American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Case Report

A New Analogue of The Cytochrome P450 Ferryl Intermediate

Albert Shteinman*

Department of Federal Research Center of Problems of Chemical Physics and Medicinal Chemistry, Russian Academy of Sciences. Acad. N.N. Semenov Avenue, Russia

***Corresponding author:** Albert Shteinman, Department of Federal Research Center of Problems of Chemical Physics and Medicinal Chemistry, Russian Academy of Sciences. Acad. N.N. Semenov Avenue, 1. Chernogolovka, Moscow region, Russia.

To Cite This Article: *Albert Shteinman*. A New Analogue of The Cytochrome P450 Ferryl Intermediate. Am J Biomed Sci & Res. 2025 25(3) AJBSR.MS.ID.003318,* DOI: 10.34297/AJBSR.2025.25.003318

Received: December 30, 2024; **Published:** January 07, 2025

Case Report

One of the most common and important natural enzymes, Cytochrome P450 (CR450), determines the oxidative transformations of endogenous and exogenous molecules in living organisms, including humans. The study of the mechanism of action of this unique enzyme and its chemical analogues and the establishment of the nature of the key oxidative intermediate is crucial for the fine organic synthesis and the successful development of two areas of human economic activity: medicine and catalysis [1].

The active center of CP450 is an octahedral Fe porphyrin complex (heme) embedded in the protein, coordinated by cysteine thiolate from a protein molecule. The undying interest in P450 is due to the fact that it is a strong oxidizer capable of hydroxylating saturated single C-H bonds and epoxidating unsaturated double C=C bonds. A unique property of CP450, as well as some non-heme oxygenase's, is the selective hydroxylation of very strong (inert) aliphatic C-H bonds, which represents an age-old dream of chemists [2]. It was found that Fe porphyrins as typical CP450 chemical models also mediate selective C–H oxidation reactions [3]. This led to the development of efficient and selective C–H oxidation catalysts and helped to get important information very useful for understanding of active intermediate of CP450. Theoretical calculations and comparison with the less reactive and, consequently, more stable related enzyme CPO played an important role in understanding of the electronic and geometric structure of active intermediate of CP450. The transient active intermediate Compound I CP450 is difficult to capture and characterize due to its extremely high reactivity. Despite the great efforts of many laboratories in the world and sophisticated spectral methods, it has eluded researchers for more than 40 years. There is still debate about the nature of its interaction with substrates and the mechanism of catalysis of oxygen transport. Formally, this mechanism is 2-electron (molecular, as opposed to one-electron, radical) [4] and the iron atom in the active intermediate CP450, in which two one-electron (radical) centers are conjugated, is formally in an effective 5-valence state.

In 2010 a report appeared from the green group [5] who for the first time managed to obtain a sufficiently pure preparation of Compound I CP450 in a fairly high concentration and carry out spectroscopic and kinetic characterization of this so-long elusive key intermediate CP450. As expected, it turned out to be an iron (IV)=O (or ferry) species with an additional oxidative equivalent delocalized on porphyrin and thiolate ligands. On the other hand, since the discovery of CP450, numerous ambitious experimental attempts have been made to uncover this mystery of Nature not only by working with various representatives of the huge family of CP450, but also by studying related iron-containing oxygenase's and their chemical models. A large role in this was played by the method of substrate probes widely used by chemists and biochemists, based on a comparison of the reactivity of the assumed active species with more studied species. Similarly, an understanding of the stoichiometric (as opposed to intimate) mechanism was achieved by spectral observation of other intermediates of the CP450 catalytic cycle. Selective hydroxylation of hydrocarbons remains the most important search problem in catalysis. The results exclude free radical diffusion, but the possibility of a short-lived substrate

radical could explain the molecular stoichiometric mechanism. The concept of the intimate mechanism CP450, called "oxygen rebound" was proposed in 1978 [6-8]. In the same 1978, it was discovered that porphyrin iron complexes reproduce many functional features of CP450, in particular, the retention of the configuration of the attacked carbon atom [6-8], a test for a molecular two-electron mechanism. In 1996, the possibility of two-electron transfer of the O atom to the C-H bond, previously known only for heme models, was shown for the first time for non-heme iron complexes [9]. Thus, simple non-heme iron complexes can serve as models of non-heme monooxygenases. Studies of the mechanisms of reactions of heme and non-heme enzymes and their chemical analogues in recent years have led to significant achievements in the field of drug development and bioinspired chemical catalysis.

At the study fulfilled 10 years ago by my international research group in the *Prof Ebbe Nordlander* lab, involving Dr *M. Mitra*, *Dr H. Nimir*, and me but not yet published we have shown that mononuclear iron complex with ligand L = N3PyPh(R)2O- [10,11] demonstrates moderate activity and excellent selectivity in alkane oxidation with m-CPBA as terminal oxidant. Introduction of t-Bu groups in the ortho- and para-positions of the phenolate donor improves oxidation efficiency significantly. Upon mixing of cooled solutions complex and m-CPBA in CH3CN-CH2Cl2 at -50°C, a green species is formed that is stable in solution up to -20°C. Table below lists mechanical substrate probes used for examination of the nature of active intermediate during bioinspired hydrocarbon oxidation catalyzed by iron complexes with similar coordination environments. Our data are very distinct from those found for complex 1 [12] $[Fe^{II}(13\text{-}TMC)]2+$ (13-TMC = 1,4,7,10-tetramethyl-1,4,7,10-tetraaza-cyclotridecane) which works via Fe-acylperoxo intermediate $(N4)Fe^{III}\text{-}O_2COR$. And opposite, the striking likeness of the substrate probe data for FeL and 2 [13] $[Fe^{III}(Me3tacn)(acac)Cl]+$ suggest that the active species in the system FeL/m-CPBA/MeCN is also $(N_4O)+\cdot Fe^{IV}=O$.

Table:

Complex	A/K	3°/2°	KIE	RC, %	Active species	Ref.
FeL	1.5	3	4.8	37-96	$(N_4O)^{+\cdot}Fe^{IV}=O$	This work
1	0.5-1	3.9-5.1	3.6-4.7	-	$(N_3O_2)^{+\cdot}Fe^{IV}=O$	[11]
2	5.7	75	8	~100	$(N_4)Fe^{III}\text{-}O_2COR$	[12]

Further study of this reaction by the stopped jet method and freezing using EPR and Mössbauer will allow us to establish the nature of the active intermediate.

This work was conceived in order to find more effective biomimetic models of natural monooxygenases capable of selective oxidation of methane to methanol.

Acknowledgement

None.

Conflict of Interest

None.

References

1. Shteinman AA (2008) Iron Oxygenases: structure, mechanism and modelling. Russ Chem Rev 77 (11): 945-966.
2. Shteinman AA, Mitra M (2021) Nonheme mono-and dinuclear iron complexes in bio-inspired CH and CC bond hydroxylation reactions: Mechanistic insight. Inorg Chimi Acta.
3. M Costas (2011) Selective C–H oxidation catalyzed by metalloporphyrins. Coord Chem Rev 255: 23-24.
4. Shilov AE, Shteinman AA (1999) Oxygen Atom Transfer into C– H Bond in Biological and Model Chemical Systems. Mechanistic Aspects. Accounts of chemical research.
5. Rittle J, Green MT (2010) Cytochrome P450 Compound I: Capture, Characterization, and C-H Bond Activation Kinetics. Science 330(6006): 933-937.
6. Groves JT, and McClusky GA (1976) Aliphatic hydroxylation via oxygen rebound. Oxygen transfer catalyzed by iron. J Am Chem Soc 98: 859–861.
7. Groves, John T (1985) Key elements of the chemistry of cytochrome P-450: The oxygen rebound mechanism. Journal of Chemical Education 62: 11.
8. John T Groves (2006) High-valent iron in chemical and biological oxidations. Inorg Biochem 100(4) :434–447.
9. Kulikova VS, Gritsenko ON, Shteinman AA (1996) Molecular Mechanism of Alkane Oxidation Involving Iron Complexes. Mendeleev Commun 6(3): 119-120.
10. Ligtenbarg AGL, Oosting P, Roelfes G, La Crois RM, Lutz M, et al. (2001) Efficient catalytic oxidation of primary and secondary alcohols using a non-heme dinuclear iron complex. Chem Commun 4: 385-386.
11. Unjaroen D, Swart M, Browne WR (2017) Electrochemical Polymerization of Iron(III) Polypyridyl Complexes through C-C Coupling of Redox Non-innocent Phenolato Ligands, Inorg Chem 56(1): 470-479.
12. Wang B, Lee YM, Clémancey M, Seo MS, Sarangi R, et al. (2016) Mononuclear Nonheme High-Spin Iron(III)-Acylperoxo Complexes in Olefin Epoxidation and Alkane Hydroxylation Reactions. J Am Chem Soc 138(7): 2426–2436.
13. Tse CW, Chow TWS, Guo Z, Lee HK, Huang JS, et al. (2014) Nonheme Iron Mediated Oxidation of Light Alkanes with Oxone: Characterization of Reactive Oxoiron(IV) Ligand Cation Radical Intermediates by Spectroscopic Studies and DFT Calculations. Angew Chem Int Ed 53(3):798-803.
14. Shteinman AA (2020) Bioinspired Oxidation of Methane. Kinetics and Catalysis 61(3): 339-359.