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β -Cyclodextrin facilitates cholesterol efflux from larval *Manduca sexta* fat body and midgut in vitro

Zeina E. Jouni*, Brandon McGill, Michael A. Wells

Department of Biochemistry and Molecular Biophysics and Center for Insect Science Biological Sciences West, P.O. Box 210 066,
The University of Arizona, Tucson, AZ 85721-0088, USA

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Abstract

The ability of 2-hydroxypropyl- β -cyclodextrin (HP β CD) and methyl- β -cyclodextrin (M β CD) to promote cholesterol efflux from [³H]cholesterol-labeled larval *Manduca sexta* fat body and midgut was tested. In fat body, both β -cyclodextrins induced a two-phase efflux of cholesterol. The first rapid phase depended on cyclodextrin concentration and was more rapid for M β CD than for HP β CD. The second, slower, phase was independent of cyclodextrin concentration and type. In midgut, only the concentration-dependent phase was observed; the rate constants are approximately 85% slower than for fat body. In both cases, a low activation energy for transfer was observed, consistent with a collision mechanism where cyclodextrin interacts directly with cholesterol in plasma membrane to affect transfer. In fat body, the second slower phase is suggestive of a second pool of exchangeable cholesterol and most likely represents transfer of cholesterol from internal membranes or different lateral domains of the plasma membrane. The lack of this second phase in midgut suggests that midgut has only a single pool of exchangeable cholesterol. Although the rates are somewhat different, the overall kinetic pattern for cyclodextrin-mediated cholesterol transfer in insect fat body closely resembles that for vertebrate cells, while the single pool behavior of the midgut is not found in vertebrate cells. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Cyclodextrins; Cholesterol; Fat body; Midgut; Efflux; *M. sexta*; Kinetic pools; Collision mechanism

1. Introduction

Cholesterol is the major sterol found in insects. Unlike vertebrates, insects are unable to synthesize cholesterol de novo, thus requiring a dietary sterol source. Cholesterol in insects is of physiological importance because it supports normal development and reproduction (Svoboda, 1999), serves as a structural component of cell membranes and as the precursor of the insect molting hormones, ecdysteroids (Grieneisen, 1994; Svoboda, 1999).

Abbreviations: 2-Hydroxypropyl- β -cyclodextrin (HP β CD); Methyl- β -cyclodextrin (M β CD).

*Corresponding author. Tel.: +1-520-621-1772; fax: +1-520-621-9288.

E-mail address: eljouniz@email.arizona.edu (Z.E. Jouni).

However, cholesterol itself is not a normal dietary sterol for phytophagous insects that consume a wide variety of phytosterols. Once absorbed phytosterols may be unchanged or converted to cholesterol or other sterols in the midgut cells (reviewed by Canavoso et al., 2001). Then they are released to lipophorin, the major lipoprotein circulating in the hemolymph (Chino and Gilbert, 1971; Jouni et al., 2002b), and are distributed to different tissues for utilization or storage in free or esterified forms (Jouni et al., 2002b). Recently, we have demonstrated that cholesterol transfer from midgut to lipophorin, from lipophorin to fat body (Yun et al., 2002) and from fat body to lipophorin (Jouni et al., 2002a) takes place primarily by a simple aqueous diffusion mechanism.

Cyclodextrins are cyclic oligosaccharides with different numbers of glucose units α -(6), β -(7) and γ -(8), and consequently possess different sized hydrophobic cavities that can accommodate non-polar molecules (Pitha et al., 1988). Cyclodextrins have been explored as tools to characterize the mechanisms by which lipophilic compounds are transferred to aqueous media (Yancey et al., 1996; Rothblat et al., 1999). This mechanism involves the collision of the acceptor particles, cyclodextrin, with the membrane, followed by the transfer of cholesterol to the acceptor (Steck et al., 1988). Exposure of mammalian cells to high concentrations of β -cyclodextrins results in cholesterol transfer rates that were more rapid than those attained with high density lipoprotein, the physiological cholesterol acceptor. The efficiency by which β -cyclodextrins mediated cholesterol transfer is related to their ability to reduce the activation energy for cholesterol incorporation into their hydrophobic cavity (Kilsdonk et al., 1995; Christian et al., 1997). Furthermore, the use of β -cyclodextrins for cholesterol transfer studies from mammalian cells demonstrated the presence of at least two kinetically distinct pools (Yancey et al., 1996). The aim of this work was to characterize cholesterol transfer kinetics in *M. sexta* fat body and midgut using β -cyclodextrins and to compare the results to those obtained with vertebrate cells.

2. Materials and methods

2.1. Materials

α -Cyclodextrin (α CD), 2-hydroxypropyl- α -cyclodextrin (HP α CD), methyl- β -cyclodextrin (M β CD), 2-hydroxypropyl- β -cyclodextrin (HP β CD), phenylmethylsulfonyl fluoride, benzamidine hydrochloride, diisopropylfluorophosphate and Grace's medium were purchased from Sigma (St. Louis, MO, USA). Falcon multi-well tissue culture plates and cell strainers were obtained from Becton Dickinson (Franklin Lakes, NJ, USA). (1,2*n*)-[3 H]cholesterol (sp. Act.=52 Ci/mmol) was purchased from Amersham Pharmacia Inc. (Piscataway, NJ, USA).

2.2. Animals

M. sexta were reared at 25–27 °C on an artificial diet prepared from wheat germ (Prasad et al., 1986). Second day fifth instar larvae (wt.=3.1–3.2 g) were fasted for 10 min then fed a small piece of diet containing 4 μ Ci of [3 H]cholesterol. Following the consumption of the labeled diet, the animals were placed on an unlabeled diet. Twenty-four hours later the fat body or midgut was dissected under cold PBS buffer (50 mM sodium phosphate, 150 mM NaCl, pH 6.5, containing 1

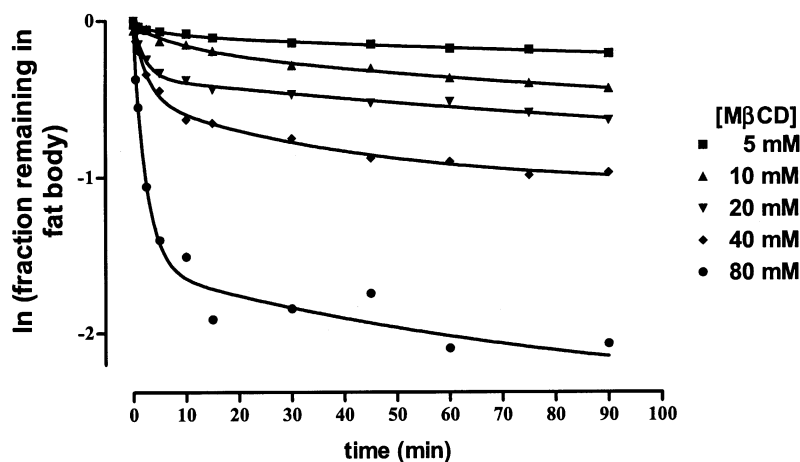


Fig. 1. Kinetics of transfer of [3 H]cholesterol from fat body to M β CD. The ln of the fraction of the initial [3 H]cholesterol remaining in the fat body is plotted as a function of time using M β CD concentrations ranging from 5 to 80 mM. At the indicated time intervals, 50 μ l of the incubation medium was used to measure the radioactivity (refer to the Experimental section for more details). Values represent averages of three determinations. The lines are polynomials and are included solely to make the different curves clear.

mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM benzamidine and 0.1 mM diisopropylfluorophosphate), washed twice with Grace's medium then used in the transfer studies.

2.3. Cholesterol transfer studies

All incubations were carried out on an orbital shaker at 45 rev./min and 28 °C unless otherwise specified. [³H]Cholesterol-labeled fat body or midgut was incubated in Grace's medium with or without the addition of cyclodextrins. At the indicated time intervals, a 50 μ l aliquot of the medium was mixed with 100 μ l of PBS buffer containing 2 mg/ml of bovine serum albumin (BSA) and centrifuged at 10 000 \times g for 10 min to remove any detached cells. Aliquots of the supernatant solution were counted for radioactivity. At the end of the incubation, the fat body or midgut was separated from the incubation medium by filtration using a cell strainer, washed three times with PBS and lysed in 2 ml of 0.1 N NaOH. Aliquots were used for the determination of protein concentrations and for radioactivity counting using a scintillation counter. Trypan blue (0.2%) exclusion tests performed during the incubation time showed that tissue integrity and cellular viability were maintained.

To test for the existence of more than one pool of cholesterol in fat body, four sets of

[³H]cholesterol-labeled fat bodies were preincubated in Grace's medium containing 40 mM HP β CD for 10 min to deplete labeled cholesterol from the rapidly exchanging pool. For one set of fat bodies transfer studies were continued as described above (control). In the other three sets, the tissues were washed three times with Grace's medium and incubated in fresh Grace's medium without HP β CD for 15, 30 and 60 min to allow for the movement of cholesterol from the slowly exchanging pool to the rapidly exchanging pool. Then the tissues were transferred to medium supplemented with 40 mM HP β CD to start a new transfer study.

2.4. Effect of temperature on cholesterol transfer

To determine the effect of temperature on transfer studies, fat body or midgut tissues and stock solutions of HP β CD were pre-equilibrated at temperatures ranging from 4 to 42 °C, and then mixed to start the experiment. At the indicated time, cholesterol transfer to 40 mM HP β CD was carried out as described above.

2.5. Influx studies

HP β CD or M β CD labeled with [³H]cholesterol were prepared by incubating the β -cyclodextrins with [³H]cholesterol-labeled fat body for 15 min. Radioactive cyclodextrins were isolated from tis-

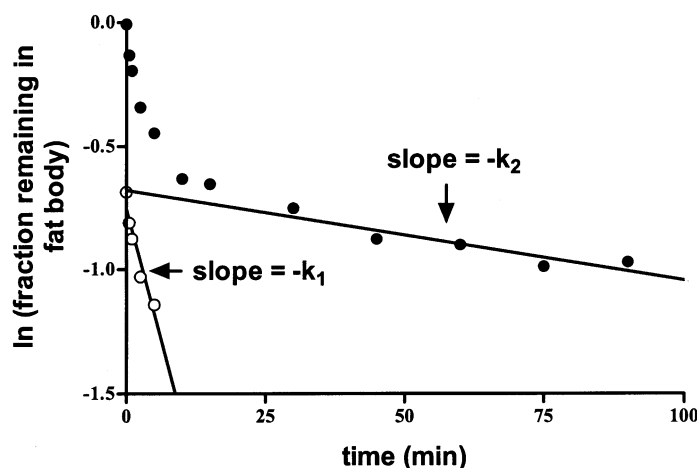


Fig. 2. Analysis of kinetic data. In this example for 40 mM M β CD, we illustrate the graphical method used to characterize the two phases of the transfer reaction. First a linear least squares analysis of the data at long times (>25 min) allows determination of k_2 and F_2 from the slope and intercept, respectively. The equation for this line is then used to calculate the contribution of the slow reaction to the transfer at shorter times and the results subtracted from the observed data. Plotting the corrected data (open circles) and using linear least squares analysis allows determination of k_1 and F_1 from the slope and intercept, respectively.

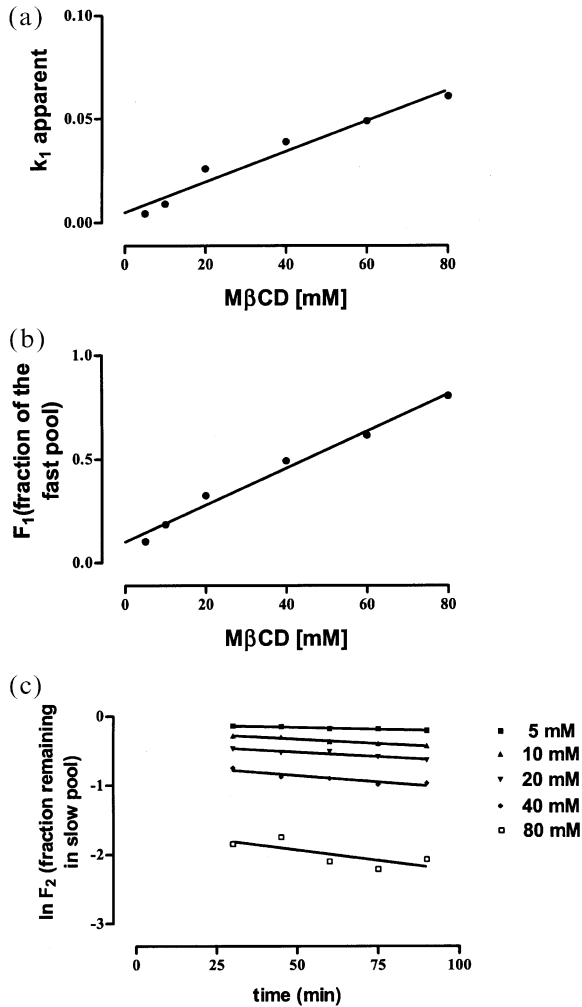


Fig. 3. Dependence of k_1 and F_1 on M β CD concentration. The apparent values of k_1 (a) and F_1 (b) derived from analysis of the data in Fig. 1 and plotted vs. the concentration of M β CD. (c) Plots of the $\ln F_2$ for the slow pool vs. incubation time. The lines represent linear least squares analyses of the data. The slope of the k_1 plot gives the true value of k_1 (see Table 1).

sues by filtration using a cell strainer and centrifuged at $10\,000\times g$ for 10 min to remove any detached cells. Labeled β -cyclodextrin was incubated with fat body for 90 min on an orbital shaker at 28 °C and the amount of radioactive cholesterol remaining in the medium was determined at different time intervals following the procedure described above. At the end of the incubation, the fat body was separated from the incubation medium washed and homogenized. Aliquots were used for the determination of protein concentrations and

for radioactivity counting using a scintillation counter.

2.6. Data analysis

Efflux studies are reported as the fraction of the initial [^3H]cholesterol remaining in the fat body or midgut as a function of time. Usually, experiments using β -cyclodextrins at high concentrations reveal at least two kinetically distinct pools of exchanging cholesterol in vertebrate cells (Rothblat et al., 1999). For that reason we chose to analyze the efflux data assuming that two parallel first-order reactions are involved.

1. The first reaction involves transfer of cholesterol from the plasma membrane to the cyclodextrin: $\text{CD} + \text{PM-C}^* \rightarrow \text{CD-C}^* + \text{PM}$; where CD = cyclodextrin; PM = plasma membrane; and C* = labeled cholesterol. This reaction would be characterized by the rate equation $v = k_1 [\text{CD}][\text{PM-C}^*]$. Because the concentration of CD is high, we assume it does not change significantly during the reaction and that this rate equation reduces to $v = k_{\text{app}}[\text{PM-C}^*]$, where $k_{\text{app}} = k_1[\text{CD}]$.
2. The second reaction involves the transfer of cholesterol from internal membranes to the plasma membrane and is characterized by the rate constant k_2 .

This simplification allows us to write the overall rate equation as $F = F_1e^{-k_1t} + F_2e^{-k_2t}$ (Yancey et al., 1996), where F_1 and F_2 represent the fraction of the reaction occurring by the fast and slow processes, respectively. A plot of $\ln F$ vs. t gives a curve whose asymptote at long time has a slope = $-k_2$. The Y intercept of this asymptote is F_2 . Using the equation of this asymptote one can calculate the contribution of the slow reaction to the transfer at each time (F_{2t}). Then a plot of $\ln(F - F_{2t})$ will yield a straight line of slope = $-k_1$. The validity of this approach is shown in Fig. 2.

2.7. Other analyses

Protein concentrations were determined using the modified Lowry method with BSA as a standard (Markwell et al., 1978). Student's unpaired t -test was used to determine the significance of differences between means.

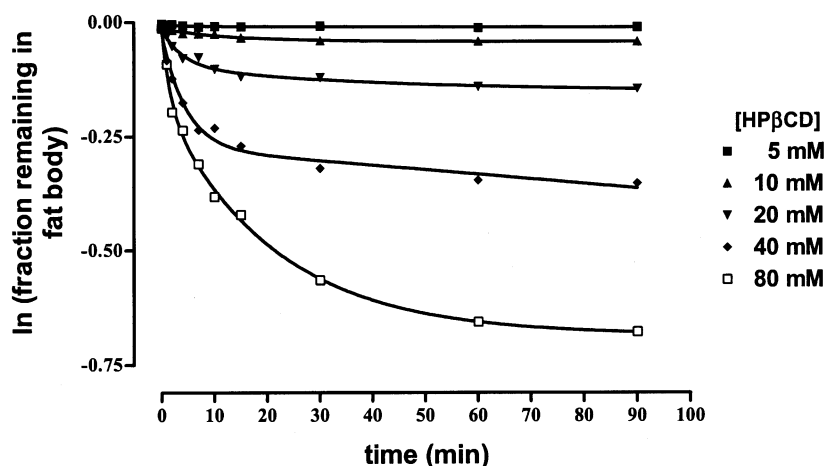


Fig. 4. Kinetics of [^3H]cholesterol transfer from fat body to HP β CD. The \ln of the fraction of the initial [^3H]cholesterol remaining in the fat body is plotted as a function of time using HP β CD concentrations ranging from 5 to 80 mM. At the indicated time intervals, 50 μl of the incubation medium was used to measure the radioactivity (refer to Experimental section for more details). Values represent averages of three determinations. The lines are polynomials and included are solely to make the different curves clear.

3. Results and discussion

3.1. Cholesterol transfer from fat body to β -cyclodextrins

Data for the time course and concentration dependence of cholesterol transfer from fat body to M β CD are shown in Fig. 1. M β CD stimulated a significant release of cholesterol from fat body. The fraction of radiolabeled-cholesterol transferred to the media increased with increasing M β CD concentration (5–80 mM) and time (0–90 min). Cholesterol transfer was biphasic involving an initial rapid phase where the majority of cholesterol was transferred to the cyclodextrin, followed by a slow phase. In Fig. 1, $\ln F$ is plotted as a function of time and the fact that the plot is not linear suggests that two parallel first-order reactions are involved. The first reaction involves transfer of cholesterol from the plasma membrane to cyclodextrin and is characterized by the apparent rate constant k_1 . The second reaction involves the transfer of cholesterol from internal membranes or from different lateral domains of the plasma membrane. This pool is characterized by the apparent rate constant k_2 .

In order to test the hypothesis that two first-order reactions are involved in the transfer of cholesterol from fat body to β -cyclodextrin, each set of the time course data was analyzed as shown

in details in Fig. 2. This plot clearly demonstrates the presence of two processes and that cholesterol is being transferred from two distinct (fast and slow) pools. Using β -cyclodextrins, Yancey et al. (1996) have demonstrated that in several vertebrate cell types and in large unilamellar cholesterol-containing vesicles, cholesterol is transferred from two kinetically distinct pools.

Further analyses of the data illustrated that the fraction of the cholesterol transferred from the fast pool (F_1) and the apparent rate constant (k_1) are both linearly dependent on the concentration of M β CD used in the experiments ($r_2 > 0.96$, Fig. 3a,b). In contrast, the fraction of cholesterol transferred from the slow pool (F_2) and its apparent rate constant (k_2) are independent of M β CD concentrations (similar slope = $-k_2$, $r_2 > 0.87$, Fig. 3c).

Similarly, the transfer of cholesterol from [^3H]cholesterol-labeled fat body to HP β CD increased with increasing the concentration of HP β CD and time (Fig. 4). At 80 mM concentration of HP β CD, approximately 25% of the initial labeled fat body cholesterol was released to the medium. Cholesterol transfer exhibited typical biphasic decay curves, also demonstrating the presence of two kinetically distinct cholesterol pools. Further analysis of these data revealed that both the apparent rate constant (k_1 apparent) and the fraction of cholesterol released from the fast pool

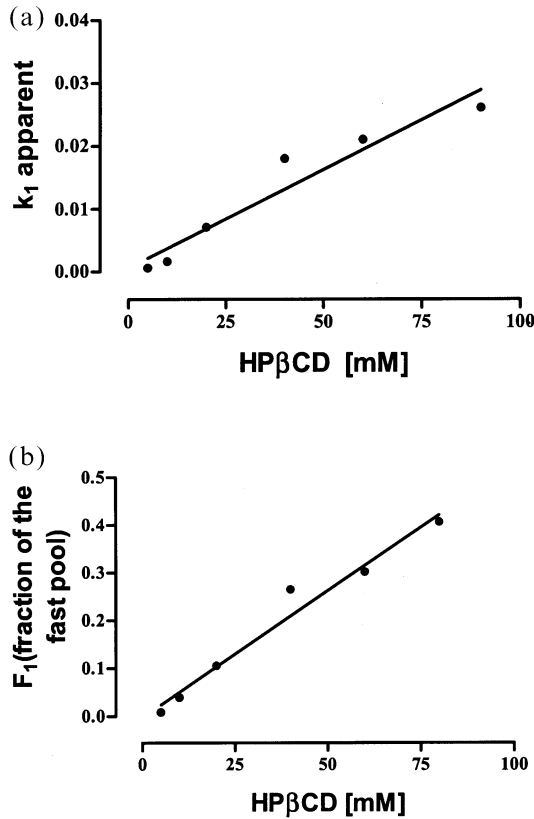


Fig. 5. Dependence of k_1 and F_1 on HPβCD concentration. The apparent values of k_1 (a) and F_1 (b) derived from analysis of the data in Fig. 4 and plotted vs. the concentration of HPβCD. The lines represent linear least squares analyses of the data. The slope of the k_1 plot gives the true value of k_1 (see Table 1).

are linearly dependent on HPβCD concentration (Fig. 5a,b), whereas F_2 and k_2 are not dependent on HPβCD concentration.

The values of k_1 and k_2 for the two pools derived from analyses of the entire data set for MβCD and HPβCD are presented in Table 1. The transfer of cholesterol to MβCD is more rapid than to HPβCD ($P < 0.002$). In the presence of equal molar amounts of MβCD and HPβCD, more cholesterol was transferred to MβCD than to HPβCD during the initial rapid phase. This is reflected by the larger values of F_1 for MβCD than for HPβCD (Figs. 3 and 5). The apparent transfer rate constants for the slow phase (k_2) are not significantly different for MβCD or HPβCD, indicating that the slow pool is independent of the type and concentration of β-cyclodextrin. The biphasic pattern observed is not unique to insect tissues, as many reports using mammalian cells as donors and β-cyclodextrins as the cholesterol acceptors showed the same biphasic pattern of efflux (Rothblat et al., 1999).

3.2. Cholesterol pools of *M. sexta* fat body

In order to confirm the presence of two pools of exchangeable cholesterol, [^3H]cholesterol-labeled fat body was incubated with 40 mM HPβCD to significantly deplete the fast pool. After 10 min, the tissues were washed to remove HPβCD, and then incubated with Grace's media alone for various times to allow replenishment of the fast pool by movement of [^3H]cholesterol from the slow pool to the fast pool. Finally, the tissues were transferred to new incubation medium containing fresh HPβCD and then new exchange kinetics data were measured (Fig. 6). The extent of cholesterol efflux during the fast phase increased with increasing replenishment time, demonstrating that cholesterol indeed had moved from

Table 1

Rate constants associated with cholesterol transfer from *M. sexta* fat body or midgut to cyclodextrins

Tissue	Rate constant	MβCD	HPβCD
Fat body	k_1 ($\text{min}^{-1} \mu\text{M}^{-1}$)	$0.74 \pm 0.04^{\text{a,d}}$	$0.31 \pm 0.04^{\text{a}}$
	k_2 (min^{-1})	$0.0023 \pm 0.009^{\text{b}}$	$0.0018 \pm 0.0003^{\text{b}}$
Midgut	k_1 ($\text{min}^{-1} \mu\text{M}^{-1}$)	$0.11 \pm 0.02^{\text{c,d}}$	$0.044 \pm 0.02^{\text{c}}$
	k_2 (min^{-1})	Too slow to measure	Too slow to measure

^a Significantly different, $P < 0.002$.

^b Not significantly different, $P > 0.1$.

^c Not significantly different, $P > 0.08$.

^d Significantly different, $P < 0.0001$.

[^3H]Cholesterol-labeled fat body or midgut was incubated with increasing concentrations (5–80 mM) of MβCD or HPβCD. At different time intervals, 50 μl of the incubation medium was used to measure the radioactivity transferred to the cyclodextrins. The rate constants correspond to the rate equation $F = F_1 e^{-k_1 t} + F_2 e^{-k_2 t}$ used in Figs. 3, 5 and 7. Values represent averages \pm S.E.M. for three to six determinations.

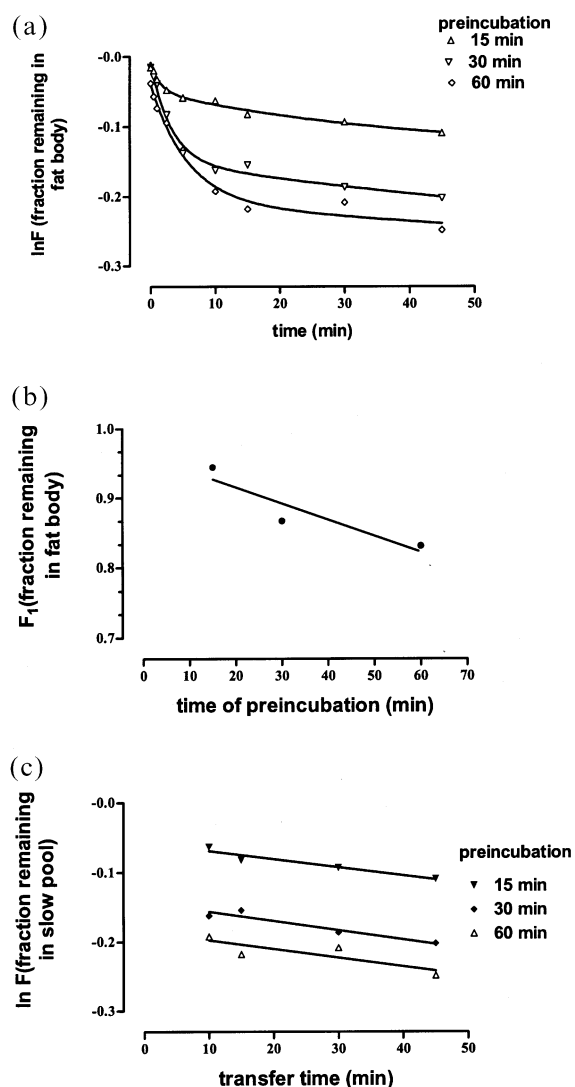


Fig. 6. Evidence for two pools of cholesterol in fat body. Fat body was incubated with 40 mM HP β CD for 10 min, then washed free of HP β CD and incubated in cyclodextrin-free media for 15, 30 or 60 min—the recovery time. Then fresh HP β CD (40 mM) was added and efflux kinetics determined as in Fig. 3. (a) Plots of the $\ln F$ for the two pools vs. time. (b) Plots of F_1 vs. recovery time min (time of preincubation), showing that the fast pool is replenished during recovery. (c) The \ln of the fraction of cholesterol transferred from the slow pool (F_2) for each recovery phase vs. transfer time min. The slopes of the lines are k_2 , which average $0.0016 \pm 0.0007 \text{ min}^{-1}$ and are not significantly different from the data presented in Table 1 ($P > 0.25$).

the slow pool to the fast pool (Fig. 6a). After 15 and 60 min of equilibrium, the size of the fast pool increased by only 5 and 16%, respectively, indicating that the movement of cholesterol from the slow pool to the fast pool is certainly slow,

since it requires several hours to completely replenish the fast pool from the slow pool (Fig. 6b). Furthermore, the rate of the slow reaction was the same as observed in the initial exchange kinetics: k_2 from initial kinetics = $0.0018 \pm 0.0003 \text{ min}^{-1}$ (Table 1); k_2 from the data in Fig. 6c = $0.0016 \pm 0.0007 \text{ min}^{-1}$, $P > 0.25$.

Our study supports the suggestion that although two kinetically distinct cholesterol pools exist, the two pools are in continuous communication and that the fast pool is being replenished from the slow pool. This observation is in agreement with our recent report that a cholesterol oxidase sensitive and accessible pool and a non-cholesterol oxidase accessible pool are present in the larval *M. sexta* fat body (Jouni et al., 2002a). It is worth mentioning that the rate constant for cholesterol efflux in the presence of 80 mM M β CD is more than 400-fold greater than that observed when lipophorin was used as a cholesterol acceptor, suggesting two different mechanisms are involved in these transfer processes.

Our data do not identify the location of the two pools. As β -cyclodextrin was able to accentuate the fast pool, it is conceivable that the location of this pool is in the plasma membrane. In mammalian systems, cholesterol released from the fast pool has been shown to be of plasma membrane origin. A recent report by Haynes et al. (2000) has demonstrated that all of the cholesterol in the fast pool and the majority of the cholesterol in the slow pool are apparently present in plasma membranes. In plasma membranes cholesterol is distributed in the inner and outer leaflet and laterally separated domains of the membrane such as caveolae which gives rise to different efflux rates. All of these locations could give rise to different pools. Regardless of the location of the pools, it is evident that cholesterol within the slow pool can exchange with the fast pool.

3.3. Cholesterol transfer from midgut to β -cyclodextrins

In contrast to the fat body, a single-second order process characterized cholesterol transfer from midgut of *M. sexta* to β -cyclodextrins, and no evidence for a slower second phase was found (Fig. 7a,b). The efflux-stimulating potential of M β CD was greater than HP β CD, demonstrating that M β CD is a better cholesterol acceptor than HP β CD. A plot of the slopes of the lines shown in Fig. 7 (data not shown), as described in Figs.

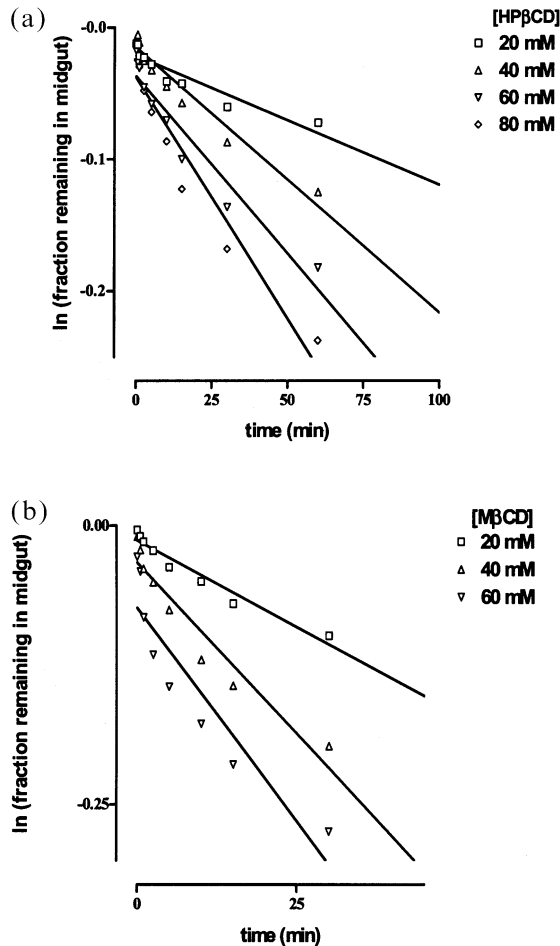


Fig. 7. Kinetics of transfer of [^3H]cholesterol from midgut to HP β CD or M β CD. The \ln of the fraction of the initial [^3H]cholesterol remaining in the midgut is plotted as a function of time using HP β CD (upper panel) or M β CD (lower panel) at concentrations ranging from 20 to 80 mM. At the indicated time intervals, 50 μl of the incubation medium was used to measure the radioactivity (refer to Experimental section for more details). Values represent averages of three determinations. The data were best fitted on linear regression equation and the calculated values of k_1 are shown in Table 1.

2 and 3, yielded second-order rates constants (k_1) of 0.11 ± 0.02 and $0.044 \pm 0.02 \mu\text{M}^{-1} \text{min}^{-1}$ for cholesterol transfer to M β CD and HP β CD, respectively (Table 1). These rates are only approximately 15% of the rates observed with fat body. Although, in mammalian systems, the cell-dependent differences between cell types exposed to phospholipid-containing acceptors are not apparent when cyclodextrins are used as cholesterol acceptors (Rothblat et al., 1999), cyclodextrins were not

able to eliminate the differences observed in *M. sexta* midgut and fat body. At this time, we do not know why cholesterol transfer from midgut behaves differently than fat body, but it is conceivable that the differences could be the result of lipid/protein membrane distribution that gives these tissues their unique functions in *M. sexta*. More studies are needed to explore this hypothesis.

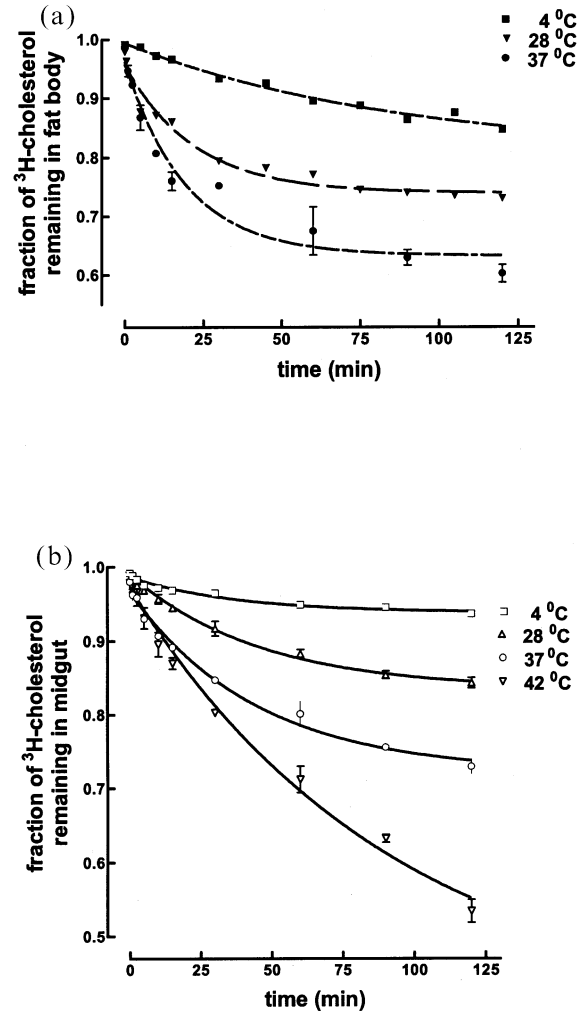


Fig. 8. Effect of temperature on the transfer of cholesterol from fat body and midgut: [a]: [^3H]Cholesterol labeled fat body tissues were incubated in Grace's medium containing 40 mM HP β CD at temperatures ranging from 4 to 37 $^{\circ}\text{C}$. [b]: [^3H]Cholesterol labeled midgut tissues were incubated in Grace's medium containing 40 mM HP β CD at temperatures ranging from 4 to 42 $^{\circ}\text{C}$. At the indicated time intervals, aliquots were used for radioactivity counting (refer to Experimental section for more details). Values represent averages \pm S.E. of 3–4 determinations.

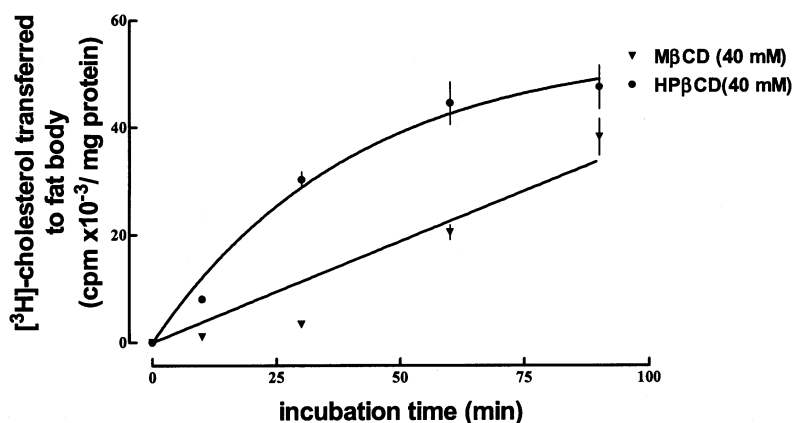


Fig. 9. Influx of [³H]cholesterol labeled-β-cyclodextrins to fat body. Fat body tissues from *M. sexta* were incubated in Grace's medium containing 40 mM of either [³H]cholesterol-labeled HPβCD (●) or [³H]cholesterol-labeled MβCD (▼) for 90 min. Cholesterol influx to fat body was determined at the indicated time intervals. Data represent averages of four determinations ± S.E.M.

3.4. Effect of temperature on cholesterol transfer

Cholesterol transfer to HPβCD was strongly temperature-dependent (Fig. 8). At 4 °C, minimal cholesterol transfer from fat body (Fig. 8a) and midgut (Fig. 8b) was observed. Using the Arrhenius equation, we calculated the activation energy for cholesterol transfer of 5.5 ± 1.3 kJ/mol from the fat body and 11.8 ± 1.3 kJ/mol from midgut. These low values are consistent with the model of collision mechanism in which the cholesterol molecule does not completely desorb from the membrane before entering the hydrophobic cavity of the cyclodextrin. Whereas, in aqueous diffusion mechanism complete desorption of cholesterol from the membrane into aqueous environment requires more energy and is characterized by an activation energy of approximately 85 kJ/mol (Yancey et al., 1996). The efficiency of an acceptor in promoting cholesterol efflux depends on its ability to diffuse through the unstirred water layer and the cell glycocalyx, and to concentrate at the cell surface (Phillips et al., 1987; Davidson et al., 1995). The closer the acceptor can come to the membrane, the shorter distance cholesterol will have to diffuse through water in order to reach the acceptor, which will greatly reduce the activation energy of the process. In vertebrate systems transfer of cholesterol to cyclodextrins are characterized by activation energies that are less than one-half of the activation energies for transfer to bulkier lipoprotein (Rothblat et al., 1999). These considerations also apply to the insect cells, because the

$t_{1/2}$ for cholesterol efflux from fat body to large acceptor particles such as lipophorin of *M. sexta* is greater than 2 h (Jouni et al., 2002a), compared to less than 10 min for MβCD at 80 mM.

We have recently reported that transfer of cholesterol from fat body to lipophorin is strongly pH-dependent with an optimum pH at 6.2, where the majority of cholesterol is transferred to lipophorin (Jouni et al., 2002a). In contrast, pH of the incubation medium had no effect on cholesterol transfer to either HPβCD or MβCD (data not shown). This is consistent with the fact that the cyclodextrins are uncharged compounds, whereas lipophorin does carry a net charge. This effect further supports the collision mechanism which is unaffected by pH (Yancey et al. 1996).

3.5. Transfer of cholesterol from β-cyclodextrins to fat body

To test the ability of cyclodextrins to release cholesterol to the fat body, influx studies were carried out using [³H]cholesterol-labeled cyclodextrins (Fig. 9). At equal molar concentration (40 mM), HPβCD transferred cholesterol more rapidly to fat body than MβCD. This is in accord with the fact that MβCD has a higher affinity for cholesterol than HPβCD, as determined from cholesterol transfer studies from fat body to cyclodextrins.

3.6. Summary

Comparison of our data-to-data for vertebrate cells (Rothblat et al., 1999) should be undertaken

with caution because our studies were carried with intact tissue. Even though larval fat body is a sheet of cells, two cells thick (Dean et al., 1985), penetration of the cyclodextrin to the surface of all cells might not be uniform. The basement membrane of the midgut might also provide a barrier. This observation is supported by the fact that adequate shaking of the incubation media, which facilitates diffusion of the acceptor to the donor membrane, was required at all β -cyclodextrin concentrations used (data not shown). Despite these differences, there are some interesting similarities exist between our results and those reported for mammalian cells. As is the case in vertebrate cells, M β CD was more effective in removing cholesterol from insect fat body than HP β CD. In vertebrate cells the half time of the fast reaction was approximately 20 s in the presence of 80 mM HP β CD (Rothblat et al., 1999), whereas for insect fat body it is approximately 20 min and for midgut approximately 280 min. The slow reaction had a half time of 15–35 min in vertebrate cells and approximately 400 min in insect fat body, and could not be observed in midgut. Although there are quantitative differences, qualitatively, these data suggest that the mechanism of cholesterol transfer, a collisional mechanism, between vertebrate or insect cells and cyclodextrins is similar. In such a mechanism the transfer occurs passively without the direct input of metabolic energy (Bell, 1984). Our preliminary data demonstrated that the electron transport chain inhibitor, azide, and the glycolytic inhibitor, fluoride, caused no inhibitory effects on the amount of cholesterol transferred to either HP β CD or M β CD (data not shown).

Not all cyclodextrins are good cholesterol acceptors from fat body or midgut, as no significant cholesterol transfer was obtained in the presence of M γ CD, HP γ CD, α CD and HP α CD at concentrations between 5 and 80 mM (data not shown). It is interesting to note that although α -cyclodextrin cannot accommodate cholesterol, it is a good diacylglycerol acceptor (Jouni et al., 2000).

β -Cyclodextrins are able to access the majority of cellular cholesterol in the fat body and midgut and significantly modify the cholesterol content of cells. This offers the interesting possibility of studying the role of cellular cholesterol in a cell system that cannot synthesize cholesterol de novo. Thus, one can predict that it should be possible to 'titrate' the cellular cholesterol content to a predetermined level in fat body and midgut and then

study a number of physiological properties of the system.

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