# Kinetics of Ganglioside Transfer between Liposomal and Synaptosomal Membranes†

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ABSTRACT: The transfer of ganglioside $GM_1$ from micelles to membranes and between different membrane populations has been examined by using a pyrene fatty acid derivative of the ganglioside. The transfer of gangliosides from micelles to membranes depends on the physical state as well as the molecular composition of the acceptor vesicles. At 30 °C, the transfer of micellar gangliosides to dipalmitoylphosphatidylcholine (DPPC) large unilameller vesicles ($T_m$ = 41.3 °C) is characterized by a rate constant of 0.01 $min^{-1}$; at 48 °C, however, the rate constant is 0.11 $min^{-1}$. Below the phase transition temperature, the activation energy is 25 kcal/mol whereas above the phase transition it is 17 kcal/mol. Similar experiments performed with synaptic plasma membranes yielded a rate constant of 0.05 $min^{-1}$ at 37 °C. The rate of transfer of ganglioside molecules, asymmetrically located on the outer layer of donor vesicles, to acceptor vesicles lacking ganglioside depends on the physical state of both the donor and acceptor vesicles. For the transfer of ganglioside from DPPC (donor) vesicles to dimyristoylphosphatidylcholine (DMPC) (acceptor) vesicles, the rates were essentially zero at 15 °C in which both vesicle populations were in the gel phase, 0.008 $min^{-1}$ at 30 °C in which DPPC is in the gel phase and DMPC is in the fluid phase, and 0.031 $min^{-1}$ at 48 °C in which both vesicle populations are in the fluid phase. The transfer of ganglioside from DPPC vesicles to synaptic plasma membranes was also dependent on the physical state of the donor vesicles and showed an inflection point at the phase transition temperature of DPPC. Finally, in the fluid phase, the addition of excess $Ca^{2+}$ (20 mM) reduced the rate constant of ganglioside transfer by only 10%, in agreement with recent studies indicating that $Ca^{2+}$ does not bind strongly to gangliosides.

Gangliosides, sialic acid containing glycosphingolipids, are normally present in the plasma membrane of mammalian cells and are particularly abundant in neuronal membranes (Ledeen, 1978; Wiegandt, 1982). They have been implicated in a variety of cell surface related processes such as in the recognition machinery of the cell, as receptors for protein hormones and bacterial toxins, and in the mechanism of viral infection.

One intriguing property of gangliosides is their apparent rapid ability to exchange between membranes. Gangliosides have been shown to be uptaken by whole cells, subcellular fractions (Callies et al., 1977; Fishman & Brady, 1976; Toffano et al., 1980), and synthetic membranes (Felgner et al., 1981), causing responses ranging from promotion of neuritogenesis, changes in cell growth rate, release of dopamine, and activation of membrane-bound enzymes (Goldenring et al., 1985; Facci et al., 1984; Leon et al., 1981). Gangliosides spontaneously transfer from micelles to phospholipid membranes leading to the formation of compositionally asymmetric phospholipid vesicles in which the glycolipid molecules are localized only in the outer leaflet of the phospholipid bilayer, thus mimicking the situation found in plasma membranes. This property of gangliosides has been used to prepare artificial membrane systems containing up to ~14 mol % (total) of asymmetrically localized gangliosides. Higher concentrations of gangliosides result in membrane solubilization and the formation of mixed ganglioside–phospholipid micelles.

In the present work, we have studied the rate of insertion of micellar ganglioside $GM_1$ into phospholipid large unilamellar vesicles and the rate of ganglioside exchange between membranes using both synthetic (unilamellar vesicles) and natural (synaptic plasma membranes) membranes. These studies have been performed spectrofluorometrically using a novel fluorescent ganglioside derivative carrying a pyrenyldecanoyl residue linked by an amide bond to the ganglioside long-chain base moiety. These studies have allowed us to examine the dependence of the rate parameters on temperature and on the physical state of the donor and acceptor membranes.

## Materials and Methods

*Pyrene-Ganglioside $GM_1$ Preparation.* $GM_1$ ganglioside, carrying a pyrenyldecanoyl residue in substitution of the natural $GM_1$ fatty acyl moiety, was prepared by Dr. S. Sonnino (Department of Biochemistry, Medical School, University of Milano, Italy) according to the original procedure of Sonnino et al. (1985). This procedure involves deacylation of the ganglioside followed by re-N-acylation of the long-chain base with pyrenyldecanoic acid, and re-N-acetylation of the sialic acid residue. The purity and integrity of the fluorescent ganglioside $GM_1$ analogue were checked by thin-layer chromatography and nuclear magnetic resonance.

*Vesicle Preparation.* Dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), and distearoylphosphatidylcholine (DSPC) were purchased from Avanti Biochemicals (Birmingham, AL) and used without further purification. The purity of all the phospholipid samples was checked by thin-layer chromatography. DPPC- and DSPC-fused unilamellar vesicles were prepared essentially as described before (Myers et al., 1984).

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DPPC or DSPC (dried from a chloroform solution and lyophilized overnight) was suspended and vortexed in 50 mM KCl containing 0.02% sodium azide at a final concentration of 50 mg/mL. The dispersions were sonicated by using a bath sonicator (Model G112 SP1G, Laboratory Supplies, Hicksville, NY) and centrifuged at 15000*g* for 60 min. During these steps, the temperature of the suspension was always maintained above the phospholipid phase transition temperature. The sonicated vesicles were then incubated for 3 weeks at 4 °C. This procedure results in the formation of a homogeneous population of large unilamellar vesicles of about 900-Å diameter (Wong et al., 1982).

DMPC large unilamellar vesicles were prepared by using a detergent dialysis technique essentially as described by Rigell et al. (1985). Typically, 5 mg of DMPC was dried from a chloroform solution and lyophilized overnight. After lyophilization, 1 mL of 50 mM KCl/0.02% sodium azide, containing 1.5% sodium cholate, was added. The suspension was vortexed and sonicated for 5 min by using a bath sonicator. Following sonication, the samples were dialyzed for 4 h against the KCl solution and then against 50 mM KCl/10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer, pH 7.0, for 40 h. When this procedure is used, a population of large unilamellar vesicles, ranging from 750 to 2000 Å in diameter, is produced (Rigell et al., 1985).

The homogeneity and size of the vesicle preparations (DPPC, DSPC, and DMPC) were checked by negative-staining electron microscopy.

*Synaptic Plasma Membranes.* Synaptic plasma membranes (SPM) were prepared following the method described by Preti et al. (1980) with only a few modifications.

Briefly, beef brain cortex homogenated in 4 volumes of ice-cold 0.32 M sucrose/1 mM potassium phosphate buffer/0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.2, was diluted to 10% (w/v) with the homogenizing solution and centrifuged at 1000*g* for 15 min. The pellet was washed twice with the homogenizing solution and centrifuged again. After this step, the pooled supernatants were centrifuged at 11500*g* for 25 min.

The resulting pellet was suspended and centrifuged 3 times under the same conditions and finally resuspended in the homogenizing solution. Aliquots corresponding to 12 g of starting fresh tissue were layered on top of a 9% (15 mL)/16% (15 mL) Ficoll gradient and centrifuged at 53500*g* for 60 min. The band at the 9%/16% interface was collected, diluted with 3 volumes of the homogenizing solution, and centrifuged at 105000*g* for 30 min. The pellet was resuspended in 20 volumes of homogenizing solution and centrifuged at 11500*g* for 25 min.

After centrifugation, the resulting pellet was hypoosmotically shocked with 1 mM potassium phosphate buffer/0.1 mM EDTA, pH 7.5, as described by Preti et al. (1980), and the suspension was centrifuged at 53500*g* for 90 min on a discontinuous sucrose gradient, 0.4 M (7.5 mL)/0.6 M (7.5 mL)/0.8 M (7.5 mL)/1 M (10 mL).

The material sedimenting between 0.8 and 1 M sucrose showing the highest specific activities of marker enzymes and the lowest content of occluded lactate dehydrogenase (LDH) activity was collected, washed 2 times with 1 mM potassium phosphate/0.1 mM EDTA, pH 7.2, and centrifuged at 90000*g* for 6 min. The resulting pellet was resuspended in 50 mM KCl and used as synaptic plasma membrane (SPM) preparation in the following experiments.

*Fluorescence Measurements.* All fluorescence experiments were performed with a Perkin-Elmer LS5 spectrofluorometer equipped with a thermostated cuvette holder. The temperature of the cuvette was controlled with a Neslab RTE-8 refrigerated bath circulator and the temperature monitored within ±0.1 °C with a Keithley digital thermometer. Excimer formation experiments were performed by measuring the fluorescence emission spectrum of pyrene-$GM_1$ as a function of time. In all the experiments, the samples were excited at 320 nm, and the emissions of monomer and dimer were taken at their maxima at 395 and 480 nm.

*Interaction of $GM_1$ Micelles with DPPC Fused Unilameller Vesicles.* The transfer of ganglioside from micelles to DPPC vesicles was followed at different temperatures by incubating ganglioside $GM_1$ micelles with DPPC fused unilamellar vesicles and following the time-dependent change of the fluorescence excimer:monomer ratio of pyrene-$GM_1$ after the donor and acceptor populations were mixed. In a typical experiment, the incubation mixture contained 88 nmol of $GM_1$ (containing 10% molar pyrene-$GM_1$) in micellar form and 1000 nmol of DPPC fused unilamellar vesicles in a final volume of 2 mL of 50 mM KCl/10 mM Tris-HCl buffer, pH 7.0. Usually, 1.9 mL of the ganglioside dispersion was thermostated in the fluorometer cuvette at the chosen temperature for at least 15 min or until the excimer:monomer ratio was proven to remain constant with time. At this point, 100 μL of DPPC fused unilamellar vesicle (FUV) preparation, preheated at the same temperature, was added. Data were collected starting 1 min from vesicle addition in order to allow equilibration of the system. Continuous mixing in the cuvette was ensured by magnetic stirring.

*$GM_1$ Transfer between Phospholipid Vesicles.* DPPC fused unilamellar vesicles containing 10% molar $GM_1$ labeled with pyrene-$GM_1$ ($GM_1$:pyrene-$GM_1$ 6:1 molar ratio) were used as ganglioside vesicle donors. These vesicles were prepared by incubation of DPPC FUVs and pyrene-$GM_1$-labeled $GM_1$ micelles at 48 °C.

The following phospholipid vesicle systems were used as ganglioside acceptors: DPPC FUVs, DSPC FUVs, and DMPC large unilamellar vesicles. The exchange of ganglioside $GM_1$ between vesicles was followed by monitoring the time dependence of the eximer:monomer ratio of pyrene-$GM_1$ at a given temperature.

In a typical experiment, the reaction mixture contained 250 nmol (as phospholipid) of donor vesicles and 250 nmol of acceptor vesicles in a total volume of 2 mL of 50 mM KCl containing 10 mM Tris-HCl buffer, pH 7.0.

Usually, 1.9 mL of donor vesicles suspended in buffer saline was thermostated in the spectrofluorometer cuvette at a given temperature for 20 min or until the excimer:monomer ratio was constant, and then 100 μL of acceptor vesicles at the proper concentration was added.

*$GM_1$ Transfer between Phospholipid Vesicles and Synaptic Plasma Membranes (SPMs).* DPPC fused unilamellar vesicles containing 10% molar $GM_1$ labeled with pyrene-$GM_1$ ($GM_1$/pyrene-$GM_1$ 6:1) were used as donors of exchangeable ganglioside, and synaptic plasma membranes were used as acceptors of $GM_1$ molecules. The time course of the excimer:monomer ratio of pyrene-$GM_1$ was recorded in order to follow the ganglioside exchange at any given temperature.

In a typical experiment, the final incubation mixture contained 180 nmol (as phospholipid) of DPPC/$GM_1$ vesicles and 120 μg (as protein) of SPMs in a total volume of 1.5 mL of 50 mM KCl/10 mM Tris-HCl buffer, pH 7.0. Usually, 1.4 mL of DPPC/$GM_1$ vesicles was thermostated at the chosen temperature until a constant excimer:monomer ratio was obtained; then 90 μL of SPM preparation was added, and the

fluorescence intensities at 395 and 480 nm were recorded as a function of time.

*$GM_1$ Transfer between Synaptic Plasma Membranes.* SPMs were labeled with pyrene-$GM_1$ and used as a source of exchangeable ganglioside. Nonlabeled SPMs were used as ganglioside acceptor membranes. The labeling of SPMs was accomplished by incubation with pyrene-$GM_1$-labeled $GM_1$ micelles. Twenty-five nanomoles of $GM_1$ ($GM_1$:pyrene-$GM_1$ molar ratio 6:1) in micelle form was incubated at 30 °C with SPMs (120 μg as protein) in a total volume of 0.5 mL of KCl/20 mM Tris-HCl buffer, pH 7.0. After 30 min, SPMs were pelleted at 15000*g* for 10 min and separated from micellar $GM_1$ which remained in the supernatant. The pellet was washed with 1 mL of 50 mM KCl and resuspended in the proper volume of KCl/Tris-HCl buffer.

For ganglioside exchange experiments, the labeled SPMs were incubated at a given temperature in the presence of nonlabeled SPMs. The incubation mixture contained 120 μg of labeled SPMs and 120 μg of nonlabeled SPM (expressed as protein) in a total volume of 1.5 mL. $GM_1$ exchange was followed by recording the time-dependent change of the excimer:monomer ratio of pyrene-$GM_1$.

*Additional Methods.* In order to eliminate vesicle fusion as a possible source of ganglioside transfer, we performed control experiments directed to evaluate the extent of vesicle fusion, if any, during the time course of the ganglioside transfer experiments. The continuous fusion assay described by Hoekstra (1984) was employed. This method relies upon the relief of the fluorescence self-quenching of octadecyl Rhodamine B chloride when membrane fusion takes place between labeled and unlabeled membrane systems; 2% octadecyl Rhodamine B chloride was incorporated into DPPC FUVs as described (Hoekstra et al., 1984), and $GM_1$ ganglioside was inserted in the vesicle outer layer after this step.

The extent of vesicle fusion was also evaluated by negative-staining electron microscopy and by following the time dependence of the light-scattering intensity of the incubation mixture, setting the fluorometer excitation and emission wavelengths at the same value of 450 nm.

None of the above methods provided any evidence of vesicle fusion during the time course of the ganglioside exchange experiments. The fluorescence emission of Rhodamine B never increased more than 1% after incubation of the samples for 1–2 h. The increase in light scattering was always less than 1%.

## Results

The excimer:monomer ratio of pyrene-$GM_1$ aqueous dispersions at increasing concentrations is reported in Figure 1. The behavior of the excimer:monomer ratio as a function of the concentration of pyrene-$GM_1$ shows a strong increase centered at $4 \times 10^{-6}$ M, indicating that the transition from the monomeric to the micellar dispersion occurs in this concentration range. At concentrations below $10^{-6}$ M, the excimer:monomer ratio is very small, indicating that the pyrene-ganglioside molecules do not undergo frequent collisions with each other, as expected when they exist in the monomeric form below their critical micelle concentration. The association of the ganglioside molecules into micellar structures results in a rather abrupt increase in the excimer:monomer ratio. The geometric constraints and increased density of the glycolipid hydrophobic chains in the micellar core lead to a compulsory interaction between pyrene groups with a concomitant increase in the excimer:monomer ratio value. The critical micelle concentration of the pyrene-labeled ganglioside falls within the range found for the unlabeled ganglioside, suggesting that

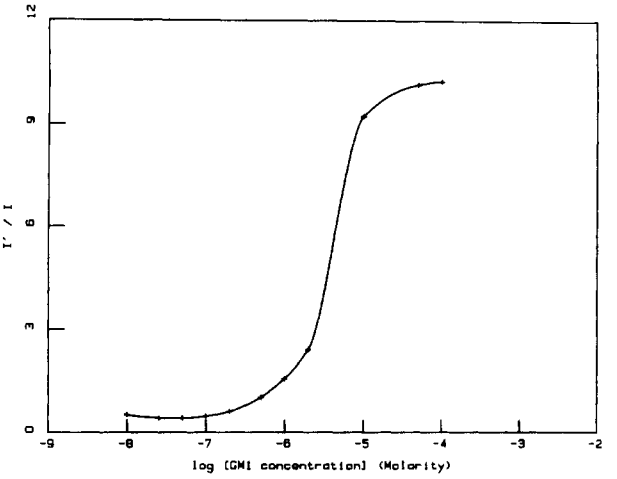


FIGURE 1: Excimer:monomer ratio of pyrene-$GM_1$ ganglioside as a function of the ganglioside concentration. The excimer fluorescence intensity ($I'$) was measured at 480 nm and the monomer fluorescence intensity at 395 nm using an excitation wavelength of 320 nm.

the presence of the pyrene label does not largely perturb the water solubility properties of the ganglioside molecule.

*Interaction of $GM_1$ Micelles with DPPC Vesicles.* Upon incubation of $GM_1$ micelles with DPPC vesicles, the ganglioside molecules insert into the outer layer of the vesicles membrane (Felgner et al., 1981). Under the conditions of these experiments (<10 mol % total ganglioside), all the ganglioside is incorporated into the phospholipid vesicles (Felgner et al., 1981, 1983). As a result of the insertion, the observed excimer:monomer ratio of an incubation mixture of pyrene-$GM_1$-labeled micelles with DPPC vesicles decreases with time.

The rate of excimer formation is a diffusion-controlled process in membrane systems. The probability of molecular collisions between two labeled molecules depends on the characteristic lateral diffusion coefficient of the membrane as well as the concentration of labeled molecules within the membrane (Galla & Hartmann, 1980). The transfer of labeled gangliosides from micelles to membranes or from a donor membrane to an acceptor membrane reduces the surface density of the labeled molecules, causing a decrease in the collision rate and therefore a smaller excimer:monomer ratio.

The time dependence of the experimental excimer:monomer ratio can be analyzed by empirically fitting the observed values to a multiple exponential decay function of the form:

$$I'(t)/I(t) = A_0 + \sum_i A_i \exp(-k_i t) \quad (1)$$

where $I'(t)/I(t)$ is the excimer:monomer ratio at time $t$, $A_0$ the excimer:monomer ratio at infinite time, and $A_i$ the amplitude factors of the exponential terms. In this study, the fits were performed by using a nonlinear least-squares procedure based upon the simplex algorithm (Nelder & Mead, 1965). Analysis of the experiments reported here revealed that in all cases studied, the data could be fitted to a single-exponential decay function. The use of two exponentials did not result in a statistically significant improvement as judged by the $F$-test criterion based upon the extra sum of squared residuals (Munson & Rodbard, 1980).

Figure 2 shows the experimental $I'(t)/I(t)$ values for the kinetics of micellar $GM_1$ insertion into DPPC vesicles as a function of temperature. Also shown in the figure are the theoretical curves obtained by fitting the data to the equation:

$$I'(t)/I(t) = A_0 + A_1 \exp(-kt) \quad (2)$$

Figure 3 shows the temperature dependence of the three parameters $A_0$, $A_1$, and $k$ obtained from the fitting procedure

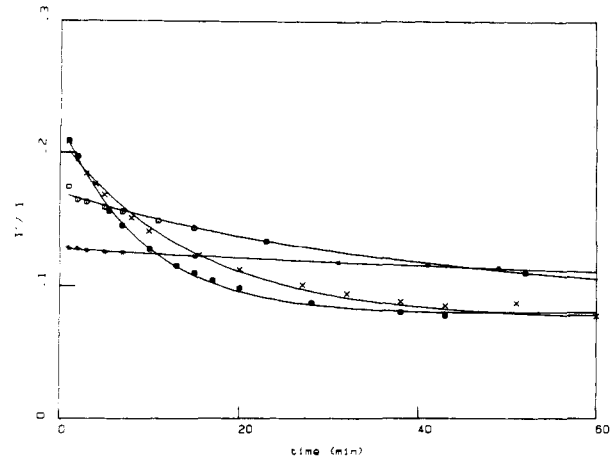


FIGURE 2: Kinetics of ganglioside $GM_1$ transfer from micelles to DPPC fused unilamellar vesicles at different temperatures. (Asterisks) 28 °C; (○) 34 °C; (×) 48 °C; (●) 54 °C. The solid lines represent the results of the fits of the experimental values to a single-exponential function. For this set of experiments, the lipid concentration was 0.5 mM, and the total ganglioside molar fraction was 0.08.

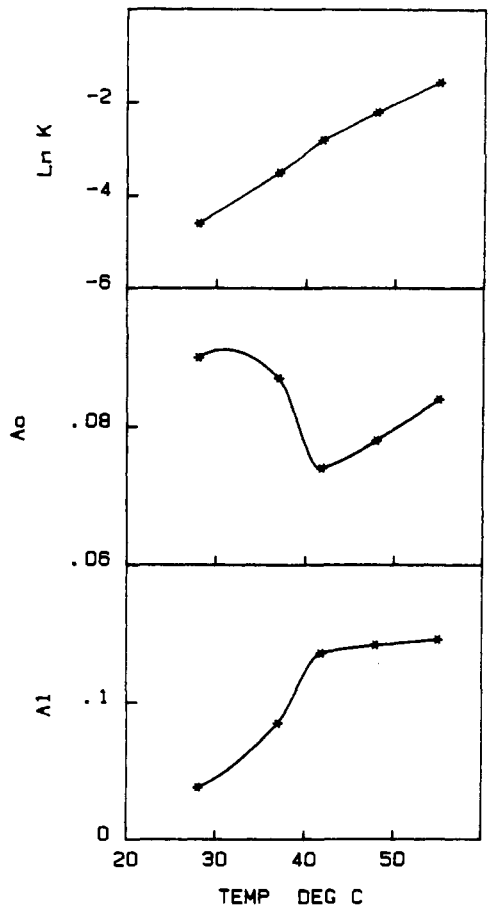


FIGURE 3: Temperature dependence of the fitted $A_0$, $A_1$, and $k$ values for the transfer of ganglioside $GM_1$ from micelles to DPPC fused unilamellar vesicles. See text for details.

outlined above. The rate constant, $k$, is strongly temperature dependent. At 30 °C, the rate constant is 0.01 $min^{-1}$ whereas at 48 °C the rate constant is 1 order of magnitude faster (0.11 $min^{-1}$). The Arrhenius plot of the rate constant shows a break at a temperature that coincides with the phase transition temperature of the acceptor vesicles ($T_m$ = 41.3 °C). Below the phase transition temperature, the activation energy for ganglioside transfer is 25 kcal/mol whereas above the phase transition temperature the value is 17 kcal/mol. These results suggest that the physical state of the acceptor membrane is

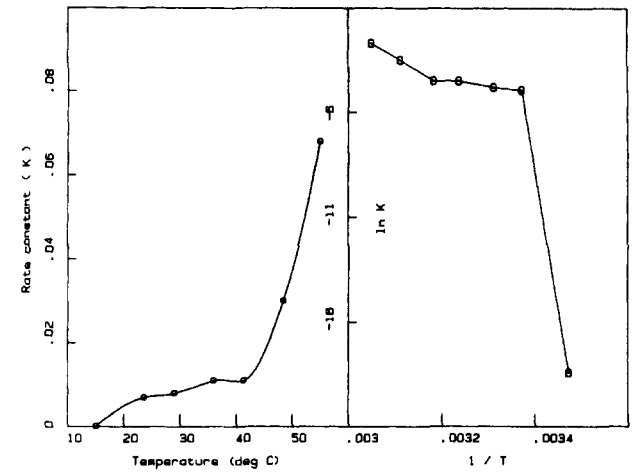


FIGURE 4: Temperature dependence of the rate constant ($k$) for the exchange of ganglioside $GM_1$ between DPPC vesicles (donor) and DMPC vesicles (acceptor).

the rate-limiting step for the transfer of micellar ganglioside to phospholipid bilayer membranes.

Figure 3 also shows the temperature dependence of $A_0$. According to eq 2, $A_0$ should be equal to the eximer:monomer ratio at infinite time, i.e., when all the ganglioside has been inserted into the DPPC bilayer. As such, the plot of $A_0$ vs. temperature should display the characteristic eximer:monomer ratio vs. temperature profile of DPPC vesicles. As shown in Figure 3, this is precisely the behavior of $A_0$, including the inflection point at the phospholipid phase transition temperature [see, for example, Galla & Hartman (1980)]. These results provide additional experimental support to the representation of the excimer:monomer ratio decay in terms of a single-exponential function.

The transfer experiments were also performed at different concentrations of acceptor vesicles. In all cases, the rate constant, $k$, was independent of concentration, indicating that the transfer of ganglioside occurs through the aqueous phase and not by micelle–vesicle collision-mediated transfer. These results are in agreement with previous observations regarding the exchange mechanism of phospholipids (Roseman & Thompson, 1980; Nichols & Pagano, 1981; Felgner et al., 1983; Storch & Kleinfeld, 1986).

*$GM_1$ Ganglioside Exchange between Phospholipid Vesicles.* The mixing of a ganglioside-containing vesicle population with a ganglioside-free vesicle population results in the spontaneous transfer and eventual equilibration of the ganglioside molecules among the entire population of vesicles. The kinetics of this process depend on the physical state of the acceptor and donor phospholipid vesicles as well as temperature. Figure 4 shows the temperature dependence of the rate constant for the exchange of ganglioside $GM_1$ between DPPC vesicles (donor) and DMPC vesicles (acceptor).

The same figure also shows the Arrhenius plot (ln $k$ vs. $1/T$) of the same experimental data. Upon increasing temperature, the plot clearly reveals the presence of two break points, at about 23 and 42 °C, respectively, corresponding to the transition temperature ($T_m$) from gel to liquid-crystalline phase of the acceptor (DMPC) and donor (DPPC) vesicles, respectively. At temperatures in which both acceptor and donor phospholipid are in the gel phase (below 23 °C), virtually no $GM_1$ exchange can be detected. Upon increasing the temperature, the acceptor vesicles (DMPC) undergo their phase transition (24 °C), while the donor phospholipid vesicles remain in the gel phase. In this temperature range (24–41 °C), the rate of ganglioside exchange remains very low and almost

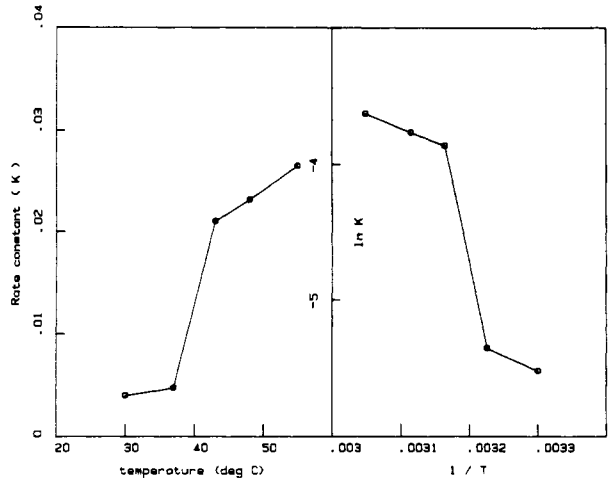


FIGURE 5: Temperature dependence of the rate constant ($k$) for the exchange of ganglioside $GM_1$ between DPPC vesicles (donor) and synaptic plasma membranes (acceptor).

constant ($k$ = 0.01 $min^{-1}$). Eventually, when the transition temperature of the donor vesicles is reached, and both the donor and the acceptor vesicle membranes are in the fluid state, the rate constant increases very rapidly with temperature. These data indicate that under these conditions the main rate-limiting step for ganglioside transfer is the ganglioside release from the donor membrane (off rate) and that this rate is strongly dependent on the physical state of the donor membrane. In particular, if both donor and acceptor vesicles are in the liquid-crystalline phase, the rate of exchange is considerably fast, whereas if they are in the solid (gel) phase the exchange rate is reduced to a minimum level.

*Effect of $Ca^{2+}$ on Rate of Ganglioside Exchange.* The presence of excess $Ca^{2+}$ (20 mM) on the rate of ganglioside exchange was examined at 54 °C. At this temperature, the vesicles are in the liquid-crystalline phase as indicated by differential scanning calorimetry (Masserini & Freire, 1986). The ganglioside transfer data reveal only a slight reduction in the rate of transfer in comparison with a calcium-free system ($k$ = 0.040 and 0.047 $min^{-1}$, respectively). The absence of a strong $Ca^{2+}$ effect is consistent with recent studies indicating that $Ca^{2+}$ only weakly associates with gangliosides and that it only has a small effect on the ganglioside lateral diffusion coefficient (McDaniel & McLaughlin, 1985; Goins et al., 1986; Masserini & Freire, 1986). The slight decrease in the rate constant is consistent with a $Ca^{2+}$-induced bilayer rigidification of the DPPC bilayer matrix in agreement with previous results.

*$GM_1$ Exchange between DPPC FUVs (Donor) and Synaptic Plasma Membranes (Acceptor).* Figure 5 shows the behavior of the rate constant for ganglioside exchange between DPPC vesicles and synaptic plasma membranes (SPMs) as a function of the incubation temperature of the system. The same figure also shows the Arrhenius plot (ln $k$ vs. $1/T$) of the same experimental data. As before, the plot shows an abrupt change at a temperature corresponding to the phase transition temperature ($T_m$) of the donor lipid (DPPC). At physiological temperatures, synaptic plasma membranes are weaker acceptors than phospholipid membranes in the fluid phase. At 37 °C, the rate constant for pyrene-$GM_1$ ganglioside transfer from DPPC to DMPC is 0.01 $min^{-1}$ whereas that from DPPC to SPMs is only 0.005 $min^{-1}$. This difference can be attributed to the lower fluidity of the synaptosomal membrane. Previously (Myers & Freire, 1985), we have shown that synaptic plasma membranes have a lower fluidity than pure phospholipid vesicles in the liquid-crystalline phase and that this lower fluidity is due to the presence of protein and cholesterol, absent in pure phospholipid vesicles. As in the case of phospholipid vesicles, the rate of exchange increased by approximately a factor of 4 upon passage of the donor membranes from the gel to the fluid phase. At 45 °C, the rate of ganglioside transfer from DPPC to SPMs was 0.02 $min^{-1}$.

*$GM_1$ Exchange between Synaptic Plasma Membranes.* As described under Materials and Methods, SPMs were loaded with pyrene-$GM_1$ and used as a source of exchangeable ganglioside. Nonlabeled SPMs were used as acceptor membranes for the glycolipid. The rate constant for ganglioside transfer increased monotonically with temperature up to 40 °C ($k$ = 0.005 $min^{-1}$ at 28 °C and $k$ = 0.04 $min^{-1}$ at 37 °C). At higher temperatures, the rate of ganglioside transfer slowed down considerably ($k$ = 0.02 $min^{-1}$ at 45 °C) probably as a result of the denaturation of the synaptosomal membrane proteins.

## DISCUSSION

The spontaneous transfer of phospholipids between different membrane populations has been examined by several authors (Roseman & Thompson, 1980; Nichols & Pagano, 1981; Correa-Freire et al., 1982; Massey et al., 1982; Storch & Kleinfeld, 1986). For phosphatidylcholines, the rate of exchange is very slow and is characterized by half-times on the order of 10–24 h whereas for fatty acids the transfer rate is significantly faster and occurs within minutes. One common characteristic of these transfer processes is that the transfer rates are independent of the concentration of acceptor vesicles, indicating that the transfer mechanism involves aqueous diffusion of the lipid rather than vesicle–vesicle collisons.

As previously observed by Felgner et al. (1983) for the transfer of micellar ganglioside to phospholipid vesicles, we have also found that the rates are independent of the concentration of acceptor vesicles, consistent with the idea that gangliosides also transfer through the aqueous phase without involving micelle–membrane or membrane–membrane collisions. It is important to note that this transfer mechanism appears to be valid for a wide variety of lipid systems including those showing very slow or very fast kinetics.

Our studies on ganglioside transfer indicate that the rate process is strongly temperature dependent and also dependent on the physical state of the donor and acceptor vesicles. For the transfer of gangliosides from micelles to vesicles, the activation energies were 25 kcal/mol below the phase transition temperature of the acceptor vesicles and 17 kcal/mol above their phase transition. The transfer of pyrene-$GM_1$ ganglioside from DPPC to DMPC vesicles was characterized by an activation energy of 14 kcal/mol above the phase transition temperature of both the donor and acceptor vesicles. On the other hand, the transfer of ganglioside between synaptic plasma membranes had an activation energy of 26 kcal/mol at physiological temperatures. These values are similar in magnitude to the ones found for other membrane systems. For example, Massey et al. (1982) have found activation energies ranging between 23 and 26 kcal/mol for the transfer of phospholipids between apolipoprotein–phospholipid recombinants. Phillips et al. (1980) have obtained activation energies of 16 kcal/mol for the transfer of cholesterol in phospholipid vesicles, and Felgner et al. (1983) have estimated an activation energy of 22.8 kcal/mol for the transfer of ganglioside $G_{t1b}$ from micelles to DPPC vesicles above their phase transition temperature.

At equivalent temperatures, the rate of ganglioside exchange depends on the fluidity and physical state of both the donor and acceptor membranes. For example, the transfer of

ganglioside from DPPC to DMPC is characterized by a time constant ($\tau = 1/k$) of 125 min at 30 °C in which the donor vesicles are in the gel phase and the acceptor vesicles are in the fluid phase and by a time constant of 30 min at 48 °C in which both populations of vesicles are in the fluid phase. It must be noted that the transfer of ganglioside from micelles to vesicles is relatively fast even below the phase transition temperature of the acceptor vesicles. The time constant for the transfer of micellar ganglioside to DPPC vesicles is 100 min at 30 °C. These results suggest that the rate of ganglioside transfer is limited by the rate at which the ganglioside molecules leave the donor vesicle. This situation is similar to the one found for other lipid molecules in which the off rate from the donor membrane has been found to be the rate-limiting step (Massey et al., 1982; Storch & Kleinfeld, 1986). Since gangliosides, due to their large carbohydrate head group, are able to interact with membrane proteins and other membrane components in a rather specific fashion, it is conceivable for cell membranes to regulate the rate of ganglioside exchange by promoting specific ganglioside interactions at the cell surface. In fact, it has been observed that certain gangliosides like $G_{t3}$ have a tissue-specific distribution (Iwamori et al., 1981; Dubois et al., 1985), strongly suggesting that these ganglioside molecules are prevented from freely exchanging between membranes.

The transfer of ganglioside from micelles to membranes or between different membrane populations obeyed a single-exponential law and could be described by a single rate constant. It should be noted that, unlike fatty acids and other phospholipids, gangliosides are unable to flip-flop between the two halves of the bilayer (Felgner et al., 1981). Under the conditions of these experiments, the membrane exchange of ganglioside occurs from the outer layer of the donor membrane to the outer layer of the acceptor membrane. This situation should be contrasted with the one observed for fatty acids (Storch & Kleinfeld, 1986) in which the exchange is accompanied by flip-flop and redistribution of the exchangeable lipid between the two bilayer halves of the donor and acceptor membranes. This latter situation gives rise to biexponential kinetics as demonstrated by Storch and Kleinfeld (1986). Another system lacking phospholipid flip-flop is that of apolipoprotein–lipid recombinants. In this case, it has also been observed that the exchange of phospholipid molecules between recombinant particles follows a single-exponential law. Under similar conditions, the magnitude of the rate constant and therefore the observed rate of transfer appear to be correlated with the aqueous solubility of the lipid. Gangliosides and fatty acids, for example, exchange relatively fast between membrane populations, whereas phospholipids and glucocerebrosides exchange over a period of days and weeks. Under physiological conditions, the effective exchange of these poorly soluble lipids requires the presence of specific exchange proteins (Wong et al., 1984).

**Registry No.** DPPC, 2644-64-6; DMPC, 13699-48-4; Ca, 7440-70-2; ganglioside $GM_1$, 37758-47-7.

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