Comparison of Catalytic Behaviors between Two 1,4-Dioxane-Degrading Monooxygenases

Fei Li, Daiyong Deng, and ***Mengyan Li***

Background/Objectives. 1,4-Dioxane (dioxane) as an emerging contaminant has drawn extensive attention from the public because of its extreme persistence, extraordinary mobility, and carcinogenic effect. Recent studies have revealed that microbes that degrade dioxane are more widespread than we previously assumed, suggesting the feasibility of monitored natural attenuation (MNA) to mitigate the dioxane contamination. To date, two bacterial enzymes, tetrahydrofuran monooxygenase (THM) and propane monooxygenase (PRM), are known for their capabilities of initiating the ring cleavage of dioxane without the inducement of extra substrates. Previous research has been dominantly done using archetypical bacterial strains that express THM or PRM. However, their catalytic behaviors have not been well characterized at enzymatic level, which is the key for advancing our knowledge on dioxane metabolism. In this study, a systematic enzymatic comparison of THM and PRM is employed to investigate their differences in biodegradation kinetics, substrate ranges, responses to co-occurring inhibitors, and isotopic fractionation.

Approach/Activities. THM and PRM are expressed in a heterologous host, *Mycobacterium smegmatis* mc^2-155, which precludes the discrepancies generated by whole cell tests. The encoding genes of THM and PRM were obtained from the well-studied strains *Pseudonocardia dioxanivorans* CB1190 and *Mycobacterium dioxanotrophicus* PH-06, respectively. After induction, cloned cells were exposed to different concentrations of dioxane with or without the presence of selected inhibitors (e.g., 1,1-DCE, 1,1,1-TCA, and TCE). The Michaelis-Menten and three inhibitory models were fitted with experimental data to evaluate the half saturation coefficients (K_m), maximum degradation velocity (V_{max}), and inhibition constant (K_I).

Results/Lessons Learned. Converging lines of evidence suggest PRM is more advantageous than THM at the field condition, because PRM exhibits a higher affinity to dioxane but less susceptibility to common co-occurring inhibitors (e.g., 1,1-DCE, 1,1,1-TCA, and TCE). Although THM has a higher V_{max} indicating a greater degradation capacity, PRM can degrade dioxane faster at the environment-relevant concentration range of dioxane (i.e., < 230 mg/L). Inhibitory kinetics analysis showed the inhibition of 1,1-DCE and1,1,1-TCA to THM and PRM are well in line with the noncompetitive inhibition mechanism, while TCE exhibits a competitive inhibition mechanism. Substrate range survey revealed both enzymes are highly specific to cyclic ether compounds (e.g., dioxane and tetrahydrofuran), and could not degrade any of the tested chlorinated volatile organic compounds (CVOCs). Furthermore, the isotopic enrichment mediated by PRM is less significant compared to that triggered by THM, suggesting dioxane attenuation potential might be underestimated using compound-specific isotope analysis (CSIA). Overall, our results indicate PRM exhibits a greater ecological fitness than THM, which may facilitate the prevalence of its hosting cells and promote the horizontal transfer of its encoded genes at sites with commingled contamination of dioxane and cVOCs. An immense need exists to investigate the distribution and dynamics of PRM genes at impacted sites to improve the evaluation of dioxane natural attenuation potentials.