



Review Article

Variants of cyclooxygenase-1 and their roles in medicine

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*Department of Chemistry and Biochemistry, E280 BNSN, Brigham Young University, Provo, UT 84602, USA***Abstract**

Acetaminophen (paracetamol) and other analgesic/antipyretic drugs such as dipyron have been postulated to act centrally through inhibition of cyclooxygenases (COXs). COX activity in lipopolysaccharide-stimulated mammalian leukocytes, microglial cells, and platelets is inhibited by these drugs at physiological concentrations. Yet purified COX enzymes are poorly inhibited by acetaminophen, particularly under conditions of high oxidant tone and elevated substrate levels. This suggests the presence of cell-specific differences that govern COX inhibition by these drugs. COX-3, a variant of COX-1, has been found in canine brain and is inhibited by acetaminophen and dipyron at physiological concentrations. Additionally, other new COX-1-derived proteins called PCOX have been identified that do not make prostaglandins but apparently bind heme and may have other enzymatic properties. Antibodies specific for these variants detect analogous proteins in human tissues. Expression of COX variants is postulated to be an integral part of the mechanism of action of analgesic/antipyretic drugs.

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Keywords: Acetaminophen; COX-3; PCOX

1. Introduction

The discovery of cyclooxygenase-2 (COX-2) [1,2] revolutionized NSAID pharmacology by providing a clear mechanism for prostaglandin regulation *in vivo* (induction by mitogens, cytokines, etc., and repression by glucocorticoids) and by providing a new target for the development of COX-2-selective drugs (see Xie et al. [3]). In our initial elucidation of this new enzyme, we demonstrated that induction of COX-2 in chicken embryo fibroblasts was exquisitely regulated by regulated intron splicing [1]. In non-dividing cells, intron-1 was retained in otherwise fully processed COX-2 mRNA. This resulted in a non-functional mRNA that was retained in the nucleus and prevented translation of functional COX-2 protein (unpublished data). However, within minutes of treatment with a mitogen, the intron is spliced out to produce functional COX-2 mRNA [1, unpublished data].

Comparison of the COX-1 and COX-2 genes demonstrated that they were extremely similar and possessed, with one exception, the same intron–exon placement [4–6]. The exception was that COX-1 genes contain an extra intron that constitutes the first intron in the gene. Thus, in COX-2

genes the first exon encodes the N-terminal hydrophobic signal peptide of the protein. However, in COX-1 genes the hydrophobic signal peptide is encoded by two exons separated by intron-1 (the extra intron) of the gene. Recently, we have found regulated intron splicing involving this intron to occur in COX-1 mRNAs. In this case, however, retention of intron-1 produces variants of COX-1, which provide potentially new targets for pharmacological intervention.

2. Cloning of COX-1 variants

Our initial observation of COX-1 variants arose through studies of COX-1 and COX-2 expression in canine tissues [7]. Dog cerebral cortex was observed to express two distinct COX-1 mRNAs of 2.6 and 1.9 kb in size. Other tissues expressed only the 2.6 kb COX-1 mRNA (Fig. 1). Libraries constructed from this RNA yielded 11 COX-1 related clones, three of which were full-length. All three of these cDNAs were found to represent fully processed mRNAs derived from the COX-1 gene except that they all retained intron-1. Intron-1 was found to be 90 nucleotides in size and represented a potential in-frame insertion.

Two of the three cDNAs encoded a protein that contained the entire COX-1 sequence with an extra 30 amino acids encoded by intron-1 inserted into its N-terminal hydrophobic signal peptide [7]. This protein was named COX-3, because

Abbreviations: COX, cyclooxygenase; NSAID, non-steroidal anti-inflammatory drug; PCOX, partial COX.

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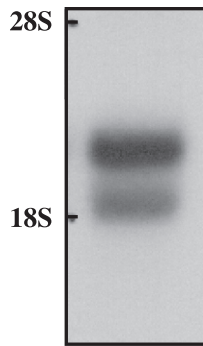


Fig. 1. Northern blot analysis. Northern blot of canine cerebral cortex poly(A) RNA ((1) 5.0 μ g; (2) 2.5 μ g) probed with 32 P-labeled canine COX-1 cDNA fragment confirmed the expression of two distinct COX-1 mRNAs of 2.6 and 1.9 kb in size (taken from Fig. 1 of Chandrasekharan et al. [7]).

it was found to possess functional cyclooxygenase activity as described below. The third cDNA, in addition to possessing this added sequence in its signal peptide, contained a deletion of sequence encoded by exons 5–8 of the COX-1 gene. This deletion represented removal of amino acids 119–337 from the COX-1 protein, which encodes a portion of the dimerization and catalytic domains. The protein encoded by this cDNA was named PCOX-1a for “partial COX.” A PCOX-1b was later identified which lacked intron-1 but contain the deletion of exons 5–8. COX-1, COX-3 and PCOX proteins are diagramed in Fig. 2.

3. COX-3 activity

Expression of COX-3 and PCOX proteins in insect cells yielded proteins that contained the 30 amino acids encoded by intron-1. This was determined using an antibody generated against a peptide sequence encoded by the 5' end of intron-1. As opposed to COX-1 whose N-terminal signal peptide is co-translationally cleaved upon translation of the nascent protein into the lumen of the endoplasmic reticulum, COX-3 and PCOX1a in dogs retain their signal peptide along with the 30 amino acids inserted in it by intron-1. This represents an addition of approximately 54 amino acids to the N-terminus of the protein. Moreover, the altered signal peptide may target the protein to specific intracellular sites. Experiments with tunicamycin demonstrated that COX-3 does at some point traffic into the lumen of the endoplasmic reticulum where it is glycosylated on asparagine [7].

COX-3 was found to be enzymatically active in synthesizing prostaglandins from arachidonic acid and possessed approximately 20% of the activity of COX-1 when expressed recombinantly in insect cells [7]. This reduced activity of COX-3 relative to COX-1 may represent an artifact of over-expression in insect cells. For example, COX-1 exhibits approximately 20% of the activity of COX-2 in the same cell system. Yet COX-1 and COX-2 in vivo possess comparable catalytic properties. Consistent with its structural

deletion, which would significantly alter portions of its peroxidase and cyclooxygenase active sites, PCOX-1a did not synthesize prostaglandins. The deletion in this protein could affect substrate binding as well as binding of endogenous oxidants and reductants required for the peroxidase/cyclooxygenase catalytic cycles. Importantly, however, the deletion does not affect the crucial proximal histidine ligand to heme, which is essential to heme binding. The tyrosine (Tyr 385 in ovine COX-1), which is involved in forming a radical that is essential to fatty acid oxygenase catalytic activity, is also unaffected. PCOX may, therefore, possess a heme-protein mediated enzymatic activity such as participation in redox reactions.

The activity of canine COX-3 was compared in insect cells with murine COX-1 and COX-2. Substrate binding was found to be indistinguishable between proteins (data not shown); however, the enzymes were found to differ significantly with regard to inhibition by analgesic/antipyretic drugs and NSAIDs.

4. Inhibition by analgesic/antipyretic drugs and NSAIDs

Analgesic/antipyretic drugs such as acetaminophen and dipyron are used extensively to treat pain and fever but have no well-defined mechanism of action. Using canine brains as their enzyme source, Flower and Vane [8], postulated that acetaminophen acted centrally by inhibiting COX enzyme. However, analyses with purified human COX isozymes as well as many studies of COX enzymes in homogenates or isolated microsomes has shown that COX-1, and particularly COX-2, are relatively insensitive to inhibition by these drugs [9]. Analysis of COX-3 showed it to be significantly more sensitive to all analgesic/antipyretic drugs tested including acetaminophen, its parental compound phenacetin, dipyron and some of dipyron's

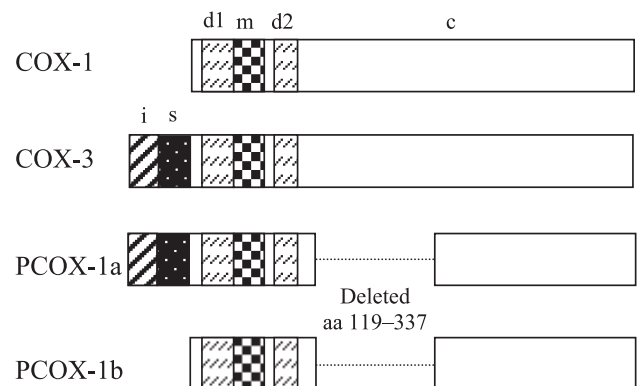


Fig. 2. Structure of COX-1 variants. A schematic representation of the domains of COX-3, PCOX-1a and PCOX-1b in comparison to COX-1. s, signal peptide; d1, dimerization domain/EGF-like domain 1; d2, dimerization domain 2; m, membrane binding domain; c, catalytic domain; i, 90-bp sequence encoded by intron 1. Amino acid numbering is according to residues in sheep seminal vesicle COX-1 (modified from Chandrasekharan et al. [7]).

breakdown products [7]. Moreover, commonly used analgesic NSAIDs including ibuprofen, diclofenac and aspirin more potently inhibited canine COX-3 than murine COX-1 or COX-2. Because these acidic NSAIDs cross the blood–brain barrier poorly, the finding of a more sensitive site of inhibition (COX-3) may explain how these drugs could exert a central effect [7].

5. Human COX-1 variants

Northern blot analysis of human mRNA was performed using an antisense oligonucleotide to intron-1 of the human COX-1 gene. As with dogs, an intron-1 containing COX-1 mRNA was detected that was most abundant in cerebral cortex and other regions of the forebrain. The intron-1 containing mRNA was also found in other tissue with heart being particularly prominent [7]. Unlike in canines, this intron-1-containing COX-1 mRNA was significantly larger than the COX-1 message (5.2 versus 2.8 kb, respectively). Polymerase chain reaction (PCR) amplification confirmed the 5.2 kb size to be due to alternative polyadenylation (unpublished data). Moreover, it also confirmed that the intron-1-containing COX-1 cDNA was fully processed except for the retention of intron-1. Initial comparisons of human intron-1 as contained in the GenBank data base revealed two sequences that were 93 and 94 nucleotides [7]. The former sequence represented an in-frame insertion, similar to intron-1 in canine COX-3 and PCOX-1a, while the 94 nucleotide sequence is out of frame. DNA sequence analysis of PCR-generated amplicons confirmed that the 94 nucleotide sequence was correct and that intron-1 in the human COX-1 sequence is out of frame with the COX-1 translation frame (Fig. 3).

To determine whether the intron-1 containing COX-1 mRNAs detected in Northern blots were capable of making proteins that contained any or all of the sequence encoded by this intron, immunoblots were performed using an antibody directed toward the sequence encoded by the 5' end of intron-11 in human. Proteins of 65-, 53-, and 50-kDa were detected with this antibody in human aorta [7]. Proteins of the same size were detected with an anti-COX-1 monoclonal antibody. Antigen competition experiments showed that detection of these proteins by anti-COX-3 antibody could be competed away using intron-1 peptide as competitor, demonstrating that these proteins likely contain a sequence encoded by intron-1. Therefore, in spite

of the fact that intron-1 is out of frame, at least part of the intron is retained in COX-1 proteins that are analogous to COX-3 and PCOX-1a in dogs. Northern blot analysis of mouse and rat mRNAs shows that rodent tissues and cells, like human, express a very high molecular weight COX-1 mRNA that contains intron-1 (unpublished data). Furthermore, intron-1 is out of frame in rodents as in human, suggesting that alternative polyadenylation is tied to intron-1 retention and potentially to the alternative processing event that overcomes the effect of the frameshift in intron-1 to produce intron-1 antibody-recognized proteins.

6. COX variants and NSAID pharmacology

The identification of COX variants opens a new chapter in NSAID pharmacology, which may answer, among other things, how analgesic/antipyretic drugs work. It has become clear that some COX-1-containing tissues in humans, such as the stomach mucosa, exhibit little to no inhibition of activity by acetaminophen [10,11]. Other tissues, such as platelets, are at least partially sensitive to this drug [12]. Expression of the COX-3 analog in platelets and other tissues provide a plausible basis for this observation. However, why COX-3 is more sensitive to these drugs remains to be determined. Structural changes evoked by alteration of the COX-1 N-terminus may result in better binding of acetaminophen to COX-3. Alternatively, altered sub-cellular localization of COX-3 may also play a role in inhibition by analgesic/antipyretic agents. Significant evidence suggests that inhibition of COX enzymes is influenced by oxidant tone. Low levels of oxidants and substrate favor COX inhibition, particularly of COX-1, whereas high levels prevent inhibition [9,13]. Altered sub-cellular environment due to an altered N-terminus may expose COX-3 and PCOXs to changed redox environments and substrate concentrations, thus affecting inhibition by acetaminophen.

The future will also reveal new roles for COX-derived proteins such as PCOXs and will demonstrate whether variants also exist for COX-2, which shares the same basic gene organization as COX-1. Our laboratory [14] and others [15,16] have identified acetaminophen-inhibitable COX activity that is associated with COX-2 expression. Through these studies, our understanding of the roles cyclooxygenases play in physiology and diseases will be expanded, and we will better understand and be able to improve the drugs that inhibit these enzymes.

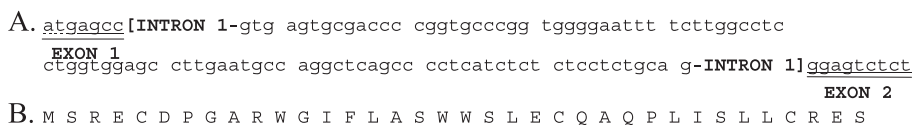


Fig. 3. Intron-1 sequence of human COX-1. (A) DNA sequence analysis established a 94-nucleotide sequence for PCR-generated amplicons of intron-1 of human COX-1. (B) Amino acid sequence from the start codon of COX-1 located six nucleotides upstream from intron-1.

Acknowledgements

The author thanks David Simpson and Joshua Tomsik for assistance in preparing this manuscript. This work was supported by NIH grant AR46688.

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