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Characterization of Chemically Modified Hyperthermophilic Enzymes for Chemical Syntheses and 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-engineered enzymes with enhanced activity and stability for the remediation of recalcitrant compounds in organic media. Organic biocatalysis may be motivated by the nature of the substrate itself, or by augmented mass transport, ease of product recovery, or novel reaction pathways afforded by the organic solvent. However, naturally-occurring enzymes are usually subject to quite limited activity and stability in such organic environments. The 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-thermally-stable in organic solvents. The premise for this study is that highly stable enzymes which are catalytically active in both water and in a range of organic solvents are optimally suited for bioremediation where substrates of interest are more soluble and may be processed with greater specificity in nonaqueous solvents. This bioprocessing system will be assessed using PCB's, DNAPL's and PAH's.

Research Progress and Implications

As of the second of year of a 3-year project, we have obtained promising results for both the demonstration of the proposed remediation strategy and fundamental understanding of the enzymatic catalysis in organic media. In one of our efforts, ligninase (LiP) was modified by poly(ethylene glycol) (PEG) and examined for the degradation of pentachlorophenol (PCP) in water-solvent mixtures. In our other efforts, basic catalytic behaviors of the chemically modified hyperthermophilic metalloenzymes including ferredoxin, hydrogenase and aldehyde oxidoreductase were examined in organic solvents.

I. Biodegradation of PCP by LiP in the Presence of Acetonitrile (MeCN): After initial screening tests with enzymes, modification methods, solvents and substrates, a model reaction system has been selected and characterized to demonstrate the proposed strategy. PCP has been selected as a model contaminant. PCP is only slightly soluble in water, the use of MeCN, however, can effectively increase PCP's solubility. The addition of 15% MeCN enhances the solubility of PCP by over one order of magnitude (from 0.06 to 0.65 mM) as a result of our study. The PEG-modification of ligninase was examined with respect to a few fundamental concerns. It was determined that, by using a free amine group titration method, 12 PEG molecules on average were attached to each LiP under the current modification method. The modification reaction is mild, and no loss in enzyme activity was observed. PEG-LiP was partially soluble in pure MeCN, while no native LiP was found dissolved in MeCN.

The modified LiP was then examined for PCP degradation in the presence of MeCN (up to 30%, v/v). Overall, the activity of PEG-LiP was higher than that of native LiP. Intrinsic activities in solutions containing 1% and 10% MeCN were determined. Interestingly, the activity of PEG-LiP in the presence of 10% MeCN is about 2.5-fold higher than that of native LiP. Experiments with PCP concentrations close to saturation with different amount of MeCN (up to 15%, v/v) were performed. Enzyme and peroxide were added into the reactor continuously by a syringe pump to reduce the enzyme inactivation effect by peroxide. The catalytic efficiency of PEG-LiP for PCP degradation in the presence of 15%

(v/v) acetonitrile was 11-fold higher than that of the native enzyme in pure aqueous buffer (480 vs. 44 mole-PCP/mole-LiPh), as a result of the augmentation in both PCP solubility and enzyme activity.

II. Modification of Hydrogenase, Pf Ferredoxin (Fd) and Aldehyde: Oxidoreductase(AOR): The modified enzymes were first characterized by EPR studies. The EPR spectra of the ^4Fe - and ^3Fe -cluster of the PEG-Fd are similar to those of native Fd in aqueous buffer, although they are not identical. It was also showed that ^4Fe cluster in the PEG-Fd becomes oxidized and converted to ^3Fe form when dissolved in toluene. These results suggested that oxidation of the Fe-S clusters could be the reason for poor stability/activity of PEG-Fd and PEG-hydrogenase in toluene. The addition of acenaphthalenide was found to be able to prohibit such an oxidation reaction. On the other hand, the hydrogenase EPR signal of the PEG-hydrogenase disappeared in toluene even in the presence of acenaphthalenide, indicating the EPR-active ^2Fe -2S cluster was oxidized. PEG-hydrogenase shows reduced EPR spectra in acetonitrile.

The catalytic behaviors of these modified enzymes were also examined in organic solvent. PEG-AOR showed enhanced (5-fold) activity for the reduction of benzyl viologen (BV) in aqueous solution, this could be in part due to the enhanced hydrophobic interaction between the PEG-AOR and substrate. Interestingly, the activity of PEG-AOR for the reduction of PEG-Fd is only ca. 17% of that of native AOR and Fd. It is suspected that the protein aggregation upon PEG-modification may be the cause for such activity loss. PEG-modified hydrogenase has a solubility of ~5 mg/ml in toluene. In the study of the catalysis of the solubilized hydrogenase in organic solvents, it was observed that addition of bulk water lead to obvious reduction sulfur dissolved in toluene. Based on this observation, a new reaction mechanism has been proposed:

Planned Activities

Efforts are underway to expand the range of enzymes, contaminants and modification methods to translate the proposed unique approach to effective remediation processes. PCB is currently being investigated as are polyaromatic hydrocarbon. Fundamental studies of the catalytic behaviors of the solubilized hyperthermophilic enzymes will be continued, and it is expected that these studies would ultimately lead to the development of high performance enzymatic catalysts for use in organic solvents.

Other Access To Information

P. Wang, C. A. Woodward, E. N. Kaufman "Poly(ethylene glycol)-Modified Ligninase Enhances Pentachlorophenol Biodegradation in Water-Solvent Mixtures." A paper submitted for publication in the journal Biotechnology and Bioengineering, April 1998.

C. H. Kim, M. w. w. Adams "Enzymatic Catalysis in Organic Solvent." 4th Annual Hyperthermophile Symposium, Del Mar, CA, May 1997.