Abstract

Human 5-Lipoxygenase (5-LOX) is a peripheral membrane-binding protein that initiates the biosynthesis of pro-inflammatory leukotrienes (LTs). These LTs have been implicated in asthma, atherosclerosis, neurodegenerative, and allergic disorders. The structure of stable 5-LOX has been solved at cryogenic temperatures. However, the conformational changes of the enzyme required for catalysis are still unknown. To elucidate these changes, we aim to solve the structure of 5-LOX at room temperature where the protein is more flexible. To achieve this, we need to grow small, uniform crystals of 5-LOX that are amenable to jetting at LCLS. One way we are attempting to do this is by co-crystallizing 5-LOX in the presence of its inhibitors. Since 5-LOX is most stable in its closed conformation, we hope that the inhibitors act by encouraging a stable conformation of the enzyme.

Methods and Materials

Results

Figure 1. Stopped-flow spectrometers are used to conduct enzyme kinetics assays. Turnovers per second represents the activity of the enzyme under when it is combined with various small molecules. Novel, significant 5-LOX inhibition was exhibited by molecules A, B, E, and F. Making them promising candidates for co-crystallization attempts.

Figure 2. Vapor Diffusion Crystallization

a. Crystals grown from hanging drop vapor diffusion with a kinetically faster variant of 5-LOX and molecule B. The crystals are small and uniform with seemingly defined edges. b. Stable-5-LOX crystals grow using sitting-drop vapor diffusion. Although these crystals are densely packed, they appear to be uniform.

Figure 3. Stable-5-LOX Batch Crystallizations

a. Stable-5-LOX microcrystals measuring between 15 and 20 microns. b. Microcrystals grown using a higher protein concentration than in Figure 3a. Overall these crystals were smaller with the largest ones being around 13 microns. c. 10-13 micron sized microcrystals of stable-5-LOX using the same protein concentration as in Figure 3a. But a higher concentration of Tacsimate.

Figure 3. Batch Crystallizations using a Kinetically Faster variant of 5-LOX (Trip)

a. Microcrystals of Trip measuring average of 13 microns. b. Trip microcrystals grown at the same concentration of protein and Tacsimate as in Figure 3a but in the presence of molecule B. The addition of the small molecule yielded crystals with a drastically different morphology. c. Microcrystals grown with molecule A in similar conditions to the crystals in Figure 3b. Although the crystals in Figures 3b and 3c were grown in the presence of different inhibitors they have similar morphologies.

Background

Asthma

- Asthma is a disease that causes difficulty breathing due to inflammation and narrowing of the airway passage.
- When the inner lining of the airways is inflamed, they can swell and produce mucus.
- This makes the airways more sensitive to certain triggers that induce asthma attacks.
- These molecules inhibit the activity of 5-LOX, blocking leukotriene formation.
- NDGA and AKBA natural product inhibitors. NDGA is a competitive inhibitor and AKBA is an allosteric inhibitor.
- CJ-13610 is a synthetic inhibitor.

5-Lipoxygenase

- 5-LOX is a peripheral membrane binding protein that initiates the biosynthesis of leukotrienes.
- 5-LOX first adds molecular oxygen to arachidonic acid (AA) to produce 5-hydroperoxyeicosatetraenoic acid, then can perform a second step to modify the intermediate to leukotriene A4 (LTA4).
- LTA4, hydrolase often converts LTA4 to leukotriene B4, a chemotactic that activates inflammatory cells.
- Or LTC4 synthase can conjugate LTA4 to reduced glutathione, forming CysLTs implicated in the response to allergens.

Inhibitors

- NDGA
- AKBA
- CJ-13610

References

- Gilbert, Nathaniel C et al. "Demystifying the Activation and Inhibition of 5-Lipoxygenase through X-ray Crystallography." Kaelie CS Bernard, Nathaniel C Gilbert, Chris J. Kuptitz, Marcia E. Newcomer

Next Steps

- Continue working to develop a protocol for developing small, uniform crystals of 5-LOX and in and out of the presence of inhibitors.
- Conducting addition kinetic assays to detect possible inhibitors of 5-LOX that encourage a stable conformation of the enzyme.
- Rotating small, uniform crystals with the X-ray free electron laser at LCLS to generate an adequate diffusion pattern of the enzyme that can be used to solve the structure at high resolution.