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# Enzymatic Ring Opening of an Iron Corrole by Plant-Type Heme Oxygenases: Unexpected Substrate and Protein Selectivities[†]

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ABSTRACT: Heme oxygenases are widely distributed enzymes involved in the oxidative cleavage of the heme macrocycle that yields the open-chain tetrapyrrole biliverdin IX, CO, and iron. For the first time, two regioisomeric iron corroles [α-CH- and γ-CH-Fe(cor)] have been utilized as artificial substrate and cofactor analogues to mammalian, plant, cyanobacterial, and bacterial heme oxygenases. The non-natural enzymatic cleavage of γ-CH-Fe(cor), catalyzed by plant-type heme oxygenases from *Arabidopsis thaliana* and *Synechocystis* sp., happens selectively at the unexpected bipyrrolic position and yields a biomimetic biliverdin-like product. The reaction is selective for this corrole regioisomer and for plant-type heme oxygenases and is the first report of an enzymatic corrole ring opening.

Metal corroles are artificial one-carbon short relatives of the ubiquitous metal porphyrins that have attracted considerable attention over the past decade (*1*, *2*). These metal complexes are special with respect to their electronic structures and, often, the noninnocent behavior of the organic ligands that tend to form magnetically stabilized radicals (*3*−*8*). The unique properties of metal corroles have more recently advanced into topical fields like catalysis, photophysics, medicinal chemistry, and others (*9*−*11*). In many of these applications, the performance of metal corroles was found to be clearly superior to that of related metal porphyrins.

Besides the electronic peculiarities, metal corroles differ from the parent porphyrin complexes mainly in their reduced symmetry (*12*). We have attempted to make use of this property variation by introducing regioisomeric iron corroles **1** and **2** as heme cofactor and substrate analogues for heme oxygenases (HOs) (Scheme 1). HOs make up a family of enzymes that catalyze the oxygen-dependent degradation of heme to biliverdin IX, CO, and iron (*13*). Three molecules of $O_2$ and seven reducing equivalents are required for this conversion. In mammals, HOs have a key function in physiological heme metabolism, whereas in certain pathogenic bacteria, HOs are used for iron utilization from heme obtained from the host (*14*−*16*). An additional role of mammalian HOs in the production of CO as a neurotransmitter has been demonstrated, and recently, bacterial HO has become a target for drug research (*17*−*20*). In contrast, higher-plant, cyanobacterial, and algal HOs are involved in the biosynthesis of open-chain tetrapyrroles that are used as the chromophores in light-sensing phytochromes or in the light-harvesting phycobiliproteins (*21*−*23*).

The majority of HOs function on heme upon the regioselective extrusion of the α-situated methine bridge (Scheme 1). Propionate-substituted metal corroles (*24*, *25*) are thought to be useful probes for this reaction. Employing α-CH- and γ-CH-Fe(cor) **1** and **2** as substrate analogues, we could speculate that the α-CH isomer **1** is transformed to ring-oxygenated or ring-opened products while the γ-CH isomer **2** should not be converted by the enzymes. Our results, however, show that in fact the opposite scenario occurs, and that a remarkable reactivity difference exists between HOs from different organisms.

First, we focused on the HOs from *Pseudomonas aeruginosa* (*15*, *26*, *27*). This bacterium is the first biological example in which two HOs with different regiospecificities exist, an α-selective HO, designated BphO, and an unusual β-/δ-selective one, named PigA (also known as HemO) (*15*, *26*). After addition of **1** and **2** to both HOs in a neutral aqueous solution, the band at 376 nm shifted to 400 nm and shoulders appeared at 535 and 620 nm, respectively (exemplarily shown for **2** and BphO in Figure S1 of the Supporting Information). A bandshift at ∼400 nm has previously been attributed to a change in equilibrium between a monomer and a μ-oxo dimer of the corrol toward the monomeric form (*25*). Therefore, we envisage a monomerization of the corrole upon interaction with the HO. This interaction was tight in both cases as proven by size-exclusion chromatography where both protein and corrol absorption eluted at the same retention time (data not shown). Addition of a reducing system, containing ferredoxin, ferredoxin-NADPH-oxidoreductase, and an NADPH-regenerating system (glucose 6-phosphate, $NADP^+$, and glucose-6-phosphate dehydrogenase), as well as an iron chelator (Tiron) resulted in immediate corrole reduction and axial coordination of histidine as indicated by the appearance of an absorbance peak at 535 nm (*25*). This reduced and bound product was stable and did not react any further (Figure S2C−F of the Supporting Information). Therefore, both iron corroles were unreactive as substrates in these HOs and may thus find use as inhibitors. The same spectral changes were observed when a sample containing enriched recombinant human HO-1 (lacking the membrane anchor) was used (data not shown), indicating that both iron corroles are unreactive not only in bacterial but also in mammalian HOs.

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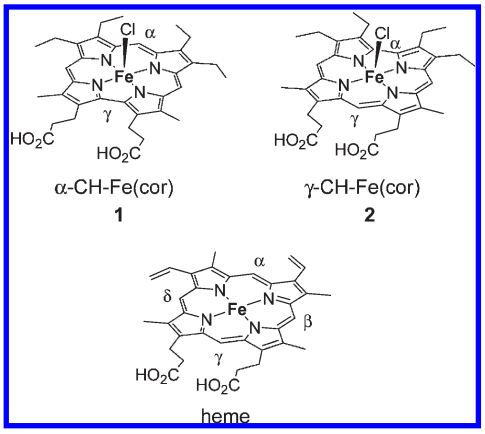
Scheme 1: Structures of Heme and Regioisomeric Iron Corroles **1** and **2** That Were Used as Substrate Analogues of HO in This Study[a]




[a]For the purposes of illustration, the nomenclature for the heme methine bridges has been adapted for the corroles as well.

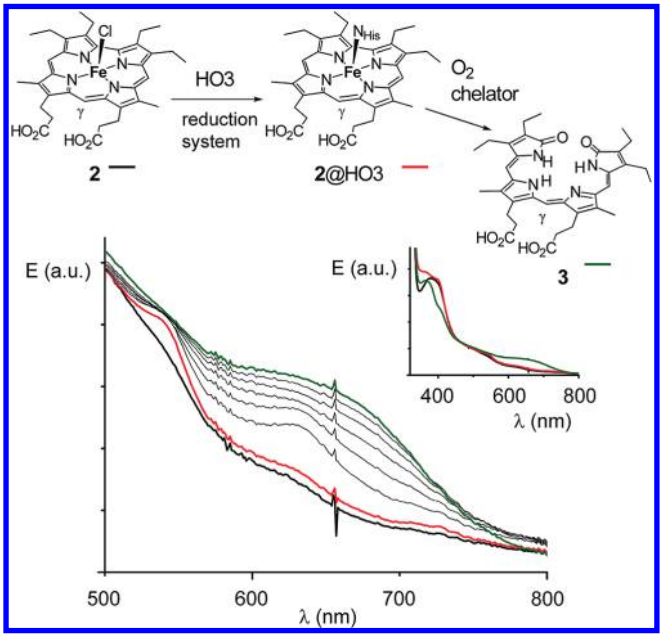



FIGURE 1: Evolution of optical spectra (detailed view; inset, full spectra) during the action of HO3 from *A. thaliana* on γ-CH-Fe(cor) **2** (bold black line), indicating initial binding and reduction to **2**@HO3 (red line) and subsequent oxidative ring cleavage to the biliverdin analogue **3** [green line; in 100 μM potassium phosphate buffer ($KP_i$) at pH 7.2 and 25 °C; for reaction details, see the text and the Supporting Information]. Spectra were recorded in 5 min intervals.

When plant α-selective HO1 (HY1), HO3, and HO4 from *Arabidopsis thaliana* and cyanobacterial HO-1 from *Synechocystis* sp. PCC6803 were investigated (*21*, *28*), the picture changed. Again, both corroles **1** and **2** reacted with the enzymes to yield stable histidine conjugates upon addition of the reducing system as shown by bands at 535 nm and shoulders at ~620 and ~720 nm in the optical spectra (*25*) (Figure 1; Figure S2A and Figure S3A,B of the Supporting Information).

Interestingly, with time, and in the presence of dioxygen, the anticipated substrate **1** did not react further (Figure S3A of the Supporting Information), while for **2**, all plant-type HOs produced a new, green product within 30 min indicated by an increase in absorption at ~670 nm (Figure 1 and Figure S3B of the Supporting Information). The product of this oxygen-dependent reaction

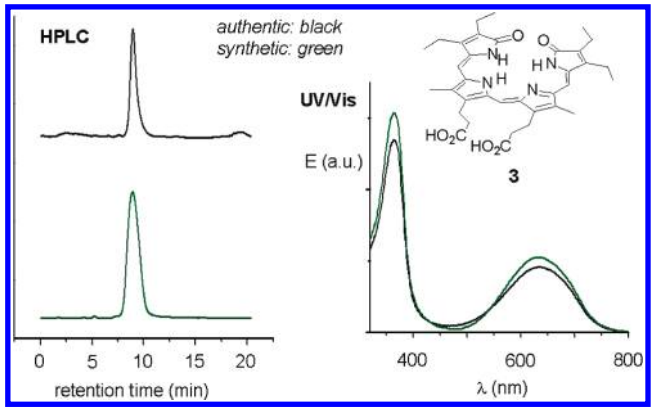



FIGURE 2: Comparison of high-performance liquid chromatography [RP-C18 column developed with acetone, 2-propanol, and 20 mM formic acid (33:33:33 by volume) and detected at 650 nm and UV−vis characteristics (in acetone, 2-propanol, and 20 mM formic acid (33:33:33 by volume)] of the product of the HO3-catalyzed oxygenation of γ-CH-Fe(cor) **2** (black lines) and the synthetic biliverdin analogue **3** (green lines).

was shown to represent the biliverdin analogue **3**. The identity of **3** was confirmed by a combination of UV−vis spectroscopy and high-performance liquid chromatography analysis in comparison with a synthetic sample (Figure 2). Biliverdin analogue **3** was the only product of the enzymatic degradation of **2**. To rule out single turnover, the reaction was performed in the presence of a 2-fold excess of **2**. Within 30 min, **2** was fully converted to **3**, revealing the catalytic nature of the reaction (Figure 1). However, the presence of an iron chelator like Tiron was always required; otherwise, the product could not be detected in the UV−vis spectrum. This resembles the situation with the natural substrate heme where an iron chelator is also required to release the Fe−biliverdin IXα complex from the active site of plant and (cyano)bacterial HOs (*26*, *28*, *29*).

During the course of our experiments, we furthermore tested whether **2** can be prereduced before binding to the enzyme and still be converted to the biliverdin analogue **3**. Interestingly, reduction of **2** by ascorbate or dithionite prior to addition of HO3 renders the γ-CH-Fe(cor) unreactive. This might be due to a nonproductive binding of the prereduced corrole to HO3 because no band at 535 nm appeared in the UV−vis spectrum (data not shown).

Additional experiments were conducted to ensure that the reaction takes place only in the presence of HO, and that no reduction of buffer-dissolved iron corroles **1** and **2** with the biological reducing system occurs. However, no conversion was observed in the absence of enzyme.

As γ-CH-Fe(cor) **2** is regioselectively cleaved at the bipyrrolic position, no CO would be expected to develop. This additional peculiarity has been independently confirmed by a myoglobin addition assay (*16*). No specific myoglobin−CO complex absorption in the UV−vis spectrum could be detected, indicating that indeed no CO is produced during the course of the reaction (data not shown). Furthermore, assays performed under anaerobic conditions stopped at the reduction step as indicated by absorption at ~535 nm. Therefore, at least one oxygen atom of the linear tetrapyrrole **3** might originate from dioxygen, rather than from water. From a balanced chemical equation, this reaction is categorized as an addition of 1 equiv of $O_2$ and 2 equiv of $H_2O$ to a molecule of γ-CH-Fe(cor) **2**, with elimination of 1 equiv of FeO(OH).

Taking the currently postulated mechanism of HO into consideration, one would speculate that the γ-CH-Fe(cor) **2** enters the active site pocket of plant-type HOs with the propionate side chains facing the solvent. Stable coordination via the axial histidine ligand requires reduction of γ-CH-Fe(cor) **2** within the enzyme. In analogy to the degradation of heme, oxygen will be required and could likely generate a short-lived peroxo species that specifically targets the bipyrrolic position that in the case of γ-CH-Fe(cor) **2** will be deeply buried in the HO active site pocket. The reason why only γ-CH-Fe(cor) **2** is converted is currently unknown. Our results appear to be peculiar and are not understood at the first glance but should be explainable on the basis of a thorough analysis of the different ligand binding and electron transfer kinetics in each case. We are currently performing detailed investigations toward this end. Another important aspect of this result, however, is the biological one in which heme oxygenases of different origins employ different substrate specificity toward the iron corroles. This would point toward a slightly different architecture of the active site of plant-type HOs compared to mammalian and bacterial HOs. On the basis of a structural model of pea HO, it has been postulated that the active site of plant HOs is more spacious than the respective enzymes from other sources because of the requirement of a second reductant (ascorbate) for full catalytic activity (*29*, *30*). If space is the only feature that allows iron corrole degradation, the chemical nature of this reaction will be addressed in the future and will be highly dependent on the crystal structure of a plant HO.

## SUPPORTING INFORMATION AVAILABLE

Detailed procedures for the preparation of **1** and **3**, protein purification, data of action of bacterial HO (BphO and PigA) and cyanobacterial HO-1 on **1** and **2**, and action of plant HO3 on **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

## REFERENCES


1. Erben, C., Will, S., and Kadish, K. M. (2000) in The Porphyrin Handbook (Kadish, K. M., Smith, K. M., and Guilard, R., Eds.) pp 233−300, Academic Press, San Diego.
2. Paolesse, R. (2000) in The Porphyrin Handbook (Kadish, K. M., Smith, K. M., and Guilard, R., Eds.) pp 201−233, Academic Press, San Diego.
3. Broering, M., Bregier, F., Consul Tejero, E., Hell, C., and Holthausen, M. C. (2007) *Angew. Chem., Int. Ed. 46*, 445–448.
4. Hocking, R. K., DeBeer George, S., Gross, Z., Walker, F. A., Hodgson, K. O., Hedman, B., and Solomon, E. I. (2009) *Inorg. Chem. 48*, 1678–1688.
5. Nardis, S., Paolesse, R., Licoccia, S., Fronczek, F. R., Vicente, M. G., Shokhireva, T. K., Cai, S., and Walker, F. A. (2005) *Inorg. Chem. 44*, 7030–7046.
6. Roos, B. O., Veryazov, V., Conradie, J., Taylor, P. R., and Ghosh, A. (2008) *J. Phys. Chem. B 112*, 14099–14102.
7. Wasbotten, I., and Ghosh, A. (2006) *Inorg. Chem. 45*, 4914–4921.
8. Ye, S., Tuttle, T., Bill, E., Simkhovich, L., Gross, Z., Thiel, W., and Neese, F. (2008) *Chemistry 14*, 10839–10851.
9. Aviv, I., and Gross, Z. (2007) *Chem. Commun.,* 1987–1999.
10. Aviv-Harel, I., and Gross, Z. (2009) *Chemistry 15*, 8382–8394.
11. Flamigni, L., and Gryko, D. T. (2009) *Chem. Soc. Rev. 38*, 1635–1646.
12. Kuck, S., Hoffmann, G., Broring, M., Fechtel, M., Funk, M., and Wiesendanger, R. (2008) *J. Am. Chem. Soc. 130*, 14072–14073.
13. Matsui, T., Unno, M., and Ikeda-Saito, M. (2010) *Acc. Chem. Res. 43*, 240–247.
14. Abraham, N. G., Drummond, G. S., Lutton, J. D., and Kappas, A. (1996) *Cell. Physiol. Biochem. 6*, 129–168.
15. Ratliff, M., Zhu, W., Deshmukh, R., Wilks, A., and Stojiljkovic, I. (2001) *J. Bacteriol. 183*, 6394–6403.
16. Wilks, A., and Schmitt, M. P. (1998) *J. Biol. Chem. 273*, 837–841.
17. Boehning, D., Moon, C., Sharma, S., Hurt, K. J., Hester, L. D., Ronnett, G. V., Shugar, D., and Snyder, S. H. (2003) *Neuron 40*, 129–137.
18. Furci, L. M., Lopes, P., Eakanunkul, S., Zhong, S., MacKerell, A. D., Jr., and Wilks, A. (2007) *J. Med. Chem. 50*, 3804–3813.
19. Otterbein, L. E., Bach, F. H., Alam, J., Soares, M., Tao, Lu. H., Wysk, M., Davis, R. J., Flavell, R. A., and Choi, A. M. (2000) *Nat. Med. 6*, 422–428.
20. Piepoli, A. L., de Salvatore, G., Lemoli, M., de Benedictis, L., Mitolo-Chieppa, D., and de Salvia, M. A. (2008) *Neurogastroenterol. Motil. 20*, 1251–1262.
21. Cornejo, J., Willows, R. D., and Beale, S. I. (1998) *Plant J. 15*, 99–107.
22. Muramoto, T., Kohchi, T., Yokota, A., Hwang, I., and Goodman, H. M. (1999) *Plant Cell 11*, 335–348.
23. Rhie, G., and Beale, S. I. (1992) *J. Biol. Chem. 267*, 16088–16093.
24. Broering, M., Bregier, F., Krueger, R., and Kleeberg, C. (2008) *Eur. J. Inorg. Chem. 35*, 5505–5512.
25. Matsuo, T., Hayashi, A., Abe, M., Matsuda, T., Hisaeda, Y., and Hayashi, T. (2009) *J. Am. Chem. Soc. 131*, 15124–15125.
26. Wegele, R., Tasler, R., Zeng, Y., Rivera, M., and Frankenberg-Dinkel, N. (2004) *J. Biol. Chem. 279*, 45791–45802.
27. Wilks, A., Black, S. M., Miller, W. L., and Ortiz de Montellano, P. R. (1995) *Biochemistry 34*, 4421–4427.
28. Gisk, B., Yasui, Y., Kohchi, T., and Frankenberg-Dinkel, N. (2010) *Biochem. J. 425*, 425–434.
29. Muramoto, T., Tsurui, N., Terry, M. J., Yokota, A., and Kohchi, T. (2002) *Plant Physiol. 130*, 1958–1966.
30. Linley, P. J., Landsberger, M., Kohchi, T., Cooper, J. B., and Terry, M. J. (2006) *FEBS J. 273*, 2594–2606.