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Marine invertebrate lipases: Comparative and functional genomic analysis

Crisalejandra Rivera-Perez *

Department of Biological Sciences, Florida International University, Miami, FL 33199, USA
Centro de Investigaciones Biologicas del Noroeste (CIBNOR), La Paz, B.C.S. 23096, Mexico

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ABSTRACT

Lipases are key enzymes involved in lipid digestion, storage and mobilization of reserves during fasting or heightened metabolic demand. This is a highly conserved process, essential for survival. The genomes of five marine invertebrate species with distinctive digestive system were screened for the six major lipase families. The two most common families in marine invertebrates, the neutral an acid lipases, are also the main families in mammals and insects. The number of lipases varies two-fold across analyzed genomes. A high degree of orthology with mammalian lipases was observed. Interestingly, 19% of the marine invertebrate lipases have lost motifs required for catalysis. Analysis of the *lid* and loop regions of the neutral lipases suggests that many marine invertebrates have a functional triacylglycerol hydrolytic activity as well as some acid lipases. A revision of the expression profiles and functional activity on sequences in databases and scientific literature provided information regarding the function of these families of enzymes in marine invertebrates.

1. Introduction

With the advent of new sequencing tools, there is a trend for sequencing genomes in model and non-model organisms. This genomic revolution enable access to genome sequences in global databases and makes possible identification and comparison of genomic variation of relevant functions in animals. One of the relevant metabolic pathways is the study of the genes involved in lipid digestion. Lipids are the major source of metabolic energy and essential molecules for the formation of cell and tissue membranes. They are important in the physiology and reproductive process of marine animals and reflect the biochemical and ecological conditions of the marine environment (Sargent et al., 1995). Among lipids, neutral lipids are particularly important since they support larval development, especially embryogenesis and metamorphosis (Sewell, 2005).

Neutral lipids are hydrolyzed by lipases (E.C. 3.1.1.3) (Jaeger et al., 1994). These hydrolytic enzymes possess multifunctional properties such as broad substrate specificity and regio-specificity (Jaeger et al., 1994). Lipases are divided into six families, based on their sequences, which is characterized by an α/β hydrolase fold (CL0028; Zinke et al., 2002). These families are the neutral (Pfam: PF00151), acid (Pfam: PF04083), lipase 2 (Pfam: PF01674), lipase 3 (Pfam: PF01764), lipase with motif Gly-Asp-Ser-(Leu) also known as GDSL (Pfam: PF00657) and hormone sensitive lipases (HSL; Pfam: PF06350) (Derewenda, 1994; Holmquist, 2000). Another small family of lipases with an important role in lipid metabolism among vertebrates and invertebrates is the adipose triglyceride lipase (ATGL; Pfam: PF01734), which function mainly in adipose tissue (Holmes, 2012). All lipases contain a consensus sequence Gly-X-Ser-X-Gly (X: any amino acid) and their catalytic mechanism is a two-step mechanism based on catalytic residues Ser, Asp/Glu and His (Ollis et al., 1992). Among other special features, lipases possess an α-helix fragment or lid which covers the nucleophilic serine and regulates access to the active site (Ollis et al., 1992). In contrast to most lipases, the ATGL family is characterized by a patatin domain and their active site is composed only of Ser and Asp (Wilson et al., 2006).

In marine invertebrates, there is a poor understanding of lipid digestion. Most previous studies on digestive lipases used crude enzyme preparations (Hervant et al., 1999; Perera et al., 2008) and only a few digestive lipases have been isolated (Slim et al., 2007; Rivera-Perez et al., 2011a,b). These enzymes are tissue specific in the digestive gland (Johnston et al., 2004; Johnston and Joel, 2005; Molschaniwskyj and Johnston, 2006; Rivera-Perez et al., 2011a), and they have been described as regio-specific and non-specific lipases. Only one intracellular lipase has been isolated and characterized in crustaceans (Rivera-Perez et al., 2011b). In contrast to mammals and insects, marine invertebrates are able to hydrolyze long-chain triglycerides (Rivera-Perez et al., 2011a; Forrellat-Barrios et al., 2004; Del Monte et al., 2002) which are associated with hydrophobicity of the lid. The lipases described so far function at pH 8.0 (Slim et al., 2007; Pasquevich et al., 2011; Rivera-Perez et al., 2011a), the same pH as human pancreatic lipase (Carriere et al., 2000). Another important characteristic of marine lipases is that they do not

* Department of Biological Sciences, Florida International University, 11200 SW 8th St, Miami, FL 33199, USA. Tel.: +1 305 348 0405; fax: +1 305 348 1986.
E-mail address: cririver@fiu.edu.

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require colipase, a small protein cofactor, to display their activity (Rivera-Perez et al., 2011a), as has been described in mammals (Carriere et al., 2000).

Given the importance of the function of lipases in lipid metabolism, this paper focuses on functional genomic analysis of marine invertebrate lipases. Lipases belonging to the seven families of lipases described earlier were found in the genome of five marine invertebrates and one fresh water invertebrate: Pacific oyster *Crassostrea gigas*, owl limpet *Lottia gigantea*, purple sea urchin *Strongylocentrotus purpuratus*, starlet sea anemone *Nematostella vectensis*, the sponge *Amphimedon queenslandica*, and water flea *Daphnia pulex*. These species represent five phylums: Cnidaria (*N. vectensis*), Echinodermata (*S. purpuratus*), Crustacea (*D. pulex*), Mollusca (*C. gigas* and *L. gigantea*) and Porifera (*A. queenslandica*). These animals differ in their organs dedicated to food digestion, as well as their source of lipids and their storage.

2. Materials and methods

2.1. Gene and protein identification of marine invertebrates

Basic Local Alignment Search Tool (BLAST) studies for the identification of invertebrate marine lipases were undertaken using web tools from the NCBI and Ensembl Metazoa using insect lipases as probes (Altschul et al., 1997). The following genomes were examined: *C. gigas* (Zhang et al., 2012), *L. gigantea* (Simakov et al., 2013), *D. pulex* (Coulbourne et al., 2011), *S. purpuratus* (Sea Urchin Genome Sequencing Consortium et al., 2006), *N. vectensis* (Putnam et al., 2007) and *A. queenslandica* (Srivastava et al., 2010). Neutral and acid lipases from these marine invertebrate genomes were identified using four *Drosophila melanogaster* protein lipase sequences as probes (GenBank accession no. **CG4979** and **CG6271** for the neutral lipases and GenBank accession no. **CG17097** and **CG7279** for the acid lipases).

Members of the lipase 2 were screened using a *Caenorhabditis elegans* lipase 2 (GenBank accession no. **NP_496693**) as the probe since *D. melanogaster* does not possess lipase 2 sequences in its genome. Lipase 3, GDSL, and hormones sensitive lipase (HSL) families were identified using tblastn analysis with *D. melanogaster* orthologues. The probes used in these screens were GenBank accession no. **CG33174** (lipase 3), **CG11029** (GDSL) and **CG11055** (HSL). To identify a small family of lipases with an emerging role in lipid mobilization, ATGL, a *D. melanogaster* probe was used (GenBank accession no. **CG5295**). This procedure produced many BLAST hits for each of the lipase families, which were individually examined and retained in the FASTA format. A record of the sequences for predicted encoded lipase proteins was maintained. These records were derived from annotated genomic sequences using the gene prediction method: GNOMON and GenomeScan and validated with the PASA (Haas et al., 2003). The relative localization of gene lipases, gene size, and exon boundaries were performed with the Ensembl genome browser (http://www.ensembl.org).

Each of the marine invertebrate gene sequences that were identified was also used in a tblastn screen of the ESTs contained in the GenBank database to identify partial sequences of lipases from marine invertebrates other than the six species with sequenced genome. The ESTs or incomplete genomic sequences were not included in the phylogenetic analyses.

The candidates were tested against the Hidden Markov Model (HMM) profile (build 2.3.2) of the lipase family domains in the Pfam HMM library in the MyHits protein database. All sequences with an E-value below 0.1, gathering cut-off above −90.0, and length above 100 amino acids were selected for further analyses. Theoretical isoelectric points and molecular weights for invertebrate marine lipases subunits were obtained with the Expasy web tools (Gasteiger et al., 2005). The presence of secretion signal peptides in predicted lipase protein sequences was inferred using the Signal P 4.1 program (Nordahl et al., 2011). The potential for subcellular localization was assessed with the ProtComp 8.0 and WoLF PSORT programs (Horton et al., 2006).

2.2. Phylogenetic analyses

Alignments of neutral lipase sequences were assembled using ClustalW (Chenna et al., 2003) in the MEGA 6 software (Tamura et al., 2007) and its default settings. Phylogenetic tree was constructed by Maximum Likelihood method based on the JTT matrix-based model (Jones et al., 1992). Statistical analysis used the Bootstrap method with 1000 replications; only values over 90% significance were shown (Felsenstein, 1985).

2.3. Gene clusters

Genomic scaffolds containing lipase genes were screened for related lipases sequences within 6 kb of their 5′- or 3′-ends. For all the genomes analyzed, the Ensembl Metazoa database was used. Two or more lipases genes separated by less than 6 kb were considered a cluster.

2.4. Motif searching

The catalytic residues of neutral and acid lipase protein sequences were inferred by sequences alignment with the well characterized human pancreatic lipase and human gastric lipase (GenBank accession no. NP_001003319, NP_001003209) (Dodson and Wlodawer, 1998). ß9 loops and lids were identified from sequences alignment with human endothelial and hepatic lipases (GenBank accession no. AAD30434, AAA59521).

2.5. Loop and lid analysis

The presence and length of loops and lids in invertebrate marine lipases were determined from sequence alignments with human pancreatic lipase (GenBank accession no. NP_001003319). The β9 loop is flanked by His residues (His_{204}–His_{224},) and the lid domain is flanked by disulfide bridges (Cys_{238}–Cys_{262}; Lowe, 2002). Identification of the potential cap domain was compared with human gastric lipase (Roussel et al., 1999).

3. Results and discussion

3.1. Differences of lipase gene number among organisms

Total number of genes across the seven lipase families in the six invertebrate lipase genomes were about two-fold different (Table 1). The sea urchin (*S. purpuratus*) and the water flea (*D. pulex*) had the most lipase genes, 46 and 45, respectively; while the sponge (*A. queenslandica*) has the fewer lipases, which is consistent with the lack of a digestive system (Lima-de-Faria, 2014) and its specialized diet (Bell, 2008).

Table 1
Representation of lipase genes from each family in the analyzed genomes from marine invertebrates and selected organisms.

Organism	Lipase families							
	Neutral	Acid	Lipase2	Lipase3	GDSL	HSL	ATGL	Total gene no.
Human	12	3	0	4	6	2	1	27
Fruit fly	31	21	0	1	2	1	1	56
C. gigas	15	2	0	3	0	1	1	21
L. gigantea	19	3	0	1	3	1	2	29
D. pulex	21	20	0	2	0	1	2	46
S. purpuratus	31	4	0	0	6	2	2	45
N. vectensis	7	6	0	3	1	1	3	21
A. queenslandica	0	1	0	2	4	1	3	11