Title: Structural Determinants of LTA4H Aminopeptidase Activation

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Leukotriene A4 hydrolase (LTA_4H) possesses opposing functions by activating both pro-inflammatory and anti-inflammatory pathways. In pro-inflammatory pathways the epoxy hydrolase (EH) activity of LTA_4H catalyzes hydrolysis of leukotriene A_4 (LTA_4) to LTB_4, a pro-inflammatory lipid mediator that contributes to diseases such as adult respiratory distress syndrome (ARDS), irritable bowel syndrome (IBS), and chronic obstructive pulmonary disease (COPD). In anti-inflammatory pathways, the aminopeptidase (AP) activity of LTA_4H catalyzes the hydrolysis of the peptide proline-glycine-proline (PGP), a chemotactic peptide reported to be associated with neutrophilic inflammation. The disease pathology associated with LTA_4H makes this enzyme an attractive target for therapeutic intervention. Several groups have targeted LTA_4H for development of therapeutics by non-selectively targeting LTA_4H EH function. However, many of these inhibitors have failed to show clinical benefit. Our therapeutic strategy is to develop anti-inflammatory compounds that selectively potentiate LTA_4H AP activity, while preserving EH activity. Previously, we evaluated enhancement of LTA_4H AP activity in kinetics assays with the small molecule 4MDM, tested 4MDM in two murine in vivo models, and determined the crystal structure of LTA_4H in complex with 4MDM. In this effort, we have synthesized new LTA_4H AP activators, evaluated the compounds kinetically and determined the 2.8 Å crystal structure of LTA_4H in complex with one of these activators. The new activator was bound in each of the 3 molecules of LTA_4H in the asymmetric unit. The 4MDM portion of the new activator bound in the same orientation as in the LTA_4H:4MDM complex structure. The presence of a heterocyclic group appended to 4MDM shifted the 4MDM group ~1.0 Å towards the LTA_4H AP active site. The methoxy group interacted with the main-chain carbonyl of Q136 and limits rotational freedom of this residue. The constraints on this residue offers insight into the differential effects of the 4MDM analog on AP activity as compared to 4MDM or ARM1. In conclusion, we have demonstrated potentiation of LTA_4H AP activity by new anti-inflammatory compounds *in vitro*, and determined the crystal structure of LTA_4H bound to one of these compounds. This structure will aid in the next round of design and synthesis of LTA_4H AP activators.