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Molecular model of the interaction between nimesulide and human cyclooxygenase-2

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Abstract

The cyclooxygenase-2 (COX-2) isoenzyme is a key target for COX-2-selective non-steroidal anti-inflammatory drugs (NSAIDs). An important difference in binding of nimesulide compared with non-selective NSAIDs appears to involve the amino acid at position 523 of the enzyme. Replacement of valine with isoleucine at this position provides access to a binding site that is larger in COX-2 than in COX-1. Nimesulide appears to exploit this enlarged binding site for establishing a number of favourable contacts with the enzyme that lead to selective inhibition of COX-2. We made these conclusions from a three-dimensional molecular model of the active site of human COX-2, constructed using the X-ray coordinates of COX-1 from sheep seminal vesicles and COX-2 from mouse fibroblasts as templates, with the aid of sequence alignment methods and molecular modelling techniques. The resulting model was refined, and the active site was probed for regions of steric and electrostatic complementarity for ligand binding. Docking studies were then undertaken with many different nimesulide conformers, a family of which could establish very favourable interactions with the NSAID binding site of human COX-2 by exploiting the extra space made available by the isoleucine/valine replacement. The stability of the resulting complexes was studied by simulating molecular dynamics.

KEY WORDS: Molecular modelling, Docking, NSAIDs, Prostaglandins, Cyclooxygenases.

Prostaglandin H_2 synthase [1], also known as cyclooxygenase (COX), is an integral membrane protein found predominantly in the endoplasmic reticulum. It is a bifunctional enzyme which first converts arachidonic acid into prostaglandin G_2 (PGG_2) by dioxygenation, and then catalyses the peroxidation of PGG_2 to prostaglandin H_2 (PGH_2).

COX activity is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) [2]. Two COX isoforms are known: constitutive COX-1, which is considered to be involved in intercellular signalling and homeostasis maintenance, and COX-2, which is mostly induced during inflammation [3].

The main differences between the primary sequences of the two human isozymes are a truncated signal peptide and an 18-amino acid insertion in the C-terminal of COX-2 (Fig. 1). Mechanisms for the cyclooxygenase and peroxidase activities are essentially the same for the two isozymes, but substrate preferences may differ [4].

Three-dimensional structure of COX

For a long time it was not possible to use molecular models of human COX based on the known structures of other haem-containing peroxidases, such as yeast cytochrome *c* peroxidase or canine myeloperoxidase. This was because of the low level of sequence identity found within this class of proteins (~20%), which suggested low topological similarity.

Further difficulties associated with the crystallization of membrane integral proteins also delayed the ability to obtain COX specimens suitable for direct study by X-ray diffraction techniques. A few years ago, using solubilization with non-ionic detergents and co-crystallization with brominated or iodinated derivatives of several NSAIDs, it become possible to obtain X-ray quality crystals of COX-1 from sheep seminal vesicles. Other workers then extended these studies to COX-2 from human cells [5] and mouse skin fibroblasts [6]. The Brookhaven Data Bank [7] makes available to the scientific community the three-dimensional structures of ovine COX-1 complexed with flurbiprofen [8], bromoaspirin [9], iodosuprofen and iodoindomethacin [10], as well as those of murine COX-2 both in an uncomplexed state and complexed with flurbiprofen, indomethacin or the selective COX-2 inhibitor SC-558 [6].

Both ovine COX-1 and murine COX-2 appear as homodimers (Fig. 2) and show three distinct folding units or protein domains: an amino terminus, which gives rise to a compact domain similar to that of epidermal growth factor; a right-handed spiral of four amphipathic

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α-helices, which probably form the membrane insertion domain; and the rest of the protein, which forms a large globular domain where catalytic activity resides.

The active site is found at the end of a long and narrow hydrophobic channel, the entrance to which is delimited by the helices of the membrane-binding domain. These helices provide a hydrophobic environment suitable for interactions with the fatty acid chains of the substrate. At the bottom of the channel is the tyrosine residue which is thought to act as the radical donor (Tyr385) [11]; midway along the channel is Arg120, which is assumed to interact with the carboxylic group of arachidonic acid (in much the same way as it interacts with the carboxylic group of the typical NSAIDs studied) [5, 7, 9, 10]; and in one of the channel walls is Ser530, the residue that is irreversibly acetylated by aspirin so that access to the channel is permanently blocked [9].

Molecular modelling of human COX-1 and COX-2

The high level of sequence conservation and functional similarity among COX-1 and COX-2 enzymes from different sources indicates very similar overall tertiary and quaternary structures. Superimposition of the three-dimensional structures of sheep COX-1 and mouse COX-2 formed the basis of our homology modelling of their human counterparts. The resulting structures were refined using the molecular mechanics program AMBER [12].

Both human COX-1 and COX-2 are monotopic membrane proteins (i.e. they integrate in just one lipid layer of the membrane; Fig. 2). Despite their chemical diversity, the 'classical' NSAIDs presumably inhibit the cyclooxygenation reaction through the common mechanism of filling the upper portion of the channel near Tyr385 and precluding access of arachidonate to the catalytic site. In the competitive or time-dependent binding process, however, several subsites might be involved, some of which are kinetically indistinguishable [5, 6, 9, 10].

The two human COX isoforms are globally very similar in the active site region. The major difference in the first shell of residues around the active centre is the replacement at position 523 of an isoleucine in COX-1 by a valine in COX-2 (Fig. 1). This position is found at the bottom of the channel that lodges arachidonic acid during the catalytic reaction and is spatially very close to the serine residue (Ser530) that aspirin acetylates [9, 13].

FIG. 1. Multiple sequence alignment [25] of COX-1 and COX-2 enzymes from different sources [26]. Amino acids for which the consensus among the different isozymes is high or moderate are coloured in red or blue, respectively. The position enclosed within a box corresponds to the only residue that is different in the first shell of amino acids lining the active site.

Molecular modelling of nimesulide and conformational search

Nimesulide (4-nitro-2-phenoxymethanesulphonanilide) [14] is a prototype of selective COX-2 inhibitors [15–17]. Its three-dimensional structure was determined by X-ray crystallography [18], and is deposited in the Cambridge Structural Database (ref. WINWUL) [19].

The conformation found in this crystal represents an energy minimum (Fig. 3), but not necessarily the conformation that the molecule adopts when it binds to its target receptor, in this case human COX-2. To explore the conformational space available to nimesulide, use was made of molecular dynamics [12] and Monte Carlo simulations [20]. These techniques allow the characterization of a much wider range of possible conformations, presumably including those of the molecule free in solution and the enzyme-bound form. For rapid intra- and intermolecular energy evaluation of each configuration in the bound state atomic affinity potentials for

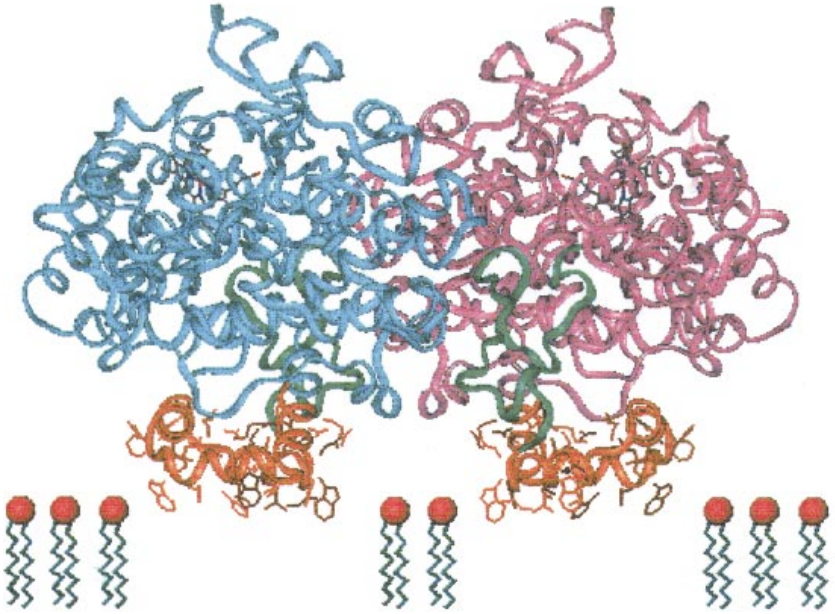

FIG. 2. Ribbon representation of the modelled human COX-2 homodimer and proposed anchoring of the enzyme in the endoplasmic reticulum membrane (represented by a few simplified phospholipid molecules). The haem groups and the hydrophobic side chains of the membrane-binding domain are shown.

carbon, oxygen, nitrogen, sulphur, and hydrogen atoms were precalculated in the putative binding site using a three-dimensional grid centred on Arg120 [20, 21]. An additional grid of electrostatic potential was calculated for scoring purposes by solving the linearized form of the Poisson–Boltzmann equation using a finite difference method [22].

Proposal of a binding mode for nimesulide in human COX-2

The binding sites of both human COX-1 and COX-2 were explored by several probes with the aid of the GRID program [21] in search of regions that could give rise to favourable interactions with the functional groups in nimesulide.

These cavities were also filled with spheres of varying sizes (between 1.4 and 4 Å radii) by means of the DOCK program [23]. Both the resulting GRID maps and the clusters of spheres generated by DOCK provided a geometric description of the volume available to the inhibitors.

Comparison of these volumes between human COX-1 and COX-2 revealed that the cavity present in COX-2 extends past the binding site of 'classical' flurbiprofen-like NSAIDs. The key difference between both enzyme isoforms is provided by one amino acid, Ile523 of COX-1, whose equivalent position (Fig. 1) is occupied by valine in COX-2. The absence of a methylene group in the side chain of valine relative to isoleucine, together with some other amino acid substitutions, appears to avoid a solution of continuity between two adjacent cavities in such a way that the binding site of COX-2 is larger and

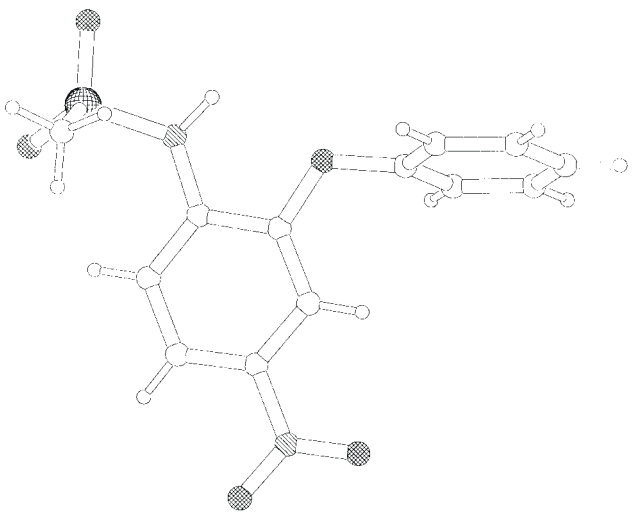

FIG. 3. X-ray crystal structure of nimesulide [18]. Hatched and criss-crossed spheres represent oxygen and nitrogen atoms, respectively; the largest atom is sulphur, and bonds involving hydrogen atoms are thinner than the rest.

Y-shaped, and discriminates in favour of some COX-2-selective inhibitors such as nimesulide (Fig. 4).

The importance of this methylene group is supported by biochemical evidence from studies with mutant enzymes that show how the profile of selective inhibition of human COX-2 by nimesulide and its analogue NS-398 is drastically altered upon mutation of Val523 to isoleucine [17, 24] or other amino acids [17]. Other evidence is provided by the recently solved X-ray complex between the selective inhibitor SC-558 and mouse COX-2, which

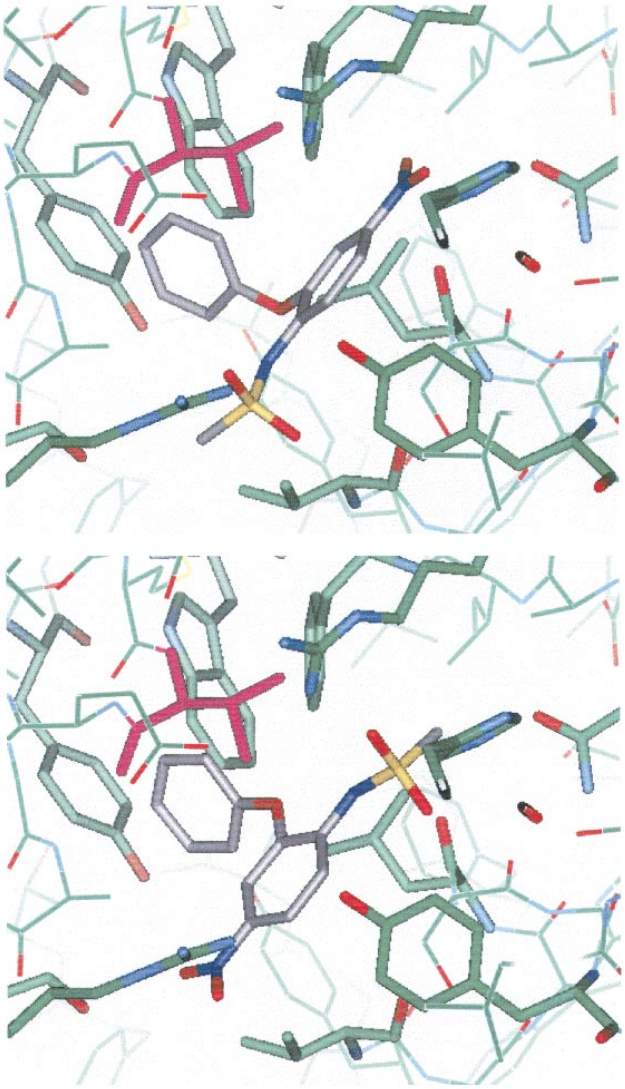

FIG. 4. Two possible orientations of nimesulide (carbon atoms in grey) in the active site of human COX-2. Val523 is coloured in magenta. Molecular dynamics simulations of both complexes appear to support the binding model shown at the bottom.

shows the phenylsulphonamide group located in this cavity [7]. This contiguous pocket appears to be inaccessible in COX-1 due to the location of the side chain of Ile523, and it remains unoccupied in the complexes of this murine COX-2 enzyme with other non-selective inhibitors [7].

Conclusions

1. Two isoforms of cyclooxygenase are known, COX-1 and COX-2.
2. The COX-2 isozyme is induced by various pro-inflammatory stimuli and is a key target for selective non-steroidal anti-inflammatory agents.
3. There are few structural differences between the two COX isoforms, but a key difference regarding nimesulide binding appears to be the amino acid at position 523.
4. Replacement of isoleucine with valine at position 523 provides access to an enlarged binding site in COX-2 that is more restricted in COX-1.
5. Nimesulide appears to exploit this enlarged binding site for establishing a number of favourable contacts with the enzyme that lead to selective inhibition of COX-2.

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