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Activity-Based Protein Profiling of Alkane Hydroxylase in *Pseudomonas putida* Strain GPo1

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Background/Objectives. Alkane hydroxylases (AHs) are widely distributed enzymes found in bacteria capable of biodegrading many fuel-associated hydrocarbon contaminants. The model AH-expressing bacterium *Pseudomonas putida* GPo1 exhibits AH activity after growth on C_3-C_{10} *n*-alkanes or after exposure to the gratuitous inducer, dicyclopropylketone (DCPK). The aim of the study was to determine whether an activity-based protein profiling (ABPP) could be used to detect AH in strain GPo1 and whether fluorescence-based detection of this enzyme by different approaches correlated with AH activity.

Approach/Activities. 1,7-octadiyne (17OD) was used as an ABPP probe for AH and AH expression in *P. putida* GPo1 was induced either by growing cells on individual *n*-alkanes or by adding DCPK to dextrose-grown cells. AH activity was determined using gas chromatography to quantify the oxidation of methyl *tertiary* butyl ether (MTBE) to *tertiary* butyl alcohol (TBA). Structural polypeptides of AH in 17OD-treated cells were fluorescently labeled using AlexaFluor 647 azide and a copper-catalyzed alkyne/azide cycloadditon reaction. Fluorescent polypeptides were separated by SDS-PAGE and visualized using IR scanning. Fluorescence from 17OD-treated cells was also quantified by flow cytometry after labeling 17OD-treated cells with AlexaFluor 488 azide.

Results/Lessons Learned. When DCPK was added to *P. putida* GPo1 growing on dextrose, the specific rates of AH-dependent MTBE oxidation increased over 5 h from <0.5 to >20 nmoles TBA generated min^{-1} mg total $protein^{-1}$. During AH induction by DCPK, subsamples of cells were exposed to 17OD and then reacted with AlexaFluor 647 azide. Several strongly fluorescent polypeptides were detected by SDS-PAGE analyses of total cell protein and IR scanning. The fluorescence associated with these polypeptides also increased during the time course of AH induction. The same fluorescent polypeptides were also detected for 17OD-treated cells grown on all *n*-alkanes tested (propane, *n*-butane, *n*-pentane, or *n*-octane). In contrast, no fluorescence or MTBE-oxidizing activity was detected in DCPK-treated cells of *P. putida* GPo12, a strain that lacks the AH-encoding OCT plasmid. Immunoprecipitation reactions conducted with solubilized whole cell extracts using either anti-AlkB, anti–AlkG, or anti-AlkT polyclonal antibodies demonstrated that three of the fluorescent polypeptides detected in DCPK- and 17OD-treated cells were AlkB (41 kDa), AlkG (19 kDa) and AlkT (46 kDa). An MS analysis of trypsin-digested polypeptides from DCPK-induced cells also confirmed that polypeptides modified by treatment with 17OD included AlkB, AlkG and AlkT. Fluorescently labeled cells were also readily detected using flow cytometry and the level of fluorescence was proportional to the levels of AH activity in defined mixtures containing varying amounts of DCPK-induced and uninduced cells. Overall, our results demonstrate that AH can be detected in *P. putida* GPo1 using a simple ABPP approach and that several AH components are covalently modified during inactivation of AH by 17OD. Our demonstration that AH can be fluorescently labeled in whole cells and that the fluorescence can be quantified by flow cytometry suggests that an ABPP approach could potentially be used to detect active AHs in environmental samples. Our results further suggest that the levels of fluorescence measured by flow cytometry could also potentially be used to predict the levels of activities of AH-expressing bacteria in the environment.