

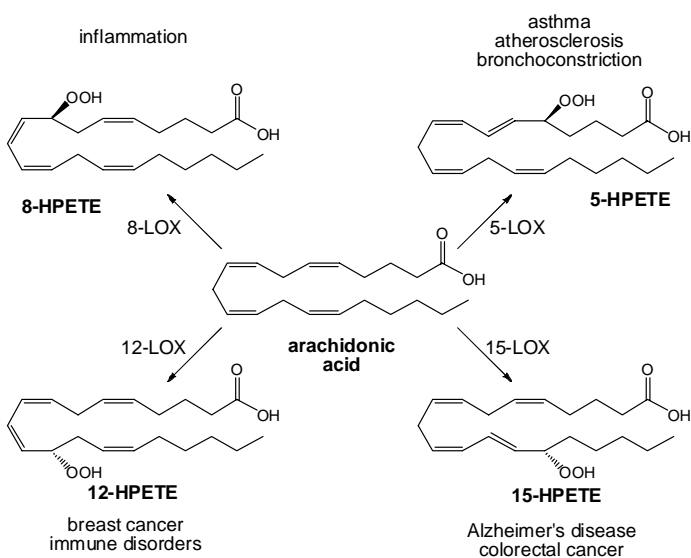
Mechanistic Studies of Lipoxygenases Using Synthetic Substrate Analogs

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Lipoxygenases (LOXs) are a class of enzymes that play key roles in a number of biological processes by mediating lipid peroxidation. These enzymes convert arachidonic acid into compounds called eicosanoids, which are involved in inflammation and have been implicated in a number of diseases such as atherosclerosis, Alzheimer's disease, and cancer. As a result, they are important therapeutic targets in the pharmaceutical industry.

Our studies focus on two particular aspects of lipoxygenases: the extremely large kinetic isotope effects (KIEs) and the control over regiochemistry of the oxidation (see Figure). The measured KIEs range from 30 to 60 in the reaction with linoleic acid and have been rationalized by invoking tunneling contributions to the rate-limiting hydrogen atom transfer during substrate radical formation. While these studies focused on linoleic acid, the reaction with arachidonic acid is of more interest since it is the natural substrate of mammalian lipoxygenases. Our laboratory has synthesized site-specifically deuterated arachidonic acid analogs and used them to measure kinetic and solvent isotope effects for the reactions of several human lipoxygenases. We found surprisingly low KIE values and also observed that the product distribution changed with deuterated substrates. These findings suggested the occurrence of isotopic sensitive branching, a process in which a large KIE affects the regio- and stereoselectivity of the enzyme.

Another goal of our study was to produce competitive inhibitors that could be used to obtain crystal structures of lipoxygenases with substrate analogs bound in the active site. Such structures are eagerly anticipated as they may provide starting points to investigate the unusually large KIEs. To this end, we have synthesized two sulfur-



containing analogs of linoleic acid. Kinetic assays have shown these compounds to be inhibitors for both soybean and human lipoxygenases and crystallographic studies are underway.