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A proposed molecular basis for the selective resveratrol inhibition of the PGHS-1 peroxidase activity

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Abstract—Docking results have enabled us to propose how resveratrol could act as a selective PGHS-1 peroxidase site inhibitor. The docking model has predicted a slightly less favorable ΔG_{bind} (−17.9 kcal/mol) of the resveratrol to the PGHS-2 peroxidase site in comparison with its corresponding binding to the PGHS-1 (−20.4 kcal/mol). The formation of hydrogen bonds among the hydroxyl groups of the resveratrol phenyl rings, the backbone of Fe-heme and the carbonyl group of Leu294 inside the PGHS-1 peroxidase site, associated with the absence of His214 in the backbone of PGHS-1, are essential features that are required to maintain the aromatic rings of the natural product parallel to the Fe-heme group and transverse to the peroxidase access channel promoting a large steric hindrance at this site and its consequent selective inhibition.

1. Introduction

Prostaglandin endoperoxide H_2 synthase (PGHS) is a homodimeric bifunctional enzyme that catalyzes the first two steps in the biosynthesis of prostaglandins from arachidonic acid. In the first step, that is, the cyclooxygenase reaction, arachidonic acid is bis-dioxygenated to form the 15-hydroperoxy-9,11-endoperoxide prostaglandin G_2 (PGG_2). In the subsequent step, that is, the peroxidase reaction, the hydroperoxide group of the PGG_2 undergoes a two-electron reduction to the corresponding alcohol, producing the prostaglandin H_2 (PGH_2)[1,2] (Fig. 1).

There are two well-characterized isoforms of PGHS, the constitutive PGHS (PGHS-1) and the inducible PGHS (PGHS-2), with ca. 60% homology in the primary sequence. The enzymes are encoded by separate genes that are expressed differentially. While PGHS-1 is widely expressed in mammalian tissues, where it presents homeostatic functions, PGHS-2 is expressed transiently upon stimulation by mitogens or other nociceptive stimuli, being involved in genesis of the inflammatory processes.[3]

In the peroxidase catalytic cycle, the initial redox reaction involves the binding of the 15-hydroxyl group of PGG_2 to the heme iron, with concomitant donation of a proton from the α (terminal)-oxygen of the peroxyl group coordinated to the heme iron at the distal His207. Subsequently, the transfer of a proton from His207 to the β-oxygen in the peroxyl group occurs which results in an acid base-catalyzed cleavage of the oxygen–oxygen bond. This reaction generates the intermediate I and the PGH_2.[4] This intermediate accepts an electron from a reducing co-substrate (Tyr385) to form the intermediate II, which is carried to the resting state ferric enzyme. The conversion of intermediate I to intermediate II can take place through the formation of a tyrosyl radical species from a tyrosine residue (Tyr385), which is required to initiate and perpetuate the cyclooxygenase reaction. Thus, the two activities of

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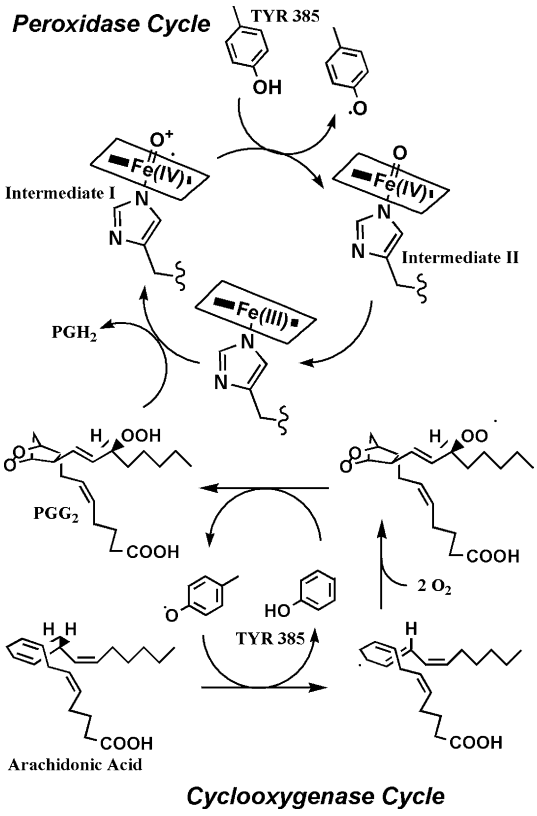

Figure 1. Tightly coupled interrelated peroxidase and cyclooxygenase activities of the PGHS. The native enzyme contains a heme subunit in the peroxidase site, usually ferriprotoporphyrin IX, with four pyrrole nitrogens bound to the Fe(III). The fifth coordination position on the proximal side of the heme is usually occupied by the imidazole group of a histidine residue.

PGHS are mechanistically interrelated, most likely through a branched-chain mechanism in which a single peroxidative turnover can sustain many cycles of the cyclooxygenase reaction[5,6] (Fig. 1).

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene, Fig. 2) is a phytoalexin found in grapes, wines, and other food products that present cardiovascular protective, cancer chemopreventive, antioxidant, and anti-inflammatory activities.

The anti-inflammatory properties of resveratrol were demonstrated by the suppression of carragenan-induced rat paw edema.[7] This effect was attributed to the blockade of prostaglandin biosynthesis, not through cyclooxygenase activity inhibition as with classical anti-inflammatory drugs, but via selective inhibition of peroxidase activity of the PGHS-1 (IC_{50} = 15 μM) in comparison with that related to PGHS-2 (IC_{50} = 280 μM).[8]

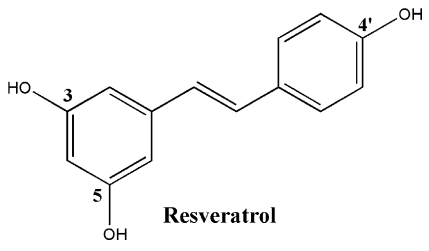

Figure 2. Molecular structure of resveratrol (3,5,4′-trihydroxy-*trans*-stilbene).

In this context, we described herein the application of docking studies to understand better the structural characteristics involved in the selective peroxidase inhibition of the PGHS-1 by the phenolic natural product, resveratrol.

2. Computational methods

2.1. Molecular structures

The study was based on two crystal structures recovered from the Brookhaven Protein Data Bank, (http://www.rcsb.org/pdb/), that is, PGHS-1 in complex with the inhibitor flurbiprofen (entry code 1EQH) and PGHS-2 in complex with SC-558 (entry code 1CX2). For the docking purposes, protein atoms of the PGHS-1 and PGHS-2 monomers A were used, the heme group being chosen to define the binding pocket. The docking experiments were carried out with this structure, after the automatic remotion of crystallization water residues, which will be here referred to as peroxidase active site. The BioMedCAche 5.0 software[9] was used for sketching, geometry optimization, and conformational search of resveratrol.[8] The minimum energy conformation was obtained first with the MM2 method, followed by application of semiempirical AM1 minimization using two dihedral angle search by step. Subsequently, the charges of resveratrol and heme group were computed using the Tripos force field with Gasteiger–Hückel method.[10]

2.2. Molecular docking

The FlexX program[11] interfaced with SYBYL 7.0 was used to dock resveratrol inside the peroxidase site of the crystallographic PGHS-1 and PGHS-2 structures. FlexX utilizes a fast algorithm for the flexible docking of small ligands into fixed protein binding sites using an incremental construction process.[12,13] The active site exploited in docking studies was defined, in the PGHS-1 and PGHS-2 monomeric forms, after a cut-off of 7.4 Å radius around the prosthetic heme group. The proposed interaction modes of the ligand with the PGHS-1 and PGHS-2 active sites were determined as the highest scored conformation (best-fit ligand) among 30 conformational and binding modes generated according to the FlexX scoring, which was represented by the structure with the most favorable binding free energy (ΔG_{bind}). FlexX uses a pure empirical scoring function similar to that developed by Rarey et al.[12] and Böhm.[14] The free binding energy of a protein/ligand complex was estimated as the sum of free energy contributions from hydrogen bonding, ion-pair interactions, hydrophobic and π-stacking interactions of aromatic groups, and lipophilic interactions. A scaling function was used to penalize deviations from the ideal geometry.

3. Results and discussion

3.1. Model validation

To ensure the efficiency of FlexX program to dock the natural ligand in the peroxidase site, we investigated previously the docking of the prosthetic heme group in the peroxidase sites of PGHS-1 and PGHS-2. The FlexX program docked it in the same pocket and aligned it in a plane similar to that occupied by the ferriprotoporphyrin IX in the crystal structure, presenting RMS values of 0.119 Å for PGHS-1 and 0.133 Å for PGHS-2.

3.2. Docking modes between resveratrol and PGHS (1 and 2) peroxidase sites

Resveratrol docking into the peroxidase active site of the PGHS isoforms furnished the ΔG_{bind} for the best-fit complexed structures with PGHS-1 and PGHS-2 of −20.4 and −17.9 kcal/mol, respectively. The heme molecular surface occupied by resveratrol was 645.6 Å in the PGHS-1 and 638.1 in the PGHS-2 (Table 1). The small heme molecular surface occupied and ΔG_{bind} difference between the two isoforms could not explain the resveratrol selectivity for PGHS-1. Graphical representations of the docking results of resveratrol are given in Figure 3 and show that it does not bind in the same manner in the two PGHS isoforms. In PGHS-2, we observe that the hydrogen bond between the two hydroxyl groups from the resorcinol ring of the resveratrol, and the His207 (NH group) and Gln203 (C=O group) residues contributes to maintaining resveratrol in a slanting position (Fig. 3A). The analysis of the molecular recognition of resveratrol by the PGHS-1 peroxide site reveals that the 3,5-hydroxyl groups attached to the phenyl ring of resveratrol interact through hydrogen bonds with the backbone of the heme in PGHS-1. Additionally, the hydrogen of the 4′-hydroxyphenyl group makes a hydrogen bond with the carbonyl oxygen atom of Leu294 (Fig. 3B), which contributes to maintaining the resveratrol in a parallel position to the heme group.

Table 1. Calculated parameters for the resveratrol binding to the PGHS-1 and PGHS-2 peroxidase sites

	PGHS-1	PGHS-2
Binding energy (ΔG_{bind}) (kcal/mol)	−20.4	−17.9
Volume (Å^3)[a]	645.6	638.1

[a] Molecular surface of resveratrol binding conformations.

3.3. Comparison of the peroxidase active sites

Observing the docking results (Fig. 3), we noticed that a difference between the carboxylic acid conformations of the heme backbone in the peroxidase site could be dictating its binding with resveratrol. After an overlapping of the heme groups of the two PGHS isoforms, which resulted in a RMS value of 0.065 from alingment of the four pyrrole nitrogens, it is clear that the variation in the disposition of the backbones A and B of PGHS-1 and PGHS-2 (Fig. 4) is not an artifice of the crystals, but arises due to hydrogen bonds between the carboxylic acid oxygens of the prosthetic group and the amino acid residues of the peroxidase site. In the backbone A of PGHS-1, the carboxylic acid group of heme moiety makes one hydrogen bond with the Thr212, while the carboxylic acid of the backbone B does not make any hydrogen bond with any amino acid residues. However, the carboxylic acid of the backbone A of PGHS-2 makes two hydrogen bonds with the Thr212 and the respective carboxylic acid group present in the backbone B makes one hydrogen bond with the Gln454.

These results have indicated to us that the variations evidenced in the backbone conformations are due to the different positions occupied by Thr212 in the active site, leading to the formation of two hydrogen bonds in the PGHS-2 and only one in the PGHS-1, besides a difference in the backbone B proximal amino acids, that is, Asp450 present in the PGHS-1 versus a Gln454 present

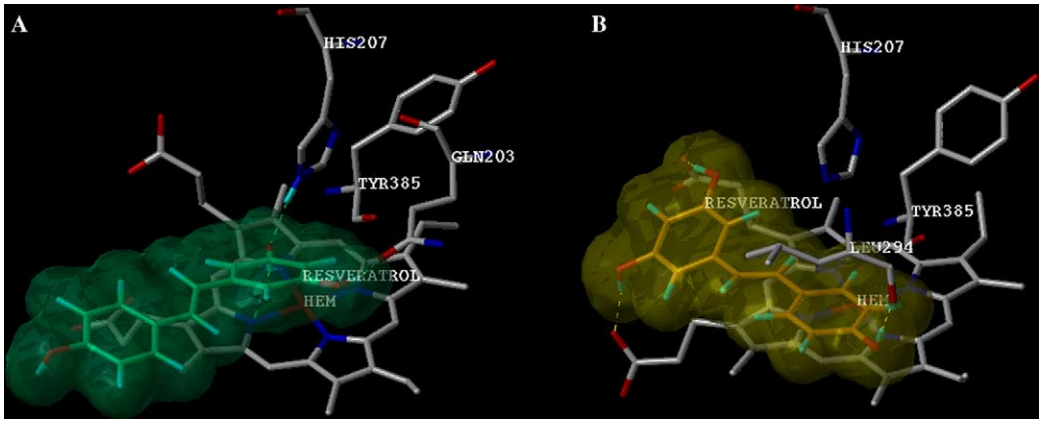

Figure 3. Molecular docking results of resveratrol with the PGHS-1 and PGHS-2 peroxidase sites. Only the main amino acid residues within 3 Å around the inhibitor are shown for clarity. (A) Resveratrol attached to the PGHS-2 peroxidase site, describing the molecular surface contour of the ligand. (B) Resveratrol attached to the PGHS-1 peroxidase site, describing the molecular surface contour of the ligand. The residues are represented as capped-sticks, and the carbon atoms of the inhibitors are shown in green-blue (resveratrol in PGHS-2) or orange (resveratrol in PGHS-1), blue (N), red (O), and green (halogen); the green-blue and yellow dashed lines represent the hydrogen bonds formed between resveratrol and the corresponding PGHS isoform.

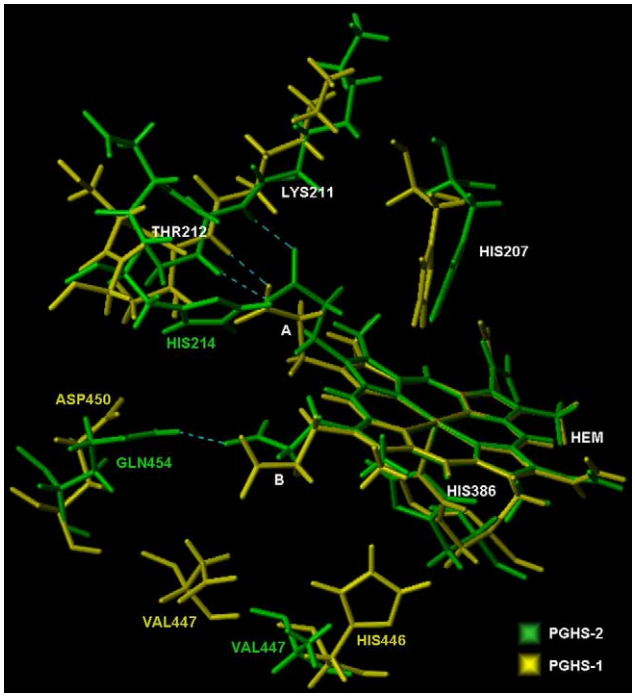

Figure 4. Molecular overlapping of the PGHS-1 (green) and PGHS-2 (yellow) prosthetic heme groups. Only the main residues that directly affect the conformation of the carboxylic acid groups, distal and proximal hystidines, and the differential hystidine 214 (PGHS-2) are shown. In white are represented the common amino acid residues of the two PGHS isoforms.

in the corresponding region of the PGHS-2. This fact determines a crucial spatial difference between the backbone B of the two isoforms once that only Gln454 amino acid residue of the PGHS-2 active is geometrically able to make a hydrogen bond with the carboxylic acid of backbone B (Fig. 4).

In addition, the presence of a His214 in the PGHS-2 produces a steric hindrance that could block the binding of resveratrol with the carboxylic acid residues of the backbones A and B of heme group, avoiding a parallel conformation adopted by the natural product in the peroxidase site of the PGHS-1 (Fig. 3).

3.4. Analyses of access pocket

As previously described in the literature, the peroxidase active site of the PGHS possesses an access pocket since the external surface up to the heme group is larger than other human peroxidases, for example, mieloperoxidases (MPO). The use of van der Waals sphere-scan program showed clearly that the heme pocket of PGHS is much more exposed to the solvent than the corresponding heme pocket of MPO, being consequently designed to accept larger substrates.[15]

Analyzing the molecular surface representations of the heme-containing pocket we can see that after binding with the PGHS-2 peroxidase site, resveratrol occupies a longitudinal disposition in the access cavity (Fig. 5A), which is dislocated to the external part of the pocket, only partially blocking the entrance of natural substrates to the prosthetic group (Fig. 5C). On the other hand, resveratrol binds transversally to the active PGHS-1 peroxidase site, completely blocking the entrance of the enzyme substrate in the pocket (Fig. 5B). By the way, different from the evidenced binding profile

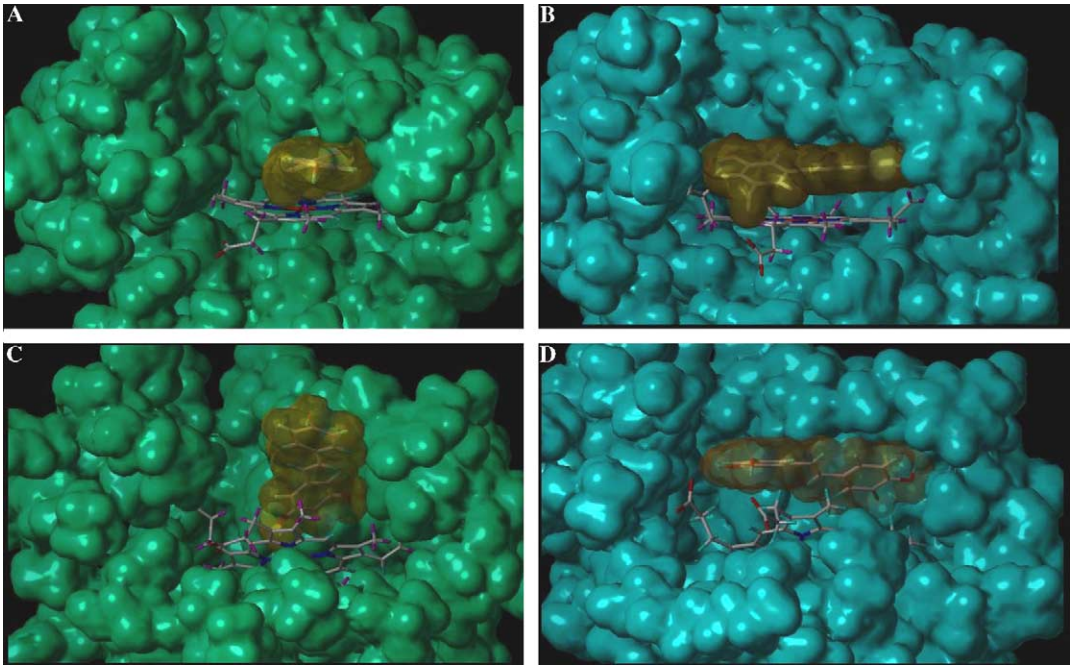

Figure 5. (A and C) Top view of resveratrol docked in access pocket with molecular surface representation in green (PGHS-2) and cyan (PGHS-2). Resveratrol bound longitudinally in PGHS-2 access pocket (A) and transversally in PGHS-1 (C). (B and D) Inferior view of access pocket. In PGHS-2, resveratrol disposal is dislocated to the external part of the pocket (B). In PGHS-2, resveratrol is totally inside of the canal (D), blocking the access of the PGG_2 to the heme group. Resveratrol is represented in molecular surface (yellow).

with PGSH-2, resveratrol is not located almost outside the PGHS-1 pocket, but totally inside in it, preventing the PGG_2 access to the heme group (Fig. 5D).

4. Conclusions

The docking results have enabled us to propose how resveratrol could act as a selective PGHS-1 peroxidase site inhibitor. In spite of us having evidenced only slight differences between the PGHS-1 and PGHS-2 binding energies, the differences between the amino acids in the two active sites lead to a differential disposal of the resveratrol inside the heme pocket, which seems to be the main reason for the selective PGHS-1 inhibition profile. The formation of hydrogen bonds among the hydroxyl groups of resveratrol phenyl rings, the Fe-heme backbones and the carbonyl group of Leu294 inside the PGHS-1 peroxidase site, and the absence of the His214 residue (present in PGHS-2) are essential for maintaining the aromatic rings of the natural product parallel to the heme group and transverse to the access pocket promoting a large steric hindrance at this site. The results described herein are in agreement with the previously related PGHS-mediated reductive profile of 5-phenyl-4-pentenyl hydroperoxide (PPHP) to 5-phenyl-4-pentenyl alcohol (PPA), which is a large substrate like PGG_2. In this assay, the substrate reduction was inhibited by resveratrol in PGHS-1 but not in PGHS-2.[16] Finally, we could evidence the strong possibility of exploring the natural stilbene template present in resveratrol in the design of new useful anti-inflammatory agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.07.028.

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