KEYNOTE PRESENTATION

Anomalous Deuterium Kinetic Isotope Effects on Fatty Acid Oxidation: Implications for Isotope Discrimination in Biology

Professor Justine P. Roth
Department of Chemistry, Johns Hopkins University
3400 N. Charles Street
Baltimore, MD 21218 USA
Phone: +1(410)516-7835
E-mail: jproth@jhu.edu

Cyclooxygenases (COX-1 and COX-2) are highly homologous tyrosyl radical-utilizing hemoproteins that possess dioxygenase as well as peroxidase activities. The products of genes on separate chromosomes, COX-1 and COX-2, control a range of physiologic and pathophysiologic functions with differing substrates and regulatory mechanisms. For example, COX-2 utilizes molecular oxygen (O_2) to convert omega-6 polyunsaturated fatty acids as well as endogenous cannabinoid ligands into prostaglandins, which are the sole precursors to all biogenic prostanoids. In addition, COX-2 is co-expressed with nitric oxide synthase in cell types where endocannabinoids are also found (unlike COX-1). Catalytic activity is enhanced by nitric oxide (NO) and the regulation has been associated with S-nitrosylation of a single cysteine in the catalytic domain of COX-2. The impact of NO in the enzyme's peroxidase and dioxygenase activities has yet to be illuminated.

This presentation will focus upon the reaction mechanisms of COX-2 relative to COX-1 as well as the structurally related rice-α-dioxygenase, another hemoprotein that utilizes a conserved tyrosyl radical in a position that is superimposable with Y371• in COX-2 (shown below). Studies of wt and Y334F COX-2, reveal anomalous deuterium kinetic isotope effects (KIEs) and similar competitive oxygen-18 KIEs of 1.020 and 1.015 on the dioxygenase catalysis with arachidonic acid (AA) and linoleic acid (LA), respectively. Anaerobic solvent isotope exchange experiments demonstrate that the reactive hydrogen of the substrate is retained at Y371 during enzyme turnover in the slow solvent exchange limit. The kinetic parameter corresponding to O_2 uptake exhibits large and/or anomalously temperature-dependent deuterium KIEs, consistent with irreversible hydrogen tunneling from Y371 to a peroxyl radical. This is the first irreversible step in dioxygenase catalysis and accounts for the regio- and stereo-specificity of product formation. The initially formed product contains a hydroperoxide moiety oxidizes the ferric protoporphyrin IX prosthetic group which is subsequently reduced by various anti-oxidants.

Deuterium and oxygen-18 KIEs observed with the wt and conformationally more flexible Y334F COX-2 variant indicate that mechanisms of regio and stereospecific product formation due to "O_2 channels" and accumulation of unpaired spin at specific positions of the bound substrates can be excluded. Instead conformational gating of Y371 re-oxidation by protein dynamics raises the possibility that endogenous cannabinoid metabolism is regulated, to a greater extent, by NO-modified forms of COX-2. The possibility that large discrimination factors result from accumulation of deuterated fatty acids, under these conditions, will be discussed.

References:

(1) “Molecular Oxygen Dependent Steps in Fatty Acid Oxidation by Cyclooxygenase-1 (COX-1).”Mukherjee, A.; Brinkley, D. W.; Chang, K.-M.; Roth, J. P. Biochemistry 2007, 46, 3975-3989.Mukherjee, A.; Brinkley, D. W.; Chang, K-M.; Roth, J. P. *Biochemistry*, **2007**, *46*, 3975-3989.

(2) “Hydrogen Tunneling Steps in Cyclooxygenase-2 Catalysis.” Danish, H. H.; Doncheva, I.; Roth J. P. *J. Am. Chem. Soc.* **2011**, *133*, 15846-15849

(3) Experimental and Computational Investigations of Oxygen Reactivity in a Heme and Tyrosyl Radical-Containing Fatty Acid α-(Di)oxygenase.” Huff, G. S.; Doncheva, I. S.; Brinkley, D. W.; Angeles-Boza, A. M.; Mukherjee, A.; Cramer, C. J.; Roth , J. P. Biochemistry 2011, 50, 7375-7389.

(4) “Catalytic Mechanism of a Heme and Tyrosyl Radical-Containing Fatty Acid α-(Di)oxygenase.” Mukherjee, A.; Angeles-Boza, A. M.; Huff, G. S.; Roth, J. P. J. Am. Chem. Soc. 2011, 133, 227-238.

(5) Liu, Y. Roth J. P. “Unmasking the Molecular Oxygen (O2) Dependent Hydrogen Transfer Step in Human Cyclooxygenase-2 (COX-2)” Liu, Y.; Roth, J. P. 2015, in revision.

Author Information:
Correspondence and requests for materials should be addressed to jproth@jhu.edu

Key Words:
deuterium kinetic isotope effects (KIEs), competitive oxygen-18 KIEs, nuclear tunneling, protein dynamics, catalysis, fatty acid oxidation mechanisms.