Insights into the Mechanism of Hydroxyethylphosphonate Dioxygenase

John T. Whitteck, Robert M. Cicchillo, and Wilfred A. van der Donk

Phosphinothricin is a phosphinate containing commercial herbicide (sold under the trade name Liberty or Basta) with sales in excess of $200 million annually. A recent revision of the biosynthetic pathway revealed that hydroxyethylphosphonate (HEP) is an intermediate that is converted to hydroxymethylphosphonate (HMP) by hydroxyethylphosphonate dioxygenase (HEPD). Further biochemical characterization has shown that HEPD is a mononuclear non-heme iron dependent enzyme that is able to catalyze this biochemically unprecedented scission of an sp^3-sp^3 carbon-carbon bond without input of exogenous electrons or utilizing cofactors. Presenting isotopologues of HEP to HEPD identified formate as the second product. Through labeling studies it was shown that C1 of HEP and the hydrogen atoms at C1 were retained in HMP.

A mechanism is proposed in which iron is anchored to the active site via 2 His/1 Glu facial triad. Binding of HEP in a bidentate fashion and binding of molecular oxygen afford an Fe(III)-superoxo complex. This complex then abstracts a hydrogen atom from C2 of HEP affording a carbon centered radical and an Fe(III)-hydroperoxo species. Radical recombination with the terminal oxygen of the Fe(III)-hydroperoxo species affords an acetal intermediate and an Fe(IV)-oxo intermediate. Collapse of the acetal forms a Horner-Wadsworth-Emmons-like carbonanion which attacks the highly electrophilic Fe(IV)-oxo species to afford HMP and formate. Release of products resets the catalytic cycle.

Synthesis of subsequent isotopologues and structural analogues of HEP have provided further mechanistic insight. Synthetic (*R*)- and (*S*)-2-^{2}H-HEP were presented to HEPD and the isotopic composition of formate was determined, thus elucidating the stereospecificity of hydrogen atom abstraction. In addition, substrate analogues (*R*)-hydroxypropylphosphonate, HMP, 1-hydroxyethylphosphonate and 1-hydroxy-2,2,2-trifluoroethylphosphonate undergo chemistry with HEPD to afford mechanistically interesting products.