Release and Transport of Lipids in the Prawn

Shin-ichi TESHIMA* and Akio KANAZAWA*
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This paper deals with the release and transport of lipids in the prawn, *Penaeus japonicus*.

The hepatopancreas obtained from the prawn after injection of palmitic acid-14C contained an abundance of radioactive phospholipids (PL) with rather large amounts of triglycerides (TG) and free fatty acids (FFA). When the in vivo prelabelled hepatopancreas, containing mainly radioactive PL and TG, was reincubated in vitro in the prawn serum, PL, TG, and FFA were released into the incubation medium. Also, the in vitro reincubation of the prelabelled hepatopancreas containing mainly TG showed that TG was the major lipid class released into the incubation medium. In addition, the present study suggests that the release of hepatopancreatic lipids in the prawn is accelerated by some factor (probably lipoproteins) of the prawn serum.

These facts can be explained by the proposal that PL, TG, and FFA are the principal lipid moieties released from the hepatopancreas into the hemolymph in the prawn, *P. japonicus*.

Recent investigations have pointed out the interesting aspects of metabolism of lipids such as sterols, long-chain polyunsaturated fatty acid, and molting hormones in crustaceans. Although the main storage site of lipids in crustaceans is recognized as the hepatopancreas, little is known about the mechanism of release and transport of lipids from the hepatopancreas to the target cells.

In a previous study, the authors have investigated the lipid composition of hemolymph of the prawn, *Penaeus japonicus*, with the suggestion that the mechanism of lipid transport in crustaceans probably differs from that in insects belonging to the same arthropods. In this study, therefore, the authors intend to clarify the mechanism of lipid release and transport in the prawn by using tracer techniques. This paper deals with the results and discussion on the above subject.

Materials and Methods

**Chemicals**

All solvents used were redistilled immediately before use. Reference lipids, undecane, cholesterol, cholesteryl oleate, tripalmitin, dipalmitin, cholesterol, phosphatidyl ethanolamine, and phosphatidyl choline were obtained from Nihon-Chromato-Kogyo Co. or Steraloids Inc. Palmitic acid-16-14C (40-60 mCi/mmol) was purchased from New England Nuclear (Massachusetts, U. S. A.). In the present study, the administration of palmitic acid-14C was carried out as a form of albumin complex. Liquid scintillation counting were obtained from Eastman Kodak Co.

**Prawns**

The prawn, *P. japonicus*, hatched in the Mitsui-Nohrin Kaiyo Sangyo Co. (Kagoshima) was transported to this laboratory and reared on the commercial diet for prawn (Evian-Kyowa, Kyowa-Hakko Kogyo Co.) until use. In this study, the male prawns, 5-6 g in body weight, were used, and the molting stage determined by the method of Drach was stage C (intermolt).

**Injection of Palmitic Acid-14C**

Palmitic acid-14C (0.5 µCi × 12) was injected to 12 prawns at the base of the fourth swimmerets and maintained in a circulating sea-water at 24°C. At 1, 2, and 4 hr after injection of palmitic acid-14C, the hepatopancreas, hemolymph, and muscle (from the part of first abdominal segment) were excised from the prawns for lipid analysis.

**Preparation of Serum and Prelabeled Hepatopancreas**

The methods for preparation of serum and prelabelled hepatopancreas were essentially similar to those used in the insects by CHINO and GILBERT. The hemolymph was centrifuged at 3000 × g for 15 min to remove the hemocytes. To 1 ml of the serum so obtained, 5 µmoles of glutathione (Kojin Co.) was added to prevent the blackening of serum. The hepatopancreas (180 mg) was carefully washed with cold 0.15 M potassium chloride.
Incubation of Prelabelled Hepatopancreas

The prelabelled hepatopancreas (180mg) was incubated for lh in 0.6ml of various incubation media such as crustacean saline, prawn serum, boiled prawn serum, and egg-albumin solution. The crustacean saline (pH 7.4) was consisted of the following salts (g/l): NaCl, 23.0; KCl, 0.964; MgCl2·6H2O, 5.0; MgSO4·7H2O, 7.27; tris, 3.0; CaCl2, 1.119; NaH2PO4, 0.138. After incubation, the hepatopancreas was thoroughly washed with the phosphate saline to remove excess palmitic acid-14C. The hepatopancreas so obtained was referred as 'in vitro prelabelled hepatopancreas' in this paper.

In addition, the hepatopancreas excised from the prawns which were injected with palmitic acid-14C and maintained for 4h was washed with 0.15 M potassium chloride in phosphate buffer. The hepatopancreas so prepared was referred as 'in vivo prelabelled hepatopancreas'.

Incubation of Prelabelled Hepatopancreas

The prelabelled hepatopancreas (180 mg) was incubated for 1 h in 0.6 ml of various incubation media such as crustacean saline, prawn serum, boiled prawn serum, and egg-albumin solution. The crustacean saline (pH 7.4) was consisted of the following salts (g/l): NaCl, 23.0; KCl, 0.964; MgCl2·6H2O, 5.0; MgSO4·7H2O, 7.27; tris, 3.0; CaCl2, 1.119; NaH2PO4, 0.138. After incubation, the hepatopancreas was washed with the phosphate saline, and the medium and washings were combined for lipid analysis.

Incubation of Prawn Serum with Palmitic Acid-14C

To ascertain the synthesis of lipid classes in the hemolymph, palmitic acid-14C (1 μCi) was incubated with 0.6 ml of the serum of prawn at 24°C for 60 min. After incubation, the lipids were extracted from the incubation mixture.

Extraction and Analysis of Lipids

Lipids were extracted from the tissue or incubation medium with chloroform-methanol-water (2: 2: 1) according to the procedure of BLIGH and DYER. Lipids were separated into lipid classes by thin-layer chromatography (TLC) on Kieselgel G (0.25 mm in thickness) with the solvents: first, isopropyl ether-acetic acid (96: 4); second, petroleum ether-ethanol-acetic acid (90: 10: 1). After TLC, the bands corresponding to reference lipid classes were located with 2, 6-dichlorofluorescein and iodine vapour, scraped with a spatula, and transferred into the scintillation vials containing 10 ml of a scintillator. The scintillator was composed of toluene (500 ml), Triton X-100 (200 ml), distilled water (100 ml), POPOP (3.2 g), and POPOP (0.24 g). A Beckman Model LS-230 liquid scintillation counter equipped with automatic external standardization was used to count radioactive samples. The efficiency of counting of radioactive lipids was approximately 85 per cent.

Results

In vivo Experiment

After injection of palmitic acid-14C into the prawn, the incorporation of radioactivity in the lipid classes isolated from the hepatopancreas, muscle, and hemolymph was examined. Crustaceans generally contain relatively large amounts of sterols as lipid classes in their tissues. Accordingly, it was preliminarily checked by TLC whether palmitic acid-14C was incorporated into sterols. The sterol fraction obtained by TLC showed very low radioactivity. The sterol fraction purified by TLC after saponification had no radioactivity. This result showed that palmitic acid-14C was not incorporated into the sterol fraction. Since the prawn, P. japonicus, lacks the sterol-synthesizing ability from lower units, no incorporation of palmitic acid into the sterol fraction is not surprising. In this study, hence, check of the radioactive incorporation into the sterol fraction was omitted.

In the hepatopancreas (Fig. 1), the injected palmitic acid-14C was highly incorporated into phospholipid (PL) and triglyceride (TG) fractions after 1 h, but poor into monoglyceride (MG), diglyceride (DG), free fatty acid (FFA) and steryl ester-hydrocarbon (SE+HC) fractions. The radioactive PL and TG increased and decreased after 2 h, respectively, and then both lipid classes
remained almost constant levels up to 4 h, whereas other lipid classes did not show so marked variation in the course of keeping period. In the muscle (Fig. 2), PL was exclusively prominent radioactive lipid class throughout the keeping period. It has been shown that the main glycerolipids of hepatopancreas were PL and TG, whereas that of muscle was PL in the prawn, *P. japonicus*3). In conjunction with these informations, it seems reasonable to conceive that the prawn accumulates lipids as TG and PL in the hepatopancreas and as PL in the muscle.

Regarding the hemolymph (Fig. 3), the main radioactive lipids was also PL throughout the keeping period, but considerable large amounts of TG and FFA were present. Since crustaceans have an open-vessel system, all tissues or organs result in bathing directly in the hemolymph. If the hemolymph itself has extremely weak or no ability for lipid synthesis from palmitic acid-14C, the radioactive lipids recovered in the hemolymph may be attributed to the lipids released from certain tissues or organs. In the prawn, therefore, some or all of the radioactive PL, TG, and FFA were suggested to play an important role in lipid release and transport.

**Table 1. Release of lipids from 'in vivo prelabelled hepatopancreas' into the medium.** Nine prawns were injected with 6 μCi of palmitic acid-14C and maintained at 24°C for 4 h, and then the hepatopancreas was excised. The prelabelled hepatopancreas (150 mg) was incubated in the medium (0.6 ml) at 24°C for 1 h.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Lipids (cpm)</th>
<th>% Release*2</th>
<th>% Distribution of radioactivity</th>
<th>SE+HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatopancreas before incubation*1</td>
<td>39,000</td>
<td>—</td>
<td>PL: 63.9, MG: 1.7, DG: 2.7, FFA: 4.6, TG: 26.8, SE+HC: 0.2</td>
<td></td>
</tr>
<tr>
<td>Incubation with the prawn serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids from the medium</td>
<td>8,580</td>
<td>22.0</td>
<td>PL: 42.4, MG: 4.2, DG: 2.9, FFA: 35.9, TG: 11.2, SE+HC: 0.5</td>
<td></td>
</tr>
<tr>
<td>Lipids from the tissue</td>
<td>22,620</td>
<td>—</td>
<td>PL: 64.6, MG: 3.1, DG: 3.4, FFA: 20.9, TG: 8.0, SE+HC: 0.1</td>
<td></td>
</tr>
<tr>
<td>Incubation with crustacean saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids from the medium</td>
<td>3,880</td>
<td>9.9</td>
<td>PL: 63.6, MG: 6.5, DG: 2.9, FFA: 16.1, TG: 10.9, SE+HC: 0.1</td>
<td></td>
</tr>
<tr>
<td>Lipids from the tissue</td>
<td>26,300</td>
<td>—</td>
<td>PL: 73.9, MG: 5.5, DG: 4.2, FFA: 10.2, TG: 7.8, SE+HC: 0.3</td>
<td></td>
</tr>
</tbody>
</table>

*1 *'in vivo prelabelled hepatopancreas (180 mg)'

*2 % Release of prelabelled hepatopancreatic lipids into the incubation medium
atic lipids were released more effectively in the prawn serum than in crustacean saline. This suggested that the prawn serum was closer to normal physiological conditions than crustacean saline. The main radioactive lipids released into the medium were PL, TG, and FFA in the prawn serum and crustacean saline. However, the release of FFA proceeded in the prawn serum more than in crustacean saline, and this assumed that the release of FFA from the hepatopancreas was stimulated by some factors in the serum. In addition, the amounts of radioactive FFA in the prelabelled hepatopancreas were found to increase after reincubation, and this supposed that a part of radioactive glycerolipids was undergone hydrolysis and then released into the medium.

**Release of Lipids from 'in vitro Prelabelled Hepatopancreas'**

To further clarify the nature of lipids released from the hepatopancreas of prawn, the prelabelled hepatopancreas was prepared by incubating in vitro the hepatopancreas with palmitic acid-14C in phosphate buffer. In this method, the prelabelled hepatopancreas containing radioactive TG as abundant lipid classes (80.3% of total radioactive lipids) was obtained. The prelabelled hepatopancreas was reincubated under various conditions, and the results are shown in Table 2 and Fig. 4. The release of lipids into the medium varied with the quality of incubation medium, as shown in Table 2. The addition of prawn serum to crustacean saline accelerated the release of lipids. Egg-albumin also showed such an effect. However, the boiling of prawn serum resulted in the loss of such an effect. The most effective release of lipids was attained when the incubation medium was consisted of the prawn serum alone. These results strongly suggested that lipoprotein(s) of the prawn serum may be concerned with the release of hepatopancreatic lipids into the hemolymph.

The radioactive lipids extracted from the medium were composed of mainly TG (71.5–89.3% of total radioactive lipids) in all incubation media, whereas other lipid classes were present as minor radioactive lipids. Although the experiment using in vivo prelabelled hepatopancreas assumed that a part of glycerolipids may be hydrolyzed in the hepatopancreas prior to the release into the medium, a marked release of FFA was not seen in the experiment using the in vitro prelabelled hepatopancreas containing abundance of radioactive TG. This resulted led to the postulation that some glycerolipids (probably PL) except TG may be hydrolyzed prior to the release from the hepatopancreas.

**Table 2. Release of lipids from 'in vitro prelabelled hepatopancreas' into the medium.**

The hepatopancreas (180 mg) of prawn was incubated in vitro with 1 μCi of palmitic acid-14C in phosphate saline (0.6 ml). The prelabelled hepatopancreas was reincubated in the medium (0.6 ml) mentioned below at 24°C for 1 h. The final volume of incubation medium was adjusted to 0.6 ml with crustacean saline.

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Lipids released into the medium</th>
<th>% Distribution of radioactive lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipids (cpm)</td>
<td>% Release*2</td>
</tr>
<tr>
<td>Hepatopancreas before incubation*1</td>
<td>334,000</td>
<td>—</td>
</tr>
<tr>
<td>Crustacean saline (0.6 ml)</td>
<td>26,600</td>
<td>7.9</td>
</tr>
<tr>
<td>Prawn serum (0.3 ml)+crustacean saline (0.3 ml)</td>
<td>52,600</td>
<td>15.7</td>
</tr>
<tr>
<td>Prawn serum (0.6 ml)</td>
<td>66,500</td>
<td>19.9</td>
</tr>
<tr>
<td>Boiled prawn serum (0.3 ml)+crustacean saline (0.3 ml)</td>
<td>7,120</td>
<td>2.1</td>
</tr>
<tr>
<td>5% Albumin in crustacean saline (0.3 ml)+crustacean saline (0.3 ml)</td>
<td>44,700</td>
<td>13.3</td>
</tr>
</tbody>
</table>

*1 'In vitro prelabelled hepatopancreas (180 mg)'
*2 % Release of prelabelled hepatopancreatic lipids into the incubation medium

Fig. 4 shows the time-course of the release of lipid classes in the incubation of prelabelled hepatopancreas with the crustacean saline containing 0.3 ml of the prawn serum. The release of TG reached almost maximum within 1 h-incubation period. Other lipid classes such as PL, FFA, and DG also seemed to be released gradually in the course of incubation times, but the quantity of...
them was very small as compared with that of TG throughout the incubation period.

**Biosynthesis of Lipid Classes from Palmitic Acid-14 C in the Hemolymph**

As stated above, the lipid components released from the hepatopancreas into the hemolymph were conceived to be mainly PL, TG, and FFA in the case of the prawn, *P. japonicus*. However, the possibility remains that PL and TG are synthesized from the released FFA in the hemolymph. As shown in Table 3, the prawn serum was capable of synthesizing PL and TG from palmitic acid-14C somewhat, but the amounts of synthesized PL and TG were extremely small. Therefore, the above-mentioned possibility was ruled out.

**Table 3.** Biosynthesis of lipid classes from palmitic acid-14C in the prawn serum. One μCi of palmitic acid-14C was incubated in vitro in 0.6 ml of the prawn serum at 24°C for 1 h. The lipids extracted from the incubation mixture were separated into the lipid classes by TLC followed by the measurement of radioactivity of individual lipid classes.

<table>
<thead>
<tr>
<th>Lipid fraction extracted</th>
<th>Radioactivity cpm × 10^3</th>
<th>% Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>34.3</td>
<td>1.9</td>
</tr>
<tr>
<td>MG</td>
<td>9.0</td>
<td>0.5</td>
</tr>
<tr>
<td>DG</td>
<td>23.3</td>
<td>1.3</td>
</tr>
<tr>
<td>FFA</td>
<td>1,680</td>
<td>94.1</td>
</tr>
<tr>
<td>TG</td>
<td>36.1</td>
<td>2.0</td>
</tr>
<tr>
<td>SE+HC</td>
<td>1.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Fig. 4. Time course of the release of radioactive lipid classes from the in vitro prelabelled hepatopancreas into the incubation medium.

The in vitro prelabelled hepatopancreas (180 mg), containing radioactive TG (268,000 cpm), PL (30,400 cpm), SE+HC (12,700 cpm), DG (11,000 cpm), FFA (7,010 cpm), and MG (2,670 cpm), was reincubated in the medium composing of the prawn serum (0.3 ml) and crustacean saline (0.3 ml) at 24°C.

Discussion

On the hemolymph lipids, there are several reports about the lobster, *Homarus americanus*¹³, crabs, *Carcinus maenas*¹⁴ and *Cancer magister*¹⁵, and crayfish, *Orconectes virilis*². In the previous study, the authors have also investigated the lipid class composition of hemolymph lipids of prawn, *P. japonicus*³, and indicated that the hemolymph lipids were composed of abundance of PL (about 65% of total lipids) with significant amounts of TG, sterols, and FFA. The informations available up to the present showed that the presence of large amount of PL was characteristic of crustacean hemolymph lipids. Generally, it has been supposed that the lipids of hemolymph reflect the aspect of lipid transport in crustaceans. Therefore, the mechanism of lipid transport in crustaceans is assumed to be different with that in insects⁴,⁵ which contain DG as main hemolymph lipids and release DG preferentially from the fat body (storage organ for lipids in insects) into the hemolymph as lipoprotein complex.

In the present study, the mechanism of lipid release from the hepatopancreas into the hemolymph was investigated on the prawn, *P. japonicus*, using tracer techniques. As a result, it was proposed that the prawn, *P. japonicus*, released PL, TG, and FFA from the hepatopancreas into the hemolymph, and also that some lipoprotein(s) of the serum may be concerned with the lipid release. The presence of lipoproteins has been demonstrated in the serum of several crustaceans; fiddler crab, *Uca pugilator*¹⁶, blue crab, *Callinectes sapidus*¹⁷, crab, *Paratelphusa hydrodromus*¹⁸,¹⁹, and crab, *C. maenas*²⁰. Regarding the lipid release in crustaceans, there are only a few reports comparable to the present study. GILBERT and O’CONNOR²¹ have supposed by in vitro experiments that PL was preferentially released from the hepatopancreas in the crayfish, *O. virilus*. ALLEN¹⁵ has investigated the lipid transport in the crab, *C. magister*, by oral administration of palmitic acid-14C followed by the lipid analysis of the hemolymph and other tissues. He
supposed that FFA and DG played a major role in the transport of lipids in the male and female, respectively, on the basis of high specific activity (dpm/mg) of both the lipid classes recovered in the hemolymph. Thus, the mechanism of lipid release and transport in crustaceans apparently seems to differ from species to species. However, it is likely that lipid metabolism of crustaceans vary with the molting stage, sex, age, and vitellogenesis. Accordingly, definite conclusion should be drawn from further investigation using various species of crustaceans and the well characterized individuals.

References