**Type Agreement:** Grant No. PCM-8402518

**Award Period:** From 8/1/84 To 1/31/87 *(Performance) 4/30/87 *(Reports)

**Sponsor Amount:**
- Estimated: 
- Funded: 

**Cost Sharing Amount:** $4,464

**Total to Date**
- $93,000

**Cost Sharing No:** G-33-355

**Title:** "Enzymatic Epoxidation and Oxygen Activation"

### ADMINISTRATIVE DATA

1) **Sponsor Technical Contact:**
   - Dr. Mary Kirtley
   - Biochemistry Program Director
   - National Science Foundation
   - Washington, DC 20550
   - (202) 357-7945

2) **Sponsor Admin/Contractual Matters:**
   - Ms. Ramona Lauda
   - Grants Official
   - National Science Foundation
   - Washington, DC 20550
   - (202) 357-9653

**Defense Priority Rating:**

**Military Security Classification:**

**RESTRICTIONS**

See Attached NSF 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:

*includes usual 6-month unfunded flexibility period.

**Sponsor I.D. #01.107.000.84.037**

**COPIES TO:**
- Project Director
- Research Administrative Network
- Research Property Management
- Accounting

**Formula OCA 4:383**
NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 02/26/91

Project No. G-33-676
Project Director MAY S W
Center No. R5820-0A0
School/Lab CHEMISTRY

Sponsor NATL SCIENCE FOUNDATION/GENERAL

Contract/Grant No. PCM-8402518
Contract Entity GTRC

Prime Contract No.

Title ENZYMATIC EPIDXIDATION & OXYGEN ACTIVATION

Effective Completion Date 870131 (Performance) 870430 (Reports)

Closeout Actions Required:

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Comments: 98-A SATISFIES THE REQUIREMENT FOR THE PATENT REPORT, SUBMITTED WITH THE FINAL REPORT. NO INVOICE REQUIRED-NSF LOC.

Subproject Under Main Project No.

Continues Project No.

Distribution Required:

- Project Director: Y
- Administrative Network Representative: Y
- GTRI Accounting/Grants and Contracts: Y
- Procurement/Supply Services: Y
- Research Property Management: Y
- Research Security Services: N
- Reports Coordinator (OCA): Y
- GTRC: Y
- Project File: Y
- Other: N

NOTE: Final Patent Questionnaire sent to PDPI. 98A
ENZYMATIC EPOXIDATION AND OXYGEN ACTIVATION

Sheldon W. May
School of Chemistry
Georgia Institute of Technology
Atlanta, Ga

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.

Mechanistic and Stereochemical Studies: Work with deutero-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 substrates. 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.
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. 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 oxidative 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.

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. 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 future. 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.
November 25, 1946

Annella K. Engel
Industry Program
National Science Foundation
Washington, D.C. 20350

Dear:

I have just been informed that the grants office at Georgia Tech has not received the enclosed report, which I prepared last fall and sent through to them. Therefore, I am sending the enclosed copy to you, and I hope in fact, you did not receive the original on account of some clerical error.

Best regards.

Very truly yours,

Sheldon W. May
Professor of Chemistry
FINAL SCIENCE FOUNDATION

FINAL PROJECT REPORT

PART I - PROJECT IDENTIFICATION INFORMATION

Program: Biochemistry Program
Project No.: Steinberg

From: To:

This Packet Contains
NSF Form 93A
And 1 Return Envelope
Heterotaxin-containing substrates: An extensive amount of work has been in this area, far beyond what we were able to visualize when the proposal was made. The results have established several heretofore unknown activities for typical HMO monooxygenase, POMHS. First, with this other substrates (e.g. aldehyde) we have discovered that POMHS readily catalyzes oxygenative 3-deoxy-D-and D-glucoside and acetyldeED products. Substrate oxygenation is a process that has not previously been observed with HMO. We have previously demonstrated that this indeed proceeds via an alternative pathway by quantitative trapping of formaldehyde and the final product pathway could produce a C-1 product at the oxidation site of the enhancer oxygen-containing substrate analogs have now been identified. First, we find that terminal oxygenation C-deoxylation (e.g., with glucose) is readily affected by POMHS and this is the way it takes place. This activity we have found to be related. Several lines of evidence suggest this reaction proceeds via the normal oxygenase POMHS pathway. We have also found that POMHS is indeed capable of terminal acrylic acid, as illustrated by the production of either succinic acids or 3-deoxy-D-gluco-sidic acid, respectively. Thus, for example, both 2 and 3-OH derivatives 2-deoxy-D-glucose in the 2 or 3- position and the normal "components" undergo oxygenative ketonization. In addition, acetylation of carbon monoxide to also produce.

"..."
ANIMAL RESPIRATION AND OXYGEN ACTIVATION

A. M. May
School of Chemistry
Georgia Institute of Technology
Atlanta, Ga.

Mechanistic and Stereochmeical Studies: Work with deuterated substrates has been completed, and has confirmed the mechanistic view outlined in a recent paper. Bacteria isolated from the stomach of guinea pigs, Deuterated aldehydes and ketones have been studied by mass spectrometry of products formed from these substrates. Furthermore, we have successfully completed an X-ray study of the inversion of configuration at the C2 position in 2-deuterated substrates. Together, our findings of correlation discrepancies between the inversion accompanying the C2 deuteration, and this work has been interpreted in terms of a simple mechanism involving the immediate enzymatic reaction. This mechanism is consistent with both 1- and 2-deuterated substrates, and shows that the reaction is catalyzed by the enzyme system.

The reaction involves a 5-7-fluorinated substrate which is converted into a fluoro-deuterated product. The fluorine at position C2 is not stable, and the 2-fluorinated product is still unclear. However, the mechanism is consistent with a simple mechanism involving the immediate enzymatic reaction. This mechanism is consistent with both 1- and 2-deuterated substrates, and shows that the reaction is catalyzed by the enzyme system.
Activities were heretofore unmeasurably slow can be clearly seen to undergo.

A similar observation has been recently reported by Groves and Watanabe on porphyrin model systems. This obviously allows for an increase in reactivity of molecules such as n-olefins which are inherently unreactive (reaction). Characterization and scoping of this imidazole reaction and the extension to other heterocycles represents an important goal for the future study. Since we can clearly pick out an imidazole ligand to Fe by X-ray crystallography. In our published work with PCO, a goal in the future will be to find the imidazole ring directly to the Fe. If so, it will provide a very interesting and generally changes through EXPS.