Diacylglycerol-carrying lipoprotein of hemolymph of the American cockroach: purification, characterization, and function

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Abstract A diacylglycerol-carrying lipoprotein (DGLP) was isolated from the hemolymph of adult male and female American cockroaches, *Periplaneta americana*. The purification procedure involved dialysis against distilled water, precipitation at low ionic concentration at pH 6.6, and separation by column chromatography on DEAE-cellulose. The final preparation was homogeneous as judged by polyacrylamide gel electrophoresis and electron microscopy. The lipoprotein comprised over 50% of the total hemolymph protein. The DGLP molecule was almost globular in shape with a diameter of approximately 160 Å. The molecular weight, determined by a sedimentation-equilibrium method, was approximately 600,000. Apoprotein of DGLP consisted of two subunits, heavy chain (mol wt 250,000) and light chain (mol wt 85,000). The total lipid content of DGLP amounted to about 50%. The lipids comprised diacylglycerol (15% of total lipid), hydrocarbons (28%), cholesterol (5%), and phospholipids (43%). *n*-Pentacosane, 3-methylpentacosane, and 6,9-heptacosadiene were identified as major hydrocarbons. Mannose (0.9%) and glucosamine (0.3%) were associated with apoprotein of DGLP. The capacity of the purified DGLP to accept diacylglycerol from both fat body and midgut was demonstrated in vitro; thus it was suggested that the same carrier molecule served to transport this lipid from storage site and absorption site. The possible multiple role of cockroach DGLP in transporting such lipids as diacylglycerol, cholesterol, and hydrocarbon from the site of storage, absorption, or synthesis is discussed.—**Chino, H., H. Katase, R. G. H. Downer, and K. Takahashi.** Diacylglycerol-carrying lipoprotein of hemolymph of the American cockroach: purification, characterization, and function. J. Lipid Res. 1981. **22:** 7–15.

Supplementary key words fat body · intestine · lipid transport · cholesterol · hydrocarbon

In addition to their role as structural components of the cell membrane and cuticle, lipids provide an important source of metabolic fuel for insects (1). Lipid reserves, in the form of triacylglycerol, are stored primarily in the fat body and are transported in the hemolymph to sites of utilization as diacylglycerol bound to lipoprotein complexes (2). Lipoproteins are involved also in the transport of lipid and protein to the developing ovary (3–7). A vitellogenin has been isolated and purified in the cockroach, *Leucophaea maderae*, (4, 7) but no primary lipid-carriers have been identified for this group of insects. Our recent study (8) has revealed that in the American cockroach, *Periplaneta americana*, diacylglycerol is the predominant lipid transported not only from the fat body but also from the gut, and has suggested that lipoprotein(s) is involved in transporting this lipid from the two organs. However, it is not known if specific lipoproteins are required for different transporting functions or if a single molecule can serve more than one physiological role.

The present report describes a procedure for the isolation and purification of a diacylglycerol-carrying lipoprotein (DGLP) from hemolymph of the adult American cockroach, and demonstrates the capacity of the purified DGLP to accept diacylglycerol from both the fat body and the midgut. We also report the physical and chemical characterization of DGLP, and demonstrate the presence of a considerable amount of hydrocarbon in DGLP, in addition to diacylglycerol.

MATERIALS AND METHODS

Animals and collection of hemolymph

Adult cockroaches were taken from colonies of *Periplaneta americana* maintained under standard conditions (9). During the collection of hemolymph, the head was supported in an absorbent tissue to prevent possible contamination of the hemolymph sample by regurgitated gut contents. An incision was

Abbreviations: DGLP, diacylglycerol-carrying lipoprotein; TLC, thin-layer chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry.

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first made by severing the metathoracic legs, and 0.5 ml EDTA-saline (0.15 M KCl, 50 mM phosphate buffer pH 6.0, 5 mM EDTA) was injected into the abdominal hemoceole. The hemolymph drained into a cooled centrifuge tube through the incision and was then centrifuged at 2,000 *g* for 5 min to remove the hemocytes. This method provided a hemocyte-free, unclotted, pure serum, although it was diluted with EDTA-saline. Procedures for injection or feeding of radiolabeled fatty acid have been described (8).

Chemicals

Standard lipids (triolein, diolein, monoolein, and oleic acid) were obtained from Sigma Chemical Co., U.S.A. and were purified by column chromatography on Florisil obtained from the same company. Chromatographically pure phosphatidylcholine and phosphatidylethanolamine were prepared from egg yolk and ox brain using silicic acid column chromatography. [1-^{14}C]Palmitic acid (50–60 mCi/mmol) was purchased from Amersham, Canada, and Daiichi Chemical Co., Japan. Myosin heavy chain was supplied by Dr. K. Yagi of Hokkaido University, thyroglobulin was purchased from Sigma Chemical Co., and RNA-polymerase (β- and β'-chain) and bovine serum albumin (monomer and dimer) from Boehringer Mannheim, West Germany. All other chemicals were of analytical grade and solvents were redistilled as appropriate. Glass-redistilled water was used throughout the experiments.

Lipid extraction, separation, and determination

Neutral lipid classes and free fatty acid were extracted from hemolymph, lipoproteins, or incubation media after the procedure of Dole (10) and separated on columns of Florisil (11). The total lipids including phospholipids were extracted from lipoprotein with chloroform–methanol 2:1 (v/v). The amounts of hydrocarbon, choresteryl ester, cholesterol, tri-, di- and monoacylglycerols, and phospholipid were determined by colorimetry, gravimetry, or scanning method; the detailed procedures have been described in earlier communications (12, 13). The efficiency of chromatographic separation was tested by subjecting an aliquot of each lipid to further separation by TLC on a silica gel plate from Merck, Germany (14). The hydrocarbon fraction obtained after Florisil column chromatography was submitted to further analysis with gas–liquid chromatography and mass spectrometry using Hitachi, model RMU-6M, equipped with Hitachi datalyser, model 002B. A glass column (1.5 m × 3 mm) containing SE-30 on Chromosorb W was run with N_2 as carrier gas, and programmed from 250°C to 300°C at 3°C/min. Since the GLC–MS data indicated the presence of an alkene together with alkanes, the alkene was separated from alkanes on a column containing 2 g silica gel impregnated with 25% (w/w) silver nitrate. The alkene thus separated was converted to trimethylsilyloxy derivative by the method of Capella and Zorzut (15), and applied to the GLC–MS system to determine the double bond positions.

Sugar, amino sugar, and amino acid

The sugars and amino sugars present in DGLP delipidated with chloroform–methanol 2:1 (v/v) were analyzed by gas–liquid column chromatography. Detailed procedures are given in an earlier communication (13). The amount of amino sugar was determined after hydrolysis of the delipidated DGLP in 4 N HCl at 100°C for 6 hr. The hydrolyzate, after dilution with 3 vol of distilled water, was passed through a column of Dowex 50. The fraction eluted with 2 N HCl was collected and submitted to quantitative determination of hexosamine by the method of Blix (16). To determine the amino acid composition of DGLP, the delipidated DGLP was hydrolyzed with 6 N HCl at 100°C for 24 hr and analyzed on an automatic amino acid analyzer (JEOL, model JLC-6AH).

Gel electrophoresis

Polyacrylamide gel electrophoresis of the purified DGLP was performed in 3.75% gel at pH 8.3. The gel was stained with Coomassie brilliant blue G-250 by a rapid staining method (17). In order to analyze the subunit structure of the apoprotein of DGLP, the delipidated DGLP was run on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis after the method of Weber and Osborn (18).

Molecular weight determination

The molecular weight of the purified DGLP (dissolved in 0.1 M KCl with 0.05 M phosphate buffer, pH 6.0) was determined by the rapid sedimentation-equilibrium method of Yphantis (19), in a Beckman model E analytical ultracentrifuge equipped with a photoelectric scanning system. The equilibrium was established at 14,000 rpm after a 24-hr run at 15°C.

Uptake of diacylglycerol in vitro

The capacity of the lipoprotein to accept lipid from fat body and midgut was demonstrated by incubating prelabeled fat body or midgut in a Ringer solution containing purified DGLP. After incubation, the radioactivity of diacylglycerol associated with DGLP was determined as an indication of the amount of this lipid released by the tissue. The dissected and well-washed fat body was prelabeled

by incubating with [1-^{14}C]palmitic acid (approximately 1×10^6 cpm/animal) for 1 hr. The subsequent procedures for the assay of diacylglycerol uptake by lipoprotein from the prelabeled fat body were similar to those employed in an earlier investigation (12). Midgut was prelabeled by feeding a solution containing [1-^{14}C]palmitic acid (1×10^6 cpm/animal) to an insect as described in an earlier report (8). Two hours after commencement of feeding, the digestive tract was exposed, ligated anteriorly to the proventriculus and at the posterior region of the intestine, and rinsed well with Ringer solution before incubation. The ligated gut sac was suspended in a small glass culture vessel (2 cm diameter, 0.5 cm depth) with ligated ends supported out of the incubation medium. The final volume of incubation medium was 1 ml.

Radioassay

Aliquots of protein fractions for radioassay were transferred to scintillation vials containing 8 ml of cocktail (100 ml of Biosolv, Beckman, U.S.A., and 42 ml of Liquifluor, New England Nuclear, U.S.A. in toluene in final volume of 1 liter). When radioassayed for lipid fractions, the samples were dissolved in 8 ml of cocktail (1 liter toluene containing 3 g PPO and 100 mg dimethyl POPOP). Radioactivity was counted on an Amersham Searle Mark III liquid scintillation counter.

RESULTS

Purification of DGLP

The DGLP was isolated and purified by a modification of the method of Chino, Murakami, and Harashima (12). In order to remove inorganic salts and EDTA that are contained in the original hemolymph, the sample was dialyzed against distilled water for about 40 min until the solution became slightly turbid. The dialyzate was centrifuged at 11,000 *g* for 10 min and, to the resulting supernatant, 8 vol of ice-cold distilled water was rapidly added. The pH was adjusted to 6.6 with 0.01 vol of 0.2 M phosphate buffer and the solution was left for 30 min in an ice bath to allow precipitation of DGLP and related molecules. The suspension was centrifuged and the precipitate was redissolved in 0.5–1.0 ml of 0.2 M phosphate buffer, pH 6.0. The solution was diluted by the addition of 3 vol of distilled water and applied to a column (7×1.1 cm) of DEAE-cellulose equilibrated with 0.05 M phosphate buffer, pH 6.0. The DGLP fraction was passed through the column with the same buffer and additional protein fractions were eluted subsequently with 0.25 M KCl in 0.05 M phosphate buffer, pH 6.0.

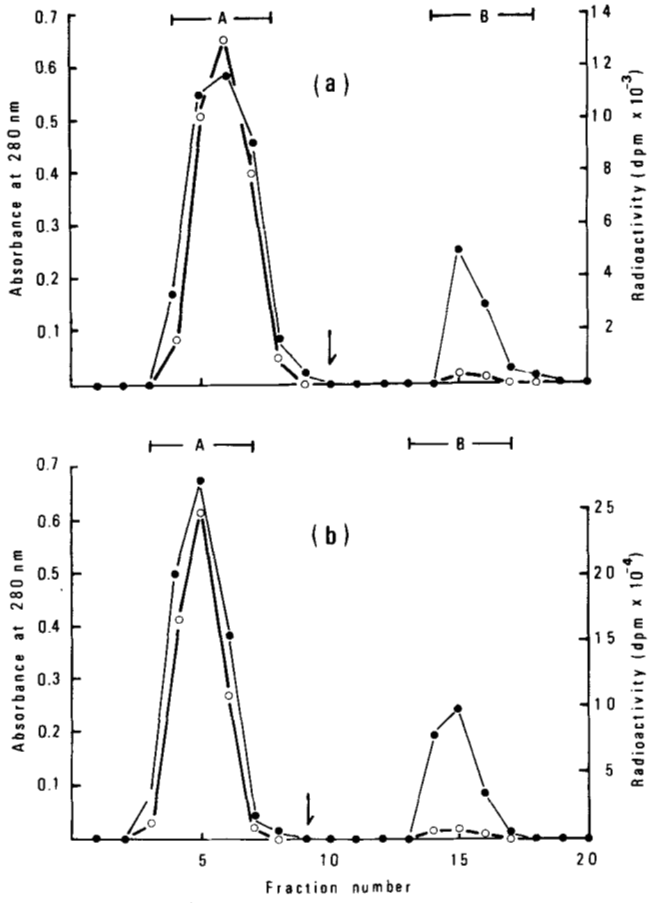

Fig. 1. DEAE-cellulose column chromatography of the fraction precipitated after adding 8 vol of chilled distilled water. (a), Original labeled hemolymph was collected and pooled from ten female cockroaches 4 hr after injection of [1-^{14}C]palmitic acid (approximately 1×10^6 cpm/animal); (b), original labeled hemolymph was collected and pooled from ten female insects 4 hr after feeding on [1-^{14}C]palmitic acid (approximately 1×10^6 cpm/animal). Fractions of 1 ml were collected in each tube. Arrow, beginning of elution with 0.25 M KCl in 0.05 M phosphate buffer, pH 6.0. Open circle, radioactivity; solid circle, absorbance at 280 nm.

A typical separation obtained from the pooled hemolymph of ten female cockroaches that had been injected previously with [1-^{14}C]palmitic acid is illustrated in **Fig. 1a.** A similar profile is obtained from the pooled hemolymph of ten female insects that were fed on [1-^{14}C]palmitic acid prior to collection of the hemolymph (Fig. 1b). It is evident from Fig. 1(a,b) that the peak of radioactivity is coincident with the peak of protein and that, irrespective of the method of administering [1-^{14}C]palmitic acid, most of the radioactivity is associated with primary protein (fraction A) which is designated as "diacylglycerol-carrying lipoprotein" (DGLP) in this paper. By con-

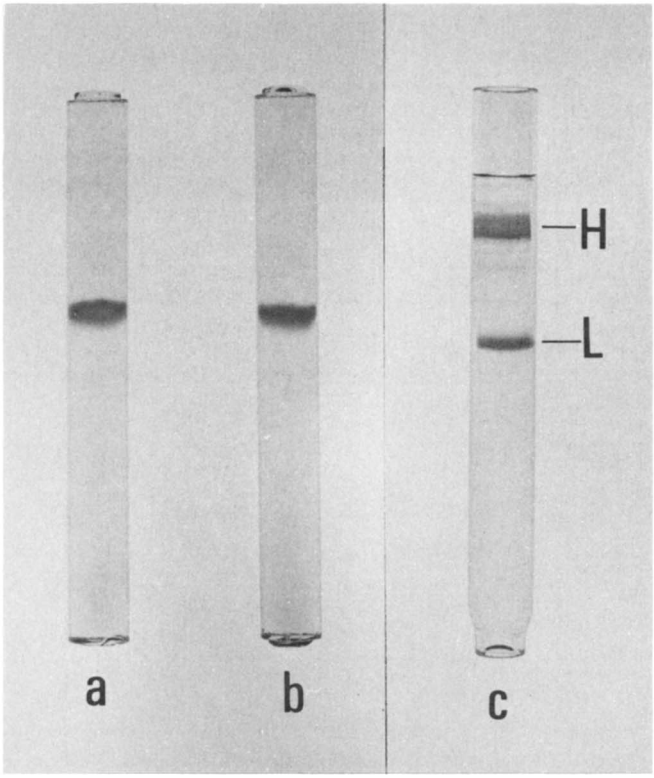

Fig. 2. Polyacrylamide and SDS acrylamide gel electrophoresis of DGLP. a, preparation from female hemolymph; b, preparation from male hemolymph; c, SDS acrylamide gel electrophoresis of the delipidated DGLP. H, heavy chain; L, light chain.

trast, the label in the second fraction (fraction B) is almost negligible. Elution profiles similar to those illustrated in Fig. 1(a,b) were obtained from the pooled hemolymph of male insects (data not shown).

The homogeneity of DGLP preparation was demonstrated by polyacrylamide gel electrophoresis of fraction A. Typical separations are shown in **Fig. 2(a,b)** and serve to demonstrate that a single lipoprotein is eluted from the column at this stage of the purification process. Electrophoretic separation of the second fraction (fraction B) eluted with 0.25 M KCl in phosphate buffer revealed a heterogeneous mixture of several proteins (no photograph shown).

Since it was demonstrated that the radiolabel of DGLP was predominantly associated with diacylglycerol (see Table 2), the efficiency of the purification procedure was tested by determining the radioactivity of the diacylglycerol fraction recovered at each step of the process during the purification of this lipoprotein from the pooled hemolymph of female insects that were fed on [1-^{14}C]palmitic acid. The results are summarized in **Table 1;** if it is assumed that all the radioactivity is associated with DGLP, the data demonstrate that the purification procedure yields a 32.7% recovery of this lipoprotein. Table 1 indicates also that the major loss of radioactivity occurs during the precipitation step under low ionic concentration (step 3).

When DGLP is prelabeled in the lipid moiety, irrespective of the method of administering [1-^{14}C]palmitic acid, most of the radioactivity is associated with the diacylglycerol fraction (**Table 2**). The predominance of diacylglycerol as the major labeled lipid is particularly evident in the DGLP taken from insects that were fed on [1-^{14}C]palmitic acid, with 94% of the label associated with the diacylglycerol fraction.

Uptake of diacylglycerol from fat body and midgut by DGLP in vitro

Earlier investigations demonstrated that fatty acid injected into the hemoceole is primarily incorporated by fat body into diacylglycerol and, subsequently, this lipid is released into hemolymph to associate with a specific lipoprotein; the release of diacylglycerol from fat body requires the presence of this lipoprotein in the extracellular medium (2, 12). The capacity of DGLP to serve this physiological role was tested by incubating prelabeled fat body in physiological saline containing purified DGLP and comparing the release of diacylglycerol under these conditions with that obtained when prelabeled fat body is incubated in physiological saline only, in fresh hemolymph, or in saline containing the protein fraction (fraction B) eluted from DEAE-cellulose column with 0.25 M KCl in phosphate buffer. The results are

TABLE 1. Summary of purification of DGLP from prelabeled hemolymph

Step or Fraction	Protein	Radioactivity of Diacylglycerol	Specific Radioactivity	Recovery of DGLP
	(mg)	*(dpm)*	*(dpm/mg protein)*	*(%)*
1. Original labeled hemolymph	18.2	1,525,200	83,800	100
2. Just before adding 8 vol of distilled water	15.6	1,217,800	78,060	79.9
3. Precipitate after adding distilled water	4.3	643,900	149,740	42.3
4. DGLP (fraction A)	3.2	499,200	156,000	32.7

The prelabeled hemolymph was prepared from ten female cockroaches that were fed approximately 1×10^6 cpm [1-^{14}C]palmitic acid per animal. Protein was determined by the method of Lowry et al. (20).

TABLE 2. Distribution of radioactivity in lipids associated with DGLP[a]

Lipid Class[b]	Prelabeled by Injection		Prelabeled by Feeding	
	Radioactivity	Percentage	Radioactivity	Percentage
	(dpm)		*(dpm)*	
Total lipids[c]	90,440	100	577,910	100
1. Hydrocarbon	16,520	18.3	1,790	0.3
2. Cholesteryl ester	357	0.4	Insignificant	
3. Triacylglycerol	1,120	1.2	468	0.1
4. Diacylglycerol	67,390	74.5	543,090	94.0
5. Monoacylglycerol	Insignificant		2,990	0.5
6. Free fatty acid	1,030	1.1	1,300	0.2
Sum of 1–6	86,417	95.5	549,638	95.1

[a] DGLP was purified from the pooled hemolymph of ten female cockroaches that received approximately 1×10^6 cpm [1-^{14}C]palmitic acid/animal by injection or feeding.

[b] Lipid classes listed were confirmed by TLC, except for monoacylglycerol and free fatty acid.

[c] Lipid fraction before separation by Florisil column chromatography. No significant radioactivity was incorporated into the phospholipid fraction.

presented in **Table 3** and demonstrate that the greatest release of diacylglycerol occurs in the presence of DGLP. The small amount of radioactivity recovered from medium that does not contain this lipoprotein is attributed to leaking as a result of fat body tissue breakdown during incubation (12). **Table 4** demonstrates that DGLP is capable also of accepting diacylglycerol from the isolated intestinal tract of adult cockroaches that were fed previously on [1-^{14}C]-palmitic acid. By contrast, the second protein fraction (fraction B) displayed no particular affinity for accepting diacylglycerol either from fat body or intestine.

Electron microscopy of DGLP

A negatively stained electron micrograph of the purified DGLP is illustrated in **Fig. 3** and demonstrates high homogeneity in terms of molecular shape and size; the molecule is almost globular with a diameter of 160 Å ± 11. This confirms the homogeneous nature of the preparation of DGLP.

Lipid composition of DGLP

As indicated in **Table 5,** the total lipid content of DGLP amounts to about 50%. Diacylglycerol represents the major acylglycerol while the triacylglycerol content is extremely low. Of particular interest is the considerable amount of hydrocarbons contained in DGLP. Analytical data of the hydrocarbons are given in **Fig. 4** and **Table 6.** An unsaturated hydrocarbon, 6,9-heptacosadiene is the major compound, and two saturated hydrocarbons, *n*-pentacosane and 3-methylpentacosane, are also present in considerable amounts. The sum of these three hydrocarbons accounts for more than 98% of the total hydrocarbons.

Phospholipids comprised phosphatidylcholine and phosphatidylethanolamine, and other phospholipids were not detected.

Amino acid composition of DGLP

The amino acid compositions of the delipidated DGLP were determined, and **Table 7** shows the data, together with those of silkworm DGLP-I for comparison. There appears to be no major difference between the two DGLPs.

Sugar and amino sugar of DGLP

GLC analysis revealed that the delipidated DGLP contained 0.9% mannose based on the protein con-

TABLE 3. Assay of uptake of diacylglycerol by DGLP from prelabeled fat body

Exp. No.	Incubation Medium	Protein Amount Used for Assay	[^{14}C]Diacylglycerol Released into Incubation Medium
		(mg)	*(dpm)*
1.	Phosphate saline[a]	0	4,890[b]
	Purified DGLP	0.60	27,110
	Fraction B	0.60	4,630
2.	Phosphate saline	0	1,080
	Fresh hemolymph	0.60	4,600
	Purified DGLP	0.43	12,780
	Fraction B	0.43	1,440

[a] 0.15 M KCl in 0.05 M phosphate buffer, pH 7.0.

[b] Release into phosphate saline represents only "artificial leaking" due to disintegration of prelabeled fat body during incubation.

Experiment 1, 150 mg prelabeled fat body containing 150,200 dpm [^{14}C]diacylglycerol in each incubation; Experiment 2, 150 mg prelabeled fat body containing 53,900 dpm [^{14}C]diacylglycerol. Incubation time, 90 min at 25°C. For details of assay method see the earlier paper (12).

TABLE 4. Assay of uptake of diacylglycerol by DGLP from prelabeled midgut

Incubation Medium	Protein Amount Used for Assay	[^{14}C]Diacylglycerol Released into Incubation Medium
	(mg)	*(dpm)*
Phosphate saline	0	1,810
Phosphate saline	0	1,730
Phosphate saline	0	1,070
Fresh hemolymph	1.8	11,210
Fresh hemolymph	1.0	4,240
Purified DGLP	1.0	9,350
Purified DGLP	0.46	6,060
Fraction B	0.34	1,490
Fraction B	0.40	864

Incubation time, 90 min at 25°C. For details see the Method section. [^{14}C]Triacylglycerol and [^{14}C]palmitic acid released into every incubation media were negligible.

TABLE 5. Lipid composition of DGLP

Component	Amount	% weight[a]
	(mg)	
Protein	95.76	50.4
Total lipids	94.08	49.6
1. Hydrocarbon	26.67	28.3
2. Triacylglycerol	1.86	2.0
3. Diacylglycerol	14.28	15.2
4. Monoacylglycerol	Not detected	
5. Cholesterol	4.75	5.0
6. Cholesteryl ester	Not detected	
7. Total phospholipids	40.29	42.8
Phosphatidylcholine		(68.4)
Phosphatidylethanolamine		(31.6)
Sum of 1–7	87.85	93.4

[a] Lipid fractions (1–7) are expressed as percentages of the total lipids. Phospholipids are expressed as percentages of the total phospholipids in parentheses.

tent, and no other sugars were detected (GLC data not shown). The presence of glucosamine was also demonstrated by GLC, and its content, determined by the method of Blix (16), was 0.3%.

Molecular weight of DGLP

It was assumed from the electron micrograph of DGLP that the molecular weight exceeded at least 500,000, and because the gel filtration method seemed inadequate to determine the molecular weight of such a large molecule, a sedimentation-equilibrium method was employed. The total lipid content and the amino acid composition of cockroach DGLP resembled those reported for silkworm DGLP-I (12). Therefore, 0.87 was taken as the specific partial volume, which had been calculated theoretically for the silkworm DGLP-I

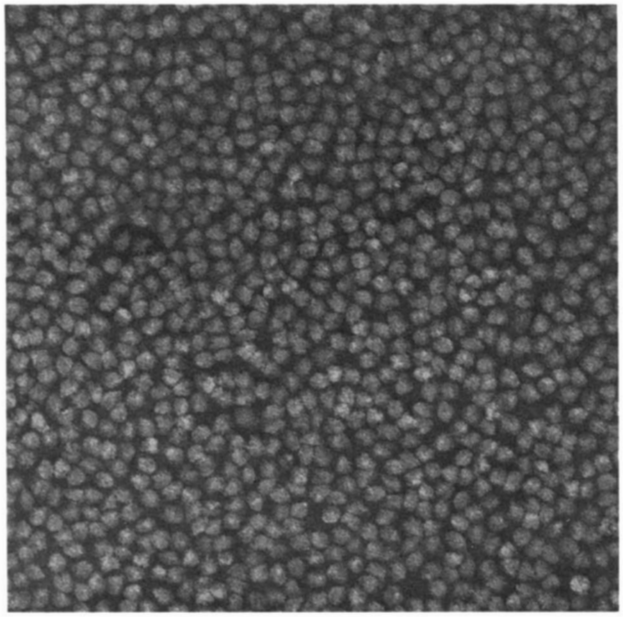

Fig. 3. Electron micrograph of DGLP negatively stained with uranium acetate and observed in a Hitachi 11B electron microscope (×150,000). The measurement of molecular size was made on 100 molecules within a certain area of the electron micrograph of 550,000 magnification.

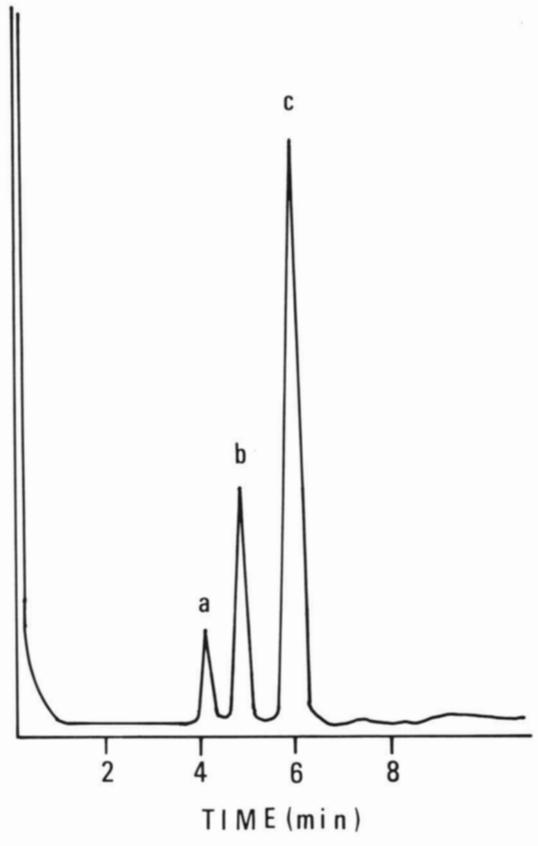

Fig. 4. Gas–liquid chromatogram of DGLP hydrocarbons. Three major fractions were identified with mass spectrometry as *n*-pentacosane (a), 3-methylpentacosane (b), and 6,9-heptacosadiene (c), respectively. Mass spectrometric data are not shown.

TABLE 6. Hydrocarbon composition of DGLP

Hydrocarbons	Content
	(%)
1. *n*-Pentacosane	7.2
2. 3-Methylpentacosane	18.1
3. 6,9-Heptacosadiene	72.3
4. Unidentified hydrocarbon	2.4

(12). Thus, the molecular weight of cockroach DGLP was estimated to be approximately 600,000.

Subunit structure of DGLP

The subunit structure of the DGLP apoprotein was determined by analysis of the delipidated DGLP on SDS acrylamide gel electrophoresis. The electropherogram illustrated in Fig. 2c clearly demonstrates that the apoprotein consists of two different subunits, heavy chain and light chain. The molecular weights of the two chains were determined by comparison with SDS acrylamide gel electrophoresis of several known molecular weight standard proteins, and were estimated to be approximately 250,000 and 85,000 for heavy chain and light chain, respectively (**Fig. 5**). The difference between the native molecular weight (600,000) and the molecular weight of apoprotein (250,000 + 85,000) approaches the total lipid content (50%).

DISCUSSION

Two DGLPs have been isolated and characterized in the silkworm, *Philosamia cynthia* (12). One of the molecules, DGLP-I, contains almost 55% diacylglycerol of the total lipid and serves as the primary vehicle for lipid transport from the fat body to the ovaries (3) and other tissues (2). The second lipoprotein, DGLP-II, contains much less diacylglycerol and has been identified as a vitellogenin which contributes to the accumulation of protein in the developing eggs (21). The present report describes the isolation and purification of another DGLP from the hemolymph of male and female American cockroaches. Polyacrylamide gel electrophoresis and electron microscopy of the final preparation demonstrate its high homogeneity (Fig. 2a, b and Fig. 3). The DGLP described herein was purified by a procedure similar to that developed for the purification of DGLP-I from *Philosamia* silkworm hemolymph (12). This similarity, together with the molecular affinity for diacylglycerol (Table 2) and its capacity to accept this lipid from incubating tissues (Table 3 and 4), suggests that this DGLP is physiologically analogous

TABLE 7. Amino acid composition of total apoprotein of DGLP

Amino Acids	Recovered Amino Acids	
	Cockroach DGLP	Silkworm DGLP[a]
	(mol/1000 mol)	
Asp	110	126
Thr	66	49
Ser	69	69
Glu	108	104
Pro	38	47
Gly	64	67
Ala	68	63
Val	84	74
Met	3	5
Ile	41	58
Leu	107	90
Tyr	30	28
Phe	47	48
His	39	28
Lys	93	107
Arg	27	37
Cys	6	

[a] From data of DGLP-I of *Philosamia* silkworm (12).

to DGLP-I of the silkworm. However, the present report extends our knowledge of DGLP in insects by demonstrating that the same molecule serves to transport the same lipid from the site of storage in the fat body and from the site of absorption in the in-

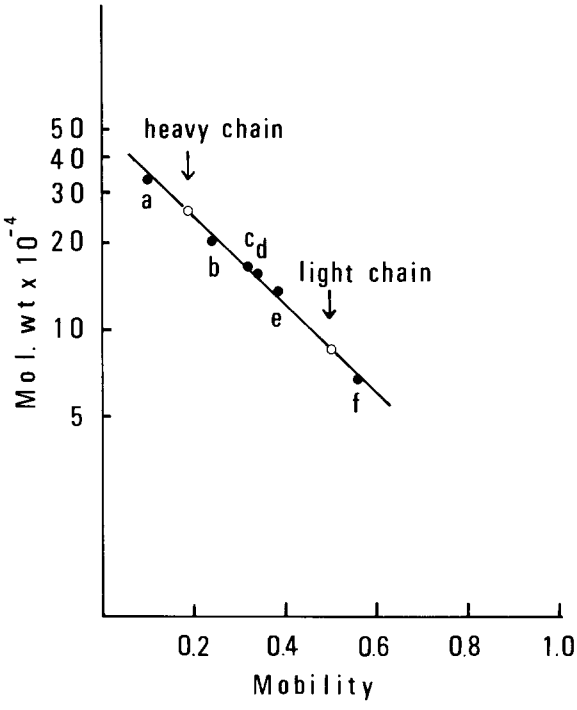

Fig. 5. The relationship of the mobilities on SDS acrylamide gel and the molecular weights. a, Thyroglobulin (330,000); b, myosin heavy chain (200,000); c, RNA polymerase β'-chain (165,000); d, RNA polymerase β-chain (155,000); e, BSA dimer (136,000); f, BSA monomer (68,000).

testine. Such an ubiquitous role for a serum lipoprotein has not been reported in any animal system. This finding also suggests that the same mechanism of diacylglycerol release and uptake is involved in the two processes, and confirms our earlier observations that the primary form in which neutral lipid is absorbed from the intestine is as diacylglycerol (8, 22).

The absolute amount of DGLP present in cockroach hemolymph appears to be very high and accounts for a major fraction of the total hemolymph protein. The data contained in Table 1 indicated that 3.2 mg of DGLP is recovered from a hemolymph sample containing 18.2 mg of protein. The percentage recovery of this protein is 32.7%; therefore the total content of this lipoprotein present in the original sample amounts to 9.79 mg (3.2/32.7 × 100). Thus, over 50% of the total hemolymph protein is represented as DGLP. The high proportion of DGLP in the cockroach contrasts with the relatively low concentration (about 10% of total hemolymph protein) of DGLP-I that has been reported in *Philosamia* silkworm (12).

Studies on the silkworm, *Philosamia cynthia*, indicated that a critical step in the purification process is the precipitation of lipoprotein from the supernatant that remains following centrifugation of the dialyzed hemolymph (12). The precipitation is facilitated by the presence of a co-precipitant which, in the silkworm, appears to be vitellogenin (12, 21). Co-precipitation is involved in the precipitation of cockroach DGLP but as this lipoprotein is precipitated also from the hemolymph of male insects, the co-precipitant is not vitellogenin. The nature of the cockroach co-precipitant has not been identified although it is possible that one (or more) component(s) of the second protein fraction (fraction B) that remains on the DEAE-cellulose column after elution of DGLP may be involved in co-precipitation.

The present study reveals that the apoprotein of cockroach DGLP consists of two non-identical subunits, heavy chain (mol wt 250,000) and light chain (mol wt 85,000). Recent observations indicate that DGLP-I of *Philosamia* silkworm also comprises heavy chain (mol wt 370,000) and light chain (mol wt 88,000).[2] Thus, it is possible that a similar subunit structure is a general feature of insect DGLPs.

The finding that cockroach DGLP contains a considerable amount of hydrocarbons in addition to diacylglycerol and cholesterol is extremely interesting. The finding supports the observation that significant radioactivity is recovered from the hydrocarbon fraction, when [1-^{14}C]palmitic acid is injected into hemoceole (Table 2). In fact, it has been reported for American cockroaches that carboxyl carbons of fatty acids are incorporated into hydrocarbons *via* elongation and decarboxylation pathway in the biosynthesis (23). The hydrocarbon compositions are essentially similar to those reported for cuticular hydrocarbons of the American cockroach; the cuticular hydrocarbons comprise heptacosadiene, *n*-pentacosane, and 3-methylpentacosane (24). Baker, Vroman, and Padmore (25) have reported the presence of hydrocarbons in hemolymph of American cockroach, and have also identified the above three compounds. It now becomes evident that these hydrocarbons are associated with the DGLP reported in this paper, and it seems very likely that DGLP serves as a carrier protein to transport these hydrocarbons from the site of synthesis to the site of deposition (cuticle), although it is still unclear where the hydrocarbons are synthesized in insects. These observations, together with our knowledge of lipid transport in insects, lead to the conclusion that the cockroach DGLP serves multiple functions in transporting such lipids as diacylglycerol, cholesterol, and hydrocarbon from the site of absorption, storage, or synthesis to the site of utilization or deposition.

The authors wish to thank Dr. T. Takazawa for performing the ultracentrifugation and Dr. S. Gasa for the GLC–MS analyses and permission to use the GLC–MS system in the Central Research Center, School of Medicine, Hokkaido University. This work was supported in part by the National Research Council of Canada and the Japan Society of the Promotion of Science through the Canada-Japan Senior Scientist Exchange Program.

Manuscript received 29 January 1980, in revised form 27 May 1980, and in re-revised form 21 July 1980.

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