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STRUCTURAL BASIS OF THE DYNAMIC MECHANISM OF LIGAND BINDING TO CYCLOOXYGENASE

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Abstract: Molecular modeling studies performed on the two cyclooxygenase (COX) isozymes suggest that the cavity at the mouth of the active site on the membrane domain that may act as an actual binding site of COX ligands. Actual docking of different inhibitors at this site provides a structural basis to explain the dynamics of COX inhibition.

An intriguing feature of cyclooxygenase (COX), is the differential inhibitory profile exhibited by different nonsteroidal antiinflammatory drugs (NSAIDs). In addition to the irreversible inhibition mode of aspirin,[1] there are NSAIDs that exhibit a time independent inhibition, in which the ligand competes reversibly with the natural substrate to form a complex enzyme-inhibitor (EI). Others display a time dependent inhibition including an initial reversible binding as described previously, which progresses to a tight irreversible to form a new complex enzyme-inhibitor (EI*).[2] This last step is a slow process with an associated energy barrier of about 10 kcal/mol.[3] Moreover, compounds may not exhibit the same behavior for both of the COX isoforms.

Knowledge of the structural features that explain this differential behavior can be useful for the design of new selective COX-2 inhibitors. Recent site directed mutagenesis studies have demonstrated the involvement of Tyr^{355} on the dynamics of the inhibition mechanism, since its mutation to Phe decreases the energy barrier associated for the formation of the complex (EI*).[3] In the present work we propose a molecular model based on the structural features of the enzyme, which is aimed at explaining the differential dynamical behavior observed among COX inhibitors. Residue numbering throughout this work refers to the ovine COX-1 sequence.

Analysis of the crystal structures of COX-inhibitor complexes[4–6] reveals a network of hydrogen bonds involving Arg^{120}, Tyr^{355}, His^{513} (Arg in COX-2) and Glu^{524} that are thought to act as a gate for ligand entrance to the COX active site. Moreover, molecular dynamics studies[7] suggest that this gate does not open easily, and that Glu^{524} side chain fluctuates to interact with either Arg^{120} or Arg^{513}. On the other hand, examination of the membrane domain of both isozymes reveals a hydrophobic cavity of the size of the COX binding site, located at the entrance of the cyclooxygenase active site. This cavity contains the larger number of nonconserved residues between the two isozymes, including (residues in parenthesis correspond to COX-2): Thr^{89} (Val), Leu^{92}(Ile),

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Ile[112] (Leu), Leu[115] (Tyr), Val[119] (Ser), and Leu [357] (Phe). Clearly, the cavities of the two enzyme isoforms exhibit qualitative differences due to the larger number of aromatic and polar residues present in COX-2. Close examination of this putative site reveals a spatial arrangement of residues that is similar to the structural features found in the COX binding site, and in fact this site exhibits a degree of symmetry with the COX binding site in respect to a plane defined by residues Arg[120] and Tyr[355] as can be shown in Figure 1. This peculiar feature may have some implications in the process of ligand recognition.

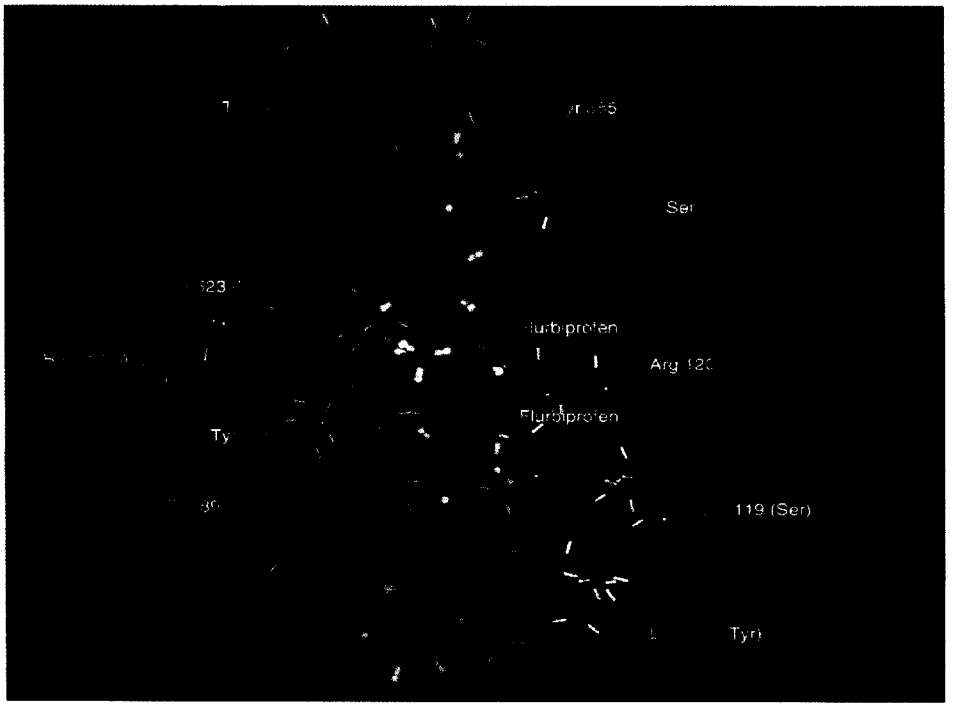
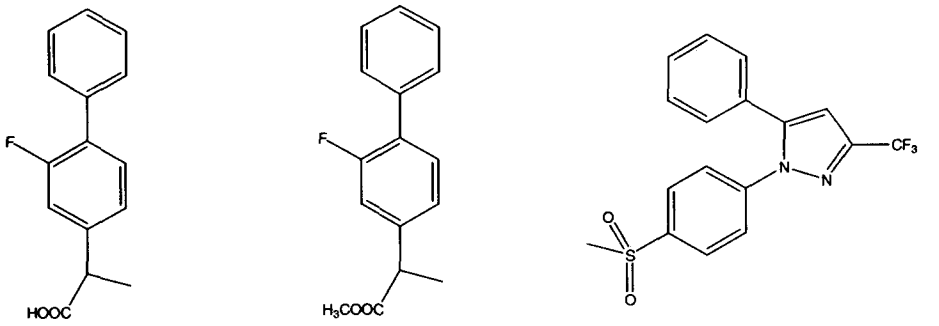


Figure 1. Comparative view of flurbiprofen bound to the cyclooxygenase binding pocket as well as to the novel putative binding site.

This novel putative NSAIDs binding site prompted us to suggest that inhibitors first bind to this cavity, where they remain until the gate to the cyclooxygenase binding site opens. This initial binding explains a competitive reversible binding step. If the residence time of the ligand is larger than the period required for the gate to open, ligands will proceed to the cyclooxygenase binding site. However, this process may be accelerated by inhibitors capable of enhancing the process of gate opening when bound to this cavity by perturbing the network of hydrogen bond at the entrance. Accordingly, inhibitors that bind only to the first site and are not capable to perturb the hydrogen bonds network, will likely exhibit a time independent inhibition mechanism. In contrast, those that bind first to the cavity and remain until the gate to the COX binding site opens, will bind in a

time dependent manner. In this case opening of the gate will be assisted by the perturbation produced by the ligand to the hydrogen bond network when bound to the first site.

As a proof of principle, docking studies of flurbiprofen (a nonselective inhibitor, time dependent in both isozymes), flurbiprofen methyl ester (a nonselective inhibitor, time independent in both isozymes) and SC58125 (a COX-2 selective inhibitor, time independent in COX-1 and time dependent in COX-2) onto this cavity were performed to both isozymes. Structures of the ligands are shown in Figure 2.



A B C

Figure 2. Structures of flurbiprofen (A), flurbiprofen methyl ster (B) and SC58125 (C)

These studies we carried out using the 3-D structure of the complex COX-2 flurbiprofen,[6] entry 3PGH of the Bookhaven databank, once the ligand was removed. The structure was minimized with a dialectric constant of 4r and a 12 Å cutoff, using the all-atom parm91 set of parameters[8] of the AMBER4.0[9] program. Initial structures of the complexes were generated manually by placing the inhibitors in the cavity using computer graphics by means of the InsightII program.[10] The complexes were geometry optimized in the same consditions as the isolated enzyme. Docking of flurbiprofen, onto the cavity was carried out with its carboxylate moiety oriented towards residues Tyr^{355} and Arg^{120} and placing the rest of the molecule facing the lipid bilayer in both isozymes. After energy minimization the resulting complexes of both isozymes are qualitatively similar as it is shown in Figure 3 for COX-1 complex.

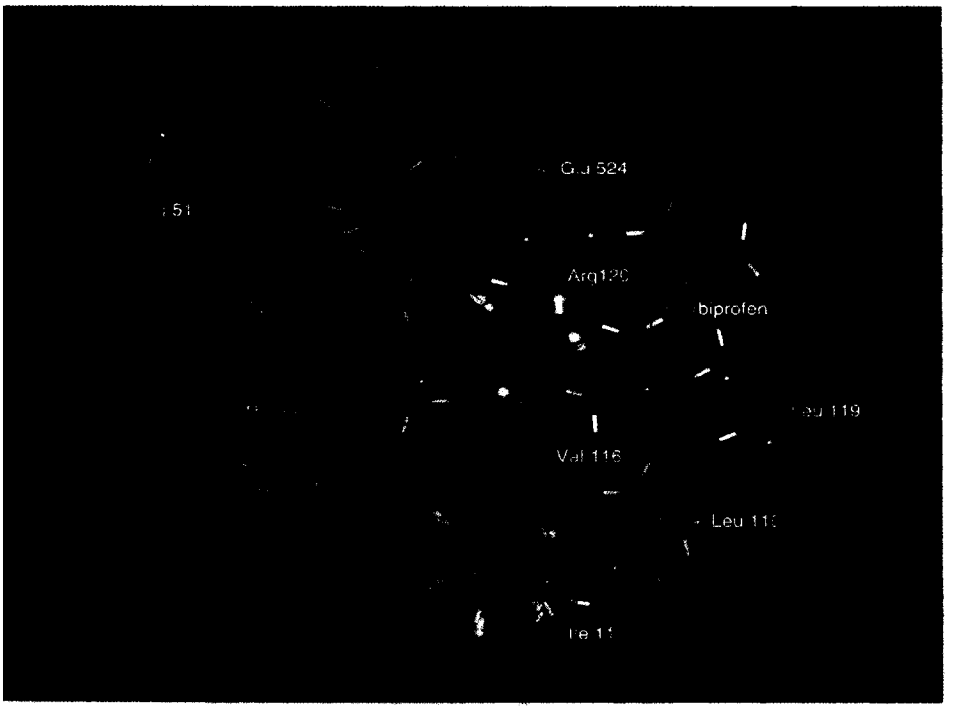


Figure 3. Complex of COX-1 with flurbiprofen bound to the novel putative binding pocket.

The main features of the complexes are: (i) a hydrogen bond interaction of the carboxylate moiety of the inhibitor with Tyr^{355}; (ii) the methyl group of flurbiprofen fits well in a hydrophobic pocket formed by Val^{116}, Val^{119} (Ser in COX2), and Leu^{115} (Tyr in COX-2), which suggests the stereospecficity of this site, since binding of the *R* enantiomer would be hampered by steric effects due to Thr^{89}(Val in COX2), (iii) the phenyl group sits in another hydrophobic pocket formed by residues Ile^{92}, Phe^{99}, Trp^{100}, Ile^{112} (Leu in COX-2) and Val^{103}.

In order to assess the stability of the two complexes we proceeded to perform molecular dynamics (MD) simulations. These were performed at a constant temperature of 300 K with the system coupled to a thermal bath. Dielectric constant was set to 4r and a cutoff of 12 Å was used to compute non-bonded interactions. In both simulations after a 300ps trajectory performed with an integration step of 1fs, complexes showed to be stable.

Docking of flurbiprofen methyl ester was performed in the same conditions of flurbiprofen. After energy minimization, the resulting complexes of both isozymes are qualitatively similar (figure not shown) for the COX-1 complex. In COX-1 the carbonyl oxygen of the ester moiety forms a hydrogen bond with Thr^{89}, which prevents the ligand to access the cyclooxygenase gate. In the case of COX-2 the carbonyl oxygen forms a hydrogen bond with the Arg^{499} side chain.

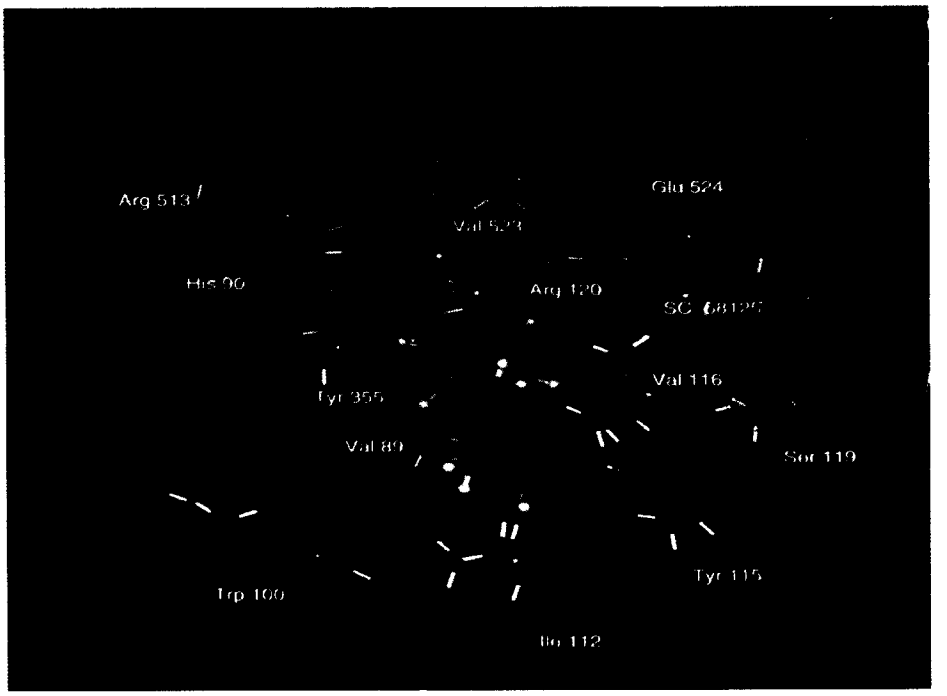


Figure 4. COX-2 SC58125 complex after minimization.

Manual docking of the selective inhibitor SC58125 was also carried out into the two enzymes in the same conditions. The inhibitors were placed with the sulfone moiety in close proximity to Arg^{120} and Tyr^{355}. The complex with COX-2 after minimization shows the ligand exhibiting two hydrogen bonds to both Tyr^{355} and Arg^{120}, respectively (Figure 4). Several attempts had to be performed in order to obtain a complex of the ligand with COX-1 exhibiting the same features as with COX-2 after minimization. This result suggests the instability of the complex later confirmed by molecular dynamics simulations. In both complexes the phenyl moiety sits in an hydrophobic pocket formed by Ile^{92}, Leu^{93}, Phe^{99}, Trp^{100}, Ile^{112}, Leu^{115}, and Leu^{357}(Phe in COX-2). Furthermore, in order to assess the stability of the two SC58125 complexes we proceeded to perform MD simulations in the same conditions as used with the two flurbiprofen complexes. In this case, the COX-2 complex appears stable during a 300 ps trajectory. In contrast, several heating schemes had to be tried with the COX-1 complex, finding in all the cases a drift of the ligand outside the pocket.

The results described in the present work in regard to the docking experiments are in full agreement with the experimental results. Modeling studies show flurbiprofen well docked into the first binding site in both COX-1 and COX-2, forming a hydrogen bond with Tyr^{355}. In these conditions it is expected that the inhibitor perturbs the hydrogen bond network and enhances the opening of the gate to the cyclooxygenase binding site, consistent with a time dependent inhibition mechanism in both isozymes as shown experimentally.[11] In contrast,

SC58125 like other highly selective inhibitors, exhibits a time dependent mechanism in COX-2 and a time independent in COX-1.[12] This is in agreement with the results of the docking studied. The ligand forms a stable complex in the cavity of COX-2 with hydrogen bonds pointing the side chains of to Tyr^{355} and Arg^{120}. Binding of the ligand to this cavity disturbs the hydrogen bond network and it is consequently expected that the inhibitor forms a tight complex in the COX binding site. In contrast, the ligand in COX-1 is displaced in regard to its position in COX-2 and as a consequence it is impaired to perturb the hydrogen bond network. In these conditions the chances of the ligand to bind into the COX binding site are expected to be much lower.

These results may help to provide new insights into COX inhibition providing a possible explanation of the not well understood differential pharmacological behavior exhibited by highly selective COX-2 inhibitors between wild type human COX-2 and double mutant H513R, I523V COX-1.[13]

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