Biochemical and Biophysical Research Communications **261,** 169–174 (1999)
Article ID bbrc.1999.1011, available online at http://www.idealibrary.com on IDEAL®

Gene Expression of a Novel Cytochrome P450 of the CYP4F Subfamily in Human Seminal Vesicles

Johan Bylund,*[,1] Niklas Finnström,† and Ernst H. Oliw*
**Division of Biochemical Pharmacology, Department of Pharmaceutical Biosciences, Uppsala University; and*
†Division of Clinical Pharmacology, Department of Medical Sciences, University Hospital, Uppsala, Sweden

Received June 14, 1999

19*R*-Hydroxyprostaglandins are major components of human seminal fluid. They are apparently formed in the seminal vesicles by NADPH-dependent *ω*2-hydroxylation. The hydroxylase is likely a cytochrome P450 (CYP), which has not been identified. To address this issue we studied gene expression of CYPs in human seminal vesicles (*n* = 4) with reverse-transcription polymerase chain reaction (RT-PCR). CYP1B1, CYP2E1, CYP2J2, CYP3A5, CYP4B1, and CYP4B1 with insertion of three nucleotides (Ser207) were detected in all subjects. RT-PCR with degenerate primers for the CYP4 family yielded a novel cDNA sequence, which was derived from a previously reported genomic sequence on chromosome 19p13.1 and present in all subjects. cDNA cloning showed that the deduced amino acid sequence consisted of 520 amino acids. Northern blot analysis demonstrated mRNA transcripts of ~2.1 and ~2.3 kb. The deduced protein showed 81.2 and 76.7% amino acid identity with the human enzymes CYP4F2 and CYP4F3. The novel CYP was designated CYP4F8. **© 1999 Academic Press**

Key Words: **CYP4B1; CYP4F8; molecular cloning; prostaglandin 19-hydroxylase.**

Cytochrome P450 (CYP) enzymes are present in many tissues and can oxidize lipids of physiological importance, e.g., bile acids, vitamin D, polyunsaturated fatty acids, prostaglandins and leukotrienes. These physiologically important enzymes often show a strict substrate specificity and tissue distribution in contrast to the versatile drug metabolizing enzymes of the liver.

A specific and physiologically important hydroxylation of prostaglandins occur in the male genital tract in man and primates. Human and primate seminal fluid contain huge amounts of 19*R*-hydroxyprostaglandins (1, 2). These metabolites are likely formed from prostaglandins by an unidentified CYP in the seminal vesicle (3). It seems likely that prostaglandin 19-hydroxylase may be position specific as 20-hydroxyprostaglandins are undetectable and only small amounts of 18-hydroxyprostaglandins are present in human semen (4). Interestingly, the ratio of prostaglandins and 19-hydroxyprostaglandins varies in human semen, and this might be due to polymorphism (4). Microsomes of human and primate seminal vesicles and NADPH metabolize PGE to 19-hydroxy-PGE only slowly, which is in sharp contrast to the biosynthesis *in vivo* (3). In addition, primate microsomes have been showed to efficiently metabolize arachidonic acid to 18*R*-hydroxyarachidonate (5). Whether this reaction is catalyzed by the prostaglandin 19-hydroxylase is unknown.

Hydroxylation of arachidonic acid and prostaglandins have been reported with many enzymes of the CYP1-4 families (6–8). Of special interest is the CYP4 family, which contains prominent *ω*1- and *ω*2-hydroxylases of fatty acids, prostaglandins and leukotrienes (9). Some of these enzymes are extrahepatic. For example, CYP4A4 is abundantly expressed in the lung of pregnant rabbits and metabolizes PGE to 20-hydroxy-PGE (7), whereas CYP4F3 is expressed in human polymorphonuclear leukocytes and oxidizes LTB_4 to 20-hydroxy LTB_4 (10).

No systematic study of expression of CYPs in human seminal vesicles has been reported. The first objective of the present study was therefore to screen human seminal vesicles with RT-PCR for a series of known human CYPs belonging to the CYP1-4 families. The prostaglandin 19-hydroxylase might, however, be a novel enzyme with very restricted localization to the seminal vesicles. Our second objective was therefore to

[1] To whom correspondence should be addressed at Division of Biochemical Pharmacology, Uppsala Biomedical Center. P.O. Box 591, SE-751 24, Uppsala, Sweden. Fax: +46 18 55 29 36. E-mail: Johan.Bylund@farmbio.uu.se.

The sequence reported in this paper was designated CYP4F8 by the P450 nomenclature committee and has been deposited in the GenBank, Accession No. AF133298.

Abbreviations used: CYP, cytochrome P450; LC–MS, liquid chromatography–mass spectrometry; LTB_4, leukotriene B_4; PGE, prostaglandin E; RT-PCR, reverse-transcription polymerase chain reaction; UTR, untranslated region.

0006-291X/99 $30.00

use the method of degenerate primers, targeted to two conserved regions of the CYP4 family, in order to identify possible expression of new CYP4 enzymes. We report as our main finding gene expression of a novel CYP designated CYP4F8 in human seminal vesicles.

EXPERIMENTAL PROCEDURES

Materials. [^{35}S]dATP (1000 Ci/mmol), [^{33}P]ddNTPs (1500 Ci/mmol), [α-^{32}P]dCTP (3000 Ci/mmol), dNTP, DNA labeling beads, Nick columns, Hybond N nylon membranes, T7 Sequencing kit and Thermosequenase kit were from Amersham Pharmacia Biotech (Solna, Sweden). TA cloning kits were from Invitrogen (Groningen, The Netherlands). AmpliTaq DNA polymerase, RNase inhibitor and oligo d(T)$_{16-18}$ were from Perkin–Elmer (Norwalk, CT). Moloney murine leukemia virus reverse transcriptase and 3′-RACE kit were from Life Technologies (Gaithersburg, MD). Primers were from Life Technologies or from Amersham Pharmacia Biotech. Microsomes of human CYP, expressed in lymphoblast cells (CYP2E1) and insect cells (CYP1B1), were from Gentest Corp (Woburn, MA). PGE_2 and 19R-hydroxy-PGE_2 were from Cayman Chemical (Ann Arbor, MI).

Human tissues. Seminal vesicles ($n = 4$) were obtained at total prostatectomia for cancer surgery at the Karolinska Hospital (Stockholm) and at the University Hospital (Uppsala), and store at −80°C. Human blood was obtained from healthy volunteers. Pieces of human adult and fetal livers, prostate glands and placentas were obtained from the University Hospital (Uppsala), and stored in −80°C.

PCR primers. The PCR primers for CYP1A1, CYP1A2, CYP1B1, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CYP4B1 and β-actin have been described and evaluated by Finnström *et al.*[2] Primers used for amplification of CYP2J2, CYP4F2 and CYP4F3 were as follows: CYP2J2, upstream primer 5′-GAGATTGACAGAGTGATTGGC-3′, downstream primer 5′-GTCCTGGCCAACTGTTCTCC-3′ (expected sizes of amplicon was 352 bp); CYP4F2, upstream primer "S4F" (5′-GGTGTTGATGACTTCCTCCAA-3′), downstream primer 5′-CACAATGTCCTGGGTGACAT-3′ (380 bp); CYP4F3, upstream primer 5′-TGGGCTGATTCACAGCTCG-3′, downstream primer 5′-GTCAAAGCTGAAGACACATTTCTGT-3′ (445 bp). We confirmed with sequencing that these primers could amplify cDNA generated from human liver RNA (CYP2J2 and CYP4F2) and RNA of human white blood cells (CYP4F3). All primer pairs were design from their genomic sequences to span at least one intron. Degenerate upstream primer 5′-CTIMGIGCIGARGYIGAYAC-3′ and downstream primer 5′-TKICCIATGCARTTCCKIGVYCC-3′ were designed based on conserved regions of 18 human, rat and rabbit cDNA sequences of the CYP4A, CYP4B and CYP4F subfamilies. These primers were expected to amplify a 451-bp fragment of a CYP4A subfamily, a 457-bp fragment of a CYP4B subfamily, and a 463-bp fragment of a CYP4F subfamily. Primers used for amplification of full coding cDNA of CYP4B1 were upstream primer 5′-TGAAGGCTAGGTGGCTGGAAC-3′ and downstream primer 5′-ATGGTAATGCCTACTTCCCTTC-3′ (1702 bp), whereas primers for amplification of full coding cDNA of CYP4F8 were upstream primer 5′-GTTGTGCGGGACAACCTTCTCCTGA-3′ and downstream primer 5′-AGGTAGGTGATGCAAAGGTAGGTGG-3′ (1639 bp). Specific primers used for RT-PCR analysis of CYP4F8 were upstream primer "S4F" and downstream primer 5′-GTGATGGATTGCGAAGATGT-3′ (445 bp).

RT-PCR. Total RNA was extracted from human seminal vesicles and white blood cells using Tri-Reagent (Sigma, St. Louis, MO) and RNeasy blood mini kit (Qiagen GmbH, Hilden, Germany), respectively, according to the manufacturers' instructions. Total RNA from human liver, prostate glands and placentas were isolated using the method of Chomczynski and Sacchi (11). The quality of the total RNA was verified by electrophoreses in agarose/formaldehyde gels. First strand cDNA was usually synthesized for 1 h at 37°C in a reaction mixture (100 μl) containing 40 μg total RNA, 1000 U reverse transcriptase, 2.5 μM oligo-dT$_{16-18}$, 1 mM dithiothreitol, 1 mM dNTP, 40 U RNase inhibitor in reverse transcriptase buffer. PCR were performed with 1 μl of the reverse transcription reaction, 10 pmol of each primer, 1.3 units of AmpliTaq DNA polymerase in a total volume of 50 μl (reaction buffer: 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTP). The quality of the cDNA preparations was verified with amplification of β-actin as a positive control. Negative controls were performed with total RNA as templates. Most PCRs were run as follows: 94°C for 2 min followed by 15–35 cycles (94°C, 45 s; 53°C, 45 s; 72°C, 60 s), and a final extension step (72°C, 10 min). PCR with degenerate primers was run as follows: 94°C for 3 min followed by 5 cycles (94°C, 2 min; 45°C, 2 min; 72°C, 2 min) and 30 cycles (94°C, 2 min; 48°C, 2 min; 72°C, 2 min) and a final extension step (72°C, 10 min). PCR for amplification of the full coding-regions of CYP4B1 and CYP4F8 were run as follows: 94°C for 3 min followed by 30 cycles (94°C, 1 min; 53°C, 1 min; 72°C, 2:30 min) and a final extension step (72°C, 10 min). PCR products were analyzed on 1.5% agarose gels, stained with ethidium bromide, and pictures were recorded with a gel camera (ImageStore 7500, Ultra violet products LTD, Cambridge, UK).

Cloning and sequencing. The PCR products were ligated with T4 DNA ligase or topoisomerase I into the TA cloning vectors pCR2.1 or pCR2.1 TOPO. Plasmid DNA was isolated from mini preparations by the alkaline lysis method (12). Sequencing of isolated plasmids and PCR products were performed with T7 DNA polymerase and [^{35}S]dATP or with cycle sequencing using ^{33}P-labeled ddNTPs.

Northern blot analysis. Ten micrograms of total RNA from two seminal vesicles was electrophoresed in a 1.0% agarose/formaldehyde gel and transferred to a nylon membrane. A 375-bp probe [nucleotides 168–542 of the CYP4F8 sequence (Fig. 3)] was labeled with the random primer method [DNA labeling beads (−dCTP)]. The labeled probe was purified with Nick columns and hybridized in QuikHyb solution (Stratagene, La Jolla, CA) at 68°C overnight. The membrane was washed, finally in 0.1× SSC/0.1% SDS at 68°C for 60 min and exposed to X-ray film.

3′-RACE. Five micrograms of total RNA isolated from seminal vesicles was transcribed according to the manufacturers' instructions. PCR was first performed with the upstream primer 5′-ATCCCCCAATCCCTACATT-3′ and a downstream specific adapter primer. The amplified products were separated on a agarose gel and purified using gel extraction kit (Qiagen). The PCR products were then reamplified with upstream primer 5′-CCAGAAGAGGTCACCTATGGCT-3′ and the same downstream primer.

Enzyme assay. 50 pmol CYP2E1 and 50 pmol CYP1B1 were incubated with 30 μM PGE_2 and 1 mM NADPH in 50 mM Tris–HCl (pH 7.4)/150 mM KCl/10 mM $MgCl_2$ at 37°C for 120 and 30 min, respectively. The samples were extracted and analyzed by LC-MS with MS/MS essentially as described (8). 19*R*-hydroxy PGE_2 was used as a reference.

Homology search. The BLAST algorithm was used for GenBank analysis (13), whereas multiple alignments were performed with the GCG program (SeqWeb version 1.0, Wisconsin Package).

RESULTS

RT-PCR screening of CYPs. Isozyme-specific primers for CYP1A1, CYP1A2, CYP1B1, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CYP4B1, CYP4F2 and CYP4F3 were used for

[2] N. Finnström, C. Bjelfman, T. Söderström, G. Smith, C. R. Wolf, and A. Rane. Expression of cytochrome P450 genes in prostate and liver as assessed by multiplex RT-PCR (submitted for publication).

RT-PCR screening of four human seminal vesicle. PCR products of the expected size were generated with primers for CYP1B1, CYP2E1, CYP2J2, CYP3A5 and CYP4B1 in all four seminal vesicles after 30 cycles as shown in Fig. 1. The PCR products were confirmed by sequencing. The other CYPs were not detected except CYP1A1 in one of the seminal vesicles. When the amplification was increased to 35 cycles, all the investigated CYPs except CYP2C9, CYP2C19 and CYP4F2 were detected in at least one sample.

All of the four amplicons of CYP4B1 consisted of a mixture of CYP4B1 and a CYP4B1 isoform with an insert of three nucleotides (AGC), which coded for Ser in position 207. This form was designated CYP4B1Ser207. To detect other possible changes, we amplified and sequenced the full coding-region of CYP4B1Ser207 and compared its sequence with CYP4B1 (14, 15). Only minor differences were noted. The coding region in CYP4B1Ser207 contained a putative Gln (CAG) in position 37, whereas one of the published sequences (14) contained Arg (CGA) in the same position. In the 3′-UTR (96–104 bp downstream from the stop codon), the sequence of CYP4B1Ser207 was in agreement with only one of the published sequences (14).

The CYPs detected in the seminal vesicles might conceivably originate from white blood cells. RT-PCR of two samples of human white blood cells revealed CYP1B1, CYP2E1 and CYP3A5 mRNA species, whereas CYP4B1 could not be detected, as previously been reported (16–18). In addition, we detected mRNA species of CYP2J2 in white blood cells. We conclude that all four human seminal vesicles contain mRNAs of CYP1B1, CYP2E1, CYP2J2, CYP3A5, CYP4B1, and CYP4B1Ser207, but some of them might originate from white blood cells in the tissue.

Recombinant human CYP2E1 and CYP1B1 were assayed for PGE_2 19-hydroxylase activity, but biosynthesis of 19-hydroxy-PGE_2 by these enzymes could not be detected by LC–MS.

RT-PCR with degenerate primers for the CYP4 family. RT-PCR with degenerate primers yielded a major band (~450 bp) which was of the expected length of a CYP4 family fragment (Fig. 2). The PCR band was cloned and sequenced. Sequence analysis revealed that it was identical to five consecutive regions of a human genomic clone, submitted to GenBank by Lamerdin *et al.*[3] It therefore seemed likely that this gene was expressed in human seminal vesicles. A comparison showed that this region of the genomic clone was related to the genomic sequences of CYP4F2[4] and CYP4F3 (19) as discussed below.

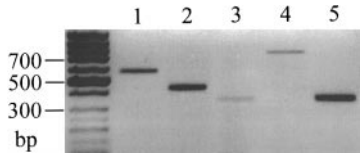

FIG. 1. RT-PCR analysis of a human seminal vesicle with isozyme-specific primers. Left lane, DNA size markers. Lane 1, CYP4B1. Lane 2, CYP3A5. Lane 3, CYP2J2. Lane 4, CYP2E1. Lane 5, CYP1B1.

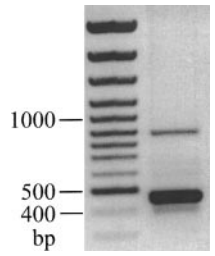

FIG. 2. RT-PCR analysis of a seminal vesicle with degenerate primers targeted to two conserved regions of the CYP4 family. The expected sizes of PCR fragments of members of the CYP4 family were 451–463 bp. Left lane, DNA size markers. Right lane, RT-PCR with degenerate primers.

To amplify the full coding-region of the cDNA, we designed primers based on the genomic sequence and on the exon-intron borders of the CYP4F2 (20) and CYP4F3 (19) genes. With these primers we obtained a cDNA of 1639 bp, which contained an open reading frame of 1560 bp. The nucleotide and the deduced amino acid sequences are shown in Fig. 3. The deduced protein was clearly related to the CYP4F subfamily and designated CYP4F8. Alignments showed that CYP4F8 had 81.2% amino acid identity with CYP4F2 and 76.7% with CYP4F3 (20, 10) (Fig. 3). The proximal heme ligand Cys^{468} was found in a typical context (21). Only two differences between the cDNA and the corresponding reported sequence were found. Most important, the cDNA contained an additional C at position 451. In addition, the cDNA contained a C instead of an A in position 1341 (Fig. 3). Those changes were confirmed with sequence analysis of amplicons generated from two different seminal vesicles.

Northern blot analysis and 3′-RACE. Northern blot analysis of the expression of CYP4F8 mRNA was performed in two different seminal vesicles. mRNA transcripts of approximately 2.1 and 2.3 kb were detected (Fig. 4). 3′-RACE was used to identify the polyadenylation sites of the CYP4F8 gene. Sequencing of amplified products generated in 3′-RACE revealed two polyadenylation sites. The poly A tails were added to nucleotides 1657 and 1840, respectively (Fig. 3). They were proceeded by polyadenylation signals, AATAAA and AGTAAA, respectively.

[3] J. E. Lamerdin, S. A. Stilwagen, M. H. Ramirez, P. McCready, H. S. Hoffman, H. Mohrenweiser, and A. V. Carrano. (1997) Identification of a CYP4F gene family at the D19S11 locus on 19p13.1, GenBank Accession No. AD000685.

[4] Genomic clone containing the CYP4F2 gene, GenBank Accession No. AC005336.

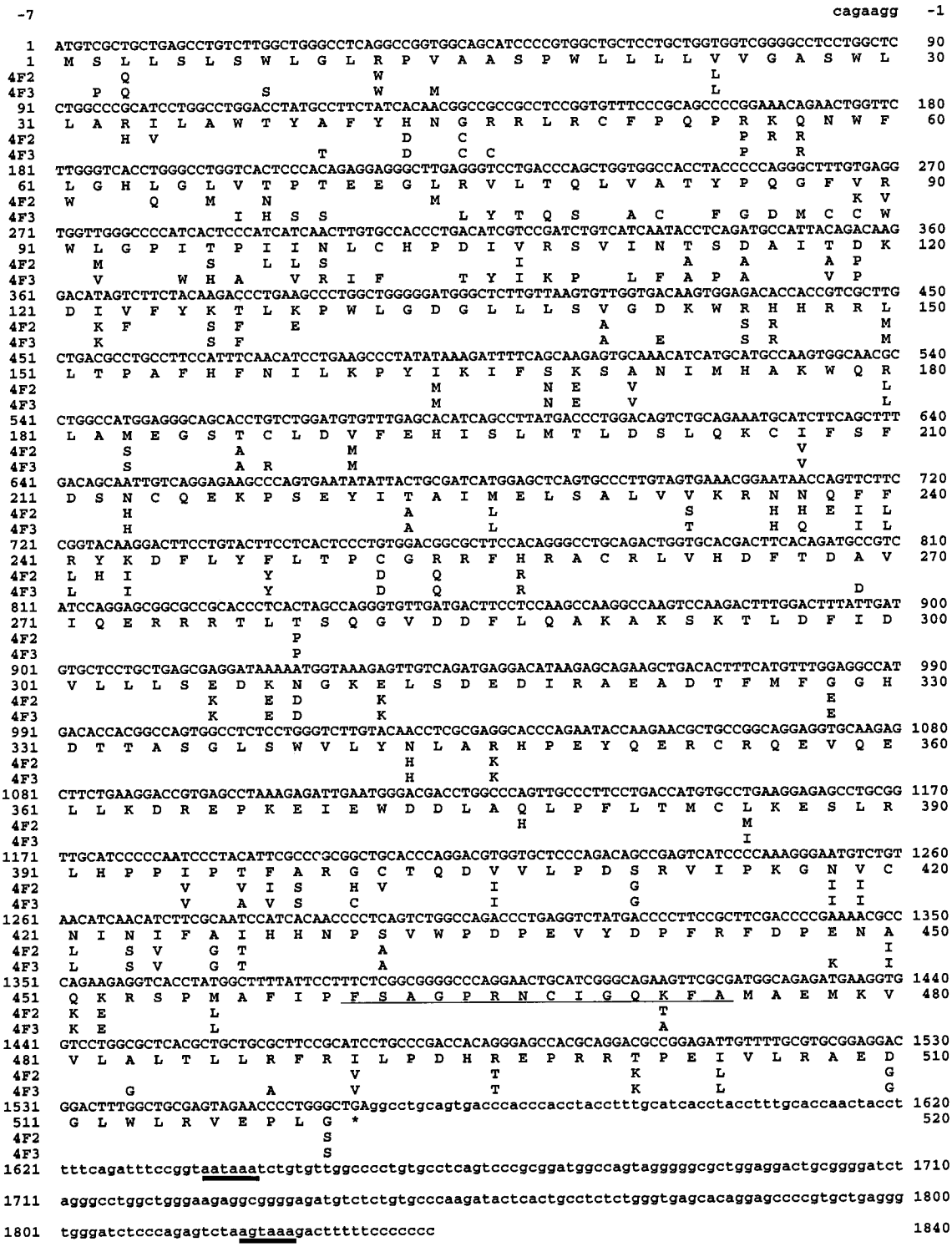

FIG. 3. Nucleotide sequence of CYP4F8 cDNA and alignment of the deduced amino acid sequences of CYP4F8, CYP4F2 and CYP4F3. The whole amino acid sequence of CYP4F8 is shown and only the amino acids of CYP4F2 and CYP4F3 which differ from those of CYP4F8 are shown. All three human enzymes have cDNA coding for 520 amino acids. The stop codon is indicated by an asterisk and the hem-binding region (amino acids 461–474) of CYP4F8 is underlined. The two polyadenylation signals are underlined in boldface.

RT-PCR of CYP4F8. CYP4F8 was detected by RT-PCR analysis in all four human seminal vesicles. We compared the expression of CYP4B1, CYP4F8 and β-actin in two seminal vesicles with RT-PCR after 15, 20, 25, 30, and 35 cycles. CYP4F8 and β-actin amplicons were detectable after 20 cycles, whereas the

CYP4B1 amplicon needed 30 cycles to be detectable (data not shown). These RT-PCR data should be interpreted with caution but they suggest that CYP4F8 mRNA might be more abundant than CYP4B1 mRNA in seminal vesicles.

RT-PCR of CYP4F8 was also performed with samples from adult liver (n = 6), fetal liver (n = 3), placenta (n = 2), white blood cells (n = 2) and prostate glands (n = 3). Amplification for 30 cycles did not generate any detectable products, whereas 35 cycles yielded amplicons of CYP4F8 in all samples of the prostate glands and in adult and fetal livers (data not shown). The identity of the amplified fragments were confirmed by sequencing. We concluded that these two tissues should be further analyzed for expression of CYP4F8.

DISCUSSION

The present investigation is the first systematic study of CYPs in human seminal vesicles. Our main finding is gene expression of a novel CYP, which was designated CYP4F8. The deduced amino acid sequence of CYP4F8 was similar to the sequences of CYP4F2 and CYP4F3 with 81.2 and 76.7% amino acid identities, respectively. mRNA of CYP4F8 was demonstrated by RT-PCR and by Northern blot analysis. CYP4F8 could not be detected with RT-PCR in human white blood cells, which suggests that CYP4F8 originates from the seminal vesicles. RT-PCR analysis also indicated that mRNA of CYP4F8 is expressed in human prostate glands and human liver. However, the higher number of cycles needed to generate positive detection of CYP4F8 in these tissues suggests lower mRNA levels compared to the seminal vesicles.

The CYP4F subfamily was discovered by Chen and Hardwick in rat hepatic tumors (22). Additional CYP4F enzymes were soon found in rats and humans (9). The two human enzymes were designated CYP4F2 and CYP4F3 (10, 20). CYP4F2 is present in liver and kidney, whereas CYP4F3 occurs in human polymorphonuclear leukocytes. CYP4F2 and CYP4F3 catalyze ω-hydroxylation of LTB_4 with an apparent K_m of 45 μM and 0.71 μM, respectively (10, 20).

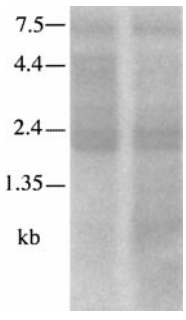

FIG. 4. Northern blot analysis of CYP4F8 in two seminal vesicles. A 375-bp cDNA sequence of CYP4F8 was labeled and used as a probe for hybridization. The numbers on the left indicate sizes of RNA markers. Two transcripts of about 2.1 and 2.3 kb were detected.

Exone/size (bp)	Introne/size (kb)	Intron borders
1 (ND)	CAGAAGgtgcca...I(0.34)....ctgcagGATGTC	-2/-1
2 (199)	GGCCTGgtgagt...II(2.2)....ttgcagGTCACT	198/199
3 (145)	CCTCAGgtactc...III(1.3)...tgtcagATGCCA	343/344
4 (54)	GGCTGGgtaagt...IV(0.09)...tactagGGGATG	397/398
5 (128)	ATGCATgtgagt...V(2.5).....ggccagGCCAAG	525/526
6 (122)	TCAGGAgtgagt...VI(0.77)...ctgcagGAAGCC	647/648
7 (271)	AGCGAGgtgggc...VII(0.32)..ctccagGATAAA	918/919
8 (67)	TTGGAGgtgagt...VIII(0.22).cctcagGCCATG	985/986
9 (130)	TGAATGgtgagt...IX(4.2)....ccttagGGACGA	1115/1116
10 (134)	CCAAAGgtgccc...X(0.09)....cgacagGGAATG	1249/1250
11 (65)	CCTGAGgtgctg...XI(0.17)...ccccagGTCTAT	1314/1315
12 (83)	GCCCAGgtgagg...XII(0.34)..ccccagGAACTG	1397/1398
13 (260)	...CGGT**AATAAA**TCTGTGTTGGCCCCTG	
13 (443)	...GTCTA**AGTAAA**GACTTTTTCCCCCCC	

FIG. 5. Exon–intron boundaries of the CYP4F8 gene. Alignment of the CYP4F8 cDNA and the genomic sequence of CYP4F8 revealed 13 exons (capital letters) and 12 introns (lowercase letters). The right column shows the intron-exon borders with nucleotide numbering from Fig. 3. Two polyadenylation signals in exon 13 are showed in boldface. ND, not determined.

We found by search of the GenBank (13) that the entire gene of CYP4F8 was present in a genomic clone,[3] which was derived from chromosome 19 (19p13.1). The CYP4F2, CYP4F3 genes and some other related genes have also been identified to this region.[5] Alignment of CYP4F8 cDNA with the CYP4F8 gene indicated 13 exons and 12 introns (Fig. 5). The CYP4F8 gene seemed to lack a TATA box in its 5′-UTR. The intron-exon borders were identical with the genes of CYP4F2 and CYP4F3. 3′-RACE revealed two polyadenylation signals located 73 and 257 nucleotides downstream from the stop codon (Figs. 3 and 5).

A physiologically relevant prostaglandin 19-hydroxylase must fulfill at least three criteria. First, the enzyme must form 19*R*-hydroxyprostaglandins as the main product (4). Second, the enzyme must be abundantly expressed in seminal vesicles. Third, its catalytic activity must be high in order to be efficiently linked to the prominent prostaglandin biosynthesis *in vivo* (1–4).

Seven CYPs may fulfill the second criterion to some extent. In addition to CYP4F8, we found gene expression of CYP1B1, CYP2E1, CYP2J2, CYP3A5, CYP4B1, and CYP4B1Ser207 in all investigated samples. As regards the first criterion we found that CYP1B1 and CYP2E1 lacked PGE_2 19-hydroxylase activity. Whether any of the other human enzymes can form 19*R*-hydroxyprostaglandins is unknown. CYP2J2 and CYP3A5 have been shown to oxidize arachidonic acid (23, 24). The catalytic activity of CYP4B1 is unclear (25) and

[5] Nelson lab home page (http://drnelson.utmem.edu/nelsonhomepage.html).

the catalytic activity of CYP4B1Ser207 remains to be determined.

Seminal vesicles have a remarkably high capacity to form prostaglandins (1–4). The fact that seminal prostaglandins are metabolized to 19-hydroxyprostaglandins *in vivo* suggests that prostaglandin 19-hydroxylase and its mRNA might be abundant. RT-PCR analysis of CYP4F8 was positive after only 20 PCR cycles, which suggests that CYP4F8 could be highly expressed in human seminal vesicles. It will be of interest to determine whether CYP4F8 can hydroxylate prostaglandins or arachidonic acid. Studies on the expression of CYP4F8 and its catalytic properties are now in progress.

ACKNOWLEDGMENTS

This study was supported by the Swedish Medical Research Council (06523 and 04496). We thank Dr. A. Rane for valuable suggestions.

REFERENCES

1. Taylor, P. L., and Kelly, R. W. (1974) *Nature* **250,** 665–667.
2. Kelly, R. W. (1997) *Prostaglandins Leukot. Essent. Fatty Acids* **57,** 113–118.
3. Oliw, E. H., Kinn, A. C., and Kvist, U. (1988) *J. Biol. Chem.* **263,** 7222–7227.
4. Oliw, E. H., and Johnsen, Ø. (1988) *Biochim. Biophys. Acta* **963,** 295–301.
5. Oliw, E. H. (1989) *J. Biol. Chem.* **264,** 17845–17853.
6. Capdevila, J. H., Zeldin, D., Makita, K., Karara, A., and Falck, J. R. (1995) *in* Cytochrome P450: Structure, Mechanism, and Biochemistry (Ortiz de Montellano, P. R., Ed.), 2nd ed., pp. 443–471, Plenum, New York.
7. Williams, D. E., Hale, S. E., Okita, R. T., and Masters, B. S. S. (1984) *J. Biol. Chem.* **259,** 14600–14608.
8. Bylund, J., Ericsson, J., and Oliw, E. H. (1998) *Anal. Biochem.* **265,** 55–68.
9. Simpson, A. E., (1997) *Gen. Pharmacol.* **28,** 351–359.
10. Kikuta, Y., Kusunose, E., Endo, K., Yamamoto, S., Sogawa, K., Fujii-Kuriyama, Y., and Kusunose, M. (1993) *J. Biol. Chem.* **268,** 9376–9380.
11. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
12. Davis, L., Kuehl, M., and Battey, J. (1994) *in* Basic Methods in Molecular Biology, 2nd ed., pp. 1–777, Appleton & Lang, Norwalk, CT.
13. Benson, D. A., Boguski, M. S., Lipman, D. J., Ostell, J., Quellette, B. F. F., Rapp, B. A., and Wheeler, D. L. (1999) *Nucleic Acids Res.* **27,** 12–17.
14. Nhamburo, P. T., Gonzalez, F. J., McBride, O. W., Gelboin, H. V., and Kimura, S. (1989) *Biochemistry* **28,** 8060–8066.
15. Yokotani, N., Sogawa, K., Matsubara, S., Gotoh, O., Kusunose, E., Kusunose, M., and Fujii-Kuriyama, Y. (1990) *Eur. J. Biochem.* **187,** 23–29.
16. Baron, J. M., Zwadlo-Klarwasser, G., Jugert, F., Hamann W., Rübben, A., Mukhtar, H., and Merk, H. F. (1998) *Biochem. Pharmacol.* **56,** 1105–1110.
17. Raucy, J. L., Schultz, E. D., Wester, M. R., Arora, S., Johnston, D. E., Omdahl, J. L., and Carpenter, S. P. (1997) *Drug Metab. Dispos.* **25,** 1429–1435.
18. Janardan, S. K., Lown, K. S., Schmiedlin-Ren, P., Thummel, K. E., and Watkins, P. B. (1996) *Pharmacogenetics* **6,** 379–385.
19. Kikuta, Y., Kato, M., Yamashita, Y., Miyauchi, Y., Tanaka, K., Kamada, N., and Kusunose, M. (1998) *DNA Cell Biol.* **17,** 221–230.
20. Kikuta, Y., Kusunose, E., Kondo, T., Yamamoto, S., Kinoshita, H., and Kusunose, M. (1994) *FEBS Lett.* **348,** 70–74.
21. Graham-Lorence, S. E., and Peterson, J. A. (1996) *Methods Enzymol.* **272,** 315–326.
22. Chen, L., and Hardwick, J. P. (1993) *Arch. Biochem. Biophys.* **300,** 18–23.
23. Wu, S., Moomaw, C. R., Tomer, K. B., Falck, J. R., and Zeldin, D. C. (1996) *J. Biol. Chem.* **271,** 3460–3468.
24. Rifkind, A. B., Lee, C., Chang, T. K. H., and Waxman, D. J. (1995) *Arch. Biochem. Biophys.* **320,** 380–389.
25. Zheng, Y. M., Fisher, M. B., Yokotani, N., Fujii-Kuriyama, Y., and Rettie, A. E. (1998) *Biochemistry* **37,** 12847–12851.