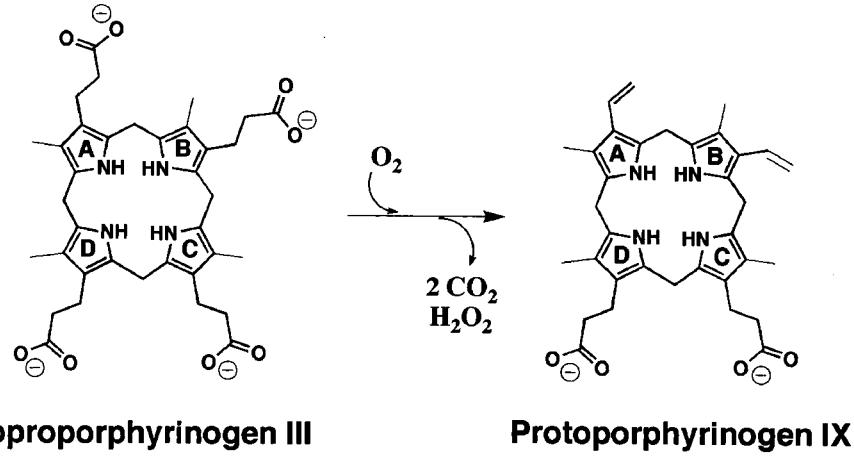

FIG. 1. **Schematic of reaction catalyzed by odCPO/Hem13p.** Propionate side chains on the pyrrole A and B rings are decarboxylated to form vinyl groups and two molecules of carbon dioxide. Molecular oxygen is converted to hydrogen peroxide, presumably via abstraction of a hydrogen atom from each of the propionate/vinyl C-β atoms.

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.

MATERIALS 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 A_{600} = 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 Tris, pH 8.0) and incubated on ice for 1 h with 1.2 ml of 10 mg/ml lysozyme prior to sonication. The membrane fraction was removed by centrifugation at 20,000 × *g* for 30 min. The supernatant was loaded onto a 1-ml Ni^{2+}-nitrilotriacetic acid column (QIAGEN Inc., Chatsworth, CA) at 4 °C. The column was washed with 40 ml of buffer containing 300 mM NaCl, 50 mM $NaPO_4$, pH 7.0, 10% (v/v) glycerol, and 2.5 mM β-mercaptoethanol, followed by elution of the protein in 30 ml of buffer containing 300 mM NaCl, 50 mM $NaPO_4$, pH 7.6, 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 Asp^6 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.

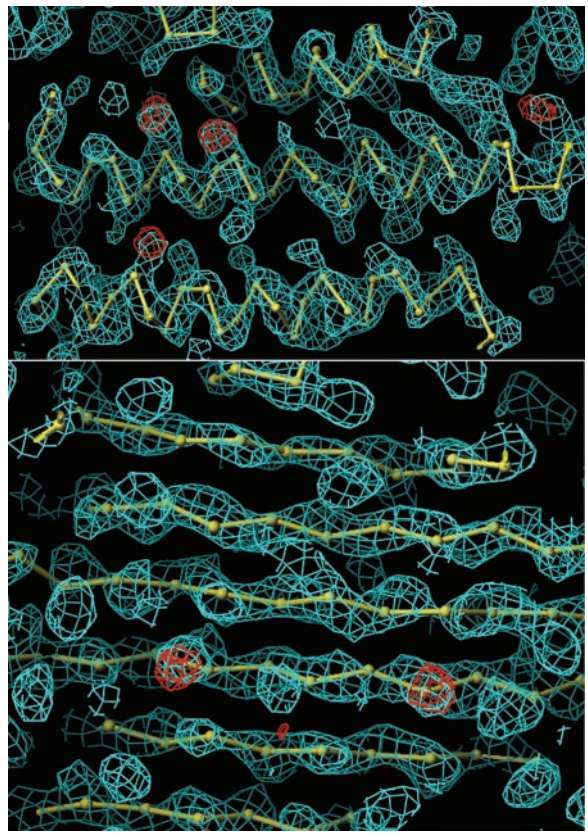

FIG. 2. **Map computed from sulfur anomalous scattering.** The single wavelength anomalous diffraction/solvent-flattened map (*cyan*; 1.3 × r.m.s.d.) was computed at 3.3-Å resolution using data collected on a rotating chromium anode. An anomalous difference map (*red*; 4 × r.m.s.d.) computed using phases derived from the refined model shows the positions of Met and Cys residues. The final refined model (*yellow*) is shown as a C-α trace. *Upper panel*, adjacent helices; *lower panel*, a region of sheet.

Data were collected from a single form C crystal using a Rigaku RAXIS-IV imaging plate area detector on a Rigaku RU-H3R rotating anode x-ray source with a chromium anode that generates useable X-rays at 2.29-Å wavelength for increased sulfur anomalous signal. The custom-designed diffraction experiment included Osmic confocal 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