2.6-Å square mean deviation [RMSD] for 492 Cα atoms; PDB entry 4IWS) and with E. coli UbiD (25% amino acid sequence identity; Z = 39.4; 2.9-Å RMSD for 452 Cα atoms; PDB entry 4IDB). The P. aeruginosa protein (30) adopts a dimeric structure similar to that of yeast FDC1. Crystallographic analysis of the oligomeric state of the E. coli protein suggests a hexamer (PDB entry 2IDB); however, the solution data remain to be reported. Amino acid sequence comparison of yeast FDC1 and the UbiD-related proteins from P. aeruginosa and E. coli indicates that conserved amino acids are distributed throughout the polypeptide (Fig. 3).

Identification of the yeast FDC1 active site. Within the eight molecules of the asymmetric unit, differences in the β2e-α5 loop (Fig. 2) and the presence of electron density near Glu285 were observed (Fig. 4a). In chains C and F, a contiguous electron density for residues 3 to 503 of yeast FDC1 allowed for unambiguous tracing of the β2e-α5 loop (Fig. 2). In other molecules of the asymmetric unit, this loop was disordered.

FIG 2 Dimeric structure of yeast FDC1. Top and side views of the yeast FDC1 dimer are shown. In one monomer, α-helices (blue) and β-strands (gold) are colored as in Fig. 1. In the second monomer, α-helices and β-strands are colored rose and green, respectively. Domains 1 to 3 and the β2e-α5 loop are indicated on the monomer at left. The reaction product 4-vinylphenol is shown as a space-filling model in each monomer.

FIG 3 Multiple-sequence alignment of UbiD proteins. Amino acid sequences of yeast FDC1 (ScFDC1; accession no. AHY75481.1), the UbiD-like PA0254 protein from P. aeruginosa (PaUbiD; accession no. WP_023115699.1), and E. coli UbiD (EcUbiD; accession no. YP_491601.1) were aligned using the MultAlin webpage. The α-helices (blue rectangles) and β-strands (gold rectangles) of yeast FDC1 are depicted above the alignment. Conserved residues are highlighted in orange, with residues in white indicating a variation from the conserved sequence. Residues in the catalytic site (red) and substrate binding site (blue) are indicated. Residues corresponding to the metal binding site of the P. aeruginosa protein are highlighted in green (30).
tron density near Glu285 observed in chains A, C, E, F, and G was modeled as 4-vinylphenol, the decarboxylation product of p-coumaric acid included in the crystallization conditions.

The position of 4-vinylphenol in a large hydrophobic pocket between a8 and the β2e-α5 loop (Fig. 4a) identifies a potential active site in domain 2 of yeast FDC1. The hydroxyl group of the reaction product interacts with Glu285, which is positioned by a charge-charge interaction with Arg175. Multiple apolar residues (Met228, Met286, Thr326, Ile330, Phe397, Phe440, Pro441, and Leu442) form the ligand binding site. Differences in the β2e-α5 loop (i.e., ordered in chains C and F and disordered in other chains) suggest a conformational flexibility of this region of the protein (Fig. 4b). In chains C and F, the β2e-α5 loop encloses the binding site and residues Val188, Ile189, and Lys190 along one side of the hydrophobic pocket to enclose the bound ligand. Because of how the β2e-α5 loop caps the active site, movement of this loop seems to be necessary for substrate entrance to the catalytic site.

In comparison to 4-vinylphenol, 3-methoxy-4-hydroxy-5-hexaprenylbenzoate, the proposed physiological substrate of UbiD in yeast (26), contains an extended polyprenyl group. The apolar binding pocket readily accommodates 4-vinylphenol, but it also appears to be large enough to fit the physiological substrate of yeast FDC1. Docking of a partial substrate analog containing a shorter, two-isoprene extension from the 4-hydroxybenzoate moiety (i.e., 3-methoxy-4-hydroxy-5-decaprenylbenzoate) suggests how the physiological substrate may fit into the active site of yeast FDC1 (Fig. 5). In this model, the 3-methoxy-4-hydroxybenzoate portion of the substrate approximates the binding mode of 4-vinylphenol in the X-ray crystal structure. Binding of this molecule orients the hydroxyl group toward Glu285, with the isoprenoid tail fitting into a pocket that extends to Trp168 (22 Å from Glu285 to Trp168) and then bends toward Trp171 and Met258 (11 Å from Trp168 to Met259). The 33-Å-long tunnel can readily accommodate the 30-carbon side chain of the physiological substrate from yeast ubiquinone synthesis.

**Biochemical analysis of yeast FDC1.** To test the enzymatic activity of yeast FDC1, ferulic acid (4-hydroxy-3-methoxycinnamic acid), p-coumaric acid (4-hydroxycinnamic acid), 3-hydroxycinnamic acid, 2-hydroxycinnamic acid, 3,4-dimethoxycinnamic acid, and 2,5-dimethoxycinnamic acid were screened as substrates. Yeast FDC1 catalyzed the decarboxylation of ferulic acid and p-coumaric acid, with comparable steady-state kinetic parameters for both substrates (Table 2).

To test the potential role of Glu285 as the catalytic residue for yeast FDC1, the E285A mutant of yeast FDC1 was generated by PCR-based site-directed mutagenesis. The E285A protein was expressed and purified as described for the wild-type enzyme. In contrast to the wild-type enzyme, the yeast FDC1 E285A mutant displayed no catalytic activity with any of the substituted cinnamic acids tested.

**DISCUSSION**

The potential uses of aromatic acid decarboxylases in bioindustrial applications drive the interest in understanding how these diverse enzymes function at the molecular level (3–10). The diverse ferulic acid, phenylacrylic acid, and phenolic acid decarboxylases in fungi, yeast, and bacteria provide a wide range of proteins for tailoring physicochemical properties and/or enzymatic activities of these useful biocatalysts (4, 6, 7, 11–22). To aid in the development of these enzymes as tools for biotransformations, we determined the X-ray crystal structure of yeast FDC1.

Structural studies of phenol acid decarboxylases define the following three general types of this enzyme: (i) dodecameric, UbiX-type flavoproteins (23, 24); (ii) the microbial lipocalin-related

<table>
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<tr>
<th>Substrate</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (nmol min&lt;sup&gt;-1&lt;/sup&gt; mg protein&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferulic acid</td>
<td>6.8 ± 0.4</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>7.2 ± 0.5</td>
<td>0.92 ± 0.17</td>
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</table>

Table 2 Kinetic analysis of yeast FDC1<sup>a</sup>

<sup>a</sup>Values are the means ± standard deviations for three experiments.
phenylacrylic acid decarboxylases (27–29); and (iii) the UbiD-like enzymes (30). The X-ray crystal structure of yeast FDC1 clearly establishes this enzyme as a member of the UbiD family of decarboxylases (Fig. 1 to 3). Although three-dimensional structures for each type of phenolic acid decarboxylase have been determined, few insights into the active sites and/or substrate binding sites of these enzymes are currently available. For example, the UbiX-type flavoproteins and the P. aeruginosa UbiD-like protein PA0254 display no detectable activity with phenolic acid substrates (23, 24, 30); however, yeast FDC1 does show decarboxylation activity with both ferulic and p-coumaric acids (Table 2). Screening of a range of substituted cinna- myl-derived substrates indicates the importance of the 4-hydroxyl group for turnover. In comparison to the structurally and biochemically characterized lipocalin-related phenylacrylic acid decarboxylases from L. plantarum, B. pumilis, Bacillus subtilis, and Enterobacter (15, 16, 19, 22), the specific activities of yeast FDC1 for ferulic acid and p-coumaric acid are approximately 1,000-fold lower than those of the microbial enzymes, but with comparable $K_m$ values. This may suggest a lack of optimized fit in the yeast FDC1 active site for these nonphysiological substrates.

Crystalization of yeast FDC1 with a bound reaction product (i.e., 4-vinylphenol) defined the location of the active site for the UbiD-related decarboxylases. In yeast FDC1, the large apolar pocket (Fig. 4a) that forms the binding site of 4-vinylphenol can easily accommodate other aromatic acids, such as ferulic acid, for decarboxylation and is consistent with earlier biochemical studies of the enzyme (21). Amino acid sequence comparison of yeast FDC1 with the UbiD-like PA0254 protein from P. aeruginosa and E. coli UbiD revealed that the substrate binding pockets of these enzymes vary, although the apolar nature of the site is generally conserved (Fig. 3). Structural studies of the UbiD-like PA0254 protein from P. aeruginosa identified a metal binding site consisting of a His and a Glu residue (Fig. 3, green shading) near the proposed active site cleft (30); however, no evidence for metal binding at the corresponding residues of yeast FDC1 was observed. Moreover, computational docking of 3-diprenyl-4-hydroxybenzoate, an analog of the physiological substrate, into the crystal structure of yeast FDC1 (Fig. 5) suggested a possible model for binding of the isoprene-derived tail of the substrate from ubiquinone synthesis. The long (~33 Å) pocket extending from the active site provides sufficient space for the 30-carbon-long isoprenoid tail of 3-methoxy-4-hydroxy-5-hexaprenylbenzoate. Comparison of the open and closed active site forms of yeast FDC1 (Fig. 4b) suggests that movement of the β2e-α5 loop serves to enclose substrates in the hydrophobic active site cavity. In addition to insights into natural and nonnatural substrate binding, the yeast FDC1 structure and subsequent functional analysis suggest an important role for Glu285 in the reaction mechanism of the enzyme.

The position of 4-vinylphenol bound to yeast FDC1 (Fig. 4a) places the substrate hydroxyl group in proximity to the carboxylate side chain of Glu285. Based on the structure, a plausible reaction mechanism for decarboxylation of aromatic acids by FDC1 can be proposed (Fig. 6). The first step of the reaction involves conversion of the phenolic acid into a quinoid. Glu285 functions as a catalytic base to abstract a proton from the substrate hydroxyl group, which begins an electron relay to yield a para-quinone intermediate. Spontaneous decarboxylation of the reaction intermediate leads to release of CO2 and formation of the final reaction product. Mutation of Glu285 to an alanine disrupts the decarboxylation of p-coumaric acid catalyzed by yeast FDC1. The overall reaction is consistent with earlier biochemical studies examining proton transfer during the decarboxylation of aromatic acid substrates (12, 17).

Sequence comparison shows that the acidic nature of the residue corresponding to Glu285 is conserved in both the P. aeruginosa and E. coli UbiD-like proteins, although an aspartate substitution occurs in the E. coli protein (Fig. 3). Similarly, the residue corresponding to Arg175, which positions Glu285 in the yeast FDC1 active site, is invariant in each of these proteins. This conservation suggests a common reaction mechanism for the UbiD family of enzymes. Interestingly, the reaction mechanism of yeast FDC1 also provides an example of convergent evolution of chemistry in enzyme families with distinct structural folds. Structural and biochemical studies of the p-coumaric acid decarboxylase from L. plantarum and the ferulic acid decarboxylase from Enterobacter sp. Px6-4, both of which contain a lipocalin fold, suggest that a catalytic glutamate is required for catalysis (22, 26).

Ultimately, structural information on the various aromatic acid decarboxylases should prove useful in the engineering of modified versions of these enzymes (42–44). Initial studies of bacterial phenolic acid decarboxylases with different substrate specificities showed that the generation of chimeric enzymes could be used to alter product profiles (44). Now, similar studies guided by three-dimensional structures become a logical next step. For example, the $K_m$ values of many aromatic acid decarboxylases fall in the millimolar range (4, 6, 7, 11–22). This may reflect the use of phenolic acid substrates that are significantly smaller than the physiological one (i.e., 3-methoxy-4-hydroxy-5-hexaprenylbenzoate) found in ubiquinone biosynthesis of yeast. Protein engineering to optimize the active site structure for binding and de-
carboxylation of hydroxycinnamates may lead to improved steady-state kinetic properties for bioindustrial applications.

ACKNOWLEDGMENTS

This work was supported by the Chinese National Science Foundation 863 Project (grant 2013AA102801 to O.Y.). Portions of this research were carried out at the Argonne National Laboratory Structural Biology Center of the Advanced Photon Source, a national user facility operated by the University of Chicago for the Department of Energy Office of Biological and Environmental Research (DE-AC02-06CH11357).

M.W.B. was an employee of Conagen, and O.Y. cofounded Conagen and Wuxi NewWay.

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