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Penaeid shrimp hemolymph lipoproteins

Gloria Yepiz-Plascencia*, Francisco Vargas-Albores, Inocencio Higuera-Ciapara

Marine Biotechnology Laboratory, Centro de Investigación en Alimentación y Desarrollo, A.C., PO Box 1735, Hermosillo, Sonora, 83000 Mexico

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Abstract

Due to their hydrophobic nature, lipids are transported in the hemolymph of shrimp by protein–lipid-complexes named lipoproteins. Since cholesterol (Ch) and polyunsaturated lipids must be provided by the diet, and they are stored mainly in the hepatopancreas; a special vehicle is necessary for their mobilization to other tissues. Two types of hemolymph lipoproteins have been isolated from penaeid shrimp. Non sex-specific lipoproteins present in males and females (LPI), and female-specific lipoproteins (LPII or Vg) that occur mainly in mature females undergoing ovarian maturation. This review focuses on current knowledge about penaeid shrimp hemolymph lipoproteins and it compares their protein and lipid constituents. These lipoproteins are of the high density and very high density types. Their lipids are predominantly phospholipids (PL), but sterols, diacylglycerols (DG), triacylglycerols, and hydrocarbons (HC) have also been found. The apolipoproteins are high molecular mass polypeptides. The LPI generally contains a fewer number of apoproteins or subunits than the LPII or Vg.

Keywords: Crustacean; Hemolymph; Lipoproteins; Penaeid; Plasma; Shrimp; Vitellogenin

1. Introduction

Lipids are a major source of energy in marine invertebrates, including shrimp; furthermore, they are involved in several essential processes for their growth, molting and reproduction. Cell membrane structure depends largely on the combination of specific lipids and proteins; additionally, lipid droplets accumulate in specific tissues

* Corresponding author. Tel.: +52-62-80-00-57, ext 524; fax: +52-62-80-00-55.
E-mail address: gyepiz@cascabel.ciad.mx (G. Yepiz-Plascencia).

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serving as energy storage. Lipids are also found in the hemolymph as water-soluble molecules formed by apoproteins and lipid moieties constituting the lipoproteins (LPs). LPs transport lipids from sites of absorption, storage or synthesis to sites of utilization. In addition to the non sex-specific lipoproteins present in females and males, female-specific lipoproteins have been described in adult female shrimp undergoing ovarian maturation. They carry lipids and proteins for egg formation that will be later used by the developing larvae after hatching from the egg. In this review, we focus on the knowledge about penaeid shrimp hemolymph lipoproteins' similarities and differences. Some comparisons are also made with other crustacean hemolymph lipoproteins; the reader is referred to a review on lipoproteins from the hemolymph and ovaries of marine invertebrates for more detailed information about crustaceans (Lee, 1991).

2. Dietary and hemolymphatic lipids

The effect of dietary lipids on the growth and maturation of crustaceans has been the subject of numerous studies. In shrimp, early studies related larvae growth, ovarian maturation, spawning capacity s and nauplii production to the quality and quantity of the lipids present in the feed (Kanazawa et al., 1977). Cholesterol is an essential component of crustaceans, since they are unable to carry out de novo synthesis of the sterol ring (Van den Oord, 1964; Zandee, 1964; Teshima and Kanazawa, 1971; Dall et al., 1990). In *Penaeus japonicus*, radiolabeled cholesterol included in the diet was first detected in the gut, then in the hepatopancreas, muscle and the rest of the body, implying a sequential transfer of cholesterol through the different tissues (Teshima and Kanazawa, 1987). The same deposition route seems to be followed by other dietary lipids as shown by studies including ^{14}C-tripalmitin (Teshima et al., 1986b) and linoleic acid-1-^{14}C in the feed (Teshima and Kanazawa, 1979). Furthermore, the inclusion of dietary phosphatidylcholine (PC) was particularly noticed in the hemolymph, but not in the hepatopancreas or muscle lipids, thus suggesting that phospholipids (PL) play a key role in the transport of other dietary lipids such as cholesterol (Ch) and triacylglycerides (TG) (Teshima et al., 1986a). It is currently accepted that dietary polyunsaturated fatty acids are essential for proper growth and maturation. In particular, linoleic acid (C18:2ω6), linolenic acid (C18:3ω3), araquidonic acid (C20:4ω6), eicosapentaenoic acid (C20:5ω3) and docosahexaenoic acid (C20: 6ω3) have been reported to play a key role in membrane integrity and flexibility (Dall et al., 1990).

Harrison (1990) reviewed the nutritional role of lipids on maturation, reproduction and embryonic development of crustaceans; nevertheless, research on specific needs per shrimp species is still in process. The relative proportion of dietary polyunsaturated fatty acids ($n-3$ and $n-6$) seems to differ between shrimp species and may be related to the ability to elongate and desaturate 18-carbon fatty acids of either the $n-3$ or $n-6$ series into long-chain highly unsaturated fatty acids (HUFA) (Xu et al., 1994). Although dietary Ch and fatty acids appear to be very important for shrimp growth, according to Chen (1993), a direct relationship between the dietary levels of PC and Ch to survival, does not seem to occur in *Penaeus monodon*.

In addition, differences in lipid concentration and tissue distribution has been found in farmed and wild shrimp and suggested to derive from the type of feed consumed by

the organisms. O'Leary and Matthews (1990) observed differences in the concentration of lipids from muscle and hepatopancreas from wild and farmed prawn *Pen. monodon*. PC concentration was higher in wild shrimp, while the opposite was found for cholesterol; however, fatty acid composition of the PCs were similar, suggesting an interaction between these two lipid components and a dependence on the dietary lipid.

As crustaceans eat, the lipids present in the feed are digested and absorbed thorough the digestive tract and transported to appropriate cells for storage or utilization. The main lipid storage organ in shrimp is the hepatopancreas (Dall et al., 1990; Muriana et al., 1993). Due to the hydrophobic nature of lipids, a special vehicle is necessary for their transport through the aqueous hemolymph; thus they associate with proteins forming lipid–protein complexes named lipoproteins or LP (Kanost et al., 1990; Lee, 1991).

3. Hemolymph lipoproteins

As in other crustaceans, shrimp hemolymph is composed of hemocytes (circulating cells) and plasma (Dall et al., 1990). Their hemolymph LPs are found outside the cells, therefore, they can be described more appropriately as plasma LPs. Shrimp as other crustaceans, arthropod and vertebrate lipoproteins can be separated into different classes according to their hydrated density. They include very low density (VLDL < 1.006 g/ml), low density (LDL 1.006–1.06 g/ml), high density (HDL 1.06–1.21 g/ml) and very high density (VHDL > 1.21 g/ml) (Teshima and Kanazawa, 1980; Lee, 1991).

Invertebrate plasma LPs appear to be have simpler apolipoprotein composition compared to vertebrate lipoproteins (Kanost et al., 1990); nevertheless, they must possess hydrophobic and hydrophilic surfaces to interact with the lipid moieties and the aqueous surrounding plasma. Two types of plasma LPs, according to their density, have been found in shrimp: high density (HDL) and very high density lipoproteins (VHDL), apparently no low density lipoproteins have yet been consistently described.

3.1. Non sex-specific lipoproteins

The isolation and purification of shrimp hemolymph LPs has heavily relied on the use of ultracentrifugation to separate them by flotation. This is due to their lower density (lipid content), compared to other proteins in the hemolymph. HDLs have been isolated from *Pen. japonicus*, *Penaeus semisulcatus*, *Penaeus vannamei* and *Penaeus californiensis*. In *Pen. japonicus*, Teshima and Kanazawa (1980) separated serum LPs by ultracentrifugation and detected two HDL and one VHDL in males and females. In these LPs, the main lipid constituent was phospholipids with 40–47% and 72–87%, for HDL_2 and HDL_3, respectively, of the total lipid composition, TG, diacylglycerols (DG), monoglycerols (MG), free fatty acids (FFA), sterols and hydrocarbons (HC) were detected in lesser proportions.

Taking into consideration the complete lipoprotein, the protein component was approximately 26% (w/w) in HDL_2, while in HDL_3 the protein component was 32%.

As expected from its density, the protein component in VHDL, with respect to lipids was higher, corresponding to 81%, however the apolipoproteins were not characterized in this study. Both LPs had the same type of lipid classes composition in females and males, the main lipid class was PL, with concentrations varying from 14% of the total lipoprotein particle in VHDL and 33% and 53% in HDL_2 and HDL_3, respectively. The predominant fatty acids present in the lipids were palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic acid (18:1), eicosamonoenoic (20:1ω9), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3), predominantly (Teshima and Kanazawa, 1980).

The HDL (LPI) from *Pen. semisulcatus* was also purified by ultracentrifugation; it is present in males and females and it is called LP1 (Tom et al., 1993; Khayat et al., 1994b). Its apoprotein and lipid composition have been recently reported (Lubzens et al., 1997). It has only one apoprotein of 110 kDa and approximately 50% lipids. As in *Pen. japonicus*, in *Pen. semisulcatus* LPI, phospholipids are the more abundant lipids (71–77% w/w), followed by Ch (18%), DG (5%) and a low concentration of TG. PC was the most abundant PL, but also phosphatidylethanolamine (PE) was found, the latter contained more HUFA than PC. In general, the fatty acids components of the lipids were 16:0, 16:1ω7, 18:0, 18:1, 18:2ω6, 20:5ω3, 22:6ω3 (Lubzens et al., 1997).

The non sex-specific HDL from the Pacific white shrimp, *Pen. vannamei*, and the yellowleg, shrimp, *Pen. californiensis*, are similar to the *Pen. semisulcatus* counterpart. *Pen. vannamei* and *Pen. californiensis* HDL have densities of 1.364 and 1.139 g/ml, respectively (Yepiz-Plascencia et al., 1995, 1998), and have one apoLP of approximately 100 kDa. The *Pen. vannamei* HDL contains approximately 57% of lipids (Ruiz-Verdugo et al., 1997); PL comprise 43%, followed by acylglycerols (AG) with 9.5%, sterols 4.8%, and traces of FFA. These shrimps LPs are also similar to other crustacean non sex-specific LPs: in the marine crustacean striped stone crab, *Charybdis feriata*, and the kuruma prawn, *Pen. japonicus*, the HDL (LP1), have densities of 1.16 and 1.18 g/ml, respectively. Similar densities were found in the freshwater crustacean *Macrobrachium rosenbergii* and the mitten crab *Eriocher japonica* LPs, with densities of 1.13 and 1.16 g/ml, respectively. In all these cases, the predominant lipids were the PL (Komatsu et al., 1993). In the freshwater crayfish *Pacifastacus leniusculus*, the HDL has a density of 1.145 g/ml (Hall et al., 1995) and was originally identified as a 1, 3-β-D-glucan binding protein (Duvic and Söderhäll, 1990).

PL are the predominant lipid class also in other crustacean lipoproteins. Early studies in the dungeness crab *Cancer magister*, identified two non sex-specific LP with high PL content, followed by Ch, DG, HD and finally TG (Allen, 1972). The same type of main lipid in the LPs was found in the spiny lobster *Panulirus interruptus* (Lee and Puppione, 1978), the crab *Cancer antennarius* (Spaziani et al., 1986) and the freshwater crab *Potamon potamius* (Stratakis et al., 1992). This is also true for *Pac. leniusculus* HDL, where polar lipids correspond to 60.9% of the total lipids, with 46.3% PC, 12.7% PE and very low concentrations of sphingomyelin (Hall et al., 1995).

Studies of shrimp HDL apolipoprotein are more recent, since early work emphasized the lipid components. One or three apolipoprotein have been reported in crustaceans for the HDL present in females and males (Table 1). The penaeid *Pen. japonicus* has three apoLPs of 180, 100 and 80 kDa (Komatsu et al., 1993), while in *Pen. vannamei* (Yepiz-Plascencia et al., 1995) and *Pen. californiensis* only a 100-kDa glycosylated

Table 1
Comparison of the apoproteins from the hemolymph lipoproteins from penaeid shrimp

Species	LPI or NSSLP (kDa)	Vg or LPII (kDa)	Vt (kDa)	Reference
Pen. japonicus		150, 105, 92, 86, 72		Vazquez-Boucard and Ceccaldi, 1986
Pen. japonicus	180, 100, 80			Komatsu et al., 1993
Pen. monodon		168, 104, 83, 74	168, 104, 83	Chen and Chen, 1993
Pen. monodon		170, 82		Chang et al., 1994
Pen. vannamei	100			Yepiz-Plascencia et al., 1995
Pen. semisulcatus	110	200, 120, 80		Lubzens et al., 1997
Pen. californiensis	100			Yepiz-Plascencia et al., 1998

Vg = vitellogenin.
Vt = vitellin.
NSSLP = non sex-specific lipoproteins.

apoLP was detected (Yepiz-Plascencia et al., 1998). Similarly in *Pen. semisulcatus*, a unique 110 kDa apoLPI was reported (Lubzens et al., 1997). ApoLPs of 100–110 kDa have also been found in other crustaceans such as the rock crab, *Can. antennarius*, (Spaziani et al., 1986), the land crab, *Pot. potamius*, (Stratakis et al., 1992) and the freshwater crayfish, *Pac. leniusculus* (Hall et al., 1995). In the case of *Can. antennarius*, there are two reports, one describing one 108 kDa (Puppione et al., 1986) and another one with three apolipoproteins of 185, 100 and 84 kDa (Spaziani et al., 1986). A recent study (Spaziani et al., 1995) actually questions the presence of female-specific lipoproteins in *Can. antennarius*, and to a lesser extent in *Callinectes sapidus* since the same HDL and its three apolipoproteins were detected in males and females. This discrepancy may be due to the methods used for the isolation of the LPs, since many times isolation by isopiknic ultracentrifugation has relied on the visual detection of light yellow colored banding (Spaziani and Wang, 1991), or the fractionation method utilized; although very unlikely, species specific factors affecting the number of apolipoproteins, cannot be completely ruled out at this time.

More detailed studies of the crustaceans, particularly about shrimp apoLP1 or HDL are very recent. The amino acid composition and N-terminal sequence (Fig. 1) of the *Pen. vannamei* apoLP were recently reported (Ruiz-Verdugo et al., 1997). The most abundant amino acids are aspartic acid/asparagine and glutamic acid/glutamine; me-

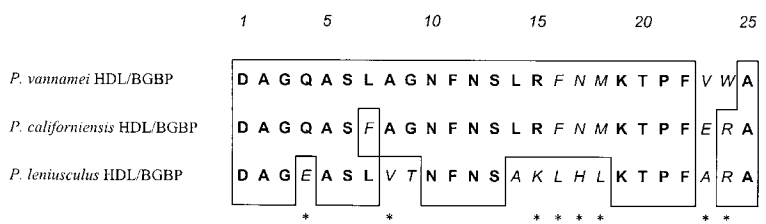

Fig. 1. N-terminal amino acid sequences from white shrimp *Pen. vannamei* (Ruiz-Verdugo et al., 1997), yellow leg shrimp *Pen. californiensis* (Yepiz-Plascencia et al., 1998) and crayfish *Pac. leniusculus* (Cerenius et al.,1994). Identical amino acid sequences are shown in bold. Asterisk mark conservative replacements.

thionine content was very low; the same occurs in *Pot. potamius* (Stratakis et al., 1992) and *Can. antennarius* (Spaziani et al., 1986) counterparts HDLs. The only complete amino acid sequence deduced from cDNA available in the scientific literature is from the freshwater crayfish, *Pac. leniusculus* (Hall et al., 1995) and published as BGBP, GenBank accession number X80687 (Cerenius et al., 1994). The *Pen. vannamei* *N*-terminal sequence has 67% homology with *Pac. leniusculus*; the similarity raises to 80% if conservative replacements are taken into consideration. We did not find any reports about crystal structure or electron micrographs of isolated shrimp HDL, however, the spiny lobster *Pan. interruptus* (Lee and Puppione, 1978) and the crab *Can. antennarius* (Spaziani et al., 1986) presented polymorphic appearance in electron micrographs and, according to the authors, may be primarily discoidal. Considering the similarities among crustaceans HDLs, it is likely that the shrimp HDL also has a discoidal shape, but this remains to be investigated.

A non sex-specific VHDL was reported from the hemolymph of one shrimp species, *Pen. japonicus* (Teshima and Kanazawa, 1980). It has a density >1.21 g/ml and contains 81% protein and 19% lipid; in particular PL corresponded to 14% of the whole VHDL particle or 69–72% of the lipids. From the same shrimp (Komatsu et al., 1993) report VHDL density of 1.265 g/ml and again PL as the predominant lipid; although low concentrations of TG were also detected.

There are reports of VHDLs in other crustaceans. VHDL from seawater striped stone crab *Cha. feriata*, freshwater prawn *M. rosenbergii*, and the mitten crab *E. japonica* have densities from 1.26 to 1.31 g/ml and contain PL, predominantly. All these VHDL have apolipoproteins with high molecular weights, but due to contamination with hemocyanin, the authors only report 370 and 170 kDa molecular mass for the freshwater prawn and mitten crab, respectively (Komatsu et al., 1993).

The *Pac. leniusculus*, VHDL (density 1.275 g/ml), contains PL (51.7%) and neutral lipids (48.1%) in approximately equal amounts. PC and PE were the components of the PL; while the neutral lipids contained HC and DG mainly (Hall et al., 1995). This VHDL has a single apolipoprotein of ~ 400 kDa, composed by two disulfide linked subunits of 210 kDa. The subunit size and amino acid composition are identical to a clotting protein previously characterized (Kopácek et al., 1993; Hall et al., 1995) and its amino acid sequence has homology to vitellogenins and von Willebrand factor, GenBank accession number AF102268 (Hall et al., 1999). In the sand crayfish *Ibacus ciliatus*, a VHDL with density of 1.27–1.29 g/ml, containing 94% protein and 6% lipids and PL as the predominant lipid was recently reported (Komatsu and Ando, 1998). It is a dimer of a 195-kDa apolipoprotein, linked by disulfide bridges and is capable of forming clots in the presence of transglutaminase and calcium. Its *N*-terminal sequence (17 amino acids) is very similar to the crayfish and spiny lobster counterparts (Doolittle and Riley, 1990) (Fig. 2).

Hall et al. (1995) characterized the HDL and VHDL from *Pac. leniusculus* plasma and found that these two proteins corresponded to previously reported proteins involved in the freshwater crayfish defense system. The same seems to be the case for the VHDL from the sand crayfish (Komatsu and Ando, 1998). In the marine shrimps, *Pen. vannamei* and *Pen. californiensis*, the HDLs or LPIs have also been identified also as a protein involved in the defense system, a beta glucan binding protein (BGBP) (Yepiz-

	1 5 10 15
I. ciliatus VHDL	**L Q P** G **L E Y Q Y R Y N G R V A** A
P. interruptus CP	**L Q P** K **L E Y Q Y** K **Y H G** I **V A** L
P. leniusculus CP/VHDL	**L** H **S N L E Y Q Y R Y S G R V A** S

Fig. 2. N-terminal amino acid sequences from sand crayfish *I. ciliatus* VHDL (Komatsu and Ando, 1998), and clotting protein (CP) from spiny lobster *Pan. interruptus* (Doolittle and Riley, 1990) and crayfish *Pac. leniusculus* (Hall et al., 1999). Identical amino acids are shown in bold.

Plascencia et al., 1998). These proteins have one apoLP of ~ 100 kDa with a very similar *N*-terminal amino acid sequence between the two of them. Furthermore, the sequence is highly homologous to the *Pac. leniusculus* BGBP a freshwater crustacean (Fig. 1). Thus, it appears that the crustacean lipoproteins are highly conserved concerning the size of the proteins and their main lipids components. Moreover, they are involved in lipid transport and the defense system. Whether the conservation is maintained in the complete amino acid sequence and/or the three-dimensional structure should await further research in these topics.

Recently, isolation and characterization of shrimp plasma LPs have been reported and thus, there is now more information available. Density, lipid classes, apolipoproteins and their amino acid composition, as wells as *N*-terminal amino acid sequences are for the first time available for shrimp LPs. Apolipoproteins sizes of the LPs from kuruma prawn *Pen. japonicus* (Teshima and Kanazawa, 1980; Komatsu et al., 1993), *Pen. vannamei* (Yepiz-Plascencia et al., 1995; Ruiz-Verdugo et al., 1997) and *Pen. semisulcatus* (Lubzens et al., 1997) are presented in Table 1. In these shrimp LPs, PLs are the most abundant lipids, in contrast to mammals and insects where TG and DG, respectively, are the most abundant lipids (Kanost et al., 1990).

3.2. Female specific-lipoproteins

In addition to the HDL common to males and females, shrimp hemolymph contains a female-specific LP which appears to be absent in non-vitellogenic females and related to vitellogenesis. Reproduction is a key element in commercial farming operations, thus, sustained research efforts have been dedicated to understanding the mechanism for ovarian maturation, since this knowledge may provide means to artificially induce reproduction.

Vitellogenin (Vg) and vitellin (Vt) are related lipoproteins (Lee, 1991), in fact Vg is considered the precursor of Vt. Vt synthesis in the ovary appears to involve the processing of a Vg precursor. Vg from different shrimp and other crustaceans is also high density LP and have been called LPII, although their density is somewhat higher, reflecting the lower lipid content compared to LPI. Vg from *Pen. semisulcatus* contains 540 mg/g of lipid compared to LPI that has 1089 mg/g. As in LPI, PC is the predominant lipid of Vg, but in addition, Vg contains small but significant TG. Both Vg and Vt are composed by three apolipoproteins with sizes of 200, 120 and 80 kDa (Lubzens et al., 1997) that are recognized by an anti-Vt antibody.

The synthesis of Vg–Vt appears to occur in the in the ovary and to a lesser extent in hepatopancreas (Lubzens et al., 1995). Although the polypeptide produced by in vitro translation of mRNA, and recognized by the anti-Vt antibody, is smaller than the size expected from the apolipoproteins, the difference in size was attributed to glycosylation occurring in the ovary (Khayat et al., 1994a; Lubzens et al., 1995). The presence of an RNA transcript of 1.0–1.1 Kb in hepatopancreas and ovary RNA, led the authors to suggest that Vg and Vt are products of the same gene (Lubzens et al., 1995) apparently expressed in ovary and hepatopancreas but not in the subepidermal adipose tissue (Fainzilber et al., 1992). However, Yano and Chinzei (1987), based in in vitro studies of ovarian maturation in *Pen. japonicus* and detection of Vt synthesis with an anti-Vt antibody, concluded that Vg is only synthesized in the ovary.

In the shrimp *Pen. japonicus*, double tracer experiments with ^{3}H-palmitic acid and ^{14}C-linolenic acid included in the feed, demonstrated labeled PC and fatty acids in hepatopancreas, but only PC was found in muscle. After bilateral eyestalk ablation to induce maturation, radiolabeled TG and PC were found in the ovaries, with concomitant decrease in the hepatopancreas labeled lipids; this was taken as proof of lipid transfer from the hepatopancreas to the ovary (Teshima et al., 1988). The Vg isolated from plasma of this shrimp is a $\sim$ 500 kDa lipoprotein, composed by subunits of 150, 105, 92, 86 and 76 kDa (Vazquez-Boucard and Ceccaldi, 1986).

In *Pen. monodon*, the female-specific hemolymph LP (Chen and Chen, 1993) was identical to the egg vitellin and formed by four polypeptides of 168, 104, 83 and 74 kDa. Based on recognition by antibodies and proteolysis mapping, the authors concluded that subunits 104 and 83 kDa are derived from the 168 precursor. Later work by Chang et al. (1994), showed that the purified Vg is a lipoglycoprotein composed by 170 and 82 kDa subunits, with density of 1.1036 g/ml. Since previous work showed a Vt with more subunits (Chang et al., 1993), and the amino acid composition of Vg and Vt are similar, they suggest that Vg is incorporated into oocytes and the largest subunit cleaved to produce the previously identified smaller subunit Vt (Chang et al., 1994).

The characterization of *Pen. vannamei* plasma Vg is less clear. An ELISA assay has been used to quantify Vg in hemolymph, but no clear indication of the subunits composition is given (Mendoza et al., 1993). Quackenbush, 1989a raised antibodies against a 97 kDa protein present in ovaries and hepatopancreas and suggested that this protein is a subunit of Vg involved in the transport of lipids from hepatopancreas to ovaries. Furthermore, eyestalk ablation induced a rapid increase of protein synthesis in hepatopancreas and ovaries and also higher concentration of the 97 kDa protein determined by ELISA, in the hemolymph (Quackenbush, 1989b). It was also shown that in vitro ovarian protein synthesis was stimulated by progesterone and estradiol, while ecdysterone, testosterone and estrogen had no effect. The utilization of a heterologous system consisting of a peptide factor from the eyestalk of crayfish *Procambarus bouveri*, stimulating yolk synthesis and a peptide factor from shrimp eyestalks inhibiting yolk synthesis yielded promising results (Quackenbush, 1992). Later on, a peptide factor from *Pro. bouveri* with inhibitory activity over Vg synthesis was characterized and this was named VIH (Vitellogenesis Inhibiting Hormone) (Aguilar et al., 1992).

Plasma Vg from *Penaeus chinensis* is a lipoglycoprotein composed by two subunits of 191 and 85 kDa (Chang and Jeng, 1995). Although vitellin is composed by five

Subunit	Protein							
170 kDa	Vitellogenin	S	I	D	L	S	Q	L	
100 kDa	Vitellogenin	S	I	D	L	S	Q	L	T
	Vitellin	S	I	D	L	S	Q	L	T
89 kDa	Vitellogenin	A	P	E	P	Q	L	V	N
	Vitellin	A	P	E	P	Q	L	V	N

Fig. 3. N-terminal amino acid sequence of vitellogenin and vitellin from freshwater prawn *M. rosenbergii* (Lee et al., 1997).

subunits (Vn1, 105, 85, 78, 58, 40 kDa) or three subunits (Vn2, 155, 85, 78 kDa), Vg and Vt amino acid compositions appear to have similarity among them and to other penaeid Vg and Vt. Therefore, it was suggested that the 191 kDa subunit is cleaved and transformed into Vt in combination with another subunit (Chang et al., 1996). Evidence from the penaeids studied thus far, indicates that Vg has less subunits than Vt. *Metapenaeus ensis* Vt is a lipoglycoprotein with similar amino acid composition to other Vg–Vt. This Vt is a 350 kDa protein formed by four subunits, with major polypeptides of 76 and 102 kDa (Qiu et al., 1997).

Knowledge about Vg in crustacean other than shrimp is also available. Vg from *M. rosenbergii* is a lipoglycoprotein with three subunits (170, 100 and 89 kDa), whereas Vt appears to have only the 100 and 89 kDa polypeptides. Interestingly, the N-terminal sequence from the 170 and 100 kDa Vg and 100 Vt subunits have exactly the same first eight amino acid sequence (Fig. 3). This also occurs with the 89 kDa subunits from both Vt and Vg (Lee et al., 1997).

In the crab *Potamon potamios*, Vg and Vt are composed by subunits with 115, 105 and 85 kDa. The Vg from some crabs also contains a 185-kDa that was proposed as a precursor for the 115 and 105 kDa proteins (Pateraki and Stratakis, 1997). Three Vg subunits have been reported with molecular masses of 190, 107 and 78 kDa for *Cal. sapidus* (Lee and Puppione, 1988) and 152, 100 and 82 kDa for *Can. antennarius* (Puppione et al., 1986).

Vitellogenin has been associated with the transport of nutrients, proteins and lipids from hepatopancreas (reserves) to the ovaries. Lipoproteins can also carry lipids to other tissues. Recently (Kang and Spaziani, 1995) showed uptake of HDL by Y-organs of the crab *Can. antennarius*; this uptake is mediated by endocytosis, dependent on de novo protein synthesis and depressed by Molt-Inhibiting Hormone (MIH). The HDL carries cholesterol necessary as a precursor for ecdysteroid synthesis.

4. Summary and concluding remarks

Several reports about non-sex specific and female-specific hemolymph lipoproteins from penaeid shrimp hemolymph are available in the scientific literature. Initial studies focused on the isolation of the lipoproteins and characterization–quantification of the

lipids. The last decade has seen major interest develop in the study of the apolipoproteins, on determining their number, molecular mass, amino acid composition, and recently, N-terminal amino acid sequences have become available for the first time. Detection of their mRNA by in vitro translation studies and immunodetection with specific anti-LP antibodies of the translation products has been used to study their synthesis. Recent publications report fewer numbers of subunits for Vg and Vt that were originally detected. This may be related to the experimental protocols used for collection of hemolymph, where an isoosmotic and quelating anticoagulant (Vargas-Albores et al., 1993) was not always used. In the absence of an appropriate anticoagulant, hemolymph lipoproteins were isolated from serum after the clot was removed, but the events leading to clot formation may have effect on the intactness of hemolymph proteins (see also Lubzens et al., 1997), for more discussion about this point).

Clotting occurs almost instantaneously after piercing the shrimps for collection of hemolymph since the injury (injections, wounds), activates hemocytes and releases the hemocyte-contained tranglutaminase necessary for polymerization of the clotting protein (Montaño-Perez et al., 1999). The hemocytes also contain a protease (Gollas-Galván et al., 1997) that could degrade serum proteins, including lipoproteins. This last problem has been controlled lately by the addition of protease inhibitors to the anticoagulant used for collecting plasma and/or during the purification steps (Yepiz-Plascencia et al., 1995; Lubzens et al., 1997; Ruiz-Verdugo et al., 1997).

The uptake by Y-organs of the HDL (Kang and Spaziani, 1995) may suggest that hemolymph LP are taken up also by tissues different than the ovary, perhaps Vg carries lipids or proteins more efficiently to the ovary and the non sex-specific LP is involved in processes common to both, males and females. The specificity of the uptake, as well as the mechanism, i.e. endocytosis or unloading at the cell membrane could be investigated once shrimp LPs are routinely isolated and an assay to follow their fate in other cells is improved or developed.

Up to now, limited information about penaeid shrimp apoLPI or apoVg amino acids sequences exists; only the N-terminal are known for a few subunits. No data are available yet about cDNA sequences and their derived amino acid sequences, but this methodology will probably become the best strategy to obtain such sequence. Given the size of these proteins, it would mean a tremendous effort to isolate enough amount of these large polypeptides in order to perform direct amino acid sequencing for the entire polypeptide. Most likely, we shall see cDNA sequences, gene localization and perhaps, insights into the mode of gene expression regulation of the shrimp hemolymph lipoproteins in the near future.

Finally, the demonstration in penaeid shrimp (Yepiz-Plascencia et al., 1998), freshwater crayfish (Hall et al., 1995, 1999) and sand crayfish (Komatsu and Ando, 1998) of plasma lipoproteins involved in the defense system, also emphasizes their dual functions and makes them interesting molecules which participate in two essential processes.

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