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Compound-Specific Isotope Analysis of Nitroaromatic Contaminant Transformations by Nitroarene Dioxygenases

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Dioxygenation is an important biochemical reaction that often initiates the mineralization of recalcitrant organic contaminants such as nitroaromatic explosives, chlorinated benzenes, and polycyclic aromatic hydrocarbons. However, to assess the extent of dioxygenation in contaminated environments is difficult because of competing transformation processes and further reactions of the dioxygenation products. Compound-specific isotope analysis (CSIA) offers a new approach to reliably quantify biodegradation initiated by dioxygenation based on changes in stable isotope ratios of the pollutant. For CSIA it is essential to know the kinetic isotope effects (KIEs) pertinent to the dioxygenation mechanism of organic contaminants. Unfortunately, the range of KIEs of such reactions is poorly constrained although many dioxygenase enzymes with a broad substrate specificity have been reported. Dioxygenase enzymes usually exhibit complex reaction kinetics involving multiple substrates and substrate-specific binding modes which makes the determination of KIEs challenging. The goal of this study was to explore the magnitude and variability of ^{13}C-, ^{2}H-, and ^{15}N-KIEs for the dioxygenation of one contaminant class, that is nitroaromatic contaminants (NACs). To this end, we investigated the C, H, and N isotope fractionation during the dioxygenation of nitrobenzene (NB), 2-nitrotoluene (2-NT), and 3-nitrotoluene (3-NT) by pure cultures, *E. coli* clones, cell extracts, and purified enzymes. From isotope fractionations measured in the substrates and reaction products, we determined dioxygenation KIEs for different combinations of the three substrates with nitrobenzene dioxygenase (NBDO) and 2-nitrotoluene dioxygenase (2NTDO). The ^{13}C-, ^{2}H-, and ^{15}N-KIEs for the dioxygenation of NB by NBDO were consistent for all experimental systems considered (i.e., *Comamonas* sp. Strain JS765, *E. coli* clones, cell extracts of *E. coli* clones, and purified NBDO). This observation suggests that the isotope fractionation during NB dioxygenation reflects the enzymatic reaction step leading to *cis*-dihydrodiol intermediates. In contrast, the observed isotope fractionation associated with the transformation of 2-NT and 3-NT by NBDO varied significantly. While 2-NT dioxygenation showed the same KIE as NB, the dioxygenation of 3-NT showed much smaller isotope fractionations than the two other substrates pointing to substrate-specific KIEs. 2-NT transformation by NBDO additionally lead to methyl-group oxidation, however this side reaction was not accompanied by noticeable isotope fractionations. Altogether, our study shows that the dioxygenation of NACs is associated with KIEs that enables one to use CSIA for assessing the extent and pathways of contaminant transformation. The current results also indicate the importance of understanding the substrate- and enzyme-specific variations in KIEs that will determine the observable contaminant isotope fractionation in the field.