

# The Importance of The Water Network Within the Leukotriene A<sub>4</sub> Hydrolase Binding Site for Aminopeptidase Activators

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The Leukotriene A<sub>4</sub> hydrolase (LTA<sub>4</sub>H) enzyme is a 69 kDa protein with two dichotomous functions in the lungs. As an epoxide hydrolase (EH), LTA<sub>4</sub>H catalyzes the hydrolysis of leukotriene A<sub>4</sub> (LTA<sub>4</sub>) to leukotriene B<sub>4</sub> (LTB<sub>4</sub>), which results in the infiltration of neutrophils. In contrast, as an aminopeptidase (AP), LTA<sub>4</sub>H promotes the resolution of neutrophilic infiltration by catalyzing the hydrolysis of the tripeptide PGP. However, recent studies suggest that the anti-inflammatory LTA<sub>4</sub>H AP activity may result from the clearance of substrates other than PGP. The AP activity of LTA<sub>4</sub>H enabled extensive characterization of the enzyme kinetics of LTA<sub>4</sub>H with peptides labeled with a *p*-nitroanilide reporter group in lieu of PGP, which cannot be monitored in a continuous enzyme assay. Activation of AP activity of LTA<sub>4</sub>H enzyme with 4-methoxydiphenylmethane (4MDM) was efficacious in promoting resolution of neutrophil infiltration in the murine cigarette smoke-induced model for emphysematous chronic obstructive pulmonary disease. Moreover, our recent study showed that 4MDM treatment consistently reduced the number of CD45+ leukocytes and CD45+CD11b+Ly6G+ neutrophil levels in LPS-exposed lungs while LTA<sub>4</sub>H activity in BALF was significantly augmented. Therefore, we published a series of 4MDM analogues that indicated different kinetic mechanisms on LTA<sub>4</sub>H AP activity, and distinct mechanisms of AP activities of three different X-*p*NA substrates in the presence of 4MDM. In this study, we determined the first new crystal complex structures of LTA<sub>4</sub>H:AMP at 2.41 Å and LTA<sub>4</sub>H:4-Me-ARM1 at 1.99 Å to demonstrate the importance of the water network within the binding site. We also determined the kinetic mechanism of AMP and 4-Me-ARM1 with Ala-*p*NA, Pro-*p*NA, and Arg-*p*NA as reporter groups.