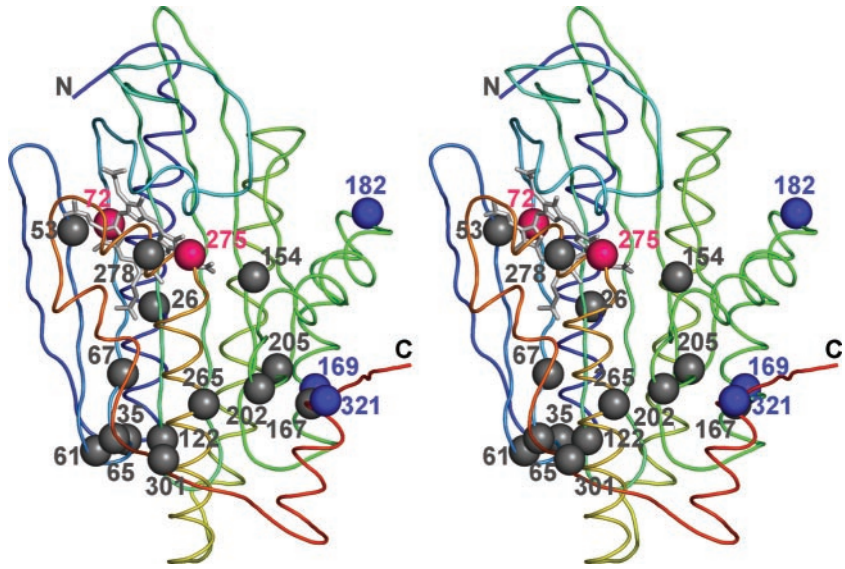

FIG. 6. **Locations of mutations identified in coproporphyria patients.** Shown is a worm representation stereo view of form II (closed) structure in the same orientation as shown in Fig. 3*A*. Sites of mutations identified in patients are shown as *spheres*. Substitutions expected to destabilize the folded protein structure are shown in *gray*. Mutations whose presumed deleterious effect is not easily explained by the structure are shown in *blue*. Mutations at the active-site cleft are shown in *magenta*. The modeled substrate molecule (*white*) indicates the approximate location of the active-site cavity.

TABLE II
Human mutations in full-length odCPO associated with coproporphyria

Yeast residue	Human residue	Human mutation (Ref.)	Comment on structure
Glu^{26}	Gln^{162}	Pro (46)	Destabilizing; residue in helix H1; incompatible with Pro
Asp^{35}	Asp^{171}	Asn (46)	Destabilizing; buried side chain; Oδ1 and Oδ2 H-bond main chain NH
Gly^{53}	Gly^{189}	Ser (47)	Destabilizing; buried residue; no room for Ser side chain
Gly^{61}	Gly^{197}	Trp (48)	Destabilizing; ϕ angle positive; no room for Trp side chain
Glu^{65}	Glu^{201}	Lys (49)	Destabilizing; lose H-bond to Arg^{263}
Gly^{67}	Ala^{203}	Thr (46)	Destabilizing; buried residue; restricted environment for Thr
Ser^{72}	Ser^{208}	Phe (50)	Active site; projects into cavity
Pro^{122}	Pro^{249}	Ser (49)	Destabilizing; buried side chain making extensive contacts
Gly^{154}	Gly^{280}	Arg (51)	Destabilizing; ϕ angle positive; no room for side chain
Gly^{167}	Ala^{293}	Thr (46)	Destabilizing; buried residue; restricted environment for Thr
Leu^{169}	His^{295}	Asp (52)	Surface-exposed residue; may destabilize helix H3–H6 packing
Asp^{182}	Gly^{308}	Val (46)	Surface-exposed residue; may destabilize loop structure
Arg^{202}	Arg^{328}	Cys (50)	Destabilizing; buried residue; H-bonds Glu^{268} and Glu^{289} of dimer partner
Thr^{205}	Arg^{331}	Trp (53)	Destabilizing; in surface pocket unable to accommodate Trp
Arg^{265}	Arg^{391}	Trp (46)	Destabilizing; buried residue making extensive contacts
Arg^{275}	Arg^{401}	Trp (46)	Active site; Trp would restrict cavity
Gln^{278}	Lys^{404}	Glu (54)	Destabilizing; H-bonds CO groups at C terminus of helix H7
Trp^{301}	Trp^{427}	Arg (48)	Destabilizing; buried at dimer interface
Thr^{321}	Arg^{447}	Cys (46)	Surface-exposed residue; no explanation

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. 4*C*), 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. 3*B*). 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. 5*B*). Instead, the top of the cleft has been closed by helix H2, for which equivalent Cα atoms move by 6–9 Å between form I and II structures (Fig. 5*D*). 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. 3*B*, 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