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Metalloporphyrin Catalyzed Oxidation of *N*-Hydroxyguanidines: A Biomimetic Model for the H_2O_2-Dependent Activity of Nitric Oxide Synthase

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Abstract—A chemical model for the H_2O_2 promoted oxidation by nitric oxide synthase (NOS) has been developed. Biomimetic oxidations were carried out using H_2O_2 and tetrakis(perfluorophenyl)porphyrinato-iron(III) chloride ($FeTPPF_{20}$) as a catalyst. Similarly to NOS our model system produces N^{δ}-cyanoornithine, citrulline and NO from NOHA and did not oxidize arginine itself. Based on these results we propose a peroxide shunt to be involved in the catalytic cycle of NOS. To the best of our knowledge this is the first chemical system that semiquantitatively mimics NOS activity.

Nitric oxide synthase (NOS) catalyzes the five-electron oxidation of arginine to citrulline (**1**) and nitric oxide (NO)[1] via the formation of *N*-hydroxyarginine (NOHA, 2) (Scheme 1).[2] NO has numerous biological actions from functioning as a key intercellular signal and defensive cytotoxin in the nervous, muscular, cardiovascular and immune system[3] to its vasodilatory and antithrombotic activity.[4] Although the mechanism of the oxidation of arginine to NOHA remains unclear, NOS oxidation of NOHA is postulated to follow a pathway usual for heme oxygenases.[5] Since simple chemical models can be useful to investigate mechanistic issues, Fukuto et al.[6] reported the use of organic and inorganic peracids to oxidize *N*-(*N*-hydroxyamidino)piperidine (**3**)[7] as a model for NOHA. Although this study shed some light on the mechanism of NOS-catalyzed oxidations, Clague et al. demonstrated[8] the inability of NOS to catalyze enzymatic reactions with peracids. The risk of over-simplification of a biological system by a chemical model can be significantly reduced using a biomimetic approach. Our good experience on metalloporphyrin-catalyzed biomimetic oxidations[9,10] prompted us to test this system for NOS-mediated reactions using model compounds and the NOHA itself.

Since our biomimetic model was previously tested on cytochrome P450 (CP450) catalyzed oxidations, a suitable NO-donor model compound oxidized by this enzyme was first investigated.[11] Choosing an iron-porphyrin suitable for modeling CP450 catalyzed oxidations[12] tetrakis (pentafluoro-phenyl)phorphirinato iron(III) chloride mediated oxidation of an aromatic amidoxime (**4**)[13] was carried out (Scheme 2). Oxidation of **4** by H_2O_2 gave nicotinamide (**5**) as the major product and also the corresponding nitrile (**6**) as a minor component (Table 1). Next, we oxidized *N*-(*N*-hydroxyamidino)piperidine (**3**), the model compound of Fukuto et al.[6] which yields almost equal amounts of the corresponding amide (**7**) and 1-piperidinecarbonitrile (**8**). Finally, H_2O_2 promoted oxidation of NOHA (**2**) gave mainly N^{δ}-cyanoornithine (**9**)[8] and also citrulline (**1**). This result is consistent with the observation of Clague et al.[8] who detected 55% of **9** and 45% of **1** in vitro for NOS. Similar to the biological system[8] H_2O_2 transformed NO to nitrite and nitrate (NO_2^-/NO_3^-) which was detected by spectrometry in a stoechiometry of 0.8 relative to **1**. Oxidation of arginine in similar conditions and even in the presence of water-soluble metalloporphyrins could not be carried out, which is also in accordance with in vitro data obtained by H_2O_2 dependent studies.[14] Fair agreement obtained for the oxidation of natural substrates (i.e., arginine and NOHA) by the model system and NOS demonstrates the biomimetic nature of our approach.

Discussions of the NOS mechanism is often based on its proposed analogy to that of the P450-catalyzed reactions.[3] Although the recent X-ray structure of the oxygenase domain of *i*NOS[15] revealed the overall fold of

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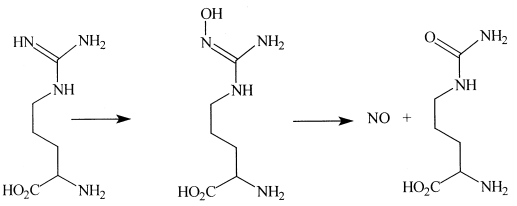
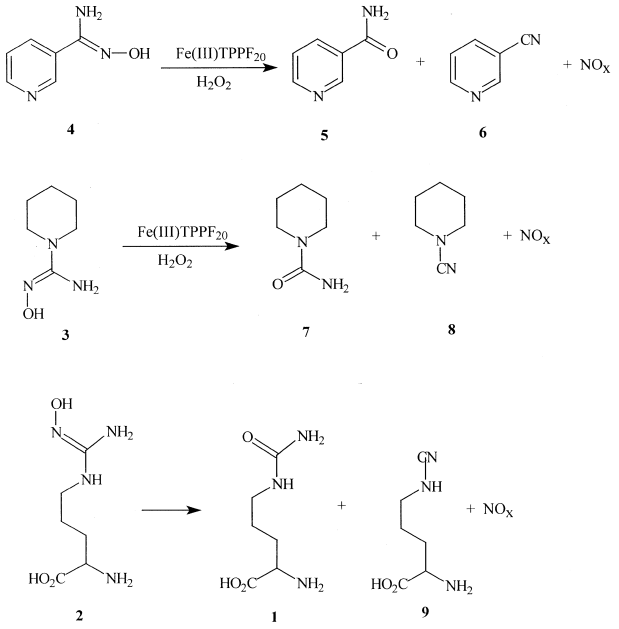


Scheme 1.



Scheme 2.

Table 1. Distribution of products obtained by $FeTPPF_{20}/H_2O_2$ effected biomimetic oxidations of **1**, **4** and **7**

N-Hydroxy	Product distribution	
Compound	Amide (%)	Nitrile
4	**5** (61)	**6** (39)
3	**7** (48)	**8** (52)
2	**1** (37)	**9** (63)
2 (in vitro)	**1** (45)	**9** (55)

this protein to be different from CP450, Mansuy et al. demonstrated[16,17] that CP450 catalyzes the NO formation from NOHA and other *N*-hydroxy compounds. P450 mediated processes involve NADPH to oxidize the substrate. NADPH is necessary to form the high valent iron *oxo* heme intermediate responsible for the oxidation of substrates, but H_2O_2 can substitute NADPH to form the iron *oxo* form directly from the resting state of the heme (peroxide shunt). Formation of high valent iron *oxo* complexes from metalloporphyrins and H_2O_2 is a potential synthetic equivalent of this process. Since product distributions are in good agreement with in vitro data, our results suggest the presence of such a peroxide shunt in the catalytic cycle of NOS as well. Considering that NOS produces H_2O_2 when it oxidizes arginine to NOHA[18] H_2O_2 mediated oxidation of NOHA cannot be ruled out as an alternative to the NADPH assisted process.

A chemical model for the biomimetic oxidation of *N*-hydroxyguanidines has been developed. This study demonstrates the ability of the $FeTPPF_{20}/H_2O_2$ system to mimic the H_2O_2 dependent action of NOS on *N*-hydroxyarginine. Comparing our results to those obtained in vitro we can propose a P450-type peroxide shunt in the catalytic cycle of NOS. Evaluation of this biomimetic approach in chemistry-based pre-screen of potential NO donor compounds is in progress.

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11. General procedure of biomimetic oxidations. *N*-Hydroxy compounds (1.1 mmol) and 0.01 molar equiv of tetrakis(perfluorophenyl)porphyrinato-iron(III) chloride ($FeTPPF_{20}$) were dissolved in 30 mL of methanol:dichloroethane (1:1) and 0.4 molar equiv of H_2O_2 was added. The reaction mixture was stirred for 30 min at room temperature and the solvent was evaporated. Formation of NO_2^- and NO_3^- oxidation products of NO were monitored spectrophotometrically. Aliquots (0.2 mL) of the reaction mixtures were mixed with 0.2 mL of 1% sulfanilamide in 4 N HCl and with 0.2 mL of 0.1% *N*-(1-naphthyl)ethylenediamine in 0.4 N HCl and their absorption at 543 nm was measured. All other products were identified by the analysis of reaction mixtures by HPLC-MS. A HPLC system equipped with Symmetry C8 (Waters) 3.9×150 column, was used for these analyses using the eluent system of eluent A (acetonitrile:water:formic acid 5:95:0.1) and eluent B (acetonitrile:water:formic acid 95:5:0.1) in a gradient of 100% A to 100% B in 20 min. Oxidation products were photometrically detected at 254 nm by setting the flow rate to 1 mL/min. Mass spectrometry studies were performed by a VG QUATTRO triplequad spectrometer (Micromass, Manchester, UK) with electrospray ionization. Product ratios were calculated on the basis of HPLC area% using authentic samples as internal standards.
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