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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),[1] is the oxidative decarboxylation of two propionate side chains of coproporphyrinogen III to form vinyl groups in the product protoporphyrinogen IX (Fig. 1) (1, 2). The enzyme from *Saccharomyces cerevisiae* is called Hem13p (3). 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-independent 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 O_2) 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 harderoporphyrinogen 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

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The atomic coordinates and structure factors (code 1tk1, 1tkl, and 1tlb) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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[1] The abbreviations used are: CPO, coproporphyrinogen oxidase; odCPO, oxygen-dependent coproporphyrinogen oxidase; r.m.s.d., root mean square deviation(s).