Crystal structure of 9S-Lipoxygenase from Enhygromyxa salina

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J. Kim *[I], J. Kim *[I]

[I]chonnam national university, Gwangju, South Korea

Lipoxygenases (LOXs) are nonheme iron-containing enzymes that catalyze dioxygenation of fatty acids with a 1,4-pentadiene structure. The LOXs can be grouped into two types according to their regiospecificity (i.e., 9-LOX and 13-LOX) which catalyze formation of 9-hydroperoxy and 13-hydroperoxy fatty acids from C18 fatty acids, respectively. The stereospecificity of LOXs is closely related to a single amino acid pair of Gly/Ala, where the glycine residue favors the (R)-form, and the alanine residue directs to yield the (S)-form (Coffa, G., & Brash, A. R. (2004). Proceedings of the National Academy of Sciences, 101(44), 15579-15584.). In contrast to several microbial 13-LOXs, microbial 9-LOX was rarely reported. We determined the crystal structure of a novel 9S-LOX from *Enhygromyxa salina* (EsLOX) that exhibits high activity for dioxygenation of linoleic acid (LIN), α-linolenic acid, γ-linolenic acid, and arachidonic acid specifically at the C9 position to S-configuration. Sequential and structural information indicates A389 as the determinant residue of Es-9S-LOX stereospecificity and this residue is located at the end of a probable oxygen tunnel. To understand its catalytic mechanism, the substrate (LIN), Fe^{2+} and OH^- were modelled into the active sites of EsLOX. LIN binds to EsLOX, where its polar carboxylate is at the entrance of, and the hydrophobic hydrocarbon chain is deep into the substrate-binding pocket with an extra space around its methyl end. This remaining space appears to afford an additional hydrocarbon unit. Indeed, arachidonic acid of 20 carbons could be successfully modelled within this substrate binding pocket. Taken together, the crystal structure of EsLOX provides a strong structural background on how the enzyme can accommodate two substrates of linoleic acid and arachidonic acid by locating their hydrocarbon chains into the pocket and oxygenate the C9 atom in an (S)-configuration specific manner.

* The authors marked with an asterisk equally contributed to the work.