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Project Title: **Characterization of Chemically Modified Hyperthermophilic Enzymes for Chemical Syntheses and Bioremediation Reactions**

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# Characterization of Chemically Modified Enzymes for Bioremediation Reactions

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## RESEARCH OBJECTIVE

Remediation processes frequently involve species possessing limited solubility in water. We are interested in novel strategies that use molecularly modified enzymes with enhanced activity and stability for the remediation of recalcitrant compounds in organic solvents. The performance of naturally-occurring enzymes is usually quite limited in such organic environments. The primary objective of the current work is to gain a fundamental understanding of the molecular and catalytic properties of enzymes that have been chemically modified so that they are catalytically-active and chemically-stable in organic solvents. The premise for this study is that stabilized and activated enzymes, which can function at harsh chemical conditions, are optimally suited for bioremediation in nonaqueous media where substrates of interest are more soluble and processed with greater efficiency. This unique strategy is examined with respect to the degradation of chlorophenols and PCBs.

## RESEARCH PROGRESS AND IMPLICATIONS

We are in the middle of the third year of a 3-year project, and this report summarizes work of the past year. We have obtained promising results both to demonstrate of the proposed remediation strategy and to reveal fundamentals of the enzymatic catalysis in organic media. In one effort, ligninase (LiP) from *Phanerochaete chrysosporium* was modified with polyethylene glycol (PEG) and examined for the degradation of pentachlorophenol (PCP) in water-solvent mixtures. In another effort, basic catalytic behaviors of the chemically modified hyperthermophilic redox enzymes including ferredoxin, hydrogenase, and aldehyde oxidoreductase were examined in organic solvents.

### I. PEG Modification of LiP and its Bioremediation Applications

LiP was modified using PEG to enhance its activity and stability for the biodegradation of PCP in the presence of acetonitrile (MeCN). MALDI-TOF mass spectrometry analysis showed that the modified enzyme has one or two PEG groups attached to each protein molecule. The modified enzyme retained 100% of its activity in aqueous solutions and showed enhanced activity in the organic solvent. The activity of the modified enzyme was found to be over twice that of the native enzyme in the presence of 10% (v/v) MeCN. The solubility of PCP was enhanced significantly by the addition of MeCN to aqueous solutions. Capitalizing on the enhanced substrate solubility and the increased activity of the modified enzyme, the catalytic efficiency of the modified LiP in solutions containing 15% MeCN was over 11-fold higher than that of the native enzyme in aqueous solutions (from 44 to 480 mol PCP/mol LiP•h).

The degradation reaction products were examined. While tetrachlorobenzoquinone (TCBQ) was the only reaction product observed in previously reported papers, our work revealed the

formation of three additional intermediates. This observation contributes to closing the mass balance between the yield of TCBQ and the disappearance of PCP. Furthermore, an additional product peak was observed by HPLC when PEG-LiP, instead of the native enzyme, was used. This result implies that the chemical modification of enzymes may affect the reaction specificity.

## II. Fundamentals of Modified Enzymes in Organic Solvents

Focus is on the basic properties of a hyperthermophilic redox protein, ferredoxin (Fd) from *Pyrococcus furiosus*, in organic solvents. This study will contribute to our knowledge of the oxidation and reduction chemistry of biocatalytic systems placed in nonaqueous reaction media.

*Thermal stability:* Studies were carried out with native and with PEG-modified Fd. Both enzymes dissolve in very polar organic solvents (e.g., DMSO) whereas PEG-Fd was also soluble in much less polar solvents including ethanol, propanol, butanol, benzene, and toluene. The thermal stability of both native and PEG-Fd is lower in DMSO compared to that in water ( $t_{1/2}$  at 80°C of 60 and <10 min, respectively). Nevertheless, PEG-Fd showed better stability in organic solvents (the  $t_{1/2}$  at 80°C increased to 30 min in toluene).

*Redox Potential:* The redox potentials of the native and PEG-Fds were determined using cyclic voltammetry. In general, little difference in redox potential was observed between the native and modified enzyme, either in aqueous solution or in solvents such as DMSO. This indicates that the integrity of the iron-sulfur cluster were not altered by PEG modification. However, organic solvent appeared to be capable of dramatically altering the redox potential of the protein. The redox potential of Fd was measured to be -690 mV in DMSO, which is much lower comparing with that in aqueous solution (-390 mV at pH 8). Since the absorption spectra of the protein remained intact in DMSO, the dramatic shift in redox potential is most likely due to the changes in cluster environment rather than structural variations. This was verified using a mutant Fd, D14S, in which a cluster-ligating aspartate is replaced by serine. The ability to change cluster potential may prove useful in allowing the kinetics of electron transfer during catalysis to be altered depending on the solvent that is used.

*Protein interactions in organic solvents:* The effect of the PEG modification on the ability of Fd to accept electrons in organic solvents from another enzyme, aldehyde ferredoxin oxidoreductase (AOR), was investigated. Information from such a study should be helpful in designing multi-enzyme redox systems to degrade recalcitrant organic pollutants. AOR oxidizes aldehydes to acids and directly reduces Fd. While there was no activity in aqueous solution when either one of the two proteins was modified with PEG (PEG-AOR or PEG-Fd), remarkably, a high rate of catalysis (50% the activity of the native proteins) was observed when both proteins were modified. However, no activity was detected with the two PEG-proteins in toluene or ethanol.

## PLANNED ACTIVITIES

Since the project and subcontracts were initiated later than the official start date, a no-cost extension is requested. Accordingly, the following activities are underway:

1. PCB degradation using hyperthermophiles. Bacteria that grow near 100°C such as anaerobic heterotrophic archaeon, *Pyrococcus furiosus*, the anaerobic heterotrophic bacterium, *Thermotoga maritima*, and the aerophilic archaeon, *Pyrobaculum aerophilum* will be investigated. Our initial tests with crude cell extracts from these organisms failed to degrade PCBs (Aroclor 1242). In ongoing experiments the same organisms are being grown in the presence of PCB analogs to determine if the relevant enzymes can be induced or if intact and active cells are required for the degradation.

2. Membrane-bound enzymes for nonaqueous biocatalysis. Due to their highly hydrophobic nature, membrane-bound enzymes are expected to be soluble in organic solvents without any modification. We will focus on the hyperthermophile *P. furiosus*. It has been shown that its membranes contain high hydrogenase activity. Surprisingly, in contrast to the well characterized cytoplasmic hydrogenase, the membrane-bound enzyme did not reduce elemental sulfur. The enzyme has been solubilized using detergent (octyl-glucoside) and its purification is in progress. Its solubility, stability and activity in various organic solvents will then be determined.
3. Degradation of PAH's and DNAPLs with modified redox enzymes. Efforts are underway to conceive bioprocessing technologies using enzymes such as LiP or HRP for the in situ degradation of organic pollutants.
4. Modify the entire AOR-Fd complex with PEG (instead of modifying the proteins separately). This approach is expected to minimize the disrupting interactions between the AOR and Fd. Effects of water/solvent mixtures will be investigated.

#### INFORMATION ACCESS

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