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Relation between positional specificity and chirality in mammalian lipoxygenases

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Sir — Lipoxygenases are a family of non-heme iron dioxygenases which catalyze the incorporation of dioxygen into unsaturated fatty acids. Lipoxygenase products are the precursors of several families of potent lipid mediators including leukotrienes, lipoxins and other eicosanoids. The nomenclature of lipoxygenases is based on positional specificity and includes the site of oxygen addition (5th, 12th or 15th carbon) in products of (5Z,8Z,11Z,14Z)-eicosate-trienoic acid (arachidonic acid). In a recent issue of *Nature Structural Biology*, an article by Gillmor and coworkers¹ describes the structure of the first mammalian lipoxygenase: rabbit reticulocyte 15-lipoxygenase. In an accompanying News and Views report, Funk and Loll² discuss the implications of this structure.

This structure closely resembles the structure of soybean lipoxygenase³ including the presence of a (β-barrel domain. Based on the structure of the enzyme with an inhibitor, the authors propose how substrate arachidonic acid is bound in the active site: a surface arginine interacts with the fatty acid carboxylate while the methyl terminus interacts with hydrophobic residues deep in the binding pocket in such a way that the correct atoms are exposed to the reactive iron site. In particular, the 15-lipoxygenase reaction proceeds through abstraction of the C13 *pro-S* hydrogen and antarafacial (on the opposite face of the plane of the olefin) addition of oxygen to the C15 carbon.

Following the original proposal of Sloane and Sigal^{4,5}, Gillmor and coworkers¹ argue that conservation of certain residues in the active site of 12-lipoxygenases would similarly position the C10 *pro-S* hydrogen for abstraction and the C12 carbon for addition of oxygen. Other residues deep in binding pocket are less bulky in 12-lipoxygenase sequences and would allow substrate arachidonic acid to bind more deeply, as required (mutagenesis of these binding pocket residues by several groups supports this idea). Gillmor and coworkers¹ deviate from previous proposals^{6,7} by suggesting that in 5-lipoxygenases an additional translation of arachidonic acid into the binding site will expose C5 for dioxygenation by equivalent active site residues. This possibility was considered by Prigge and coworkers⁸ who concluded that: “Further translation of the fatty acid will not produce [5-]lipoxygenase products with the correct stereospecificity of hydrogen abstraction and oxygen addition.” That is, the product of a 5-lipoxygenase working in the manner proposed by Gillmor and coworkers¹ will have chirality (*pro-R* abstraction, R center formed) opposite to that of observed product (*pro-S* abstraction, S center formed).

To achieve product of the correct chirality in such a 5-lipoxygenase, arachidonic acid would have to be bound quite differently than in 12- and 15-lipoxygenases: conservation of nearby residues argues against this kind of explanation. Instead, if arachidonic acid binds to 5-lipoxygenases in the opposite direction (with its methyl and C termini exchanged), the product will have correct chirality (Fig 1*a, b*). This model involves burying the arachidonic acid carboxylate in the binding pocket of 5-lipoxygenases, in agreement with the observation that altered carboxylate fatty acids are not substrates for 5-lipoxygenases, and are substrates for 12- and 15-lipoxygenases. Poor reactivity of 5-lipoxygenase with linoleic acid as substrate is easy to explain if one assumes the carboxylate is fixed in the binding pocket and the 5th carbon is exposed to the reactive site. Linoleic acid does not have a reactive olefin at the 5th carbon as arachidonic acid does (Fig 1*c*); instead, the first linoleic acid olefin is at the 9th carbon.

References

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Fig. 1.

a, Stereospecificity of oxygen addition and hydrogen abstraction for 15- 12- and 5-lipoxygenase reactions are shown with arachidonic acid as the substrate. Arrows denote the locations of dioxygen addition (dashed arrows are below the plane of the page and solid arrows are above the plane of the page). **b**, When arachidonic acid is arranged in an opposite orientation, the 5-lipoxygenase reaction in panel (b) becomes spatially identical to the 15-lipoxygenase reaction in panel (a). Note that although linear conformations of arachidonic acid are shown here, the same chirality arguments also apply to U-shaped conformers. **c**, Linoleic acid is shown in the same orientation as the arachidonic acid in panel (b). If the carboxylate is fixed in the 5-lipoxygenase binding pocket, the unreactive 5th carbon of linoleic acid will be aligned with the reactive iron site.