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Unprecedented Overmetabolism of a Porphyrinogen Substrate by Coproporphyrinogen Oxidase

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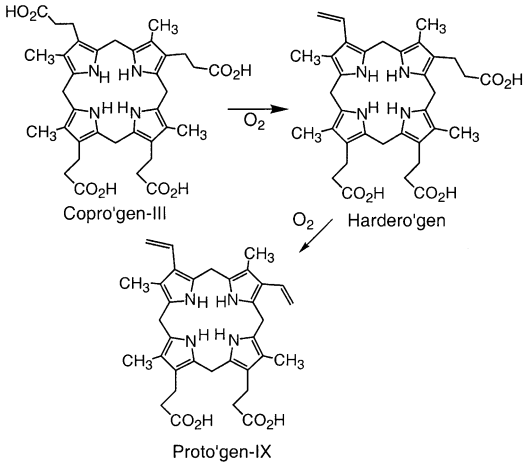
Abstract—Harderoporphyrinogen-I is metabolized by avian hemolysate preparations of coproporphyrinogen oxidase to give a trivinylic product; this unprecedented 'overmetabolism' of the porphyrinogen substrate provides strong support for a proposed model of the active site of this poorly understood enzyme.

Coproporphyrinogen oxidase (EC 1.3.3.3), a key enzyme in the heme biosynthetic pathway, converts coproporphyrinogen-III into protoporphyrinogen-IX via the tripropionate intermediate harderoporphyrinogen (Scheme 1).[1–3] The enzyme selectively converts the propionate residues at the A and B rings into vinyl moieties, a process that formally involves both an oxidation and a decarboxylation, while leaving the C and D ring propionate units untouched. The mechanism for this conversion remains completely unknown.[3] Copro'gen oxidase derived from aerobic organisms requires molecular oxygen, but no cofactors have been identified and most investigations strongly indicate that metal ions are not critical components.[4] Nonetheless, some success has been reported in determining the stereoselectivity of this conversion[1] and in elucidating the substrate binding requirements for this enzyme.[3]

In addition to its critical role in heme biosynthesis, copro'gen oxidase has medicinal significance in that defects in this enzyme lead to a disease of porphyrin metabolism known as Hereditary Coproporphyria. Acute porphyrias of this type may be induced by illness or emotional stress, or may result from certain drug treatments or by chemical exposure. The disease may result in the overproduction and accumulation of porphyrins, and this can lead to symptoms such as skin photosensitivity and neurological disorders.[5]

In previous work, we developed a model for the active site of copro'gen oxidase based a series of studies on the metabolism of substrate analogues.[3,6] Coprogen-III is the natural substrate for this enzyme and copro'gen-IV is known to also be an excellent substrate[3] but the other two type isomers, copro'gen-I and copro'gen-II, are not metabolized by copro'gen oxidase (Chart 1).[3,7] Further investigations allowed the presence of three critical sites to be deduced (Fig. 1).[3] At site X in the model, a propionate unit is required for binding, while at region Z steric factors can tolerate small nonpolar units such as methyl or vinyl, but not a larger propionate group. Region Y represents the catalytic site where oxidative decarboxylation of a correctly positioned propionate moiety takes place. In an attempt to rigorously test this model, we selected harderoporphyrinogen-I (**1**; Scheme 2) as a probe for the active site of copro'gen oxidase. Although copro'gen-I is not a substrate for copro'gen oxidase, the hypothetical first formed product



Scheme 1. Metabolism of Coproporphyrinogen-III.

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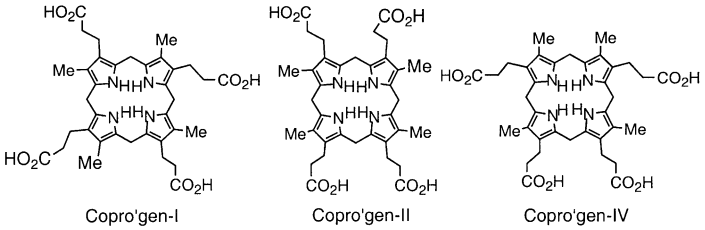


Chart 1.

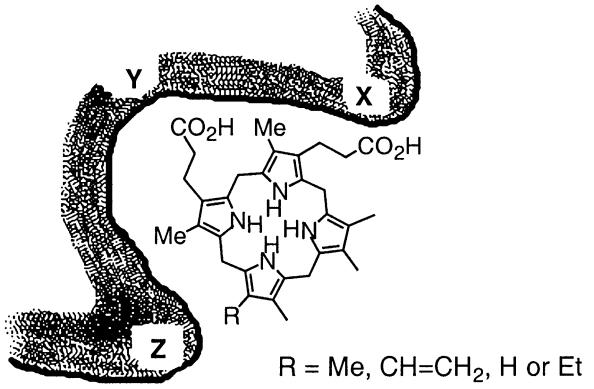
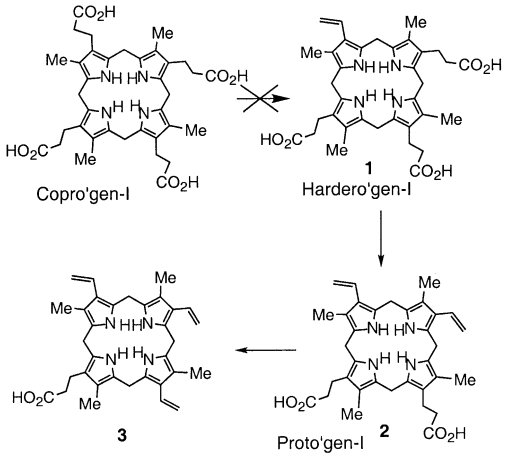


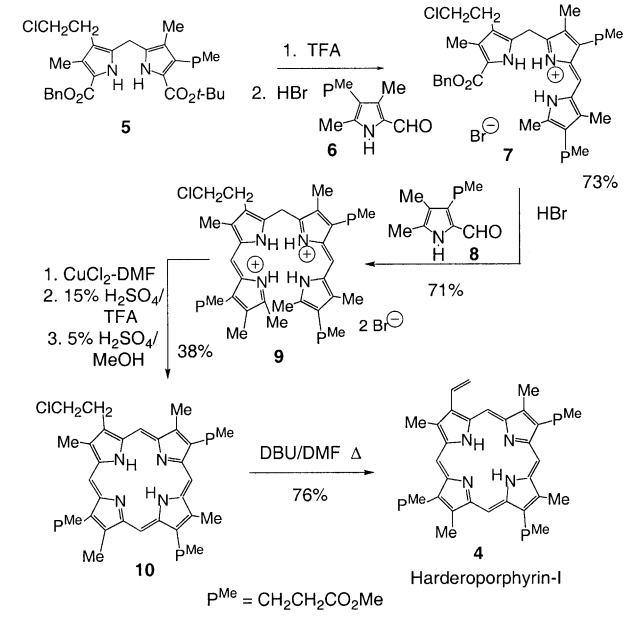
Figure 1. Model for the active site of coproporphyrinogen oxidase.



Scheme 2.

from its metabolism would be hardero'gen-I. Ironically, our model predicts that hardero'gen-I should be an excellent substrate for this enzyme and furthermore the divinylic product, proto'gen-I (**2**), should be further processed to give a unique trivinylic product **3** (Scheme 2). This would represent an unprecedented over-metabolism of the substrate[8] as no natural or synthetic substrate has been observed to produce a third vinyl side chain.[3] Hence, the results from a study of this type would provide a stringent test for the validity of the substrate binding model.

Porphyrinogens are unstable and are usually generated as needed from the corresponding porphyrin methyl esters by hydrolysis with 25% HCl and reduction with 3% sodium amalgam.[3] Hence, our initial target was harderoporphyrin-I trimethyl ester (**4**). This was synthesized



Scheme 3.

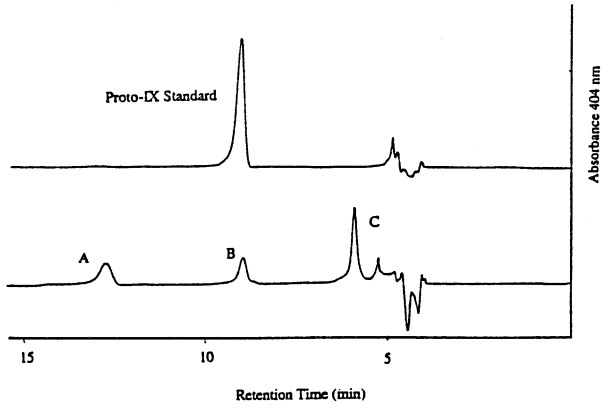


Figure 2. HPLC of the esterified porphyrin extract from an incubation of **1** with chicken red cell hemolysates showing the formation of protoporphyrin-I (peak B) and a trivinylic product **3** (peak C). Peak A = residual harderoporphyrin-I trimethyl ester. Protoporphyrin-I overlaps with endogenous protoporphyrin-IX[3] and the percent formation of this metabolite was calculated by subtracting the quantities of protoporphyrin observed in zero time control incubations. HPLC analyses were performed on a 5 μ partisil column (25 cm ×4.6 mm id) eluting with 25% ethyl acetate-cyclohexane (v/v) at a flow rate of 1 mL/min.

by established chemistry using the tripyrrene-*a*,*c*-biladiene approach.[3,5,9,10] The vinyl moiety is too fragile to survive the synthesis and hence was introduced in the form of a chloroethyl unit. Treatment of dipyrrylmethane

5[3] with TFA and condensation with pyrrole aldehyde **6**[11] in the presence of HBr afforded the tripyrrane benzyl ester **7** (Scheme 3). Cleavage of the benzyl ester with TFA–HBr[9b] and condensation with a second pyrrole aldehyde **8**[11] afforded the *a,c*-biladiene **9**. Cyclization with copper(II) chloride in DMF at room temperature,[9c] followed by demetallation with 15% sulfuric acid-TFA and reesterification, afforded the chloroethylporphyrin **10** in 38% yield. Dehydrohalogenation with DBU in DMF afforded the required vinylporphyrin **4**.

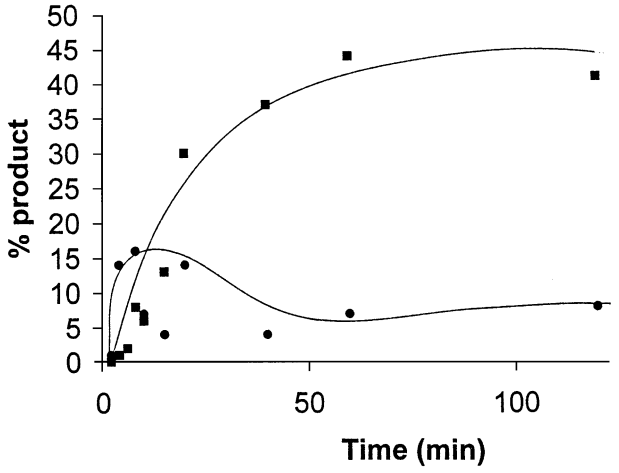


Figure 3. Time course study for the incubation of harderoporphyrinogen-I with chicken red cell hemolysates showing the % protoporphyrin-I (●) and trivinylic porphyrin product (■) formation versus time.

Following ester cleavage and reduction with 3% sodium amalgam, porphyrinogen **1** was incubated with chicken red cell hemolysates (CRH).[3,12] The porphyrin products were submitted to Fischer esterification, and subsequent analysis of the metabolites by TLC and HPLC demonstrated that two new porphyrins were being generated from **1** and the polarities (as assessed by R_f values or retention times, respectively) were consistent with di- and monocarboxylate products of the type anticipated (Fig. 2). The minor dicarboxylate product, presumably protoporphyrin-I (**2**), was seldom observed to accumulate much beyond 10% of the isolated products. However, the major monocarboxylate product **3** was observed to accumulate to a far greater extent and reached an asymptote at approximately 60% conversion (Fig. 3).

In order to provide further evidence for the formation of **3**, a preparatative experiment[3,6] was carried out by incubating hardero'gen-I with CRH for 5 h. Following extraction and esterification with 5% sulfuric acid-methanol,[3] the porphyrin products were purified on silica gel by flash chromatography eluting with 1:10 ethyl acetate/toluene containing 5% triethylamine. The 300 MHz proton NMR spectrum of the porphyrin methyl ester strongly supported the expected structure (Fig. 4). The presence of three vinyl units, giving rise to multiplets at 6.1–6.5 (3H) and 8.2–8.4 ppm (6H), was confirmed and the lack of symmetry in the macrocycle gave rise to five 3H singlets for the 4 porphyrin methyls and the single methyl ester between 3.6–3.8 ppm. The *meso*-protons afforded a 2H singlet and two 1H singlets in the downfield region between 10.1 and 10.3 ppm,

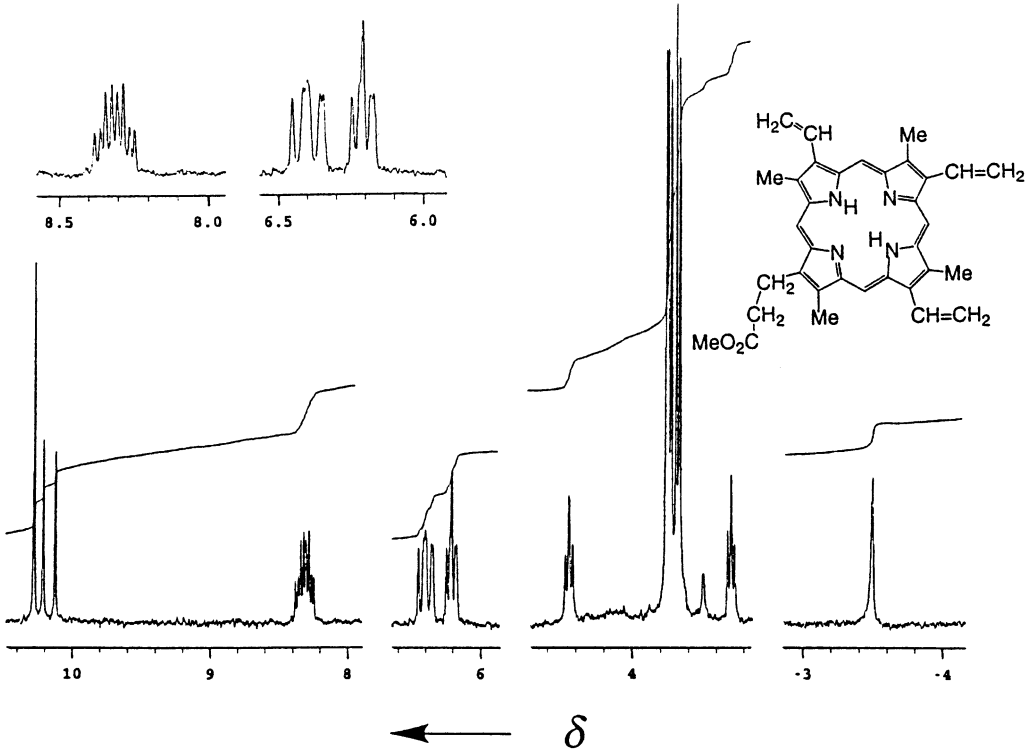


Figure 4. 300 MHz proton NMR spectrum of the trivinylic product formed by incubating harderoporphyrinogen-I with CRH after conversion to the corresponding porphyrin methyl ester.

while the internal NHs gave rise to an upfield 2H singlet at −3.5 ppm due to the porphyrin's diatropic ring current. Finally, the presence of two 2H triplets at 3.3 and 4.4 ppm confirmed the presence of one residual propionate side chain. High-resolution FAB mass spectrometry provided additional confirmation for the trivinyl product (Calcd for $C_{34}H_{34}N_4O_2+H$: 531.2760. Found: 531.2758).

The observation that harderoporphyrinogen-I is converted into a trivinylic product[13] provides strong evidence in support of our proposed model for the active site of copro'gen oxidase. Further investigations are underway to more fully elucidate the binding requirements of this poorly understood enzyme.

Acknowledgements

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References and Notes

1. (a) *Biosynthesis of Tetrapyrroles*, Jordan, P. M., Ed.; Elsevier: London, 1991. (b) Akhtar, M. In *The Biosynthesis of Tetrapyrrole Pigments*; Wiley: Chichester (Ciba Foundation Symposium 180), 1994; pp 130–155.
2. (a) Cavaleiro, J. A. S.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans 1* **1974**, 1188. (b) Jackson, A. H.; Jones, D. M.; Philip, G.; Lash, T. D.; Battle, A. M. del C.; Smith, S. G. *Int. J. Biochem.* **1980**, *12*, 681.
3. Lash, T. D.; Mani, U. N.; Drinan, M. A.; Hall, T.; Zhen, C.; Jones, M. A. *J. Org. Chem.* **1999**, *64*, 464 and references cited therein.
4. Medlock, A. E.; Dailey, H. A. *J. Biol. Chem.* **1996**, *271*, 32507.
5. Moore, M. R. *Int. J. Biochem.* **1993**, *25*, 1353.
6. Lash, T. D.; Hall, T.; Mani, U. N.; Jones, M. A. *J. Org. Chem.* **2001**, *66*, 3152.
7. (a) Porra, P. J.; Falk, J. E. *Biochem. J.* **1964**, *90*, 69. (b) Al-Hazimi, H. M. G.; Jackson, A. H.; Ryder, D. J.; Elder, G. H.; Smith, S. G. *J. Chem. Soc., Chem. Commun.* **1976**, 188. (c) Buldain, G.; Hurst, J.; Frydman, R. B.; Frydman, B. *J. Org. Chem.* **1977**, *42*, 2953. (d) Buldain, G.; Diaz, L.; Frydman, B. *J. Org. Chem.* **1977**, *42*, 2957. (e) Battersby, A. R.; Hamilton, A. D.; McDonald, E.; Mombelli, L.; Wong, O.-H. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1283. (f) Al-Hazimi, H. M. G.; Jackson, A. H.; Knight, D. W.; Lash, T. D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 265.
8. However, the overmetabolism of uroporphyrinogens by uro'gen-III methylase, an early enzyme in the pathway leading to Vitamin B_{12}, to give pyrrocorphins has been reported: Muller, G.; Schmiedl, J.; Schneider, E.; Sedlmeier, R.; Wörner, G.; Scott, A. I.; William, H. J.; Santander, P. J.; Stolowich, N. J.; Fagerness, P. E.; Mackenzie, N. E.; Kriemler, H.-P. *J. Am. Chem. Soc.* **1986**, *108*, 7875. Scott, A. I.; Warren, M. J.; Roessner, C. A.; Stolowich, N. J.; Santander, P. J. *J. Chem. Soc., Chem. Commun.* **1990**, 593.
9. (a) Baptista de Almeida, J. A. P.; Kenner, G. W.; Rimmer, R.; Smith, K. M. *Tetrahedron* **1976**, *32*, 1793. (b) Smith, K. M.; Craig, G. W. *J. Org. Chem.* **1983**, *48*, 4302. (c) Smith, K. M.; Minnetian, O. M. *J. Chem. Soc., Perkin Trans. 1* **1986**, 277.
10. Smith, K. M. In *The Porphyrin Handbook*, Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic: San Diego, 2000; Vol. 1, p 119.
11. Lash, T. D.; Mani, U. N.; Lyons, E. A.; Thientanavanich, P.; Jones, M. A. *J. Org. Chem.* **1999**, *64*, 478.
12. Chicken blood provides a convenient source for copro'gen oxidase.[3]
13. Hardero'gen-I also affords the trivinylic product in incubations with a preparation of cloned human copro'gen oxidase,[4] demonstrating that the model is valid for both the human and avian versions of this enzyme. The *Escherichia coli* JM109 culture into which the human gene for copro'gen oxidase had been cloned[4] was a gift from Dr. H. A. Dailey, University of Georgia, Athens, GA.