Crystal Structure of the Oxygen-dependant Coproporphyrinogen Oxidase (Hem13p) of Saccharomyces cerevisiae*

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Coproporphyrinogen oxidase (CPO) is an essential enzyme that catalyzes the sixth step of the heme biosynthetic pathway. Unusually for heme biosynthetic enzymes, CPO exists in two evolutionarily and mechanistically distinct families, with eukaryotes and some prokaryotes employing members of the highly conserved oxygen-dependent CPO family. Here, we report the crystal structure of the oxygen-dependent CPO from Saccharomyces cerevisiae (Hem13p), which was determined by optimized sulfur anomalous scattering and refined to a resolution of 2.0 Å. The protein adopts a novel structure that is quite different from predicted models and features a central flat seven-stranded antiparallel sheet that is flanked by helices. The dimeric assembly, which is seen in different crystal forms, is formed by packing of helices and a short isolated strand that forms a β-ladder with its counterpart in the partner subunit. The deep active-site cleft is lined by conserved residues and has been captured in open and closed conformations in two different crystal forms. A substrate-sized cavity is completely buried in the closed conformation by the ~8-Å movement of a helix that forms a lid over the active site. The structure therefore suggests residues that likely play critical roles in catalysis and explains the deleterious effect of many of the mutations associated with the disease hereditary coproporphyria.

Heme is one of the most common prosthetic groups of proteins in both prokaryotes and eukaryotes. This abundance reflects essential roles in energy metabolism, stress response, oxygen transport, and signal transduction. Accordingly, heme is essential in all organisms tested; heme biosynthetic enzymes have been highly conserved throughout evolution; and mutations in these enzymes cause several human diseases.

The sixth step in the biosynthesis of heme, which is catalyzed by coproporphyrinogen oxidase (CPO), is the oxidative decarboxylation of two propionate side chains of coproporphyrinogen III to form vinyl groups in the product protoporphyrinogen IX. This reaction is carried out by the enzyme from Saccharomyces cerevisiae, which is called Hem13p. In plants, this step of the heme biosynthetic pathway is also required for the production of chlorophyll.

CPO is unusual among heme biosynthetic enzymes in that evolution has selected two very different and unrelated enzymes to catalyze the same reaction (4). Some prokaryotes encode oxygen-independent CPO enzymes, which, as shown by the crystal structure of Escherichia coli oxygen-dependent CPO (5), are radical S-adenosylmethionine enzymes (6, 7) that utilize both a [4Fe-4S] cluster and S-adenosylmethionine as cofactors. In contrast, the oxygen-dependent CPO (odCPO) enzymes (Hem13p) encoded by eukaryotes and some prokaryotes employ a very different mechanism. There are reports of requirements for copper (8) and manganese (9), although other studies found no metal ion or other cofactor dependence (except O2) for odCPO activity (10–12). Regardless of mechanistic details, the first decarboxylation has been shown to be the rate-limiting step for the overall reaction, and transient formation of the 3-carboxyl intermediate harderporphyrinogen has been demonstrated (13, 14).

Mature odCPO is an ~35-kDa protein that exists as a stable ~70-kDa dimer in solution (3, 9, 11, 12, 15–17). It is located in the mitochondria of higher eukaryotes (1, 12, 15, 18, 19), but resides in the cytosol of S. cerevisiae (16). Despite these different intracellular localizations, the mature protein sequence has been highly conserved, with the only significant difference being the presence or absence of mitochondrial targeting sequences that are removed during import (20). Mutations of odCPO cause the autosomal dominant disease hereditary coproporphyria, with >20 different odCPO mutations identified in afflicted families (21).

In an effort to better understand the biochemical basis for catalytic activity and the deleterious effect of clinically identified mutations, we determined the crystal structure of yeast (S. cerevisiae) odCPO/Hem13p in two different crystal forms at
resolutions of 2.0 and 2.4 Å. The enzyme adopts a unique fold that presents two independent active sites on the dimeric structure that are revealed by deep clefts lined with evolutionarily conserved residues. In one crystal form, the cleft is open to bulk solvent, whereas in the other form, movement of two helices closes the cleft entrance to leave a cavity that is the size and shape of a substrate molecule. Finally, mapping of the mutations associated with coproporphyria indicates that most of these changes will destabilize the protein structure or distort the active-site cavity.

Mattterials and Methods

Expression and Purification of odCPO/Hem13p—A cDNA encoding S. cerevisiae Hem13p was amplified using PCR and cloned into the expression vector pET16B. Protein was expressed in E. coli strain BL21(DE3) pLysS (Novagen, Madison, WI) grown in LB medium at 37°C. Induction with 500 μM isopropyl-β-D-thiogalactopyranoside at A600 = 0.5 was followed by growth for 4 h before harvesting by centrifugation and storing of pellets at −80°C. Cell pellets from 6 liters of culture were resuspended in 50 ml of lysis buffer (100 mM NaCl and 50 mM imidazole, and 2 mM β-mercaptoethanol. Fractions containing purified histidine-tagged odCPO/Hem13p were dialyzed against 4 liters of 20 mM Tris, pH 7.5, 2% isopropyl alcohol, and 0.2 M sodium acetate) was mixed with 300 mM NaCl, 50 mM NaPO4, pH 7.0, 150 mM imidazole, and 2 mM β-mercaptoethanol. Fractions containing purified histidine-tagged odCPO/Hem13p were dialyzed against 4 liters of 20 mM Tris, pH 7.5, and 5% (v/v) glycerol; concentrated to 25 mg/ml using Centriprep concentrators (Amicon, Inc., Beverly, MA); and used in the crystallization trials.

Crystallization—Form C crystals, named because the protein was cleaved during crystallization, grew with bipyramidal morphology in sitting drops at 21°C. The reservoir solution (20% polyethylene glycol 3000, 0.1 M HEPES, pH 7.5, and 0.2 M sodium acetate) was mixed with an equal volume of protein solution in the drop. A washed form C crystal ran with an apparent molecular mass of 30 kDa on SDS-polyacrylamide gel (a weaker band was also seen at 10 kDa). N-terminal sequencing of protein from a form C crystal revealed that the first five residues in the protein were DPRNL, indicating that the crystallized protein was cleaved at Asp5 and suggesting that 60–70 of the C-terminal residues had also been removed.

The form I crystals grew with rod morphology in sitting drops at 4, 13, and 21°C. The reservoir solution was 18% polyethylene glycol 8000, 0.1 M HEPES, pH 7.5, 2% isopropyl alcohol, and 0.2 M sodium acetate. Drops were equal parts protein and reservoir solutions. The form II crystals grew with plate morphology in sitting drops at 21°C. The reservoir solution was 2.2 M ammonium sulfate and 0.1 M Tris, pH 8.5. The drops were a 2:1 mixture of protein and reservoir solutions.

X-ray Data Collection—All data were collected from crystals maintained at 100 K. Crystals were suspended in rayon loops and plunged into liquid nitrogen. Form C crystals were transferred directly from the crystallization drop to liquid nitrogen. Form I and II crystals were first transferred to a cryoprotectant solution prior to cooling. Cryoprotectant for form I crystals was composed of 10% (v/v) glycerol added to the reservoir solution, and that for form II crystals was composed of 15% (v/v) glycerol added to a 1:1 mixture of reservoir solution and 3.5 M aqueous ammonium sulfate.

Data were collected from a single form C crystal using a Rigaku RU-300 rotating anode x-ray source with a chromium anode that generates useable X-rays at 2.98 Å wavelength for increased sulfur anomalous signal. The custom-designed diffraction experiment included Osmic conical optics to focus the x-ray beam and a helium path enclosure to minimize air absorption and scattering between the sample and detector (22). High-resolution data for refinement were collected from a second form C
RESULTS AND DISCUSSION

Structure Determination—Crystals of histidine-tagged yeast odCPO/Hem13p were grown in three different space groups. One of these crystal forms is a truncated protein that underwent limited proteolysis during crystallization to yield a protein that started at Asp⁶, as indicated by N-terminal sequencing of protein from a washed crystal. SDS-PAGE analysis gave a molecular mass of ~30 kDa, suggesting that protein in these crystals was also cleaved at the C terminus to remove the last ~60–70 residues. We refer to these crystals as form C (cleaved). Protein from the other two crystal forms (forms I and II) migrated similarly to full-length odCPO/Hem13p on SDS-polyacrylamide gel. N-terminal sequencing indicated that form II crystals were processed at the N terminus to start at Ala³.

We were unable to prepare crystals of selenomethionine-substituted odCPO/Hem13p that diffracted with sufficient strength to allow reliable phase determination. Structure determination was therefore approached using intrinsic anomalous scattering from the eight sulfur atoms in the truncated molecule of form C crystals. This crystal form offered the advantage of a relatively high fraction of sulfur atoms. Data were

<table>
<thead>
<tr>
<th>Crystallographic data and refinement</th>
<th>Crystal</th>
<th>C</th>
<th>I</th>
<th>II</th>
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<td>Source beamline X8C resulted in a model of good stereochemistry and agreement with x-ray terms (see Table I).</td>
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<td>Source. Data were collected from a single form II crystal using an imaging plate area detector on a rotating anode x-ray source with a copper anode. Crystallographic statistics are given in Table I.</td>
<td></td>
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<td>Data collection⁴</td>
<td>RAXIS-Cr</td>
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<tr>
<td>Data processing⁶</td>
<td>d*TREK DENZO</td>
<td>DENZO</td>
<td>DENZO</td>
<td></td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>38.5–2.50 (2.59–2.50)</td>
<td>30.0–1.90 (1.97–1.90)</td>
<td>30.0–2.00 (2.07–2.00)</td>
<td>30.0–2.40 (2.55–2.40)</td>
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<td>No. reflections measured</td>
<td>222,966</td>
<td>661,152</td>
<td>486,505</td>
<td>478,664</td>
</tr>
<tr>
<td>No. unique reflections</td>
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<td>23,022</td>
<td>53,840</td>
<td>87,606</td>
</tr>
<tr>
<td>Completeness (%)</td>
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<td>97.0 (95.6)</td>
<td>98.4 (97.5)</td>
<td>93.9 (90.7)</td>
</tr>
<tr>
<td>∆θ/dθ</td>
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<td>&gt;20 (6.0)</td>
<td>19 (3.2)</td>
<td>15 (2.5)</td>
</tr>
<tr>
<td>Mosaicity</td>
<td>0.4°</td>
<td>0.58°</td>
<td>0.47°</td>
<td>0.83°</td>
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<td>Rmerge (%)</td>
<td>0.057 (0.383)</td>
<td>0.072 (0.580)</td>
<td>0.057 (0.583)</td>
<td>0.077 (0.309)</td>
</tr>
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Refinement statistics

| Resolution (Å) | 30.0–1.90 (1.97–1.90) | 30.0–2.00 (2.05–2.00) | 30.0–2.40 (2.46–2.40) |
| Rcryst (%) | 0.228 (0.251) | 0.207 (0.258) | 0.208 (0.248) |
| Robs (%) | 0.231 (0.290) | 0.254 (0.301) | 0.282 (0.350) |
| Protein residues | 8–265 | 8–265 | 8–265 |
| r.m.s.d. bonds (Å) | 0.011 | 0.016 | 0.021 |
| r.m.s.d. angles | 1.44 (1.38) | 1.50 (1.47) | 1.83 (1.72) |

* Data were collected on Rigaku RAXIS-IV detectors mounted on rotating anode sources with chromium (RAXIS-Cr) and copper (RAXIS-Cu) targets. Other data were collected on ADSC Quantum-4 CCD area detectors at beamlines X8C and X25 at the Brookhaven National Laboratory National Synchrotron Light Source (NSLS).

* Data processing was performed using DENZO/SCALEPACK (44) or d*TREK (45).

* Rcryst = Σ||Fo| − |Fc||/Σ|Fo|, where |Fo| is the observed and |Fc| is the calculated structure factor amplitude.

* Rmerge is the same as Rcryst, calculated with a randomly selected test set of reflections (5% of the total) that were never used in refinement calculations.

* 219 residues were modeled. Disordered regions of the protein that could not be modeled are positions 42–50, 92–106, and 192–206.

* The full-length protein has been modeled as a single chain in all molecules in the asymmetric unit. For form II, residues 91–110 have only diffuse density and were assigned zero occupancies.

* Values for non-Gly and non-Pro residues only.

Crystal and from a form I crystal at the National Synchrotron Light Source. Data were collected from a single form II crystal using an imaging plate area detector on a rotating anode x-ray source with a copper anode. Crystallographic statistics are given in Table I.

Structure Determination and Refinement—The form C crystal structure was determined by sulfur single wavelength anomalous diffraction phasing. SOLVE (23) was used to locate seven sulfur positions using 3.3-Å data and to calculate phases based on this solution. The M value was 0.27, and the overall Z score was 19. Density modification was performed with RESOLVE (24) to produce an electron density map that allowed building of several strands and helices. Phases from this refined model were used to calculate an anomalous difference Fourier map that confirmed the sulfur substructure and found an eighth site. SOLVE and RESOLVE were rerun, fixing the eight sulfur positions, to give a slightly improved map (see Fig. 2). Refinement and rebuilding using 1.9-Å data collected at National Synchrotron Light Source beamline X8C resulted in a model of good stereochemistry and agreement with x-ray terms (see Table I).

The form I and II crystal structures were determined by molecular replacement using the program MOLREP (25) with the CCP4i interface (26). The refined form C model was used to determine the form I structure, which has six molecules in the asymmetric unit. The refined form I model was subsequently used to determine the form II structure, which has six molecules in the asymmetric unit. In both cases, model building with the program O (27) and refinement with REFMAC5 (28) were straightforward (see Table I).

Crystallization and refinement were straightforward (see Table I).
collected using a rotating anode generator fitted with a chromium anode, appropriate optics, and a helium x-ray path (22). The single wavelength anomalous diffraction/solvent-flattened map (Fig. 2) was of sufficient clarity to allow building of an initial model that was subsequently refined to an $R$ factor/$R_{free}$ of 22.8/23.1% using 1.9-Å data collected from another form C crystal at a synchrotron (Table I). This refined model starts at Arg8 and ends at Arg 265. A total of 219 ordered residues are included in the model, and three flexible loops and the 63 C-terminal residues have been omitted; the ordered residues are

**Fig. 3. Structure of odCPO/Hem13p.**

A, ribbon diagram. The chain is color ramped from blue (N terminus) to red (C terminus). Shown is a stereo view of odCPO/Hem13p with secondary structural elements and N/C termini labeled. A and Figs. 4–6 were made using PyMOL (available at www.pymol.org). B, amino acid sequence. The sequences of *S. cerevisiae* Hem13p and human odCPO are shown, with secondary structural elements of the Hem13p crystal structure above. Residues invariant across an alignment of 10 diverse odCPO sequences after alignment with ClustalW (55) are shown on a magenta background. The sequences used in the analysis were from *S. cerevisiae, Drosophila melanogaster, Homo sapiens, Aplysia californica, Mus musculus, Nicotiana tabacum, Synechocystis sp. PCC6803, E. coli, Ralstonia solanacearum, and Agrobacterium tumefaciens* strain C58. Residues that expose at least 10 Å² of accessible surface area to the active-site cavity of the form II structure are indicated with green dots. Residues that lose surface area upon dimer formation are indicated with blue squares. Black diamonds indicate positions of mutations identified in cases of coproporphyria (Table II).
Arg⁸–Ala¹⁴, Gly¹３¹–Lys⁹¹, Asp¹⁰⁷–Lys¹⁹¹, and Gly²⁰⁷–Arg²⁶⁵.

The anomalous scattering of sulfur atoms was used in the classic structure determination of crambin (29). Since then, sulfur anomalous scattering has contributed to a number of structure determinations (30), although only a few of the new structures have resulted from sulfur anomalous scattering data collected on a rotating anode source rather than at a synchrotron (31–34). The primary limitation of this approach is the small anomalous signal obtained from sulfur with conventional copper anode targets. In view of this, it has been suggested that use of a chromium anode, which more than doubles the $f^\ast$ of sulfur, might allow a general approach to crystal structure determination without the need to prepare heavy atom derivatives, modified protein, or synchrotron radiation (22). To the best of our knowledge, this is the first report of a new protein structure determined using sulfur anomalous scattering data collected on a chromium target rotating anode generator. Our experience shows that this approach can work well for a 30-kDa protein that contains eight ordered sulfur atoms and diffracts to 2.5-Å resolution on a rotating anode source.

The structures of form I and II crystals, both of which contain the full-length protein, were subsequently determined using molecular replacement starting with the form C structure. These models were refined at resolutions of 2.0 Å (form I) and 2.4 Å (form II). The $R$ factors/$R_{free}$ values are 20.7/25.4% (form I) and 20.8/28.2% (form II), and the other statistics also indicate that the models have been refined appropriately (Table I).

**Structure Description**—The crystallographic refinements resulted in models for the form I and II structures that start from the first ordered residue (Pro⁴ (form I) and Ala³ (form II)) and extend to the C terminus. odCPO/Hem13p forms a seven-stranded antiparallel $\beta$-sheet that is relatively flat and is covered on both sides by helices (Fig. 3). This structure is essentially identical in all of the crystal forms. Consequently, the form C structure will not be discussed further because, al-

![The odCPO/Hem13p dimer.](image-url)
though this form was important for structure determination of the full-length protein, it lacks more than half of the residues that are visible in the form I and II crystals and does not form the physiologically relevant dimer (discussed below). The two monomers in form I crystals overlap with a root mean square deviation (r.m.s.d.) of 0.6 Å on all pairs of Cα atoms, and the six monomers in form II crystals overlap on each other with r.m.s.d. of 0.7–1.0 Å over all Cα atoms. Form I and II monomers overlap with a r.m.s.d. of –1.5 Å on all pairs of Cα atoms except those before Arg and between Gln and Lys, inclusive. The 12 N-terminal residues project in very different directions in the two crystal forms, apparently because of different lattice constraints. The Gln–Lys loop appears to move significantly between the two crystal forms, in part because, as discussed below, helix H2 serves as a lid that moves to cover the active-site cavity in the form II structure.

odCPO/Hem13p crystallizes as a dimer in which the β-sheets face each other and project above their surrounding helices (Fig. 4). This is consistent with multiple reports that the enzyme is dimeric in solution (3, 9, 15, 16). The dimeric interface buries a total of 2638 Å² of solvent-accessible surface area and includes 11 direct hydrogen bonding interactions between protein atoms. There are also five water molecules buried at the interface, although most of the contact surface is hydrophobic. The 28 residues that lose accessible surface area upon dimerization are derived from helices H5, H7, H8, and H9; strands S1, S2, S3, and S8; and connecting segments. At the center of the interface, strand S8 forms an antiparallel β-ladder with its
2-fold related partner. The relevance of the crystallographic dimer is indicated by its extensive surface area, the location of conserved residues at the interface (Fig. 4C), and the observation that the same dimer is formed by the two monomers in the form I asymmetric unit and the six monomers in the asymmetric unit of form II crystals. The dimeric arrangement appears to be important for structure at the active site since many residues that stabilize dimer formation are close to residues that line the active-site cleft, and failure to dimerize would likely destabilize the active-site conformation.

Active-site Cleft/Cavity—The odCPO sequences have been highly conserved throughout evolution; 76 of the residues (23%) are invariant between 10 highly diverged species that range from cyanobacteria to man (Fig. 3B). The invariant residues are mostly buried in the hydrophobic core or at the dimer interface, whereas the surface-exposed invariant side chains are primarily centered about a deep cleft of the form I crystal structure that appears to house the enzyme active site (Fig. 5). The cleft is sandwiched between one face of the β-sheet (strands S3–S7) and helices H7–H9. These secondary structural elements pack against each other at the base of the cleft. Residues from helix H4 also contribute to the base and to one side of the cleft, and helix H2 is positioned above the cleft in the form I structure, rather like a raised lid.

Remarkably, the active-site cleft is not open to bulk solvent in the form II crystal structure (Fig. 5B). Instead, the top of the cleft has been closed by helix H2, for which equivalent Ca atoms move by 6–9 Å between form I and II structures (Fig. 5D). Helix H8 also moves by ~4 Å, and these two helices pack tightly against each other in the form II conformation to close the active-site cleft. As a consequence of this conformational change, the form II active site is completely sequestered from bulk solvent and encloses a cavity that approximates the size and shape expected for the substrate molecule. The 30 residues that expose accessible surface area to the cavity are indicated with green dots in Fig. 3B, and the importance of the cavity for enzymatic function is further supported by the observation that 18 of these residues are invariant. The completely buried active site explains why substitutions throughout the tetrapyrrole macrocycle limit catalytic turnover (35–37).

Because of the striking match between the dimensions of the enclosed cavity of the form II structure and a substrate molecule, we have modeled coproporphyrinogen into this space. It appears that the few close contacts that result in the model might be relieved by relatively minor changes such as adjustment of side chain rotamer angles. The substrate molecule modeled in this way would also occupy an unimpeded position in the form I structure, and it is included in Fig. 5 for illustrative purposes. We are cautious about proposing specific contacts based upon this crude modeling exercise, although the...
model does suggest some tentative conclusions about substrate binding. The bound tetrapyrole appears constrained to lie with the macrocycle plane oriented approximately as shown in Fig. 5. The specific locations of propionate side chains are not obvious, although candidate binding partners include the two invariant positively charged side chains exposed to the cavity, Arg$^{135}$ and Arg$^{275}$. Other candidate propionate ligands include the invariant polar side chains of Ser$^{72}$, Ser$^{117}$, His$^{131}$, and Asn$^{133}$, as well as a number of main chain amide groups. We speculate that the central NH groups of the four pyrrole rings of the substrate are coordinated by the invariant side chain of Asp$^{374}$, which is the only invariant carboxylate side chain exposed to the cavity and is located near the middle of one face. This possibility is attractive since uroporphyrinogen decarboxylase, which catalyzes the preceding step in heme biosynthesis and provides the best model for cyclic tetrapyrole binding by an enzyme, uses an invariant aspartate side chain to coordinate all four pyrrole NH groups of its substrate (38). Precisely determining the substrate binding geometry and locating the specific sites of oxygen binding and the decarboxylation reaction, which are not currently apparent, will be a priority for our future experiments.

Comparison with Other Structures—As expected, the structure is very different from that of the unrelated oxygen-independent CPO, which is a monomeric two-domain protein whose most prominent feature is a curved parallel $\beta$-sheet (5). Based upon sequence analysis, it was predicted that odCPO/Hem13p would fold as two T-fold domains (39); T-folds are composed of four sequential antiparallel $\beta$-strands and a pair of antiparallel helices between the second and third strands (40). However, the observed odCPO/Hem13p structure is quite different from this prediction. Furthermore, T-fold proteins have a distinctive mode of assembly in which three to five protein subunits form rings of 12–20 $\beta$-strands, and two of these rings pack face to face (40). This results in assembly around a distinctive central tunnel (T-fold for tunnel-fold). Thus, odCPO/Hem13p does not possess significant similarity to the T-fold proteins in the structure of the monomer or in higher order assembly.

A search with DALI (41) found just one structure (LMAJ006828) with significant similarity ($Z = 39$) to odCPO/Hem13p. LMAJ006828 is a hypothetical protein from Leishmania major that was determined by the Structural Genomics of Pathogenic Protozoa Program (depts.washington.edu/sgpp) at 1.4Å resolution and was recently deposited in the Protein Data Bank (code 1VJU). There is no corresponding publication currently available for this structure, and the Protein Data Bank header describes it as having unknown function. LMAJ006828 has the same topology and forms the same dimer as odCPO/Hem13p; 37% of the residues are identical to their structural counterparts in yeast odCPO/Hem13p, including all but 4 of the 76 invariant residues indicated in Fig. 3B. This high level of structural and sequence similarity strongly implies that LMAJ006828 is a coproporphyrinogen oxidase. LMAJ006828 residues are disordered between strands S2 and S3, indicating that this structure is in an open configuration. Indeed, it seems likely that the disordered conformation for the helix H2 loop corresponds to the fully open state, whereas the form I structure seen for yeast odCPO/Hem13p, in which the opening to the active-site cleft is only $-8$ Å between atom centers, is better viewed as midway between the fully open and fully closed conformations.

Clinically Identified Mutations—A number of mutations have been identified that encode the full-length odCPO protein, yet appear to cause hereditary coproporphyria. Because of the high degree of sequence identity (52%) between yeast Hem13p and human odCPO, we have mapped these 19 mutations onto the Hem13p structure (Fig. 6 and Table II). The deleterious effect of most of these mutations can be explained as likely caused by a decrease in stability. These “destabilizing” residues are scattered over the structure, including at the dimer interface. We are unable to explain the deleterious effect of mutation at the surface-exposed positions 169, 182, and 321 (yeast Hem13p numbering), although it is impossible to rule out an effect on stability or perhaps mediation of an as yet unidentified protein-protein contact. In contrast, the mutations at Ser$^{72}$ and Arg$^{275}$ are especially suggestive. These residues are both invariant, and although these substitutions seem unlikely to greatly perturb stability, they would alter the size, shape, and polarity of the active-site cavity.

In summary, the structure explains how many of the mutations give rise to coproporphyria and supports the model that the enzymatic reaction proceeds in an isolated cavity that is formed by conformational change upon binding substrate. If the reaction intermediate is able to reposition within the active-site cavity, this architecture would explain why the first decarboxylation, on the pyrrole A ring, is rate-limiting and rapidly followed by decarboxylation of the pyrrole B ring propionate (13, 14). One potential advantage of the substrate-induced conformational change is that it might generate a specific pathway and binding site for the molecular oxygen cofactor, such as seen for cholesterol oxidase (42, 43). This is an attractive possibility since it would provide a mechanism to protect the highly oxygen-sensitive substrate and product from inappropriate oxidation.

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