Comparative Biochemistry and Physiology, Part D 7 (2012) 340–350

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part D

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

Conservation of group XII phospholipase A_2 from bacteria to human

Timo J. Nevalainen [a,*], João C.R. Cardoso [b]

[a] Department of Pathology, University of Turku and Turku University Hospital, Turku, Finland
[b] Comparative Molecular Endocrinology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005–139 Faro, Portugal

ARTICLE INFO

Article history:
Received 11 May 2012
Received in revised form 18 July 2012
Accepted 18 July 2012
Available online 31 July 2012

Keywords:
GXII PLA_2
Conserved domain
pfam06951
Evolution
Prokaryotes
Eukaryotes

ABSTRACT

Vertebrate group XII phospholipases A_2 (GXII PLA_2, conserved domain pfam06951) are proteins with unique structural and functional features within the secreted PLA_2 family. In humans, two genes (GXIIA PLA_2 and GXIIB PLA_2) have been characterised. GXIIA PLA_2 is enzymatically active whereas GXIIB PLA_2 is devoid of catalytic activity. Recently, putative homologues of the vertebrate GXII PLA_2s were described in non-vertebrates. In the current study a total of 170 GXII PLA_2 sequences were identified in vertebrates, invertebrates, non-metazoan eukaryotes, fungi and bacteria. GXIIB PLA_2 was found only in vertebrates and the searches failed to identify putative GXII PLA_2 homologues in Archaea. Comparisons of the predicted functional domains of GXII PLA_2s revealed considerable structural identity within the Ca^{2+}-binding and the catalytic sites among the various organisms suggesting that functional conservation may have been retained across evolution. The preservation of GXII PLA_2 family members from bacteria to human indicates that they have emerged early in evolution and evolved via gene/genome duplication events prior to Eubacteria. Gene duplicates were identified in some invertebrate taxa suggesting that species-specific duplications occurred. The analysis of the GXII PLA_2 homologue genome environment revealed that gene synteny and gene order are preserved in vertebrates. Conservation of GXII PLA_2s indicates that important functional roles involved in species survival and were maintained across evolution and may be dependent on or independent of the enzyme's phospholipolytic activity.

1. Introduction

Phospholipase A_2 (PLA_2) represents a large family of enzymes and enzyme-like proteins characterized in organisms ranging from bacteria to vertebrates (Dennis et al., 2011). They are proposed to have emerged very early in evolution (Nevalainen et al., 2012). According to the calcium ion dependency as to their catalytic activity, PLA_2 members are classified in three broad categories of calcium-dependent secreted PLA_2s ($sPLA_2$) and cytosolic PLA_2s and calcium-independent cytosolic PLA_2s (Dennis et al., 2011). Secreted PLA_2s are the most abundant and they are synthesized by many different cell types, secreted in various body fluids and participate in important physiological and pathological functions, such as digestion of dietary phospholipids, inflammatory reaction, antimicrobial defence and venom toxicity (Nevalainen et al., 2000, 2008; Fry et al., 2009; Murakami et al., 2010, 2011).

According to their molecular structure $sPLA_2$s have been subdivided into 18 groups (G) (GIA, GIB, GIIA, GIIB, GIIC, GIID, GIIE, GIIF, GIII, GV, GIX, GX, GXIA, GXIB, GXIIA, GXIIB, GXIII and GXIV) (Six and Dennis, 2000). Recently, a novel classification system was proposed for $sPLA_2$s (Nevalainen et al., 2012) based on their conserved structural domains (Finn et al., 2008; Marchler-Bauer et al., 2011), and they were restricted to two general distinct categories, the cd-collection and pfam-collection. The origin of $sPLA_2$s of both collections was traced back to bacteria and members of the two collections were proposed to have shared common ancestry and evolved independently under distinct evolutionary pressures (Nevalainen et al., 2012). The cd-collection comprises members of GI, GII, GIII, GV, GX and GXI PLA_2s and the pfam-collection GXII and GXIV PLA_2s. Within $sPLA_2$s, the cd-collection members were the most diverse and five sub-families were identified. Two sub-families constitute the pfam-collection, pfam09056 (GXIV PLA_2s) conserved from bacteria to fungi and animals and pfam06951 (GXII PLA_2s) which was previously reported to be vertebrate specific but was also identified in non-vertebrate metazoans and non-metazoan eukaryotes introducing novel challenges in the study of the origin and evolution of $sPLA_2$s (Nevalainen et al., 2012).

In vertebrates, two GXII PLA_2s (GXIIA and GXIIB PLA_2s) that became functionally divergent after a gene duplication event have been reported. A GXIIA PLA_2 and a catalytically inactive GXII PLA_2-like protein (GXIIB PLA_2) were cloned from human (Gelb et al., 2000; Rouault et al., 2003) and also from other tetrapods and teleosts (Hillier et al., 2004; Carninci et al., 2005; Leong et al., 2010). Sequence comparisons revealed that with the exception of the histidine-aspartic acid (HD) dyad amino acid motif of the histidine catalytic site, the vertebrate GXII PLA_2s comprise a unique group and share scarce sequence and structural similarities with the other $sPLA_2$ members. The signature motifs of GXII PLA_2s within

Abbreviations: PLA_2, phospholipase A_2; $sPLA_2$, secreted phospholipase A_2.

* Corresponding author at: Department of Pathology, University of Turku, Kiinamyllynkatu 10, FIN-20520 Turku, Finland. Tel.: +358 40 7274215; fax: +358 2 3337459.
E-mail addresses: timneva@utu.fi (T.J. Nevalainen), jccardo@ualg.pt (J.C.R. Cardoso).

1744-117X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbd.2012.07.002

the Ca^{2+}-binding site (GSPLF in human GXIIA PLA_2) and the histidine (CCXXHDXC), and aspartic acid (CD) catalytic sites are localised in the central part of the molecule, whereas in the other PLA_2s such as the human GIII (cd04704) and GIIA PLA_2 (cd00125) they are closer to the N-terminus. Furthermore, the histidine and aspartic acid catalytic sites are further apart in GIII and GIIA than in GXIIA PLA_2s (Fig. 1). The spacing of cysteine residues and putative disulfide bonds are also distinct from the other $sPLA_2$s (Gelb et al., 2000).

Functional characterisation of GXIIA PLA_2 revealed that its catalytic activity is relatively low in comparison with the other $sPLA_2$ members. While GXIIA PLA_2 is strongly expressed in human heart and skeletal muscle, kidney and pancreas (Gelb et al., 2000), the enzymatically inactive GXIIB PLA_2 is mainly expressed in liver, small intestine and kidney in both human and mouse (Rouault et al., 2003). GXII PLA_2s are suggested to mediate their physiological roles in part via alternative mechanisms independent of their catalytic activity (Gelb et al., 2000; Ho et al., 2001; Rouault et al., 2003), which at present are poorly characterised. In tetrapods, GXIIA PLA_2 signalling is also associated with embryonic development and in amphibians ectodermal GXIIA PLA_2 induces ectopic olfactory structures by blocking bone morphogenetic protein signalling independent of PLA_2 hydrolytic activity (Muñoz-Sanjuán and Brivanlou, 2005).

The present study aims to understand the origin and functional evolution of the GXII PLA_2 members by the identification of their putative vertebrate homologues in non-vertebrate species. Based upon the conserved sequence annotation of the vertebrate pfam06951 (GXII PLA_2) members, genes and transcripts for GXII PLA_2s were retrieved from a wide variety of organisms ranging from bacteria to unicellular and multicellular eukaryotes using *in silico* database searches, and the homologue functional sites, which contain the catalytic and Ca^{2+}-binding sites, were identified and putative functions considered. Homologues of the vertebrate GXIIA PLA_2 were characterised in several invertebrates, non-metazoan eukaryotes, fungi and bacteria suggesting that they have emerged early in evolution via gene and genome duplication events. GXIIB PLA_2s were exclusively found in vertebrates. Considerable conservation within the GXII PLA_2 functional domains across evolution was identified and comparisons of the gene environments of vertebrate and invertebrate members suggested lack of conservation within the non-vertebrate homologue regions while the gene linkage is maintained in the vertebrate radiation. The identification of pfam06951 members in non-vertebrate eukaryotes and prokaryotes makes it possible to reconstruct the evolutionary history of GXII PLA_2 and will contribute to a better understanding of their function and discovery of novel physiological roles which may have been maintained across evolution.

2. Material and methods

2.1. Database mining and data collection

GXII PLA_2 sequences were retrieved from publicly available protein databases of NCBI (http://www.ncbi.nlm.nih.gov) and Swiss-Prot (http://www.expasy.ch/sprot/) using the Basic Local Alignment Search Tool (BLASTp) algorithm (Altschul et al., 1997) and default settings. Database searches were performed using the mature peptide sequences of the human GXIIA PLA_2 (Q9BZM1) and GXIIB PLA_2 (Q9BX93). In addition, bacterial PLA_2 and PLA_2-like protein sequences were identified at the NCBI Microbial and Eukaryotic Genome database (http://www.ncbi.nlm.nih.gov/genome). Searches covered all completed genomes at the database (2019 bacterial, 106 archaeal and 268 eukaryotic genomes, January 2012 release) and also available EST data using the tBLASTn and sequence matches with e-value <10 were retrieved and their sequences analysed. The deduced protein sequences were obtained using the BCM Search Launcher (http://searchlauncher.bcm.tmc.edu/seq-util/Options/sixframe.html) and compared with available homologue data.

2.2. Gene sequence database searches

Putative vertebrate and invertebrate GXII PLA_2 genes were retrieved from the NCBI and ENSEMBL (www.ensembl.org) databases. Searches in Ensembl were performed using a similar strategy described above to search the available lamprey (*Petromyzon marinus*), teleost (Atlantic cod, *Gadus morhua*; tetraodon, *Tetraodon nigroviridis*; fugu, *Takifugu rubripes*; medaka, *Oryzias latipes*; stickleback, *Gasterosteus aculeatus* and zebrafish, *Danio rerio*), frog (*Xenopus tropicalis*), lizard (*Anoles carolinensis*) and chicken (*Gallus gallus*) genomes using the BioMart tool to identify the Ensembl Gene ID homologues. Similarly, the genomes of the tunicate *Ciona intestinalis*, nematode *Caenorhabditis elegans*, fruit-fly *Drosophila melanogaster* and unicellular yeast *Saccharomyces cerevisiae* were also investigated.

2.3. In silico sequence annotations

The conserved domains of the PLA_2 retrieved were deduced from NCBI Conserved Domains Database CDD-27036 PSSMs (http://www.ncbi.nlm.nih.gov/cdd). The result includes an alignment between the query and the search model consensus sequence, the expect-value for the alignment, the identity (name) of conserved domain and

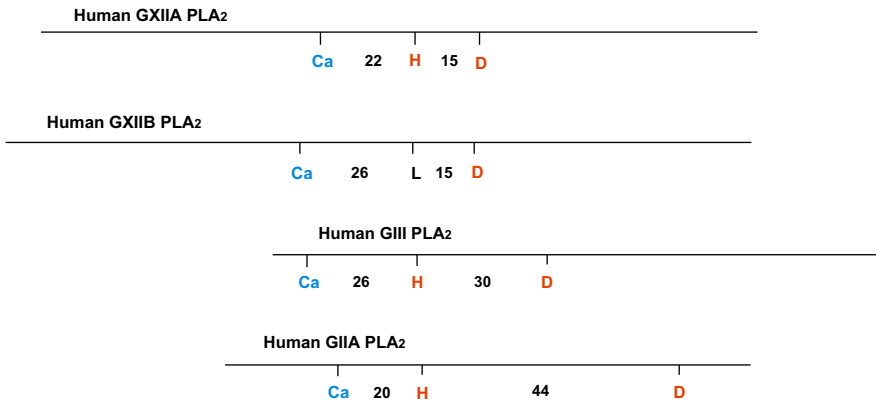

Fig. 1. Comparison of the locations of the Ca^{2+}-binding site (Ca) and histidine (H) and aspartic acid (D) catalytic sites of human GXIIA (Q9BZM1, conserved domain pfm06951) and GXIIB (Q9BX93, pfam06951) PLA_2s with those of human GIII (Q9NZ20, cd04704) and GIIA (P14555, cd00125) PLA_2s. The horizontal lines represent the mature protein chains of GXIIA PLA_2 (167 amino acid residues, aa), GXIIB PLA_2 (175 aa), GIII PLA_2 (141 aa) and GIIA PLA_2 (124 aa). The metal binding amino acids of the Ca^{2+}-binding site are glycine, proline and phenylalanine in GXIIA PLA_2, glycine, tyrosine and leucine in GXIIB PLA_2, two glycines in GIII PLA_2 and histidine and two glycines in GIIA PLA_2. The distances between the Ca^{2+}-binding and catalytic sites are indicated by the number of amino acids residues between the respective sites.