

α -Cyclodextrin extracts diacylglycerol from insect high density lipoproteins

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Abstract α -Cyclodextrins are water-soluble cyclic hexamers of glucose units with hydrophobic cavities capable of solubilizing lipophiles. Incubating α -cyclodextrin with high density lipophorin from *Manduca sexta* or *Bombyx mori* resulted in a cloudy, turbid solution. Centrifugation separated a pale yellowish precipitate. Thin-layer chromatography analysis of the lipid extract of the precipitate showed that the major lipid was diacylglycerol, while KBr density gradient analysis of the supernatant demonstrated the presence of a lipid-depleted very high density lipophorin. Transfer of diacylglycerol from lipophorin to cyclodextrin was specific to α -cyclodextrin and was not observed with β - or γ -cyclodextrins. pH had no effect on diacylglycerol transfer to α -cyclodextrin. However, the transfer was strongly dependent on the concentration of α -cyclodextrin and temperature. Increasing the concentration of α -cyclodextrin in the incubation mixture was associated with the formation of increasingly higher density lipophorins. Thus, at 20, 30, and 40 mM α -cyclodextrin, the density of *B. mori* lipophorin increased from 1.107 g/ml to 1.123, 1.148, and 1.181 g/ml, respectively. At concentrations greater than 40 mM, α -cyclodextrin had no further effect on the density of lipophorin. α -Cyclodextrin removed at most 83–87% of the diacylglycerol present in lipophorin. Temperature played an important role in altering the amount of diacylglycerols transferred to α -cyclodextrin. At 30 mM α -cyclodextrin, the amount of diacylglycerol transferred at different temperatures was 50% at 4°C, 41% at 15°C, 20% at 28°C, and less than 3% at 37°C. We propose that diacylglycerol transfers to α -cyclodextrin via an aqueous diffusion pathway and that the driving force for the transfer is the formation of an insoluble α -cyclodextrin–diacylglycerol complex.—Jouni, Z. E., J. Zamora, M. Snyder, W. R. Montfort, A. Weichsel, and M. A. Wells. α -Cyclodextrin extracts diacylglycerol from insect high density lipoproteins. *J. Lipid Res.* 2000. 41: 933–939.

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Cyclodextrins are produced from starch by the action of cyclodextrin glycosyltransferase. Structurally, cyclodextrins are cyclic oligosaccharides consisting of 6, 7, or 8 d-glucopyranosyl units connected by α -(1 \rightarrow 4) glycosidic linkages and are referred to as α -, β -, and γ -cyclodextrins,

respectively (1). In the donut-shaped cyclodextrins, the pyranosyl rings of the cyclodextrins are arranged so that the hydroxyl groups point outward creating a hydrophobic inner surface. The number of glucose units in the cyclodextrin determines the size of this hydrophobic pocket and, in turn, which lipids the cyclodextrin solubilizes (2). Many factors affect the solubilization of lipids by cyclodextrins, including the concentration of cyclodextrins, the characteristics of the guest molecules (hydrophobicity and size), and the reaction conditions. The inclusion complexes can contain one or more molecules of cyclodextrins and can contain one or more entrapped “guest” molecules. As part of an ongoing study of lipid transport in insects, we investigated whether α -, β -, or γ -cyclodextrins could remove lipids from the insect lipoprotein, lipophorin. Lipophorin is a high density lipoprotein found in the hemolymph of insects and functions as a reusable shuttle that transports lipids through insect hemolymph (3, 4). The major transported lipid is diacylglycerol but little is known about the mechanism by which diacylglycerol is transferred between lipophorin and tissues. We report in this paper that α -cyclodextrin is able to remove diacylglycerol from lipophorin. The ability of α -cyclodextrin to modify the diacylglycerol content of lipophorin may be an important tool in investigating the mechanism of diacylglycerol transfer between lipophorin and tissues.

METHODS

Materials

2-[Morpholino]-ethanesulfonic acid (MES) and 3-[N-morpholino]-propanesulfonic acid (MOPS) were from Amersham (Piscataway, NJ). α -Cyclodextrin, hydroxypropyl α -cyclodextrin,

Abbreviations: MES, 2-[morpholino]-ethanesulfonic acid; MOPS, 3-[N-morpholino]-propanesulfonic acid; PMSF, phenylmethylsulfonic acid; K_{eq} , equilibrium constant defined in terms of concentrations of reactants and products present at equilibrium; DPG, dipalmitoyl diacylglycerol.

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[³H]8,9 oleic acid, phenylmethylsulfonyl fluoride (PMSF), and Grace's medium were from Sigma (St. Louis, MO).

Insects

Bombyx mori of the polyvoltine race N₄ (a gift from Dr. Yamashita, Nagoya University) were fed on an artificial diet prepared from mulberry leaves (Yakult Corp., Tokyo, Japan). *Manduca sexta* were fed on an artificial diet prepared from wheat germ (5). All insects were reared at 25–27°C with an 18-h light, 6-h dark cycle.

Lipophorin isolation

High density lipoproteins were isolated from the hemolymph of third or fourth day fifth instar *M. sexta* or *B. mori* by density gradient ultracentrifugation (5, 6). Briefly, the hemolymph density was adjusted to 1.31 g/ml (8.9 g KBr in 20 ml), placed in a Quick-Seal centrifuge tube, and overlaid with 20 ml of 0.9% NaCl. The tubes were centrifuged at 50,000 rpm in a VTi 50 rotor at 5°C for 18 h. One-ml fractions were collected, absorbance at 280 nm and 450 nm was determined, and the densities of the fractions were measured. Fractions containing lipophorin were dialyzed against PBS buffer (150 mM NaCl, 2 mM EDTA, and 0.5 mM benzamidine hydrochloride, 50 mM sodium phosphate, pH 6.5). The solution was then concentrated using a 100 kD molecular mass cut centricon filters (Amicon, Beverly, MA). The purity of lipophorin preparations was confirmed by SDS-PAGE analysis, using either a linear (7.5%) or gradient (4–15%) SDS-PAGE prepared as described by Laemmli (7). All gels were stained with Coomassie Brilliant Blue R-250.

Transfer studies

Lipophorin was incubated with α -cyclodextrin on an orbital shaker at 100 rpm for 15 min at room temperature. The turbid reaction mixture was centrifuged at 10,000 rpm for 10 min. A sample of the supernatant was subjected to density gradient ultracentrifugation and analyzed as described above. Another sample of the supernatant was extracted 3 times with Folch (methanol–chloroform 1:2 (v:v)) and the combined lipid extracts were separated on TLC plates. Diacylglycerol spots were identified with iodine vapor, collected and extracted with Folch, dried under N₂ gas, resuspended in hexane, and used for the determination of diacylglycerols. A vanillin-based neutral lipid assay was used to measure diacylglycerol content, using diolien as a standard (8). The precipitate was washed several times with PBS and extracted three times with dichloromethane and the extracts were used for the determination of diacylglycerols. When mentioned, organic phases were separated on TLC plates and diacylglycerols were collected and used as mentioned above.

Effect of pH and temperature

For these studies, 30 mM α -cyclodextrin and 3 mg/ml of *M. sexta* lipophorin were used. To study the effect of pH, lipophorin was dialyzed into a buffer containing MES (0.01 M), MOPS (0.01 M), Trizma base (0.5 mM), and NaCl (0.15 M) of different pH values between 5 and 7. For the effect of temperature on transfer studies, stock solutions of α -cyclodextrin and lipophorin (PBS buffer) were pre-equilibrated to temperatures ranging from 4°C to 37°C, then mixed to start the experiment.

Lipophorin concentration dependence

The effect of increasing concentrations of *M. sexta* lipophorin (1.5, 3, 5, and 7 mg/ml) was determined using a constant concentration of α -cyclodextrin (30 mM).

Transfer of [³H]diacylglycerol to α -cyclodextrin

B. mori larvae were starved for 1 h then fed a cube of diet (0.5 cm × 0.5 cm × 0.5 cm) containing [³H-n-8,9]oleic acid. After

consumption of the labeled diet, the insects were switched to a normal diet for 1 h. Then hemolymph was collected in a solution containing phenylthiourea, benzamidine (0.5 mM), and PMSF (0.5 mM) in PBS (9). [³H]diacylglycerol-labeled *B. mori* lipophorin was isolated as described above and incubated with 40 mM α -cyclodextrin on an orbital shaker for 15 min, then the turbid reaction mixture was centrifuged at 10,000 rpm for 10 min. A sample of the supernatant was subjected to density gradient ultracentrifugation and density, radioactivity, and absorbances at 280 and 452 nm along the gradient were determined. Another sample of the supernatant was extracted 3 times with methanol–chloroform 1:2 (v:v) and the extract was used for the determination of diacylglycerols, as described above. The pellet was washed several times with PBS and then extracted 3 times with dichloromethane for the determination of diacylglycerols.

Analysis of the composition of the α -cyclodextrin–diacylglycerol complex

To isolate the complex, 5 mg/ml *M. sexta* HDLp was incubated in 40 mM α -cyclodextrin at room temperature on an orbital shaker for 15 min, then the turbid reaction mixture was centrifuged at 10,000 rpm for 10 min. The pellet was washed several times with PBS, resuspended in 1 ml PBS, and a 300- μ l sample was extracted three times with dichloromethane. The organic phases were combined, dried under nitrogen gas, and used for the determination of diacylglycerols, as described above. An aliquot of the aqueous phase was used to determine α -cyclodextrin concentration using anthrone (10) and α -cyclodextrin was used as a standard. Data are reported as the ratio of μ moles of α -cyclodextrin/ μ moles of diacylglycerols.

Modeling the structure of the α -cyclodextrin–diacylglycerol complex

A model of dipalmitoyl diacylglycerol (DPG) solvated by α -cyclodextrin was built using the coordinates for dipalmitoyl diacylglycerol (11) and α -cyclodextrin (12). An initial model was built by hand on an SGI graphics computer, using the Insight II software package MSI (Molecular Simulations Inc., San Diego, CA). Three rings of cyclodextrin were sufficient to cover the DPG molecule while stacking atop one another. The complex was energy-minimized using the Discover module in Insight II and the cvff forcefields, resulting in a model with reasonable stereochemistry, extensive van der Waals contacts and hydrogen bonds, but no disallowed high-energy contacts.

Analyses of data

Transfer studies are reported as μ g diacylglycerol transferred to α -cyclodextrin or were calculated as the percentage of diacylglycerol transferred to α -cyclodextrin. In some cases, the data were analyzed by linear or non-linear regression using GraphPad Prism (GraphPad Software, Inc).

Protein analysis

Protein concentrations were determined using a modified Lowry method with bovine serum albumin as a standard (13).

Statistical analysis

For statistical analysis, Student's unpaired *t*-tests were used to determine the significance of differences between means.

RESULTS AND DISCUSSION

α -Cyclodextrin removes diacylglycerol from lipophorin

Addition of 40 mM α -cyclodextrin to *B. mori* [³H]diacylglycerol-labeled lipophorin resulted in a turbid reaction

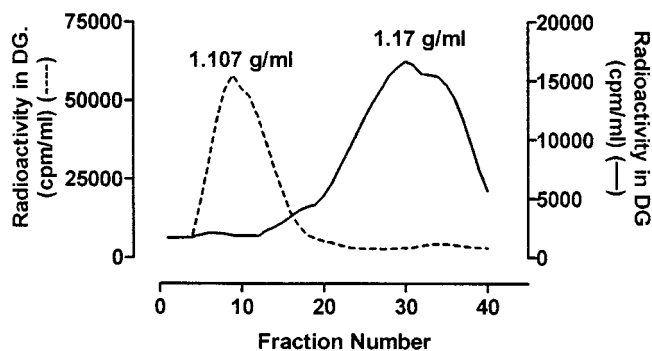


Fig. 1. Effect of α -cyclodextrin on the density of *B. mori* lipophorin. Lipophorin was incubated with 40 mM α -cyclodextrin. After removal of the precipitate, the lipophorin in the supernatant was subjected to KBr density gradient ultracentrifugation. Fractions from the KBr gradient were analyzed for radioactivity and density; lipophorin before incubation (dashed line); lipophorin after incubation with α -cyclodextrin (solid line).

mixture with the spontaneous formation of a pale yellowish precipitate. This precipitate was recovered by centrifugation at 10,000 *g* for 10 min. TLC analysis of the lipid extract of the precipitate showed that diacylglycerols accounted for the majority of the lipid present, along with small amounts of carotenoids and phospholipids (less than 7% combined). About 67% of the radiolabeled diacylglycerols present in the original lipophorin preparation were precipitated with α -cyclodextrin. Incubation with α -cyclodextrin caused a shift in the density of the radioactive peak (diacylglycerol) relative to control (Fig. 1). Both peaks shown in Fig. 1 corresponded to lipophorin as confirmed by SDS-PAGE and immunoblotting using anti-lipophorin-I and -II antibodies (data not shown).

α -Cyclodextrin concentration dependence

Fig. 2 shows the effect of increasing concentrations of α -cyclodextrin on the density of *B. mori* lipophorin. Increasing the concentration of α -cyclodextrin in the incubation mixture was associated with production of a higher density lipophorin. Untreated *B. mori* lipophorin has a density of 1.107 g/ml and addition of 10 mM α -cyclodextrin did not cause a significant density shift. However, at 20, 30, and 40 mM α -cyclodextrin, the density of lipophorin progressively increased to 1.123, 1.148, and 1.181 g/ml, respectively. At the higher α -cyclodextrin concentrations, the peak of lipophorin was broader, suggesting the presence of subfractions of lipophorin with a wide range of densities. No significant differences in the absorbances at 452 nm in the untreated and α -cyclodextrin-treated lipophorin were observed, indicating that α -cyclodextrin does not extract carotenoids from lipophorin. In addition, no loss of lipophorin protein was observed at any α -cyclodextrin concentration used. This observation is consistent with the work of Ohtani et al. (14), who showed that α -, β -, and γ -cyclodextrins failed to bind to polypeptides in significant amounts. Similar results were obtained using *M. sexta* HDLp (data not shown).

The amount of diacylglycerol removed from either *B.*

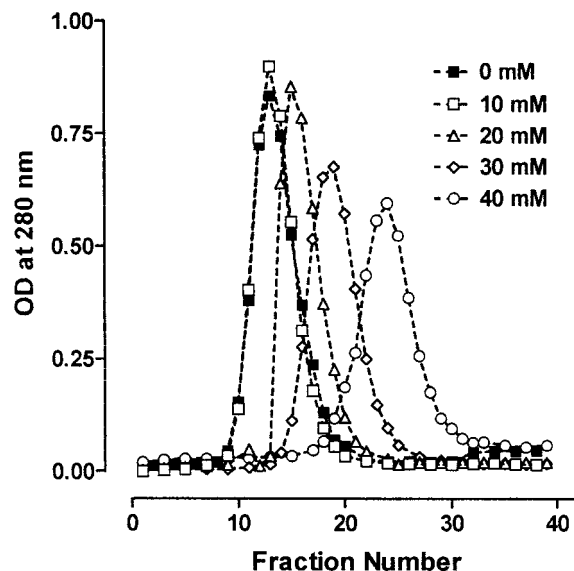


Fig. 2. Effect of α -cyclodextrin concentration on diacylglycerol removal from lipophorin. *B. mori* lipophorin (5 mg) was incubated with increasing concentrations of α -cyclodextrin. After removal of the precipitate, the lipophorin in the supernatant was subjected to KBr density gradient ultracentrifugation. KBr gradient fractions were analyzed for protein at 280 nm; (■) control; (□) 10 mM; (△) 20 mM; (◇) 30 mM; and (○) 40 mM α -cyclodextrin.

mori or *M. sexta* lipophorin by increasing concentrations of α -cyclodextrins is presented in Table 1. Although at 10 mM α -cyclodextrin, lipophorin lost 4–7% of its diacylglycerol content, this amount was not enough to cause a density shift of the particle (Fig. 2). This observation is consistent with the report of Soulages, van Antwerpen, and Wells (6), which showed that at least a 10% change in diacylglycerol content was required to obtain a noticeable shift in the density of the particle. At all concentrations of α -cyclodextrin there was not a significant difference in the amount of diacylglycerol removed from the two different sources of lipophorin. The large amount of diacylglycerol transferred to α -cyclodextrin indicates that most of the transfer is from the core diacylglycerol pool because the surface pool of diacylglycerol is less than 5% of the diacylglycerol in lipophorin (6). At this time, it is not clear

Table 1. Effect of increasing α -cyclodextrin (α -CD) concentration on transfer of diacylglycerol (DG)

α -CD	Amount of DG in <i>M. sexta</i> HDLp	Amount of DG in <i>B. mori</i> HDLp
mM	μ g	μ g
0	3421.4 \pm 239.1	3268.2 \pm 255
10	3287.2 \pm 162.1	3030.4 \pm 147
20	2335.2 \pm 25.4 ^a	2296.4 \pm 53 ^a
40	561.1 \pm 53.2 ^a	622.7 \pm 71 ^a
60	342.8 \pm 117.3 ^a	326.3 \pm 11 ^a

Five mg of either *B. mori* or *M. sexta* lipophorin (HDLp) was treated with the indicated concentration of α -cyclodextrin and the amount of diacylglycerol remaining in solution was determined.

^a Significantly different from 0 mM and 10 mM α -cyclodextrin.

whether diacylglycerol efflux from HDLp occurs from the core pool via the surface pool of diacylglycerol that is in equilibrium with the core pool, or through a totally different location on HDLp. We consistently observed that about 15% of the lipophorin–diacylglycerol is not transferred to α -cyclodextrin, which might suggest a “structural” role for this small pool of diacylglycerol. A structural role for diacylglycerol has been proposed previously (6).

Specificity for α -cyclodextrin

The α -, β -, and γ -cyclodextrins have different cavity sizes of 5, 6, and 8 Å, respectively, and some specificity in the interaction of the different cyclodextrins with lipophorins might be expected (14). To determine transfer specificity, *M. sexta* lipophorin was incubated with 30 mM α -, β -, and γ -cyclodextrins for 15 min and the extent of diacylglycerol transfer was determined. Neither β - nor γ -cyclodextrins were able to cause any significant transfer of diacylglycerol from lipophorin whereas under these conditions α -cyclodextrin caused a 28% transfer of diacylglycerol from lipophorin. The same results were obtained when longer incubation times of 2 h were used. The inability of β - and γ -cyclodextrins to extract diacylglycerol from HDLp was also confirmed using up to 60 mM concentrations of the corresponding cyclodextrins and longer incubation times of 2 h. These results suggest that the cavity size is one important factor in determining diacylglycerol transfer to cyclodextrins.

Lipophorin concentration dependence

The amount of diacylglycerol transferred to α -cyclodextrin increased with increasing the concentration of lipophorin in the incubation media (Fig. 3). Nonlinear least squares analysis of the transfer data demonstrated a saturation behavior at high concentrations of lipophorin and this was confirmed by linearity of the double reciprocal analysis of the data (Fig. 3, inset).

Effect of pH on diacylglycerol transfer

pH values of the reaction mixture ranging from 5 to 7 had no effect on the amount of diacylglycerol transferred to 30 mM α -cyclodextrin (Fig. 4). The same results were obtained when different concentrations of α -cyclodextrin were used. Likewise, the ionic strength of the reaction mixture ranging from 0 to 2 mM NaCl exhibited no effect on the ability of α -cyclodextrin to extract diacylglycerol from lipophorin (data not shown).

Effect of temperature on diacylglycerol transfer

Temperature played a critical role in determining the amount of diacylglycerol transferred to α -cyclodextrin (Table 2). At 30 mM α -cyclodextrin the amount of diacylglycerol transferred to α -cyclodextrin increased from less than 3% at 37°C to 50% at 4°C. Assuming that the reaction is, $\text{Lp-DG} + \text{CD} \rightleftharpoons \text{Lp} + \text{CD-DG}$, where Lp-DG is lipophorin–diacylglycerols, Lp is diacylglycerols-depleted lipophorin, CD is cyclodextrin, and CD-DG is cyclodextrin–diacylglycerols, the K_{eq} at 4, 15, and 28°C can be calculated from the data in Table 2. A van't Hoff plot of these

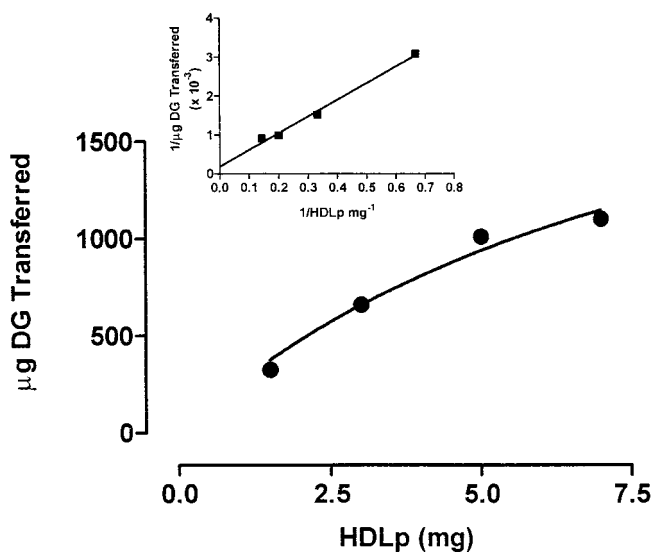


Fig. 3. Effect of increasing concentration of *M. sexta* lipophorin on diacylglycerol transfer. Increasing concentrations of lipophorin were incubated with 40 mM α -cyclodextrin and the amount of diacylglycerol present in the precipitate was determined. In the main figure, the line was determined by non-linear least squares analysis assuming a simple saturable process. The inset shows a double reciprocal plot of the same data. From either plot, it was determined that the maximal amount of diacylglycerol transferred was 5,350 μg . Values represent averages \pm standard deviation for four determinations. Standard deviations are smaller than the symbols.

data (Fig. 5) has a slope of 4600 ± 1100 and a Y intercept of -20 ± 3.8 . These numbers translate into $\Delta H^\circ = -38.2 \pm 9.1$ kJ/mol and $\Delta S^\circ = -166 \pm 31.6$ J/°mol. The values for ΔH° and ΔS° are consistent with a reaction that proceeds well at low temperature and poorly at high temperature. However, if the equilibrium written above were the one being studied, one would expect hydrophobic interactions to play a critical role in complex formation. Hydrophobic

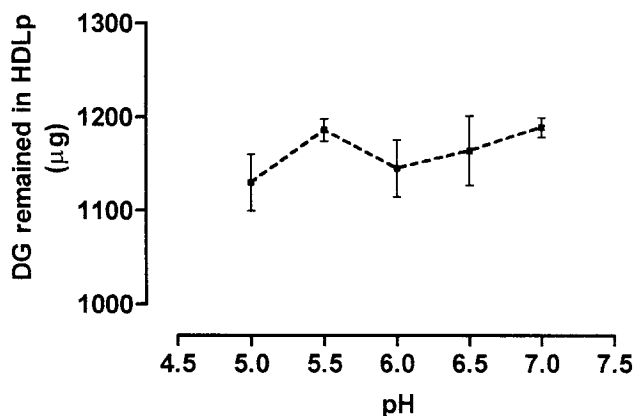


Fig. 4. Effect of pH on diacylglycerol transfer. α -Cyclodextrin was incubated with *M. sexta* lipophorin at pHs ranging between 5 and 7. After the reaction, the precipitate was removed and the amount of diacylglycerol remaining in solution was determined. Values represent averages \pm standard deviation for six determinations from two experiments.

Table 2. Effect of temperature on the amount of diacylglycerol associated with HDLp after treatment with α -cyclodextrin

Temperature	Diacylglycerol Remaining in HDLp
$^{\circ}\text{C}$	μg
4	776.0 ± 91.5
15	915.5 ± 205.9
27	1122.7 ± 92.1
37	1540.3 ± 26.5

Three mg of *M. sexta* HDLp containing 1552 μg of diacylglycerol in one ml were treated with 30 mM α -cyclodextrin at the indicated temperatures and the amount of diacylglycerol (DG) (μg) remaining in solution was determined.

interactions are usually not favored at low temperature and favored at high ionic strength. As this is not the case, we assume that the equilibrium involved is the formation of the precipitated complex, which should indeed have an unfavorable ΔS° . The addition of α -cyclodextrin to a lipophorin solution is followed instantly by the formation of the precipitated inclusion complex, which would also be consistent with the concept that the driving force for this process is the formation of the precipitate.

Previous studies have employed different techniques for the preparation of diacylglycerol depleted lipophorin. The *in vitro* incubation of insect hemolymph lipid transfer particle (LTP), *M. sexta* lipophorin, and human low density lipoprotein resulted in the formation of a stable diacylglycerol-depleted very high density lipophorin species (15). This is a laborious method to prepare diacylglycerol-depleted very high density lipophorin because it requires the purification of LTP and the isolation of reaction products. Another technique used to manipulate the diacylglycerol content of lipophorin takes advantage of the actions of phospholipase

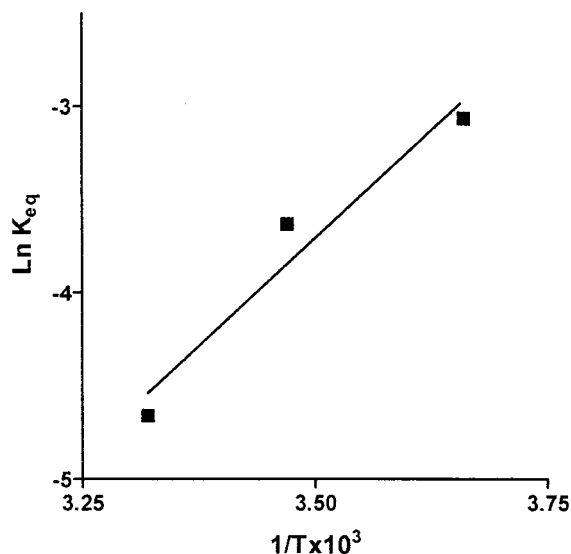


Fig. 5. Van't Hoff plot of the data in Table 2. The slope of the line is 4600 ± 1100 and the Y intercept is -20 ± 3.8 and the correlation coefficient (r^2) = 0.95. These numbers translate into $\Delta H^{\circ} = -38.2 \pm 9.1$ kJ/mol and $\Delta S^{\circ} = -166 \pm 31.6$ J/ $^{\circ}$ mol.

A_2 or triacylglycerol lipase on lipophorin (16). The method resulted in contaminated products and necessitated an additional purification step. Thus, it is clear that α -cyclodextrin is a superior tool to manipulate diacylglycerol content of lipophorin.

Model for the α -cyclodextrin–diacylglycerol complex

Quantitative analysis of the α -cyclodextrin–diacylglycerol complex indicated the presence of a molar ratio of α -cyclodextrin:diacylglycerol of 3.3 ± 1.3 ($n = 10$). To address whether a chemically reasonable, water-soluble complex could form between α -cyclodextrin and diacylglycerol, we examined possible arrangements using computer modeling. After modeling and energy minimization to relieve bad contacts, a model was found with diacylglycerol completely surrounded by, and completely filling, three cyclodextrin rings (Fig. 6), which is consistent with the experimental results obtained. The computer-modeling complex has no interior cavities, and no disfavored contacts be-

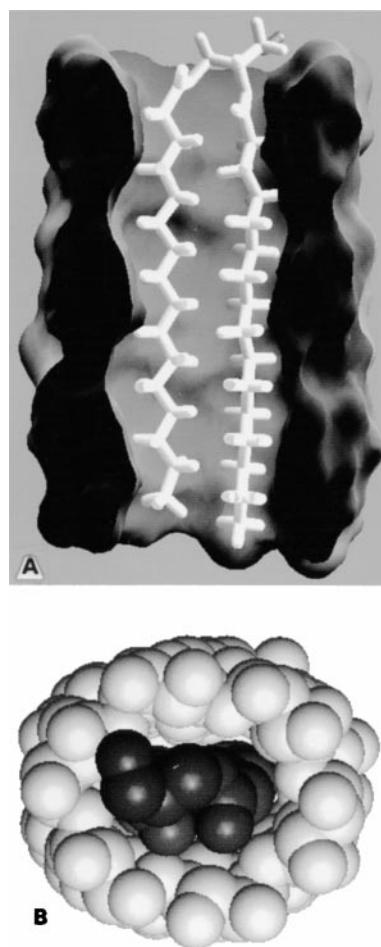


Fig. 6. Model of the α -cyclodextrin–diacylglycerol complex. A: Side view of the complex with the diacylglycerol shown as a stick model and the α -cyclodextrin is represented by a sliced-through solid surface. The figure was made with program GRASP (20). B: End-on view with all non-hydrogen atoms of the complex shown as black spheres for diacylglycerols and white spheres for cyclodextrin. The figure was made with the program Insight II.

tween the rings and the lipid moiety. Numerous potential hydrogen bonds exist between the dextrin rings even though these distances were not restrained during the minimization. Hydrophilic groups, suggesting the units would be water-soluble, dominate the exterior surface. The model provides an attractive molecular explanation for the ratio of α -cyclodextrin:diacylglycerol found in the isolated complex and deduced from the data in Fig. 6. This model is characterized by oval, torous-shaped α -cyclodextrin molecules with radii $13 \times 17 \text{ \AA}$ with interior cavity dimensions of $4 \times 7 \text{ \AA}$. Three such molecules stacked on top of each other would produce a cylinder 23 \AA long. Such a cylinder could just accommodate a single molecule of diacylglycerol, which is about 20 \AA long. When DPG is embedded in the hydrophobic cavity of the cylinder, the entire complex is 27 \AA long, which includes the hydrophilic head of DPG.

The reason the complex precipitates is less obvious. One possible explanation is that the individual cyclodextrin/lipid units could stack end-to-end through hydrogen bonding between cyclodextrin rings. Such a mechanism of hydrogen bond formation and precipitation would most likely be highly cooperative, as once the first hydrogen bond between complexes formed, the rest would be optimally aligned to form also. Additional hydrophobic surface area (from the ends of the complexes) might also be buried in this arrangement resulting in the precipitation of the strands.


Mechanism of diacylglycerol transfer

There are three possible mechanisms by which lipids could be transferred from donors to acceptors: *i)* receptor-mediated transfer (17); *ii)* a collision mechanism (18); or *iii)* an aqueous diffusion mechanism (19). Receptor-mediated transfer of diacylglycerols to α -cyclodextrin does not apply because there are no receptors present. A collision mechanism is also unlikely to account for the transfer of diacylglycerols to α -cyclodextrins, because high ionic strength and high temperature usually favor collisional process, which is clearly not the case here.

Thus, the most likely mechanism by which this transfer occurs is via an aqueous diffusion pathway. Although the aqueous solubility of diacylglycerol is expected to be quite low, there will still be a finite amount of diacylglycerol in solution. The formation of the α -cyclodextrin–diacylglycerol complex leads to precipitation of the complex, which provides the driving force for extraction of diacylglycerol from lipophorin. Why this complex should be insoluble is not obvious, especially considering that a somewhat analogous complex between cholesterol and β -cyclodextrin is soluble.

Summary and conclusions

We have shown for the first time that α -cyclodextrin can remove diacylglycerol from lipoproteins. α -Cyclodextrin is an excellent tool to modify the composition of lipophorin. In vitro formation of functional particles that have the same protein and lipid composition as nascent lipophorin is extremely important, as their purification

from eggs is laborious. Furthermore, the formation of intermediate density lipophorins is of equal importance. In vivo, these lipophorin particles are metabolized so fast that they do not occur. Formation of these particles in vitro would give us the opportunity to study their stability, composition, structure, morphology, and the rate at which they are metabolized. Preliminary data indicate that α -cyclodextrin-treated lipophorin (diacylglycerol-depleted lipophorin) is a more efficient system to export diacylglycerol from fat body tissues of *M. sexta* than is diacylglycerol-loaded lipophorin (Z. E. Jouni and M. A. Wells, unpublished results). In the future, it will be interesting to determine whether conditions can be found in which diacylglycerol can be transferred from α -cyclodextrin to lipoproteins or membranes. 

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REFERENCES

1. Pitha, J., T. Irie, P. B. Sklar, and J. S. Nye. 1988. Drugs solubilizers to aid pharmacologists: amorphous cyclodextrin derivatives. *Life Sci.* **43**: 493–502.
2. Bender, M. L., and M. Komiyama. 1978. Cyclodextrin Chemistry. Springer-Verlag, Berlin.
3. Chino, H., and R. Downer. 1979. The role of diacylglycerol in absorption of dietary glyceride in American cockroach, *Periplaneta americana*. *Insect Biochem.* **9**: 379–382.
4. Soulages, J. L., and M. A. Wells. 1994. Lipophorin: the structure of an insect lipoprotein and its role in lipid transport in insects. *Adv. Protein Chem.* **45**: 371–415.
5. Prasad, S. V., R. O. Ryan, J. H. Law, and M. A. Wells. 1986. Changes in lipoprotein composition during larval-pupal metamorphosis of an insect, *Manduca sexta*. *J. Biol. Chem.* **261**: 558–562.
6. Soulages, J. L., R. R. van Antwerpen, and M. A. Wells. 1996. Role of diacylglycerol and apolipoprotein-III in regulation of the physicochemical properties of the lipophorin surface: metabolic implications. *Biochemistry.* **35**: 5191–5198.
7. Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of the bacteriophage T4. *Nature.* **227**: 680–685.
8. Holwerda, D. A., J. Van Doorn, and A. M. T. Beenackers. 1977. Characterization of the adipokinetic and hyperglycaemic substances from the locust *Corpus cardiacum*. *Insect Biochem.* **7**: 151–157.
9. Tsuchida, K., and M. A. Wells. 1988. Digestion, absorption, transport and storage of fat during the last larval stadium of *Manduca sexta*. Changes in the role of lipophorin in the delivery of dietary lipid to the fat body. *Insect Biochem.* **18**: 263–268.
10. Van Handel, E. 1965. Estimation of glycogen in small amounts of tissue. *Anal. Biochem.* **11**: 256–265.
11. Han, G. W., J. R. Ruble, and B. M. Carven. 1994. The crystal structure of 1,2 dipalmitoyl-*sn*-glycerol at 123K. *Chem. Phys. Lipids.* **71**: 219–228.
12. Mikami, B., M. Sato, T. Shibata, M. Hirose, S. Aibara, Y. Katsube, and Y. Morita. 1992. Three-dimensional structure of soybean beta-amylase determined at 3.0 angstroms resolution: preliminary chain tracing of the complex with alpha-cyclodextrin. *J. Biochem.* **112**: 541–546.
13. Markwell, M. A. K., S. M. Hass, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87**: 206–210.
14. Ohtani, Y., T. Irie, K. Uekama, and K. Fukunaga. 1989. Differential effects of α -, β -, and γ -cyclodextrins on human erythrocytes. *Eur. J. Biochem.* **186**: 17–22.

15. Ryan, R. O., A. N. Wessler, H. M. Price, S. Ando, and S. Yokoyama. 1990. Insect lipid transfer particle catalyzes bidirectional vectorial transfer of diacylglycerol from lipophorin to human low density lipoprotein. *J. Biol. Chem.* **265**: 10551–10555.
16. Kawooya, J. K., D. J. van der Horst, M. C. van Heusden, B. L. J. Brigot, R. van Antwerpen, and J. H. Law. 1991. Lipophorin structure analyzed by in vitro treatment with lipases. *J. Lipid Res.* **32**: 1781–1788.
17. Oram, J. F. 1983. The effects of HDL subfractions on cholesterol homeostasis in human fibroblasts and arterial muscle cells. *Arteriosclerosis*. **3**: 420–432.
18. Steck, T. L., F. J. Kezdy, and T. Lange. 1988. An activation-collision mechanism for cholesterol transfer between membranes. *J. Biol. Chem.* **263**: 13023–13031.
19. Johnson, W. J., F. H. Mahlberg, G. H. Rothblat, and M. C. Phillips. 1991. Cholesterol transport between cells and high-density lipoproteins. *Biochim. Biophys. Acta.* **1085**: 273–298.
20. Nichols, A., R. Bharadwaj, and B. Honig. 1993. GRASP—graphical representation and analysis of surface properties. *Biophys. J.* **64**: A166.