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Minireview

Prostaglandin H synthase: Implications for membrane structure

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Abstract

The crystal structure of the membrane protein prostaglandin H synthase (PGHS) provides strong evidence for the existence of monotopic membrane proteins: PGHS seems to interact with the membrane via a motif of amphipathic helices positioned parallel to the plane of the membrane. The orientation of this unique membrane binding motif is fixed in space by an epidermial growth factor(EGF)-like module on its amino-terminal end and by the catalytic domain at its carboxy-terminal end. The catalytic domain of PGHS has a high structural homology to other mammalian heme peroxidases.

Key words: Membrane protein structure; Cyclooxygenase; Peroxidase; X-ray structure

1. Introduction

Arachidonic acid, a 20-carbon unsaturated essential fatty acid, is a natural component of membrane phospholipids and is released as a free fatty acid by stimuli-activated phospholipases A_2 [1]. The bifunctional enzyme prostaglandin H_2 synthase (PGHS) catalyzes the first committed step in the conversion of arachidonic acid, via a free radical mechanism, into prostaglandin H_2 (PGH_2), the precursor of all prostaglandins and thromboxanes [1,2]. The resulting arachidonate derivatives are powerful modulators of many physiological processes including vasodilation, vasoconstriction, blood clotting, and inflammation. Prostanoid biosynthesis and PGHS activity are implicated in pathophysiology of cancer, arthritis and cardiovascular disease [3–5] and PGHS is the primary target for nonsteroidal anti-inflammatory drugs [2].

PGHS was classified many years ago as a membrane protein on the basis of biochemical experiments: detergents are needed to extract PGHS from biological membranes and to maintain the enzyme in a solubilised state [2,6]. The nature of the substrate, arachidonic acid, made this hypothesis seem quite reasonable. Free arachidonate would most likely stay associated with the lipid bilayer and therefore the active site of PGHS should have some access to the hydrophobic milieu of the membrane. When amino acid sequences became available [7,8], attempts were made to identify the portions of the enzyme which interact with the membrane. The assignment of the most hydrophobic segment(s) as transmembrane was based on the use of standard methodologies [10,11]; the different models proposed either one or two transmembrane segments [2,7,9]. The three-dimensional structure of the ovine PGHS isoenzyme-1 [12] showed clearly that the observed PGHS fold is not consistent with any model postulating transmembrane segments. The best model for the interaction of PGHS with the lipid bilayer involves the insertion of a motif of amphipathic helices into the membrane, creating a monotopic membrane protein as defined by Blobel [13].

2. The PGHS fold

PGHS-1 exists, in the asymmetric unit of the crystal, as a symmetric dimer (Fig. 1a). The overall fold of PGHS can be describe as having a tripartite domain structure (Fig. 1b). The amino-terminal portion begins with an epidermal growth factor(EGF)-like module (as defined by Campbell and Bork [14]), continues on with the membrane-binding domain, then ends with a large catalytic domain containing the two PGHS active sites: the cyclooxygenase and the peroxidase active sites. The cyclooxygenase active site, which catalyses the formation of prostaglandin G_2 (PGG_2) from arachidonic acid, resides at the apex of a long hydrophobic channel extending from the membrane-binding domain to the center of the molecule [12]. This observation is quite consistent with the above-mentioned hypothesis that the enzyme should interact with the hydrophobic core of the bilayer. In contrast, the peroxidase active site, which catalyzes the reduction of PGG_2 to PGH_2, is located on the other side of the molecule at the heme binding site (Fig. 1b), away from the membrane-binding domain. The catalytic domain of PGHS-1 has a striking structural homology to the mammalian peroxidase, myeloperoxidase [12,15], a soluble protein. This observation strongly suggests that PGHS evolved from a soluble peroxidase ancestor, acquiring not only an internal, hydrophobic active site, but

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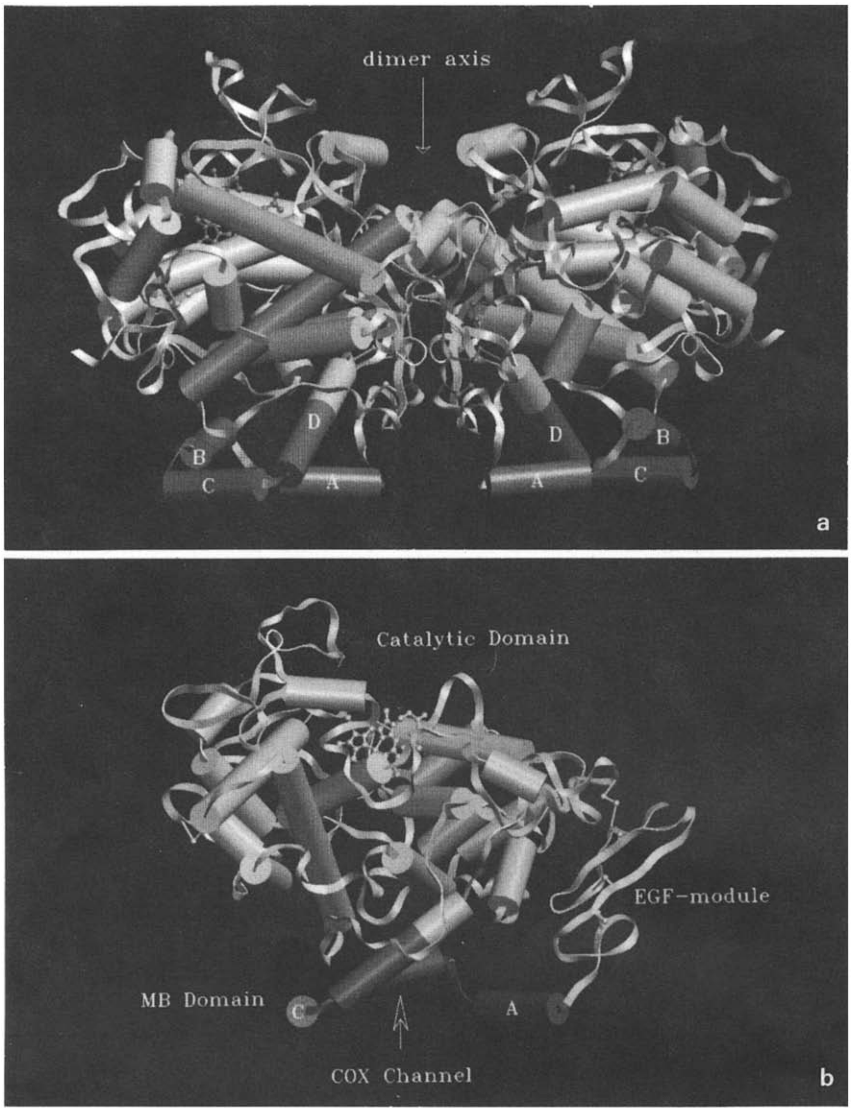

Fig. 1. In (a), a schematic ribbon representation of the PGHS dimer with tubes representing α-helices. The two-fold symmetry axis is denoted by an arrow. In (b), the left-hand monomer of (a) is rotated 90° about the 2-fold axis and viewed from the dimer interface to show the domain structure of the enzyme. The general structural features, EGF-like module, cyclooxygenase active site channel (COX channel), membrane-binding (MB) domain and catalytic domain, are labeled. Note the positions of the helices labeled A, B, and C in the dimer.

also the EGF-like module and the membrane-binding domain to allow direct interaction with the lipid bilayer.

The functions of EGF-like modules are unknown, but they are thought to serve as structural building blocks that initiate or maintain protein–protein interactions and, perhaps, protein–membrane interactions [14]. These modules, containing two small two-stranded β-sheets held together by three intradomain disulfide bonds, are found in many extracellular proteins, blood clotting factors and cell-surface membrane proteins. The EGF-like module in PGHS is very similar to murine EGF [16] and to the second EGF-like module of Factor Xa [17]. The observed differences between the structures are due to the different lengths between the loops formed by the three disulfide bridges in the EGF-like modules. Least squares superposition of the 6 cysteine residues (using $C\alpha$, $C\beta$, and $S\gamma$ atoms) from any pair of EGF-like structures gives a RMS deviation on the order of 1.3–1.4 Å for the 18 atoms [12,17].

The EGF-like module is oriented with its carboxy-terminus just at the beginning of helix A in the membrane-binding domain and thus would be positioned close to the membrane surface. Three related observations are of interest here. First, Kohda and Inagaki [18] have presented evidence that the carboxy-terminal portion of murine EGF can interact strongly with micelles of phopholipid analogs. Second, the homology between the amino-terminal domain of PGHS and EGF extends beyond the boundaries of sequence homology usually seen in EGF-like modules [14], persisting up to the

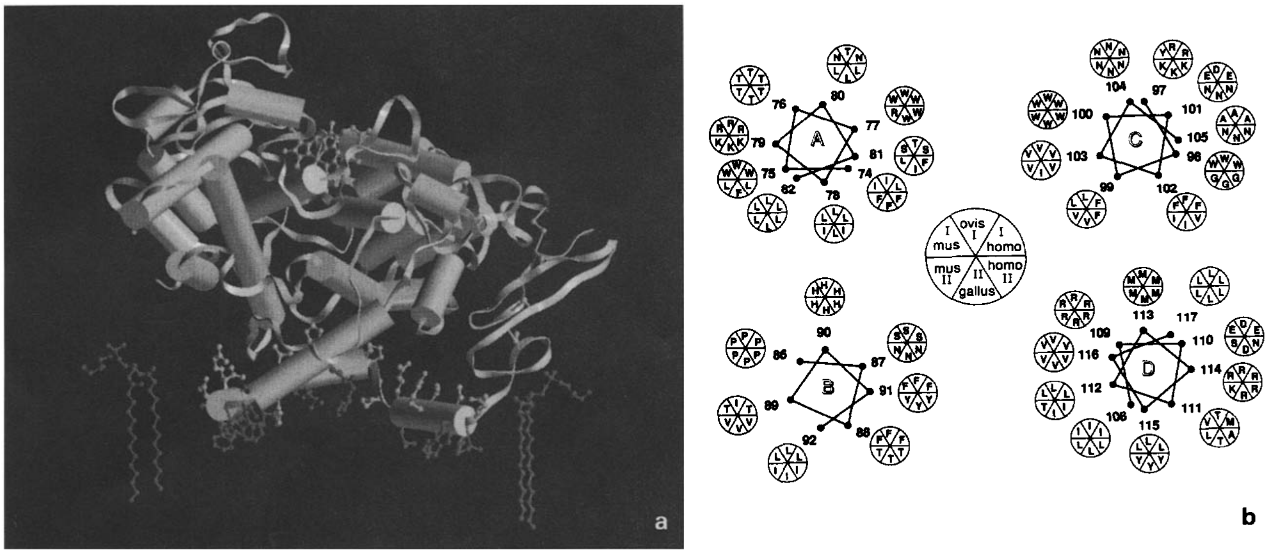

Fig. 2. In (a), a schematic of the PGHS monomer shown in Fig. 1a with residues in the membrane binding domain highlighted (light gray for polar, and dark gray for hydrophobic) to reveal the hydrophobic surface of the membrane binding motif. The conformation for the dimyristoyl phosphatidylglycerol molecules (DMPG) shown flanking the monomer was taken from Pascher et al. [40]. DMPG molecules are shown next to the membrane binding domain to denote roughly how PGHS might sit in the bilayer. In (b), a series of helical wheels for helices A, B, C and D are shown; the residues represented here are taken from the 6 published sequences for PGHS [7,34–38]. The 'pie' in the center of the panel shows which residues from which PGHS sequence are at each position in helices A, B, C and D.

Leu78–Arg79 dipeptide in PGHS, which falls in the middle of helix A [12]. Lastly, another mammalian peroxidase, thyroid peroxidase, also exhibits high sequence homology with myeloperoxidase in the catalytic domain [14,19]. However, thyroid peroxidase is a membrane protein, but unlike PGHS, thyroid peroxidase apparently has an EGF-like module at the carboxy-terminal portion of the sequence followed immmediately by a putative transmembrane anchor [19]. These observations support the hypothesis that EGF-like modules may be directly involved with protein–membrane interactions when they preceed a membrane-binding domain.

The membrane binding domain, which follows the EGF-like module, is formed by a motif comprising three amphiphilic helices (A, B, C) which lie approximately in a plane (Fig. 1). The beginning of helix D is also amphipathic but this fourth helix is more of a transitional structure in the motif as it connects the three first helices to the catalytic domain. The membrane-binding motif is held rigidly in position by the transitional helix D at its carboxy-terminus and by the EGF-like module at its amino-terminus. Moreover, the EGF-like module itself is anchored not only by the intradomain disulfide bond Cys37–Cys159, but also through extensive intersubunit interactions [12]. The surfaces of the helices A, B and C that face away from the catalytic domain are composed of hydrophobic residues (Fig. 2a). In the dimer (Fig. 1a), the two membrane-binding motifs therefore create an extensive hydrophobic surface along one side of the enzyme [12].

3. Structural aspects of membrane interaction

From the three-dimensional structure of PGHS, we have proposed a model for its insertion into the membrane [12] where the protein interacts with the membrane through the insertion of the membrane-binding motifs into the bilayer (Fig. 2a), in a manner suggested for surface-active peptides [20,21]. The protein, however, would not span the lipid bilayer and therefore PGHS is a monotopic membrane. The existence of monotopic membrane proteins have been postulated quite some time ago [13,22,23], but PGHS would be the first example where the three-dimensional structure has been determined. From biochemical data examples of such membrane proteins are quite scarce: cytochrome b_5 [24] seems to form a monotopic interaction under some experimental conditions and pyruvate oxidase [25] is linked to the membrane through an amphipathic peptide at the carboxy-terminus. The extremely small number of clearly established monotopic membrane proteins led to the conclusion that they would be rare, perhaps for a variety of reasons [26]. These include the strong perturbation in the bilayer structure they would induce and the possible lack of insertion machinery for that type of protein structure. The PGHS structure, on the other hand, suggests a more prosaic explanation: the observed 'rarity' of monotopic membrane proteins may be due to the difficulty in identifying them by their sequence. The identification of PGHS as a membrane protein from biochemical experiments and the lack of a sequence 'signature' for identifying putative monotopic membrane motifs essentially led to the wrong conclusions. The structure of PGHS offer thus the first opportunity to describe some of the structural characteristics of a monotopic membrane-binding motif.

Over the past decade, several methods have been published for identifying putative transmembrane helices by virtue of the amino acid distribution [10,11]. With the determination of the bacterial photosynthetic reaction center structure [27,28], the sequence analysis of well-defined transmembrane helices could be made [28,29]. Using this limited structural information, others [30,31] have expanded the scope of the sequence analyses to identify and characterize of putative transmembrane helices in many membrane proteins of unknown tertiary structure. For example, Landolt-Marticorena et al. [31] have recently suggested that a consistent distribution of amino acids exists in transmembrane segments and their flanking regions for putative type I single span membrane proteins: (1) the hydrophobic region is dominated by aliphatic residues (Leu, Ile, Val), (2) the boundaries of the hydrophobic region are often demarcated by aromatic residues (Phe, Tyr, Trp), and (3) the immediate flanking regions can be rich in Arg or Lys. The primary structure of a transmembrane helix would then vary in a predictable fashion as the helix reaches the membrane surface and enters into the hydrophobic milieu. As shown by earlier work [30], the cytoplasmic side of these putative transmembrane helices tend to have a greater concentration of cationic residues. The question now arises as to what might be the primary structure characteristics of a membrane-active helical motif, as we propose, where the helices bind with their axes parallel to the membrane surface. Would they mimic transmembrane helices and show a distribution of amino acid residues across the helix similar to that found along transmembrane helices or do they have a primary sequence structure similar to 'generic' amphipathic helices on the surface of soluble proteins?

White and colleagues [21,32,33] have provided an appropriate framework for analyzing membrane structure by studying the partitioning of small tripeptides into the lipid bilayer. They departed from a representation of the membrane as a single hydrocarbon slab and divided the membrane into three phases: a hydrophobic core region, an interfacial region and a solvent region. They also introduced the notion of a variable interfacial hydrophobicity scale IFH(h) that describes the free energy for membrane interaction which depends on the level of satisfied hydrogen bonds. The variable h describes the state of hydrogen bond formation for a particular side chain (i.e. $h = 0$, for no H-bond formation; $h = 1$, when all possible H-bonds are formed). In other words, this scale allows one to take into account the effects of hydrophobicity and the potential for H-bond formation for a particular residue. In the hydrophobic core of the bilayer, residues like Trp, Tyr, Thr and Ser would have an unfavorable IFH(h) if all potential bonds could not be made (i.e. h near 0.0). When a residue moves toward the interface, where all possible H-bonds could be formed, the IFH($h = 1$) then becomes very favorable for binding of a peptide at the membrane interface. They further stress that residues with a large surface will have a favorable interaction with the interface even if they are charged (for example Arg or Lys), provided that the charged group remains near the aqueous phase. To enter the hydrophobic core of the bilayer, a residue must therefore be part of a secondary structure element where nearly all possible main chain hydrogen bonds are satisfied. The experimentally derived IFH(h) scale therefore predicts the residues which could interact with the interfacial and hydrophobic regions of the bilayer, depending on the state of H-bond formation White and Wimley [33] also suggested that an α-helix provides an excellent secondary structural element for interaction with the bilayer interfacial region. Given their observations, the amino acid composition across a membrane-embedded helix should mimic what others have found for transmembrane helices.

A comparison of the monotopic helices of three PGHS-1 sequences [7,34,35,] and three PGHS-2 sequences [36–38], seen in Fig. 2b, clearly shows the strong tendency toward the conservation of the amphipathic nature of the membrane-binding motif. From this sequence database, it is possible to create a 'consensus' helix for the membrane-binding motif represented by a helical wheel divided into four different sectors about helix axis: (a) an upper sector that would interact either with the solvent or with the protein and may therefore not have a unique sequence composition, (b) a lower or hydrophobic sector, thought to interact with the hydrophobic core of the membrane, and, finally, (c) two intermediate sectors that describe the transition from the exterior to the interior of the membrane through the interfacial region defined by the lipid head groups. Fig. 3 summarizes the distribution of the residues found in these four sectors as seen in helices A, B and C of the membrane-binding motif.

On a qualitative level, this approach fits very well with the expected distribution for the residues involved in membrane-helix interaction, as predicted by the Jacobs and White model, particularly with regard to the localization of the aromatic residues Tyr and Trp in the interfacial regions. Furthermore, the distribution of amino acid residues across the censensus helix (Fig. 3) is very similar to what was found by Landolt-Marticorena et al. [31] for transmembrane helices (see above). The consensus helix may also allow one to approximate the localization of the helices relative to the membrane. The helices in the membrane-binding motif have an average diameter of 14–16 Å. The residues of the Sector 2 should be in the interface with the charged group facing the solvent or

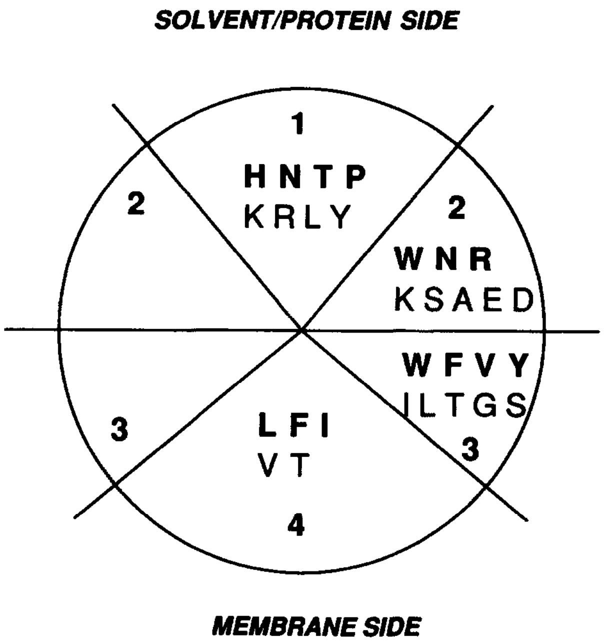

Fig. 3. The series of helical wheels for helices A, B and C shown in Fig. 2b have been reduced to a single censensus helix and the helix was then divided into 4 spatial sectors. The amino acids that make up the majority of the residues in each sector are shown on the upper line and rarer substitutions are on the lower line.

interacting with the charged headgroup of the lipids. Given the diameter of the helices, the lowest sector (Sector 4 in Fig. 3) should interact with the hydrophobic core of the membrane, whereas the Sector 3 should be at the transition between the core and the interfacial region.

These observations, however preliminary, show again that the general principles that govern the interaction of membrane proteins with the lipid bilayer that were proposed by Singer and colleagues over thirty years ago [23,39], are still valid; but it also shows that the possibilities for satisfying these conditions are vaster than we have sometimes thought.

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