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# Overexpression of Human Prostaglandin G/H Synthase-1 and -2 by Recombinant Vaccinia Virus: Inhibition by Nonsteroidal Anti-inflammatory Drugs and Biosynthesis of 15-Hydroxyeicosatetraenoic Acid

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**SUMMARY**

Human prostaglandin G/H synthase (hPGHS)-1 and hPGHS-2, key enzymes in the formation of prostanoids from arachidonic acid, were expressed at high levels in COS-7 cells using a T7 RNA polymerase/vaccinia virus expression system. The open reading frame of hPGHS-2 cloned into vaccinia virus without its natural 5′ and 3′ untranslated regions directed only low levels of hPGHS-2 enzyme activity in COS-7 cells. High-level hPGHS-2 expression was achieved by appending the 3′ untranslated region of hPGHS-1 to the hPGHS-2 open reading frame, with subsequent expression of the hybrid mRNA using vaccinia virus. Enzymatically active recombinant hPGHS-1 and hPGHS-2 were present as glycosylated proteins in the microsomal fraction prepared from infected cells, whereas recombinant hPGHS-1 and hPGHS-2 prepared from the microsomal fraction of cells treated with tunicamycin, an inhibitor of *N*-linked glycosylation, were enzymatically inactive. The major prostanoid products formed by microsomes from COS-7 cells containing either recombinant hPGHS-1 or hPGHS-2 after incubation with arachidonic acid were prostaglandins $D_2$ and $E_2$, with lower levels of prostaglandin $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$. A range of potencies were observed for various nonsteroidal anti-inflammatory drugs as inhibitors of prostaglandin $E_2$ synthesis by hPGHS-1 and hPGHS-2. Recombinant hPGHS-1 and hPGHS-2 both produced 15- and 11-hydroxyeicosatetraenoic acid (HETE) from arachidonic acid, with 15-HETE production by hPGHS-2 being stimulated 5-fold by preincubation with aspirin. Chiral phase high performance liquid chromatography analysis showed that aspirin-treated hPGHS-2 produced 15(*R*)-HETE, with no detectable 15(*S*)-HETE.

PGHS (EC 1.14.99.1) catalyzes the conversion of arachidonic acid to $PGH_2$, the common precursor for the formation of prostanoids (1, 2), which can act as mediators of numerous biological responses through their interaction with prostanoid receptors. NSAIDs exert most of their anti-inflammatory, analgesic, and antipyretic activity through inhibition of PGHS. Until recently only one form of PGHS, originally isolated from ovine seminal vesicles and designated PGHS-1, had been characterized (3–5). More recently the gene and cDNA sequences for a second form of PGHS (PGHS-2) have been cloned, sequenced, and characterized from chicken, mouse, and human sources (6–11). In a variety of cell types, PGHS-2 is rapidly and readily inducible by a number of agents, including mitogens, endotoxin, hormones, cytokines, and growth factors (6, 9, 10, 12–14). This induction of hPGHS-2 may play a central role in mediating the rapid onset of the inflammatory response, whereas the constitutive expression of hPGHS-1 in most cell types may provide prostanoids for the normal physiology of cells and tissues. Recently, DeWitt and co-workers (15) reported that murine PGHS-1 and PGHS-2 are differentially inhibited by certain NSAIDs. Selective and potent inhibitors of the two forms of PGHS would aid in the evaluation of the contributions of these enzymes to physiological and pathological conditions.

To study the characteristics of PGHS-1 and PGHS-2, it is critical to establish a source of the individual enzymes. To date, cloned PGHS-1 and PGHS-2 from human, mouse, and sheep sources have been expressed in limited amounts by transient expression in transfected mammalian cell lines (6, 7, 10, 15–17). The baculovirus system has been used to overexpress

**ABBREVIATIONS:** PGHS, prostaglandin G/H synthase; DNBPG, (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-phenylglycine; GC, gas chromatography; hPGHS, human prostaglandin G/H synthase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HETE, hydroxyeicosatetraenoic acid; MS, mass spectrometry; NSAID, nonsteroidal anti-inflammatory drug; ORF, open reading frame; PCR, polymerase chain reaction; PG, prostaglandin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; UTR, untranslated region; VV, vaccinia virus; TK, thymidine kinase; HPLC, high performance liquid chromatography.

murine PGHS-2, resulting in the production of a 68-kDa protein, which corresponds to the molecular mass of the nonglycosylated mature form of PGHS-2 (18). Expression of sheep PGHS-1 in Sf9 insect cells using a baculovirus system yielded high-level expression of a largely nonglycosylated protein with a lower specific activity than that of PGHS-1 transiently expressed in COS cells (19). This suggests that post-translational glycosylation of PGHS is essential in obtaining high specific activity enzyme. The VV expression system is widely used to produce large amounts of proteins that undergo correct post-translational modifications, in a broad range of mammalian host cells (20–22). Therefore, we have used this system for the high-level expression of hPGHS-1 and hPGHS-2, and an initial pharmacological profile of the two enzymes is presented.

## Materials and Methods

**Growth and maintenance of cell lines.** COS-7 and 143B TK$^-$ cell lines were obtained from the American Type Culture Collection (American Type Culture Collection numbers 1651 and CRL8303, respectively) and grown as monolayer cultures in a humidified environment of 6% $CO_2$ at 37°, in Dulbecco's modified Eagle's medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum, 10 mM glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin, 100 μg/ml gentamicin (GIBCO-BRL, Missassauga, Ontario, Canada), and 25 mM HEPES, pH 7.4. For 143B TK$^-$ cells, 25 μg/ml 5-bromo-2′-deoxyuridine was also added to the culture medium.

**Construction of recombinant VV transfer vectors.** The hPGHS-1 cDNA (16) was a gift from Dr. C. Funk (Vanderbilt University, Nashville, TN). VV vectors (20, 22) were obtained from Dr. B. Moss (National Institutes of Health, Bethesda, MD). The hPGHS-1 cDNA insert was isolated from plasmid pcDNA1::hPGHS-1 (16) as a 2.3-kilobase *Bam*HI-*Eco*RV fragment and was ligated into the *Bam*HI and *Stu*I sites of the multiple cloning site of the VV transfer vector pTM1 (22) to create the plasmid pTM1-hPGHS-1.

The ORF of hPGHS-2 was generated by reverse transcriptase-PCR of RNA isolated from human osteosarcoma 143B cells (American Type Culture Collection number CRL 8303), using oligonucleotides based on the published sequence (6). The hPGHS-2 reverse transcriptase-PCR product was subcloned into the *Eco*RV site of plasmid vector pKS (Stratagene, La Jolla, CA) and designated pKS::hPGHS-2orf (data not shown). A VV transfer vector containing hPGHS-2, designated pTM1-hPGHS-2-orf, was constructed by replacing the ATG methionine start codon of the hPGHS-2 cDNA with the ATG methionine start codon encoded within the *Nco*I restriction site in the VV transfer vector pTM1 (22), using the following complementary synthetic oligonucleotides: hPGHS-2–148, 5′-CATGCTCGCCCGCGCCCTGCTGCTGTG CGCGGTCCTGGCGC-3′; hPGHS-2–149, 5′-CAGGACCGCGCACA GCAGCAGGGCGCGGGCGAG-3′.

The annealed oligonucleotides hPGHS-2–148 and hPGHS-2–149 encode a modified *Nco*I site spanning an ATG methionine start codon, the first 14 codons of hPGHS-2, the first nucleotide of the 15th codon of PGHS-2, and an *Hae*II site encoded by the last five nucleotides of oligonucleotide hPGHS-2–148. A hPGHS-2 cDNA fragment was prepared by digesting the plasmid pKS::hPGHS-2orf with *Hae*II and *Bam*HI, yielding a 1.9-kilobase DNA fragment that encodes amino acids 14–604 of hPGHS-2. The annealed oligonucleotides hPGHS-2–148 and hPGHS-2–149 and the *Hae*II-*Bam*HI hPGHS-2 cDNA fragment were ligated into the VV transfer vector pTM1 between the *Nco*I and *Bam*HI sites to create plasmid pTM1-hPGHS-2-orf.

A second VV hPGHS-2 transfer vector, designated pTM1-hPGHS-2-3′fl, was constructed and contained the hPGHS-2 ORF and the hPGHS-1 3′ UTR attached to the 3′ end of the hPGHS-2 ORF. The hPGHS-1 3′ UTR was prepared by PCR using the plasmid pCDNA::hPGHS-1 (16) as template and the following oligonucleotides as amplification primers: hPGHS-1–156, 5′-CTAGCTAGCTAGAAT TCGGGGCAGGAAAGCAGCATTCT-3′; hPGHS-1–157, 5′-TCGAT CGATCGAGGATCCGGGCGAATTTTATTAGCTTCA-3′.

Oligonucleotide hPGHS-1–156 codes for an 11-nucleotide clamp sequence and an *Eco*RI restriction site, followed by the first 21 nucleotides after the TGA stop codon in the hPGHS-1 cDNA sequence (16). Oligonucleotide hPGHS-1–157 codes for a 12-nucleotide clamp sequence, a *Bam*HI site, and a 21-nucleotide sequence complementary to the last 21 nucleotides of the hPGHS-1 3′ UTR (16). The PCR was carried out using a kit from Cetus-Perkin Elmer (Montreal, Quebec, Canada), according to the manufacturer's instructions. After the PCR, the 784-base pair amplification product was digested with *Bam*HI and *Eco*RI to remove the clamp sequences. The resulting 749-base pair DNA fragment was then ligated to the 3′ end of hPGHS-2 in the plasmid pTM1-hPGHS-2-orf, which had previously been cleaved with *Eco*RI and *Bam*HI, to create plasmid pTM1-hPGHS-2–3′fl.

**Growth and selection of recombinant VV.** Transfer of the hPGHS genes from the recombinant plasmids to VV strain WR was achieved by using a standard homologous recombination method (23). Briefly, the homologous recombination was carried out by first infecting approximately $1 \times 10^6$ COS-7 cells with $5 \times 10^5$ plaque-forming units of wild-type VV for 2 hr at 37°. Two hours after infection, cells were transfected with 10 μg of each of the plasmids pTM1-hPGHS-1, pTM1-hPGHS-2-orf, and pTM1-hPGHS-2–3′fl, using a calcium phosphate precipitation procedure according to the manufacturer's instructions (Stratagene). Four hours after application of the calcium phosphate-DNA precipitates, medium was replaced with fresh growth medium. Two days later, recombinant viruses were collected from the infected cells and plaque-purified on 143B TK$^-$ cells overlaid with 1% agarose in selective growth medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and containing 25 μg/ml 5-bromo-2′-deoxyuridine). Viral plaques were amplified and analyzed for integration of hPGHS-1 and hPGHS-2 sequences into the viral genome by dot-blot hybridization, using radioactively labeled DNA probes generated from hPGHS-1 and hPGHS-2 DNA sequences. After three rounds of plaque purification and amplification, three recombinant VV were generated and designated VV:hPGHS-1 (VV containing the hPGHS-1 ORF and 3′ UTR), VV:hPGHS-2-orf (VV containing the hPGHS-2 ORF), and VV:hPGHS-2–3′fl (VV containing the hPGHS-2 ORF and hPGHS-1 3′ UTR). The recombinant VV:TF7-3 (20), which contains a bacteriophage T7 RNA polymerase gene under the control of a VV promoter, was obtained from Dr. B. Moss.

**Preparation of microsomal protein fractions from infected cells.** Monolayer cultures of confluent COS-7 cells were coinfected with either VV:hPGHS-1 or VV:hPGHS-2 and the helper VV:TF7-3, each at a multiplicity of infection of 10. At various times after infection, cells were harvested by scraping, washed twice with phosphate-buffered saline, resuspended in lysis buffer (100 mM Tris, pH 7.4, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 2 μg/ml soybean trypsin inhibitor), and then disrupted using a microsonic cell disruptor (Cole-Parmer). Samples were centrifuged for 10 min at 10,000 × *g* at 4° and the resulting supernatant was centrifuged for 90 min at 100,000 × *g* at 4°. The 100,000 × *g* microsome fraction was resuspended in 100 mM Tris, pH 7.4, 10 mM EDTA, to yield a protein concentration of 0.5–3 mg/ml. Protein concentrations were determined using the Bio-Rad protein assay (Richmond, CA) with bovine serum albumin as standard. Using a similar procedure, microsomes were prepared from the human histiocytic lymphoma cell line U-937 (American Type Culture Collection number 1593). A stock solution of 1 mg/ml tunicamycin (24) (Boehringer Mannheim, Laval, Quebec, Canada) was prepared in 10 mM NaOH. Where indicated, tunicamycin was added to cultures 1 hr after infection, to a final concentration of 10 μg/ml.

**PGHS assays.** PGHS activity in microsomes was measured by determining the level of $PGE_2$ synthesis from arachidonic acid. Briefly, each assay contained 1–6 μg of protein in a total volume of 250 μl containing 100 mM Tris·HCl, pH 7.4, 10 mM EDTA, supplemented with 2 μM hematin, 0.5 mM phenol, and 1 mM reduced glutathione. For

experiments involving NSAIDs, reaction mixtures were then incubated with the test compound or dimethylsulfoxide vehicle (5 μl) for 5 min. The reaction was then initiated by the addition of arachidonic acid to a final concentration of 2 μM, followed by incubation at room temperature for 40 min. Reactions were terminated by the addition of 1 N HCl to a final concentration of 0.1 N. Before analysis of $PGE_2$, samples were neutralized by the addition of 1 N NaOH. Levels of $PGE_2$ in samples were quantified by a $PGE_2$ radioimmunoassay (NEN-DuPont, Montreal, Quebec, Canada), according to the manufacturer's instructions.

In a parallel set of experiments, a profile was determined by GC/MS for several of the potential PG products that could be produced by the microsomes in the presence of arachidonic acid, as described above. PGHS assays of microsomes prepared from VV-infected cells were mixed with an equal volume of acetonitrile and centrifuged at 14,000 × *g* for 10 min to precipitate protein, and 50 μl of the supernatant were removed for PG determination. GC/MS determination of the methoxime, pentafluorobenzyl ester, trimethylsilyl ether derivatives of $PGD_2$, $PGE_2$, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$, using tetradeuterated internal standards, resonance electron-capture ionization, and selected ion-monitoring detection, was accomplished according to previously reported procedures (25, 26).

NSAIDs studied included diclofenac [2-[(2,6-dichlorophenyl)amino] benzeneacetic acid], meclofenamic acid [2-[(2,6-dichloro-3-methylphenyl)amino]benzoic acid], zomepirac [5-(*p*-chlorobenzoyl)-1,4-dimethylpyrrole-2-acetic acid], and piroxicam [4-hydroxy-2-methyl-3-(pyrid-2-yl-carbamoyl)-2*H*-1,2-benzothiazine 1,1-dioxide], which were purchased from Sigma. Indomethacin [1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid], (*RS*)-ibuprofen [α-methyl-4-(2-methylpropyl)benzeneacetic acid], and aspirin (2-acetoxybenzoic acid) were purchased from Cayman (Ann Arbor, MI). Sulindac sulfide (L-612,835) was obtained from the Merck sample collection.

**Assays for 15-HETE production.** For the measurement of 15-HETE synthesis, microsomal preparations (10–30 μg of protein) were resuspended in 100 mM Tris·HCl, pH 7.4, 10 mM EDTA, to a final volume of 200 μl. Samples were then incubated at room temperature in the presence of aspirin (final concentration, 100 μM) or ethanol vehicle (2 μl). Reduced glutathione (2 μl) and phenol (2 μl) were then added to final concentrations of 1 mM and 0.5 mM, respectively. Hematin (1 μl; final concentration, 0.5 μM) or dimethylsulfoxide vehicle was then added, and the reaction was initiated by the addition of arachidonic acid (2 μl) or ethanol vehicle. After incubation at room temperature for 30 min, reactions were stopped by addition to 1 ml of methanol containing 0.25 nmol of $PGB_2$, as an internal standard for HPLC analysis. Reaction products were extracted into chloroform (1 ml), dried under a stream of nitrogen, and resuspended in 200 μl of HPLC solvent. One hundred-microliter samples were analyzed by reverse phase HPLC on a Nova-Pak $C_{18}$ column (Waters, Mississauga, Ontario, Canada) using an isocratic solvent system of methanol/water/acetic acid (75:25:0.01), at a flow rate of 1 ml/min (27). The eluant was monitored for 15-HETE at 234 nm, using a model 994 photodiode array system (Waters). In this system, the retention time of an authentic standard of 15-HETE (Cayman) was 14 min. Products were quantitated by comparison of peak areas with those of a standard curve of 15-HETE, with correction for minor differences in extraction efficiency determined using the $PGB_2$ internal standard.

The *R*- and *S*-enantiomers of 15-HETE were identified using a chiral phase HPLC method, as reported previously (28, 29). Briefly, microsomal protein prepared from COS-7 cells coinfected with VV:TF7-3 and VV:hPGHS-2 was preincubated for 2 hr in the presence of 100 μM aspirin. The synthesis of 15-HETE was then initiated by dilution of the aspirin-treated microsomes into a solution containing 100 mM Tris·HCl, pH 8.0, 5 mM EDTA, 1 mM phenol, 1 μM hematin, and 20 μM arachidonic acid, and the solution was incubated at room temperature for 10 min. The reaction products were extracted into ether, dried under nitrogen, resuspended in an excess of ethereal diazomethane, and incubated for 1 hr at room temperature to form the HETE methyl ester (28, 29). The ethereal diazomethane-treated mixture was dried under nitrogen and resuspended in hexane, and the 15-HETE methyl ester was purified by normal phase HPLC on a μPorasil column (Waters) using hexane/isopropanol/acetic acid (100:1:0.1) as the eluting solvent, at a flow rate of 1 ml/min. The stereochemistry of the isolated 15-HETE methyl ester was determined by chiral phase HPLC on a Bakerbond DNBPG chiral column (J. T. Baker, Phillipsburg, NJ) in hexane/isopropanol (100:0.5), at a flow rate of 0.5 ml/min (28, 29).

**Immunoblot analysis.** Protein samples were mixed with 0.5 volume of SDS sample buffer [20 mM Tris·HCl, pH 6.8, containing 0.4% (w/v) SDS, 4% glycerol, 0.24 M β-mercaptoethanol, and 0.5% bromphenol blue], boiled for 5 min, and subjected to SDS-PAGE on 9- × 10-cm precast 10% Tris-glycine acrylamide gels (Novex, San Diego, CA). Proteins were electrophoretically transferred to nitrocellulose membranes using an immunoblot transfer apparatus (Novex), according to the manufacturer's instructions. Nonspecific sites on the membrane were blocked with 3% dry skim milk powder in 20 mM Tris·HCl, pH 7.5, 500 mM NaCl, 0.1% (v/v) Tween 20, for 1 hr at room temperature, and the membranes were washed twice in 20 mM Tris·HCl, pH 7.5, containing 500 mM NaCl and 0.05% (v/v) Tween 20, for 5 min. Blots were then incubated with a 1/1000 final dilution of either an anti-ram seminal vesicle PGHS antiserum (Cayman) or a PGHS-2 antipeptide antiserum (Cayman) in 20 mM Tris·HCl, pH 7.5, containing 500 mM NaCl and 0.05% (v/v) Tween 20. Immunoblot analysis was carried out using either the ECL detection system (Amersham, Oakville, Ontario, Canada) or $^{125}$I-Protein A detection (30) (NEN-DuPont). Purified PGHS-1 and PGHS-2 from ram seminal vesicles and sheep placenta, respectively, were purchased from Cayman.

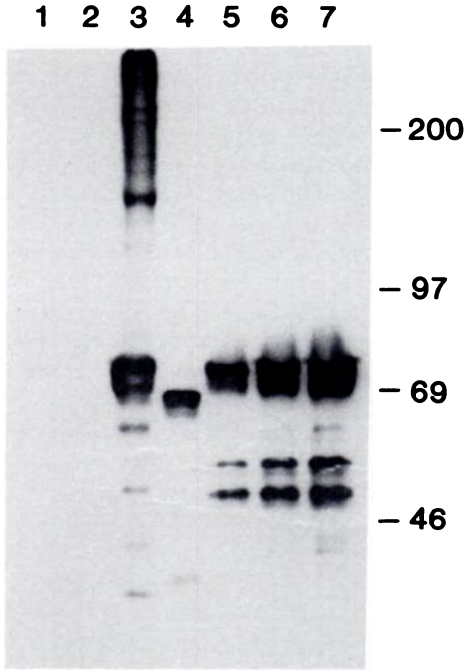


**Fig. 1.** Immunoblot analysis of VV-directed production of hPGHS-1 in COS-7 cells. COS-7 cells were infected with the indicated viruses, grown for 24 hr, harvested, and then used for the preparation of microsomes. Proteins were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with an anti-ram seminal vesicle PGHS-1 antiserum, with detection by chemiluminescence. *Lane 1*, 10 μg of microsomal protein from U-937 cells; *lane 2*, 10 μg of microsomal protein from COS-7 cells infected with VV:TF7-3; *lane 3*, 10 μg of microsomal protein from COS-7 cells infected with VV:TF7-3 and VV:hPGHS-1; *lane 4*, 10 μg of microsomal protein from tunicamycin-treated COS-7 cells infected with VV:TF7-3 and VV:hPGHS-1; *lanes 5*, *6*, and *7*, 50, 100, and 200 ng, respectively, of purified sheep PGHS-1. The positions of molecular weight standards are indicated.

TABLE 1

**Comparison of recombinant hPGHS-1 and hPGHS-2 expression in microsomes prepared from COS-7 cells and PGHS-1 expression in U-937 cells**

| Cell source for microsome preparation[a] | VV[b] | PGHS activity[c] | |
|---|---|---|---|
| | | −AA | +AA |
| | | ng of $PGE_2$/mg of protein | |
| U-937 | None | 0.1 | 20 |
| COS-7 | VV:TF7-3 | 0.12 | 0.37 |
| COS-7 | VV:TF7-3 + VV:hPGHS-1 | 2.1 | 1500 |
| COS-7 (tunic.) | VV:TF7-3 + VV:hPGHS-1 | 0.1 | 0.1 |
| COS-7 | VV:TF7-3 + VV:hPGHS-2-orf | 2.1 | 14 |
| COS-7 | VV:TF7-3 + VV:hPGHS-2-3′fl | 92.5 | 3300 |
| COS-7 (tunic.) | VV:TF7-3 + VV:hPGHS-2-3′fl | <1 | <1 |

[a] COS-7 cell cultures were grown to confluency and then infected with recombinant VV. In certain COS-7 cultures tunicamycin (tunic.) was added 1 hr after infection, to a final concentration of 10 μg/ml. Twenty-four hours after infection, cells were harvested by scraping and disrupted by sonication in lysis buffer containing 100 mM Tris·HCl, pH 7.4, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 2 μg/ml soybean trypsin inhibitor. Microsomes were prepared by high speed centrifugation as described in Materials and Methods. For comparison, microsomes were also prepared from unstimulated cultures of U-937 cells.

[b] The recombinant viruses are VV:TF7-3 (VV containing the bacteriophage T7 RNA polymerase gene under the control of a VV promoter), VV:hPGHS-1 (VV containing the hPGHS-1 cDNA ORF and 3′ UTR), VV:hPGHS-2-orf (VV containing the hPGHS-2 ORF), and VV:hPGHS-2-3′fl (VV containing the hPGHS-2 ORF and the hPGHS-1 3′ UTR).

[c] PGHS activity was determined by measuring the formation of $PGE_2$ by radioimmunoassay in the absence (−AA) or presence (+AA) of 20 μM arachidonic acid as substrate.

## Results

**Expression of hPGHS-1 recombinant VV.** The hPGHS-1 cDNA, previously defined by sequence analysis, encodes a 599-amino acid protein containing a putative 23-amino acid leader peptide and four potential glycosylation sites (16). The hPGHS-1 cDNA, including its 3′ UTR sequence, was cloned into the multiple cloning site of the VV transfer vector pTM1 (22), generating pTM1-hPGHS-1. In this vector the hPGHS-1 sequence is located downstream from a T7 RNA polymerase promoter sequence and is flanked by TK sequences (20, 22). Homologous recombination between pTM1-hPGHS-1 and wild-type VV resulted in recombination of the T7 promoter sequence and the hPGHS-1 cDNA sequence into the VV genome at the TK locus, to generate VV:hPGHS-1 (see Materials and Methods). The VV system used here requires coinfection with two recombinant viruses (20, 22). One recombinant virus, VV:TF7-3, contains the bacteriophage T7 RNA polymerase gene under the control of the VV P7.5 promoter (20). The second recombinant virus contains the hPGHS sequences flanked by the T7 promoter.

To examine the hPGHS-1 protein expressed by VV:hPGHS-1, we chose the COS-7 monkey kidney cell line. This cell line almost completely lacks detectable endogenous PGHS activity (16) and is therefore a convenient cell system to assess the expression of PGHS protein introduced by viral infection. COS-7 cells were either infected with the recombinant VV:TF7-3 or coinfected with VV:TF7-3 and VV:hPGHS-1. Twenty-four hours after infection, cells were harvested, microsomal membrane fractions were prepared by ultracentrifugation, and PGHS levels were measured by enzyme assays and immunoblotting procedures. As shown in Fig. 1, the expected 72-kDa protein corresponding to glycosylated hPGHS-1 was seen only in cultures coinfected with VV:TF7-3 and VV:hPGHS-1, and it co-migrated with purified ram seminal vesicle PGHS-1. No band corresponding to PGHS was observed in mock-infected cultures (Fig. 1, *lane 2*), whereas a band corresponding to PGHS-1 in microsomes prepared from unstimulated U-937 cells was detected after a 12-fold longer exposure of the autoradiogram shown in Fig. 1. In tunicamycin-treated COS-7 cells infected with VV:TF7-3 and VV:hPGHS-1, an immunoreactive band was detected at 69 kDa, presumably representing nonglycosylated hPGHS-1 (Fig. 1).

We next demonstrated that VV:hPGHS-1 was expressing active enzyme by measuring the production of $PGE_2$ from arachidonic acid in microsomal fractions of infected cells (Table 1). The PGHS activity in COS-7 cells coinfected with VV:TF7-3 and VV:hPGHS-1 was >1500-fold higher than that in mock-infected COS-7 cells and was approximately 75-fold higher than in microsomes prepared from the human histiocytic lymphoma cell line U-937 (Table 1). The GC/MS profiles of prostanoids produced by the mock-infected cells and cells coinfected with VV:TF7-3 and VV:hPGHS-1 were consistent with these data (Fig. 2). Significant levels of eicosanoids were not produced in the mock-infected cells, whereas $PGE_2$ and $PGD_2$ were the most abundant PGs produced in the coinfected cells (Fig. 2). Treatment of cells with tunicamycin, an inhibitor of

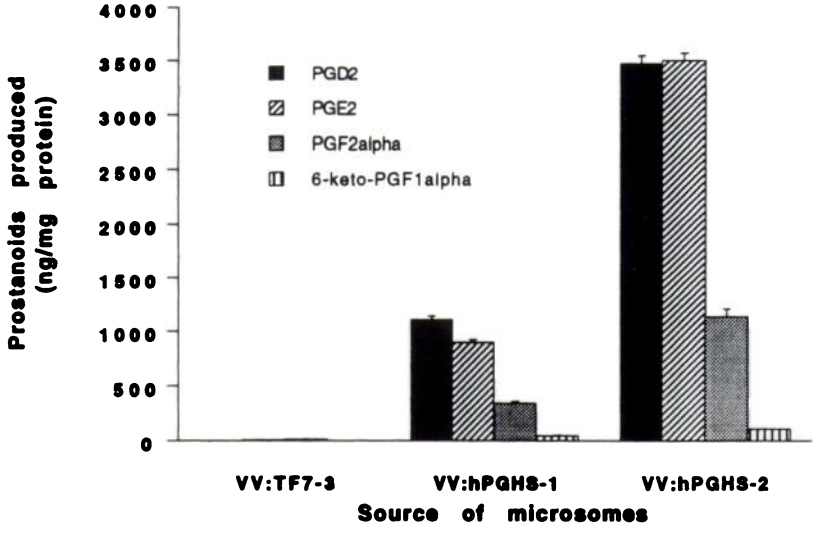


**Fig. 2.** GC/MS analysis of PGHS products from microsomes of COS-7 cells infected with either VV:TF7-3, VV:hPGHS-1, or VV:hPGHS-2-3′fl. Microsomal fractions were assayed for PGHS activity as described in Materials and Methods. The reactions were terminated by the addition of an equal volume of acetonitrile, and an aliquot of the supernatant was analyzed by GC/MS. The values represent the average and standard error for four reactions. No significant production of PGs was detected in microsomes that had been inactivated by heating at 100° for 5 min. The amount of PGs produced in PGHS assays carried out in the absence of arachidonic acid was <1% of that detected in the presence of arachidonic acid.

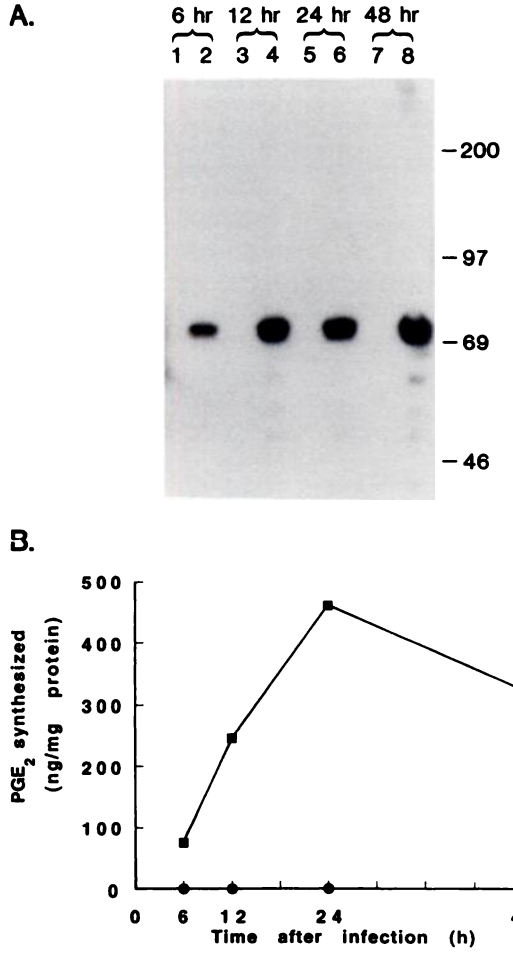


Fig. 3. Time course of hPGHS-1 protein and enzyme activity produced by recombinant VV:hPGHS-1. A, Immunoblot analysis of COS-7 cells infected with either VV:TF7-3 (*lanes 1, 3, 5,* and *7*) or VV:hPGHS-1 and VV:TF7-3 (*lanes 2, 4, 6,* and *8*). After infection, cells were cultured for the times indicated and were harvested, and microsomes were prepared. Ten micrograms of microsomal protein/lane were separated by SDS-PAGE and transferred to nitrocellulose. Immunoblotting was performed using an anti-ram seminal vesicle PGHS antiserum, with detection by $^{125}$I-Protein A. Positions of molecular weight standards are indicated. B, PGHS activity in microsomes prepared from cells infected with VV:hPGHS-1 and VV:TF7-3 (■) or VV:TF7-3 (●). At various times after infection, microsomes were prepared and PGHS activity was determined by assaying the synthesis of $PGE_2$, using 10 $\mu$M arachidonic acid as substrate. $PGE_2$ concentrations were determined by a specific $PGE_2$ radioimmunoassay.

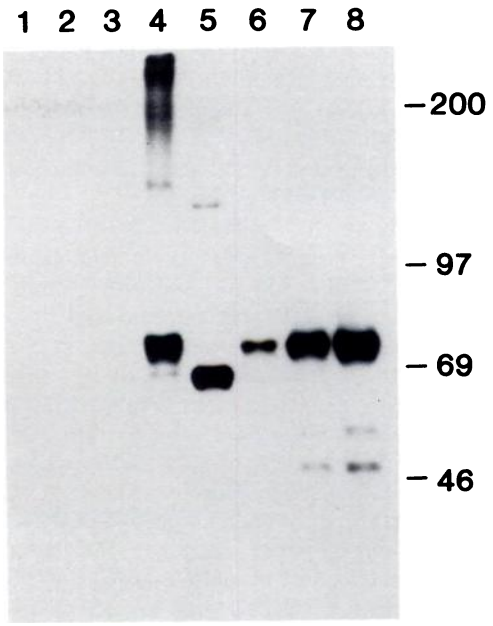


Fig. 4. Immunoblot analysis of VV-directed production of hPGHS-2 in COS-7 cells. After a 24-hr infection, cells were harvested, microsomal cell fractions were prepared, and microsomal proteins were separated by SDS-PAGE. Immunoblotting was performed using an anti-murine PGHS-2 antipeptide antiserum, with detection by chemiluminescence. *Lane 1*, 1 $\mu$g of microsomal protein from U-937 cells; *lane 2*, 1 $\mu$g of microsomal protein from COS-7 cells mock-infected with VV:TF7-3; *lane 3*, 1 $\mu$g of microsomal protein from COS-7 cells infected with VV:TF7-3 and VV:hPGHS-2-orf; *lane 4*, 1 $\mu$g of microsomal protein from COS-7 cells infected with VV:TF7-3 and VV:hPGHS-2-3′fl; *lane 5*, 1 $\mu$g of microsomal protein from tunicamycin-treated COS-7 cells infected with VV:TF7-3 and VV:hPGHS-2-3′fl; *lanes 6, 7,* and *8*, 100, 300, and 500 ng, respectively, of purified sheep PGHS-2.

*N*-linked glycosylation (24), at the time of viral infection yielded microsomal fractions lacking PGHS activity (Table 1), although these microsomes contained an equivalent amount of immunoreactive PGHS-1 protein with a 3–4-kDa reduction in apparent molecular mass, as assessed by immunoblot analysis (Fig. 1). Fig. 3 demonstrates that the synthesis of recombinant hPGHS-1 in COS-7 cells was detected at 6 hr after infection by both immunoblot analysis and activity measurements. Whereas the amount of hPGHS-1 protein continued to increase up to 48 hr after infection, enzyme activity began to decline after 24 hr. For subsequent experiments, cells were harvested 24 hr after infection. Our immunoblot analyses detected several lower molecular mass bands in COS-7 microsomes containing hPGHS-1 and in the purified ram seminal vesicle PGHS-1 preparation, presumably representing proteolytic breakdown products (Figs. 1 and 3).

**Expression of hPGHS-2 recombinant VV.** The hPGHS-2 cDNA sequence codes for a polypeptide of 604 amino acids that is 61% identical to hPGHS-1 (6). Features of hPGHS-2 deduced from its cDNA sequence include an 18-amino acid signal peptide and four potential *N*-linked glycosylation sites (6). In addition, residues of hPGHS-1 proposed either to be at the active site or to be involved in heme coordination are conserved in hPGHS-2 (6). A VV containing the hPGHS-2 ORF was constructed and designated VV:hPGHS-2-orf. The hPGHS-2 3′ UTR was not used in this construction because it contains 12 copies of a motif found in many immediate-early genes and shown to confer enhanced mRNA degradation (6). Even without the hPGHS-2 3′ UTR, only low levels of PGHS activity were obtained when COS-7 cells were coinfected with recombinant viruses VV:hPGHS-2-orf and VV:TF7-3 (Table 1). Northern blot analysis comparing total RNA extracted from COS-7 cells infected with VV:hPGHS-2-orf and VV:TF7-3 showed that the recombinant hPGHS-2 mRNA transcript did not accumulate in infected cells (data not shown). Because of the previous suggestion that the ORF of hPGHS-2 may contain mRNA instability elements (6), we attempted to stabilize the hPGHS-2 mRNA by attaching the 3′ UTR sequence of hPGHS-1 to the hPGHS-2 ORF, in a VV designated VV:hPGHS-2-3′fl. The ability of VV:hPGHS-2-3′fl to direct high-level PGHS expression in COS-7 cells was determined 24 hr after infection by assaying PGHS activity in microsomes prepared from cells that had been coinfected with VV:TF7-3 and VV:hPGHS-2-3′fl (Table 1). Levels of PGHS activity

expressed by VV:hPGHS-2-3'fl were approximately 2-fold higher than those obtained with VV:hPGHS-1 and 165-fold higher than the PGHS activity in U-937 microsomes (Table 1). As with PGHS-1, GC/MS data confirmed $PGE_2$ as the most abundant prostanoid produced in the VV:TF7-3- and VV:hPGHS-2-3'fl-coinfected cells (Fig. 2). Immunoblot analysis using a PGHS-2 antipeptide antiserum detected a predominant band at 74 kDa, which co-migrated with authentic PGHS-2 purified from sheep placenta (Fig. 4). In microsomes from tunicamycin-treated COS-7 cells infected with VV:hPGHS-2-3'fl, a single band of 69 kDa was detected and presumably represents the nonglycosylated form of hPGHS-2. No PGHS activity was detected in these microsomes despite high levels of PGHS-2 protein in the microsomes (Fig. 4; Table 1). The amount of hPGHS-2 in microsomes prepared from COS-7 cells infected with VV:hPGHS-2-3'fl and VV:TF7-3 was approximately 4% of the total microsomal protein (data not shown).

**NSAID inhibition of recombinant hPGHS-1 and hPGHS-2.** The ability of seven standard NSAIDs to inhibit PGHS activity in microsome preparations from COS-7 cells containing recombinant hPGHS-1 and hPGHS-2 was determined (Fig. 5; Table 2). For the compounds tested, the rank orders of potencies against hPGHS-1 and hPGHS-2 were essentially the same. Although the $IC_{50}$ values determined against hPGHS-1 and hPGHS-2 ranged from 0.1 $\mu$M to >300 $\mu$M, the $IC_{50}$ values for diclofenac, meclofenamic acid, indomethacin, sulindac sulfide, and zomipirac against hPGHS-1 and hPGHS-2 demonstrated only up to a 3-fold selectivity for either enzyme. The selectivity of ibuprofen and piroxicam could not be determined in these assays because the $IC_{50}$ values of these compounds against hPGHS-2 were >300 $\mu$M. None of the compounds tested was highly selective as an inhibitor of either hPGHS-1 or hPGHS-2.

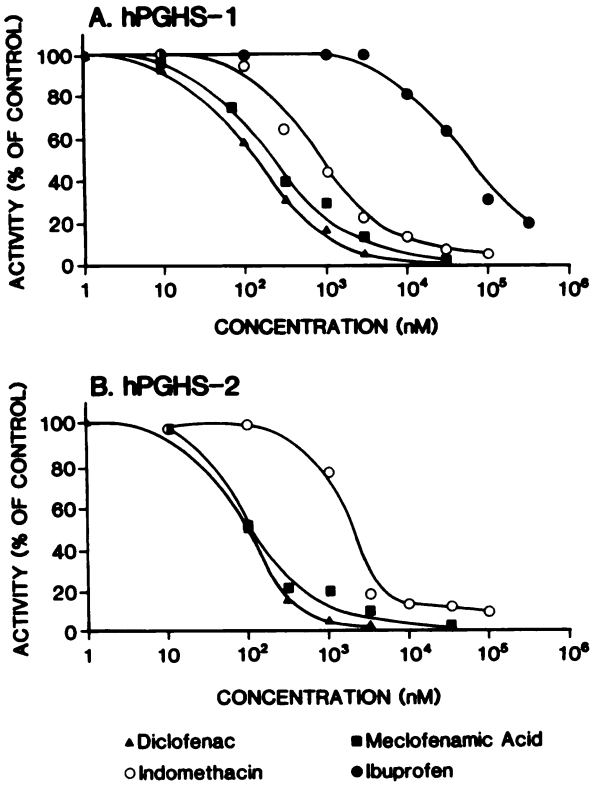


**Fig. 5.** Dose-response curves for inhibition of hPGHS-1 and hPGHS-2 activities by NSAIDs. $PGE_2$ synthesis by microsome preparations from COS-7 cells expressing hPGHS-1 or hPGHS-2, in the presence of 2 $\mu$M arachidonate, were measured by radioimmunoassay as described in Materials and Methods. Levels of $PGE_2$ synthesized in these experiments were confirmed by HPLC-MS. The maximal activities in the absence of inhibitor (100%) for PGHS-1 and PGHS-2 were 300 and 2300 ng of $PGE_2$ synthesized/mg of protein, respectively.

TABLE 2

**Inhibition of hPGHS-1 and hPGHS-2 activity by NSAIDs**

PGHS activities in microsomal preparations from COS-7 cells expressing hPGHS-1 or hPGHS-2 were determined by measuring the synthesis of $PGE_2$ using a specific $PGE_2$ radioimmunoassay. Microsomes were preincubated with the test compound for 5 min at room temperature, in the absence of added arachidonic acid. Reactions were initiated by the addition of arachidonic acid to a final concentration of 2 $\mu$M.

| Inhibitor | $IC_{50}$[a] | |
|---|---|---|
| | hPGHS-1 | hPGHS-2 |
| | $\mu$M | |
| Diclofenac | 0.12 | 0.10 |
| Meclofenamic acid | 0.28 | 0.10 |
| Indomethacin | 0.60 | 1.4 |
| Sulindac sulfide[b] | 5.2 | 10.7 |
| Zomepirac | 15 | 22 |
| (*RS*)-Ibuprofen | 60 | >300[c] |
| Piroxicam | 300 | >300[c] |

[a] Values represent the average of two or three experiments.
[b] The active metabolite of sulindac.
[c] For values shown as >300, the test compound displayed <20% inhibition at concentrations up to 300 $\mu$M.

TABLE 3

**15-HETE synthesis by recombinant hPGHS-1 and hPGHS-2**

| Source of microsomes[a] | 15-HETE synthesis[b] | |
|---|---|---|
| | −Aspirin | +Aspirin |
| | ng of 15-HETE/assay | |
| COS7/mock | <0.1 | <0.1 |
| COS-7/hPGHS-1 | 24.3 ± 0.1 | 26.5 ± 0.2 |
| COS-7/hPGHS-2 | 12.3 ± 1.0 | 60.5 ± 3.2 |

[a] Microsomal fractions were prepared from COS-7 cells mock-infected with VV:TF7-3 or COS-7 cells infected with VV:TF7-3 and VV:hPGHS-1 or VV:TF7-3 and VV:hPGHS-2.
[b] Microsomes were preincubated for 30 min with 100 $\mu$M aspirin and then incubated with 20 $\mu$M arachidonic acid for 30 min, as described in Materials and Methods. Samples were analyzed by reverse phase HPLC, with monitoring of the eluant at 234 nm. The area of the peak co-migrating with an authentic 15-HETE standard was integrated and the mean and standard error for two experiments are shown. Approximately 3-fold more hPGHS-1 activity than hPGHS-2 activity was used in this assay.

**15-HETE synthesis by hPGHS-1 and hPGHS-2.** The formation of 11-HETE and 15-HETE by a lipoxygenase activity of PGHS has been reported previously (31–33). More recently, 15-HETE synthesis from arachidonic acid has been observed after incubation of aspirin-treated COS-1 cells expressing murine PGHS-2, but not in the absence of aspirin pretreatment (15). In the same system, synthesis of 15-HETE was not observed in either untreated or aspirin-pretreated COS-1 cells expressing murine PGHS-1 (15). After incubation of microsomes from COS-7 cells containing either hPGHS-1 or hPGHS-2 with arachidonic acid and HPLC analysis of reaction products, similar levels of a metabolite corresponding to the retention time of 15-HETE were observed (Table 3). This reaction product was not observed using microsomes from mock-infected COS-7 cells (Fig. 6; Table 3). Preincubation of microsomes containing hPGHS-2 with aspirin resulted in a 5-fold increase in the synthesis of this metabolite (Fig. 6; Table 3). The identity of the aspirin-induced metabolite produced by hPGHS-2 as 15-HETE was confirmed by coelution on HPLC

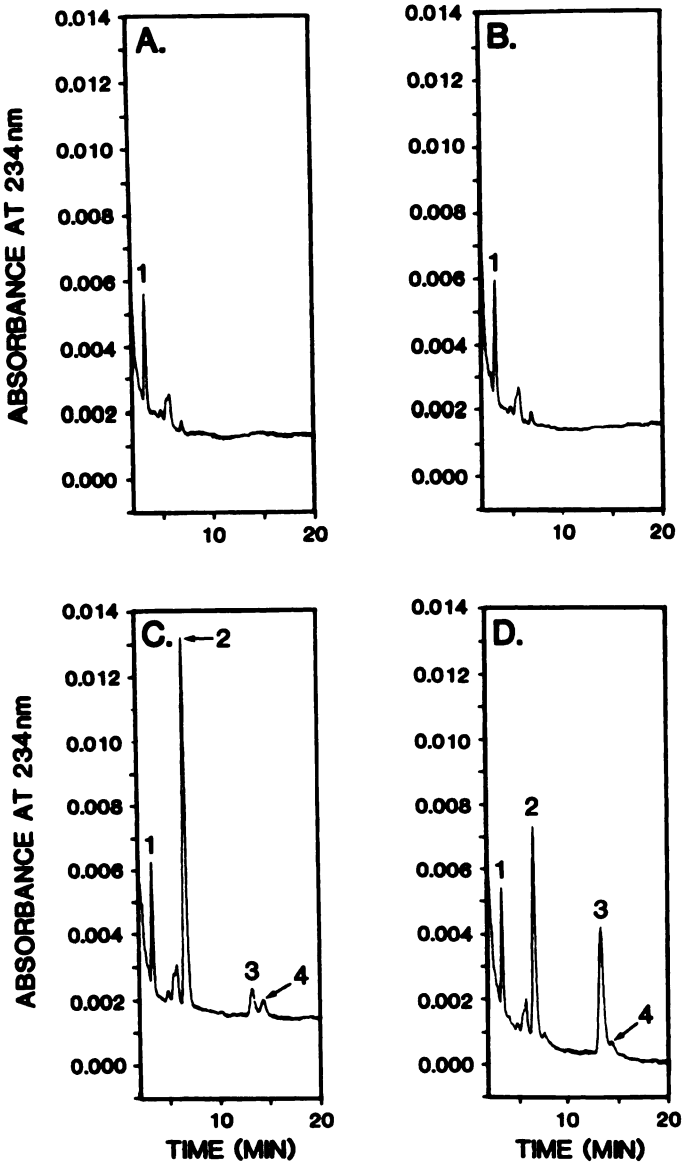


Fig. 6. Endogenous and aspirin-stimulated synthesis of 15-HETE by microsomes containing hPGHS-2. Microsomal fractions from COS-7 cells mock-infected with VV:TF7-3 (A and B) or COS-7 cells infected with VV:TF7-3 and VV:hPGHS-2 (C and D) were preincubated in the absence (A and C) or presence (B and D) of 100 $\mu$M aspirin for 30 min, followed by incubation with 20 $\mu$M arachidonic acid for 30 min, as described in Materials and Methods. Samples were analyzed by reverse phase HPLC and the eluant was monitored at 234 nm. *Peak 1* is 0.25 nmol of $PGB_2$, which was added to samples before HPLC analysis, as an internal standard. *Peaks 2, 3,* and *4* were identified as 12-hydroxy-5,8,10-heptadecatrienoic acid, 15-HETE, and 11-HETE, respectively, based on their coelution with authentic standards and HPLC-MS analysis.

with authentic 15-HETE (Cayman). In addition, the three arachidonic acid metabolites produced by untreated and aspirin-treated COS-7 microsomes containing hPGHS-2 shown in Fig. 6 were characterized by HPLC/continuous-flow liquid secondary ion MS to be 12-hydroxy-5,8,10-heptadecatrienoic acid, 15-HETE, and 11-HETE (data not shown). These identities were further corroborated by the fact that the mass spectra in negative ion mode included fragments for 15-HETE ($m/z$ 219) and 11-HETE ($m/z$ 167) that were characteristic for the position of oxygenation. In contrast to results with microsomes containing hPGHS-2, aspirin pretreatment of microsomes from COS-7 cells containing hPGHS-1 did not result in an increase in 15-HETE production (Table 3). Whereas aspirin pretreatment had a differential effect on 15-HETE synthesis by hPGHS-1 and hPGHS-2 under the same conditions, aspirin exhibited similar potencies for the inhibition of $PGE_2$ synthesis by both enzymes (data not shown). At a concentration of 10 $\mu$M, indomethacin completely inhibited endogenous 15-HETE synthesis by hPGHS-1 and hPGHS-2 and aspirin-stimulated 15-HETE synthesis by hPGHS-2 (data not shown). Because the stereochemistry of the 15-HETE produced by PGHS-2 is not known (15, 34), we examined the enantiomeric composition of the 15-HETE produced by aspirin-treated hPGHS-2-containing microsomes using chiral phase HPLC (28, 29). Microsomes prepared from COS-7 cells expressing recombinant hPGHS-2 were incubated in the presence or absence of 100 $\mu$M aspirin and the 15-HETE produced was converted to its methyl ester and purified by normal phase HPLC (Fig. 7A). A 5-fold increase in the amount of the methyl ester of 15-HETE was observed after aspirin treatment of hPGHS-2 microsomes. When analyzed by chiral phase HPLC (28, 29), only a metabolite corresponding to the retention time of the methyl ester of 15($R$)-HETE was detected (Fig. 7C). The identity of the 15($R$)-HETE enantiomer was confirmed by coinjection of the derivatized product with a 1:1 mixture of authentic $R$- and $S$-isomers of 15-HETE (Fig. 7D).

## Discussion

There has been considerable interest aroused in the possibility that isozyme-selective inhibitors of PGHS may have improved properties, compared with current NSAIDs, and may have a reduced ability to induce mechanism-based side effects. To develop such inhibitors, an adequate supply of hPGHS-1 and hPGHS-2 is required. In most cells PGHS-2 is a short-lived product (14, 18, 35), making it necessary to establish sources other than normal cell lines for the expression and analysis of these products. An initial attempt to produce active recombinant sheep PGHS-1 in a baculovirus expression system resulted in a large quantity of low specific activity enzyme (19), indicating that it is critical that the expressed PGHS undergoes all of the post-translational processing that occurs in normal cells. In particular, incomplete or inappropriate glycosylation has been suggested to be the cause of the low specific activity of the PGHS-1 used in those studies (19). In contrast, limited quantities of high specific activity murine, sheep, and human PGHS-1 and murine PGHS-2 have been prepared by transfection of COS cells with SV40-based expression vectors (6, 7, 10, 15–17). Those studies suggest that COS cells carry out the appropriate post-translational modifications of PGHS-1 and PGHS-2 required for high specific activity.

In the present work we have developed a high-level expression system for both hPGHS-1 and hPGHS-2, using a VV expression system in COS-7 cells. As a result of these studies a number of novel findings have been obtained, including information on the relationship between glycosylation and enzymic activity, the potential instability of the hPGHS-2 mRNA corresponding to the ORF, and the lipoxygenase activity of the enzyme. Thus, tunicamycin-treated cells produced hPGHS-1 and hPGHS-2 of lower molecular mass that were enzymically inactive, indicating that post-translational modifications, in particular glycosylation, are essential to obtain active enzyme. Recent studies using site-directed mutagenesis

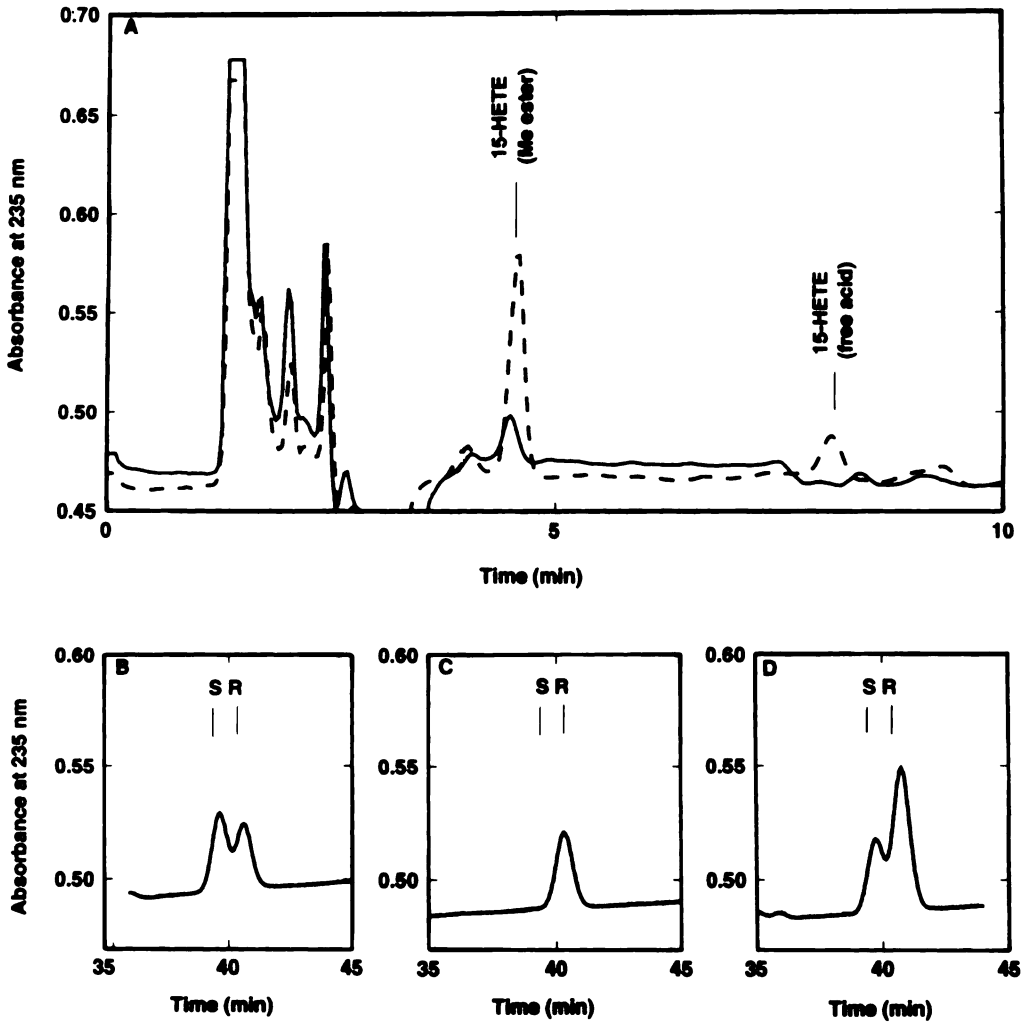


Fig. 7. Aspirin-treated COS-7 microsomes containing hPGHS-2 synthesize 15(*R*)-HETE but no detectable 15(*S*)-HETE. A, Purification by normal phase HPLC of methyl-esterified reaction products produced by untreated (——) or aspirin-treated (- - -) COS-7 microsomes containing hPGHS-2. The conditions for the aspirin treatment of microsomes, the synthesis of 15-HETE by microsomes, the methyl ester derivatization of 15-HETE, and the HPLC analysis are described in Materials and Methods. The retention times of authentic 15-HETE methyl ester (4.5 min) and 15-HETE (8.0 min) are indicated. B, C, and D, Identification of enantiomers of 15-HETE methyl ester derivatives by chiral phase HPLC. B, Separation of 60 ng of authentic methyl esters of 15(*S*)- and 15(*R*)-HETE on a DNBPG chiral phase column. C, Chiral phase HPLC separation of the methyl ester derivative of 15-HETE synthesized by aspirin-treated hPGHS-2. Sixty nanograms of the methyl ester derivative of 15-HETE isolated by normal phase HPLC, as shown in A, were chromatographed on a DNBPG chiral phase column. D, Coinjection of 60 ng of authentic methyl ester derivatives of 15(*S*)- and 15(*R*)-HETE and 60 ng of the methyl ester derivative of 15-HETE produced by aspirin-treated hPGHS-2 microsomes, on a DNBPG chiral phase column. In B, C, and D the retention times of authentic 15(*S*)-HETE methyl ester (39.3 min) and 15(*R*)-HETE methyl ester (40.3 min) are indicated.

have shown that three of the four potential *N*-glycosylation sites in ovine PGHS-1 are glycosylated, with glycosylation of one of the sites ($Asn^{410}$) being required for expression of the cyclooxygenase and peroxidase activites (36). However, enzymatic deglycosylation of mature PGHS-1 does not abrogate either its cyclooxygenase or peroxidase activities, suggesting that glycosylation is necessary for the protein to attain a mature active conformation but is not necessary for it to maintain activity (36). Thus, although PGHS-1 and PGHS-2 are expressed in cells treated with tunicamycin, the lack of glycosylation of these proteins prevents the correct post-translational processing necessary for them to attain enzymic activity.

Limited quantities of high specific activity murine PGHS-2-containing but not hPGHS-2-containing microsomes have been prepared by transient transfection of COS cells (6, 7, 10, 15–17). The inability to obtain recombinant hPGHS-2 with high specific activity in transfected COS cells was attributed to instability of the hPGHS-2 mRNA, possibly due to sequences within the hPGHS-2 ORF (6). The present data support the observation that mRNA transcripts containing the hPGHS-2 ORF are unstable. What was particularly interesting in the present study was that the hPGHS-1 3′ UTR was able to confer stability to the hPGHS-2 mRNA. This could be because the PGHS-1 3′ UTRs from human, sheep, and mouse are characterized by having an unusually high degree of sequence conservation and the potential for formation of secondary structure (16, 37). In contrast, the PGHS-2 3′ UTRs from chicken, mouse, and human are characterized by a large number of Shaw-Kamen sequences, which have been suggested to play a role in determining mRNA instability (6, 8–11).

Our studies with seven common NSAIDs revealed that up to a 3-fold selectivity in enzyme inhibition occurred with the hPGHS isozymes, whereas the murine PGHS isozymes have been reported to display up to a 30-fold differential sensitivity

to NSAID-mediated inhibition (15). For example, the $IC_{50}$ values reported for indomethacin range from 4.9 to 8.1 $\mu$M for murine PGHS-1 and from 130 to 160 $\mu$M for murine PGHS-2 (15). In our studies the $IC_{50}$ values for indomethacin against hPGHS-1 and -2 were 0.60 $\mu$M and 1.4 $\mu$M, respectively. The differences in the NSAID-mediated inhibition between the human and murine PGHS isozymes may reflect species differences, because the murine PGHS-2 and hPGHS-2 are 88% identical at the amino acid level. In addition, because certain NSAIDs are known to show time-dependent inhibition (15), the methods of assaying NSAID-mediated inhibition of PGHS isozymes may contribute to the observed differences in the $IC_{50}$ values between the human and murine PGHS isozymes. The sensitivity of the murine PGHS isozymes to NSAIDs was assayed by measuring oxygen consumption, using an oxygen electrode and measuring the reaction rate immediately after mixing of the drug and enzyme (15). Our measurements of NSAID inhibition of the hPGHS isozymes were performed by first preincubating the enzyme with drug for 5 min and then measuring product formation at a single time point, using a specific radioimmunoassay for $PGE_2$. To determine whether differences in the primary amino acid sequences of PGHS isozymes from various species contribute to the differences in NSAID-mediated inhibition of these enzymes, a direct comparison of the enzymes, in which factors such as time dependence of drug inhibition are examined, is required.

PGHS-1 is inhibited irreversibly by aspirin through acetylation of $Ser^{530}$ (3–5). Site-specific mutagenesis studies have indicated that this conserved serine residue is not involved in catalysis but that acetylation of this residue creates "bulk" close to the active site, preventing access of substrate to the activated tyrosyl radical that initiates the initial hydrogen abstraction (17, 38). An unusual finding with hPGHS-2 in our studies was that aspirin treatment caused the enzyme to metabolize arachidonate primarily to 15(*R*)-HETE instead of $PGH_2$. Similar observations have been seen in cultured ovine tracheal epithelial cells, which synthesize 15-HETE after incubation with arachidonate, with aspirin pretreatment enhancing this synthesis approximately 5-fold (34). The induction of aspirin-enhanced 15-HETE synthesis in these cells was dependent on the presence of growth-promoting supplements in the culture medium (34). The 15-HETE-forming activity in both untreated and aspirin-treated cells was inhibited by indomethacin and immunoprecipitated by an anti-PGHS antiserum, consistent with PGHS being responsible for the production of 15-HETE under both sets of conditions (34). Our results demonstrate that both hPGHS-1 and hPGHS-2 are capable of synthesizing 15-HETE in addition to $PGH_2$. In the presence of aspirin, as reported previously (15, 34), 15-HETE synthesis is enhanced after acetylation of the enzyme by aspirin. In contrast, acetylation of hPGHS-1 does not result in the stimulation of 15-HETE synthesis. In addition, both enzymes produced a small amount of 11-HETE after oxygenation of arachidonic acid. This is consistent with the proposed mechanism for PGHS, which postulates hydrogen abstraction at C13 followed by preferential oxygenation at position C11 (2, 39). The results would suggest that the active site of PGHS-2 differs somewhat from that of PGHS-1, in that insertion of bulk by acetylation of the serine residue of hPGHS-2 does not block access of arachidonic acid to the catalytic site. Instead, a conformational change in the binding of arachidonic acid may occur, such that C15 is preferentially oxygenated, rather than C11, resulting in the synthesis of 15-HETE (34).

The present work describes high-level expression systems for hPGHS-1 and hPGHS-2 that are suitable for producing amounts of enzymes to fully characterize putative inhibitors. The products of the reactions of hPGHS-1 and hPGHS-2 have been characterized and, in addition, data on seven standard NSAIDs have been obtained, indicating that it is possible to screen novel compounds using this enzyme source. Potential NSAIDs can be profiled with respect to their selectivity, intrinsic potency, dependence on substrate concentration, potential for time-dependent inhibition, and reversibility of inhibition. It will be of interest to determine whether isozyme-selective inhibitors developed through such procedures will show therapeutic advantages over conventional NSAIDs, which appear to show little selectivity for either enzyme.

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