Prostaglandin E Synthase, a Terminal Enzyme for Prostaglandin E2 Biosynthesis

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Introduction

Biosynthesis of prostanoids involves oxidation and subsequent isomerization of membrane-derived arachidonic acid (AA) via three sequential enzymatic reactions. The initial step of this metabolic pathway is the stimulus-induced liberation of AA from membrane glycerophospholipids by phospholipase A2 (PLA2) enzymes. The released AA is sequentially metabolized to prostaglandin (PG) G2 and then to PGH2 by either cyclooxygenase (COX)-1 or COX-2. PGH2 is then converted to various bioactive PGs (thromboxane (TX) A2, PGD2, PGE2, PGF2, and PGI2) by the respective terminal prostanoïd synthases, which have different structures and exhibit cell- and tissue-specific distributions.

It is now believed that segregated utilization of COX-1 and COX-2 occurs in the distinct PG-biosynthetic pathways, even when they co-exist in the same cell. Generally, the constitutive COX-1 is mainly utilized in the immediate PG biosynthesis, which occurs within several minutes after stimulation with Ca2+ mobilizers, whereas the inducible COX-2 is an absolute requirement for delayed PG biosynthesis, which lasts for several hours following various stimuli. Although the precise molecular mechanisms underlying the functional segregation between the two COXs have still remained obscure, subtle differences in their subcellular location and enzymatic properties can account for this event. It has been shown that COX-1 is located in the endoplasmic reticulum (ER) and perinuclear membranes, whereas COX-2 resides predominantly in the perinuclear envelope: this difference may influence the availability of the substrate AA released by distinct PLA2 enzymes to each COX (Morita et al., 1995; Reddy and Herschman, 1997) In addition, in vitro enzymatic analyses have pointed that effective catalysis by COX-2 can proceed at low hydroperoxide levels that are insufficient to sustain catalysis by COX-1 (Kulmacz and Wang, 1995; Murakami et al., 1999). It is therefore conceivable that COX-2-mediated
Table 1. Properties of PGES enzymes

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Structural properties</th>
<th>Expression</th>
<th>Subcellular localizations</th>
<th>COX preference</th>
<th>Specific characteristics</th>
<th>In vivo functions*</th>
</tr>
</thead>
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<tr>
<td>mPGES-1</td>
<td>MAPEG family</td>
<td>inducible</td>
<td>perinuclear membrane</td>
<td>COX-2</td>
<td>trimer formation</td>
<td>inflammation, pain, fever, cancer</td>
</tr>
<tr>
<td>mPGES-2</td>
<td>thioredoxin homology domain</td>
<td>constitutive</td>
<td>Golgi, cytosol</td>
<td>COX-1, COX-2</td>
<td>cleavage of N-terminal hydrophobic region dimer formation</td>
<td>unknown</td>
</tr>
<tr>
<td>cPGES</td>
<td>Hsp90 cochaperone p23</td>
<td>constitutive</td>
<td>cytosol</td>
<td>COX-1</td>
<td>complex formation with Hsp90 and CK2</td>
<td>unknown</td>
</tr>
</tbody>
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*mIn vivo functions revealed by analyses on gene-disrupted mice.

catalysis is preferred in particular cellular situations where AA supply is limited (Shitashige et al., 1998; Murakami et al., 1999). As an alternative possibility, it has been proposed that the two COX isoforms display distinct functional coupling with individual terminal prostanoid synthases. This concept was initially suggested for coupling between COXs and PG synthase (PGES) enzymes (Tanioka et al., 2000; Murakami et al., 2000), and subsequently between COXs and other terminal prostanoid synthases (Ueno et al., 2001).

Until now, three proteins that catalyze the conversion of PGH2 to PGE2, rather specifically have been identified; namely membrane-bound PGES (mPGES)-1 (Jakobsson et al., 1999; Murakami et al., 2000), mPGES-2 (Tanioka et al., 2002), and cytosolic PGES (cPGES) (Tanioka et al., 2000). In this article, we focus on the expression and functions of these three PGES enzymes in the context of COX coupling, which have been further supported by several in vivo studies using COX isozyme-selective inhibitors and gene-manipulated mice. The characteristic properties of these PGES enzymes (mPGES-1, mPGES-2 and cPGES) are summarized in Table 1.

mPGES-1

Preferred utilization of COX-2 over COX-1 in the PGE2-biosynthetic pathway was initially reported in rat peritoneal macrophages, which produced TXA2 and PGD2 through COX-1 in the A23187-induced immediate response and PGE2 and PGD2 through COX-2 in the lipopolysaccharide (LPS)-induced delayed response (Narita et al., 1998; Brock et al., 1999). PGE2 production by osteoblasts occurred predominantly through COX-2 irrespective of the co-presence of COX-1 (Chen et al., 1997; Murakami et al., 1997). In a rat inflammatory model, COX-2-selective inhibitors reduced the accumulation of PGE2 but not of other PGs (Harada et al., 1996). These observations predicted the presence of a particular terminal PGs that is coupled with COX-2 in marked preference to COX-1.

It is now apparent that mPGES-1 represents a long sought, COX-2-preferential PGES, mPGES-1 is a glutathione (GSH)-requiring perinuclear protein belonging to the MAPEG (membrane-associated proteins involved in glucosamoid and GSH metabolism) family (Jakobsson et al., 1999; Murakami et al., 2000). mPGES-1 constitutes a trimer in the crystal (Murakami and Kudo, 2004). As in the case of COX-2, expression of mPGES-1 is induced by proinflamatory stimuli and is down-regulated by anti-inflamatory glucocorticoid. Induction of mPGES-1 expression has also been observed in various systems in which COX-2-derived PGE2 has been implicated to play a critical role, such as inflammation, fever, pain, female reproduction, tissue repair, and cancer (Thoren et al., 2003). Stimulus-inducible expression of mPGES-1 is controlled by the transcription factor Egr-1, which binds to the proximal GC box in the mPGES-1 promoter leading to mPGES-1 transcription (Narita et al., 2002). Studies employing connasection of mPGES-1 and either COX isozyme as well as those with small interfering RNA or antisense technology to knockdown mPGES-1, have revealed that mPGES-1 is functionally coupled with COX-2 in marked preference to COX-1 (Murakami et al., 2000; Kamei et al., 2004). Colocalization of COX-2 and mPGES-1 in the same perinuclear membrane may allow their efficient functional coupling. Nonetheless, Coupling between COX-1 and mPGES-1 can also occur if AA is abundantly supplied by explosive activation of cytosolic PLA2 (cPLA2) (Murakami et al., 2000).

Crucial involvement of mPGES-1 in various pathophysiological events has been clarified by studies using mPGES-1 knockout mice (Uematsu et al., 2002; Trebino et al., 2003; Engblom et al., 2003; Boulet et al., 2004; Kamei et al., 2004). mPGES-1 accounted for the majority of inducible PGES activity in various tissues of LPS-treated mice (Boulet et al., 2004; Kamei et al., 2004) and peritoneal macrophages isolated from mPGES-1-null mice produced minimal PGE2 in response to LPS (Uematsu et al., 2002; Kamei et al., 2004). In the collagen- or the collagen antibody-induced arthritis model, mPGES-1-deficient mice developed milder arthritis than did wild-type mice (Trebino et al., 2003; Kamei et al., 2004). Similar arthritic phenotypes have been observed in mice lacking cPLA2 (Hegen et al., 2003), COX-2 (Myers et al., 2001) or the PGE receptor EP4 (Rocca et al., 1999), thus revealing a metabolic flow of the cPLA2/COX-2/mPGES-1/EP4 pathway leading to the development of inflammatory
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arthritis. The results seen in the collagen-induced arthritis model might be influenced in part by inadequate proximal lymphocyte-mediated responses, since COX-2-deficient mice exhibited altered helper T cell development, a process reversed by PGE2 (Rocca et al., 1999).

Anogenital-induced paw edema was markedly reduced in mPGES-1-deficient mice as compared to replicate wild-type mice (Trebino et al., 2003). This deficit in edema was accompanied by a marked reduction in the infiltration of white blood cells into the inflamed site. Likewise, migration of macrophages following peritoneal injection of thioglycollate was strikingly reduced in mPGES-1-null mice relative to replicate wild-type mice (Kamei et al., 2004). Formation of inflammatory granulation tissue and attendant angiogenesis in the dorsum induced by subcutaneous implantation of a cotton thread was significantly reduced in mPGES-1 knockout mice as compared with wild-type mice (Kamei et al., 2004). In this model, mPGES-1 deficiency was also associated with reduced induction of vascular endothelial cell growth factor (VEGF) in the granulation tissue. Thus, mPGES-1-derived PGE2, in cooperation with VEGF, may play a critical role in the development of inflammatory granulation and angiogenesis.

PGE2 is known to be a mediator of inflammatory pain. Peripheral pain nociception, as assessed by acetic acid writhing test, was significantly reduced in mPGES-1-deficient mice relative to wild-type mice (Trebino et al., 2003; Kamei et al., 2004). This phenotype was particularly evident when these mice were primed with LPS, where the stretching behavior and the peritoneal PGE2 level of knockout mice were far less than those of wild-type mice (Kamei et al., 2004), being consistent with elevated expression of COX-2 and mPGES-1 in response to LPS. The basal (i.e. LPS-nonprimed) writhing response was also partially reduced in mPGES-1-null mice (Trebino et al., 2003; Kamei et al., 2004), where COX-1-mPGES-1 coupling takes place. In a neuropathic pain model prepared by spinal nerve transaction, mPGES-1-null mice failed to exhibit mechanical allodynia and thermal hyperalgesia (Murakami et al., 2004).

Genetic inactivation of COX-2, but not COX-1, resulted in reduction of PGE, levels in the central nervous system (CNS), in association with impaired LPS-induced febrile response (Li et al., 1999). Likewise, mPGES-1 knockout mice showed no fever and no central PGE2 synthesis after peripheral injection of LPS (Engblom et al., 2003). Treatment of rats with LPS led to an increase in the expression of mPGES-1 in blood vessels, especially in veins and venules, in the whole brain (Yamagata et al., 2001). Thus, cerebral vascular endothelial cells express components enabling blood-borne cytokines to stimulate the synthesis of PGE2, whose small size and lipophilic property allow it to pass across the blood-brain barrier and to diffuse into the CNS neurons, thereby evoking the febrile response. In contrast, wild-type and mPGES-1 mutant mice showed similar psychogenic stress-induced hyperthermia, diurnal temperature variations, and reduced motor activity following injection of turpentine (an aseptic cytokine-induced pyresis inducer) (Sethi et al., 2005). This indicates that mPGES-1 is functionally dissociated from the stress-induced hyperthermia, circadian temperature regulation, and the inflammation-induced activity depression.

COX-2 plays a critical role in the development of colorectal cancer and likely other types of cancer. Pharmacological or genetic inactivation of COX-2 led to suppression of cell growth and survival as well as reduction of tumor size, invasion and metastasis (Oshima et al., 1996). Disruption of cPLA2 (Takaku et al., 2002) or the PGE receptor EP2 (Sonoshita et al., 2001) also resulted in a reduced incidence of gastrointestinal polyps, providing strong evidence for a link between PGE2 signaling and oncogenesis. Tumorogenic potential of mPGES-1 has been suggested by the observations that transfection of mPGES-1 in combination with COX-2, but not with COX-1, into HEK293 cells led to cellular transformation with a concomitant increase in PGE2 (Munakami et al., 2000), that the COX-2/mPGES-1-overexpressed cells formed a number of large colonies in soft agar culture and were tumorigenic when implanted into nude mice (Kamei et al., 2003), and that transgenic mice overexpressing both COX-2 and mPGES-1 developed metastases, hyperplasia and tumorous growth in the glandular stomach with heavy macrophage infiltration (Oshima et al., 2004).

It can be thus concluded that mPGES-1 is involved in various types of pathology including inflammation, pain hyperalgesia, fever, and cancer. Notably, the absence of gross abnormalities in ductus arteriosus closure immediately after birth, which is markedly impaired in COX-1/COX-2-double (Lotin et al., 2001) and EP4 (Segi et al., 1998) knockout mice, and in female reproduction, where EP2 is involved in the ovulation step (Hizaki et al., 1999), implies the compensatory participation of other PGESs in these physiological events. These facts, together with its inducible property during inflammation and other pathogenesis, agree well with the proposal that mPGES-1 represents a target for the treatment of various inflammatory diseases that will spare important physiological systems in which other PGs are involved.

mPGES-2

The second membrane-associated form of PGES, mPGES-2, has a catalytic glutaredoxin/thioredoxin-like domain and is activated by various thiol reagents (Tanikawa et al., 2002). This enzyme is synthesized as a Golgi membrane-associated protein, and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cysteine enzyme (Tanikawa et al., 2002; Mutai et al., 2003). Cotransfection of mPGES-2 with either COX isozyme demonstrated that mPGES-2 could be coupled with both COX-1 and COX-2 (Munakami et al., 2003). Expression of mPGES-2 is rather constitutive in various cells and tissues and is not elevated appreciably during inflammation or tissue damage. However, a considerable increase of mPGES-2
expression is observed in human colorectal cancer, in which mPGES-1 is also overexpressed (Murakami et al., 2003).

Crystallization of mPGES-2 reveals that it forms a dimer and is attached to lipid membrane by anchoring the N-terminal section (Yamada et al., 2005). Two hydrophobic pockets connected to form a V shape are located in the bottom of a large cavity. The geometry suggests that the SH of Cys pockets connected to form a V shape are located in the bottom and is attached to lipid membrane by anchoring the N-2- COX-1-, but not COX-2-, derived PGH2 experiments indicated that cPGES is capable of converting Cyspockets and its endoperoxide moiety interacts with the SH of Cys 2. The fold of mPGES-2 is quite similar to those of GSH-dependent hemapoietic PGD synthase, except for the two large loop sections.

cPGES

Cytosolic PGES (cPGES) is a GSH-requiring enzyme constitutively expressed in a wide variety of cells and is identical to p23, a co-chaperone of heat shock protein 90 (Hsp90) (Tanioka et al., 2000). Cotransfection and antisense experiments indicated that cPGES is capable of converting COX-1, but not COX-2, derived PGH2 to PGE2 in cells, particularly during the immediate PGE2-biosynthetic response elicited by Ca2+-evoked stimuli (Tanioka et al., 20). Localization of cPGES in the cytosol may allow coupling with proximal COX-1 in the ER in preference to distal COX-2 in the perinuclear envelope. Although the expression of cPGES is constitutive and is unaffected by proinflammatory stimuli in most cases, some exceptions have been reported. Administration of IL-1 into the mouse cortex via intraparenchymal microinjection led to an increase in PGE2, which was accompanied by elevated expression of cPGES as well as that of COX-2 and mPGES-1 with different kinetics (Moore et al., 2004). In pregnant female mice, cPGES was strongly detected in the stroma underlying the luminal epithelium surrounding the implanting blastocyst at the implantation site and in decidualized cells under artificial decidualization (Ni et al., 2003).

cPGES is directly associated with and phosphorylated by casein kinase 2 (CK2), resulting in marked reduction of Km for the substrate PGH2 (Kobayashi et al., 2004). In activated cells, CK2-directed phosphorylation of cPGES occurs in parallel with increased cPGES enzymatic activity and PGE2 production, and these processes are facilitated by interaction with Hsp90 (Kobayashi et al., 2004). cPGES, CK2, and Hsp90 form a stoichiometric complex of 1:1:1 immediately after cell activation. In this context, Hsp90 may act as an essential scaffold protein that brings cPGES and CK2 in proximity, thereby spatially allowing their efficient functional interaction under physiological condition. Pharmacologic inhibition of CK2 or Hsp90 or mutation of two CK2-directed phosphorylation sites on cPGES results in poor activation of cPGES, indicating that the tertiary complex formation and attendant phosphorylation are the tertiary complex formation and attendant phosphorylation are the necessary proteins for cPGES to act in cells. These results provide the first evidence that the cellular function of the eicosanoid-biosynthetic enzyme is under the control of a molecular chaperone and its client protein kinase.

Concluding Remarks

It has become apparent that there are multiple PGES enzymes in mammalian cells and that they display distinct functional coupling with upstream COX enzymes. Distinct PGES enzymes may control the spatial and temporal production of PGE2 in different aspects of pathophysiology in particular tissues and cells. Therefore, understanding the regulatory mechanisms for each PGES is of considerable importance. Although COX-2 inhibitors have reduced gastrointestinal toxicity as compared with traditional NSAIDs, there are also some adverse effects associated with this new group of drugs (Crotford et al., 2000). For instance, specific inhibition of COX-2 blocks the production of renal and systemic PGI2 (McAdam et al., 1999), thereby causing altered excretion of sodium, edema, and elevated blood pressure (Stichtenoth and Frolich, 2000). In addition, specific inhibition of COX-2 alters the balance between platelet-derived thromboxane A2 and endothelium-derived PGI2, leading to increases in the risk of thrombosis due to altered vascular tone (Crotford et al., 2000; Bombardier et al., 2000). Thus, more selective modulation of the prostanooid pathway appears to be desirable. From this standpoint, mPGES-1 represents an attractive novel target for therapeutic intervention for patients with various inflammatory diseases and cancer.

References


