Kinetic, Spectroscopic and Structural Investigations of the First Coordination Sphere Mutant Asn694Gly of Soybean Lipoxygenase-1

Erika N. Segraves[1], Maksymilian Chuszcz[2], Viola C. Ruddat[1],

Wladek Minor[2], Theodore R. Holman[1]

[1]*Chemistry and Biochemistry Department, University of California, Santa Cruz,*
[2]*Department of Molecular Physiology and Biological Physics, University of Virginia*

ABSTRACT. Lipoxygenase (LO) is a mononuclear, non-heme iron dioxygenase that catalyzes the incorporation of molecular oxygen into a 1,4-*cis*, *cis*-pentadiene fatty acid to form hydroperoxide products. In plants, soybean LO isoform-1 (sLO-1) oxygenates linoleic acid to produce a 13(S)-hydroperoxide which affects plant senescence, while in humans, the lipoxygenase products of arachidonic acid are implicated in asthma, heart disease and various cancers. Two soybean lipoxygenases, sLO-1 and sLO-3, and one rabbit lipoxygeanse (15-rLO) have been crystallized. The five residues that serve as ligands for the iron-binding site have been shown to be highly conserved across plant and mammalian lipoxygenases, however, a key difference arises in the sixth amino acid ligand of the active site. 15-sLO-1 is the best characterized lipoxygenase with an Asn ligation. Utilizing MCD, Solomon and coworkers determined that the Asn was highly flexible and could vary in bond distance depending on experimental conditions. The flexibility and importance of the Asn was confirmed when it was mutated to a histidine, N694H, which manifested only a 6-coordinated species, due to the strong ligation of the histidine. In addition, the ligation of the His lowered the reactivity of the enzyme due to the fact that the iron's reduction potential was lowered and hence its rate of hydrogen abstraction lowered. This critical importance of the sixth ligand reinforces the need to generate additional mutants at this site to get a clearer picture of its role in the catalytic mechanism. In our continuing examination of the role of the Asn694 position in soybean lipoxygenase-1 (sLO-1), we present in the current poster kinetic, spectroscopic and structural characterization of a point mutation, Asn694Gly (N694G), that contains an active site with two iron-bound water ligands and a dramatically lowered enzymatic activity.