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The enigma of coproporphyrinogen oxidase: How does this unusual enzyme carry out oxidative decarboxylations to afford vinyl groups?

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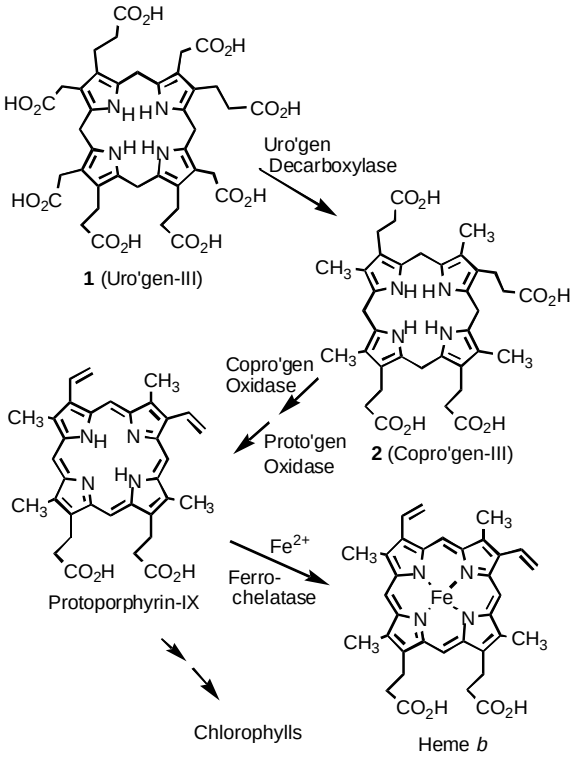
Abstract—A new mechanism is proposed to explain how coproporphyrinogen oxidase performs two oxidative decarboxylations on a porphyrinogen substrate without the aid of cofactors or metal ions in the presence of molecular oxygen.

1. Introduction

Metalloporphyrins play critical roles in all living organisms, and in mammals iron porphyrins (hemes) are crucial components of hemoglobin (oxygen transportation), myoglobin (oxygen storage), cytochromes (electron transport), cytochrome oxidase (reduction of molecular oxygen), catalase (decomposition of hydrogen peroxide), peroxidases (oxidation of organic molecules with peroxide), nitric oxide synthases, etc.[1] In mammals, the biosynthetic pathway leading to heme *b* (protoheme) involves a sequence of eight enzymatic steps starting from glycine and succinyl CoA.[1] The first macrocyclic intermediate, uroporphyrinogen-III (**1**), is generated as a nonconjugated hexahydroporphyrin (Scheme 1). This octacarboxylic acid undergoes a series of four decarboxylations, mediated by uroporphyrinogen decarboxylase in the cytoplasm, to afford coproporphyrinogen-III (copro'gen-III; **2**),[2,3] and this species is then acted upon by the mitochondrial enzyme coproporphyrinogen oxidase to give protoporphyrinogen-IX (proto'gen-IX; **3**).[4] Subsequent dehydrogenation by protoporphyrinogen oxidase gives protoporphyrin-IX and iron insertion then produces heme *b*.[1] Protoporphyrin-IX is also the precursor to the chlorophylls, which fulfill essential roles in photosynthesis.[1] One of the least well understood steps in the pathway leading to the hemes and chlorophylls concerns the conversion of **2** to **3** by copro'gen oxidase.[5,6] The process involves the stepwise degradation of the A and B ring propionic acid side chains to generate the divinylic porphyrinogen **3** via



Scheme 1. Later stages of heme biosynthesis.

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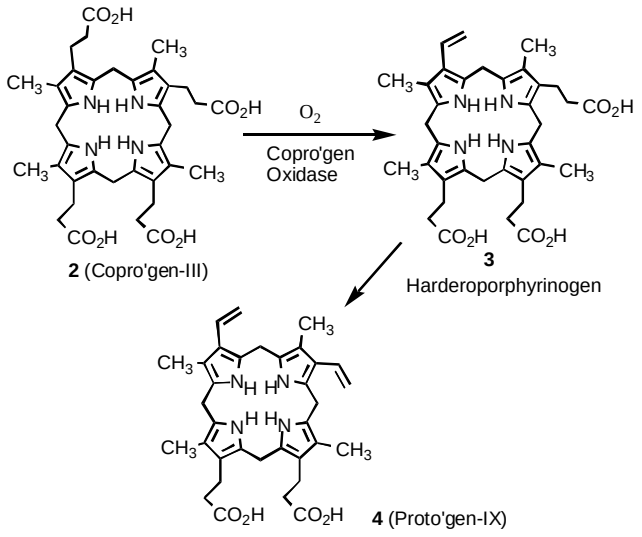
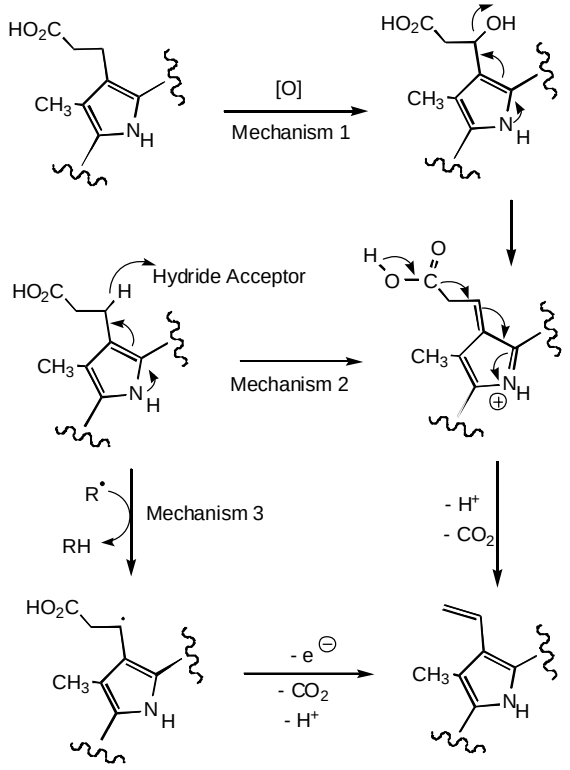


Scheme 2. Stepwise oxidative decarboxylation of coproporphyrinogen-III.

harderoporphyrinogen (**4**) (Scheme 2).[7] Detailed investigations using substrate analogues have established the requirements for substrate recognition and metabolism and this has allowed the development of an active site model for the enzyme.[6,8,9] However, the mechanism by which the propionate side chains are converted to vinyl groups is presently poorly understood. In aerobic organisms, the enzyme has an obligatory requirement for molecular oxygen but no cofactors have been identified.[1,6,10] In addition, the human enzyme has been conclusively shown not to be a metalloprotein.[11]

2. Results and discussion

Recently, a crystal structure of an oxygen-dependent form of coproporphyrinogen oxidase from *Saccharomyces cerevisiae* was published and a substrate-sized cleft or cavity was identified and tentatively assigned as the active site.[12] Unfortunately, this achievement has not yet allowed the catalytic action of copro'gen oxidase to be determined and the mechanism for the oxidative decarboxylation of the propionic acid side chains remains a mystery. Two main mechanisms have previously been proposed to explain the oxidative decarboxylation reaction. In the first proposal, an oxidation takes place to afford a hydroxypropionate unit and this undergoes sequential loss of water and carbon dioxide (pathway a, Scheme 3).[5,13] In the alternative mechanism, an unspecified hydride acceptor is postulated and this generates the same exocyclic vinylene intermediate prior to loss of CO_2 (pathway b).[5,14] Both mechanisms are consistent with the observation that all three hydrogens on the vinyl moiety derive from the propionate grouping,[5,15] although it is unclear how either one of these processes could occur. Some evidence for the presence of porphyrins with hydroxypropionate side chains (e.g., **5**) in natural sources has been presented,[7b,16] and an acrylate porphyrin related to coproporphyrin-III, the so-called S411 porphyrin (**6**), has been isolated from



Scheme 3. Previously proposed mechanisms for the oxidative decarboxylation of the propionate side chains of copro'gen-III.

meconium[17] (see Fig. 1). However, these porphyrins may be artifacts and the porphyrinogen related to S411 is not a substrate for copro'gen oxidase.[18] Anaerobic organisms use a structurally unrelated form of copro'gen oxidase that incorporates a 4Fe–4S cluster with an *S*-adenosylmethionine in close proximity, and it has been proposed that the oxidative decarboxylation occurs via a radical decarboxylation pathway under these circumstances (pathway c, Scheme 3).[19] This is far less likely to be the case for the metabolism of **2** by the aerobic enzyme found in higher organisms.

The fact that the mechanistic action of one of the key enzymes in a primary metabolic pathway is not understood is surprising and an appreciation of this has recently led to increased interest in copro'gen oxidase.[6,8,9,12,19,20] In addition, studies in this area also have clinical implications as defects in any one of the

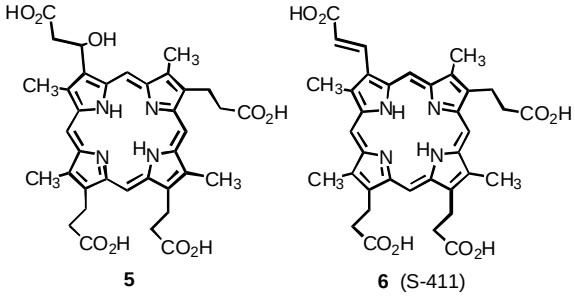
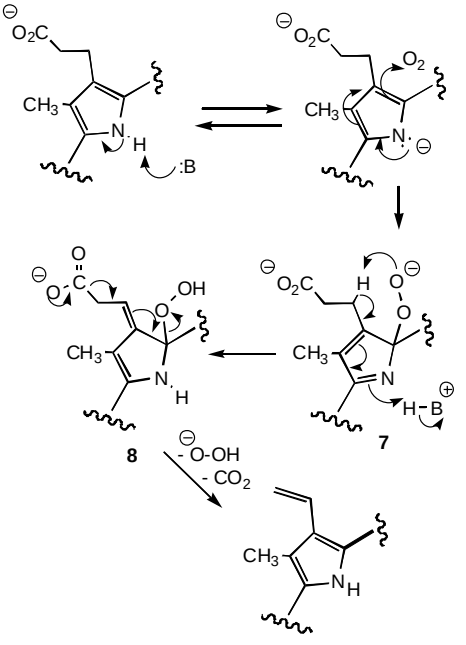


Figure 1. Naturally occurring porphyrins with potential relevance to the heme biosynthetic pathway.

enzymes in the heme pathway leads to diseases known as porphyrias. Porphyrias can arise from genetic defects or due to the effects of environmental toxins, and may produce pathological symptoms that include skin photosensitivity and lesions, liver damage, and neurological problems.[21]

Given that cofactors and metal ions play no role in the activity of the aerobic form of copro'gen oxidase, the following mechanism is postulated to explain the action of copro'gen oxidase based on simple chemical principles (Scheme 4). Initial deprotonation of the pyrrole NH would give an azacyclopentadienyl anion, and this could react with oxygen to produce the 2*H*-pyrrole peroxide anion **7**.[22] Abstraction of a side chain proton via a six-membered ring transition state would be expected to produce the exocyclic double bond in **8**, and this species is set up to eliminate carbon dioxide and hydrogen peroxide to give the vinyl pyrrole derivative. For the enzymic degradation of copro'gen-III, formation of CO_2 has been confirmed by using a carbon-14 labeled substrate,[23] and the generation of peroxide has been noted in a recent report.[20] This mechanism bears some resemblance to the proposed mechanism for the urate oxidase reaction.[24] Urate oxidase (UOX) catalyzes the conversion of urate to 5-hydroxyisourate using molecular oxygen without the involvement of cofactors or transition metal ions and the consumed O_2 is also converted to H_2O_2.[24] It has been proposed that the initial reaction involves general base catalysis to generate a dianion that reacts with O_2. However, beyond this point the mechanisms appear to diverge. Although, a structural relationship between UOX and copro'gen oxidase has been suggested previously,[25] the proposal that copro'gen oxidase is a T-fold protein has been shown to be incorrect.[12]



Scheme 4. New mechanism for the base-catalyzed oxidative decarboxylation of the propionate side chains of coproporphyrinogen-III.

The porphyrinogen intermediates cannot be planar but may exist in several strain-free conformations.[26] As has been observed for the related calix[4]pyrroles, porphyrinogens are likely to alter their conformations in response to anion binding or neutral molecule interactions.[27,28] It is intriguing to note that copro'gen-III has recently been co-crystallized with the previous enzyme in the pathway, uro'gen decarboxylase, and shown to favor a 'bowl' or 'domed' conformation where the pyrrole NHs hydrogen bond to a conserved aspartate residue.[29] This arrangement in copro'gen oxidase would allow the substrate to associate with an anionic residue that could initiate the base-catalyzed oxidative decarboxylation (Fig. 2). We have previously developed an active site model for copro'gen oxidase based upon the requirements for the arrangement of peripheral substituents,[6,8,9] and this model has been adapted to incorporate an anion binding interaction. The proposed configuration shown in Figure 2 would facilitate deprotonation of a pyrrolic nitrogen, thereby triggering reaction with molecular oxygen. Although a deeply buried substrate sized cavity was identified at the dimer interface in the crystal structure of oxygen-dependent copro'gen oxidase,[12] it is not possible at this time to assign the precise region that is involved in these proposed interactions. Indeed, it is not clear that a dimeric structure is necessary for enzymic activity,[30] and more structural information will be required to fully elucidate the mechanism of the oxidative decarboxylation of copro'gen-III.

3. Conclusion

The action of coproporphyrinogen oxidase has been a mystery for more than 40 years, but recent investigations are starting to reveal how this enzyme carries out the conversion of copro'gen-III to proto'gen-IX. This highly conserved enzyme,[11] which is found in all aerobic organisms, appears to carry out oxidative decarboxylations in the presence of molecular oxygen without any conventional cofactors or metal cations, and has previously defied all attempts to develop a detailed understanding of its catalytic role. The new mechanism proposes that the enzyme uses deprotonated pyrrole units as azacyclopentadienyl anions that react with oxygen at the α-positions, and that subsequently intra-

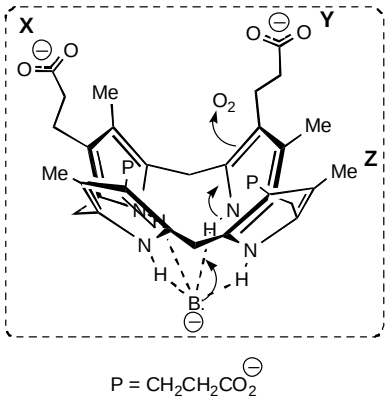


Figure 2. Modified active site model for coproporphyrinogen oxidase.

molecular deprotonation of the propionate side chain forms an external vinylene moiety. This can undergo loss of carbon dioxide and hydrogen peroxide to form a vinyl grouping. The new mechanism is consistent with all the available data for copro'gen oxidase and this proposal is the first to properly take into account the reactivity of pyrrolic structures.

Acknowledgments

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