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Net Mass Transfer of Galactosylceramide Facilitated by Glycolipid Transfer Protein from Pig Brain: A Monolayer Study†

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ABSTRACT: A net mass transfer of galactosylceramide (GalCer) and galactosyldiacylglycerol (GalDG) is catalyzed by the glycolipid transfer protein from pig brain. GalCer and GalDG are transferred from a monolayer to phosphatidylcholine vesicles in the subphase or from a glycolipid monolayer to a phosphatidylcholine monolayer. No transfer of phosphatidylcholine is measured under these conditions. It is found that the glycolipid transfer protein functions as a carrier and that glycolipid is bound to less than 50% of the transfer protein. The presence of lipid-free proteins fits with the proposed mechanism of net mass transfer. The glycolipid transfer is influenced by the fluidity of the lipid interface and by the matrix lipid of the interface. GalCer transfer is stimulated in the presence of GalDG.

Proteins which facilitate the transfer of phospholipids between membranes in vitro have been widely found in various cells [for reviews, see Zilversmit et al. (1976), Kader (1977), and Akeroyd & Wirtz (1982)]. Phosphatidylcholine-specific phospholipid transfer protein, phosphatidylinositol transfer protein, and nonspecific lipid transfer protein have been purified. The mechanisms of lipid transfer facilitated by these proteins have been studied (Demel et al., 1984). The phosphatidylcholine transfer protein acts mainly as an exchange protein (Demel et al., 1973; Helmkamp et al., 1976) when both membranes contain phosphatidylcholine. Under certain conditions, a small percentage of net transfer can be found (Devaux et al., 1977; Wirtz et al., 1980).

For the phosphatidylinositol transfer protein, which binds primarily phosphatidylinositol but also phosphatidylcholine,

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transfer of phosphatidylinositol from a monolayer to a phosphatidylcholine vesicle has been demonstrated (Demel et al., 1977). However, no net mass transfer could be demonstrated since the phosphatidylinositol transfer is accompanied by phosphatidylcholine transfer in the reverse direction (Zborowsky & Demel, 1982). A glycolipid-specific lipid transfer protein was purified to apparent homogeneity from pig brain (Abe et al., 1982). The protein has a molecular weight of about 20000 and an isoelectric point of about 8.3. The protein facilitates the transfer of various sphingoglycolipids [such as galactosylceramide (GalCer), glucosylceramide, lactosylceramide, globotriaosylceramide, globotetraosylceramide, globopentaosylceramide, sialosyllactosylceramide, and sulfatide], various glyceroglycolipids [such as galactosyldiacylglycerol (GalDG), glucosyldiacylglycerol, digalactosyldiacylglycerol, and diglucosyldiacylglycerol], and 1-*O*-(β-D-galactosyl)-*N*-[10-(1-pyrenyl)-9-decenoyl]sphingosine (K. Yamada, A. Abe, and T. Sasaki, submitted for publication; Abe et al., 1984). The transfer of lipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, cholesterol, and cholesteryl oleate, is not at all facilitated by the transfer protein. Furthermore, the transfer of dimannosyldiacylglycerol is not facilitated by the protein. Moreover, the transfer of [^{3}H]GalCer and 1-*O*-(β-D-galactosyl)-*N*-[10-(1-pyrenyl)-9-decenoyl]sphingosine from donor liposomes to acceptor liposomes of phosphatidylcholine has been measured.

Since liposomes formed of nontransferable lipids can function as excellent acceptor membranes, it has been assumed that the protein most likely facilitates a net mass transfer of glycolipid molecules from donor to acceptor membrane. When the protein was incubated with phosphatidylcholine–cholesterol liposomes containing 3.5 mol % of [^{3}H]GalCer, it was found that 1 mol of the protein bound about 0.13 mol of [^{3}H]GalCer (A. Abe and T. Sasaki, submitted for publication). If we assume the glycolipid–protein complex as an intermediate of the transfer reaction, the presence of large portions of glycolipid transfer protein in a lipid-free state fits well with the proposed mechanism of net mass transfer of glycolipid molecules in which a lipid-free transfer protein is essential in the course of the transfer reaction.

In this paper, the mechanism of glycolipid transfer reaction facilitated by glycolipid transfer protein was studied by the use of monomolecular lipid film spread at the air–water interface. The net mass transfer of [^{14}C]GalCer from monolayer to either phosphatidylcholine vesicles or phosphatidylcholine monolayer has been demonstrated. Furthermore, results obtained during the course of the experiments on the transfer mechanism show that the GalCer transfer is influenced by the presence of other membrane lipids.

Materials and Methods

Soybean phosphatidyl[*methyl*-^{14}C]choline (specific activity 40 mCi/mmol) was obtained by the method of Stoffel et al. (1971). [1-^{14}C]Stearoylgalactosylsphingosine ([^{14}C]GalCer) was prepared by coupling [1-^{14}C]stearoyl chloride with 1-β-D-galactosylsphingosine (Sigma, St. Louis, MO) according to the method of Dubois et al. (1980). [1-^{14}C]Stearoyl chloride was prepared from [1-^{14}C]stearic acid (57.6 mCi/mmol) (Amersham, U.K.) and oxalyl chloride by the method of Okuyama et al. (1969). 3-(*O*-β-D-[6-^{3}H]Galactosyl)-*sn*-1,2-diacylglycerol (34 mCi/mmol) was prepared according to the method of Radin (1972) by sodium [^{3}H]borohydride (New England Nuclear, Boston, MA) reduction of the aldehyde, which was prepared by oxidation of galactosyldiacylglycerol with galactose oxidase (Sigma, St. Louis, MO). Galactosyldiacylglycerol was prepared from spinach leaves as described by Galliard (1974). Phosphatidylcholine was prepared from rat liver by the method described previously (Sasaki & Sakagami, 1978). Phosphatidylcholine from rat liver had the following fatty acid composition (in mass % where *n* represents the number of carbon atoms in the chain): 16:0, 14.3%; 18:0, 37.3%; 18:1 (*n* − 9), 2.8%; 18:2 (*n* − 6), 9.8%; 20:2 (*n* − 6), 1.6%; 20:3 (*n* − 6), 4.1%; 20:4 (*n* − 6), 18.6%; 22:3 (*n* − 3), 0.8%; 22:4 (*n* − 6), 3.9%; 22:6 (*n* − 6), 3.7%. GalCer containing non-hydroxy fatty acids was obtained from Sigma (St. Louis, MO). Trivial names of the glycolipids used were GalCer for galactosylceramide (Galβ1→1Cer) and GalDG for galactosyldiacylglycerol (3-[Galβ1-]-*sn*-1,2-diacylglycerol).

A clear vesicle suspension of egg phosphatidylcholine–4 mol % phosphatidic acid (lipid concentration 10 mM) is formed by a 1-min sonication of hand-shaken liposomes (de Kruijff et al., 1975).

Preparation of Glycolipid Transfer Protein. The protein was purified from pig brain by a modification (A. Abe and T. Sasaki, unpublished results) of the method described previously (Abe et al., 1982). The purified glycolipid transfer protein gave a single band with an estimated molecular weight of 22000 on gel electrophoresis in the presence of sodium dodecyl sulfate according to the method of Weber & Osborn (1969). Protein was determined by the method of Bensadoun & Weinstein (1976).

Interfacial Measurements. (*A*) *Transfer of Lipid from the Interface.* For the measurement of lipid transfer from the monolayer to vesicles in the subphase or the reverse transfer from vesicles to monolayer, a Teflon trough was used; 5.4 × 5.8 cm wide and 0.5 cm deep, volume 15 mL. Monomolecular layers were formed at the air–water interface from a chloroform–methanol solution (9:1 v/v). A 1.5 × 1.5 cm extended corner with a hole of 0.8 cm was used to add protein and vesicles to the subphase. The subphase is extensively stirred with a stirring bar. The trough was filled with 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 0.1 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM dithiothreitol (DTT). The surface pressure was determined with a recording Beckman LM500 electrobalance using a sand-blasted platinum plate, 1.96 cm wide and 1 cm high. The surface radioactivity was measured with a gas-flow detector (Nuclear Chicago 8731) with a micromil window; 150 μg/cm^2, 4.2 × 1.3 cm. The gas used was helium–1.3% butane. All experiments were performed in a thermostated box at 25 °C.

(*B*) *Transfer of Lipid to the Interface.* The transfer of [^{14}C]phosphatidylcholine from vesicles in the subphase to the monolayer produced only a small change in surface radioactivity. Therefore, the monolayer was collected at the end of the experiment. Underneath a monolayer of unlabeled GalCer–GalDG (8:92 molar ratio), 1 μmol of [^{14}C]phosphatidylcholine vesicles (containing 5 × 10^6 cpm) and 100 μg of glycolipid transfer protein were injected. After 2.5 h of incubation, the subphase was washed with 1 L of buffer. The buffer was injected and removed at opposite sides of the trough, both at a rate of 20 mL/min. The radioactivity in the subphase dropped to the background value. The monolayer was collected by suction into a counting vial (Rietsch et al., 1977). The collected volume was measured by weight, and a sample was taken from the subphase to correct the interfacial radioactivity. Instagel (Packard) was used as a counting solution.

(*C*) *Transfer of Lipids between Two Separate Monolayers.* A Teflon trough was used, 5 × 7 cm wide and 0.5 cm deep; the volume was 19 mL. The surface of the trough was sep-

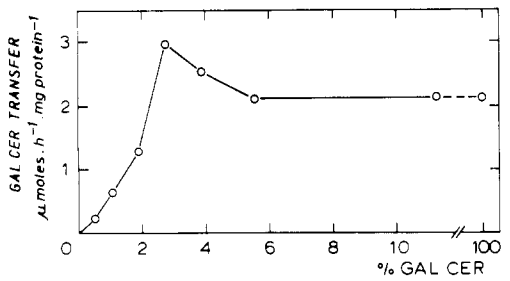

FIGURE 1: Effect of [^{14}C]GalCer content in rat liver phosphatidylcholine monolayers on the rate of [^{14}C]GalCer transfer to egg phosphatidylcholine–4% phosphatidic acid vesicles, catalyzed by glycolipid transfer protein. The monolayer (8.3 nmol of total lipid) consisted of rat liver phosphatidylcholine and various mole percentages of [^{14}C]GalCer as indicated at an initial surface pressure of 30 mN m^{-1}. The subphase contained 1 μmol of egg phosphatidylcholine–4% phosphatidic acid vesicles and 50 μg of glycolipid transfer protein in 10 mM Tris-HCl buffer (pH 7.4), 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT.

FIGURE 2: Effect of cholesterol content in rat liver phosphatidylcholine–3 mol % [^{14}C]GalCer monolayers on the rate of [^{14}C]GalCer transfer to egg phosphatidylcholine–4% phosphatidic acid vesicles, catalyzed by glycolipid transfer protein. The monolayer (8.3 nmol of total lipid) consisted of rat liver phosphatidylcholine, various mole percentages of cholesterol as indicated, and 3 mol % [^{14}C]GalCer at an initial surface pressure of 30 mN m^{-1}. The subphase contained 1 μmol of egg phosphatidylcholine–4% phosphatidic acid vesicles and 50 μg of glycolipid transfer protein in 10 mM Tris-HCl buffer (pH 7.4), 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT.

arated into two equal parts by a solid Teflon bar, 0.9 cm wide, screwed to the sides. At one side, a monomolecular film was formed of labeled lipid and at the other side of unlabeled lipid. The surface radioactivity was measured alternately on one side and the other. The surface pressure was measured with a platinum plate, 0.18 cm wide and 1 cm high. Removal of this plate gives an insignificant change in surface concentration. Glycolipid transfer protein (378 μg) was injected in the subphase. After 7 h, the monolayers were collected, and a sample of the subphase was taken as described above.

RESULTS

The transfer of GalCer from one membrane to another was studied by use of a monomolecular film of [^{14}C]GalCer at the air–water interface and egg phosphatidylcholine vesicles in the subphase, measuring the change in surface radioactivity. Fifty micrograms of glycolipid transfer protein (GL-TP) and 1 μmol of phosphatidylcholine vesicles were injected underneath a monomolecular layer of [^{14}C]GalCer with a surface pressure of 30 mN m^{-1}. The injection of GL-TP or vesicles in the subphase did not influence the surface pressure. The observed decrease in surface radioactivity of the pure GalCer monolayer was only 0.05% min^{-1}. This equals a transfer rate of 2.15 nmol of GalCer h^{-1} (mg of GL-TP)$^{-1}$ (Figure 1). Decreasing the surface pressure or increasing the protein concentration did not enhance the transfer rate from this monolayer. A decrease of the interfacial GalCer concentration by mixing with rat liver phosphatidylcholine to concentrations between 6 and 100 mol % GalCer revealed a constant transfer rate of 2.15 nmol of GalCer h^{-1} (mg of GL-TP)$^{-1}$ (Figure 1). A maximum transfer rate of 2.95 nmol of GalCer h^{-1} (mg of GL-TP)$^{-1}$ was found at a concentration of 3 mol %. Further reduction of the GalCer concentration below 3 mol % resulted in a decrease of the transfer rate (Figure 1). The addition of 50 μg of GL-TP to the subphase of a phosphatidylcholine monolayer containing 3 mol % [^{14}C]GalCer in the absence of phosphatidylcholine vesicles did not cause a measurable decrease in surface radioactivity. In the absence of a monomolecular lipid film, the protein collected at the interface to give a pressure of 11.2 mN m^{-1}.

To establish whether the fluidity of the lipid interface determines the transfer of GalCer, the fluidity-reducing effect of cholesterol was studied. To monolayers of rat liver phosphatidylcholine containing 3 mol % [^{14}C]GalCer were added increasing amounts of cholesterol. The presence of 8.3 mol % cholesterol caused a decrease in the rate of GalCer transfer to phosphatidylcholine vesicles of 29% (Figure 2). The addition of 20 and 35 mol % cholesterol caused a decrease in the GalCer transfer rate of 73% and 88%, respectively. The importance of GalCer distribution in a fluid lipid layer is supported when rat liver phosphatidylcholine is replaced by dipalmitoylphosphatidylcholine. The transfer rate of GalCer from a monolayer of dipalmitoylphosphatidylcholine containing 5 mol % [^{14}C]GalCer to egg phosphatidylcholine vesicles is reduced to 0.4 nmol h^{-1} (mg of GL-TP)$^{-1}$. Addition of increasing amounts of GalDG to monolayers of rat liver phosphatidylcholine containing [^{14}C]GalCer caused an increase in the rate of GalCer transfer to phosphatidylcholine vesicles (Table I). The transfer rate of GalCer from a GalDG monolayer is greatly enhanced compared to rat liver phosphatidylcholine monolayers (Table I).

Table I: Effect of GalDG Content of the Monolayer on the Rate of [^{14}C]GalCer Transfer from the Monolayer to Vesicles[a]

monolayer (mol %)			[^{14}C]GalCer transfer [nmol h^{-1} (mg of protein)$^{-1}$]
[^{14}C]GalCer	GalDG	rat liver PC	
2.7	0	97	2.95
2.4	26	72	4.95
4.3	47	49	7.20
8.3	92	0	8.50

[a] The transfer of [^{14}C]GalCer from the monolayer to phosphatidylcholine vesicles was measured by the initial change in surface radioactivity. The monolayer consisted of [^{14}C]GalCer, GalDG, and rat liver phosphatidylcholine (PC) at the indicated values at an initial surface pressure of 30 mN m^{-1}. The subphase contained 1 μmol of egg phosphatidylcholine–4% phosphatidic acid vesicles and 50 μg of glycolipid transfer protein in 10 mM Tris-HCl buffer (pH 7.4), 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT.

GalCer is transferred from a mixed monolayer of [^{14}C]-GalCer–GalDG (molar ratio 8:92) at a rate of 8.5 nmol of GalCer h^{-1} (mg of GL-TP)$^{-1}$. In the presence of 100 μg of GL-TP, there is nearly 90% transfer of GalCer from the monolayer to phosphatidylcholine vesicles within 2 h. It has been shown by the liposome–liposome assay system (K. Yamada, A. Abe, and T. Sasaki, unpublished results) that GalCer and GalDG are transferred nearly equally well by the GL-TP. To establish whether this is also the case under the conditions used in this study, the transfer of [^{3}H]GalDG from the monolayer to phosphatidylcholine vesicles is measured. After an incubation with 100 μg of GL-TP for 2 h, the monolayer was collected, and a sample from the subphase was taken. It showed that more than 90% of the [^{3}H]GalDG was transferred to the subphase. It can be concluded that a transfer of nearly 90% of both glycolipids takes place from a mixed monolayer

of GalCer–GalDG (molar ratio 8:92) to phosphatidylcholine vesicles in the subphase. This transfer is accompanied by a significant drop in surface pressure of about 4.5 mN m^{-1}. This could indicate that a net transfer of glycolipid from monolayer to vesicles takes place. The fact that no further drop in surface pressure is seen can be due to increased vesicle, or protein, adsorption at lower surface pressures or protein-catalyzed transfer of phosphatidylcholine to the monolayer. The following experiments were performed to establish whether there is only transfer of glycolipid from the monolayer or whether there is also transfer of phosphatidylcholine in the reverse direction. ^{14}C-Labeled phosphatidylcholine vesicles were injected underneath a monolayer of unlabeled GalCer–GalDG (molar ratio 8:92), of 32.8 mN m^{-1}, as described under Materials and Methods. In the absence of transfer protein, 1.02 nmol of phosphatidylcholine vesicles collected at the interface. This value did not decrease during the washing procedure of the subphase, as described under Materials and Methods, as the vesicle concentration in the subphase dropped to nearly zero. If the GalCer–GalDG monolayer with an initial surface pressure of 32.2 mN m^{-1} was incubated in the presence of 100 μg of GL-TP and 1 μmol of [^{14}C]phosphatidylcholine vesicles for 2.5 h, 3.35 nmol of phosphatidylcholine collected at the interface. This value is higher than the blank value obtained above but lower than the 8.3 nmol of glycolipid which is transferred to the vesicles in the subphase. During the transfer of GalCer–GalDG there was a drop of 4.4 mN m^{-1} in the surface pressure. Therefore, the blank experiment in the absence of protein was performed also at a lower pressure, 15.7 mN m^{-1}. Injection of vesicles now caused a rapid increase in surface pressure to 25.8 mN m^{-1} while 2.97 nmol of phosphatidylcholine collected at the interface. This value is comparable with the value obtained in the previous experiment in the presence of protein. The decrease in surface pressure apparently caused an increased aspecific adsorption of phosphatidylcholine. It is concluded that there is no transfer of phosphatidylcholine from the vesicles to the monolayer as a result of glycolipid transfer to the subphase.

To avoid adsorption of phosphatidylcholine vesicles to the interface, the transfer between two separate monolayers can be studied. Since both donor and acceptor membranes consist of only 4.21 nmol of lipid, a high protein concentration is required to achieve a reasonable transfer rate. Monolayer I consists of [^{14}C]GalCer–GalDG (molar ratio 8:92) and monolayer II of rat liver phosphatidylcholine. Both monolayers have an initial pressure of 30 mN m^{-1}. After injection of 126 μg of GL-TP in the subphase, the reaction was followed by alternately measuring the surface radioactivity of monolayers I and II for 7 h. The surface radioactivity of monolayer I decreased rapidly, and the surface radioactivity in monolayer II increased very slowly. At the end of the experiment, the monolayers were collected, and a sample was taken from the subphase. Of the radioactivity, 48.6% was still present in monolayer I, 6.7% was transferred to monolayer II, and 44.6% was present in the subphase. It is obvious that the glycolipid present in the subphase is bound by the protein. To account for the ^{14}C label distribution, after equilibration with the transfer protein, it was calculated that the transfer protein could contain maximally 0.5 mol of glycolipid per molecule of protein. It is possible that the transfer of glycolipid from monolayer I to monolayer II is limited by an increase in surface pressure in monolayer II during the course of the incubation.

An experiment similar to the previous one is described in Figure 3. Monolayer I consists of [^{14}C]GalCer–GalDG (molar ratio 8:92) and monolayer II of rat liver phosphatidylcholine. The initial surface pressure of monolayer I is 30 mN m^{-1}, and that of monolayer II is 20 mN m^{-1}. After injection of 378 μg of GL-TP in the subphase, there was a fast initial drop in the surface radioactivity of monolayer I due to the binding of glycolipids by the protein. The surface pressure of monolayer II increased gradually to 32.7 mN m^{-1}, and there was also a small increase in the surface radioactivity. Reduction of the surface pressure of monolayer II by removing some of the monolayer lipid after 2 h 30 min, 3 h 45 min, and 5 h 30 min, to 20, 20, and 15 mN m^{-1}, respectively, enhanced the transfer of glycolipid. After 7 h, the monolayers were collected, and a sample from the subphase was taken. Of the radioactivity, 23.3% was still present in monolayer I, 20.8% was the total amount that was collected from monolayer II, and 55.8% was present in the subphase. This experiment shows that 20.8% of the glycolipid originally present in monolayer I is transferred to monolayer II. From the radioactivity distribution, it can be calculated also in this experiment that the ratio of bound lipid to protein is less than 0.50.

In the following experiment, described in Figure 4, monolayer I consists of unlabeled GalCer–GalDG (molar ratio 8:92) and monolayer II of ^{14}C-labeled soybean phosphatidylcholine; 378 μg of GL-TP was injected into the subphase. The ex-

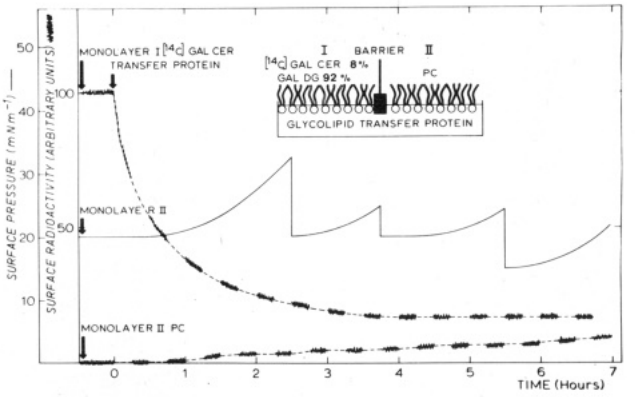

FIGURE 3: Transfer of [^{14}C]GalCer between two separate monolayers. Monolayer I consists of 4.2 nmol of [^{14}C]GalCer–GalDG (molar ratio 8:92); monolayer II consists of 4.2 nmol of rat liver phosphatidylcholine (PC). The subphase contained 378 μg of glycolipid transfer protein in 10 mM Tris-HCl buffer (pH 7.4), 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT. The surface radioactivities of monolayers I and II are alternately measured. The surface pressure of monolayer II is indicated.

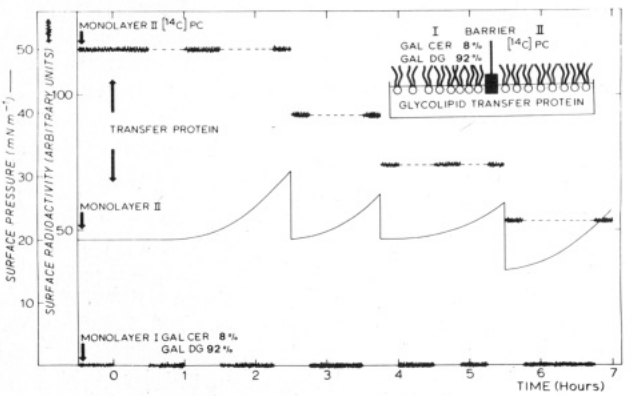

FIGURE 4: Transfer of [^{14}C]phosphatidylcholine between two separate monolayers. Monolayer I consists of 4.2 nmol of GalCer–GalDG (molar ratio 8:92); monolayer II consists of 4.2 nmol of ^{14}C-labeled soybean phosphatidylcholine (PC). The subphase contained 378 μg of glycolipid transfer protein in 10 mM Tris-HCl buffer (pH 7.4), 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT. The surface radioactivities of monolayers I and II are alternately measured. The surface pressure of monolayer II is indicated.

periment was performed exactly in the same way as the previous experiment. There was absolutely no change in the surface radioactivity of monolayer I or monolayer II. Reduction of the surface pressure of monolayer II in the same way as described in the previous experiment, after 2 h 30 min, 3 h 45 min, and 5 h 30 min, did automatically cause a drop in the surface radioactivity of monolayer II but did not increase the surface radioactivity of monolayer I (Figure 4). The monolayers were again collected, and a sample was taken from the subphase after 7 h. The radioactivity distribution showed that 4.5% of the radioactivity was present in the subphase, which means that the protein hardly binds phosphatidylcholine, and only 0.4% of the radioactivity was found in monolayer I. This experiment shows clearly that, although glycolipid is transferred to the phosphatidylcholine monolayer, there is no transfer of phosphatidylcholine to the glycolipid monolayer.

Discussion

It seems obvious that for the formation of a lipid–glycolipid transfer protein complex the protein is capable of extracting from the interface only those [^{14}C]GalCer molecules which are present in a fluid environment. A monolayer of 95 mol % dipalmitoylphosphatidylcholine–5 mol % [^{14}C]GalCer is a poor interface for the transfer reaction, probably because this monolayer is not fluid enough for the transfer protein. The addition of increasing amounts of cholesterol to rat liver phosphatidylcholine reduces the transfer rate of [^{14}C]GalCer enormously (Figure 2). Incorporation of up to 3 mol % [^{14}C]GalCer in rat liver phosphatidylcholine monolayers results in an increasing transfer rate of [^{14}C]GalCer (Figure 1). However, higher concentrations of GalCer do not result in an increase in the transfer rate. [^{14}C]GalCer in a monolayer containing GalDG from spinach may be present in an environment very favorable for extraction by the transfer protein. Although both GalCer and GalDG can be transferred by the protein, GalCer is transferred at higher rates from the monolayer containing GalDG (Table I). From a monolayer containing 92 mol % GalDG and 8 mol % [^{14}C]GalCer, the transfer rate of GalCer has increased 4-fold compared to a monolayer containing rat liver phosphatidylcholine instead of GalDG.

The transfer of GalCer and GalDG from the monolayer to phosphatidylcholine vesicles did not result in a transfer of phosphatidylcholine in the reverse direction as determined by labeling the vesicle phosphatidylcholine. In the absence of transfer protein, at an initial surface pressure of 32.8 mN m^{-1}, 1.02 nmol of vesicle phosphatidylcholine adsorbed at the interface without giving rise to a measurable change in surface pressure. Such liposome adsorption has been noticed before (Zborowsky & Demel, 1982). At an initial pressure of 15.7 mN m^{-1}, 2.97 nmol of vesicle phosphatidylcholine collected at the interface, while the pressure rose to 25.8 mN m^{-1}. This amount of phosphatidylcholine is not significantly different from the amount that collected at the interface in the presence of the glycolipid transfer protein. The decrease in surface pressure of 4.5 mN m^{-1} in the presence of transfer protein also indicates that net mass transfer occurs. A further drop in pressure is apparently abolished by the adsorption of vesicle phosphatidylcholine.

Clear proof of net mass transfer is also provided by measuring the transfer between a glycolipid monolayer and a phosphatidylcholine monolayer. Glycolipid (GalCer and GalDG) was transferred to the phosphatidylcholine monolayer while no transfer of phosphatidylcholine to the glycolipid monolayer could be measured (Figures 3 and 4). The transfer is also indicated by the pressure increase in the phosphatidylcholine monolayer. This pressure increase hampers further glycolipid transfer. Reduction in surface pressure stimulates the transfer reaction (Figure 3). Such pressure changes were not seen in the presence of phosphatidylcholine transfer protein (Demel et al., 1973), or phosphatidylinositol transfer protein (Zborowsky & Demel, 1982), where no net mass transfer occurred. The glycolipid transfer protein was found to bind GalCer but not phosphatidylcholine. Net mass transfer can be visualized since only a small part of the transfer protein was found to contain glycolipid.

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Registry No. GalCer, 85305-88-0; cholesterol, 57-88-5; dipalmitoylphosphatidylcholine, 2644-64-6.

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