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Activity-Based Protein Profiling as a Novel Molecular Diagnostic Tool

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Background/Objectives. Activity-based protein profiling (ABPP) is a proteomics method that enables the detection of catalytically active enzymes in complex mixtures. Although this approach has been widely used in mammalian cell studies, its application to the study of microorganisms involved in environmentally important reactions and in environmental samples is currently limited. This presentation will summarize our recent research into the use of ABPP to detect, identify and quantify monooxygenase enzymes involved in the biodegradation of several environmental contaminants including fuel hydrocarbons, solvents and 1,4-dioxane (14D). Our long-term aim is to develop quantitative ABPP approaches that will enable detection of key monooxygenase in environmental samples and can be used to predict rates of monooxygenase-catalyzed reactions in environmental samples.

Approach/Activities. Our ABPP studies typically involve the mechanism-based inactivation of monooxygenase enzymes using simple terminal diyne probes. The *in vivo* inactivation of these enzyme by diynes results in the formation of a covalent enzyme-probe adduct that retains an unreacted terminal alkyne group. This adduct can then be labeled with azide-containing reporter tags using a simple alkyne/azide cycloaddition “click” reaction. Bacterial cells containing the labeled monooxygenases can then be analyzed by a variety of methods including SDS-PAGE, mass spectrometry, fluorescence microscopy and flow cytometry/fluorescence activated cell sorting.

Results/Lessons Learned. Our research using pure cultures of model bacteria has demonstrated that the ABPP approach is widely applicable and can be used to selectively label and identify well-characterized monooxygenases in strains that grow on either ammonia, methane, alkanes, alkenes, or aromatics. In the case of *Nitrosomonas europaea,* our kinetic studies have demonstrated that diynes are mechanism-based inactivators that specifically target ammonia monooxygenase (AMO). The active-site containing component of this enzyme (AmoA) can be fluorescently labeled after treatment of cells with a range of terminal diynes. Confirmation that AmoA undergoes covalent modification during diyne inactivation has been shown using both in-gel and on-bead proteolytic digestion and mass spectrometric analyses of the resulting peptide fragments. Our recent analyses indicate there is a direct relationship between the amount of fluorescent AmoA detected by SDS-PAGE/ IR scanning and AMO activity. Fluorescence microscopy also demonstrates that the ABPP approach is effective in labeling whole cells of *N. europaea*. In the case of the *n*-alkane-oxidizing bacterium *Pseudomonas putida* GPo1, we have demonstrated that several components of the membrane-bound alkane hydroxylase (AH) can be detected in alkane-grown cells or in cells in which AH activity has been specifically induced by a gratuitous inducer, DCPK. Analyses of DCPK-induced cells have also shown that induction of AH activity can be readily detected by flow cytometry. In the case of the isobutane- and 14D- oxidizing strain *Rhodococcus rhodochrous* ATCC 21198, our ABPP approach has detected substrate-dependent co-expression of both propane monooxygenase and a soluble methane monooxygenase (sMMO)-like enzyme that is implicated in 14D degradation. We have also used ABPP to monitor and verify induction of the expression of this enzyme by 14D. A quantitative analysis of the fluorescent-derivative of this enzyme in SDS-PAGE analyses suggests the limit of detection is ~1 ng of protein or ~1 $x10^4$ cells. We have also demonstrated that this sMMO-like enzyme can be detected in soil microcosms containing either native isobutane-oxidizing bacteria or augmented ATCC 21198.