Comparative Biochemistry and Physiology, Part B 169 (2014) 51–62

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Four cDNAs encoding lipoprotein receptors from shrimp (*Pandalopsis japonica*): Structural characterization and expression analysis during maturation

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ARTICLE INFO

Article history:
Received 30 October 2013
Received in revised form 18 December 2013
Accepted 19 December 2013
Available online 31 December 2013

Keywords:
Decapod
Pandalopsis japonica
Lipophorin
Reproduction
Vitellogenin

ABSTRACT

As in all other oviparous animals, lipoprotein receptors play a critical role in lipid metabolism and reproduction in decapod crustaceans. Four full-length cDNAs encoding lipoprotein receptors (Paj-VgR, Paj-LpR1, Paj-LpR2A, and Paj-LpR2B) were identified from *Pandalopsis japonica* through a combination of EST screening and PCR-based cloning. Paj-LpR1 appears to be the first crustacean ortholog of insect lipophorin receptors, and its two paralogs, Paj-LpR2A and Paj-LpR2B, exhibited similar structural characteristics. Several transcriptional isoforms were also identified for all three Paj-LpRs. Each expression pattern was unique, suggesting different physiological roles for these proteins. Paj-VgR is an ortholog of vitellogenin (Vg) receptors from other decapod crustaceans. A phylogenetic analysis of lipoproteins and their receptors suggested that the nomenclature of Vgs from decapod crustaceans may need to be changed. A PCR-based transcriptional analysis showed that Paj-VgR and Paj-LpR2B are expressed almost exclusively in the ovary, whereas Paj-LpR1 and Paj-LpR2A are expressed in multiple tissues. The various transcriptional isoforms of the three Paj-LpRs exhibited unique tissue distribution profiles. A transcriptional analysis of each receptor using tissues with different GSI values showed that the change in transcription of Paj-VgRs, Paj-LpR2A and Paj-LpR1 was not as significant as that of Vgs during maturation. However, the transcriptional levels of Paj-LpR2B decreased in ovary at maturation, suggesting that their transcriptional regulation is involved in reproduction.

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1. Introduction

As in all oviparous species, vitellogenesis in decapod crustaceans involves nutrient deposition and yolk formation to ensure an ample energy supply. Yolk proteins are lipoprotein complexes that are conjugated to carbohydrates and carotenoid pigments (Wallace et al., 1967). The major protein component of yolk protein is vitellogenin (Vg). Genes encoding Vg have been isolated from various decapods, and two copies of Vg genes have been isolated from several species, including *Pandalopsis japonica*, *Metapenaeus ensis*, and *Penaeus monodon* (Kung et al., 2004; Tiu et al., 2009; Jeon et al., 2010). The hepatopancreas appears to be the principal production site for Vg in the suborder Pleocyamata, which includes brachyurans, astacideans, and carideans (Okuno et al., 2002; Tsutsui et al., 2004), whereas the hepatopancreas and ovary contribute equally to Vg production in Dendrobranchiata, including penaeid shrimp (Raviv et al., 2006; Tiu et al., 2006). Interestingly, the Vg genes isolated from decapod crustaceans exhibit the highest level of similarity to lipophorins (Lps) from insects, while hemolymph clotting proteins (CPs) are clustered together with insect Vg genes (Cheng et al., 2008; Jeon et al., 2010). In insects, Lp is the main insect hemolymph lipoprotein. It is involved in lipid transport in various tissues, and in providing lipids and other yolk precursors from the fat body to the ovaries (Rodenburg and Van der Horst, 2005; Swevers et al., 2005). In insects, Vg and Lp genes are internalized into growing oocytes (Machado et al., 1996; Fan et al., 2002).

Lipoproteins are internalized by membrane-bound receptors, including Vg receptors (VgRs) and Lp receptors (LpRs) (Rodenburg and Van der Horst, 2005). Genes encoding VgRs have been identified in several insect species, including *Aedes aegypti* (Sappington et al., 1996), *Periplaneta americana* (Tufail and Takeda, 2005), and *Balttella germanica* (Ciudad et al., 2006). Similar numbers of LpR genes have been isolated from several insect species, including *Locusta migratoria* (Dantuma et al., 1999), *Galleria mellonella* (Lee et al., 2003), and *B. germanica* (Ciudad et al., 2007). Although both VgR and LpRs belong to the low-

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1096-4959/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpb.2013.12.005

density lipoprotein (LDL) receptor (LDLR) superfamily, which harbors conserved motifs, including a ligand-binding domain (LBD), epidermal growth factor (EGF) precursor domain, O-linked sugar domain (OLSD), transmembrane domain (TMD), and cytoplasmic domain, the two types of receptors can be clearly distinguished from each other based on the conserved domain organization of each type. The phylogenetic and functional relationships among lipoproteins and their receptors have not been clearly established in decapod crustaceans.

Since the first putative VgR cDNA was isolated from *P. monodon* (Tiu et al., 2008), three additional full-length and partial cDNA sequences encoding crustacean lipoprotein receptors have been added to the GenBank database (http://www.ncbi.nlm.nih.gov/). Based on their domain organization, all four lipoprotein receptors appear to be orthologs of insect VgRs. Homologs of LpRs have not been identified in decapod crustaceans.

Pandalopsis japonica is an important species due to its commercial value and as a model system for molting and reproduction studies because it survives for long periods after eyestalk ablation (Lee et al., 2011; Jeon et al., 2012). Further, its evolutionary importance cannot be understated, as Caridea (true shrimp) can be used for comparative studies with other decapod crustaceans, including Brachyura (crabs), Astacidea (lobsters and crayfish), and Dendrobranchiata (penaeid shrimp). In the present study, we identified four distinct full-length cDNAs encoding lipoprotein receptors (Paj-LpR1, Paj-LpR2A, Paj-LpR2B, and Paj-VgR), and we investigated their primary structure and transcriptional characteristics, which are related to ovarian maturation.

2. Materials and methods

2.1. Experimental animals

Live shrimp (*P. japonica*) were purchased from a local seafood market and acclimatized in circulating aerated seawater for 7 days at 4 °C before dissection. The shrimp were fed daily with squid and polychaetes. The day/night lengths were maintained at 12 h/12 h. Before dissection, the animals' wet body and gonad weights were measured. The isolated tissues were directly frozen in liquid nitrogen and stored at −70 °C before use for total RNA extraction. The individual gonad-somatic index (GSI = [gonad weight/total body weight] × 100) was calculated as described previously (Jeon et al., 2010).

2.2. Cloning of full-length cDNAs encoding four lipoprotein receptors (Paj-LpR1, Paj-LpR2A, Paj-LpR2B, and Paj-VgR)

A cDNA contig database of *P. japonica* was established by a commercial next-generation sequencing service (Macrogen Inc., Seoul, Korea) as described previously (Jeon et al., 2012). Using the LpR1sequence from *Bombyx mori* (**NP_001104808**) as bait, the cDNA database was screened using the Basic Local Alignment Search Tool (BLAST) program (http://ncbi.nlm.nih.gov). To isolate the full-length cDNA sequence, fragment analysis by PCR and RACE was performed as described in Supplementary data 2. Degenerate and sequence-specific primers were designed using the IDTSciTools program (http://eu.idtdna.com/analyzer/applications/oligoanalyzer/default.aspx) targeting conserved amino acid residues to extend the sequences (Supplementary data 1). The primers used in this experiment were commercially synthesized by Bioneer Co. (Daejeon, Korea). Since it was not possible to obtain full-length cDNA with a single reaction, fragmental sequences were also obtained by PCR with sequence-specific and degenerate primers (Supplementary data 1 and 2). 5′-RACE and walking were carried out using a CapFishing™ full-length cDNA isolation kit and DNA Walking SpeedUp™ Premix Kit according to the manufacturer's protocol (Seegene, Seoul, Korea). 3′-RACE was performed using sequence-specific forward primers and previously used 3′-RACE primers, which harbor a lab-designed linker sequence (Lee et al., 2011). The fragmental sequences were joined together and the single transcript was reconfirmed by RT-PCR.

Total RNA was isolated from the ovary and hepatopancreas, which are considered to be the major production sites for lipoprotein receptors in arthropods (Tufail and Takeda, 2009). Genomic DNA was removed by DNase I (Promega, Madison, WI, USA) treatment prior to cDNA synthesis. cDNAs were synthesized as described previously (Jeon et al., 2011). The reactions (20 μL) contained 1 μL of cDNA (200 ng/μL), 2 μL of sequence-specific primers (4 pM) (Supplementary data 1), 0.1 μL of Ex Taq Hot Start Version (Takara Bio Inc., Shiga, Japan), 2 μL of dNTPs (2.5 mM each), and 2 μL of 10× buffer. The PCR conditions were 1 min at 94 °C followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. 18S rRNA primers were used as a positive control (Supplementary data 1). The reactants were separated by 1.5% agarose gel electrophoresis and stained with ethidium bromide. Those amplicons of the expected sizes were purified using an AccuPrep Gel Purification Kit (Bioneer Co.), ligated into the TA plasmid vector with a pGEM-T Easy Cloning Kit (Promega), and transformed into *Escherichia coli* DH5-alpha competent cells. The cDNAs were sequenced with an ABI Biosystems 3730 sequencer (Applied Biosystems, Foster City, CA, USA).

2.3. Bioinformatic analysis

Nucleotide and amino acid sequence similarities were analyzed using BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/). The full-length, deduced amino acid sequences were aligned using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and the domain organization of each lipoprotein receptor was predicted by the SMART algorithm (http://smart.embl-heidelberg.de/) (Letunic et al., 2012). Phylogenetic trees for the lipoproteins and their receptors were constructed by the minimal evolution method using Molecular Evolutionary Genetics Analysis (MEGA5) software (Tamura et al., 2011). All annotated sequences were retrieved from the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/).

Analyzed cDNA sequences included the Insect LpRs of *Bombyx mori* (BAE71406), *Aedes aegypti* (AF355595), *Locusta migratoria* (AJ000010) and *Tribolium castaneum* (XP_967944); the insect VgRs of *Tribolium castaneum* (XM_963810), *Anopheles gambiae* (EAA06264), *Aedes aegypti* (AAK15810) and *Blattella germanica* (CAJ19121); the crustacean ovarian LpRs of *Marsupenaeus japonica* (BAH57291) and *Penaeus semisulcatus* (AAL79675); the crustacean VgRs of *Macrobrachium rosenbergii* (ADK55596) and *Penaeus monodon* (ABW79798); the vertebrate LDLRs of *Mus musculus* (CAA45759), *Sus scrofa* (AAC17444), *Homo sapiens* (AAA56833) and *Bos taurus* (XP_874020); the vertebrate VgRs of *Oncorhynchus mykiss* (CAD10640), *Anguilla japonica* (BAB64337) and *Morone americana* (AAO92396); the vertebrate VLDLRs of *Homo sapiens* (AAA61344), *Mus musculus* (AAA59384) and *B. taurus* (NP_776914); the insect Vg of *Apis melifera* (NP_001011578), *Bombus ignites* (ACQ91623), *Nasonia vitripennis* (XP_001307388) and *Culex quinquefasciatus* (XP_001857967); the maxillopod Vg of *Tigriopus japonicus* (ACJ12892) and *Lepeophtheirus salmonis* (ABU41134); the decapod CP of *Marsupenaeus japonicus* (ABK59925) and *Penaeus monodon* (ABW77320); the mollusk Vg of *Crassostrea gigas* (BAC22716) and *Haliotis discus* (BAF98238); the vertebrate Vg of *Gallus gallus* (AAA49139), *Oryzias latipes* 1 (BAB79696), *O. latipes* 2 (BAB79591), *Anguilla japonica* 1 (AAR82899) and *A. japonica* 2 (AAR82898); the vertebrate apoB of *Rattus norvegicus* (NP_062160) and *H. sapiens* (NP_000375); the insect apoLp-II/I of *Locusta migratoria* (CAB51918), *Nilaparvata lugens* (BAG75121) and *C. quinquefasciatus* (XP_001849310); the decapods Vg of *Scylla paramamosain* (ACO36035), *Callinectes sapidus* (ABC41925), *Pandalopsis japonica* 1 (ACU51164), *P. japonica* 2 (KF731996), *Litopenaeus vannamei* (AAP76571), *Marsupenaeus japonicus* 1 (BAB01568) and *M. japonicus* 2 (BAD98732). The insect LDLR of *B. mori* (BAG12564) and the

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