MS53.O04

Structure basis of Leukotriene C4 Synthase and its isophtalate inhibitors

H. Ago[1], N. Okimoto[3], Y. Kanaoka[4], G. Morimoto[3], Y. Ukita[1], H. Saino[2], M. Taiji[3], M. Miyano[2]

[1]RIKEN SPring-8 Center, Hyogo, Japan, [2]Aoyama Gakuin University, Department Chemistry and Biological Science, Tokyo, Japan, [3]RIKEN Quantitive Biology Center, Kobe, Japan, [4]Harvard Medical School, Brigham & Women's Hospital, Boston, USA

Leukotriene (LT) C4 synthase (LTC4S) is a key enzyme for the production of cysteinyl leukotrienes, LTC4, LTD4 and LTE4, which are relevance to asthma and allergy. LTC4S catalyzes the conjugation of glutathione (GSH) to LTA4. The crystal structure of LTC4S complex with GSH revealed the active sites locate at the interfaces of adjacent monomer in trans-membrane homo-trimer [1]. The unique U-shaped GSH binds the inner hydrophilic interface cavity and the amphiphilic LTA4 was proposed to bind in the hydrophobic V-shaped crevasse on the interface of the trimer hydrophobic surface. Two essential arginine residues was proved to exert as acid-base catalysis at the both sides of two substrates in highly regio- and stereo-selective manner enzymatically and crystallographically [2]. The architecture of the catalysis with Arg104 and Arg31 residues is the unique among various Glutathione-S-transferases. Arg104 activates and stabilizes the thiorate anaion of the bound GSH and Arg31 stabilize and activate epoxy of the other substrate LTA4, and each mutation causes not only reduction of activity but also each substrate binding affinity substantially. Furthermore, the putative LTA4 binding position was confirmed using the anomalous signal of selenium of the bound seleno-dodecylmaltoside (Se-DDM) at the V-shaped crevasse. To identify leads for novel therapeutics, we attempted to search competitive inhibitors against the unique shaped GSH binding site to discriminate the GSH binding sites of other GSTs that accommodate only its extending backbone conformer [3]. Hierarchical in silico screenings of 6 million compounds provided 300,000 dataset for docking, and after energy minimization based on the crystal structure of LTC4S, 111 compounds were selected as candidates for a competitive inhibitor to glutathione. 5-(5-Methylene-4-oxo-4,5-dihydrothiazol-2-ylamino) isophthalic acid moiety was identified to inhibit LTC4 formation both an enzyme assay and a whole-cell assay. Finally, 5-((Z)-5-((E)-2-methyl-3-phenylallylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino) isophthalic acid was found to be the most potent inhibitor with 1.9 µM of IC50, and in the whole-cell assay to inhibit LTC4 synthesis with cell permeability in a concentration dependent manner.

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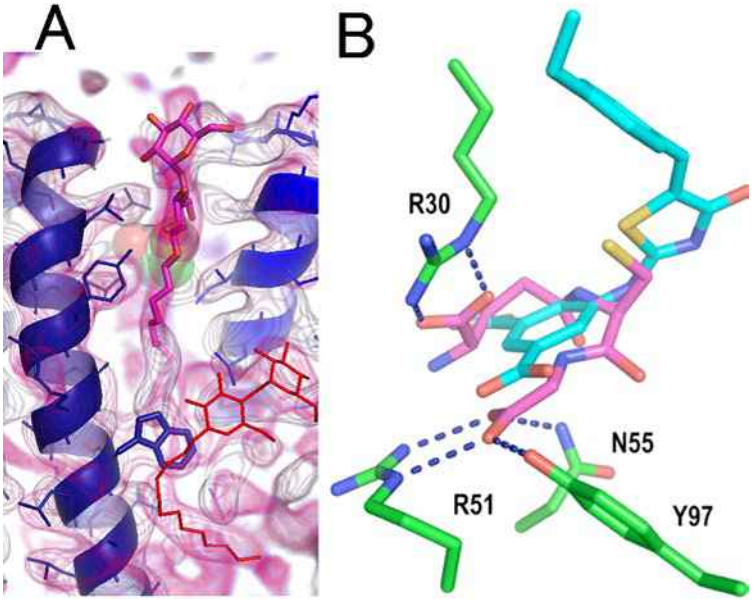

Keywords: LTC4S, membrane protein, isophthalic acid