

PROJECT ADMINISTRATION DATA SHEET

ORIGINAL REVISION NO. _____

Project No. G-33-627 (R5932-PA0) GTRC/~~CR~~ DATE 6 / 5 / 85

Project Director: S. W. May School/~~LES~~ Chem

Sponsor: DHHS/PHS/NIH/NIGMS

Type Agreement: Grant No. 1-R01-GM33950-01

Award Period: From 8/1/85 To 7/31/86 (Performance) 10/31/86 (Reports)

Sponsor Amount: This Change Total to Date

Estimated: \$ _____ \$ 104,005

Funded: \$ _____ \$ 104,005

Cost Sharing Amount: \$ 6,094 Cost Sharing No: G-33-386

Title: Metallo Monooxygenase Catalysis

ADMINISTRATIVE DATA

OCA Contact John Schonk x4820

1) Sponsor Technical Contact:

2) Sponsor Admin/Contractual Matters:

Warren Jones

Diana O'Donovan

NIGMS

NIGMS

National Institute of Health

National Institute of Health

Bethesda, MD 20250

Bethesda, MD 20250

301/496-7621

301/496-7621

Defense Priority Rating: N/A Military Security Classification: N/A

(or) Company/Industrial Proprietary: N/A

RESTRICTIONS

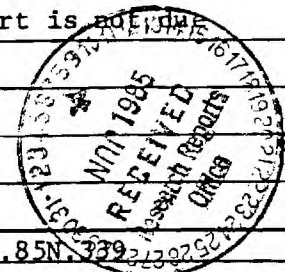
See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval - Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of \$500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

No funds may be expended after 7/31/86 even though the final report is not due until 10/31/86.



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SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 11/24/86

Project No. G-33-627

School/~~Dept~~ Chem.

Includes Subproject No.(s) N/A

Project Director(s) S. W. May

GTRC / ~~XXX~~

Sponsor DHHS/PHS/NIH/NIGMS

Title Metallo Monooxygenase Catalysis

Effective Completion Date: 7/31/86

(Performance) 10/31/86

(Reports)

Grant/Contract Closeout Actions Remaining:

None

~~Final Invoice~~ or Final Fiscal Report

Closing Documents

Final Report of Inventions

Govt. Property Inventory & Related Certificate

Classified Material Certificate

Other _____

Continues Project No. _____

Continued by Project No. G-33-694

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- A. Jones
- R. Embry

G 33-627

SECTION IV PROGRESS REPORT SUMMARY		GRANT NUMBER GM-33950	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Sheldon W. May		PERIOD COVERED BY THIS REPORT	
NAME OF ORGANIZATION Georgia Institute of Technology		FROM 8/1/86	THROUGH 7/31/87
TITLE (Repeat title shown in item 1 on first page) Metallo Monooxygenase Catalysis			
(SEE INSTRUCTIONS)			

Publications

A. G. Katopodis, K. Wimalasena, J. Lee, and S. W. May, "Mechanistic Studies on Non-Heme Iron Monooxygenase Catalysis: Epoxidation, Aldehyde Formation, and Demethylation by the ω -Hydroxylation System of *Pseudomonas oleovorans*", Journal of the American Chemical Society, 106, 7928-7935 (1984).

S. W. May and A. G. Katopodis, "Oxygenation of Alcohol and Sulphide Substrates by a Prototypical Non-Haem Iron Monooxygenase: Catalysis and Biotechnological Potential", Enzyme Microb. Technol., vol. 8, 17-21 (1986).

Progress Report

The objectives of this research program, focusing on the monooxygenase system from *P. oleovorans* which carries out epoxidation and hydroxylation of simple aliphatic hydrocarbon substrates (POEHS) are to extend and amplify the mechanistic information we have on the chemical pathway of the reaction, to extend our investigations to the design and evaluation of novel substrates, to focus on interactions at the active site critical to catalysis, and to carry out initial feasibility studies for applying physical techniques to this monooxygenase. The following paragraphs summarize our progress during this first year of the project.

Mechanistic and Stereochemical Studies: Work with deuterio-olefins has now been completed, and has confirmed the mechanistic view outlined in Scheme I of the proposal. Deuterium migration concomitant with aldehyde formation has now been fully confirmed by mass spectral analysis of products produced from 1,1-diD-1-octene. Furthermore, we successfully completed synthesis of cis-1-D-1-octene and confirmed the 70% inversion of configuration we reported with the trans-1-D-1-octene. Taken together, our finding of corresponding inversion of olefinic geometry together with the D migration accompanying aldehyde formation fully confirm our mechanistic hypothesis, and this work has now been published in a full paper in J.Am.Chem.Soc.

Turning to fluorinated substrate analogs, synthesis of 1-F-1-octene, 1,1-diF-1-octene and 2-F-octene have been completed. Characterization of the enzymatic products formed from these substrates is currently being carried out by gc/ms, with CI ms proving essential since complex results are being obtained. We have preliminary evidence for formation of hydroxyoctanoate from both the 1,1-diF- and the 2-F substrates, but the sequence of chemical steps giving rise to this product is still unclear. Model studies on authentic fluorinated epoxides, possible immediate enzymatic products, are currently being carried out. Assay work with both 1-fluoro-octane and 1-H-perfluoro-octane has been completed using the F electrode; both compounds are good sub-

strates. Assay rates show linear dependence on monooxygenase present, absolute dependence on the presence of all components, and a sigmoidal dependence on FeRd, exactly as seen in the standard gc assays. A problem in this work has been the deactivating effect of F substitution which makes product identification studies much more difficult. With our discovery of striking activation of POEHS by imidazole, we now plan to reexamine the F compounds under conditions of much higher turnover.

We have found that 1,7-octadiyne is a potent time-dependent inactivator of POEHS. Inactivation exhibits characteristics of mechanism-based irreversible inhibition, and further detailed studies on this and other mechanism-based inactivators are planned. An aspect of the work on these inactivators which we did not anticipate in the proposal is that with the various new catalytic competences we are now finding for POEHS (see below), mechanism-based inactivators represent tools of choice for demonstrating that these quite distinct substrate classes all undergo reaction at a common active site. For the coming year we will reexamine F- and Me- olefins in the presence of POEHS activators, further characterize the acetylenic inactivator, and exploit the newly-discovered catalytic competences of POEHS to examine other mechanism-based substrate analogs.

Heteroatom-containing substrates: An extensive amount of work has been done in this area, far beyond what we were able to visualize when the proposal was written in 1983. The results have established several heretofore unknown activities for our prototypical NHI monooxygenase, POEHS. First, with thioether substrates (e.g. heptyl methyl sulfide) we have discovered that POEHS readily catalyzes oxygenative conversion to both sulfoxide and S-dealkylation products. Sulfide oxygenation exhibits the enzymological characteristics of the normal oxygenative pathway of POEHS, is kinetically facile, and is inhibited by our suicide substrate, 1,7-octadiyne. Studies with a series of thioether substrates revealed that partitioning between the sulfoxide and S-demethylation products is a function of substrate structure. Thus, octyl methyl sulfide is oxidized to the chiral sulfoxide, while p-methoxy phenethyl sulfide gives only the S-demethylation product. S-dealkylation is a process that has not previously been observed with monooxygenases, so we unequivocally demonstrated that this indeed proceeds via an oxygenative pathway by quantitative trapping of formaldehyde and the thiol; a simple displacement pathway would produce a C-1 product at the oxidation state of methanol.

Several types of oxygen-containing substrate analogs have now been investigated. First, we find that terminal oxygenative O-demethylation (e.g. with 1-methoxyoctane) is very readily effected by POEHS, and this is the most kinetically facile activity we have found to date. Several lines of evidence establish that this reaction proceeds via the normal oxygenative POEHS pathway. Strikingly, we have also found that POEHS is indeed capable of non-terminal oxyfunctionalization, as illustrated by the production of either secondary alcohols or ketones via oxygenative O-demethylation of branched alkyl methyl or branched vinyl methyl ethers, respectively. Thus, for example, both 2- and 3-methoxy alkanes readily undergo O-demethylation to the 2- or 3-alcohols, and the vinyl ether, 2-OMe-1-octene, undergoes oxygenative ketonization. In all cases, as expected for an oxygenative process, formaldehyde is also produced stoichiometrically. In related work, we also discovered the facile oxygenation of terminal alcohols to aldehydes by POEHS, again carefully establishing all the enzymological characteristics of the reaction.

In our view, these results are highly significant. We have now demonstrated the first example of chiral sulfoxidation by any enzyme system of this type. Coupled with our mechanistic and stereochemical results with olefinic substrates, analysis of the stereochemistry and mechanism of sulfur oxygenation will provide important insight into the chemistry of catalysis. Secondly, our discovery of the heretofore unrecognized competence of POEHS for oxygenation of non-terminal moieties significantly expands the menu of capabilities for this prototypic monooxygenase. This not only allows much more flexibility in the design of new substrates or inhibitors, but it also suggests a greatly expanded synthetic potential for this and other closely related bacterial hydrocarbon hydroxylating enzymes. Our working hypothesis for the mechanism of action of POEHS postulates that the substrate and binding sites of this enzyme are arranged in a manner necessitating oxygen attack at the terminal carbon. It now becomes clear that the reactivity of POM can be directed to positions other than the terminus of a straight chain, given an appropriate balance of binding and inherent reactivity of the functionality undergoing initial electron transfer to the active site metal. Elaboration of these aspects of our new substrate classes represents our goal for the coming year in this aspect of the program.

Biochemistry Focusing on the Catalytic Site: Within the past few months we have worked out a new isolation procedure for POM which utilizes FPLC. This will facilitate eventual EXAFS studies where the most difficult biochemical impediment is the requirement for large amounts of enzyme at high concentration. As stated in our proposal, we will draw heavily on our experience with PAH in attempting to apply EXAFS to POEHS. Our next EXAFS run at Brookhaven is scheduled for July; at that time we will be examining E, ES and E-pterin PAH samples. We therefore foresee running our first POEHS sample during the following beam period allotted to us.

An important finding which we have made recently is that POEHS catalysis is strikingly accelerated by imidazole, presumably via interaction with the active site iron. Stimulation is such that molecules whose activities were heretofore unmeasurably slow can be clearly seen to undergo reaction. A similar observation has been recently reported by Groves and Watanabe with an iron porphyrin model epoxidation system. This obviously allows us to reexamine reactivity of molecules such as F-olefins which are inherently unreactive toward oxygenation. Characterization and scoping of this imidazole activation and its possible extension to other heterocycles represents an important goal for the coming year. Furthermore, since we can clearly pick out an imidazole ligand to Fe by EXAFS, as demonstrated in our published work with PCD, a goal in the future will be to see whether the imidazole binds directly to the Fe. If so, it will provide a handle for examining ligation and geometry changes through EXAFS.