model does suggest some tentative conclusions about substrate binding. The bound tetrapyrrole 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^{274}, 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 tetrapyrrole 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 β-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 β-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 β-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 1.4-Å 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; 263/291 Cα atoms superimpose with an r.m.s.d. of 1.0 Å for the monomer, and 526/582 Cα atoms superimpose with an r.m.s.d. of 1.5 Å for the dimer. Note that this dimer is not the same as implied by the asymmetric unit defined in the Protein Data Bank code 1VJU entry, although it is revealed by application of crystallographic symmetry operators and has a much larger interface than the other lattice contacts of the deposited structure. The high level of structural similarity is reflected in the amino acid sequence; 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. 3*B*. 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 conformation. 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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Note Added in Proof—Since acceptance of this manuscript, another report of a protein structure determination by sulfur single wavelength anomalous diffraction phasing using a chromium rotating anode has been published on-line (Chen, L., Chen, L.-R., Zhou, X. E., Wang, Y., Kahsai, M. A., Clark, A. T., Edmondson, S. P., Liu, Z.-J., Rose, J. P., Wang, B.-C., Meehan, E. J., and Shriver, J. W. (2004) *J. Mol. Biol.*, in press.

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