My Favorite Protein: Cyclooxygenase

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The goal of pain management has been sought after for many years, meaning people are constantly striving to improve function, enabling individuals to participate in day-to-day activities. One specific aspect of this field lies within the area inflammation. Various diseases such as rheumatic disease and arthritis cause a great deal of pain due to inflammation. For this reason, the mechanism of this disorder needed to be determined so anti-inflammatory drugs could be developed in order to aid individuals with such a disease.

In the 1930's, the prostaglandin was determined to be a major component in pain and inflammation, allowing the mode of action of pain management to begin to take shape. Prostaglandins were discovered to be mediators in inflammation that can be found in virtually all tissues and organs. "They are autocrine and paracrine lipid mediators which act upon platelet, endothelium, uterine and mast cells among others."[1] After this became known, the mechanism for these inflammation mediators was determined in order to try to find a way to inhibit the cycle. In doing this a fascinating enzyme was discovered called cyclooxygenase (otherwise known as COX) which would aid in their journey to anti-inflammatory drug discovery due to the fact that this enzyme catalyzes this conversion of arachidonic acid to PGG_2, an vital step in the prostaglandin cycle.

Now prostaglandins are synthesized in the cell from arachidonic acid. This component is released from membrane phospholipids by phospholipase A_2, which is activated by various stimuli.

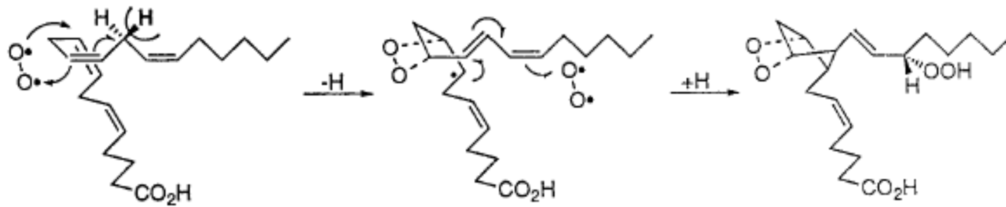

Figure 1: Chemical steps in the conversion of arachidonic acid to PGG_2 [2]

"The oxygenation of arachidonic acid is initiated by a protein oxidizing agent tyrosyl radical. This is formed by the enzyme removing the 13-pro-S-hydrogen, which generates a pentadienyl radical. The trapping of the carbon radical at C-11 with O_2 produces a peroxyl radical generating a cyclic peroxide and carbon centered radical at C-8. The C-8 radical adds to the double bond at C-12 generating the bicyclic peroxide and an allylic radical. Trapping of the carbon radical at C-15 with O_2 generates a peroxyl radical which is reduced to PGG_2 by Tyr-385 which regenerates the Tyr-385 radical for the next round of cyclooxygenase catalysis." [2]

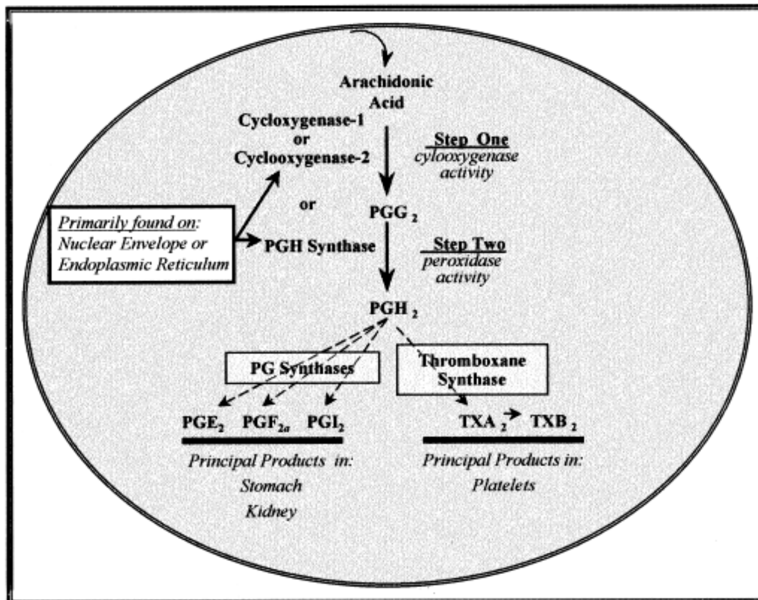

Figure 2: Diagram of Prostnoid Metabolism [1]

After PGG_2 is formed, peroxidase activity catalyzes the conversion of this unstable intermediate to another called PGH_2. The PGH_2 intermediate is then passed into either the cyclooxygenase or lipoxygenase pathways to form prostaglandin, thromboxane or leukotriene. The cyclooxygenase pathway produces thromboxane, prostacyclin and prostaglandin D, E and F, while the lipoxygenase pathway synthesizes leukotrienes.[1] After these prostaglandins

are produced, they are released through the prostaglandin transporter on the cell's plasma membrane.

It is known that without cyclooxygenase performing the initial conversion, prostaglandins could not be formed. Therefore attention turned to discovering more information about this important enzyme. In 1990 it was noted that there is a possible existence of two different cyclooxygenase enzymes. These two isomers are known as COX-1 and COX-2. COX-1, prostaglandin endoperoxide H synthase 1, is formed in platelets and functions to maintain tissue homeostasis, where COX-2 otherwise known as prostaglandin endoperoxide H synthase 2 principally mediates inflammation at sites such as atheromatous plaques and neoplasms. Other aspects that differentiate the two COX enzymes are factors such as the different chromosome the cyclooxygenases are found on, their range of expression, as well as their protein structure which came to great importance when creating anti-inflammatory drugs.

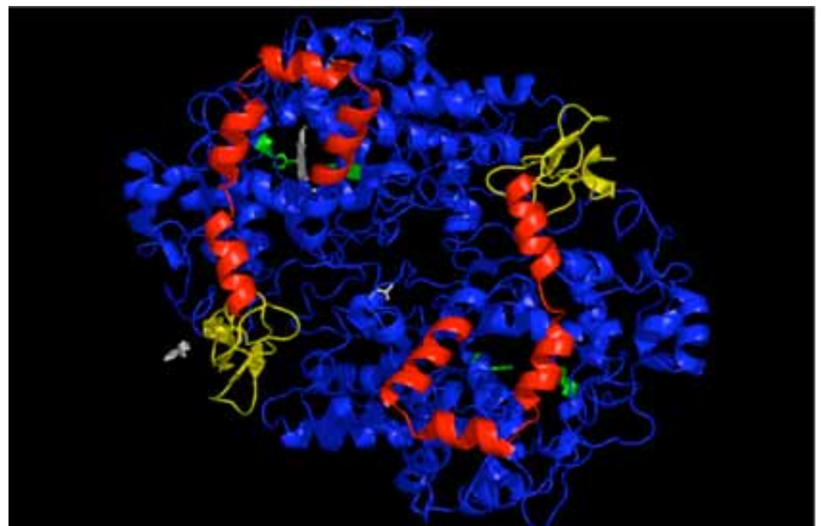

Figure 3: N-terminal domain depicted in yellow, the membrane binding motif depicted in red and catalytic domain in blue. (F7)

It was determined by x-ray crystallography at a resolution of 3.0 Å that the cyclooxygenase enzyme existed as a homodimer with 587 amino acid residues per chain thus yielding a molecular weight of 67230 daltons[3]. The R and R_{free} factors of the cyclo-oxygenase structures were denoted to be 0.321 and 0.308 respectively, showing that over refinement was not an issue with the structural analysis . Upon investigation of the

homodimer, it was determined that within a single monomer of this structure, three domains existed; the N-terminal epidermal growth factor domain, the membrane binding motif and the C-terminal catalytic domain as seen in Figure 3. Collectively, these domains are made up of 25 alpha helices, seven 3_{10} helices, four beta sheets as well as five disulfide bonds which contribute to the interface binding of the two individual monomers to complete the enzyme. In addition, it has been noted that by viewing the crystal structures of the COX enzyme four carbohydrate sites (occupied by n-acetyl-d-glucosamine) were discovered as well as the positioning of a peroxidase catalytic site, which is occupied by a heme group in the structures analyzed.

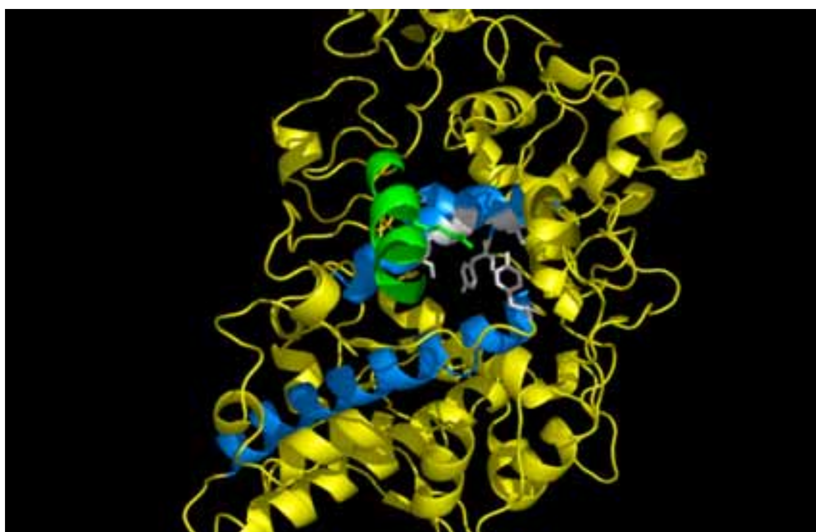

Figure 4: View of COX hydrophobic pocket indicated by blue helices, including membrane binding helicies in green in addition to pertinent catalytic residues Try355, Try 385, Ser 530, Arg 120, and Val 349 (F8)

However the fascinating focal point of this enzyme is within a hydrophobic channel created by helices C and D which interact with the membrane as well as helices 6, 8, and 17 which lead to the cyclooxygenase active site of the enzyme as seen in Figure 4.[4] In addition, the positioning of the amino acids themselves creates two hydrophilic channels where the product of the enzymatic activity is released. Various important residues that affect the catalytic activity of this also reside in this narrow hidden pocket. For example, Try 385 in its radical form is thought to be responsible for abstracting a proton from arachidonic acid during its conversion to PGG2,

where Ser 530, located near the active site, affects binding of arachidonic acid or substrate. Arg 120, also located in this hydrophobic pocket is found to anchor the carboxylate of the substrate to form an ion pair, and Ileu/Val 349 controls the accessibility of exit channels of the final products of this enzymatic activity.

As noted earlier, two isoforms of the cyclooxygenase enzyme exist. These two isoforms share a sequence identity of 60% denoting that the overall structures of the enzyme isoforms are highly conserved. Differences lie within the terminal regions of the structures where COX-1 has an insertion of eight residues on its N-terminus end in comparison to COX-2 which as an eighteen residue insertion on its C-terminus end. In addition, there are two lone substitutions within the hydrophobic pocket active site of the enzyme. Val 523 and 434 in COX-2 are substituted from the original Ileu 523 and 434 residues found in COX-1, as seen in Figure 5. This alters the shape and size of the hydrophobic region by 0.7Å, thus creating regulatory specificity of the enzyme.[4,5]

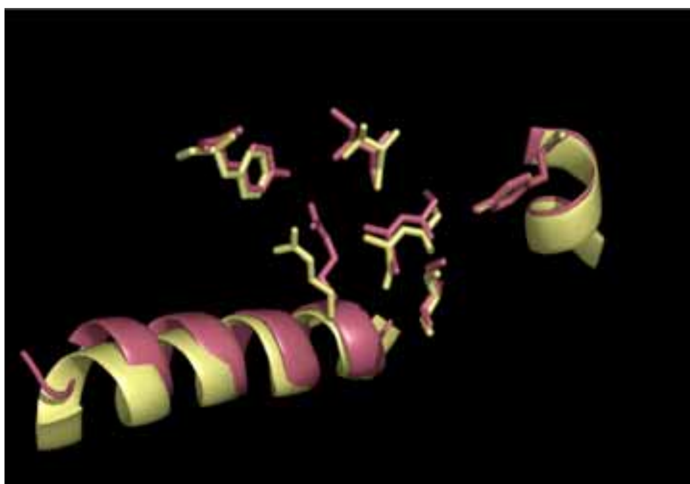

Figure 5: Overlay of COX-1, raspberry, and COX-2, gold, hydrophobic pocket and significant residues. (F9)

In both isoforms of the COX enzyme, inhibition mainly focuses upon the binding of or interaction with arachidonic acid. In one case of cyclooxygenase inhibition, COX-2 uses arginine 120 in its active site to form a hydrogen bond with arachidonate. [6] The formation of this weak bond is located below a reactive serine which transfers its acetyl group. The presence of this group sterically hinders cyclization and oxygenation of

arachidonate to PGG_2, thus resulting in permanent inhibition of the enzyme.[6] In addition, inhibition of the COX-2 enzyme is contributed to a side chain of valine which opens up a hydrophobic side pocket within the COX-2 active site; an aspect which is not exhibited in COX-1. This change in the COX-2 active site alters the interactions of arachidonate with Try 385 and the other important residues involved in the chemical conversion of arachidonic acid to PGG2, thus forming a target area to produce COX-2 inhibition. In either case, the binding of inhibitor does not disrupt the overall structure to cause inhibition; they affect the chemical reaction of the substrate in order to halt prostaglandin synthesis.

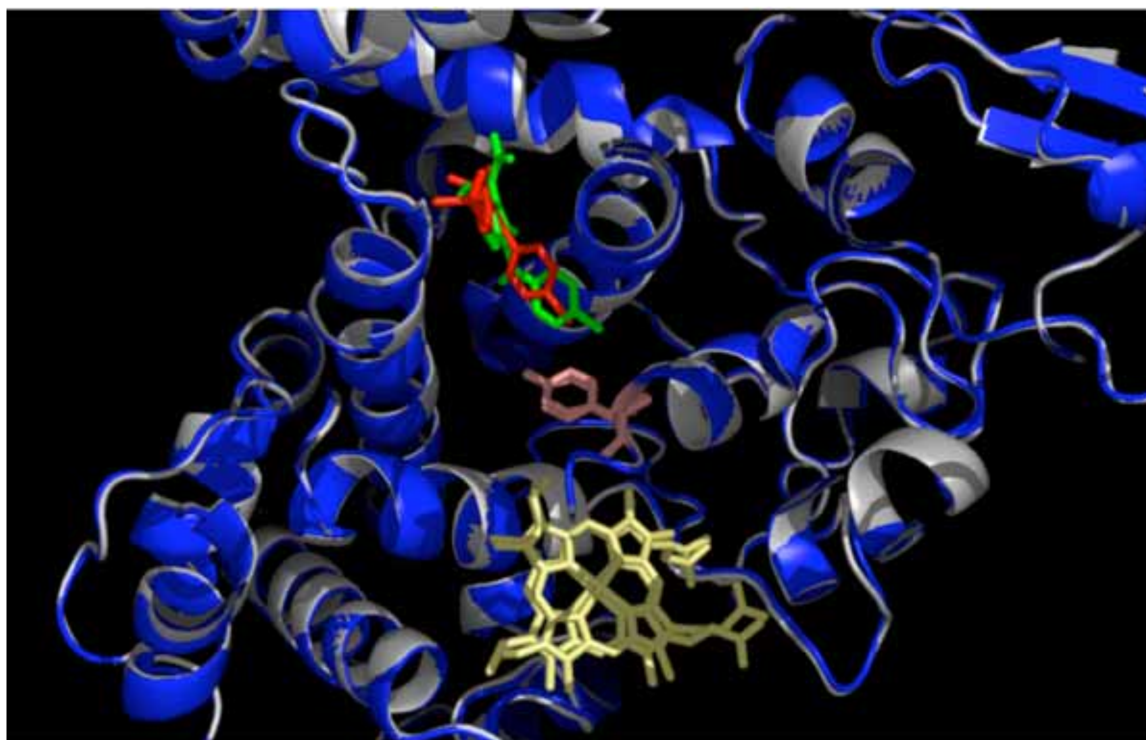

Figure 6: Comparison of COX-2 with Non-Selective inhibitor indomethacin, green/blue, and COX-2 with Selective inhibitor, red/silver, in relation to the significant residue Try 385 and Heme which is in place of arachidonic acid (F5)

Based on the information gathered from the different COX enzymes throughout the years, a specific category of drugs used to treat inflammation was created called non

steroidal anti-inflammatory drug (NSAIDs). NSAIDs work by inhibiting cyclooxygenase activity of prostaglandin synthase. Traditionally, these non-selective drugs targeted the cyclooxygenase enzyme in general such as naproxen, ibuprofen, and indomethacin which inhibited both the COX-1 and COX-2 isoforms.[6] This resulted in a decrease in inflammation however an increase in gastrointestinal complications was experiences due to the various roles COX-1 plays in different cells. Nevertheless, the slight differentiations of the COX-1 and COX-2 enzymes allowed NSAIDs to be refined in order to target the inflammation aspect of the disease with less side affects. These drugs were known as selective COX-2 inhibitors, such as SC-558, celecoxib, and rofecoxib. A comparison of non-selective inhibitor, idomethacin within the COX-2 enzyme versus a selective inhibitor SC-558 within the COX-2 enzyme can be seen in Figure 6[6].

All in all a great amount of information was able to be obtained from the structure of this particular enzyme. Incite was gained in the inflammation world due to facts obtained such as residue changes all the way to substrate interactions. Due to this discovery, an entire class of drugs to help individuals with inflammation disorders was able to be created based on the information gathered from this type of analysis.

References

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2. Marnett, Lawrence and Scott W Rolinson. *The Journal of Biological Chemistry*, 1999, 274:33, 22903-22906.
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5. Luong, C. et. al. *Nature Structural Biology.* 1996, 3, 927 – 933.
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Guideline to Pymol Presentation to go along with discussion:

F7 – Shows Domains of the COX enzyme
F1 – Shows the Secondary structure where alpha helices, beta sheet, 3_{10} helices and disulfide bonds are denoted
F12 – Shows b-factor values
F11 – Showing active site for inhibitor
F2- A general view of the hydrophobic pocket to orient where it is located in the protein
F8- A close up view of the hydrophobic pocket for important residue analysis
F3 – shows overlay of COX-1 versus COX-2
F9 – Shows the important residue conversion from COX-1 versus COX-2
F4 –Non-selective Inhibitor within the hydrophobic pocket
F6 –Selective Inhibitor within the hydrophobic pocket with labeled interactions to show that hydrogen bonding is not occurring, primarily hydrophobic interactions
F5 – Shows Selective versus non-selective Inhibitor interactions.