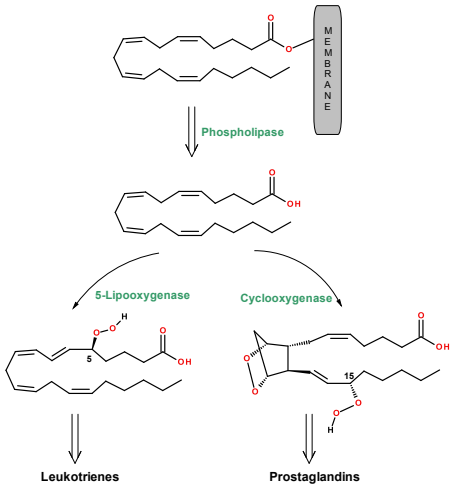
Chem 162/262 (Kahn, 2012) Assignment #1: (Due Apr 18)

Enzymes: Mechanisms & Inhibition

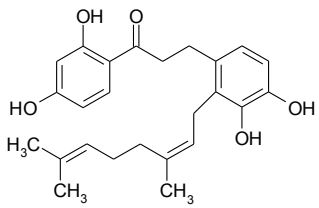
1. Inflammation is a complex process that at the tissue level is mediated by prostaglandins and leukotrienes. These molecules are synthesized from arachidonic acid. The synthesis of prostaglandins from arachidonic acid is catalyzed by cyclooxygenease, a well-characterized anti-inflammatory target. The synthesis of leukotrienes from arachidonic acid is catalyzed by 5-lipoxygenase. Despite the fact that leukotrienes mediate inflammation and hypersensitivity reactions, 5-lipooxygenase has not been successfully targeted in humans. However, some anti-inflammatory 5-lipooxygenase inhibitors have been identified from oriental medicinal plants.



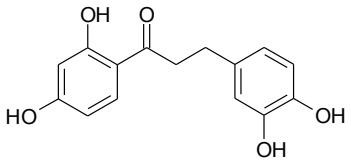
Cells contain significant concentrations (ca. 5 mM) of membrane-bound arachidonic acid but the level of free arachidonic acid is quite low (ca. 1 μM). External stimuli mediate activation of phospholipases via increased intracellular calcium. The action of phospholipases releases arachidonic acid from membranes and increases the concentration of free arachidonic acid 100-fold. The free arachidonic acid is then converted to pro-inflammatory leukotrienes and prostaglandins.

AA-induced ear edema in mice is a useful disease model for inflammation. In a typical assay, 2 mg of arachidonic acid, dissolved in acetone, is topically applied to the right ear while acetone only is applied to the left ear. One hour after the application of arachidonic acid, the thickness of both ears is measured; the difference is a measure of inflammation. To study the effect of anti-inflammatory drugs, some mice are pretreated with compounds that may inhibit lipooxygenase.

a) A new selective inhibitor of 5-lipooxygenase from Indonesian plant *Artocarpus communis* was reported to suppress AA-induced ear edema with IC_{50} value of 50 nM. The compound binds to the same form of enzyme that arachidonic acid binds. Sketch the Michaelis-Menten plot and the double reciprocal plot in the presence of 0, 5, and 50 nM concentration of the inhibitor when the concentration of arachidonic acid (K_m = 10 μM) is varied from 0 to 200 μM. Make sure to label your axes and lines completely. Only qualitative analysis is required.



b) Assume that the compound shown on the left forms a ternary complex with the enzyme and the substrate ($K_{i'}$= 10 nM), and also binds to the free enzyme (K_i = 200 nM). Sketch the double reciprocal plot in the presence of 0, 5, and 50 nM concentrations of the inhibitor. Make sure to label your axes and lines completely. Only qualitative analysis is required.



c) Which compound is likely to be stronger inhibitor of 5-lipoxygenase under physiologically relevant conditions? Justify your answer.

2. In their famous 1971 PNAS paper Michael Page and William Jencks proposed that enzymes may work as "entropy traps". Based on simple thermodynamic arguments they arrived to the conclusion that enzymes can accelerate bimolecular reactions by as much as 10^8.

a) Explain the "entropy trap" concept of Page and Jencks in your own words.

b) Many scientists now believe that while the basic idea behind the "entropy trap" concept remains valid, the 10^8 value largely overestimates rate acceleration that could be achieved by simply binding two reactants of the bimolecular reaction to the enzyme. Discuss reasons why the 10^8 estimate might be wrong.

c) Is the concept of "entropy trap" relevant to designing enzyme inhibitors as potential drugs? Discuss why.

3. Before the crystal structure of orotidine 5'-monophosphate decarboxylase (ODCase) was solved in 2000, three alternative mechanisms for the decarboxylation of OMP were considered likely. While the atomic-level analysis of this enzyme has helped to disprove some of these mechanisms, the molecular mechanism of catalysis by this highly proficient enzyme remains largely unsolved.

1. Draw (with appropriate chemical structures) the three alternative mechanisms that were considered prior to availability of the crystal structure. If you cannot find out from the literature what these three mechanisms were, you can come up with your own hypotheses.

2. Using molecular visualization software (SYBYL, PyMOL, any of your choice), prepare an image of the ODCase active site showing the bound nucleobase and the residues that are in direct contact with the nucleobase. You do not need to show the sugar nor 5'-phosphate moieties. Please do not use images that somebody else has made.

3. Discuss how the crystal structures of ODCase complexed with inhibitors disprove some of the mechanisms that you outlined earlier.