

COX-1 And COX-2 Enzymes Synthesize Prostaglandins and Are Inhibited by NSAIDS (Nonsteroidal Anti-inflammatory Drugs)

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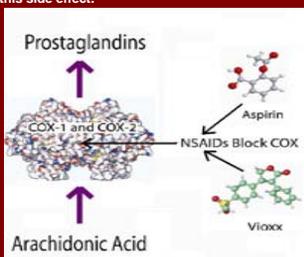


Abstract

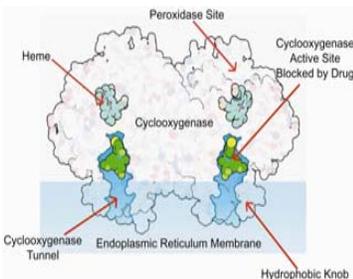
Prostaglandin Hormone Synthases (COX-1 and COX-2) are enzymes that produce prostaglandins. Prostaglandins are responsible for fever, pain, and inflammation, but also the maintenance of the lining of the stomach and prevention of ulceration. COX-1 is found mainly in the gastrointestinal lining, and COX-2 at sites of inflammation. NSAIDS (Nonsteroidal anti-inflammatory drugs) such as aspirin, ibuprofen, naproxen, and flurbiprofen inhibit both COX-1 and COX-2, and are taken regularly by over 33 million Americans for pain and inflammation. Some 10%-50% of these users suffer gastrointestinal side effects from the inhibition of COX-1 which protects the stomach lining. To solve this problem a series of drugs were created that inhibited only COX-2: Vioxx, Bextra, Celebrex, and Aleve. These drugs do not have the unwanted side effects, but some have been linked to increased numbers of heart attacks and strokes. We are studying the interaction of COX enzymes with NSAIDS and COX-2 inhibitors to see how the enzymes are inhibited from catalyzing prostaglandins. We are also studying models of the normal COX substrate, arachidonic acid, and NSAIDS and COX-2 inhibitors. We are designing models of the active sites of COX-1 and COX-2 using pdb files 1Q4G and 1PXX, as well as models of the normal COX substrate (arachidonic acid), NSAIDS, and COX-2 inhibitors to better understand the actions and side-effects of NSAIDS and COX-2 inhibitors.

Introduction

COX-1 and COX-2 are very similar in structure (60- 65% of the sequence is conserved), however they are expressed in different parts of the body. The enzymes convert arachidonic acid, a fatty acid in cell membranes, into prostaglandins, modified fatty acids attached to a ring of five carbons. COX stands for cyclooxygenase meaning that it is an enzyme that oxidizes a substrate. The prostaglandins that the two enzymes produce are identical, but get converted into different paracrine hormones - hormones that only work in the immediate area they are created. They are not shipped all over the body like most hormones. COX-1 and COX-2 have two active sites: a cyclooxygenase site, and a peroxidase site which activates the heme groups that participate in the cyclooxygenase reaction. The cyclooxygenase site opens into the membrane of the smooth endoplasmic reticulum. Cyclooxygenase inhibitors bind into the cyclooxygenase site of the enzyme, effectively inhibiting both that site and the peroxidase site. Because COX-1 and COX-2 are so similar, most NSAIDS inhibit both enzymes. Inhibiting COX-1, which protects your stomach, can create problems (such as abdominal pain, diarrhea, bloating, heartburn, and ulcers) if used over a long period of time. However, many people with chronic pain such as arthritis need to inhibit COX-2's for months or years on end. COX-2 selective inhibitors were considered wonder drugs for a time, but then Vioxx and Bextra were linked to increased numbers of heart attacks. They were taken off the market for a while, but were recently put back on because many patients would rather have a risk for heart trouble than have pain or stomach problems. Celebrex and Aleve are under investigation to see if they too have this side effect.

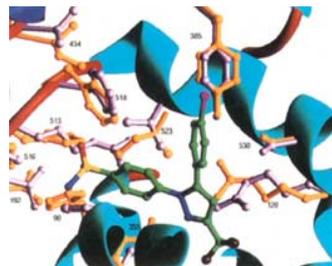


(1) Structure



Cyclooxygenases are dimers (consisting of two identical individual proteins) that attach to each other by the hydrophobic points on their surfaces. The proteins are embedded on the endoplasmic reticulum membrane, anchored by hydrophobic knobs. The enzyme has two active sites. Located deep inside the enzyme is the cyclooxygenase active site, which the substrate enters through a tunnel that extends down into the plasma membrane. When the first reaction has occurred, the product of the reaction moves to the second site (near the Heme molecule) where the peroxidase reaction occurs. The product of this reaction is then released into the cytosol.

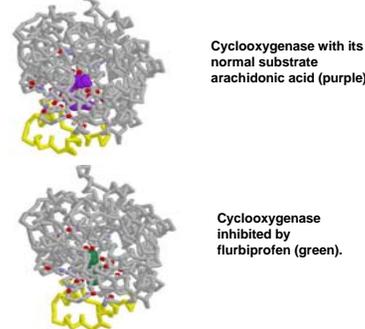
(2) COX-1 vs. COX-2 Structure



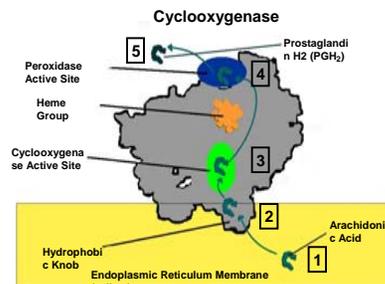
In the picture above, COX-1 (orange) and COX-2 (lavender) are superimposed around SC-558. SC-558 (Celebrex (celecoxib)) is shown bound to COX-2 (carbon, green; nitrogen, blue; sulfur, yellow; oxygen, red; fluorine, dark brown; bromine, magenta) which is super-imposed on structurally homologous residues of COX-1 from its complex with flurbiprofen (omitted for clarity). The blue ribbon is the carbon backbone. The larger NSAID binding pocket in COX-2 is clearly visible. Access to this 'side pocket' is restricted in COX-1 because of the larger isoleucine at 523.

(3) Cyclooxygenase Active Sites

In the pictures below, the heme is orange, the hydrophobic knob is yellow, and the amino acids in the Cyclooxygenase active site are colored in CPK (red for oxygen, blue for nitrogen, and gray for carbon).



(4) Chemical Process



1. Phospholipids in the membrane are broken down by phospholipase A2 when triggered by first messengers, including neurotransmitters, neuromodulators, and neurohormones. One of the products of this break down is arachidonic acid.
2. Assisted by side chains located within the channel, arachidonic acid enters COX through the hydrophobic knob that anchors the protein into the membrane.
3. The arachidonic acid goes into the cyclooxygenase active site, which adds two oxygen molecules to the arachidonic acid, turning it into prostaglandin endoperoxide (PGG2).
4. Prostaglandin endoperoxide (PGG2) goes to the peroxidase active site where the heme group helps remove one oxygen atom from the peroxide form.
5. The prostaglandin endoperoxide (PGG2), after having one oxygen atom removed, becomes prostaglandin H2 (PGH2) which leaves the COX enzyme and regulates blood clots, pain sensitivity, and allergic reactions.

(5) Drugs

The three drugs below are all nonsteroidal anti-inflammatory drugs (NSAIDS). The first two NSAIDS, aspirin and ibuprofen, are called nonselective COX inhibitors since they affect both COX-1 and COX-2 substantially (note their high COX-2/COX-1 effect ratios). The third, Vioxx, is a type of NSAID called a selective inhibitor since it targets only the COX-2 enzyme (note its low effect ratio).

NSAIDS and How They Inhibit COX	COX-2/COX-1 Effect Ratios:	PDB Image of NSAID
Aspirin Developed in 1997 by Felix Hoffmann. (http://www.aspirin-foundation.com/) How it Works: The acetyl group (green) attached to the salicylic acid (blue) reacts with the amino acid Serine 530 present in both COX-1 and COX-2, blocking the binding of the arachidonic acid substrate.	166	
Ibuprofen Developed in 1961 by a team led by Stewart Adams. (http://www.ibuprofen-foundation.com/what-ibuprofen-is-not) How it Works: Ibuprofen (yellow) occupies a COX1 and COX2 active site, blocking the path of the arachidonic acid substrate.	15	
COX-2 specific Celebrex (celecoxib) Developed in the 1990's by G.D. Searle & Co. and Pfizer Inc. (http://www.pfizer.com/cro/cro/cro/celebrexresearch) How it Works: COX-2 inhibitors like Celebrex exploit the fact that the active site of COX-2 is about 20% larger than that of COX-1.	<.001	

(6) Conclusions

- COX-1 and COX-2 are enzymes that synthesize prostaglandin, a molecule involved in signaling pain and maintaining the lining of the stomach.
- The enzymes are 60-65% similar and create identical prostaglandins, but they are expressed in different areas of the body.
- The slight difference between the enzymes allows certain drugs to be selective for one and not the other, inhibiting COX-2 (which would otherwise promote inflammation), and ignoring COX-1 (which protects the gastrointestinal lining).
- The reason Vioxx causes heart attacks and strokes isn't certain. The COX-1 enzyme encourages blood clotting while the COX-2 enzyme inhibits clotting. Drugs like Vioxx that block COX-2 enzymes and allow COX-1 enzymes may increase blood clotting, an excess of which can lead to coronary events like heart attacks and strokes.