Lipophorin Receptor: The Insect Lipoprotein Receptor

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Keywords
Low-density lipoprotein receptor, lipophorin, lipophorin receptor, insects.

The low-density lipoprotein receptor (LDLR), one of the best characterized cell-surface receptors, mediates cholesterol homeostasis and other functions in mammals. The members of the LDLR superfamily are structurally related and characterized by distinct functional domains. Insect lipoprotein receptor is called lipophorin receptor (LpR) and belongs to the LDLR superfamily. Here, we review the structural and functional aspects of lipophorin receptors.

Receptors are proteins, either embedded on cell surface or soluble within the cytoplasm, that recognize and bind with specific molecules producing a specific effect in the cell. A molecule which binds to a receptor and induces downstream signaling is called a ligand. The receptor–ligand interaction is a key element for the functioning of all biological systems. The low-density lipoprotein receptor (LDLR) is the prototype of a large family of structurally homologous cell-surface receptors and functions as endocytic and signaling receptor in a wide variety of cellular processes. These receptors bind to their ligands (such as lipoprotein) and internalize them through receptor-mediated endocytosis. In mammals, the crucial role of LDLR is the clearance of cholesterol-rich low-density lipoproteins (LDL) from the bloodstream by transporting them into the cell [1]. Once inside the cell, the cholesterol is stored, used or removed from the cell. Mutations in the LDLR genes disrupt the receptors' ability to remove cholesterol from the blood – the accumulation of cholesterol causes atherosclerosis (hardening of arteries), responsible for the majority of cardiovascular diseases. Due to this reason it has been the most extensively studied cell-surface receptor so far. However, apart from cholesterol metabolism, lipoprotein receptors have also been recognized for often unrelated roles such as

cellular signal transducers [2,3]. The LDLR family members can be found in species ranging from nematodes to insects to mammals, indicating that the genes have originated from an ancestor gene during evolution. The purpose of this article is to introduce one of the evolutionary ancient families of cell-surface proteins and their molecular characterizations in insects.

Box 1.

Drs. Michael S Brown and Joseph L Goldstein were awarded the Nobel Prize in Physiology/Medicine in 1985 for their outstanding work on LDLR.

Unlike mammalian blood, insect hemolymph contains a single class of low- to high-density lipoproteins called lipophorin (Lp), which consists of protein and lipid moieties. Lp has an approximate native molecular weight of 730 kDa and consists of two different subunits. They are 230–250 kDa apolipophorin I (apoLp I) and 50–90 kDa apolipophorin II (apoLp II)[1]. The main function of the Lp is to deliver lipids throughout the insect body for metabolism and storage. The fat body, which is an insect analog of vertebrate liver and adipose tissue, serves a key role in lipid metabolism, being the site of lipid storage and transport. The Lp carries mainly diacylglycerol and phospholipid. The delivery of these lipids to the target tissues by Lp occurs with-out concurrent degradation of the protein part of Lp particle. The lipid-depleted Lp can reload the lipid for the next delivery and thus Lp functions as a reusable shuttle. The lipophorin receptor (LpR), which is on the outer cell surface, binds to Lp and is sequestered through receptor-mediated endocytosis. After the process, the receptor returns to the cell surface by a process called receptor recycling[2]. The lipids taken up by the cell are then used for various metabolic activities. Biochemical and molecular characterizations of insect LpRs reveal that they are members of the LDLR gene family. The first molecular characterization of the insect LpR was reported in locust, *Locusta migratoria* followed by mosquito (*Aedes aegypti*), wax moth (*Galleria mellonella*), silkworm (*Bombyx mori*), cockroaches (*Blatella germanica* and *Leucophaea maderae*), fruit fly (*Drosophila melanogaster*), honey bee (*Apis mellifera*) and beetle (*Tribolium castaneum*).

[1] Apoprotein: The polypeptide moiety of a protein is called apoprotein, whereas the non-polypeptide parts such as lipid, metal ions, etc., are called prosthetic group. Together they are known as holo-protein.

[2] Receptor Recycling: Receptor-ligand complexes enter the cell by endocytosis at clathrin-coated pits, where the receptor molecules cluster on the cell surface. Bound lipoprotein particles are subsequently released in the low-pH milieu of the endosome, and the receptors then return to the cell surface in a process called receptor recycling. The neutral pH causes the receptor to revert to its native conformation, ready to receive another lipoprotein particle.

Biological Significance of LpR

As noted above, Lps are insect proteins mainly involved in lipid transport, acting as a shuttle for lipids between different tissues. Lipid is used as a major energy source for development as well as other metabolic and physiological processes (e.g., flight). In addition, during vitellogenesis[3], Lp transports lipids and other yolk precursors from the fat body to the ovaries and thus plays a critical role in egg development. Lp is incorporated into the cells mainly through the receptor-mediated endocytosis and thus LpRs play a vital role in the overall physiology of insects.

[3] Vitellogenesis: The process by which the yolk is formed and accumulated in the ovum as a source of nutrients for the egg development. Vitellogenin and lipophorin are the major yolk protein precursors in insects. It is an essential process to sustain reproduction.

Structure of LpR Proteins

The insect LpRs which are the core members of the LDLR family are characterized by the presence of a signal peptide followed by five structural and functional domains, which are discussed here (*Figure* 1).

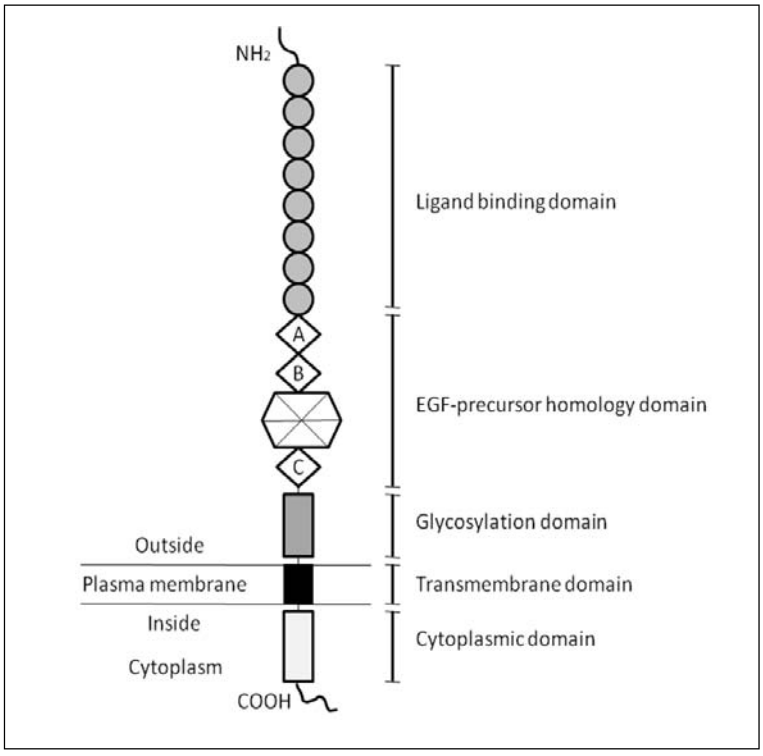

Figure 1. Structure of lipophorin receptor.

Ligand Binding Domain (*LBD*)

LBD is responsible for the interactions between the receptor and its ligand. This N-domain consists of an average of 350 amino acids and eight cysteine-rich repeats, each with six conserved cysteine residues, known as LDLR class A repeats. These repeats form disulphide bonds which are essential for the ligand-receptor binding process. The LBD is characterized by the conserved sequence of acidic amino acids (C**D**XXX**D**CX**D**X**SDE**; X indicates any amino acid) and these acidic residues are involved in the utilization of calcium ions. It is well known that the ligand binding domain of LDLR binds to Ca^{2+} which is critical for the correct folding, and disulphide formation of LpR. All insect LBDs contain eight cysteine-rich repeats except a mosquito fat body LpR variant and a German cockroach LpR, both of which have seven repeats.

Epidermal Growth Factor Precursor Domain (*EGFD*)

The receptor-ligand complex, internalized by the cell via endocytosis, moves into the endosome where a drop in pH detaches the ligand from the receptor, which is then recycled back to the cell surface. The EGFD mediates the acid-dependent dissociation of the receptor and its recycling back to the cell surface. It consists of three cysteine-rich repeats known as growth factor repeats (EGF A, B and C) and is characterized by the tetra peptides YWTD/(Y/F) WXD motif that forms a β-propeller domain.

O-linked Glycosylation Domain

The third domain, a short stretch of less than 50 amino acids, is rich in serine and threonine which provides potential sites for *O*-linked glycosylation[4]. However, the two mosquito LpR variants, expressed in fat body and ovary, have 63 and 257 residues, respectively. In this region, insect LpR isoforms differ by the insertion/deletion of many amino acids. In silkworm the LpR1 in comparison to other isoforms has an additional 27 amino acids in the glycosylation domain. The role of this domain is poorly understood though it was reported to protect the receptor from

[4] Glycosylation: The process of attaching sugar moities to proteins on the serine/threonine residues (*O*-linked glycosylation) or on the asparagine residue (N-linked glycosylation). These modifications can change the properties of the proteins significantly.

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Locust     RHYLHRNVTSMNFDNPVYRKTTEDQFSLEKNQYQPQ-RIYPATVGEEAHEPLTSPGTNDYV
Silkworm1  RHYVHHNVTSMNFDNPVYRKTTEDQFALEKNGYAPGSKLYPSTVGEEAQEPLNKPNTEFV
Silkworm2  RHYVHHNVTSMNFDNPVYRKTTEDQFALEKNGYAPGSKLYPSTVGEEAQEPLNKPNTEFV
Silkworm3  RHYVHHNVTSMNFDNPVYRKTTEDQFALEKNGYAPGSKLYPSTVGEEAQEPLNKPNTEFV
Silkworm4  RHYVHHNVTSMNFDNPVYRKTTEDQFALEKNGYAPGSKLYPSTVGEEVRALTAMECTLNIQNSLR
Mosquito   KHHVHRNSTSMNFDNPVYRKTTEDQFSLEKN--LPN-RMYPSTVGEEAQEPLNRPGTNDFV
Waxmoth    RHYVHRNVTSMNFDNPVYRKTTEDHFALEKNGYAPGSKLYPSTVGEEAQEPLNTSGTNDFV
Cockroach  RHYLHRNVTSMNFDNPVYRKTTEDQFSLEKNQYQPQ-RIYPATVGEEAQEPLTSPGTNDYV
Beetle     RKYVKRNMTSMNFDNPVYRKTTEDQFSLEKNQYPPS-RPYLSTVGEEAQQPLTGANNQNDNV
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Figure 2. Clustal X† alignment of cytoplasmic domain of insects LpR. The internalization signal, NPVY is shown in bold and the specific amino acids of silkworm LpR4 are in bold and underlined. Aserisks indicate conserved residues.

† It is a computer program for analyzing multiple sequence and profile alignments.

denaturation during recycling, and modulate the proteolytic cleavage of ectoderm at the cell surface. It is marked by many additions and deletions and is the most structurally divergent among LDLR family members.

Transmembrane Domain

The transmembrane domain lies at the carboxyl-terminal end of the *O*-linked sugar domain. A hydrophobic region of 20–22 amino acid residues represents the transmembrane domain of the receptor. It plays an important role in receptor functions of several recycling passes between the plasma membrane and various endocytic vesicles. It forms a transmembrane α-helix and functions as a membrane anchor. The deletion of this membrane leads to secretion of truncated receptors from the cells.

Cytoplasmic Domain

This domain constitutes approximately 60 amino acid residues with a signal for receptor internalization via coated pits[5] containing a consensus tetrapeptide, Asn-Pro-Xaa-Tyr (NPXY). This internalization signal has been conserved for 350 million years among vertebrate LDLRs and this indicates that this motif may have originated from a common ancestor of both vertebrates and insects. The NPXY motif is also known to function as a docking site for several cytosolic adapter proteins[6] involved in physiological and signaling events. The cytoplasmic tail of the silkworm LpR4 contains 18 unique amino acids compared to LpR1-3.

[5] Coated pits: They are regions of the cell membrane specialized in receptor-mediated endocytosis. Their cytoplasmic surface is coated with a bristle-like structure made of protein called clathrin. During the first steps of endocytosis, clathrin-coated pits are internalized to form clathrin-coated vesicles.

[6] Adaptor Protein: A protein molecule that mediates protein–protein or protein–lipid interactions in signal transduction pathways. For example, phos-photyrosine binding (PTB) domains are components of cytoplasmic docking proteins that bind lipoprotein receptors through NPXY motifs.

Species	Accession Number*
Locusta migratoria (migratory locust)	CAA03855
Aedes aegypti (yellow fever mosquito)	AAK72954
Galleria mellonella (wax moth)	ABF20542
Bombyx mori (silk moth)	BAE71406
Blattella germanica (German cockroach)	CAL47125
Leucophaea maderae (Madeira cockroach)	BAE00010
Drosophila melanogaster (fruit fly)	AAQ22563
Apis mellifera (honey bee)	XP_395858.3
Tribolium castaneum (red flour beetle)	XP_967944

* **Accession Number:** A unique identification number given to a DNA or protein sequence in the public sequence databases as given below. (All three databases share the data.)
GenBank: It is an open access sequence database maintained by National Center for Biotechnology Information (NCBI), USA.
EMBL: Another open access sequence database called Europeon Molecular Biology Laboratory.
DDBJ: The Asian open access sequence database called DNA Databank of Japan.

Table 1. Reference information of insect LpRs.
(Details can be accessed through NCBI/EMBL/DDBJ)

Genomic Structure of LpR

Unlike vertebrate LDLR, the exon/intron organization of LpR is not available except in the silkworm, *B. mori*. The silkworm LpR1 (*Bm*LpR1) consists of 16 exons separated by 15 introns[7] spanning 122 kb. Other isoforms (LpR2, 3 and 4) have 15 exons[8] separated by 14 introns. The first and second introns are larger than other introns spanning 21.3 and >65 kilobase, respectively. It is also shown that all these isoforms are generated from a single gene by alternative splicing[9] and *Bm*LpR gene is located on chromosome 5. The 45 kb human LDLR gene consists of 18 exons and 17 introns and is located on chromosome 19. Although the functional domains of LpR are highly similar to the vertebrate LDLR, the genomic structure is strikingly different in terms of huge intron size in insects. Thus, *Bm*LpR gene is at least two times bigger than the human lipoprotein receptor gene.

Alternative Splicing

Alternative splicing of pre-mRNAs generates multiple transcripts

[7] Intron: A segment of a gene situated between exons that is removed before translation of messenger RNA and does not function in coding for protein synthesis.

[8] Exon: A segment of gene that codes information for protein synthesis that is transcribed to messenger RNA.

[9] Alternative splicing refers to the production of multiple transcript isoforms from a single gene, due to variations in the splicing reaction of pre-mRNA. Hence, one gene can produce many proteins.

which may encode proteins with functional differences. It is a common phenomenon among members of the LDLR gene superfamily and most of these splice variants are found to be functional proteins. Like LDLR, LpRs exist in variant forms arising from differential splicing from a single gene. In silkworm *B. mori*, four alternatively spliced isoforms, LpR1, LpR2, LpR3 and LpR4 are found, which are differentially expressed in tissue/stage-specific manner. The LpR4 is unique as it is expressed only in the brain and central nervous system and has 18 unique amino acids in the cytoplasmic domain. Mosquito, wax moth, honey bee, and cockroach have shown two LpR isoforms each.

LpR Gene Expression

The vitellogenin receptor (VgR), which is highly homologous to LpR in structure and a member of LDLR superfamily, is restricted to females as ovary is the sole tissue expressing it. These receptors bind to their respective ligands, vitellogenin (Vg) and lipophorin (Lp) and they are called yolk protein precursors. However, chicken has only one receptor which can take up both Lp and Vg. LpR gene expression has been seen in males and females alike in tissues such as fat body, ovary, testis, gut, Malpighian tubules, brain and silk glands. The tissue and stage-specific expression of silkworm, *Bm*LpR isoforms, LpR1-4 has been reported [4]. The LpR1 expression was detected in all tissues with dominant expression in ovary and brain, and high transcript levels of LpR2 was seen in pupal tissues, whereas LpR3 was less abundant than other isoforms. Interestingly, the LpR4 receptor variant was expressed exclusively in the brain and central nervous system. In mosquito, two LpR variants, each specific to fat body and ovary have been reported. In general, insect LpR mRNA was detected throughout the ovarian development but increased after the onset of vitellogenesis with an exception in honey bee, *A. mellifera*, where high transcript levels of LpR were observed in virgin queens. The transcript size of insect LpR ranges from 3–6 kb with an average open reading frame (ORF)[10] of 2808 kb coding for ~ 936 amino acids. Treatment with juvenile hormone increased the LpR protein in the

[10] ORF: An open reading frame is a portion of a DNA molecule translated into amino acids which contains no stop codons.

German cockroach. In *Drosophila*, female sterility occurs due to a defective receptor. Lower amount of lipophorin was observed in the growing oocytes of RNA interference (RNAi)-treated German cockroach. This is because the knocked down LpR is unable to bind and take up Lp into the oocytes. In addition, the functional characterization of LpR proteins through ligand binding that showed that they can bind to Lp *in vitro*. The native molecular weight of LpR proteins ranges from 97 to 140 kDa with post-translational modifications, especially glycosylation.

Future Work

The low-density lipoprotein receptor gene family is an evolutionarily conserved group of cell-surface receptors produced by mammals and other organisms. Initially they were thought to be endocytic receptors that mediate the uptake of lipoproteins. But recent findings have shown that these receptors have other roles in a range of cellular processes. Among other activities, members of this family act as signal transducers in neuronal migration processes, and regulate synaptic plasticity of the brain [3]. Sequence analysis indicates the presence of several phosphorylation sites in the cytoplasmic domain of LpRs and they are believed to be involved in signal transduction cascades [4]. However, so far there has been no report supporting the signal transducing functions of lipophorin receptors in insects. As noted above, we have recently obtained a potential candidate lipophorin receptor for possible signaling functions [5]. The silkworm LpR4 is a unique receptor variant form as it is expressed only in the brain and central nervous system and possesses 18 unique amino acids in the cytoplasmic domain as compared to other isoforms. A putative signal transduction motif in the 18 amino acids of the cytoplasmic domain of LpR4 [5] indicates that it might be involved in signaling functions. This needs to be investigated. Studies elucidating diverse functions are a must for lipophorin receptors of insects. It will change our ideas about the role of these receptors as mere endocytic receptors to being multifunctional cell-surface proteins.

Suggested Reading

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