



Comparative and functional genomics of lipases in holometabolous insects

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ABSTRACT

Lipases have key roles in insect lipid acquisition, storage and mobilisation and are also fundamental to many physiological processes underpinning insect reproduction, development, defence from pathogens and oxidative stress, and pheromone signalling. We have screened the recently sequenced genomes of five species from four orders of holometabolous insects, the dipterans *Drosophila melanogaster* and *Anopheles gambiae*, the hymenopteran *Apis mellifera*, the moth *Bombyx mori* and the beetle *Tribolium castaneum*, for the six major lipase families that are also found in other organisms. The two most numerous families in the insects, the neutral and acid lipases, are also the main families in mammals, albeit not in *Caenorhabditis elegans*, plants or microbes. Total numbers of the lipases vary two-fold across the five insect species, from numbers similar to those in mammals up to numbers comparable to those seen in *C. elegans*. Whilst there is a high degree of orthology with mammalian lipases in the other four families, the great majority of the insect neutral and acid lipases have arisen since the insect orders themselves diverged. Intriguingly, about 10% of the insect neutral and acid lipases have lost motifs critical for catalytic function. Examination of the length of lid and loop regions of the neutral lipase sequences suggest that most of the insect lipases lack triacylglycerol (TAG) hydrolysis activity, although the acid lipases all have intact cap domains required for TAG hydrolysis. We have also reviewed the sequence databases and scientific literature for insights into the expression profiles and functions of the insect neutral and acid lipases and the orthologues of the mammalian adipose triglyceride lipase which has a pivotal role in lipid mobilisation. These data suggest that some of the acid and neutral lipase diversity may be due to a requirement for rapid accumulation of dietary lipids. The different roles required of lipases at the four discrete life stages of holometabolous insects may also contribute to the diversity of lipases required by insects. In addition, insects use lipases to perform roles for which there are no correlates in mammals, including as yolk and male accessory gland proteins.

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1. Introduction

Lipids are essential for all forms of life but they perform a diverse and unique array of functions in insects. As in other organisms they are key components of membrane and energy storage systems and the starting materials for the synthesis of many secondary metabolites. However they have also been enabling for many adaptations that are distinctive to all or parts of the Class Insecta. Phenomena such as metamorphosis, life cycle and caste diversity, diapause, cuticular hydrocarbons, precursors for pheromone synthesis (Beenakkers et al., 1985; Canavoso et al., 2001; Hahn and Denlinger, 2007) and many of the 'eukaryote extremophile' ecological niches occupied by individual insect species all depend to varying degrees on adaptations of lipid metabolism.

Lipid accumulation and mobilisation is particularly important for the radical reconstruction of body plan and biochemistry in holometabolous (that is, having distinct egg, larval, pupal and adult stages) insects. Lipid accumulation during their larval stages is primarily used to support metamorphosis during the pupal stage and, in many instances, to support the demanding flight and reproductive activities of non-feeding adult stages. In the feeding stages there is a carefully regulated balance between lipid and fatty acid material obtained from the diet and *de novo* synthesis of fatty acids from dietary carbohydrates. For many insects, polyunsaturated fatty acids are an essential requirement which must be obtained through the digestion of dietary lipids (Canavoso et al., 2001). Different insect species have evolved to utilise nearly every type of available nutrient from plant phloem through to decaying meat, which in turn requires a diversity of lipid digestive and metabolic systems.

The main lipids in insects are triacylglycerides (triacylglycerols, TAGs), which are used both for energy storage and for storage of

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fatty acid precursors for semiochemicals; diacylglycerides (DAGs), the main form of transported lipid; phospholipids, which are involved in membrane structure; and hydrocarbons and wax esters which are incorporated into or on the surface of the cuticle to minimise evaporative water loss (Gilby, 1965; Fast, 1967; Stanley-Samuelson et al., 1988). Large depots of lipids are stored in the insect fatbody, predominantly in the form of TAG (Canavoso et al., 2001). In adults, this lipid material may be mobilised as energy for flight and in females it is also used as egg yolk material and to provide energy for the developing embryo (Troy et al., 1975; Kawooya and Law, 1988). Lipophorin, the major lipoprotein found in the insect hemolymph, serves as a reusable, non-internalised shuttle to deliver lipid material from the fat body to these sites (Canavoso et al., 2001; van der Horst et al., 2002). DAG is the predominant neutral lipid transported in insects (Soulages and Wells, 1994) but other neutral lipids such as triacylglycerol and long-chain hydrocarbons, free fatty acids and phospholipids are also transported in some insects. This involvement of DAG and lipophorin in insect lipid transport differs fundamentally for the situation in mammals where TAG and free fatty acids are used heavily at different points in the process and different sets of carrier molecules are also involved (Olson, 1998).

The tools of modern genomics provide us with powerful new probes for investigating the enzymology underpinning the uniqueness and diversity of insect lipid metabolism. In this paper we apply these tools to one of the key groups of enzymes involved, namely the lipases. Lipases, generally defined as triacylglycerol hydrolases (EC 3.1.1.3), cleave the carboxylester linkages in TAG, in particular, but also in DAG, galactolipids and phospholipids. Key steps in lipid uptake, transport and utilisation are thus controlled by lipases.

Most lipases from all organisms can be divided into six families defined by sequence relationships within the α/β hydrolase fold superfamily of proteins. These are the neutral (PF00151), acid (PF04083), lipase2 (PF01674), lipase3 (PF01764), GDSL (PF00657) and hormone sensitive lipases (PF06350) (Derewenda, 1994; Holmquist, 2000). All six families appear to use the same, two-step, reaction mechanism based on a catalytic triad of residues (usually Ser-His-Asp/Glu) that generates a charge relay system and a highly nucleophilic serine. Another small family of lipases with an emerging role in lipid mobilisation in insects, the adipose-triglyceride-lipase (ATGL) family, also have an N-terminal domain with a predicted α/β hydrolase fold and an active site serine residue but they also include a distinctive patatin domain (PF01734) (Zimmerman et al., 2004). Furthermore, while some phospholipid hydrolysing activities are contained within the six families above, most phospholipases so far studied lie in a different family, the phospholipase A₂ family (EC 3.1.1.4), which sits in a different structural superfamily and uses a different catalytic mechanism (Yuan and Tsai, 1999).

The information currently available on the biochemistry of the lipases from the six main families described above is almost entirely obtained from mammalian enzymes. The limited empirical structural data indicate that the six families share the same basic α/β hydrolase fold consisting of an eight-stranded mostly parallel α/β structure. Loop insertions and extra domains create enzymes with different sizes (ranging from 25 to over 100 kDa). These enzymes do not require cofactors for catalysis and have a similar catalytic mechanism, with the catalytic triad contained in conserved loops and a conserved oxyanion hole formed by backbone amide hydrogen atoms from the residue adjacent to the catalytic nucleophile (usually serine) and in residues located between the third β -strand and second helix (Holmquist, 2000).

The GDSL family of lipases can hydrolyse fatty acids from TAGs, DAGs, monoacylglycerols (MAGs), and from the *sn*-2 position of

phospholipids, and many will also hydrolyse carboxylester or thiolester substrates (Akoh et al., 2004). The lipase2 and lipase3 family members can hydrolyse TAGs or carboxylesters with varying fatty acid constituents (Holmquist, 2000). The hormone-sensitive lipases hydrolyse TAGs and cholesterol esters (Holm et al., 1988) but are under significant hormonal and neuronal control through reversible cAMP-dependent phosphorylation of a serine residue (Belfrage et al., 1984). The neutral lipases are so named for their ability to hydrolyse neutral lipids such as TAGs, DAGs and MAGs, but members of this family are also known to hydrolyse the *sn*-1 position in phospholipids and galactolipids (Derewenda, 1994; Carrière et al., 1998). The acid or abhydrolipase family members are so named because the founding member, the gastric lipase of mammals, was found to function at acidic pH whereas the activity of human pancreatic lipase from the neutral lipase family has a pH optimum of 8 (Carrière et al., 2000). The acid lipases predominantly hydrolyse TAGs and cholesterol esters (Gargouri et al., 1989).

This study compiles the sequence and functional information available for insect lipases to build a picture of the roles these enzymes play in the distinct physiology of these organisms. The focus is on the six lipase families above and the sequence data are substantially based on the genomes of five holometabolous species. These are the fruit fly *Drosophila melanogaster*, the malarial mosquito *Anopheles gambiae*, the silkworm *Bombyx mori*, the honey bee *Apis mellifera* and the flour beetle *Tribolium castaneum*. These species represent the four largest insect orders: Diptera (*Drosophila*, *Anopheles*), Lepidoptera (*Bombyx*), Hymenoptera (*Apis*) and Coleoptera (*Tribolium*). Although all five species are holometabolous they have very different sources of, and uses for lipids. *D. melanogaster* eats yeasts and nectar of fermenting fruit, *A. gambiae* larvae consume algae and adults feed on plant nectar, with blood meals for females only, *A. mellifera* eats nectar and fermented pollen, *B. mori* is phytophagous and *T. castaneum* eats cereal grains. *T. castaneum* rarely flies but adults of the other four are all frequent and expert fliers.

2. Materials and methods

2.1. Gene detection and annotation

All the lipase sequences from the thoroughly annotated *D. melanogaster* genome (<http://flybase.bio.indiana.edu>) were already available in the Pfam database (<http://www.sanger.ac.uk/CGI-bin/Pfam>; Finn et al., 2007), as were 17 sequences from other insect species.

To identify neutral and acid lipase predicted proteins from the *A. gambiae* genome, a TBLASTN analysis was performed using four *D. melanogaster* lipase protein sequences as probes (accession numbers CG4979 and CG6271 for the neutral lipases and CG17097 and CG18284 for the acid lipases). A similar TBLASTN screen was used for *A. mellifera* (<http://www.ngsc.bcm.tmc.edu/projects/honeybee>) except that CG6283 was used to identify neutral lipases and CG6753, CG11598 and LIP1 (CG7279) were used to identify the acid lipases. Some *A. mellifera* lipases were also obtained from NCBI (<http://www.ncbi.nlm.nih.gov/BLAST>) which used GNOMON to predict gene structures. For *T. castaneum* (<http://www.bioinformatics.ksu.edu/BeetleBase>) the TBLASTN screen used CG6431 to identify neutral lipases and an *Anopheles* acid lipase protein sequence AgamA1 (encoded by http://www.ncbi.nlm.nih.gov/Genbank/Genbank.fcgi?acc=XM_312606) to identify acid lipases; gene structures were then predicted using Genscan (Burge and Karlin, 1997) or Softberry (<http://www.softberry.com/berry.phtml>). Lipases from *B. mori* were obtained using homology searching against the predicted proteome of *B. mori* (<http://silkworm.genomics.org.cn/index.jsp>) using a Batch BLASTP analysis (Altschul et al., 1997) and using all the *D. melanogaster* neutral and acid lipase protein sequences as probes. In this paper, insect lipase names, other

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