Identification of CYP4F8 in Human Seminal Vesicles as a Prominent 19-Hydroxylase of Prostaglandin Endoperoxides*

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A novel cytochrome P450, CYP4F8, was recently cloned from human seminal vesicles. CYP4F8 was expressed in yeast. Recombinant CYP4F8 oxygenated arachidonic acid to (18R)-hydroxyarachidonate, whereas prostaglandin (PG) D2, PGE1, PGE2, PGF2α, and leukotriene B4 appeared to be poor substrates. Three stable PGH2 analogues, 9,11-epoxyethano-PGH2 (U-44069), 11,9-epoxyethano-PGH2 (U-46619), and 9,11-diazo-15-deoxy-PGH2 (U-51605) were rapidly metabolized by ω2- and ω3-hydroxylation. U-44069 was oxygenated with a V_{max} of –260 pmol min^{-1} pmol P450^{-1} and a K_{m} of ~7 μM. PGH2 decomposes mainly to PGE2 in buffer and to PGF2α by reduction with SnCl2. CYP4F8 metabolized PGH2 to 19-hydroxy-PGF2α, which decomposed to 19-hydroxy-PGE2 in buffer and could be reduced to 19-hydroxy-PGF2α with SnCl2. 18-Hydroxy metabolites were also formed (~17%). PGH2 was metabolized to 19- and 18-hydroxy-PGH2 in the same way. Microsomes of human seminal vesicles oxygenated arachidonate, U-44069, U-46619, U-51605, and PGF2α similar to CYP4F8. (19R)-Hydroxy-PGE2 and (19R)-hydroxy-PGE2 are the main prostaglandins of human seminal fluid. We propose that they are formed by CYP4F8-catalyzed ω2-hydroxylation of PGH2 and PGH2 in the seminal vesicles and isomerization to (19R)-hydroxy-PGE by PGE synthase. CYP4F8 is the first described hydroxylase with specificity and catalytic competence for prostaglandin endoperoxides.

Human and primate seminal fluid contains conspicuous amounts of prostaglandin (PG)E1, PGE2, (19R)-hydroxy-PGE1, and (19R)-hydroxy-PGE2 (1–4). PGs are formed by PGH synthase of seminal vesicles, but the mechanism of biosynthesis of (19R)-hydroxy-PGE has not been resolved. Many properties of a tentative PG 19-hydroxylase can be deduced from analysis of PG compounds in semen. First, (19R)-hydroxy-PGE1 and (19R)-hydroxy-PGE2 are equally abundant. 18-Hydroxy-PGE and small amounts of Δ18- and Δ19-PGE compounds are also present (5–7). The PG 19-hydroxylase will thus be expected to oxygenate C-18 and C-19, whereas the Δ18- and Δ19-PGE compounds might be formed by typical cytochrome P450-catalyzed desaturations (5–8). Second, semen analysis suggests that PG 19-hydroxylase must be closely linked to PGH synthase. The ratios of PGE1 to (19R)-hydroxy-PGE1, and PGE2 to (19R)-hydroxy-PGE2 change little in ejaculates obtained at long or short time intervals (4). The ratios are reproducible in each subject, but vary considerably between normal men (4, 8–9). A majority can be defined as “rapid” hydroxylators with PGE/(19R)-hydroxy-PGE ratios below 0.4 (8).

It seems likely that 19-hydroxylation of PGs is catalyzed by cytochrome P450 of seminal vesicles (4), but the enzyme has not been convincingly demonstrated. Microsomes of primate and human seminal vesicles and NADPH only slowly metabolized PGD2 to 19-hydroxy-PGE2, which differed from the biosynthesis in vivo (1–4). In contrast, microsomes of monkey seminal vesicles and NADPH rapidly metabolized arachidonic acid to (18R)-HETE (10). This left us with an enigmatic microsomal preparation, which rapidly catalyzed 18-hydroxylation of arachidonate and only slowly catalyzed 19-hydroxylation of PGD2.

What is the biological function of seminal PGs in humans? Little can be stated with absolute certainty. Seminal fluid contains an unprecedented high concentration of PGs. Exposure of sperm to PGs is not required for in vitro fertilization (11). It therefore seems likely that these PGs contribute to fertility by ensuring maximum efficiency in vivo, but the mechanism is unknown. PGs have a wide spectrum of biological effects mediated through activation of G-protein-coupled prostanoid receptors (12). Seminal PG compounds may have immunosuppressive actions in the female genital tract, induce tolerance to sperm antigens, promote sperm survival, and contribute to the acrosome reaction (3, 13, 14). Targeted disruption of PGH synthase and prostanoid receptor genes has shown that PGs are of physiological importance in rodent reproduction (12, 15, 16).

Knowledge of the biosynthesis of (19R)-hydroxy-PGE in male genital organs might provide tools to study their role in reproduction. In pursuit of the tentative PG 19-hydroxylase, we recently performed a systematic study of cytochrome P450 mRNA of human seminal vesicles (17). mRNA of a novel enzyme, CYP4F8, appeared to be abundantly expressed in all samples examined. The objective of the present study was to identify the function of this novel human cytochrome P450.

EXPERIMENTAL PROCEDURES

Materials—Arachidonic acid (99%), linoleic acid (99%), bis(trimethylsilyl) trifluoroacetamide, and (2S,3S)-(+)-phenylpropionic acid (97%) were from Sigma. (19R)-Hydroxy-PGE2, PGH2, and PGH2 were obtained from Cayman Chemical Co., Inc. (Ann Arbor, MI). PGH2 was also prepared as described (18), stored in acetone at ~80 °C, and checked for
Fig. 1. Analysis of 18-HETE formed from arachidonic acid by CYP4F8. A, LC-MS analysis with selective ion monitoring of two ions, m/z 319 for HETEs and epoxides of arachidonic acid and m/z 337 for dihydroxyeicosatrienoic acids. The trace shows the combined ion intensities. The main peak (marked 18-HETE) was identified by MS/MS (MS/MS 319 → full scan). The mobile phase was MeOH/H2O/ acetic acid (80:20:0.01). B, steric analysis of 18-HETE methyl ester by GC-MS after hydrogenation as the (2S)-phenylpropionic acid derivative (selective ion monitoring at m/z 325) (10).

Table 1

Hydroxylation of different substrates by CYP4F8

| Substrate | Product formation* |
|-----------|-------------------|
| U-44069   | 154 pmol min⁻¹ pmol P450⁻¹ |
| U-51605   | 104 pmol/min |
| PGH₂       | 23 pmol/min |
| U-46619   | 3 pmol/min |
| PGE₂⁻a    | <0.1 pmol/min |
| PGE₁⁻a    | ND |
| PGE₁⁻b    | ND |

* Formation of 19- and 18-hydroxy metabolites during incubations of CYP4F8 with 10 µM substrate was estimated by LC-MS.

by growing the yeast in adenine- and uracil-deficient medium. To achieve higher expression levels, the yeast cells were first grown to high density with glucose as the main energy source; thereafter, galac- tose was added to induce expression. In brief, the procedure was as follows. Transformed yeast cells were grown to a density of ~30 × 10⁶ cells/ml in S/G1 medium (containing per liter: casamino acids 1 g, yeast nitrogen base 7 g, glucose 20 g, tryptophan 20 mg). The cells were then diluted to ~2.5 × 10⁶ cells/ml and grown for 22–24 h in YPGE medium (containing per liter: yeast extract 10 g, bactopeptone 10 g, glucose 5 g, ethanol 16 g). Galactose (2%) was added, and the cells were harvested 16 h later by centrifugation (22). The cell walls were disrupted with glass beads, and the micromolar fraction was obtained by differential centrifugations at +4 °C (20,000 × g for 10 min and 100,000 × g for 60 min). The pelleted microsomes were homogenized in 0.05 M Tris-HCl, 20% glycerol, and 1 mM EDTA (pH 7.4) and stored at –80 °C. Cytochrome P450 and cytochrome P450 reductase were measured as described (22). Control microsomes were prepared from yeast transfected with the V60 plasmid without an insert. Protein was determined as described (22, 23).

Experimentation—Yeast microsomes (50 µg, ~2 pmol of CYP4F8) or microsomes of human seminal vesicles (1–2 mg/ml) were incubated with 1 µM NADPH and 5–200 µM substrate in a total volume of 100 µl of 0.1 M KHPO₄ and 2 mM EDTA (pH 7.4) for 45 s to 30 min at 37 °C. For GC-MS analysis, 25 pmol of CYP4F8 was used. Substrates were added in <1 µl of solvent. The reactions were terminated with 4 volumes of ethanol. Incubations with PGE₁ were also terminated by addition of buffered SnCl₂ (18). The metabolites were extracted on a Sep-Pak C₈ column with ~90% recovery (24).

Kinetics—CYP4F8 (3 pmol in 0.3 ml) was preincubated with 5, 10, 50, and 200 µM U-44069 in triplicate for 2 min at 37 °C. NADPH (1 mM) was added, and the reaction was stopped after 45 s. The biosynthesis of hydroxy metabolites of U-44069 was quantified by LC-MS analysis using selective ion monitoring of the carboxylate anion (m/z 235). 19-Hydroxy-PGB₂ was added as an internal standard in some experiments. Standard curves were constructed from known amounts of U-44069 (selective ion monitoring of the carboxylate anion at m/z 249). We assumed that U-44069 and its hydroxy metabolites yielded the same response, as the increase in the hydroxy metabolites corresponded to the decrease in U-44069. Vₘₐₓ and Kₘ were calculated according to the Lineweaver-Burk plot. The rate of biosynthesis of 19- and 18-hydroxy metabolites of endoperoxide analogues, PGD₂, PGE₁, PGE₁, PGE₂, PGF₁α, and PGH₁ by CYP4F8 (2 pmol; 1–5 min at 37 °C) was estimated at a fixed substrate concentration (10 µM). Metabolite formation was estimated by LC-MS using standard curves of parent compounds or by percent conversion of substrate.

LC-MS Analysis—Equipment for LC-MS analysis was as described (24). The column contained octadecasila silica (5 µm, 250 × 2 mm; Chromasil C₅, 100 Å, Phenomenex Inc., Torrance, CA) and was eluted at 0.2 ml/min. The mobile phase was CH₃OH/H₂O/acetic acid (80:20:0.01) for analysis of monohydroxy metabolites of arachidonic acid. Hydroxy-PGs and PGs were analyzed by three different systems: a system with a linear gradient from CH₃OH/H₂O/acetic acid (60:40:0.01) to 100% methanol in 23 min and two isocratic systems (CH₃OH/H₂O/acetic acid (80:40:0.01) and CH₃CN/H₂O/acetic acid (35:65:0.01)). The

The coding region of CYP4F8 was amplified with a sense primer (5’-TTGGGATCCAAAATGTCGCTGCTGAGC-3’) that contained a BamHI linker sequence, three A res- idues to increase the expression efficiency (21), and 23 base pairs of the translation start site of CYP4F8 cDNA. The antisense primer (5’-CTGACTATTCGCAGGGTTCTACGCG-3’) contained 19 base pairs of the end of the coding sequence, the stop codon, and an EcoRI linker sequence. Polymerase chain reaction was performed with Pfu polymerase using a full-length cDNA clone of CYP4F8 (17) as template with an annealing temperature of 60 °C. The polymerase chain reaction product was subcloned into the V60 yeast expression vector at the BamHI and EcoRI sites. The expression of CYP4F8 was carried out with the S. cerevisiae strain WIR, which has been genetically modified to also overexpress the yeast reductase (20). A galactose-inducible promoter in the plasmid and in the yeast genome, respectively, was used to control the expression. After transformation of the plasmid into the WIR yeast strain by a lithium acetate method, selection of clones was
FIG. 2. LC-MS analysis of hydroxy metabolites of U-44069 formed by recombinant CYP4F8. A, the metabolites were analyzed by selective ion monitoring of the carboxylate anions at m/z 365, and the ion intensity is shown. The mobile phase was CH₃OH/H₂O/acetic acid (60:40:0.01). The first eluting peak (marked 19-OH-U-44069) was found by both LC-MS and GC-MS to contain the 19-hydroxy metabolite of 9,11-epoxymethano-PGH₂, and the second peak (marked 18-OH-U-44069) was found to contain the 18-hydroxy metabolite. B, shown are the results of the biosynthesis of hydroxy metabolites of U-44069 at different substrate concentrations. Data are the means ± S.D. of triplicate determinations. The inset shows a Lineweaver-Burk plot of 1/[S (μM)] versus 1/V (pmol min⁻¹ pmol P450⁻¹).

RESULTS

Expression of CYP4F8 in Yeast—The yield of CYP4F8 was 26–62 pmol/mg of microsomal proteins, and the yield of cytochrome P450 reductase was >10-fold higher (430–850 pmol/mg). The reduced CYP4F8-CO complex had an absorption maximum at 449.6 nm. Control microsomes did not contain appreciable amounts of cytochrome P450 and did not metabolize any of the substrates discussed below.

Hydroxylation of Fatty Acids—Recombinant CYP4F8 and NADPH metabolized arachidonic acid to one major metabolite (Fig. 1A). This metabolite was identified as 18-HETE by LC-MS analysis. The MS/MS analysis of the carboxylate anion (m/z 319 → full scan) yielded a mass spectrum that was identical to that of authentic 18-HETE. Characteristic signals were noted at m/z 291 (319-58, loss of CH₃CH₂CHO) and 217 (261-44, loss of CO₂) (24). Steric analysis by GC-MS showed that peak I contained 19-hydroxy-PGF₁α, that peak II contained 19-hydroxy-PGE₁ (with trace amounts of 19-hydroxy-PGD₁ on the right shoulder), and that peak III contained 18-hydroxy-PGE₁, PGE₂α, PGE₂, and PGD₂ eluted as marked.

FIG. 3. LC-MS analysis of PG compounds formed during incubation of CYP4F8 with PGH₂. A, CYP4F8 was incubated with PGH₂, and its metabolites were then allowed to decompose in buffer, and formation of hydroxy-PGs and PGs was analyzed by LC-MS. MS/MS analysis suggested that peak I contained 19-hydroxy-PGF₁α, that peak II contained 19-hydroxy-PGE₁ (with trace amounts of 19-hydroxy-PGD₁, on the right shoulder), and that peak III contained 18-hydroxy-PGE₁, PGE₂α, PGE₂, and PGD₂ eluted as marked.

TABLE II

| Substrate | CYP4F8 | Seminal vesicles | Seminal fluid |
|-----------|--------|----------------|--------------|
| PGH₁ | 78 | 22 | 90 | 10 |
| PGH₂ | 83 | 17 | 92 | 8 |
| U-51605 | 56 | 44 | 55 | 45 |

* 19- and 18-hydroxy-PGE of human seminal fluid.
Hydroxylation of PGs and Leukotriene B<sub>4</sub>—Recombinant CYP4F8 was incubated with PGD<sub>2</sub>, PGE<sub>1</sub>, PGE<sub>2</sub>, PGF<sub>2</sub>α, and leukotriene B<sub>4</sub>, and the formation of hydroxylated metabolites was analyzed by LC-MS with MS/MS. These compounds were all poor substrates, and metabolites could not be identified in many experiments (Table I). However, using a high substrate concentration (100 μM) and long incubation times, small amounts of 19-hydroxy metabolites of PGE<sub>2</sub> and PGF<sub>2</sub>α could be identified by LC-MS analysis and by comparison with authentic standards. Cytochrome b<sub>5</sub> at the same concentration as CYP4F8 did not augment the oxygenation of PGE<sub>1</sub> and PGE<sub>2</sub>. Hydroxylation of PGH<sub>2</sub> Analouges—CYP4F8 was found by LC-MS to metabolize U-44069 to two hydroxy metabolites at a ratio of ~6:1 (Fig. 2A). LC-MS analysis was consistent with 19- and 18-hydroxy metabolites. GC-MS analysis of the trimethylsilyl ether methyl ester derivative of the main metabolite showed a mass spectrum at a C-value of 25.1 with a strong signal at m/z 117 (H<sub>3</sub>CH-CH-O<sup>-</sup>-Si(CH<sub>3</sub>)<sub>3</sub>). The C-value of the trimethylsilyl ether methyl ester derivative of U-44069 was 22.8. The difference in the C-values of the metabolite and the parent compound and the mass spectrum of the metabolite suggested that it was 19-hydroxy-(9,11-epoxymethano)-PGH<sub>2</sub> (25). The minor metabolite showed a mass spectrum with a strong signal at m/z 131 (H<sub>3</sub>CH-CH<sub>-</sub>-O<sup>-</sup>-Si(CH<sub>3</sub>)<sub>3</sub>) at a C-value of 24.9 and was identified as 18-hydroxy-(9,11-epoxymethano)-PGH<sub>2</sub> (25).

The hydroxylation of U-44069 at 37 °C was linear with time for at least 6 min and linear with protein (0.1–1.6 mg/ml). Cytochrome b<sub>5</sub> was without effect. K<sub>m</sub> and V<sub>max</sub> values were estimated by a Lineweaver-Burk plot. The apparent K<sub>m</sub> was ~7 μM, and the V<sub>max</sub> ~260 pmol min<sup>-1</sup> pmol P450<sup>-1</sup> (Fig. 2B).

CYP4F8 also efficiently metabolized U-46619 and U-51605 (Table I). LC-MS analysis suggested that the main products of U-46619 were 19- and 18-hydroxy metabolites (Table II). The structures were confirmed by GC-MS analysis of the trimethylsilyl ether methyl ester derivatives. The mass spectrum of the main metabolite showed a characteristic signal at m/z 117 and had a C-value of 25.6. The mass spectrum of the other metabolite showed a characteristic signal at m/z 131 and had a slightly smaller C-value of 25.4, whereas the C-value of U-46619 was 22.7. The two metabolites were thus identified as 19-hydroxy-(11,9-epoxymethano)-PGH<sub>2</sub> and 18-hydroxy-(11,9-epoxymethano)-PGH<sub>2</sub> (25). The latter was partially separated by HPLC into two equal components with identical MS/MS spectra, conceivably stereoisomers at C-18, but this was not further investigated. In U-51605, the endoperoxide oxygens are replaced by a N=N- group, and the hydroxy group at C-15 has been deleted. LC-MS with triple MS (m/z 347 → m/z 319 → full scan) suggested that CYP4F8 metabolized U-51605 to 19- and 18-hydroxy metabolites (Table II). U-51605 and its 19- and 18-hydroxy metabolites were too unstable for GC-MS analysis.

Hydroxylation of PGH<sub>1</sub> and PGH<sub>2</sub>—Recombinant CYP4F8 was incubated with 10 μM PGH<sub>2</sub> and 1 mM NADPH at 37 °C for 10 min, and the products were analyzed by LC-MS (Fig. 3). Under these conditions, PGH<sub>2</sub> will decompose in buffer to PGF<sub>2</sub>α, PGE<sub>2</sub>, and PGF<sub>2</sub>α, as shown in Fig. 3 and to (12S)-hydroxyheptadecatrienoic acid (data not shown) (18). Hydroxy metabolites of PGH<sub>2</sub> will decompose to hydroxy metabolites of PGs in the same way. The first eluting product (peak I) was identified as 19-hydroxy-PGF<sub>2</sub>α by its MS/MS spectrum (MS/MS 369 → full scan). Peak II mainly contained 19-hydroxy-PGF<sub>2</sub>α (MS/MS 367 → full scan), but trace amounts of 19-hydroxy-PG<sub>2</sub>α eluted on the right shoulder of the peak as judged by MS/MS analysis (due to the relative intensity at m/z 251). The formation of 19-hydroxy-PGF<sub>2</sub>α was confirmed by GC-MS analysis as described below. 18-Hydroxy-PG<sub>2</sub>α was identified in peak III by MS/MS analysis, which showed a characteristic signal at m/z 273 (loss of 2H<sub>2</sub>O and CH<sub>2</sub>CH=CHO). PGF<sub>2</sub>α, PGE<sub>2</sub>, and PGD<sub>2</sub> eluted after 18, 24, and 29 min, respectively.

The structure of the main hydroxy metabolite of PGH<sub>2</sub> was confirmed by GC-MS analysis. PGF<sub>2</sub>α compounds that were formed during incubation with PGH<sub>2</sub> and CYP4F8 were converted to PGF<sub>2</sub>α compounds by alkali treatment. A polar PGF<sub>2</sub>α metabolite was purified by HPLC and analyzed by GC-MS (trimethylsilyl ether methyl ester derivative). The mass spectrum and the C-value of 26.1 was identical to those reported for 19-hydroxy-PG<sub>2</sub>α (4, 5).

An experiment with chemical reduction of products confirmed that PGH<sub>2</sub> was metabolized to 19-hydroxy-PG<sub>H</sub><sub>2</sub>. PGH<sub>2</sub> was incubated with CYP4F8 for 2 min, and one-half of the incubation was terminated with buffered SnCl<sub>2</sub>, whereas the other half was terminated with ethanol. LC-MS analysis of the sample reduced with SnCl<sub>2</sub> showed two major peaks (Fig. 4A). 19-Hydroxy-PGF<sub>2</sub>α was present in peak I, and 19-hydroxy-PGF<sub>2</sub>α and 18-hydroxy-PGF<sub>2</sub>α were present in peak II. In contrast, the second sample yielded 19-hydroxy-PGE<sub>2</sub>α as the main product due to decomposition of 19-hydroxy-PHG<sub>2</sub> in aqueous ethanol (Fig. 4B). Small amounts of 18-hydroxy-PGE<sub>2</sub>α were present in peak III in both chromatograms (Fig. 4B).

PGH<sub>1</sub> was metabolized by CYP4F8 in the same way as PGH<sub>2</sub>. The main metabolites decomposed in buffer to 19-hy-
As shown in Table I, U-44069 and U-51605 appeared to be the best substrates of CYP4F8. PGH$_2$ was metabolized at a lower rate. However, PGH$_2$ is unstable, and our in vitro conditions may not mimic in vivo biosynthesis. It is noteworthy that PGH$_2$ and the stable endoperoxide analogues were metabolized more efficiently than PGE$_2$, PGF$_{2\alpha}$, PGD$_2$, and PGE$_1$.

**Human Seminal Fluid**—18-Hydroxy-PGE compounds in pooled seminal fluid ($n = 12$) were analyzed by LC-MS. 18-Hydroxy-PGE compounds were present in slightly lower relative amounts in seminal fluid compared with those formed by hydroxylation of PGH$_1$ and PGH$_2$ by CYP4F8 in vitro (Table II). 18-HETE could not be detected in these samples by LC-MS.

**Human Seminal Vesicles**—Microsomes of seminal vesicles ($n = 3$) and NADPH metabolized U-44069 to 19- and 18-hydroxy metabolites at a ratio of $\approx 6:1$ as judged by LC-MS analysis (Fig. 5A). U-44069, U-46619, and U-51605 were metabolized to 19- and 18-hydroxy metabolites in the same relative amounts as by CYP4F8 (Table II). To determine whether PGH$_2$ was metabolized to 19-hydroxy-PGH$_2$ by microsomes of seminal vesicles, we repeated the experiments described above with SnCl$_2$. Treatment with buffered SnCl$_2$ yielded significant formation of 19-hydroxy-PGF$_{2\alpha}$, whereas 19-hydroxy-PGE$_2$ was the main product formed in aqueous ethanol (Fig. 5B). Small amounts of 18-hydroxy-PGE$_2$ were detected in some experiments. The yield of 19-hydroxy metabolites of PGH$_2$ was rather poor. Microsomal fractions of seminal vesicles may contain PGE synthase and other PGH$_2$-metabolizing enzymes. For example, PGH$_2$ was also transformed to 6-keto-PGF$_{1\alpha}$ in some experiments. As previously reported, microsomes of human seminal vesicles only slowly convert PGE$_1$ and PGE$_2$ to their 19-hydroxy metabolites (4). Attempts to compare the biosynthesis of 19-hydroxy-PGE$_2$ from PGE$_2$ with the biosynthesis of 19-hydroxy-PGH$_2$ from PGH$_2$ were unsuccessful due to the low metabolism of PGE$_2$.

Arachidonic acid was metabolized to 18-HETE by CYP4F8 as described above, and we confirmed that microsomes of human seminal vesicles and NADPH also formed this metabolite (Fig. 5C). Small amounts of 15-HETE (retention time of 23 min) and 11-HETE (retention time of 25 min) were also identified by LC-MS, but these HETEs can also be formed by PGH synthase of seminal vesicles (27).

**DISCUSSION**

mRNA of a new enzyme, CYP4F8, was recently discovered in human seminal vesicles (17). We have expressed CYP4F8 in yeast and report as our main finding that recombinant CYP4F8...
metabolizes PGH₂ and PGH₃ to 19-hydroxy-PGH₂ and 19-hydroxy-PGH₃, respectively. Three stable PGH₂ analogues were also substrates, whereas PGG₂, PGE₂, and PGF₂α were metabolized poorly (Table I). Our results suggest that PGH₁ and PGH₂ are endogenous substrates for CYP4F8, which thus can be named PGH₁-19-hydroxylase. CYP4F8 is the first described hydroxylase with pronounced specificity for prostaglandin endoperoxides.

9,11-Epoxymethano-PGH₂ (U-44069) was the best substrate of recombinant CYP4F8. U-44069 was converted to 19- and 18-hydroxy metabolites at a ratio of 6–1, with a Vₘₐₓ of ~7 µM and a Vₘₐₓ of ~260 pmol min⁻¹ pmol P450⁻¹. This Vₘₐₓ value is remarkably high for a mammalian cytochrome P450 (20, 22).

Both microsomes and recombinant CYP4F8. Both metabolized U-44069, U-46619, U-51605, and arachidonic acid to virtually the same profile of 19- and 18-hydroxy metabolites (Table II). Both preparations also oxygenated PGH₂ to 19-hydroxy metabolites and to small amounts of 18-hydroxy metabolites. These observations suggest that CYP4F8 is present in seminal vesicles. Human and primate semen contains >95% of the 19R-stereoisomers of 19-hydroxy-PGs (1–4). It remains to be determined whether CYP4F8 oxidizes PGH₂ to the 19R-stereoisomer of 19-hydroxy-PG₂. However, we confirmed that CYP4F8 metabolizes arachidonate to 19-hydroxylase with pronounced specificity for prostaglandin endoperoxides.

In summary, our results suggest that CYP4F8 of human seminal vesicles catalyzes αζ-hydroxylation of PGH₁ and PGH₂, which will lead to biosynthesis of the two main PGs of human seminal fluid, (19R)-hydroxy-PGE₁, and (19R)-hydroxy-PG₂. CYP4F8 is the first described enzyme with both specificity and kinetic competence for hydroxylation of PGH₁ and PGH₂.

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