Understanding Activation and Inhibition of Leukotriene A_4 Hydrolase Aminopeptidase by 4MDM-ARM1 Hybridized Modifiers

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The Leukotriene A4 hydrolase (LTA4H) is a unique zinc metalloenzyme enzyme with opposing bi-functional activities. The epoxy hydrolase activity (EH) of LTA4H, converts LTA4 to LTB4, a well-known pro-inflammatory mediator, but the aminopeptidase activity (AP) of LTA4H clears proline-glycine-proline (PGP), a pro-inflammatory chemotactic peptide. Our recent studies showed that activation of the AP activity of LTA4H with 4MDM was efficacious in promoting the resolution of neutrophil infiltration in the murine cigarette smoke-induced model for emphysematous chronic obstructive pulmonary disease. Haeggström and his colleagues published data with 4-(4-benzylphenyl)thiazol-2-amine (ARM1) as a new ligand for LTA4H with potential anti-inflammatory properties. Numao's study and our recent paper showed that PGP does not induce inflammation and the biology of LTA4H AP activity is independent of PGP. Therefore, we focused on the development of small molecules that showed differential effects on Ala-pNA hydrolysis. Our previous studies revealed the effect of 4MDM and ARM1 hybridized modifiers on the activation of LTA4H AP activity. We studied the kinetic mechanism of LTA4H in the presence of 2-Me-ARM1, 2-OMe-ARM1, and 2-CF3-ARM1 to determine kcat/Km, α, β, and KX. We showed that modulators with different size substituents change the AP activity from inhibition to activation. To further understand the effect of different substituents on AP activity, we determined the first X-ray crystal structure of the LTA4H:2-OMe-ARM1 complex at 1.68 Å. Our previously published LTA4H:4-OMe-ARM1 structure showed that 4-OMe-ARM1 has a lower B-factor with more structured beta sheets and extended helices. In contrast, in the LTA4H:2-OMe-ARM1 complex, the displacement of 2-OMe-ARM1 hinders the secondary structure and pushes water molecules to the catalytic zinc atom, which correlated with a higher B-factor and KX. The para-substituted analog, 4- OMe-ARM1, demonstrated a relatively higher β value over the ortho-substituent, 2-OMe- ARM1. The data suggest that the methoxy group in ortho- and para-substituents demonstrated the opposite effect on the LTA4H AP activity by modulating KX and β values.