SWITCHING THE REACTION SPECIFICITY OF MAMMALIAN 15-LIPOXYGENASE BY IN SILICO MUTATIONS

P. Saura,[a,b] L. Masgrau,[b] J. M. Lluch,[a,b] and À. González-Lafont*[a,b]

[a]*Departament de Química and* [b]*Institut de Biotecnologia i de Biomedicina (IBB), Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
e-mail: Patricia.Saura@uab.cat

The regio- and stereospecific catalysis of lipoxygenases (LOXs) is a key feature to understand how each LOX isoform manages to generate a different lipid mediator, with quite different (even opposite) physiological roles, from a common substrate, arachidonic acid (AA). The proximity of one particular AA methylene to the Fe(III)-OH- cofactor has been used as an structural feature that determines the regioselectivity of the rate-determining H-abstraction step. In this study we explore the molecular basis of rabbit 15-LOX-1 reaction specificity by means of two different *in silico* mutations that correspond to: i) modification of residues at the bottom of the active site cavity (Ile418) in accordance with mutagenesis experiments and, ii) modification of residues which hinder the evolution of AA during the reactive process (Leu597 and Ile663).

Molecular dynamics simulations and quantum mechanics/molecular mechanics calculations have been carried out on the Ile418Ala and Leu597Ala/Ile663Ala mutants of rabbit 15-LOX-1:AA. The lower average energy barriers for H_{10}-abstraction versus H_{13}-abstraction in both mutants confirm the change of regioselectivity from a 15-lipoxygenating wild type enzyme (H_{13}-abstraction favored) into a 12-lipoxygenating mutant enzyme (H_{10}-abstraction favored). However, the analysis of the generated productive mutant structures shows that the H_{10}/H_{13}-OH distances are neither the unique nor the most relevant factor for explaining the molecular basis of 15-LOX-1 regioselectivity. So, our in silico calculations provide a new mechanistic basis for the experimental "triad concept". In the wild type enzyme,[1] which is predominantly 15-lipoxygenating, H_{10} abstractions, but not the H_{13} ones, are sterically hindered by the bulky conserved residues Leu597 and C-terminal Ile663. However, in its Ile418Ala mutant arachidonic acid slides in deeper into the binding pocket, in such a way that C_{10} moves to the region occupied by C_{13} in wild type, and H_{10}-abstraction can occur without steric disturbance, the mutant becoming predominantly 12-lipoxygenating in agreement with experiment. When those two bulky residues are mutated *in silico* to smaller ones in the Leu597Ala/Ile663Ala mutant, H_{10}-abstraction is not impeded anymore and regioselectivity is also clearly inverted.

1) P. Saura, R. Suardíaz, L. Masgrau, J.M. Lluch, À. González-Lafont. *ACS Catalysis* **2014**, *4*, 4351-4363