The Incorporation of ^{18}O into Phthalate in the Enzymatic Formation of 4,5-dihydro 4,5-dihydroxy phthalate: Isotope labeling and LC-MS/MS studies

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Phthalate dioxygenase oxidizes phthalate to 4,5-dihydrodihydroxy diol. The reaction in catalyzed by a two component system that has been isolated from Pseudomonas cepacia as shown in the scheme. Electrons are transferred from NADH through a flavoprotein reductase containing FMN and a plant-type ferredoxin which transfers electron to a Rieske[2Fe-2S] center in the oxygenase. The later passes electrons to a mononuclear ion center that catalyzes the oxidation of phthalate to dihydrodiol. Phthalate undergoes dihydroxylation where the two oxygen atoms were inserted in the C-H bond of the aromatic ring to give 4,5-dihydro-4,5-dihydroxy phthalate. We have examined whether both atoms of oxygen incorporated derive from a single O_2, and whether any ring hydrogens exchange with solvent. The isotope labeling experiments indicates that the both oxygen incorporated into the product are derived from O_2. We used a mixture of ^{18}O and ^{16}O to ascertain whether the incorporated oxygens derived from the same molecule or not. The LCMS/MS spectrum showed two major parent ion peaks at 199 and 203 corresponding to ^{16}O and ^{18}O incorporation and there was no indication for both ^{16}O and ^{18}O incorporation. The fragmentation patterns clearly confirm the parent ions 199 and 203, suggesting that the incorporated oxygen comes from same oxygen molecule and not from the different molecules. The fragmentation patterns of the daughter ions 183, 159, 139, 115 and 95 of ^{18}O incorporated product (M^{-1} , 203) were identical to the fragmentation patterns 181, 155, 137, 111 ands 93 of the ^{16}O except for the increase in mass due to ^{18}O atoms. Reaction with deuterated phthalate showed that no ring hydrogens exchange. Indeed, when the DHD dehydrogenase and NAD^+ are added, only NADD, stereospecifically labeled in the pro-R position, is found. The finding of no exchange of either oxygen or hydrogen restricts what possible mechanisms can be valid. Thus no hydrogen atoms are abstracted, implying that an electron is removed from the substrate to initiate the oxygenation. Steady state kinetics showed no KIE, also consistent with these results.