Prostaglandin F$_{2\alpha}$ Formation from Prostaglandin H$_2$ by Prostaglandin F Synthase (PGFS): Crystal Structure of PGFS Containing Bimatoprost†,‡

Junichi Komoto,§ Taro Yamada,§ Kikuko Watanabe,‖ David F. Woodward,⊥ and Fusao Takusagawa*,§

Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Avenue, Lawrence, Kansas 66045-7534, Division of Applied Life Science, Graduate School of Integrated Science and Art, University of East Asia, 2-1 Ichinomiya-gakuencho, Shimonoseki, Yamaguchi 751-0807, Japan, Department of Biological Sciences, Allergan, Inc., 2525 Dupont Drive (RD-2C), Irvine, California 92612

Received September 13, 2005; Revised Manuscript Received December 23, 2005

ABSTRACT: Prostaglandin H$_2$ (PGH$_2$) formed from arachidonic acid is an unstable intermediate and is efficiently converted into more stable arachidonate metabolites by the action of enzymes. Prostaglandin F synthase (PGFS) has dual catalytic activities: formation of PGF$_{2\alpha}$ from PGH$_2$ by the PGH$_2$ 9,11-endoperoxide reductase activity and 9$\alpha$,11$\beta$-PGF$_2$ (PGF$_{2\alpha\beta}$) from PGD$_2$ by the PGD$_2$ 11-ketoreductase activity in the presence of NADPH. Bimatoprost (BMP), which is a highly effective ocular hypotensive agent, is a PGF$_{2\alpha}$ analogue that inhibits both the PGD$_2$ 11-ketoreductase and PGH$_2$ 9,11-endoperoxide reductase activities of PGFS. To examine the catalytic mechanism of PGH$_2$ 9,11-endoperoxide reductase, a crystal structure of PGFS[NADPH + BMP] has been determined at 2.0 Å resolution. BMP binds near the PGD$_2$ binding site, but the $\alpha$- and $\omega$-chains of BMP are locate on the $\omega$- and $\alpha$-chains of PGD$_2$, respectively. Consequently, the bound BMP and PGD$_2$ direct their opposite faces of the cyclopentane moieties toward the nicotinamide ring of the bound NADP. The $\alpha$- and $\omega$-chains of BMP are involved in H-bonding with protein residues, while the cyclopentane moiety is surrounded by water molecules and is not directly attached to either the protein or the bound NADPH, indicating that the cyclopentane moiety is movable in the active site. From the complex structure, two model structures of PGFS containing PGF$_{2\alpha}$ and PGH$_2$ were built. On the basis of the model structures and inhibition data, a putative catalytic mechanism of PGH$_2$ 9,11-endoperoxide reductase of PGFS is proposed. Formation of PGF$_{2\alpha}$ from PGH$_2$ most likely involves a direct hydride transfer from the bound NADPH to the endoperoxide of PGH$_2$ without the participation of specific amino acid residues.

PGF$_{2\alpha}$, one of the earliest discovered and most common prostaglandins, is actively biosynthesized in various organs of mammals (1–7) and exhibits a variety of biological activities, including contraction of pulmonary arteries (8–11). PGF$_{2\alpha}$ is mainly synthesized directly from PGH$_2$ by PGH$_2$ 9,11-endoperoxide reductase (12–15). A small amount of PGF$_{2\alpha}$ is also produced from PGE$_2$ by PGE$_2$ 9-ketoreductase (16, 17). A PGF$_{2\alpha\beta}$ epimer has been reported to...
Aldo-keto reductase family, PGFS, NADPH, PGH₂, PGD₂, NADP⁺, NSAIDs, PGF₂α, PGF₂β, BMP, Flurbiprofen, Ketoprofen, IMN, prostaglandin amides, cyclooxygenase-2 (COX-2), endoperoxide reductase, 9,11-endoperoxide reductase, 11-ketoreductase.

PGF synthase (PGFS) was first purified from bovine lung (15, 27). PGFS has dual functions; it catalyzes the formation of PGF₂α from PGH₂ by PGH₂ 9,11-endoperoxide reductase in the presence of NADPH and also catalyzes the formation of PGF₂αβ from PGD₂ by PGD₂ 11-ketoreductase (28). However, this enzyme does not catalyze the reduction of PGE₂ (15) (Chart 1).

Interestingly, this enzyme exhibits reductase activities toward various carbonyl compounds, such as 9,10-phenanthrenequinone (PQ), p-nitrobenzaldehyde, and p-nitrocatechol (15). Although the PGD₂ 11-ketoreductase activity is competitively inhibited by PQ, the PGH₂ 9,11-endoperoxide reductase activity is not inhibited by PQ, suggesting that the PGD₂ and PGH₂ binding sites are different (15). PGFS belongs to the aldo-keto reductase family on the basis of its substrate specificity, molecular weight, and amino acid sequence (29, 30). Human 3α-hydroxysteroid dehydrogenase isosforms have been systematically named AKR1C1–AKR1C4, and PGFS, 3α-hydroxysteroid dehydrogenase type 2, is named AKR1C3 (31). We proposed a catalytic mechanism for the PGD₂ 11-ketoreductase activity of PGFS on the basis of complex structures of PGFS[NADP⁺ + PGD₂] and PGFS[NADPH + rutin] (32), which is similar to the basic mechanism of the aldo-keto reductase family (33). Our mechanism uses the catalytic triad (D50⋯K84⋯Y55 H-bonding) instead of the catalytic tetrad (D50⋯K84⋯Y55⋯H117 H-bonding) for donation of a proton from Y55 to PGD₂ (33). Also, in our mechanism, H117 might act as a general acid at a low pH to donate a proton to PGD₂.

Recent reports suggest that the PGF activity is high in gastrointestinal tumors, and nonsteroidal anti-inflammatory drugs (NSAIDs) protect against the progression of gastrointestinal tumors (34–40). There is a growing body of evidence which shows that NSAIDs may also protect against a variety of other cancers, including prostate carcinoma and, most recently, leukemia (34–40). For example, treatment of leukemia HL-60 cell line cells with indomethacin (IMN) inhibits the growth of HL-60 cells, and overexpression of PGFS in myeloid cells promotes proliferation, suggesting that IMN inhibits PGFS (41–43). Indeed, a crystal structure of PGFS containing IMN has been determined (44). PGFS is inhibited by NSAIDs such as Suprofen (IC₅₀ = 0.6 μM), Flurbiprofen (IC₅₀ = 0.8 μM), Ketoprofen (IC₅₀ = 3.8 μM), and IMN (IC₅₀ = 4.1 μM) (45), and these NSAIDs have preventive activity against the progression of gastrointestinal tumors. Therefore, specific inhibitors of PGFS might be important anticancer drugs.

Recently, it has become apparent that there are neutral lipids of several existing fatty acids (46–55). These neutral lipids occur in the form of an amide, ether, or ester, which replaces the invariant carboxylic acid moiety. Anandamide and 2-arachidonyl glycerol are examples of neutral lipids that are substrates for cyclooxygenase-2 (COX-2). The resultant products are prostaglandin amides (prostamides) or glyceryl esters (49, 51). Reduction of prostamides produces biologically active prostamide F₂α. Bimatoprost (BMP) is a synthetic structural analogue of PGF₂α in which the charged carboxylic acid group is replaced with a neutral ethylamide substituent.

Structurally, BMP is therefore a prostaglandin amide or prostamide and exhibits biological activity similar to that of prostamide F₂α (56, 57). BMP is of particular interest because it is clinically the most efficacious ocular hypotensive agent reported to date, its activity exceeding that of both timolol and latanoprost (58, 59). The ocular hypotensive activity has been suggested to be due to enzymatic hydrolysis to a free acid metabolite that would behave as an authentic prostanoïd FP receptor agonist (60), but this is controversial (61). Also, BMP in-

---

1 Abbreviations: AKR, aldo-keto reductase; BMP, bimatoprost; IMN, indomethacin; NADP, either NADP⁺ or NADPH; NSAIDs, nonsteroidal anti-inflammatory drugs; PGD₂, prostaglandin D₂; PGF₂αβ, 9α,11β-prostaglandin F₂; PGF₂α, 9α,11β-prostaglandin F₂; PGFS, prostaglandin F synthase; PGFS[NADPH + BMP], NADPH- and bimatoprost-bound PGFS; PGFS[NADP⁺ + PGD₂], NADP⁺- and PGD₂-bound PGFS; PGH₂, prostaglandin H₂; PQ, 9,10-phenanthrenequinone.
Prostaglandin F₂α Formation by Prostaglandin F Synthase

Table 1: Crystallographic Statistics for PGFS[NADPH + BMP]a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>unit cell dimensions</td>
<td>a = 44.27 Å, b = 78.16 Å, c = 48.89 Å, β = 102.0°</td>
</tr>
<tr>
<td>resolution (Å)</td>
<td>2.0</td>
</tr>
<tr>
<td>total no. of observations</td>
<td>117502</td>
</tr>
<tr>
<td>no. of unique reflections</td>
<td>19525</td>
</tr>
<tr>
<td>completeness (%)</td>
<td>88.7 (63.3)</td>
</tr>
<tr>
<td>R(ecut) (outer shell)</td>
<td>0.054 (0.126)</td>
</tr>
<tr>
<td>no. of protein non-hydrogen atoms</td>
<td>2563</td>
</tr>
<tr>
<td>cofactor</td>
<td>1 NADPH (48 atoms)</td>
</tr>
<tr>
<td>substrate/inhibitor</td>
<td>1 BMP (30 atoms)</td>
</tr>
<tr>
<td>no. of solvent molecules (H₂O)</td>
<td>109</td>
</tr>
<tr>
<td>resolution range (Å)</td>
<td>20–2.0</td>
</tr>
<tr>
<td>total no. of reflections used in R(ecut)</td>
<td>1923</td>
</tr>
<tr>
<td>R(ecut) (outer shell)</td>
<td>0.221 (0.241)</td>
</tr>
<tr>
<td>R(free) (outer shell)</td>
<td>0.281 (0.292)</td>
</tr>
<tr>
<td>rmsd for bond distances (Å)</td>
<td>0.007</td>
</tr>
<tr>
<td>rmsd for bond angles (deg)</td>
<td>1.2</td>
</tr>
<tr>
<td>rmsd for torsion angles (deg)</td>
<td>26</td>
</tr>
<tr>
<td>most favored region (%)</td>
<td>94.0</td>
</tr>
<tr>
<td>of the Ramachandran plot</td>
<td>6.0</td>
</tr>
<tr>
<td>additional allowed region (%)</td>
<td>44.2 (outer shell)</td>
</tr>
<tr>
<td>of the Ramachandran plot</td>
<td>24.0 (outer shell)</td>
</tr>
</tbody>
</table>

a Space group P2₁. The M₄ of the subunit is 35 530. The number of complexes in the unit cell is 2. V_C = 2.43 Å³. The percentage of solvent content 47.5%, a R(ecut) = Σ(|F_o| - |F_c|)/Σ|F_o|. b Outer shell is 2.0–2.1 Å resolution. c R(ecut) = Σ|F_o| - |F_c|/Σ|F_o|.

Hibits formation of PGF₂α from PGH₂ (IC₅₀ = 6 μM) and formation of PGF₂α from PGD₂ (IC₅₀ = 5 μM) by PGFS (62).

Here we describe crystal structures of a human lung PGFS ternary complex, PGFS[NADPH + BMP]. On the basis of the structure, we propose a catalytic mechanism for formation of PGF₂α from PGH₂ by PGFS.

EXPERIMENTAL PROCEDURES

Crystallization. Recombinant human lung PGFS was purified from Escherichia coli HB101 containing a pUC-hLuFS plasmid encoding the human lung PGFS sequence. The enzyme was purified to electrophoretic homogeneity using methods previously described (63). The hanging drop method of vapor diffusion was employed for crystallization of the enzyme. BMP was dissolved in 95% ethanol and added to the crystallization solution just before the crystallization was set up. Thick plate-shaped crystals of PGFS-[NADPH + BMP] suitable for X-ray diffraction studies were grown in a solution containing 1.0 mM BMP, 1.0 mM NADPH, 0.14 M NaCl, 50 mM MES buffer (pH 7.0), and 26% (w/v) PEG 8000 with a protein concentration of 7 mg/mL at 4 °C. The crystals were grown for 14 days.

Data Measurement. The crystal (~0.3 mm × 0.2 mm × 0.1 mm) of PGFS-[NADPH + BMP] in a hanging drop was soaked with a nylon loop and dipped into a cryoprotectant solution for 10–15 min, before it was frozen in liquid nitrogen. The cryoprotectant solution was composed of the original mother liquor containing 18% ethylene glycol. The frozen crystal was transferred onto a Rigaku RAXIS Iic imaging plate X-ray diffractometer with a rotating anode X-ray generator as an X-ray source (Cu Kα radiation at 50 kV and 100 mA). The X-ray beam was focused to 0.3 mm by confocal optics (Osmic, Inc.). The diffraction data were measured up to 2.0 Å resolution at ~180 °C. The data were processed with DENZ0 and SCALEPACK (64). The statistics are given in Table 1.

Crystal Structure Determination. The unit cell dimensions and space group of the PGFS[NADPH + BMP] crystal indic-
PGH₂ 9,11-Endoperoxide Reductase Activity (PGH₂ → PGF₂α) Assay. The PGH₂ 9,11-endoperoxide reductase activity was assayed under the same conditions that were used for the PGD₂ 11-ketoreductase activity except that 80μM [1-14C]PGH₂ (0.1μCi) was used as a substrate in place of 1.5 mM [3H]PGD₂ and that the incubation time was 1 min at 30°C.

RESULTS AND DISCUSSION

Overall Structure. The crystallographic refinement parameters (Table 1), final 2Fo −Fc maps, and conformational analysis by PROCHECK (68) indicate that the crystal structure of the PGFS[NADPH + BMP] complex has been determined with acceptable statistics. As shown in Figure 2, the protein structure of PGFS displays the characteristic fold of the AKR superfamily (69), an (α/β)₈ barrel with three associated large loops. The β-strands (β1–β8) form the cylindrical core of the barrel and are surrounded by α-helices (α1–α8), while the accompanying loops (loop 4, loop 7, and loop 9) partially cover the C-terminal end of the barrel. In addition to the (α/β)₈ core structure, there are two β-strands (B1 and B2) from the N-terminus sealing the N-terminal end of the barrel and two α-helices (H1 and H2) from the C-terminal part of the molecule packed by the side of the barrel. These additional β-strands and α-helices are conserved in other AKR structures (69).

Cofactor (NADPH) binds in a deep cavity at the C-terminal end of the barrel and extends across the barrel closer to the core. The bound NADPH is heavily involved in H-bonds with amino acid residues in the loops connecting β-strands and α-helices. The nicotinamide moiety is located within the enzyme near the center of the barrel, while the adenine moiety is exposed on the outer surface of the protein.

BMP in the Active Site. As shown in Figure 3, a BMP molecule is located above the bound NADPH and is surrounded by loop 1, 2, 4, 7, and 9. Although the two hydroxyl groups (O₉ and O₁₁) of the cyclopentane moiety are pointed to the nicotinamide ring of the bound NADPH, there is no significant interaction between them. Each of the O₉ and O₁₁ hydroxyl groups of the cyclopentane moiety is involved in two H-bonds with two water molecules (w₂ and w₅) and another water molecule (w₄), respectively. The ethylamide α-chain is deep within the active site cavity, while the phenyl ω-chain is located above the α-chain. O₁ and N₁ in the α-chain participate in H-bonds with O₂ of S118 and w₁, respectively. Although O₁₀ of Y319 can be near N₁ in to form a H-bond, there is no H-bond between them because of the presence of the ethyl moiety. The hydroxyl group O₁₅ in the ω-chain

FIGURE 2: Ribbon drawings of PGFS[NADPH + BMP]: (A) side view and (B) top view. The α-helices and β-strands in the α/β-base are colored aquamarine and magenta, respectively. The loops are colored yellow with numbers. The bound NADPH and BMP are colored cyan and light pink, respectively. The topology of the polypeptide is ~B1(7–9)-B2(15–17)-β1(19–22)-(loop 1)-α1(32–44)-β2(48–50)-(loop 2)-α2(58–70)-β3(80–85)-(loop 3)-α3(92–106)-β4(113–116)-(loop 4)-α4(144–156)-β5(162–166)-(loop 5)-α5(170–177)-β6(188–192)-(loop 6)-α6(200–208)-β7(212–216)-(loop 7)-α7(239–248)-H1(252–262)-β8(266–270)-(loop 8)-α8(274–284)-H2(290–297)-(loop 9). B1 and B2 are extra β-strands that are not involved in the β-barrel structure component.
participates in a H-bond with O₆ of S129 and w₄. Therefore, the polar groups of α- and ω-chains (tails) are directly connected to the protein with H-bonds, while the cyclopentane moiety (head) is associated with water molecules, suggesting that the cyclopentane moiety is movable in the active site cavity. Although the polar groups of BMP are involved in H-bonds, BMP is bound to the protein by mainly hydrophobic interactions. There are seven aromatic residues (Y24, W86, F139, W227, F306, F311, and Y319) surrounding the bound BMP. W227 is located over the cyclopentane moiety of BMP as if it seals the bound BMP into the active site cavity.

Since BMP is an analogue of PGF₂α, a PGF₂α molecule can be completely superimposed on the bound BMP and forms the same H-bonds that were observed for BMP, except that O₁ of the carboxyl group forms a H-bond with O₄ of Y319 and water (w₁). This modeling suggests that the product PGF₂α from PGH₂ might bind to the same site as BMP before being released from the active site cavity. An inhibition study supports this conclusion because BMP inhibits the 9,11-endoperoxide reductase activity as well as the 11-ketoreductase activity of PGFS (62).

**A Comparison of the PGD₂ and BMP Binding Sites.** Although the crystal structure of PGFS[NADPH + BMP] is not isomorphous to that of PGFS[NADP⁺ + PGD₂], the peptide fold is identical within experimental error. Significant movements are only observed in the side chains of W227 and F311. These aromatic residues are two of seven residues surrounding BMP, suggesting that the PGFS structure is relatively rigid. The NADP⁺ and NADPH binding sites in the PGFS structure are the same within experimental error (rmsd = 0.22 Å), and their H-bonding networks are also the same. Although PGD₂ and BMP bind to a similar site, there are several significant differences between their binding schemes. The α- and ω-chains of BMP are located on the ω- and α-chains of PGD₂, respectively, indicating that the BMP and PGD₂ binding schemes are flipped. The bound BMP and PGD₂ direct their opposite faces of the cyclopentane moieties toward the nicotinamide ring of the bound NADP (Figure 4).

PGFS has an oxyanion hole in the active site. In the PGFS[NADPH + BMP] structure, the carbonyl oxygen (O₁₁) of PGD₂ is pulled into the oxyanion hole and forms H-bonds with O₉ of Y55 and N₆₂ of H117. On the other hand, no part of BMP is near the oxyanion hole in the PGFS[NADPH + BMP] structure. A water (w₆) is located in the oxyanion hole and forms H-bonds with Y55 and H117. Consequently, the positions of the cyclopentane moieties of BMP and PGD₂ are significantly different, suggesting that the catalytic sites of 11-ketoreductase and 9,11-endoperoxide reductase in PGFS are in different locations in the active site cavity.
A Possible Catalytic Mechanism. BMP inhibits the PGD$_2$ 11-ketoreductase activity as well as the PGH$_2$ 9,11-endoperoxide reductase activity (62). From the crystal structures of PGFS[NADP$^+$ + PGD$_2$] and PGFS[NADPH + BMP], the PGD$_2$ 11-ketoreductase inhibitory activity of BMP is quite obvious because the two molecules bind to a similar region in the active site cavity. Since BMP is a PGF$_2$R analogue and also inhibits the PGH$_2$ 9,11-endoperoxide reductase reaction, it is reasonable to assume that PGH$_2$ and PGF$_2$R bind to the BMP binding site. On the basis of this assumption, we can build model structures of PGFS[NADP$^+$ + PGF$_2$R] from PGFS[NADPH + BMP] and another model structure of PGFS[NADPH + PGH$_2$] by forming the endoperoxide between O$_9$ and O$_{11}$ (Figure 5). The endoperoxide moiety of PGH$_2$ in the model structure is too distant to transfer a hydride directly from the bound NADPH. In PGFS[NADPH + BMP], the cyclopentane moiety of BMP is surrounded by water molecules (w$_2$–w$_5$) and does not directly attach to the protein through H-bonding, suggesting that the cyclopentane moiety is movable in the active site cavity. As shown in Figure 5, the cyclopentane moiety of BMP can be moved toward the bound NADPH without breaking the H-bonds between the chains of PGH$_2$ and the protein. In this model structure, O$_9$ of PGH$_2$ is brought near the bound NADPH and is able to receive the pro-$R$ hydrogen from the bound NADPH.

On the basis of these two PGFS[NADPH + PGH$_2$] model structures, we propose a putative catalytic mechanism for the PGH$_2$ → PGF$_2$R reaction. PGH$_2$ binds at the BMP binding site found in the crystal structure (model 1, colored magenta in Figure 5), and the cyclopentane–endoperoxide moiety swings toward the bound NADPH (model 2, colored yellow in Figure 5) without breaking the H-bonds of the $\alpha$- and $\omega$-chains. In this geometry, O$_9$ of PGH$_2$ can be located within 3.5 Å of C$_4$ of NADPH, the pro-$R$ hydrogen of NADPH can be directly transferred to O$_9$ of PGH$_2$, and the O$_9$–O$_{11}$ bond is broken in a concerted fashion (Figure 6A). The negatively charged O$_{11}$ receives a proton from the solution to complete the reaction, and the resulting PGF$_2$R is released from the active site cavity. The proposed catalysis occurs only if the endoperoxide moiety of PGH$_2$ is oriented correctly relative to the bound NADPH, whose location and orientation did not vary in the three crystal structures (PGFS[NADP$^+$ + PGD$_2$], PGFS[NADPH + rutin], and PGFS[NADPH + BMP]).

Obviously, the proposed mechanism for formation of PGF$_2$R from PGH$_2$ is different from that of formation of PGF$_2$R from PGD$_2$ proposed on the basis of PGFS[NADP$^+$ + PGD$_2$] and PGFS[NADPH + rutin] structures (32). In PGF$_2$R formation, a PGD$_2$ binds to the active site, carbonyl oxygen O$_{11}$ is in the oxyanion hole, and the carbonyl carbon (C$_{11}$) directs its $re$ face toward the nicotinamide ring of the...
bound NADPH. Y55 (or H119) donates a proton to O11 of PGD2, and the pro-R hydrogen of NADPH is transferred directly to C11 of PGD2 to form PGF2αβ (Figure 6B).

Y55 is the essential amino acid residue for the PGD2 11-ketoreductase activity, while it does not participate in the proposed PGH2 9,11-endoperoxide reductase activity. Therefore, the Y55F mutated enzyme was prepared, and the PGD2 11-ketoreductase and PGH2 9,11-endoperoxide reductase activities were measured. The Y55F mutated enzyme loses the PGD2 11-ketoreductase activity but retains the PGH2 9,11-endoperoxide reductase activity, indicating that the formation of PGF2αβ from PGD2 and the formation of PGF2α from PGH2 utilize the different catalytic mechanism.

Since no specific amino acid residue directly facilitates the hydride transfer in the proposed catalytic mechanism of PGH2 9,11-endoperoxide reductase, the hydride transfer rate would be slower than that of the PGD2 11-ketoreductase reaction facilitated by a general acid catalysis. Indeed, the catalytic rate (kcat = 5.7 × 10^-3 s^-1) and efficiency (kcat/Km = 2.28 × 10^2 M^-1 s^-1) of PGH2 9,11-endoperoxide reductase are lower than those of PGD2 11-ketoreductase (kcat = 1.42 × 10^-2 s^-1, kcat/Km = 9.47 × 10^3 M^-1 s^-1) (30).

PGF2α Binding Site and Mode. The proposed catalytic mechanism assumes that PGF2α binds to the BMP binding site in the same binding mode which is different (flipped mode) from that of PGD2 found in the crystal structure of PGFS[NADP^+ + PGD2]. It is possible that the bulky ethylamide and phenyl groups at the α- and ω-chains, respectively, might prevent the cyclopentane moiety of BMP from entering deeply into the active site cavity as observed in PGD2 binding and induce BMP to have a mode flipped compared to the PGD2 binding mode. To examine this possibility, the following three model structures were built: (1) BMP bound to the BMP binding site in the same mode as PGD2, (2) BMP bound to the PGD2 binding site in the same mode as PGD2, and (3) BMP bound to the PGD2 binding site in the same mode as BMP (flipped mode).

Model 1 was built by flipping the bound BMP, and the conformations of α- and ω-chains were varied to superimpose the α- and ω-chains on the α- and ω-chains of the bound BMP, respectively. Note that O11 is far from the oxyanion hole (the bound water w6 position). Models 2 and 3 were built by placing O11 or O8 of BMP, respectively, in the oxyanion hole; each BMP model structure was rotated at O11 or O9, and the conformations of α- and ω-chains were varied to superimpose them on the bound PGD2 in the PGFS-[NADP^+ + PGD2] structure. Since the active site cavity of PGFS is relatively large, BMP fits well in the active site.
cavity in the three model structures without short contacts. Thus, the modeling studies suggest that the BMP binding site and mode found in the PGFS[NADPH + BMP] structure would not be due to the bulky groups attached to the α- and ω-chains.

Since BMP and PGF2α are structurally very similar, PGF2α could bind to PGFS in a manner similar to that of BMP in the model structures; i.e., it could bind to two different sites (PGD2 and BMP binding sites) in two different binding modes (PGD2 and flipped modes). However, PGFS does not catalyze the reduction reaction from PGD2 to PGF2α or from PGE2 to PGF2α (28). Both PGD2 and PGE2 are structurally quite similar and have a carbonyl group. One of the characteristic structural features of PGFS is the presence of an oxyanion hole in the active site of 11-ketoreductase. The carbonyl oxygen (O11) of PGD2 is placed in the oxyanion hole in the PGFS[NADPH + BMP] structure, and thus, the carbonyl group is reduced to a hydroxyl group to produce 9α,11β-PGF2α (PGF2α), but not 9α,11α-PGF2α (PGF2α). On the other hand, if PGE2 were to bind to the PGD2 binding site in the flipped mode to place carbonyl O9 in the oxyanion hole, the carbonyl group (C9=R) would be reduced to PGF2α. However, PGE2 is not reduced to PGF2α by PGFS, indicating that PGE2 and PGF2α do not bind to the PGD2 binding site in the flipped mode. Therefore, the catalytic activity of PGFS indicates that the “real” PGF2α binding site must be shifted from the PGD2 binding site (i.e., O9 or O11 is not in the oxyanion hole) and thus eliminates the model 2 and 3 structures. Although the cyclopropene moiety of BMP (PGF2α) in model 1 is shifted from that of PGD2, O9 and O1 are not pointed toward the nicotinamide ring of the bound NADPH, suggesting that the model 1 structure is not suitable for PGF2α production. Since the BMP binding site found in the PGFS[NADPH + BMP] structure is shifted from that of PGD2 but O9 is near C9 of the nicotinamide ring of the bound NADPH, it is reasonable to conclude that PGF2α and BMP bind to the same site in the same mode (flipped mode).

ACKNOWLEDGMENT

We express our thanks to Professor Richard H. Himes for a critical reading of the manuscript and very valuable comments.

REFERENCES

Prostaglandin F₂α Formation by Prostaglandin F Synthase


