Comparative Biochemistry and Physiology, Part B 174 (2014) 45–52

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

# Novel membrane-associated prostaglandin E synthase-2 from crustacean arthropods☆

Kristella Hansen, Külliki Varvas, Ivar Järving, Nigulas Samel *

*Department of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia*

ARTICLE INFO

*Article history:*
Received 14 March 2014
Received in revised form 29 May 2014
Accepted 30 May 2014
Available online 16 June 2014

*Keywords:*
Membrane associated prostaglandin E synthase-2
Cyclooxygenase
PGES gene
Crustacean
Arthropod

ABSTRACT

Prostaglandins (PG) have been shown to play important physiological roles in insects and marine invertebrates, yet the knowledge of their biosynthetic pathways is often lacking. Recently, we described cyclooxygenases in two amphipod crustaceans, *Gammarus* sp. and *Caprella* sp. In the present study, we report the cloning and characterization of prostaglandin E synthases (PGES) from the same organisms. The amphipod membrane-bound PGES-2-type enzymes share about 40% of the amino acid sequence identity with human mPGES-2, contain a conserved Cys110–x–x–Cys113 motif and have very low heme-binding affinity. The recombinant enzymes purified in the absence of dithiothreitol specifically catalyze the isomerization of $PGH_2$ into $PGE_2$. The PGES activity is increased in the presence of reduced glutathione and inhibited with a sulfhydryl group inhibitor. We assume that the amphipod mPGES-2, unlike in their mammalian counterparts, is responsible for $PGE_2$ synthesis, not only *in vitro* but also *in vivo*.

© 2014 Elsevier Inc. All rights reserved.

## 1. Introduction

Prostaglandins (PGs) are well-known lipid mediators in vertebrates, and they have also been shown to play important regulatory roles in insects and other arthropods. In mammals, the biosynthesis of PGs occurs through multiple enzymatically regulated reactions. The process is initiated through the release of arachidonic acid (AA) from membrane phospholipids by the hydrolytic action of phospholipase $A_2$. The released AA is further metabolized into the unstable endoperoxide intermediate $PGH_2$ by the actions of PG endoperoxide synthase, also called cyclooxygenase (COX). Two distinct COX isozymes exist, COX-1 and COX-2, which are differently regulated. Once formed, the $PGH_2$ intermediate is converted to various prostanoids by specific $PGH_2$ isomerases and reductases (Smith et al., 2011).

In mammals, prostaglandin E synthase (PGES, EC 5.3.99.3), which isomerizes COX-derived $PGH_2$ specifically to $PGE_2$, occurs in three structurally and biologically distinct forms (Kudo and Murakami, 2005). Cytosolic PGES (cPGES) is a glutathione (GSH)-dependent enzyme constitutively expressed in a wide variety of cells, and is functionally linked to COX-1 to promote immediate $PGE_2$ production (Tanioka et al., 2000). The two membrane-bound PGES enzymes have been designated as mPGES-1 and mPGES-2. mPGES-1 is a GSH-dependent perinuclear protein that is induced by proinflammatory stimuli, and that converts COX-2-derived $PGH_2$ to $PGE_2$ (Murakami et al., 2000). mPGES-2 is initially synthesized as a Golgi membrane-associated protein and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme (Watanabe et al., 1999; Tanikawa et al., 2002). This enzyme is constitutively expressed in various cells and tissues and is functionally coupled with both COX-1 and COX-2 (Murakami et al., 2003). Recently, it was reported that macaque mPGES-2 exists in two forms, as heme-free and heme-bound enzymes, that the heme-free enzyme catalyzes the formation of $PGE_2$ from $PGH_2$, and that the heme-bound mPGES-2 is a GSH-dependent protein which catalyzes $PGH_2$ degradation to 12(S)-hydroxy-5,8,10(Z,E,E)-heptadecatrienoic acid (HHT) and malondialdehyde (MDA). As the heme-free recombinant mPGES-2 converts to the heme-bound form if free heme is available, it was proposed that macaque mPGES-2 is a $PGE_2$ synthase *in vitro* but not *in vivo* (Takusagawa, 2013).

$PGE_2$ is the most common prostanoid in terrestrial and marine invertebrates and its physiological roles in reproduction, ion transport, immunity and defense reactions have been reported (Stanley, 2000;

*Abbreviations:* AA, arachidonic acid; GSH, glutathione; GST, glutathione S-transferase; COX, cyclooxygenase; HHT, 12(S)-hydroxy-5,8,10(Z,E,E)-heptadecatrienoic acid; HPLC, high performance liquid chromatography; MDA, malondialdehyde; Ni-NTA, nickel nitrilotriacetic acid; PGES, prostaglandin E synthase; cPGES, cytosolic prostaglandin E synthase; mPGES, membrane-associated PGE synthase; PG, prostaglandin; pHMB, p-Hydroxymercuribenzoate; RACE, rapid amplification of cDNA ends; UTR, untranslated region.

☆ Database linking: The mRNA sequences of amphipod mPGES-2 have been submitted to the GenBank database under the accession no. KC832830 (*Caprella* sp.) and no. KC832831 (*Gammarus* sp.), and the protein sequences under accession no.AGO64144 (*Caprella* sp.) and no.AGO64145 (*Gammarus* sp.).

* Corresponding author. Tel.: +372 620 4376; fax: +372 620 2828.
*E-mail address:* nigulas.samel@ttu.ee (N. Samel).

http://dx.doi.org/10.1016/j.cbpb.2014.05.004
1096-4959/© 2014 Elsevier Inc. All rights reserved.

Rowley et al., 2005; Stanley, 2011). The occurrence of $PGE_2$ has been shown in various marine arthropods, e.g. in the ovaries of the shrimp *Penaeus monodon* (Wimuttisuk et al., 2013), the kuruma prawn *Marsupenaeus japonicus* (Tahara and Yano, 2004), and the crab *Oziotelphusa senex senex* (Reddy et al., 2004), as well as in the previtellogenic ovary of the prawn *Macrobrachium rosenbergii* (Sagi et al., 1995). $PGE_2$ was identified in the secretory products of the parasitic copepod crustacean *Lepeophtheirus salmonis* (Fast et al., 2004) and in the blood cells of the shore crab *Carcinus maenas* (Hampson et al., 1992).

Although COX genes have been identified in all vertebrate animals investigated, there is little information about PG biosynthesis in lower animals and plants. In vitro biosynthesis of typical mammalian prostaglandins in invertebrates via the COX pathway was first reported in the soft coral *Gersemia fruticosa* (Varvas et al., 1994). To date, COX enzymes have been cloned and characterized in the soft corals *G. fruticosa* and *Plexaura homomalla* (Koljak et al., 2001; Valmsen et al., 2001; Valmsen et al., 2004) and in two amphipod crustaceans, *Gammarus* sp. and *Caprella* sp. (Varvas et al., 2009). COX has also been cloned and identified in the shrimp *P. monodon*, although the enzyme activity has not been examined (Wimuttisuk et al., 2013). In addition, the first non-animal COX was recently identified in the red alga *Gracilaria vermiculophylla* (*Rhodophyta*). The algal COX has only about 20% identity with human COX-1 and COX-2 and, unlike its mammalian counterparts, expresses easily in prokaryotic *Escherichia coli* cells as a highly active and fully functional enzyme (Kanamoto et al., 2011; Varvas et al., 2013).

There is also bioinformatic evidence available of a possible COX pathway in different invertebrates. Using genome database analysis, COX genes have been reported in the primitive chordates *Ciona savignyi* and *Ciona intestinalis* (Järving et al., 2004), in the crustaceans *Daphnia pulex*, *Homarus americanus*, and *Petrolisthes cinctipes* (Heckmann et al., 2008), and in the human body louse *Pediculus humanus corporis* (Varvas et al., 2009). However, homologs of mammalian COX genes have not been identified in completely sequenced insect genomes of *Drosophila* sp., *Aedes aegypti*, *Anopheles gambiae*, *Apis mellifera*, *Bombyx mori*, *Tribolium castaneum* or others.

On the other hand, PGES-like sequences are common in arthropod genomes. While more than 30 predicted mPGES-2-like sequences have been identified in insects and other arthropod genomes so far, there is little information about the catalytic activity of corresponding proteins.

Here we describe the molecular cloning and characterization of functional mPGES-2 enzymes in the aquatic arthropod crustaceans *Gammarus* sp. and *Caprella* sp. Both enzymes specifically catalyzed the isomerization of $PGH_2$ (produced with algal COX) into $PGE_2$ *in vitro*. The protein and gene structures of amphipod mPGES-2 are briefly analyzed.

## 2. Materials and methods

### 2.1. Materials and reagents

[$^{14}$C]AA was obtained from Perkin Elmer. The oligonucleotides were purchased from DNA Technology (Denmark). Restriction enzymes were obtained from MBI Fermentas. All other chemicals, if not mentioned otherwise, were obtained from Sigma-Aldrich. The crustacean samples were collected from the coast of the Kanagawa prefecture in Tokyo Bay and contained the red alga *G. vermiculophylla* and the small amphipod crustaceans *Gammarus* sp. and *Caprella* sp., which inhabit the macro algae community. The samples were stored at −80 °C until RNA isolation.

### 2.2. RNA isolation and cDNA cloning

Total RNA was extracted from tissue homogenate using SDS–guanidinium precipitation. The method is previously described by Su and Gibor (1988) and Koljak et al. (2001). mRNA was prepared from the total RNA using an oligo(dT)-cellulose column and purification kit (Qiagen). The first strand cDNA was prepared using an oligo(dT)-adapter primer (Song et al., 1993).

Partial cDNA sequences coding amphipod mPGES-2 were obtained using nested PCR and two pairs of degenerative primers (Supplementary Table S1). 5′-RACE was accomplished using a 5′-RACE Kit (Roche Diagnostics). 3′-RACE was accomplished using the first strand cDNA prepared with an adapter-linked oligo(dT) primer. All the PCR products were cloned into the pGEM-T Easy vector (Promega), amplified in *E. coli* and sequenced.

cDNAs encoding the crustacean mPGES-2 proteins were amplified by PCR using the proofreading Phusion polymerase (Finnzymes). Primers carried BamHI restriction sites at their respective 5′ ends, and upstream primers carried additional $His_6$-tags for the further purification of the recombinant proteins.

The amplified fragments were digested with BamHI, purified and cloned into the corresponding sites of the pET11-a vector (Novagen). The primers used for amplification of full-length and N-terminally truncated variants of gammarid and caprellid mPGES-2 are given in Supplementary Table S2.

### 2.3. Expression and purification of the recombinant enzymes

*E. coli* BL21(DE3)RP cells expressing the recombinant amphipod $His_6$-mPGES-2 were cultured in a 100 ml LB medium (containing 100 μg/ml ampicillin) alone or containing 0.2 mM $FeCl_3$ and 1.5 mM δ-aminolevulinate at 20 °C for 16 h following the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside at 0.6 of $OD_{600}$. The cells were harvested by centrifugation. All purification procedures were carried out at 4 °C. Recombinant proteins were purified with a nickel nitrilotriacetic acid (Ni-NTA) column, using a batch purification method (Qiagen). The cells were suspended with 3 ml of buffer A (30 mM potassium phosphate buffer, 1 M NaCl, 0.1 mM GSH, 1 mM phenylmethanesulfonylfluoride and 0.01% octyl β-D-glucopyranoside, pH 8.0) and were disrupted by sonication for $5 \times 5$ s followed by centrifugation at $16000 \times g$ for 10 min. After the addition of 20 mM imidazole, the supernatant was gently mixed with 0.5 ml Ni-NTA His·Bind slurry for 2 h. The protein-resin complex was packed into a column and washed with buffer B (30 mM potassium phosphate buffer, 1 M NaCl, 0.1 mM GSH, pH 8.0) containing 100 mM imidazole and 0.01% octyl β-D-glucopyranoside, and subsequently with buffer B (containing 100–135 mM imidazole). The enzyme was eluted with buffer B supplemented with 300 mM imidazole. The concentration of protein solution and the removal of imidazole were accomplished using continuous diafiltration through a MWCO 30 kDa filter (Pall Co.). Since the purified protein was relatively unstable and tended to aggregate easily, the filtration was carried out in the presence of 30 mM potassium phosphate buffer (pH 8.0), containing 1.5 M NaCl, 0.1 mM GSH, 30% glycerine and 0.03% octyl β-D-glucopyranoside (buffer C), in which protein aggregation was minimal.

The purity of the enzyme preparations was estimated by 15% SDS-PAGE and Coomassie Blue staining. Protein concentrations were determined by SDS-PAGE, using a bovine serum albumin calibration curve, created according to protein band intensities and GeneTools software (Syngene). In a Western blot analysis, the proteins were separated by 15% SDS-PAGE and transferred to a nitrocellulose membrane (Millipore). $His_6$-mPGES-2 proteins were detected using a monoclonal anti-polyHistidine (mouse IgG2a isotype, Sigma).

### 2.4. Heme binding measurements

The heme content and heme binding to the purified amphipod protein were determined spectrophotometrically (Shimadzu UV-1601) at 240–600 nm. Heme binding to mPGES-2 was examined with titration analysis. 2.5 μl of freshly prepared 100 μM hemin in dimethyl sulfoxide was added to 0.5 ml of 8 μM purified protein solution in buffer C. The

Download English Version:

https://daneshyari.com/en/article/1975314

Download Persian Version:

https://daneshyari.com/article/1975314

Daneshyari.com