Characterization of human glutathione-dependent microsomal prostaglandin E synthase-1

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**ABSTRACT**

Prostaglandins (PGs) are lipid mediators, which act as local hormones. PGs are formed in most cells and are synthesized *de novo* from membrane-released arachidonic acid (AA) upon cell activation. Prostaglandin H synthase (PGHS) -1 or 2, also referred to as COX-1 and COX-2, metabolize AA to PGH₂, which is subsequently converted in a cell-specific manner by downstream enzymes to biologically active prostanoids, i.e. PGE₂, PGD₂, PGF₂α, PGI₂ or TXA₂. PGHS-1 is constitutively expressed in many cells and is mainly involved in housekeeping functions, such as vascular homeostasis, whereas PGHS-2 can be induced by proinflammatory cytokines at sites of inflammation. Prostaglandin E synthase (PGES) specifically catalyzes the conversion of PGH₂ to PGE₂, which is a biologically potent prostaglandin involved in several pathological conditions; including pain, fever, inflammation and possibly some forms of cancers and neurodegenerative diseases.

mPGES-1 was initially identified as a homologue to microsomal glutathione transferase-1 (MGST1) with 37% identity on the amino acid sequence level and referred to as MGST1-like 1 (MGST1-L1). Based on the properties of MGST1-L1, regarding size, amino acid sequence, hydrophathy and membrane localization, the protein was identified as a member of the MAPEG-superfamily (membrane-associated proteins in eicosanoid and glutathione metabolism). The superfamilly consists of 16-18 kDa, integral membrane proteins with typical hydropathy profiles and diverse functions. The MAPEG family comprises six human members, which in addition to mPGES-1 are; 5-lipoxygenase activating protein (FLAP), leukotriene C₄ synthase (LTC₄S), MGST1, MGST2 and MGST3. MGST1-2 and -3 are glutathione transferases as well as glutathione-dependent peroxidases, while FLAP and LTC₄S are crucial for leukotriene biosynthesis.

Human mPGES-1 was cloned and characterized as a 16 kDa, inducible GSH-dependent microsomal PGE synthase. Northern blot analysis of mPGES-1 mRNA demonstrated a low expression in most tissues, medium expression in reproductive organs and a high expression in two cancer cell lines (A549 and HeLa). A549 cells had been used earlier as a model system to study PGHS-2 induction by the proinflammatory cytokine IL-1β and mPGES-1 was also induced by IL-1β in these cells. A protein of similar size was detected in microsomes from sheep vesicular glands, which are known to contain a highly efficient microsomal PGES, indicating that the long-sought membrane bound PGES. Furthermore, a time study of PGHS-2 and mPGES-1 expression revealed a coordinate induction of these enzymes, which was correlated with increased PGES activity in the microsomal fraction. Tumor necrosis factor-α (TNF-α) also induced mPGES-1 in these cells and dexamethasone was found to counteract the effect of these cytokines on mPGES-1 induction. A method based on RP-HPLC and UV-detection was developed to efficiently quantify PGES activity. A small set of potential mPGES-1 inhibitors were tested and NS-398, Sulindac sulfide and LTC₄ were found to inhibit PGES activity with IC₅₀-values of 20 µM, 80 µM and 5 µM, respectively.

The human mPGES-1 gene structure was investigated. The mPGES-1 gene span a region of approximately 15 kb, is divided into three exons and is localized on chromosome 9q34.3. A 862 bp fragment directly upstream of the translation start site exhibited promoter activity when transfected in A549 cells. The putative promoter is GC-rich, lacks a TATA box at a functional site and contains numerous potential transcription factor binding-sites. Two GC-boxes, two tandem Barbie-boxes and an aryl hydrocarbon response element were identified. The putative promoter region of mPGES-1 was transcriptionally active and reporter constructs were regulated by IL-1β and phenobarbital.

The expression of mPGES-1 was investigated in synovial tissues from patients suffering from rheumatoid arthritis (RA). Primary synovial cells obtained from patients with RA were treated with IL-1β or TNF-α. Both cytokines were found to induce mPGES-1 mRNA from low basal levels to maximum levels after 24 hours and the induction by IL-1β was inhibited by dexamethasone in a dose-dependent manner. The protein expression of mPGES-1 was also induced by IL-1β with a linear increase up to 72 h. In contrast, the PGHS-2 induction demonstrated an earlier peak expression (4-8 h). Furthermore, the protein expression of mPGES-1 was correlated with increased microsomal PGES activity. In these biochemical experiments any significant contribution of cytosolic PGES or other cytosolic or non-inducible membrane bound PGE synthases was ruled out.

A purification protocol for mPGES-1 was developed. Human mPGES-1 was expressed with a histidine tag in *Escherichia coli*, solubilized by Triton X-100 and purified by a combination of hydroxyapatite and immobilized metal affinity chromatography. mPGES-1 catalyzed a rapid GSH-dependent conversion of PGH₂ to PGE₂ (170 µmol/min mg). The enzyme, also displayed a high GSH-dependent activity against PGO₂₃, forming 15-hydroperoxy PGE₂ (250 µmol/min mg). In addition, mPGES-1 possessed several other activities; glutathione-dependent peroxidase activity towards cumene hydroperoxide, 5-HpETE and 15-hydroperoxy-PG-E₂, as well as conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) to GSH. These activities likely reflect the relationship with other MAPEG enzymes. Two-dimensional crystals of purified mPGES-1 were obtained and a 10 Å projection map was determined by electron crystallography. Hydrodynamic studies were also performed on the mPGES-1-Triton X-100 complex to investigate the oligomeric state of the protein. Electron crystallography and hydrodynamic studies independently demonstrated a trimeric organization of mPGES-1.

Together with other studies published to date, mPGES-1 has been verified biologically as a drug target and the next step in this validation process requires specific inhibitors to be tested in animal disease models.

To Gunilla

In memory of my mother

*Imagination is more important than knowledge.*

*Albert Einstein*
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INTRODUCTION

Historical background
Almost a century ago, in 1913, Battez & Boulet reported a lowered blood pressure and contraction of the urinary bladder in dogs after injections from extracts of the human prostate gland (23). In 1930, two gynecologists, Kurzrok & Lieb discovered that human semen contracted and relaxed the human uterus during artificial insemination (171). Goldblatt (95,96) and von Euler (412,413) extended this research and discovered that human semen contained a substance that lowered the blood pressure and stimulated the smooth muscle of the uterus. The substance appeared to be an amphipathic acid and was termed “prostaglandin” by von Euler (411), since its biological activity seemed to originate from the accessory genital glands. Twenty-four years later, Bergström and Sjövall isolated the prostaglandins E and F (30,31) and soon thereafter, the structures of these compounds were solved (29). Mass spectrometry was the key method for these discoveries. In 1964, Bergström and van Dorp and collaborators, independently discovered that prostaglandins originated from C\textsubscript{20} polyunsaturated fatty acids (28,389). They incubated homogenates of the sheep vesicular gland with [\textsuperscript{3}H]-labeled arachidonic acid (AA) and observed an enzymatic conversion to PGE\textsubscript{2}. The biosynthetic pathway for the formation of prostaglandins was not known, but the three incorporated oxygens were found to derive from molecular oxygen (263,307) and Bengt Samuelsson postulated the involvement of a cyclic endoperoxide in the formation of these compounds (307). In 1973, Hamberg and Samuelsson with coworkers isolated two short-lived endoperoxides in the biosynthesis of prostaglandins, which subsequently led to a series of discoveries of new metabolic products from this pathway, such as thromboxane (104,105) and prostacyclin (221,405). At that time, a high glutathione-dependent prostaglandin E synthase activity was also found in microsomes from bovine and sheep vesicular glands (223,264,363).

Other arachidonic acid metabolites were soon structurally elucidated, such as the leukotrienes (LTs), LTC\textsubscript{4} (106), LTD\textsubscript{4} and LTE\textsubscript{4} (187,230). Two separate pathways for leukotriene and prostaglandin formation were discovered, namely the 5-lipoxygenase (5-LO) pathway (39,40) and the prostaglandin H synthase (PGHS) pathway (28,389,390), respectively (Fig. 1). All metabolites that originate from arachidonic acid or other C\textsubscript{20} polyunsaturated fatty acids are commonly called eicosanoids due to their related structures.
Figure 1. Overview of the 5-LO and PGHS pathways. LTA₄ and PGH₂ can be further metabolized by specific enzymes into various leukotrienes and prostaglandins, besides the depicted LTC₄ and PGE₂. The enzyme abbreviations shown in bold are members of the MAPEG superfamily.

**BIOSYNTHESIS OF EICOSANOIDS**

**Eicosanoids**
The term eicosanoid originates from the Greek word for 20 (eikosi) and implies products derived from polyunsaturated fatty acids with 20 carbon atoms, i.e., prostaglandins, leukotrienes, thromboxanes, lipoxins and other related compounds. Mammalian cells do not contain the enzymes responsible for introducing double bonds beyond carbon number 9 in polyunsaturated fatty acids and therefore, linoleic acid (18:2 ω6) and linolenic acid (18:3 ω3) are essential fatty acids that need to be obtained through the diet. Linoleic acid can be elongated and desaturated to yield dihomo-γ-linolenic acid (20:3 ω6) and arachidonic acid (20:4 ω6), while linolenic acid can be converted to eicosapentaenoic acid (20:5 ω6). Of these fatty acids, arachidonic acid is the most abundant in human cells. The C₂₀ polyunsaturated fatty acids are metabolized into various eicosanoids (Fig. 2).
Release of arachidonic acid

Most arachidonic acid is stored in the cell membranes, esterified in the sn-2 position of phospholipids (137). Under normal conditions, the level of free arachidonic acid is low, but upon stimulation, arachidonic acid is released by phospholipases (PLs). Phospholipases and acyl-CoA transferases determine the concentration of free arachidonic acid through hydrolysis and re-esterification of phospholipids (97,137,335). Phosphatidylcholine (PC) may account for up to 50% of the total content of phospholipids and the inositol-containing lipids for 5-8%. Phospholipase A₂ and D can utilize PC as a substrate, while phospholipase C specifically hydrolyzes inositol-containing lipids (59). Many PLA₂ enzymes are active within the cell or in the close vicinity and have distinct, but interconnected roles in arachidonic acid release. Phospholipase activity is regulated by several mechanisms including: G-protein coupled receptors (59), phosphorylation (186) and activation by divalent cations (166). So far, 19 enzymes with PLA₂ activity have been identified and cloned in mammals (see reviews) (19,121,167). A classification of the PLA₂s that has historically been utilized, divides them into three groups: secretory (sPLA₂), cytosolic Ca²⁺-dependent (cPLA₂), and cytosolic Ca²⁺-independent (iPLA₂).

Phospholipases

Secreted forms of phospholipase A₂

Secretory PLA₂s comprise the largest group of PLA₂ enzymes and consist of low molecular weight (14-17 kDa), disulfide-linked Ca²⁺-requiring enzymes (mM range) with a highly conserved catalytic site and Ca²⁺-binding loop (167). Several of the sPLA₂ enzymes have been reported to take part in a number of biological processes.
such as inflammation and host defense. Secretory PLA$_2$-IIA is the most widely distributed isozyme in humans and rats. Secretory PLA$_2$-IIA is abundantly expressed in human tissues related to the immune response (166,322). Moreover, sPLA$_2$-IIA can be induced in a wide variety of cells by pro-inflammatory cytokines and lipopolysaccharides (LPS) (3,61,172,238,275,356). This induction is suppressed by anti-inflammatory glucocorticoids (249,313).

Secretory PLA$_2$-V is closely related to sPLA$_2$-IIA and is widely expressed in human tissues, with the highest expression in the heart (53). Secretory PLA$_2$-V is also induced by pro-inflammatory stimuli in several immune cells like mast cells, macrophages, and type 2 helper T (T$_H$2) cells (18,312), and appears to substitute for sPLA$_2$-IIA in some cells (15,287,312).

Studies with specific sPLA$_2$ inhibitors, antibodies, antisense nucleotides and transfection studies have revealed that several of the sPLA$_2$ isozymes have the capacity to regulate cellular arachidonic acid release through distinct mechanisms where arachidonic acid is provided to PGHS-1 in the immediate phase and to PGHS-2 in the delayed phase of cellular prostaglandin formation (235,236,245).

Both sPLA$_2$-IIA and sPLA$_2$-V are heparan-binding enzymes that display significant affinity for cell surface heparan sulfate proteoglycan (HSPG) (242,245). The intracellular sorting route, referred to as the HSPG-shuttling pathway, concentrates sPLA$_2$-IIA into restricted intracellular compartments, which allows colocalization with perinuclear arachidonic acid metabolizing enzymes such as PGHS and 5-LO for efficient eicosanoid production (237). However, sPLA$_2$-V can also act on the plasma membrane surface independently of HSPG (109,237).

In addition to these two sPLA$_2$s, sPLA$_2$-X has also been shown to stimulate arachidonic acid release when added exogenously to different cell lines (33,110). Although, sPLA$_2$-X has a low affinity for cell surface HSPG, it can release arachidonic acid spontaneously in the absence of stimuli (33,110). Secretory PLA$_2$-X is expressed in the intestine, colon, stomach and testis (68) and has also been detected in the immune organs (110).

**Cytosolic Ca$^{2+}$-dependent phospholipase A$_2$**

The cytosolic PLA$_2$-sub family consists of high molecular weight PLA$_2$s (cPLA$_2$s) (>60 kDa) (57,166,276,347), of which cPLA$_2$$\alpha$ (group IVA) has received the most attention. Ca$^{2+}$ and phosphorylation tightly regulate cPLA$_2$$\alpha$ activity. Cytosolic PLA$_2$$\alpha$ is the only known PLA$_2$ enzyme with a marked preference for arachidonic acid over other fatty acids in the sn-2 position (57,75,111,166). Cytosolic PLA$_2$$\alpha$ is constitutively expressed in most tissues and cells, with the exception of mature B and T lymphocytes (93,121,186). Furthermore, the expression of cPLA$_2$$\alpha$ is increased by pro-inflammatory cytokines and growth factors and can be prevented by glucocorticoids (128,193,313). Submicromolar concentrations of Ca$^{2+}$ is required to facilitate translocation of cPLA$_2$$\alpha$ from the cytosol to the nuclear membrane or endoplasmatic reticulum, rather than being necessary for catalysis (124,251,291). This translocation is essential for the initiation of arachidonic acid release (57,83,316).

There seems to be a connection between the secreted forms of PLA$_2$s and the cytosolic PLA$_2$s. In support for this, certain forms of sPLA$_2$s (IB or IIA) can
activate cPLA2α and induce arachidonic acid release via the M-class sPLA2 receptor-mediated pathway (87,118,135). On the other hand, in mouse macrophage-like cells and rat-fibroblastic cells, cPLA2α is required for the induction and activation of sPLA2-V (16,18,330) and sPLA2-IIA, respectively (172,173).

**Cytosolic Ca²⁺-independent phospholipase A₂**
A cytosolic Ca²⁺-independent PLA₂ (iPLA₂) was cloned and purified in 1996 (14,180,364). iPLA₂ shows no strict specificity for sn-2 fatty acids and is fully active in the absence of Ca²⁺, but its role in biological events is not fully elucidated (364). However, iPLA₂ is proposed to play function in phospholipid remodeling through deacylation of phospholipids (408).

**Other phospholipases**
Arachidonic acid can also be released through the phospholipase C (PLC)-pathway by cleavage of phosphatidyl inositol-bisphosphate (PIP₂) into inositol trisphosphate (IP₃) and diacylglycerol (DAG) (294) with subsequent metabolism of DAG by diglyceride lipase and release of arachidonic acid (17,26). Phospholipase D (PLD) can cleave phosphatidylcholine (PC) into phosphatidic acid (PA) and choline. Subsequently, PA can be metabolized by PLA₂ into lysoPA and arachidonic acid (364). Alternatively, PA is metabolized by phosphatidic acid phosphohydrolase (PAP) into DAG (375). DAG can then be further metabolized by diglyceride lipases into arachidonic acid (26).

**Functional coupling of PLA₂s and PGH synthases**
Pharmacological and biochemical studies of PLA₂ isozymes and PGH synthases have been performed on various cells in order to identify the enzymes responsible for efficient transfer of arachidonic acid in different phases of prostaglandin biosynthesis. (172,245,252,330). PGHS-1 seems to be limited to the immediate phase of prostaglandin formation, while PGHS-2 is a prerequisite for the delayed phase, which lasts for several hours (172,235,240,252).

The importance of cPLA₂ for efficient PG-formation was demonstrated by the use of cPLA₂ inhibitors (172,239,245,252,300) and by studies using cPLA₂ knock-out mice (88,385). The inducible sPLA₂-IIA and sPLA₂-V have been demonstrated to participate in both immediate and delayed PG-biosynthesis by the use of antibodies, inhibitors and antisense oligonucleotides (172,252,330,358). Cotransfection studies of several PLA₂s with PGHS-1 or PGHS-2, demonstrated that sPLA₂-IIA, sPLA₂-V and cPLA₂ was functionally linked with PGHS-1 and PGHS-2 in the immediate response and predominantly with PGHS-2 in the delayed response, whereas iPLA₂ was preferentially linked with PGHS-1 in the immediate response (235,236,245). Furthermore, the sPLA₂ enzymes can act on neighboring cells to induce arachidonic acid release. Functional coupling of sPLA₂-V and PGHS-1 has also been seen during immediate PGD₂-formation (287).
METABOLISM OF ARACHIDONIC ACID

Free arachidonic acid is metabolized through oxygenation by three enzymatic pathways in mammals. The prostaglandin H synthase (PGHS) pathway produces prostaglandins and thromboxanes via the prostaglandin endoperoxides PGG2 and PGH2, the lipoxygenase pathway produces leukotrienes and certain hydroperoxy acids (HpETEs) and the monooxygenase pathway (cytochrome P-450 enzymes) leads to a series of epoxy- and hydroxy-acid derivatives (see reviews) (48,334).

Biosynthesis of prostaglandins

Prostaglandins are lipid mediators, which act as local hormones on adjacent cells. They are formed in most cells and are synthesized de novo from membrane-released arachidonic acid upon cell activation by mechanical stress, cytokines, growth factors or certain hormones. Both cPLA2s and sPLA2s can be translocated to the endoplasmatic reticulum (ER) and Golgi apparatus after cell activation. Arachidonic acid is then released and presented to one of the forms of prostaglandin H synthase (PGHS), which metabolize AA to PGH2. PGH2 can subsequently be converted in a cell-specific manner by downstream enzymes to biologically active prostanoids, i.e. PGE2, PGD2, PGF2α, PGI2 or TXA2 (Fig. 3). In many cases, only one prostanoid is produced in a given cell type. For example, thromboxane A2 synthase (TXAS) is present in platelets, prostacyclin synthase (PGIS) is present in endothelial cells, PGF2α synthase (PGFS) is present in the uterus, two types of PGD2 synthase (PGDS) are found in brain and mast cells, respectively and microsomal PGE synthase-1 (mPGES-1) is present in activated cells involved in inflammation (synoviocytes) (353).

PGH-synthase
Prostaglandin H synthase (PGHS; EC 1.14.99.1), also called prostaglandin endoperoxide synthase and cyclooxygenase (COX), is a membrane bound heme-
dependent bis-oxygenase (COX) and peroxidase. PGHS catalyzes the oxidation of arachidonic acid with two molecules of O\textsubscript{2}, forming the endoperoxide, PGG\textsubscript{2}. The 15-hydroperoxide of PGG\textsubscript{2} is subsequentially reduced by the peroxidase (POX) activity of PGHS to PGH\textsubscript{2} (126,205,337,339).

Two isoforms of PGHS have been found in mammals, PGHS-1 (74,214,422) and PGHS-2 (168,417). PGHS-1 is constitutively expressed in most tissues and cells and is considered to be a housekeeping enzyme. In contrast, PGHS-2 is inducible by various stimuli such as hormones, cytokines and mitogens. Both PGHS-1 and PGHS-2 are glycosylated heme-containing homodimers with two catalytic sites. The enzymes are located on the luminal surfaces of the endoplasmatic reticulum (ER) and on the inner and outer membranes of the nuclear envelope (229,349). PGHS-1 and 2 are not integral membrane proteins, but rather anchored to the lipid bilayer through hydrophobic surfaces of their amphipathic helices. Considerable research has focused on the structure, function and physiology of the PGHS isoforms since they are the targets of nonsteroidal anti-inflammatory drugs (NSAIDs), which account for billions of dollars in sales for the pharmaceutical industry (392). PGHS-1 and PGHS-2 have very different expression profiles in several physiological processes (120,337). The PGHS isozymes are also involved in pathological processes. PGHS-1 is involved in thrombosis (273,274), while PGHS-2 mainly takes part in inflammation, pain and fever (64) and some forms of cancer (189,406). It has also been suggested that PGHS-2 plays a role in neurological disorders like Alzheimer’s (211) and Parkinson’s diseases (344).

Gene structure and expression of PGHS-1
The cDNA of PGHS-1 was first cloned from sheep vesicular glands, a rich source of this enzyme (74,214,422), and shortly thereafter, the human gene and primary structure was characterized (423). The human PGHS-1 gene consists of 11 exons, spans a region of approximately 22 kb and is transcribed as a 2.8 kb mRNA coding for a protein with an apparent molecular mass of 70 kDa. The protein is made up of 599 amino acids, including a glycosylation signal and a peptide of 23 amino acids, which is cleaved off in its mature form.

The PGHS-1 promoter region lacks a functional TATA or CAAT box and is GC-rich, which is consistent with a housekeeping gene. Several putative transcriptional regulatory elements are found in the promoter region, such as two sp1 motifs, two AP- sites, an NF-IL6 motif and a GATA.

Gene structure and expression of PGHS-2
The inducible PGHS isozyme was discovered in chicken and mouse fibroblasts in response to src and tumor-promoting phorbol esters (TPA) (168,417). The isozyme was named PGHS-2 and subsequent cloning work revealed that the human gene is about 8.3 kb long with 10 exons and is transcribed as 2.8, 4.0 and 4.6 kb mRNA variants (127,148). PGHS-2 is also a glycoprotein, of which the mature form contains 587 amino acids. PGHS-1 and –2 display about 60% sequence identity on the amino acid level, while sequence identity among orthologs from different species varies between 85-90% (336). A number of potential transcription regulatory elements have been identified in the 5’-flanking region of the PGHS-2 genes, including a TATA box.
Two NF-κB, three sp1, two AP-2, one NF-IL6, one CRE and one E-box binding site have been found (136). PGHS is distantly related to a peroxidase family that split up before divergence between mammals and avians.

PGHS-2 is responsible for PG production in several physiological and pathological conditions and there are a number of studies on PGHS-2 gene expression in various tissues and cells associated with these conditions (for reviews, see) (72,119,120,337). The PGHS-2 gene expression is particularly responsive to proinflammatory mediators such as IL-1, TNFα, INFγ, LPS and 12-O-tetradecanoylphorbol 13-acetate (TPA), but also growth factors, hormones and oncogenes. PGHS-2 gene expression can be regulated by some shared signaling pathways between the NF-κB site, NF-IL6 motif, CRE and E-box in the PGHS-2 promoter (362). Also, three mitogen-activated protein kinase (MAPK) cascades, ERK1/2, JNK/SAPK and p38 contribute to the induction of PGHS-2 either independently or in a concerted manner (354).

**Regulation of PGHS-2 expression**

Gene expression of PGHS-2 is mediated by several receptor-dependent signaling processes (Fig. 4). The Toll-like-receptor 4 (TLR4) has been shown to be the receptor responsible for LPS-mediated signaling (159,421). LPS-induced cellular activation of NF-κB in endothelial and monocytic cells has been shown to be associated with activation of JNK, ERK1/2 and p38 (100). Interestingly, the IL-1 signaling pathway in mammals is very similar to the Toll signaling pathway in *Drosophila* and activation of these receptors induces the recruitment of an adapter molecule called myeloid differentiation factor 88 (MyD88) (113,298). MyD88 recruits IL-1 receptor-activated kinase (IRAK), which interacts with TNF receptor associated factor 6 (TRAF6). This complex can then interact with NF-κB-inducing kinase (NIK). In turn, NIK activates the IκB kinase complex (IKK) that phosphorylates IκB. This phosphorylation leads to an ubiquitin-proteasome-mediated degradation and release of activated NF-κB. However, the TNF-receptor signaling is not mediated by TRAF6, but instead by TRAF2. The signaling pathways of IL-1, TNF and LPS, converge after TRAF2 and TRAF6. Another adapter protein, called ECSIT, is linked to the signaling pathway from the TLR4 and IL-1 receptors to MEKK.

The MAPK cascade is a very important signaling pathway for PGHS-2 expression and consists of three different subgroups of kinases (ERK, JNK/SAPK, and p38). The ERKs are mainly activated by growth factors and oncogenes, including v-Ras and v-Src. The JNK/SAPK and p38 pathways are activated by proinflammatory cytokines, LPS and environmental stress, like ultraviolet light, ionizing radiation and oxidative stress. When the MAP kinases are activated, they phosphorylate transcription factors, which then regulate gene expression. The proinflammatory cytokine IL-1β is a well-known inducer of PGHS-2 gene expression, which signals through JNK/SAPK and p38 MAPK (99). The PGHS-2 gene has been shown to be an important Ras target since oncogenic mutations in Ras and overexpression of PGHS-2 is found in many forms of human cancers, including breast cancer and colorectal carcinoma (327,355).
Figure 4. Signaling of PGHS-2 gene induction.

Dexamethasone is a common anti-inflammatory steroid, which binds to the glucocorticoid receptor (GR) and activates transcription of a number of genes via glucocorticoid response elements (GREs) (25). Dexamethasone has been found to be an efficient suppressor of inflammatory-induced PGHS-2 expression. No GREs are found in the 5'-flanking region of human PGHS-2, but the mechanism of glucocorticoid-mediated repression involves suppression of AP-1 and NF-κB-dependent transcription (13,314). Dexamethasone can also act through other post-transcriptional mechanisms of repression and treatment of A549 cells with dexamethasone only reduced the transcription rate of PGHS-2 by 40%, while the mRNA and protein levels were completely repressed (257,258).
dexamethasone-dependent repression involved a reduced PGHS-2 mRNA half-life with shortening of the average length of the poly(A) tails.

Activated MAPK has also been reported to regulate gene expression at the post-transcriptional level by mRNA stabilization of PGHS-2 (52,410).

**Biological functions**

PGHS-1 and PGHS-2 have striking differences in tissue expression and regulation, therefore it is postulated that they have different physiological functions. Confocal fluorescence imaging microscopy and immunohistological techniques have revealed differences in subcellular localizations of PGHS-1 and PGHS-2. Both PGHS-1 and PGHS-2 are localized in the endoplasmatic reticulum, but PGHS-2 is more concentrated in the nuclear envelope (229).

PGHS-1 is present in nearly all cells under basal conditions (62) and is expressed at high levels in specific tissues, such as vascular endothelia, monocytes, platelets, renal collecting tubules and seminal vesicles. PGHS-1 produces prostaglandins responsible for homeostatic functions, such as regulation of renal blood flow, maintenance of the gastric mucosa and platelet function. Studies of PGHS-1 (-/-) mice have revealed the biological role of PGHS-1. PGHS-1 (-/-) mice have reduced platelet aggregation, are more sensitive to radiation injury, but have no gastric pathology as would be expected, since PGHS-1 was thought to protect the gastric mucosa (132,179). However, when selective PGHS-2 inhibitors were given to PGHS-1 deleted mice, the mucosa protection was significantly decreased (231). Thus, both PGHS-1 and PGHS-2 seems to be involved in gastric mucosa protection. Furthermore, PGHS-1 seems to be important in reproduction, since PGHS-1 (-/-) pairings lead to few offspring (288).

PGHS-1 is not normally induced, but cell-lines that undergo differentiation and mimic the developmental process have increased expression of PGHS-1 (362). Shear stress has also been reported to increase PGHS-1 expression in human umbilical vein endothelial cells (HUVEC) (268) and this together with pharmacological studies (281), suggests a role for PGHS-1 in atherosclerosis.

PGHS-2 is not normally present in most tissues, but is inducible by proinflammatory cytokines, growth factors, hormones and exogenous stimuli in many cell types, like synoviocytes, endothelial cells, chondrocytes, osteoblasts and monocytes (336). However, constitutive PGHS-2 expression has been found in several tissues, like the kidney, lung epithelial cells and intact thyroid tissue (10,114,254,295). Also, tissues that are exposed to constant mechanical stress, such as bone, blood vessels and brain (electrical stimulation) express PGHS-2 constitutively (362). PGHS-2 deficient mice have more profound phenotypic changes as compared to PGHS-1 (-/-) mice. Female PGHS-2 deficient mice have deficient reproductive functions including ovulation, fertilization and implantation (76,227). Phenotype changes in PGHS-2 (-/-) mice have also been observed in peritonitis, cardiac fibrosis, renal nephropathy and failure of ductus arteriosus closure (55,178). PGHS-2 seems to play an important role in tumorigenesis. Supression of tumorigenesis in PGHS-2 (-/-) mice have confirmed epidemiological studies, demonstrating that NSAIDs suppress the incidence of colon cancer (270). However, the effect of NSAIDs on tumorigenesis cannot entirely be explained by inhibition of
PGHS-1 or PGHS-2. Studies with PGHS-1 and PGHS-2 double knockout mice have revealed novel mechanisms for NSAID activities in tumorigenesis, independent of PGHS expression (426).

**PGHS catalysis**

Each PGHS-homodimer contains one heme per subunit, which is necessary for both the COX and POX reaction (218,267). The COX and POX activities occur at separate but functionally interactive sites within the enzyme (342). The peroxidase reaction is necessary to activate the cyclooxygenase reaction. A hydroperoxide reacts with the heme-iron and initiates a two-electron oxidation, which yields an enzyme state with an oxyferryl-heme radical cation, called Compound I. The radical undergoes intramolecular migration from the heme group to Tyr385, which creates intermediate II (338,376). Already by 1967, Hamberg & Samuelsson proposed the basic steps in the PGHS mechanism (103). The postulated mechanism remains virtually the same today, however, recent structural studies of PGHS have revealed important functions in the mechanism, which can be broken down into four steps (91). The carboxyl group of AA interacts with Arg120, which leads to a proper positioning of AA in the COX channel (200,277). The tyrosyl radical (Tyr385) initiates the COX reaction by abstracting the 13proS hydrogen from AA (103,377,378). This step has been shown to be the rate-limiting step of the COX reaction. Subsequently, an 11R-peroxyl radical is formed in the presence of O2. The 11R-peroxyl radical then attacks carbon 9 and forms the endoperoxide with following isomerization of the radical to carbon 8. A major reconfiguration of the substrate is necessary for ring closure between carbon 8 and 12, during or immediately after formation of the endoperoxide bridge. The ω-end of the substrate will have to move much closer to the carboxyl group in this conformational change. The 11R-peroxyl radical is believed to swing “over” carbon 8 with an R-side attack on carbon 9 through the rotation of the bond between carbon 10 and 11, which brings carbon 12 closer to carbon 8 for ring closure. This would also lead to repositioning of carbon 15 for an additional attack of O2, thus forming the 15S-peroxyl radical. The catalytic cycle of COX is completed by the 15S-peroxyl radical, which abstracts the hydrogen from Tyr385, thereby regenerating the tyrosyl radical and producing PGG2 (91). The 15-hydroperoxyl group of PGG2 is thereafter reduced to PGH2 by the POX activity of PGHS. The crystal structure of PGHS does not reveal any direct pathway for PGG2 to travel through the protein from the COX to the POX site. PGG2 is believed to exit the COX site through the opening in the membrane binding domain and travel around the surface of the protein to get to the POX site (342).

The PGHS POX reaction occurs at a heme-containing active site, close to the protein surface, partially exposed to solvent (170,277). The POX reaction is considered to be the second step in the formation of PGH2, but the peroxidase activity is absolutely required for COX activation (337,338). Initially, other peroxides, besides PGG2 are needed to start the COX reaction. Neither the identity nor the source of the hydroperoxide needed for the initial heme oxidation in vivo is known. However, as soon as the first catalytic cycle of the COX reaction has started, it can continue independently of the POX cycle (163). Both PGHS-1 and –2 have been shown to reduce a number of peroxides with a marked preference for secondary alkyl
hydroperoxides and PGG$_2$ is probably the physiologically most important substrate (176,196).

There are some differences in the rate of POX activities between PGHS-1 and –2 with no obvious structural explanation (196). The COX activity of PGHS-2 is activated at a lower concentration of hydroperoxide than for PGHS-1, possibly related to a low peroxide state of the relevant cell (169,340).

Both the POX and COX activities of PGHS are suicide inactivated during catalysis by a breakdown of active enzyme intermediates. The exact mechanism for the suicide inactivation and protein modification associated are not known, but probably involves reaction of amino acid radicals with molecular oxygen (342).

Arachidonic acid is the best substrate for PGHS-1 and –2, but both enzymes can also oxygenate closely related fatty acids in intact cells, such as linoleic acid (18:2 w6), dihomo-γ-linolenic acid (20:3 w6) and eicosapentaenoic acid (20:5 w3). PGHS-2, but not PGHS-1 has also been shown to convert the endocannabinoids 2-arachidonylethanolamide into the precursors for prostaglandin glycerol esters and prostaglandin ethanolamides (164,165).

The difference in substrate specificity between PGHS-1 and –2 could explain the reason for two isozymes. Interestingly, both isozymes have been found to produce small amounts of other products, besides PGG$_2$ from AA, due to different conformers in the COX active site (11R-HpETE, 11S-HpETE, 15R-HpETE, 15S-HpETE) (372,416). However, little is known about the role of these alternative metabolites in physiology.

The first three-dimensional structure of a PGHS-enzyme was published in 1994 (ovine PGHS-1 complexed with the NSAID, flurbiprofen) (277) and soon thereafter, the crystal structure of human and murine COX-2 was elucidated (170,199). Drug interactions with PGHS have been extensively studied by the use of crystallography. The PGHS isoforms were found to be structurally homologous and quite superimposable. However, there are some structural differences between the COX site of PGHS-1 and –2. The active site of COX-1 and –2 does not share the exact same surrounding amino acids and the size of the main channel is slightly larger in PGHS-2 (~20%). These differences have made it possible to design selective drugs against PGHS-2.

**NSAID action**

PGHS-1 and –2 are the major targets for NSAIDs. Structural data of the PGHS isoforms have revealed that NSAIDs inhibit the fatty acid substrate binding at the COX site. There are two major classes of NSAIDs; the classical NSAIDs, which inhibit both PGHS-1 and –2 and the PGHS-2 selective inhibitors (73,234). Most of the NSAIDs inhibit PGHS-1 and –2 by a reversible competitive inhibition. However, acetylsalicylic acid inhibits PGHS by a rapid, reversible binding followed by a covalent modification of Ser530 (91,342,392).

Furthermore, acetylsalicylic acid has been reported to directly inhibit the NF-κβ signaling pathway through Iκβ kinase and prevent the expression of PGHS-2 (50). This might explain the anti-inflammatory properties of NSAIDs, which are independent of PGHS inhibition (419).
PROSTAGLANDIN E SYNTHASE

Prostaglandin E synthase (PGES) (EC 5.3.99.3) specifically converts the endoperoxide moiety of prostaglandin endoperoxides into the 9-keto-11-hydroxy conformation found in PGE₂. PGE₂ is the most common prostanoid and mediates a number of biological responses through certain prostanoid receptors (see prostanoid receptors). Originally, microsomes from sheep and bovine seminal vesicles were found to contain high glutathione (GSH)-dependent PGES activity (223,264,363). Many attempts have been made to isolate microsomal PGES, with limited success, mainly due to the instability of the enzyme. Several cytosolic glutathione transferases have also been found to possess PGES activity. Two anionic glutathione transferases with GSH-dependent PGES activity were purified from human brain (265). However, these activities were later shown to not be specific for PGE₂ formation, but also produced PGD₂ and PGF₂α from PGH₂ (51,384). Two other cytosolic glutathione transferases of the Mu-class have been purified from human brain cortex, which demonstrated significant GSH-dependent PGES activities (32). Another cytosolic GSH-dependent PGES (cPGES/p23) that is functionally linked with PGHS-1 was isolated from rat brain after LPS-treatment (367). There are several reports about a membrane-bound GSH-independent PGES in heart, spleen and uterus, isolated from rat and bovine (396,397). Recently, the cDNA corresponding to the microsomal GSH-independent monkey protein was cloned and purified and is now referred to as mPGES-2 (365).

This thesis is based on the characterization of human microsomal glutathione S-transferase-1-like 1 (MGST1-L1), which was cloned and identified in 1999 by Jakobsson et al. (143) and will thus be discussed in detail later (see discussion). Orthologs of MGST1-L1 have also been cloned from several other species (84,182,202,244). The membrane-bound PGE synthases are designated as mPGES and the cytosolic PGE-synthases as cPGES.

mPGES-2

A membrane-bound GSH-independent PGES expressed in heart, spleen and uterus, has been isolated from rat and bovine (396,397) and the corresponding monkey protein was recently expressed, purified and referred to as mPGES-2 (365). The cDNA for monkey mPGES-2 encodes a 33-kDa protein with the consensus region of glutaredoxin and of thioredoxin. The human mPGES-2 gene was localized on chromosome 9q33-q34, close to the genes of PGHS-1, mPGES-1 and lipocalin-type PGDS. Purified mPGES-2 displayed a Kₘ and Vₘₐₓ of 28 µM and 3.3 µmol min⁻¹ mg⁻¹, respectively with a pH-optimum between 6-7 (365). Recombinant mPGES-2 was activated by several SH-reducing reagents such as dithiothreitol, GSH and β-mercaptoethanol, in order of decreasing effectiveness. Northern blot analysis demonstrated that mPGES-2 mRNA was mainly localized in various regions of the brain and heart, but not in genital organs, as compared to mPGES-1 (365). More recently, transfection studies of mPGES-2 and PGHS-1 or PGHS-2 demonstrated that mPGES-2 promoted PGE₂ production in the immediate and delayed responses, with modest preference to PGHS-2 (241). mPGES-2 demonstrated a constitutive
expression in many cells and was not induced during tissue inflammation. However, protein expression of mPGES-2 was elevated in human colorectal cancer.

**Cytosolic PGES**

Cytosolic PGES (cPGES) is identical to p23, a 23 kDa, heat shock protein 90 (Hsp 90)-binding protein, originally implicated as a cofactor for the chaperone function of Hsp90 (134,147). cPGES was initially identified as a cytosolic, LPS-induced, GSH-dependent PGES with \( K_m \) and \( V_{\text{max}} \)-values of 14 \( \mu \)M and 190 nmol min\(^{-1}\) mg\(^{-1}\), respectively (367). In a more recent study, cPGES was shown to be activated in the presence of the Hsp 90-complex, Mg\(^{2+}\) and ATP (366). Unlike typical GSTs (305), cPGES not only showed negligible activities against several cytosolic GST substrates, including CDNB, but was even inhibited by them (367). These properties are similar to those of hematopoietic PGDS, which belongs to the \( \sigma \)-class of GSTs (151). The homology between cPGES and other cytosolic GSTs, including hematopoietic PGDS is low (~20%), but they all share a conserved tyrosine near the N-terminus (Tyr9), which serves to stabilize the GSH thiolate in many cytosolic GSTs. Mutation of this amino acid abrogated the activity of cPGES, suggesting the same function (367).

Cotransfection studies of cPGES and PGHS-1 in HEK293-cells have shown that these enzymes are functionally linked and associated with maintenance of tissue homeostasis (367). However, less efficient biosynthesis of PGE\(_2\) has been seen in KAT-50, a well differentiated thyroid epithelial cell line, expressing PGHS-2 and cPGES (107). The role of cPGES in vivo is not fully understood and studies with cPGES (-/-) mice will reveal its implications in physiology and pathophysiology. Recent data indicates that cPGES may play an important role during implantation, decidualization (260) and parturition (212).

**GSTs**

Several cytosolic GSTs have been reported to convert PGH\(_2\) into PGE\(_2\), PGD\(_2\) and PGF\(_{2\alpha}\), non-specifically (51,265,384). Two cytosolic glutathione transferases of the Mu-class, purified from human brain cortex demonstrated specific GSH-dependent PGES activities (32). The recombinant GSTM2-2 and 3-3 catalyzed the conversion of PGH\(_2\) to PGE\(_2\) at the rates of 282 and 923 nmol min\(^{-1}\) mg\(^{-1}\), with an apparent \( K_m \) of 140 and 1500 \( \mu \)M, respectively, at the optimal pH of 8. The human GSTM2-2 and 3-3 are mainly expressed in the brain (301) and the rat counterpart of GSTM3-3 is localized in the thalamus and hypothalamus (146).
CATABOLISM OF PGE$_2$ AND FORMATION OF PGF$_2$

The main pathway for catabolism of eicosanoids is initiated by oxidation of the 15(S)-hydroxyl group, catalyzed by NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) (144), followed by reduction of the $\Delta^{13}$ double bond, catalyzed by NADPH/NADH-dependent $\Delta^{13}$-ketoprostaglandin reductase (13-PGR) (Fig. 3) (8). These enzymes are ubiquitously expressed in mammalian tissues (359) and the degradation products of these enzymes (15-ketoprostaglandins and 13,14-dihydro-15-ketoprostaglandins) have significantly reduced biological activities (7). There are two types of 15-PGDHs, type I is NAD$^+$-dependent and selective towards eicosanoids (144), while type II can use both NAD$^+$ and NADP$^+$ and has a broader substrate specificity (194). Since the type II enzyme has much higher $K_m$ for prostaglandins, compared to type I, it is not believed to play an important role in the catabolism of eicosanoids (359). The eicosanoids can be further metabolized, in several steps by $\beta$- and $\omega$-oxidation of the side chains into various dinor- and tetranor products (Fig. 3) (308). For example, $\beta$-oxidation shortens the $\alpha$-chain of various prostaglandins into the corresponding C18-homologues (102) and $\omega$-oxidation of prostaglandins is performed by cytochrome P-450 enzymes (45,46,269). Actually the most abundant prostaglandins in the semen are 19R-hydroxy-PGE$_1$ and 19R-hydroxy-PGE$_2$ (368).

PGE$_2$ can also be metabolized non-enzymatically to PGA$_2$, which can be further isomerized enzymatically at $\Delta^{10}$ of PGA$_2$ to $\Delta^{11}$, by PGCS, thus forming PGC$_2$ (Fig. 3) (149). PGCS has been found in serum or plasma from human, rabbit, pig, dog, rat and cat (149) and the cat enzyme has also been partially purified.

PGF synthases

PGF$_2$ is formed via three pathways from PGH$_2$, PGE$_2$ or PGD$_2$ by PGH 9-, 11-endoperoxide reductase, PGE 9-ketoreductase, or by PGD 11-ketoreductase, respectively (Fig. 3) (393). These activities are dependent on NADH or NADPH. Furthermore, a 16.5 kDa, microsomal GSH-dependent PGF$_{2\alpha}$ synthase was partially purified from sheep vesicular glands (43) and recently, a novel GSH-activated, LPS-inducible PGF$_{2\alpha}$ synthase was detected in various cells, with the highest activity found in lung (250). These two PGF$_{2\alpha}$ synthases specifically converted PGH$_2$ to PGF$_{2\alpha}$. Several GSH-(S) transferases also have the capacity to produce PGF$_{2\alpha}$ from PGH$_2$ (51).

PGE 9–ketoreductase can convert PGE$_2$ specifically to PGF$_{2\alpha}$ in the presence of NADH or NADPH. PGE 9–ketoreductase is a cytosolic enzyme, purified from chicken heart (184), human (404) and bovine placenta (153). The enzyme is a member of the aldo-keto reductase (AKR) superfamily (409), based on the broad substrate specificity, size, cofactors and sequence identities. Furthermore, a carbonyl reductase, detected in human brain (403) and a 20$\alpha$-hydroxysteroid dehydrogenase (HSD), were found to be identical with PGE 9–ketoreductase (11,315).
PROSTAGLANDIN TRANSPORT

PGH$_2$ is synthesized in the lumen of the ER by PGHS-1 or PGHS-2 (229) and is then believed to diffuse through the membrane, where it is further metabolized by terminal enzymes (302,363). The synthesized prostaglandins diffuse across the plasma membrane into the extracellular compartment, driven by pH and the membrane potential (319). However, metabolic clearance of prostaglandins requires energy-dependent uptake across the plasma membrane, with following catabolism. Local signal termination of prostaglandins must be achieved to prevent undesired effects. Several types of prostaglandin transporters that are involved in the re-uptake of prostaglandins have been characterized. PGT is a lactate/prostaglandin exchanger and is expressed in PGHS-containing cells (197). Another prostaglandin transporter, called organic anionic transporter (OATP), has lower affinity and specificity for prostaglandins (319). Also, a liver-specific OAT (LST-1) has been cloned from human (1).

PROSTANOID RECEPTORS

The biological activities of prostaglandins are mediated through the binding of specific G-protein coupled rhodopsin-type receptors (GPCR) with seven transmembrane domains (255). These receptors originate from different genes and have been cloned from various species. The receptors have been classified into 8 types with several subtypes, based on their responsiveness to various agonists/antagonists (256) and include: the PGD receptor (DP) (37), four subtypes of the PGE receptor (EP$_{1-4}$) (22,89,290,420), the PGF receptor (FP) (2), the PGI receptor (IP) (36) and the TXA receptor (TP) (123) (Table 1). There are several splice variants of the EP$_3$, FP and TP receptors (278,285,289), which differ only in their C-terminal ends. Furthermore, a novel DP receptor was characterized as CRTH2 (Chemotactrant Receptor-homologous molecule expressed on T-Helper type 2 cells) and reported to be involved in allergic inflammation and rhinitis (122).

Functionally, the prostanoid receptors can be divided into three groups: the relaxant, contractile and inhibitory receptors. The DP$_1$, EP$_2$, EP$_4$ and IP receptors are called the “relaxant” receptors, since they signal through a $G_s$-mediated intracellular increase of cyclic adenosine monophosphate (cAMP). The EP$_1$, FP and TP receptors signal through a $G_q$-mediated increase in intracellular calcium and are thus called the “contractile” receptors and the EP$_3$ and DP$_2$ receptors are “inhibitory” receptors that couple to $G_i$ and decreases cAMP-formation. Most of the prostanoid receptors are located at the plasma membrane, but some have also been found at the nuclear envelope (34,35). The roles of prostaglandins in various physiological and pathophysiological events have been investigated in mice, deficient in each of the prostanoid receptors. A summary of the results from gene-disruption studies is given in table 2. The EP$_{1-4}$ receptors are clearly involved in many pathological conditions.
### Table 1: Properties of prostanoid receptor subtypes.

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>G protein</th>
<th>Signaling</th>
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<tbody>
<tr>
<td>DP₁</td>
<td>Gₛ</td>
<td>cAMP↑</td>
</tr>
<tr>
<td>DP₂ (CRTH2)</td>
<td>Gₛ/α</td>
<td>cAMP↓</td>
</tr>
<tr>
<td>EP₁</td>
<td>Unidentified</td>
<td>[Ca²⁺]↑</td>
</tr>
<tr>
<td>EP₂</td>
<td>Gₛ</td>
<td>cAMP↑</td>
</tr>
<tr>
<td>EP₃</td>
<td>Gᵢ, Gₛ, Gₐ</td>
<td>[Ca²⁺]↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cAMP↑</td>
</tr>
<tr>
<td>EP₄</td>
<td>Gₛ</td>
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<td>FP</td>
<td>Gₐ</td>
<td>[Ca²⁺]↑</td>
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<td>IP</td>
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<td>[Ca²⁺]↑</td>
</tr>
<tr>
<td>TP</td>
<td>Gₛ, Gᵢ, Gₛ</td>
<td>[Ca²⁺]↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cAMP↓</td>
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</tbody>
</table>

### Table 2: Major phenotypes of prostanoid receptor deficient mice.

<table>
<thead>
<tr>
<th>Disrupted gene</th>
<th>Phenotypes</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>DP</td>
<td>Decreased allergic response in bronchial asthma</td>
<td>(208)</td>
</tr>
<tr>
<td>EP₁</td>
<td>Decreased tumor formation in colon</td>
<td>(395)</td>
</tr>
<tr>
<td>EP₂</td>
<td>Impaired ovulation and fertilization</td>
<td>(125,158,373)</td>
</tr>
<tr>
<td></td>
<td>Impaired regulation of blood pressure</td>
<td>(12,158,373,427)</td>
</tr>
<tr>
<td></td>
<td>Loss of bronchodilation</td>
<td>(325)</td>
</tr>
<tr>
<td></td>
<td>Impaired bone metabolism</td>
<td>(191)</td>
</tr>
<tr>
<td>EP₃</td>
<td>Impaired febrile response to pyrogens</td>
<td>(387)</td>
</tr>
<tr>
<td></td>
<td>Impaired gastric mucosal integrity</td>
<td>(361)</td>
</tr>
<tr>
<td></td>
<td>Impaired regulation of blood pressure</td>
<td>(12)</td>
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<td>Decreased inflammatory pain and swelling</td>
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THE MAPEG-SUPERFAMILY

The MAPEG (membrane associated proteins in eicosanoid and glutathione metabolism)-superfamily was defined according to enzymatic activities, sequence motifs and structural properties (141,142). The MAPEG-members are 16-18 kDa membrane-bound proteins with similar hydropathy profiles, which indicate that they have 3-4 membrane-spanning regions and a similar topology (Fig. 5A) (142). Multiple sequence alignment of the human MAPEG members demonstrates six strictly conserved amino acids (Fig. 5B). The family was found to consist of six human proteins, including 5-lipoxygenase activating protein (FLAP), leukotriene C₄ synthase (LTC₄S), MGST1, MGST2, MGST3 and MGST1-L1. Several members have also been identified in plant, i.e. (Arabidopsis thaliana, Oryza sativa and Ricinus communis), fungi (Aspergillus nidulans) and bacteria (Synechosystis, Escherichia coli and Vibrio cholerae).

Based on multiple sequence alignments, the MAPEG-family can be divided into four subgroups. FLAP, LTC₄S and MGST2 belong to one group and are crucial for leukotriene biosynthesis. MGST3, together with members in plant and fungi make up one group, while Escherichia coli and Vibrio cholerae belong to yet another group and MGST1 and MGST1-L1 make up the last group. MGST1, 2- and -3 all have glutathione transferase and glutathione –dependent peroxidase activities, possibly related to detoxification and protection against oxidative stress.

FLAP is hypothesized to act as a substrate provider for 5-lipoxygenase (5-LO) and is necessary for efficient 5-LO activity (77,201,215). LTC₄S specifically catalyzes the conjugation of LTA₄ (the 5-LO product) with GSH, thus forming LTC₄ (174,261,402). MGST2 and MGST3 have also been found to possess LTC₄S activities (139,140), but the activity of MGST3 was modest. Human MGST2 expression was localized in the liver, endothelial cells and lung membranes (320), while LTC₄S was mainly localized in lung membranes, platelets, eosinophils and mast cells. LTC₄S is specific for LTA₄, but MGST2 and MGST3 have broader substrate specificity. MGST2 and MGST3 can also catalyze GSH-dependent peroxidase activity against 5-HpETE, with apparent Kₘ of 7 µM and 21 µM, respectively (140). Furthermore, MGST2, but not MGST3 can catalyze conjugation of GSH to CDNB, implying that MGST2 and MGST3 are involved in detoxification and oxidative stress. MGST1 has wide substrate specificity and is broadly expressed, with the highest concentration in the liver (225). The substrates for MGST1 include halogenated arenes, like CDNB, various polyhalogenated hydrocarbons (5) and lipid hydroperoxides (224,233), suggesting a role in protection against oxidative stress (232). LTA₄ and other epoxides are poor substrates for MGST1 (226,345). Interestingly, LTC₄ is a tight-binding inhibitor of MGST1, but the function for this is not known (21). An overview of the MAPEG members in eicosanoid metabolism is presented in figure 1.
Figure 5A. Hydropathy plots of the human MAPEG members, based on the alignments in 5B.
### Figure 5B.

Multiple sequence alignments of the human MAPEG members. The groups are based on sequence similarity derived from a larger multiple sequence analysis made by Jakobsson *et al.* (141).

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|M| :Same within a group
|M| :Same in all
|M| :Same in two groups
AIMS OF THE PRESENT INVESTIGATION

mPGES-1 was initially identified as a homologue to microsomal glutathione transferase-1 (MGST1) with 37% identity on the amino acid sequence level. Before the function of mPGES-1 was known, it was referred to as MGST1-like 1 (MGST1-L1). Based on primary structure alignment and hydropathy plot studies, mPGES-1 was discovered to belong to the MAPEG-superfamily. mPGES-1 was found to specifically catalyze the conversion of PGH2 to PGE2 in the presence of glutathione. The aim of this study was to biochemically characterize human mPGES-1 and explore its relation to PGHS-2 in various pathological conditions.
METHODOLOGY

Common methods
I performed the following methods in the original articles; cell culture (paper I, II, III), subcellular fractionation (paper I, II, III, IV), gel electrophoresis and Western blotting (paper I, II, III, IV), Northern blotting (paper I), reverse phase high performance liquid chromatography (RP-HPLC), (paper I, II, IV, V), UV-spectroscopy (paper V), protein determination (paper I, II, III, IV, V) and ultracentrifugation (paper I, II, III, IV, V).

Assay development
In paper I, we used [3H]-PGH2 to measure PGES activity, however, with unsatisfactory quantitative results. Due to the unstable nature of PGH2, a quantitative and reproducible PGES assay was needed. Therefore, we developed a PGES assay, based on RP-HPLC and UV detection (paper II). Several compounds were tested as potential internal standards and 11β-PGE2 was found to be the most suitable candidate. 11β-PGE2 eluted with almost baseline separation from PGE2 and did not interfere with non-enzymatically formed PGF2α or PGD2 (paper II, fig. 1). 11β-PGE2 and PGE2 demonstrated identical UV absorbance properties at 195 nm. Equal amounts of 11β-PGE2 and PGE2 (as determined by GC/MS) were compared before and after solid phase extraction to test the recovery of the PGES assay (~90 %). To optimize the quantitative analysis and minimize interference by non-enzymatic degradation products, a stop-solution containing FeCl2 was added. FeCl2 terminates the reaction and converts PGH2 into mainly 12-HHT and malondialdehyde. The substrate (PGH2) was kept on CO2-ice until use to keep the non-enzymatic activity low. Solid phase extraction was performed immediately after terminating the reaction, since prostaglandins are more stable in organic solvent. Analysis was then performed, using RP-HPLC and UV-detection at 195 nm.

A purification protocol for recombinant human 6-histidine tagged (His6) mPGES-1 was developed. Human His6-mPGES-1, expressed in E. coli BL21(DE3) was purified in two steps by hydroxyapatite and immobilized metal affinity chromatography (Paper V). First, His6-mPGES-1 was completely solubilized in 4% Triton X-100, with preserved enzymatic activity in the solubilized extract. The solubilized extract was mixed with hydroxyapatite and after a 10-min incubation on ice, was subjected to a short centrifugation pulse. The supernatant, containing the unbound fraction was cleared by centrifugation and filtration. The cleared fraction was then loaded on a 1-ml HiTrap 10 chelating column (Amersham Biosciences), charged with Ni2+. The remaining unspecifically bound proteins were removed by a wash step of 60 mM imidazole and the purified histidine-tagged protein was finally eluted with 350 mM imidazole as a single peak. The eluted protein was immediately desalted on a HiPrep 26/10 desalting column (Amersham Biosciences). Human His6-mPGES-1 was purified to apparent homogeneity with a yield of 1.0-3.5 mg per liter of BL21(DE3) expression culture, when purifying from
whole cell lysate (Paper V, fig. 4). An important finding for successful purification was that GSH and glycerol had a stabilizing function for solubilized mPGES-1 (therefore included in all the buffers used for purification).

**Hydrodynamic studies**

To determine the molecular mass of mPGES-1, we performed hydrodynamic studies in presence of Triton X-100 (Paper V). The sedimentation coefficient of the mPGES-Triton X-100 complex was determined by the use of sucrose gradients. Purified mPGES-1 and marker proteins with known sedimentation coefficients were added on top of a 5-20 % sucrose gradient containing GSH and Triton X-100. The samples were then subjected to ultracentrifugation at 160,000 x g_{av} for 45 h at 20ºC. Fractions were collected and PGES activity, refractive index and protein content were measured (Paper V, fig. 9A). After plotting the activities and protein content, the sedimentation coefficient of mPGES-1 could be calculated by linear regression. The partial specific volume of the mPGES-Triton X-100 complex was determined by density equilibrium centrifugation. Purified mPGES-1 was added to a 20-50 % sucrose gradient containing GSH and Triton X-100. The tubes were centrifuged at 246,000 x g_{av} at 20ºC until equilibrium had been reached (72 h). Fractions were collected and the refractive index, i.e. sucrose content, was plotted against PGES activity (Paper V, fig. 9B). The sucrose content of the fraction with the highest PGES activity corresponded to the density of the mPGES-Triton X-100 complex and the density was inverted to yield the partial specific volume. The Stokes radius was determined by the use of gel exclusion chromatography. Purified mPGES-1 was loaded on a Sephacryl S-300 HR column together with marker enzymes (high molecular weight gel filtration calibration kit). The samples were eluted, collected and analyzed by measuring PGES activity and absorbance at 280 and 405 nm (Paper V, fig. 9C). The square root of –log K_{av} values were plotted against the known Stokes radii of the marker enzymes and the Stokes radius of the mPGES-Triton X-100 complex was obtained.

The sedimentation coefficient, partial specific volume and Stokes radius were then substituted into the Svedberg equation (Paper V, Eq. 1) and the molecular weight of the mPGES-1-Triton X-100 complex was calculated. To determine the amount of bound detergent in the complex, protein content and UV-absorbance was measured on the eluted fractions from the immobilized metal ion affinity column. The resulting weight of the mPGES-1-Triton X-100 complex agreed with a trimeric quaternary structure.
RESULTS

Paper I
Human mPGES-1 was cloned and characterized as a 16 kDa, inducible, GSH-dependent, microsomal PGE synthase. The tissue distribution of mPGES-1 was analyzed by Northern blot and high expression was found in two cancer cell lines, A549 and HeLa cells. mPGES-1 was also expressed at intermediate levels in reproductive organs. A rabbit anti-human antiserum was raised against the amino acids 59-74 of mPGES-1 and Western blot analysis specifically recognized a 15-to 16-kDa protein in the membrane fraction of bacteria expressed mPGES-1. PGES activity was measured after incubation with $[^3H] $-PGH$_2$ and analyzed by RP-HPLC with radioactivity detection. The membrane fraction contained a high GSH-dependent PGES-activity (0.25 µmol min$^{-1}$ mg$^{-1}$). A549 cells had been used earlier as a model system to study PGHS-2 induction by the proinflammatory cytokine IL-1$\beta$ and mPGES-1 was also induced by IL-1$\beta$ in these cells. Western blot analysis also detected a 16-kDa protein in sheep vesicular glands.

Paper II
In the following study, protein expression of PGHS-2 and mPGES-1 was further investigated in A549 cells after IL-1$\beta$ treatment. A time study of PGHS-2 and mPGES-1 expression revealed a coordinate induction of the enzymes. The observed induction was correlated with increased PGES activity in the microsomal fraction. Tumor necrosis factor-α (TNF-α) also induced mPGES-1 in these cells and dexamethasone was found to completely suppress the effect of both cytokines on mPGES-1 induction. A method based on RP-HPLC and UV-detection was developed to measure PGES activity. This method was used to screen for potential inhibitors of mPGES-1, including a small set of NSAIDs, stable PGH$_2$ analogues and cysteinyl leukotrienes. NS-398, Sulindac sulfide and LTC$_4$ were found to inhibit PGES activity with IC$_{50}$-values of 20 µM, 80 µM and 5 µM, respectively.

Paper III
In order to investigate the gene structure of mPGES-1, a P1 clone containing the gene for mPGES-1 was isolated and characterized. The mPGES-1 gene was localized on chromosome 9q34.3, spanning a region of approximately 15 kb and divided into three exons. The transcription start of the PGES gene has not been identified, but a 682 bp fragment directly upstream of the translation start site exhibited promoter activity when transfected in A549 cells. The putative promoter is GC-rich, lacks a TATA box at a functional site and contains numerous potential transcription factor binding-sites. Two GC-boxes, two tandem Barbie-boxes and an aryl hydrocarbon response element (AHR) were identified. The putative promoter region of mPGES-1 was transcriptionally active and reporter constructs were regulated by IL-1$\beta$ and phenobarbital.
Paper IV

Since PGE\textsubscript{2} had been demonstrated to play an important role in several pathological conditions like rheumatoid arthritis (RA), it was important to study the role of mPGES-1 in these conditions. Primary synovial cells were obtained from synovial tissues derived from patients suffering from RA. These cells were treated with IL-1\textbeta or TNF-\alpha and the expression of mPGES-1 and PGHS-2 were investigated by Northern and Western blot. Both cytokines induced mPGES-1 mRNA from a low basal level to a maximum level at 24 h. Moreover, the IL-1\textbeta induction was inhibited by dexamethasone in a dose-dependent manner. The protein expression of mPGES-1 was induced by IL-1\textbeta with a linear increase up to 72 h. PGHS-2 was also induced, however with an earlier peak expression (4-8 h). The protein expression of mPGES-1 correlated with PGES activity and demonstrated a 3-5-fold increase after IL-1\textbeta treatment. No PGES activities were found in the cytosolic fractions nor in the absence of GSH, ruling out any contribution of cytosolic PGE synthases and GSH-independent PGE synthases.

Paper V

In order to study the structure and biochemical function of mPGES-1, the protein was overexpressed as an N-terminal 6-histidine tag fusion protein in \textit{E.coli} BL21(DE3). Bacterial recombinant histidine-tagged mPGES-1 was solubilized with Triton X-100 and purified by a combination of hydroxyapatite- and immobilized metal affinity chromatography. mPGES-1 catalyzed a rapid GSH-dependent conversion of PGH\textsubscript{2} to PGE\textsubscript{2} and demonstrated a high \(k_{cat}/K_M\) (\textit{paper V, Table I}). mPGES-1 could also catalyze several other activities; GSH-dependent conversion of PGG\textsubscript{2} to 15-hydroperoxy PGE\textsubscript{2}, glutathione-dependent peroxidase activity towards cumene hydroperoxide, 5-HpETE and 15-hydroperoxy-PGE\textsubscript{2}, as well as conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) to GSH (\textit{paper V, Table II}).

Two-dimensional crystals of purified mPGES-1 were prepared and a projection map was determined by electron crystallography. Furthermore, hydrodynamic studies were performed on the solubilized mPGES-1 detergent complex (Triton X-100) to investigate the oligomerization of the protein. These two methods independently demonstrated a trimeric organization of mPGES-1.
DISCUSSION

Identification of mPGES-1

Human, microsomal, GSH-dependent prostaglandin E synthase (mPGES-1) was initially discovered as an EST-tag by database searches of enzymes involved in eicosanoid and glutathione metabolism. The EST-clone was found to code for a protein with 37% sequence homology to microsomal glutathione S-transferase-1 (MGST1), thus it was referred to as MGST1-like 1 (L1). The gene coding for MGST1-L1 was also independently identified as a p53 induced gene (PIG12), but no function was described (279). Based on the properties of MGST1-L1 regarding size, amino acid sequence, hydropathy and membrane localization, it was identified as a member of the MAPEG superfamily. In 1997, Burgess and Reddy reported on a 16.5 kDa, microsomal GSH-dependent PGF$_2\alpha$ synthase in sheep vesicular glands (43) and since the PGF$_2\alpha$ synthase possessed certain properties similar to other MAPEG members, PGH$_2$ was tested as a substrate for several of the members, including MGST1-L1. MGST1-L1 did not produce any PGF$_2\alpha$, but showed a specific formation of PGE$_2$. Hence, MGST1-L1 was referred to as microsomal PGES-1 (paper I). An antiserum raised against an internal peptide of mPGES-1 also recognized a protein of similar size in microsomes from sheep vesicular glands, which are known to contain a highly efficient microsomal PGES, indicating that mPGES-1 was the long-sought membrane bound PGES (Paper I).

Biochemical characterization of mPGES-1

*Basal expression of mPGES-1*

We examined the expression of mPGES-1 in human tissues, and Northern dot blot analysis of mPGES-1 mRNA demonstrated a low expression in most tissues, medium expression in reproductive organs and a high expression in two cancer cell lines (A549 and HeLa) (Paper I). A low basal expression of mPGES-1 has also been observed in several rat tissues, but high constitutive expression was seen in the stomach (202). A high expression of mPGES-1 was also found in the thymus of rat as well as epithelial cells of the human thymus (297). The highest constitutive expression of mPGES-1 in mouse was observed in the ovary, urinary bladder and kidney (98). Furthermore, mPGES-1 has been detected in conjunction with ovulation and fertilization (84,183). Impaired fertility was seen in mice lacking the EP$_2$ receptor (125,158) and PGHS-2 (76,227). Furthermore, mPGES-1 has been implicated in bone metabolism as well as certain metabolic bone diseases like osteoporosis (54,244,303). mPGES-1 may therefore participate in normal physiology, especially in renal function, bone metabolism, reproduction and maintenance of the gastrointestinal mucosa. However, it should be noted that mPGES-1 null mice do not demonstrate any phenotypic changes in these organs and functions, suggesting that other non-inducible PGE synthases can substitute for mPGES-1 in normal physiology (374,381).
Regulation of mPGES-1 expression

A549 cells were reported to produce more PGE₂ after cytokine treatment (133,217) and fibroblasts treated with LPS or platelet derived growth factor (PDGF) contained high microsomal PGES activity (157,207). Since A549 cells were earlier used as a model system for PGHS-2 induction by IL-1β and mPGES-1 mRNA was highly expressed in these cells, it was logical to study mPGES-1 protein expression in this model.

Non-treated A549 cells had a low expression of mPGES-1, but the protein expression was induced significantly by IL-1β (Paper I). The basal expression of mPGES-1 in rat was also very low, but could be dramatically induced by LPS in various organs (202,244,418). These data suggested that the induction of PGHS-2 and mPGES-1 could be co-regulated. We examined this possibility in a time study of PGHS-2 and mPGES-1 expression in A549 cells. A coordinate induction of PGHS-2 and mPGES-1 protein expression was observed in these cells after treatment with IL-1β with a linear increase up to 72h (Paper II). Various proinflammatory stimuli have been shown to co-ordinately induce mPGES-1 and PGHS-2 in several types of cultured cells, often associated with increased PGE₂ production (Paper II and IV) (202,244,346). Furthermore, induction of mPGES-1 was seen after β-amyloid treatment of astrocytes (311) and hormonal stimulation of ovarian follicles (84). A summary of mPGES-1 induction in various tissues and cells is presented in table 3.

Glucocorticoids are known to reverse inflammatory processes, and cytokine-induced mPGES-1 expression was abolished by dexamethasone in A549 cells (Paper II). Inhibition of mPGES-1-induction by dexamethasone has also been reported in other cell types, such as macrophages and synoviocytes (Paper IV) (244). Even though mPGES-1 and PGHS-2 appear to be co-regulated in many cases, their rate of induction differ in some cell systems, indicating separate regulatory mechanisms of induction (Paper IV). Co-expression of PGHS-2 and mPGES-1 have been observed in various pathological conditions and diseases; including pain, fever, inflammation, cancer and Alzheimer’s disease, suggesting a functional link between these enzymes for efficient prostaglandin production (Paper II and IV), (78,202,244,374,381,418).
### Table 3: Induction of mPGES-1 in various tissues and cells.

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<td>Mouse, osteoblastic cells</td>
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**Functional coupling of mPGES-1 and PGHS-2**

The reports about coordinate induction of mPGES-1 and PGHS-2 implicated that they were functionally linked ([Paper II and IV](#)), (244). In support for this, AA-treated HEK293 cells, co-transfected with mPGES-1 and PGHS-2 produced several times more PGE₂, compared to when mPGES-1 or PGHS-2 were expressed alone (244). Furthermore, *in vivo* studies of rats demonstrated that PGHS-2 inhibitors reduced formation of PGE₂ more efficiently compared to other prostaglandins (112). Also, several reports have demonstrated co-localization of mPGES-1 and PGHS-2 in the endoplasmatic reticulum and perinuclear membrane (182,244,418). This is in agreement with our studies of IL-1β induced A549 cells, where the only PGES activity was found in microsomes and only in the presence of GSH ([paper II](#)). Recently, mPGES-1 was also shown to specifically convert PGG₂ to 15-hydroperoxy PGE₂, suggesting an alternative pathway for PGE₂ biosynthesis ([paper V](#)). Although this has not been observed *in vivo*, it implies yet another functional link between PGHS-2 and mPGES-1.

However, our study on synoviocytes isolated from patients with RA, suggest that mPGES-1 and PGHS-2 expression is regulated by different mechanisms ([paper IV](#)). IL-1β increased the expression of mPGES-1 in a linear manner up to 72h, while the expression of PGHS-2 reached a maximum already after 4-8h. The IL-1β induced PGHS-2 expression seems to involve NF-κβ, but the mPGES-1 promoter does not contain any such NF-κβ site. Furthermore, the 3’-region of mPGES-1 lacks the AUUUA instability sequences found in the PGHS-2 gene. Also, cytokine-induced expression of mPGES-1 was recently demonstrated to be regulated by Egr-1 (early growth response-1) (253), mediated by the p38 mitogen–activated protein kinase pathway (299), implying
that PGHS-2 and mPGES-1 can be induced by the same kind of stimuli, but use different signaling pathways.

**Gene structure and expression of mPGES-1**

The primary structures of human, mouse and rat mPGES-1 demonstrate a high degree of amino acid sequence homology (>80%). The gene organization of human mPGES-1 is similar to MGST1, but differs from other MAPEG members that contain additional exons ([paper III](#)) (156). The putative promoters of both mPGES-1 and MGST1 are GC-rich, lacks a TATA-box at a functional site and contain several potential transcription binding sites. A number of potential transcription factor binding-sites were identified in the promoter of mPGES-1, *i.e.* two GC-boxes, two tandem Barbie-boxes and an aryl hydrocarbon response element (AHR) ([paper III](#)). The putative promoter region of mPGES-1 was shown to be transcriptionally active and could be induced by IL-1β and down-regulated by phenobarbital. If physiologically relevant, the mPGES-1 gene is the first gene known to be repressed by phenobarbital.

However, the tandem Barbie-boxes were recently shown not to be involved in the down-regulation of mPGES-1 by phenobarbital (79). Phenobarbital is believed to act on a *cis*-acting PB-Responsive Enhancer Module (PBREM) element (130), but the mechanism behind this down-regulation has not been elucidated. Also, Sp1 and Sp3 were shown to be responsible for basal expression of mPGES-1, but only Sp1 was needed for basal MGST1 expression (79).

There are several reports that indicate that both PGE₂ and PGD₂ participate in regulation of sleep and wakefulness. PGD₂ has been shown to be the most potent endogenous sleep-promoting substance (386), while PGE₂ has been suggested to counteract the effect of PGD₂ (115,116). PGE₂ is also present at higher levels in the brain during wakefulness (92). Phenobarbital is an effective sedative and anticonvulsant and since we found that it reduced mPGES-1 promoter activity, it may indicate that mPGES-1 is involved in sleep and wakefulness ([paper III](#)). However, this remains to be investigated and mPGES-1 null-mice will be a useful tool to study this hypothesis.

The gene and primary structure of mouse mPGES-1 were recently characterized (253). The organization of the mouse mPGES-1 gene was similar to the human mPGES-1 gene, regarding exons/introns and lack of a functional TATA box. Several consensus *cis*-acting elements were found within 1.8 kb upstream of the mouse mPGES-1 gene, including C/EBPα and –β, AP-1, two tandem GC-boxes, three putative glucocorticoid response elements and two progesterone response elements (253). The tandem GC-boxes in the mPGES-1 promoter were shown to play a major role in regulating its inducible transcription. Electro mobility shift assay (EMSA) studies of the mouse mPGES-1 promoter region indicated that the GC-boxes did not bind Sp1 or Sp3, but bound an inducible zinc finger protein, called Egr-1, instead. Egr-1 was demonstrated to be a key transcription factor in regulating the inducible expression of mPGES-1. Cytokine-induced mPGES-1 expression was demonstrated to be regulated by Egr-1, mediated by the p38 mitogen–activated protein kinase pathway (299). Interestingly, no binding site for NF-κβ, CRE or E-box has been found in the mPGES-1 promoter, as seen
in PGHS-2 induction. Thus, it indicates that the mechanisms for induction of mPGES-1 and PGHS-2 are different.

**Steady state kinetics of mPGES-1**

mPGES-1 specifically converts PGH₂ to PGE₂, but only in the presence of GSH, which is absolutely required for activity and GSH can not be substituted by other thiols (paper V), (271). Purified, bacteria expressed, mPGES-1 demonstrated an efficient catalytic conversion of PGH₂ with a $V_{\text{max}}$ value of 170 µmol min⁻¹ mg⁻¹ at 37°C (paper V, Table I and II). The apparent $K_M$ for mPGES-1 was 0.16 mM and is comparable to $K_M$ for other PG-synthases (paper V, Table IV). Furthermore, in line with our data, Lazarus et al. observed a $K_M$ of 130 µM for mouse mPGES-1, expressed in *Escherichia coli* (182). Also, our preliminary data suggest a high $K_M$ for PGH₂ in IL-1β-induced A549 cells (unpublished). The apparent $K_M$ for GSH was 0.7 mM, determined at a concentration of 400 µM PGH₂ (paper V, Table I).

In 1974, Samuelsson et al. proposed that PGE₂ could be formed through isomerization of PGH₂ or by isomerization of PGG₂ to 15-hydroperoxy-PGE₂, with subsequent reduction to PGE₂ (309). In the search for additional activities, we investigated whether the unstable intermediate PGG₂ could be isomerized by mPGES-1. Incubation with PGG₂ actually demonstrated an even more efficient conversion of PGG₂ to 15-hydroperoxy-PGE₂ ($V_{\text{max}}$: 250 µmol min⁻¹ mg⁻¹) in the presence of GSH, as compared to PGH₂ (paper V, Table I and II). The importance of PGG₂ as an alternative substrate for mPGES-1 will be further discussed below.

Independently from our study, Ouellet et al. reported on the purification of mPGES-1 expressed in a baculovirus system (271). They also observed a high PGES-activity, but a lower $K_M$ compared to us, in the presence of dodecylmaltoside ($V_{\text{max}}$: 38 µmol min⁻¹ mg⁻¹, $K_M$: 14 µM at 0°C). The discrepancies in activity and $K_M$ for mPGES-1 may depend on differences in the enzyme activity assay, the temperature and the type of detergent used. Other explanations can be differences in post-transcriptional modifications and lipid composition between prokaryotic and eukaryotic cells. There are actually several putative phosphorylation sites in the mPGES-1 sequence that might be important for modulation of mPGES-1 (unpublished).

**Glutathione as cofactor**

Reduced glutathione (GSH) is a $\gamma$-glutamylcysteinylglycine-tripeptide, which is quite abundant within cells (~ 5 mM). GSH is essential for maintaining the structure of red blood cells and for keeping hemoglobin in the ferrous state, but is also involved in detoxification reactions with hydrogen peroxide and organic peroxides. GSH is absolutely required for mPGES-1 activity (paper I), with an apparent $K_M$ of 0.7 mM (paper V, Table I), but also has a stabilizing effect on solubilized mPGES-1. Furthermore, GSH could not be substituted by other SH-reducing agents for mPGES-1 activity (paper V) and does not seem to be oxidized during mPGES-1 catalysis (271).
Speculations on PGE₂ biosynthesis

mPGES-1 efficiently catalyzed a GSH-dependent conversion of PGG₂ to 15-hydroperoxy-PGE₂ (15-HpPGE₂) (paper V, Table II). This activity suggests an alternative pathway for the synthesis of PGE₂ (paper V, fig 10). Either, PGG₂ is converted into 15-hydroperoxy-PGE₂ by mPGES-1 with subsequent reduction to PGE₂, non-enzymatically or enzymatically by glutathione-dependent peroxidases (9,262) or alternatively by the POX activity of PGHS-1/-2.

Since the two active sites of PGHS are spatially well-separated, PGG₂ has to diffuse from the COX site to the other side of the enzyme to reach the POX pocket (Fig. 6A) (337). This may not be the most efficient route for the unstable PGG₂ metabolite. An alternative pathway for PGE₂ production could occur through shuttling of the intermediate between PGHS and mPGES-1. Possibly, PGG₂ is transferred to mPGES-1 instead of diffusing to the POX pocket. mPGES-1 could then convert PGG₂ to 15-hydroperoxy-PGE₂, which is subsequently shuttled back to PGHS-2, where the reduction to PGE₂ occurs (Fig 6B).

Biochemical topology studies on MGST1 have demonstrated that the active site is located on the cytoplasmic side of the ER (6). According to the “positive-inside rule” and topology predictions of prokaryotic MAPEG members (unpublished), the N- and C-terminals are located on the periplasmic side of prokaryotic cells, which corresponds to the luminal side of the ER in eukaryotic cells (67,333,414). In line with this, hydropathy plots of the MAPEG members indicate that the N- and C-terminals of each protein are located on the same side of the membrane (Fig. 5A) (141). Furthermore, mutational work on the hydrophilic loops of LTC₄S (175) and affinity studies of MK-886 on FLAP (203) indicate that the loops responsible for activity are located on the same side of the membrane. Together, these data implies that the active site of mPGES-1 could be located on the cytoplasmic side of the ER.

FLAP is involved in the transfer of AA to 5-LO and is necessary for efficient 5-LO activity (77,201,215). In analogy with FLAP, interaction between PGHS-2 and mPGES-1 could hypothetically facilitate more efficient PGE₂ biosynthesis. Transfection of FLAP into certain cell systems has been reported to increase PGHS-2 expression and PGE₂ biosynthesis (24). However, FLAP has no PGH₂ metabolizing capacity (unpublished results).
Figure 6. Hypothetical pathways for PGE\textsubscript{2}-biosynthesis.

**Speculations on mPGES-1 catalysis**
Several mechanisms have been proposed for the GSH-dependent conversion of PGH\textsubscript{2} to PGE\textsubscript{2}. These mechanisms include nucleophilic attack by the thiolate anion of glutathione. In 1971, Lands et al. proposed a mechanism with a GSH-assisted hydride shift (177). However, this reaction involves the unstable intermediate, thiohemiketal. More likely, the nucleophilic thiolate anion will attack the peroxide oxygen on C-9, thus forming an adduct of GSH (or enzyme cysteine thiol) and PGH\textsubscript{2}, with subsequent enzyme-assisted deprotonation of C-9, producing PGE\textsubscript{2} and thiolate anion (Fig. 7A). Possibly, another GS\textsuperscript{−} (or enzyme cysteine thiolate) in solution could act as a base in the second step. This is in agreement with the proposed mechanism of hematopoietic PGDS (151). The mechanism for mPGES-1 could also proceed in a concerted manner by abstraction of the proton on C-9 by a glutathione or enzyme cysteine thiolate, followed by isomerization into PGE\textsubscript{2} (Fig. 7B). These mechanisms of mPGES-1 involve activation of reduced GSH, which is in line with our observation of a Meisenheimer complex with mPGES-1 (unpublished results). A tyrosine has been suggested to activate GSH in several cytosolic GSTs (305), hematopoietic PGDS (151) (α-class cytosolic GST), cPGES/p23 (367) and also in LTC\textsubscript{4}S (Tyr93) (175). However, mutation of the highly conserved Tyr117 in mPGES-1 did not affect catalytic activity (244) and neither did mutations of tyrosines in MGST1 inhibit catalysis (399). The amino acids responsible for GSH-binding and catalytic activity in mPGES-1 and MGST1 have not yet been identified. However, N-ethylmaleimide (NEM) activates MGST1 and inhibits mPGES-1, implying that a Cys or Ser participates in the mechanism of MGST1 and mPGES-1 (Paper V), (399). Recently, Watanabe et al. demonstrated that Cys110 is essential in the active site of mPGES-2 (398). A mechanism involving Cys110 was proposed, similar to the one in
figure 7A where Cys110 attacks the peroxide linkage at C-9, instead of activated GSH. Arg110 is strictly conserved in all MAPEG members and when it was replaced with Ser in mPGES-1, the catalytic function was abrogated, implying an essential role (244). In LTC₄S, Arg51 has been proposed to function as a proton donor in the opening of the LTA₄ epoxide for conjugation with GSH (175). However, mutation of the corresponding amino acid in mPGES-1 (Arg70) did not affect the PGES-activity.

![Figure 7A](image)

![Figure 7B](image)

**Figure 7.** Proposed mechanisms for PGE₂ formation by mPGES-1.

_Inhibition of mPGES-1_

We tested some common NSAIDs, stable PGH₂-analogues and cysteinyll leukotrienes in vitro as putative inhibitors of mPGES-1. The mPGES-1 activity was inhibited by LTC₄, NS-398 (a specific PGHS-2 inhibitor) and sulindac sulfide with IC₅₀-values of 5, 20 and 80 µM, respectively (Paper V). mPGES-1 was also inhibited by MK-886, a FLAP inhibitor with an IC₅₀-value of 3 µM (202). MK-886 binds to the AA-binding region of FLAP, which is highly conserved in LTC₄S and mPGES-1 and could possibly be involved in the binding of eicosanoids (203). The motif ERXXAXXNXXD/E might represent a consensus sequence for interaction with AA and/or other eicosanoids (202). Furthermore, 15-deoxy-Δ₁₂,₁₄-PGJ₂, arachidonic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were recently reported to inhibit mPGES-1 with similar IC₅₀-values (0.3 µM) (282). This observation suggests a novel mechanism of action for
the anti-inflammatory effects of DHA, EPA and 15-deoxy-Δ^{12,14}-PGJ_2. The main target for 15-deoxy-Δ^{12,14}-PGJ_2 is believed to be the peroxisome proliferator-activated receptor-γ (PPAR-γ) (145,292), but can also inhibit IL-1β-induced PGE_2 formation by a PPAR-γ-independent way in rheumatoid synoviocytes (379). The PPAR-γ-independent anti-inflammatory effect of 15-deoxy-Δ^{12,14}-PGJ_2 could at least partially be explained by inhibition of mPGES-1.

15-deoxy-Δ^{12,14}-PGJ_2 is formed from PGHS-2 derived PGD_2 through a series of dehydration reactions (Fig. 3). The presence of 15-deoxy-Δ^{12,14}-PGJ_2 in vivo has been demonstrated by immunohistochemistry on LPS-treated macrophages and macrophages in atherosclerotic plaques (329). Formation of 15-deoxy-Δ^{12,14}-PGJ_2 has also been reported during resolution of inflammation in carrageenin-induced pleurisy in rats (94).

Furthermore, the activity of mPGES-1 was not inhibited by CDNB, a common substrate for GSTs (244), in contrast to cPGES (367).

**Purification of mPGES-1**

Previous attempts to isolate GSH-dependent membrane-bound PGES were only partially successful (223,264,363). The main obstacle was the instability of the protein, which lost activity rapidly after purification. Recently, we managed to purify human mPGES-1 by a combination of hydroxyapatite and immobilized metal affinity chromatography (Paper V). The expression system of histidine-tagged mPGES-1 was quite efficient for a membrane-bound protein, as seen by the amount of pure enzyme recovered and purification factor (up to 2% of the total membrane protein).

Earlier purification attempts of mPGES-1 demonstrated that Triton X-100 was a suitable detergent for solubilization, but higher concentrations (>1.5%) led to a decreased recovery of activity (363). However, recombinant histidine-tagged mPGES-1 was completely solubilized by 4% Triton X-100, with preserved enzymatic activity in the solubilized extract when GSH and glycerol were included in the buffer (Paper V, fig. 2A).

Another purification protocol of mPGES-1, based on hydroxyapatite column chromatography, has been described (271). One percent diheptanoylphosphatidylcholine (DHPC) was used for solubilization of Sf-9 cell membranes and gave 72% recovery of the mPGES-1 activity. Since DHPC is rather expensive, the purification of mPGES-1 was conducted in the presence of 1% octylglucoside instead. These expression systems and purification protocols for mPGES-1 provide useful tools for future crystallization and mechanistic studies.

**Quaternary structure of mPGES-1**

2-D crystallization attempts on MGST1 have previously been successful (117) with increasingly higher resolutions of the projection structure (317,318). Purified histidine-tagged mPGES-1 in 1% Triton X-100, was successfully crystallized by adding phospholipids prior to reduction of the detergent content (Paper V). Triton X-100 was used for solubilization of bacteria expressed mPGES-1 and fortunately, the same detergent was suitable for 2-D crystallization. Another important factor for successful crystallization of
mPGES-1 was the use of crystallization conditions similar to those successful for MGST1. A 10 Å projection structure of mPGES-1 was obtained after several steps of image processing (Paper V, fig. 8). The molecular weight of his6-mPGES-1 in relation to the unit cell size implied that mPGES-1 forms a trimer in the crystal. Optimization of the 2-D crystallization conditions will hopefully lead to a high resolution structure of mPGES-1 in the future.

We have performed hydrodynamic studies on the mPGES-1-Triton X-100 complex (Paper V). To determine the molecular mass of the complex, the sedimentation coefficient, partial specific volume and Stokes radius were measured. A sedimentation coefficient of 4.1 S, partial specific volume of 0.891 cm³/g and a Stokes radius of 5.09 nm were obtained and the Svedberg equation was then used to calculate the molecular mass of the mPGES-1-Triton X-100 complex, which was found to be 215 000. The detergent content of the mPGES-1-Triton X-100 complex was 2.8 g Triton X-100 /g protein and after subtracting the values for the detergent content our calculations match with a trimeric quaternary structure (Paper V). Thus, two independent methods indicate that mPGES-1 has a trimeric quaternary structure. This is in line with studies on the closely related MGST1, which also has been demonstrated to be a trimer (41,117,198,400). The quaternary structure of FLAP and LTC₄S remains to be determined.

**Additional activities of mPGES-1**

**Glutathione dependent peroxidase activity**

Since MGST1, -2 and -3 are GSH–dependent peroxidases (142), mPGES-1 was tested with several peroxide substrates. mPGES-1 was found to reduce 15-hydroperoxy-PGE₂ to PGE₂ in presence of GSH, albeit at a low catalytic rate (0.04 µmol min⁻¹ mg⁻¹) (Paper V, Table II). Non-enzymatic production of PGE₂ from 15-hydroperoxy-PGE₂ was significant. GSH is present intracellularly at mM concentrations under normal physiological conditions and might be sufficient for non-enzymatic reduction of 15-hydroperoxy-PGE₂ (341). Alternatively, GSH-dependent peroxidases or PGHS-1/-2 could reduce 15-hydroperoxy-PGE₂ to PGE₂. In addition, mPGES-1 showed a modest GSH-dependent activity against 5-HpETE (0.04 µmol min⁻¹ mg⁻¹), but catalyzed the GSH-dependent peroxidase activity towards cumene hydroperoxide more efficiently (0.17 µmol min⁻¹ mg⁻¹) (Paper V, Table II). In fact, this activity was in the same range as with recombinant rat MGST1 (401). These data indicate that mPGES-1 might be involved in protection against oxidative stress, but since the peroxidase activities of mPGES-1 are low compared to the PGES activity, they probably reflect the relationship with other MAPEG enzymes.

**Glutathione transferase activity**

Several proteins with PGES-activity also have GST-activity towards CDNB, like the two anionic forms of cytosolic PGH-E isomerases (265) and the cytosolic Mu-class glutathione transferases M2-2 and M3-3 (32). Since several of the MAPEG members
also possess glutathione transferase activity (141,142,225), it was of interest to study GST-activity on mPGES-1. mPGES-1 catalyzed a small, but significant CDNB-GSH conjugating activity (0.8 µmol min⁻¹ mg⁻¹) (Paper V, Table II). However, no GST-activity against LTA₄ was detected with mPGES-1 (unpublished). The GST-activity of mPGES-1 might also reflect the relationship with other MAPEG enzymes.

**mPGES-1 in pathology**

The collective data from knock-out and pharmacological studies of PGHS-2 (76,190,192,227,231,270) and EP-receptors (125,158,216,303,321,387,394) clearly suggest involvement of PGE₂ in various pathological conditions. Several enzymes with PGES activity have been identified and cloned, but mPGES-1 is the most active, inducible PGES enzyme reported thus far (Paper V, Table IV), (271) and constitutes a highly probable candidate for PGE₂ production in pathology. mPGES-1 null mice demonstrated a normal phenotype regarding fertility and number of offspring (374,381), unlike the PGHS-2 null mice, which showed deficient renal and reproductive functions (76,227). Likely, other proteins with PGES activity are involved in fertilization and development. Importantly, the mPGES-1-deficient mice completely lost the capability to produce PGE₂ over baseline levels after LPS-treatment in vivo (381). Recently, Trebino et al. studied mPGES-1 null mice in several pathological models and observed a marked reduction of inflammatory responses and pain, as compared with wild-type mice (374).

**Inflammation**

The classical signs of acute inflammation are pain, swelling, heat generation and local reddening and loss of function (dolor, tumor, calor, rubor and functio laesa). NSAIDs diminish pathological symptoms related to inflammation by inhibition of prostaglandin formation (206,228,391). PGE₂ is formed in large quantities at sites of inflammation and can mediate several pathological features of inflammation (181). PGE₂ is a potent vasodilator (407) and acts synergistically with histamine and bradykinin to increase microvascular permeability with edema as a consequence (70,284). These vascular changes also result in local warmth, erythema and inflammatory pain.

Trials with PGHS-2 specific inhibitors have confirmed the dominant role of PGHS-2 in producing proinflammatory prostaglandins (64). Furthermore, genetic deletion of PGHS-2 prevents the development of autoimmune arthritis (248). Both PGHS-2 and mPGES-1 are induced by proinflammatory stimuli, like IL-1β, TNFα and LPS both in vitro (Paper I, II and IV) (244,346) and in vivo (78,202,244,374,381,418), implying an important role for these enzymes in inflammatory conditions. Also, induced mPGES-1 expression was inhibited by the anti-inflammatory glucocorticoid dexamethasone (Paper II and IV), (202,244). mPGES-1 was recently demonstrated to be overexpressed in synovial tissues from patients with RA (Westman, 2003, submitted). Furthermore, Trebino et al. studied the pathogenesis of collagen-induced arthritis in mPGES-1 deficient mice. They demonstrated the importance of mPGES-1 in both acute and chronic PGE₂-dependent inflammation in vivo (374). These data clearly strengthen
the importance of mPGES-1 in inflammatory processes. mPGES-1 represents an important, “overlooked” drug target for the treatment of inflammatory disease. This is probably due to the seemingly opposed modes of action by PGE_2. For instance, PGE_2 is both proinflammatory and promotes resolution of inflammation (20). Furthermore, PGHS-2 derived prostanoids, like 15-epi-lipoxins and resolvins have been demonstrated to promote resolution of inflammation (94,188,323). Inhibition of mPGES-1 will thus theoretically spare the beneficial prostanoids, including physiologically important PGE_2.

Pain
Prostaglandins are involved in pain as shown by the antinociceptive effect of NSAIDs and studies with exogenously added prostaglandins, which are able to induce hyperalgesia and allodynia (161). The importance of PGE_2 in inflammatory pain has also been demonstrated using selective anti-PGE_2 antibodies that inhibit pain sensitization, edema and hyperalgesia in rats (220,280). Peripheral nociceceptor terminals are sensitized by PGHS-2 derived PGE_2 and produce localized pain hypersensitivity (210). Peripheral inflammation also affects the neighboring tissue and causes pain hypersensitivity (secondary hyperalgesia) and can also cause diffuse muscle and joint pain, fever, fatigue and anorexia (69). These illness symptoms were thought to occur by a brain-regulating mechanism involving nerve impulses from the injured region through the spinal cord to the brain (415). However, Samad et al. (306) and Ek et al. (78) independently proposed that nerve impulses are not involved, but it is the proinflammatory cytokine, IL-1β that signals the brain about local inflammation. PGHS-2 seems to be involved in these central nervous system (CNS) responses, since it is induced by IL-1β in the spinal cord neurons, elevating PGE_2 levels in the cerebrospinal fluid (306). Knock-out studies of prostanoïd receptors have shown that IP and EP_3 are the major prostaglandin receptors, mediating enhanced acetic acid-induced writhing response in LPS pre-treated mice, i.e. in endotoxin-enhanced inflammatory nociception (382). Furthermore, spinal EP_1 and EP_3 receptors have been demonstrated to mediate PGE_2-induced allodynia and hyperalgesia, respectively (216). Recently, knock-out studies demonstrated that mPGES-1 is involved in mediating acute pain in inflammatory processes (374). Acetic acid-induced pain was reduced by 50% in mPGES-1-null mice, similar to NSAID-treated control mice. These data implicate an important role for mPGES-1 in inflammatory pain.

Fever
Fever is a CNS-controlled rise of body temperature in response to infection or inflammation (310). At the site of inflammation, activated immune cells produce proinflammatory cytokines, including IL-1, IL-6, and TNF-α, which reach the bloodstream and target the brain (80). Fever can be suppressed by NSAIDs, implying that prostaglandins are involved in fever generation. Indeed, PGE_2 has been shown to play a critical role in the CNS where it acts on EP_3 receptors (387). Studies using selective PGHS-2 inhibitors and knock-out mice have shown that PGHS-2, but not PGHS-1, is involved in suppression of PGE_2-formation, associated with a decrease in fever (47,190).
The role of mPGES-1 in fever was recently elucidated (78,418). Intravenous injection of rats with IL-1β or LPS induced mPGES-1 messenger RNA in vascular cells throughout the brain (78,418). PGHS-2 was also induced with a more transient up-regulation of mRNA. Furthermore, *in situ* hybridization and immunohistochemistry revealed a co-localization of mPGES-1 and PGHS-2 after IL-1β treatment, indicating an efficient PGE2 production. mPGES-1 mRNA was also co-expressed with mRNA coding for the interleukin-1 receptor (IL-1 R) in blood brain barrier endothelial cells (78). Furthermore, recent data on mPGES-1 (-/-) mice have confirmed that this enzyme is critical for fever generation (Engblom et al., 2003, submitted). The current mechanism of PGE2 synthesis in fever-mediation is believed to proceed through a series of steps; circulating IL-1β binds to IL-1 receptors on the luminal surface of endothelial cells, resulting in PGHS-2 and mPGES-1 expression, followed by PGE2 synthesis. Due to the amphipathic properties of PGE2, it can then diffuse into the parenchyma where it binds to prostaglandin-sensitive neurons in the ventromedial preoptic area (VMPO), leading to fever (82).

**Rheumatoid arthritis**

Rheumatoid arthritis (RA) is a common chronic inflammatory and destructive joint disease with a prevalence of 0.5-1%. Typical symptoms are joint swelling and pain caused by the inflammatory process, eventually leading to destruction of the joint (343). The cause of RA is not entirely understood, but it has been speculated whether the disease can be triggered by infectious agents (332). RA is regarded as an autoimmune disease and there is a strong association to a number of autoantibodies (351). Proinflammatory cytokines, like TNF-α, IL-1 and IL-6 play a central role in pathogenesis of RA (71,85). Several proinflammatory cytokines like IL-1β and TNF-α are known to induce the production of PGE2 in RA at sites of inflammation (Paper IV) (296). There is evidence for PGE2 as a mediator of inflammation in arthritis (63). PGE2 affects tissue remodeling at sites of chronic inflammation and can also modulate the immune system. PGE2 is also involved in inflammatory angiogenesis (27), bone destruction (296) and induction of matrix metalloproteinases (MMPs) in RA (213,324).

During the last couple of years, a number of reports have described the importance of PGHS-2 and mPGES-1 in the pathology of RA and models of RA. High expression of PGHS-2 has been seen in RA (66,152,185,331) and PGHS-2-specific inhibitors clearly have a great impact on pain and inflammation (64,155). Induced co-expression of PGHS-2 and mPGES-1 was recently seen in human synovial cells after treatment with proinflammatory cytokines (Paper IV) (162). Several models of RA have implicated the importance of mPGES-1. For instance, induction of mPGES-1 was seen in a rat-adjuvant induced arthritis model (58,202). This arthritis model was also demonstrated to have a systemic effect, inducing mPGES-1 in the endothelial cells along the blood-brain barrier and in the parenchyma (81). The unambiguous involvement of mPGES-1 in RA was demonstrated in a collagen-induced arthritis model, using mPGES-1 (-/-) mice (374). Recently, another report demonstrated that mPGES-1 is
overexpressed in synovial tissues from patients with RA (Westman et al., 2003, submitted).

Cancer
There is clinical, biochemical and genetic evidence for the importance of PGHS-2-derived PGE₂ in the development of colorectal cancer and also possibly other cancers (406). A number of epidemiological studies indicate that chronic use of NSAIDs lowers the incidence and mortality rate for colorectal cancer in humans and in animal models (204,283,326). PGHS-2 is highly expressed in various transformed cell lines and tumor tissues (154,355) and treatment with selective PGHS-2 inhibitors reduces the adenoma burden in both humans (350) and animals (286). Also, overexpression and antisense suppression have demonstrated that PGHS-2 is involved in the progression of several cancer forms (195,380).

More direct evidence for the importance of PGHS-2 and PGE₂ in colorectal tumorigenesis was found in gene targeting studies. In a human model for familial adenomatous polyposis, gene disruption of PGHS-2 (270) or the EP₂ (348) receptor resulted in reduction of the number of intestinal polyps. In another model, disruption of the genes for EP₁ (395) or EP₄ (247) suppressed the development of carcinogen-induced colorectal cancer. Furthermore, gene disruption of cytosolic PLA₂ also lead to reduced polyposis in Apc mutant mice (129,360). Angiogenesis, important for tumor progression, was markedly suppressed in EP₃ (-/-) mice, in a model that mimics tumor-stromal angiogenesis (4).

Both PGHS-2 and mPGES-1 are needed for efficient PGE₂-biosynthesis. This has been shown in a human embryonic kidney cell line (HEK293) and when co-transfected with mPGES-1 and PGHS-2, but not PGHS-1, showed an aggressive growth and aberrant morphology (244). In a following study, co-expression of mPGES-1 and PGHS-2 resulted in colony formation in soft agar culture and tumor formation when implanted into nude mice (150).

However, when HEK293 cells were incubated with PGE₂ for at least two weeks, no change in morphology was seen (244). This is contradictive to what has been seen in human colorectal carcinomas where PGE₂ treatment led to increased growth, motility and change in morphology, possibly mediated through the EP₄ receptor (327,328) and must be further investigated.

Since mPGES-1 was found to possess other activities besides PGES activity, other metabolites produced by mPGES-1 could possibly contribute to procarcinogenic effects (Paper V). For example, the biological function of 15-hydroperoxy-PGE₂ is not known and is a possible candidate.

mPGES-1 was also overexpressed in colon cancer, lung cancer and endometrial carcinoma (138,424,425). mPGES-1 was demonstrated to be overexpressed in >80% of the human colon and lung tumors and adenomas, but there were differences in the degree of expression between mPGES-1 and PGHS-2 and some tumors expressed very little of these enzymes (424,425). Recently, mPGES-1 was found to be overexpressed in >50% of human gastric cancer cells (van Rees et al., 2003, in press). However, in
contrast to PGHS-2, mPGES-1 was not induced by phorbol 12-myristate 13-acetate (PMA) or IL-1β, indicating different regulatory mechanisms for PGHS-2 and mPGES-1 expression. Further studies on mPGES-1 are necessary to elucidate its implications in carcinogenesis.

**Alzheimer's disease**

There are three major pathologies that characterize Alzheimer's disease (AD): senile plaques, neurofibrillary tangles and inflammation (101). One of the most critical events for the onset of Alzheimer's disease is the deposition of β-amyloid in the brain. In the search for β-amyloid-induced genes in rat astrocytes, mPGES-1 mRNA was identified by a cDNA subtraction technique, suggesting a potential role in the development of Alzheimer's disease (311). Many epidemiological studies indicate that chronic use of NSAIDs delays the onset of Alzheimer's disease (42,101). This is probably due to inhibition of the increased PGHS-2 expression and PGE2 production found in patients with Alzheimer's disease (160,222,272). Also, IL-1β-induced PGHS-2 expression and PGE2-secretion was inhibited by dexamethasone in human neuroblastoma cells, suggesting a coupling of glial derived IL-1β and increased PGHS-2 expression in neuronal cells in chronic degenerative diseases, like Alzheimer's disease (131).

**Atherosclerosis**

Inflammation has been found to play a central role in the events that leads to erosion of atherosclerotic plaques (388). In fact, markers of inflammation are related to increased risk of cardiovascular disease (293). Macrophages participate in the inflammatory process and synthesize MMPs, which are capable of degrading plaque constituents. Increased expression of MMP-2 and MMP-9 has been found in human plaques in association with macrophages (90). Induction of MMP-2 and MMP-9 is activated by a PGE2/cAMP-dependent pathway (60). In agreement, colocalization of PGHS-2 and mPGES-1 was recently seen in symptomatic atherosclerotic plaques, coupled with induction of metalloproteinases, resulting in plaque rupture and clinical symptoms (56). Also, a specific PGHS-2 inhibitor (NS-398) decreased production of MMPs, which was reversed by PGE2. Furthermore, the involvement of PGHS-2 in early atherogenesis was recently confirmed in low-density lipoprotein (LDL)-receptor deficient mice (44). Another report describes the importance of PGHS-1-derived prostaglandins in acceleration of atherogenesis in LDL-receptor knockout mice (281). Thus, both PGHS-1 and –2 seem to be involved in atherosclerosis and an evaluation of the effects of various selective PGHS-2 inhibitors on plaque progression in humans should follow. The beneficial effects of a selective mPGES-1 remain to be studied.

**mPGES-1 as a drug target**

PGHS-2 specific inhibitors have reduced gastrointestinal toxicity compared to less selective PGHS-2 inhibitors, but other unwanted side effects exist (64). Specific PGHS-2 inhibition can lead to edema and elevated blood pressure due to altered excretion of
sodium (352). Specific PGHS-2 inhibition also leads to inhibition of renal and systemic PGI₂ (49,209), which may alter the balance between platelet-derived thromboxane A₂ and endothelial-derived PGI₂, leading to altered vascular haemostasis and perhaps to an increased risk of thrombosis (38,65). Thus, a specific mPGES-1 inhibitor is desirable, sparing beneficial prostanoids that participate in resolution of inflammation and important physiological systems in which other prostaglandins participate.

Other effects of a specific mPGES-1 inhibitor in inflammatory conditions may include conversion of PGH₂ (shunting) to other enzymatically or non-enzymatically produced prostanoids (Fig. 3) or novel anti-inflammatory products (94). For example, cells that express PGDS could “shunt” PGH₂ into PGD₂, which can be non-enzymatically metabolized into 15-deoxy-Δ₁₂,₁₄-PGJ₂. This shunting could thus lead to an even more efficient anti-inflammatory effect through inhibition of mPGES-1, since 15-deoxy-Δ₁₂,₁₄-PGJ₂ is an inhibitor of mPGES-1 and also has anti-inflammatory properties. Studies with specific inhibitors of mPGES-1 on various cell systems and mPGES-1 (-/-) mice are needed to further elucidate any shunting effects. Shunting may also be a disadvantage if large amounts of TXA₂ are produced.

Another advantage with specific inhibition of mPGES-1 was seen in mPGES-1 (-/-) mice, which demonstrated a normal phenotype, suggesting that other PGE synthases substitute for mPGES-1 in normal physiology (374,381).

Conclusions

Our data cover characterization of human mPGES-1 from initial identification to purification, 2-D crystallization and kinetic characterization, including discovery of new activities. Human mPGES-1 was cloned and characterized as a 16 kDa, inducible, GSH-dependent, microsomal PGE synthase and was identified as a member of the MAPEG superfamily. mPGES-1 possessed several activities besides PGES-activity, like GSH-dependent peroxidase activities and GSH-transferase activities, probably representing the evolutionary relationship to other MAPEG members. We observed that mPGES-1 was induced by proinflammatory cytokines and this induction was prevented by dexamethasone, suggesting a role in inflammatory processes. mPGES-1 was found to play an important role in RA where it was induced by IL-1β and TNF-α in an experimental setting. Several studies of mPGES-1 and PGHS-2 indicate that they are functionally linked and can be induced by the same kind of stimuli, but with different signaling pathways. PGHS-2 and mPGES-1 play an important role in a number of pathological conditions and diseases like inflammation, pain, fever, RA, cancer, atherosclerosis and neurodegenerative diseases.

mPGES-1 is a very interesting, novel drug target due to its seemingly specific involvement in pathophysiology. Continued research is required, especially on the structure of the protein and on the catalytic mechanism. The aim is to produce specific and effective inhibitors to be tested in various clinical trials.
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