Biochimica et Biophysica Acta, 687 (1982) 195–203
Elsevier Biomedical Press

BBA 71124

A RAT BRAIN CYTOSOL PROTEIN WHICH ACCELERATES THE TRANSLOCATION OF GALACTOSYLCERAMIDE, LACTOSYLCERAMIDE AND GLUCOSYLCERAMIDE BETWEEN MEMBRANES

KEIKO YAMADA and TERUKATSU SASAKI *

Department of Biochemistry, Sapporo Medical College, South-1, West-17, Sapporo, 060 (Japan)

(Received October 23rd, 1981)

Key words: Lipid transfer protein; Galactosylceramide; Lactosylceramide; Glucosylceramide; Liposome; (Rat brain)

The cytosol fraction of rat brains accelerated the transfer of [^{3}H]galactosylceramide, [^{3}H]lactosylceramide, and [^{3}H]glucosylceramide from donor to acceptor liposomes, which contained one of these glycosphingolipids as a constituent. All the three glycosphingolipid transfer activities were eluted from a Sephadex G-75 column at an identical position; from a K_{av} value of 0.377 a molecular weight of 18000 was estimated for the transfer protein. The glycosphingolipid transfer reactions were characterized by the use of the active protein fraction obtained by the Sephadex chromatography. The translocation of each of the three [^{3}H]glycosphingolipids from the donor liposomes to the acceptor liposomes was shown by thin-layer chromatography of the lipids extracted from the acceptor liposomes, which were separated from the donor liposomes after the incubation. In all three glycosphingolipid transfers, it was found that the transfer rates were highest when liposomes lacking in glycosphingolipids were used as an acceptor. Addition of one of the three glycosphingolipids to the acceptor liposomes as a constituent at 1 mol% resulted in a decrease in the measured rates of all three glycosphingolipid transfers: the effect was strongest with galactosylceramide, intermediate with glucosylceramide, and weak with lactosylceramide. In all three glycosphingolipid transfer assays, larger amounts of glycosphingolipids were exchanged between liposomes when the assay was performed using liposomes containing 10 mol% of a glycosphingolipid instead of using liposomes containing 1 mol% of the glycosphingolipid; the results suggest that phospholipids in the liposomes participate in some unknown way in the lipid transfer mediated by the glycosphingolipid transfer protein. The Sephadex G-75 fraction of the glycosphingolipid transfer protein did not facilitate the transfer of [^{3}H]phosphatidylethanolamine from the donor to acceptor liposomes.

Introduction

The translocation of phospholipids among intracellular membranes is mediated by the activity of phospholipid exchange proteins [1–8]. The mechanism of intracellular translocation of glycosphingolipids remains to be elucidated. It has been presumed that glycosphingolipids are present on the outer side of plasma membranes [9–11], whereas the major site of glycosphingolipid biosynthesis has been shown to be the Golgi complex [12–14]. Metz and Radin [15] reported the isolation of glucosylceramide uptake protein from the cytosol of bovine spleen. The protein accelerated the transfer of glucosylceramide from liposomes to red cells and vice versa. Since several phospholipid exchange proteins with different lipid specificities are involved in the intracellular exchange of phospholipids [1–3,6], we are interested to examine

* To whom correspondence should be addressed.

activities for the transfer of various glycosphingolipids in the cytosol of various organs.

In this paper we wish to show the presence in rat brain of a protein which accelerates the transfer of lactosylceramide, galactosylceramide, and glucosylceramide from donor liposomes to acceptor liposomes.

Materials and Methods

Materials. Preparations of concanavalin A-Sepharose 2B and [6-^{3}H]galactosylglucosylceramide (38.6 μCi/μmol) were previously described [16]. Galactosylceramide containing α-hydroxy fatty acids was purchased from Sigma Chemical Company (St. Louis). The glycolipid was tritium-labeled (40.9 μCi/μmol) by the galactose oxidase/sodium boro[^{3}H]hydride method according to the procedure described by Radin [17]. 1-Acyl-2-[9,10-^{3}H]oleoyl-*sn*-glycero-3-phosphocholine (2.01 mCi/μmol) was prepared as described previously [18]. [^{3}H]Phosphatidylethanolamine (1 mCi/μmol) was prepared from *Salmonella anatum* grown in a medium containing [2-^{3}H]-glycerol. [^{3}H]Glucosylceramide (8.0 mCi/μmol) and [^{14}C]cholesteryloleate (52.5 μCi/μmol) were obtained from New England Nuclear (Boston). Trypsin (diphenylcarbamoyl chloride treated) was obtained from Sigma Chemical Company (St. Louis), and soybean trypsin inhibitor was purchased from Worthington Biochemical Corporation (Freehold).

Assay of glycosphingolipid transfer activities. Glycosphingolipid transfer activities were measured by determining the transfer of a class of [^{3}H]glycosphingolipids from concanavalin A-reactive liposomes to concanavalin A-nonreactive liposomes in a volume of 0.2 ml. The method is a modification of our assay for phospholipid exchange activities [16]. In the assay procedure A (precipitation method) [16], the liposomes were prepared, unless otherwise stated, from a lipid mixture containing phosphatidylcholine, phosphatidylinositol, cholesterol, and a class of glycosphingolipids in molar ratios of 90:10:50:1; [^{3}H]glycolipid was used in the preparation of the concanavalin A-reactive liposomes; the reactive liposomes contained [^{14}C]cholesteryloleate (0.36 nmol/100 nmol of phospholipids) as well as dimannosyldiacylglycerol (6.75 nmol/100 nmol of phospholipids). [^{14}C]Cholesteryloleate was added as a non-exchangeable marker and served as a reference to correct for incomplete recovery of the donor liposomes. In the assay procedure B (filtration method) [16], cholesterol was not added as a constituent of the liposomes and the reactive liposomes [19] contained Manα1 → 4Manβ1 → 4Glc-ceramide [20] in place of dimannosyldiacylglycerol at 1.15 nmol/100 nmol of phospholipids. The trihexosylceramide was used in the preparation of the liposomes to achieve a better separation of the donor and acceptor liposomes in the assay procedure B. In both assay procedures, the extent of the glycosphingolipid transfer from the donor to acceptor liposomes was measured from the ^{3}H/^{14}C ratios (X) of the reactive liposomes recovered from the assay mixture by binding to concanavalin A. Percentage of a [^{3}H]glycosphingolipid transferred is calculated as $(1 - XY) \times 100\%$, where Y is the ^{14}C/^{3}H ratio of the reactive liposomes recovered from the assay mixture that was incubated in the absence of glycosphingolipid transfer proteins. The amount of a glycosphingolipid exchanged in the assay was calculated by the method described by Hellings et al. [21]. It seems that the dimannosyldiacylglycerol and the trihexosylceramide, which were added as a constituent of the donor liposomes, are non-exchangeable under the assay conditions we used because the separation of the donor and acceptor liposomes by concanavalin A was not affected even after a marked progress of the glycosphingolipid transfer reaction.

Preparation of rat brain cytosol fraction and Sephadex G-75 gel filtration of the fraction. Male rats of the Wistar strain, 60–70 days of age, were killed by decapitation. The whole brains were washed with ice-cold 10 mM sodium phosphate (pH 7.0) containing 0.15 M NaCl and 1 mM dithiothreitol, and homogenized in 3 vol. of the same medium with four strokes of a Teflon pestle in a glass homogenizer. The homogenate was centrifuged at $15000 \times g$ for 20 min. The supernatant fraction was centrifuged at $10^5 \times g$ for 60 min. In this report, the $10^5 \times g$ supernatant fraction is designated as cytosol fraction. A portion of the cytosol fraction (67.5 mg protein in 10 ml) was subjected to gel filtration on a column (2 cm × 140

cm) of Sephadex G-75. Chromatography was performed in the same medium containing 0.02% sodium azide. Flow rate was 15 ml/h. 4-ml fractions were collected. Fractions containing the glycosphingolipid transfer protein were combined. The protein of the combined Sephadex G-75 fraction was used in the characterization of the glycosphingolipid transfer activities.

Effect of trypsin digestion on the galactosylceramide transfer activity of the Sephadex G-75 fraction. The Sephadex G-75 fraction (17.7 μg protein) was incubated in the presence of trypsin (protein to trypsin ratio = 4) at pH 7.0 and 25°C for 20 and 60 min in a volume of 330 μl. After the addition of a 3-fold weight excess of soybean trypsin inhibitor, the galactosylceramide transfer activity was determined by the assay procedure B using 50 μl and 100 μl aliquots. Samples incubated in the presence of simultaneously added trypsin and soybean trypsin inhibitor did not differ from the untreated Sephadex G-75 fraction.

Identification of the lipid transferred to the acceptor liposomes in the galactosylceramide, lactosylceramide, and glucosylceramide transfer reactions. In these experiments, the standard incubation mixture of the assay procedure A was scaled up 5-fold. Each incubation mixture contained 31.6 μg protein of the Sephadex G-75 fraction. The mixtures were incubated at 25°C for 60 min. After the incubation, the donor and acceptor liposomes were separated by the concanavalin A-mediated precipitation of the concanavalin A-reactive liposomes. Lipids were extracted from the donor and acceptor liposome fractions. The lipids were quantitatively applied to a precoated silica gel plate (0.25 mm thick) (Merck). The plate was developed in chloroform/methanol/water (65:25:4, by vol.). Radioactive areas were located by fluorography: the plate was sprayed with 7% (w/v) 2,5-diphenyloxazole in acetone [22] and exposed to Kodak X-Omat R film for 19–22 days at −75°C. Lipids were located by charring with sulfuric acid.

Results

Glycosphingolipid transfer activity in rat brain cytosol was measured by determining the transfer of a class of [^{3}H]glycosphingolipids from donor liposomes to acceptor liposomes. The donor and acceptor liposomes contained the glycosphingolipid as a constituent at a molar ratio of glycosphingolipid to phospholipid of 1:100. In an attempt to find out a protein which facilitates glycosphingolipid transfer from the donor to acceptor liposomes, we first examined the galactosylceramide transfer activity in the brain extract of suckling rats (21 days after birth), which are at the growth-stage most active in myelination. Although it was found that the cytosol fraction of suckling rat brains contains protein(s) exhibiting galactosylceramide transfer activity, we soon became aware that adult rat brain was a better source of the protein(s). Therefore, all the results presented in this paper were obtained by the use of adult rat brains as a material. The cytosol fraction prepared from adult rat brains facilitated the transfer of [^{3}H]galactosylceramide, [^{3}H]glucosylceramide, and [^{3}H]lactosylceramide in a manner dependent on the amount of the fraction up to 380 μg protein

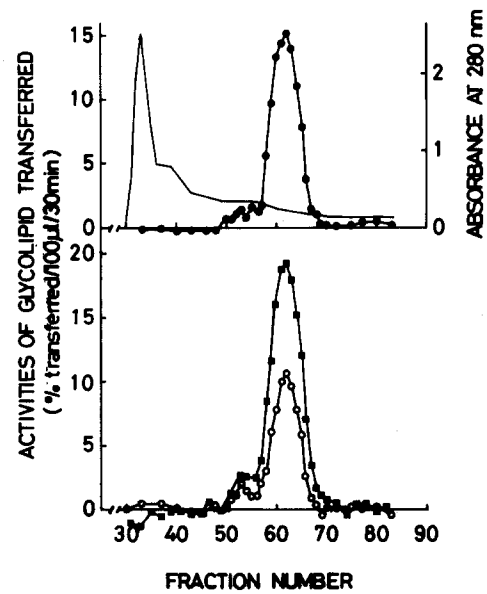

Fig. 1. Elution pattern of the glycosphingolipid transfer protein from a Sephadex G-75 column. The chromatography was performed as described in Materials and Methods. Aliquots (100 μl) of each fraction were assayed for galactosylceramide transfer activity (○), lactosylceramide transfer activity (●), and glucosylceramide transfer activity (■) by the assay procedure A: incubation was at 25°C for 30 min. Elution of protein (————) was determined by measuring absorbance at 280 nm. Fractions 60–64 were combined for use in the following experiments.

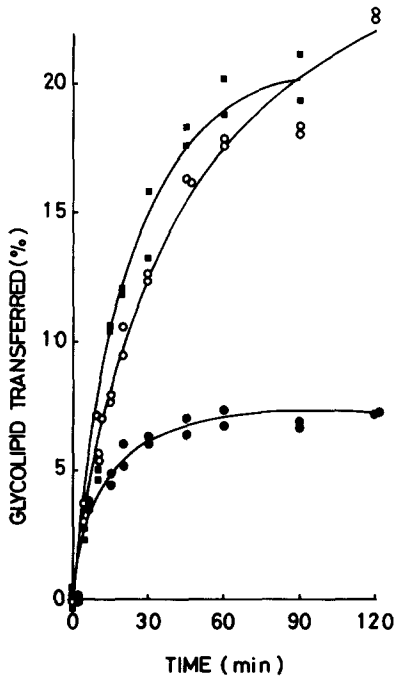

Fig. 2. Time courses of galactosylceramide, lactosylceramide, and glucosylceramide transfer reactions accelerated by the Sephadex G-75 fraction. The glycosphingolipid transfer activities were assayed in duplicate under the standard conditions by the assay procedure B. Incubation was at 25°C for the indicated time. Each incubation mixture of galactosylceramide, lactosylceramide, and glucosylceramide transfer assays contained 3.16 μg protein, 1.89 μg protein, and 1.26 μg protein, respectively, of the Sephadex G-75 fraction. Where superimposition of values occurs, the symbols are inserted side by side for clarity. ○, galactosylceramide transferred; ●, lactosylceramide transferred; ■, glucosylceramide transferred.

under the standard assay conditions by the procedure A. The brain cytosol accelerated the transfer of the three glycosphingolipids at an order of the rates: glucosylceramide transfer > lactosylceramide transfer > galactosylceramide transfer. The three glycosphingolipid transfer activities of rat brain cytosol were about 0.3–0.6 nmol of glycosphingolipid exchange between liposomes/30 min per mg protein.

The cytosol fraction of adult rat brains was subjected to gel filtration on a Sephadex G-75 column to estimate the molecular weight of the glycosphingolipid transfer protein and to obtain a protein preparation suitable for further characterization of the transfer activities. All the three glycosphingolipid transfer activities were eluted from the G-75 column at an identical position with a K_{av} of 0.377; an elution position of a protein with a molecular weight of about 18000 (Fig. 1). On the Sephadex G-75 chromatogram, a small shoulder of the three activities was found immediately ahead of the main peak of the activities; the K_{av} value (0.26) of the shoulder suggests a molecular weight of 30000. The phosphatidylcholine exchange activity in the brain cytosol was eluted from the G-75 column at around fraction No. 60 (data not shown), a position where a protein with a molecular weight of 21000 was eluted. However, no phosphatidylethanolamine exchange activity was found in the G-75 fractions in spite of the fact that the exchange activity was assayed by the use of liposomes containing 1 mol% of phosphatidyl-ethanolamine to make the assay highly sensitive to low activity.

The properties of the glycosphingolipid transfer activities were examined by the use of a protein

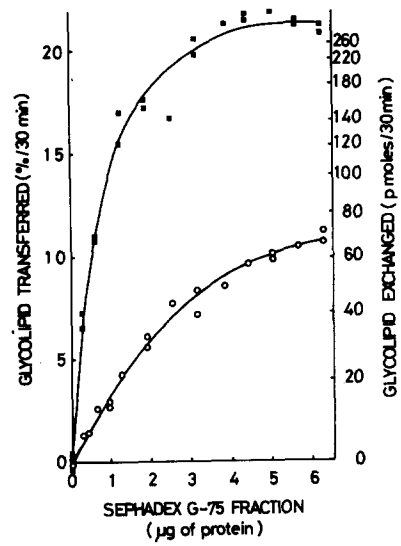

Fig. 3. Effect of various amounts of the protein of the Sephadex G-75 fraction on the galactosylceramide and glucosylceramide transfers between liposomes. The glycosphingolipid transfer activities were assayed in duplicate under the standard conditions by the assay procedure B. Incubation was at 25°C for 30 min. Each incubation mixture contained the indicated amount of protein of the Sephadex G-75 fraction. In the calculation of the amounts of glycosphingolipid exchanged, it was assumed that 22% of the [^{3}H]glycosphingolipids were transferred from the donor to acceptor liposomes at the equilibrium point. ○, galactosylceramide transferred; ■, glucosylceramide transferred.

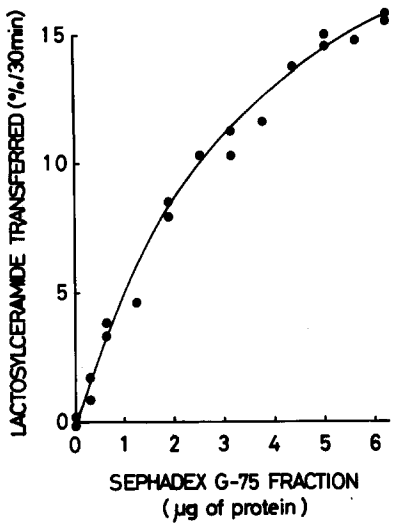

Fig. 4. Effect of various amounts of the protein of the Sephadex G-75 fraction on the lactosylceramide transfer between liposomes. The activity was assayed in duplicate under the standard conditions by the assay procedure A. Incubation was at 25°C for 30 min. Each incubation mixture contained the indicated amount of protein of the Sephadex G-75 fraction.

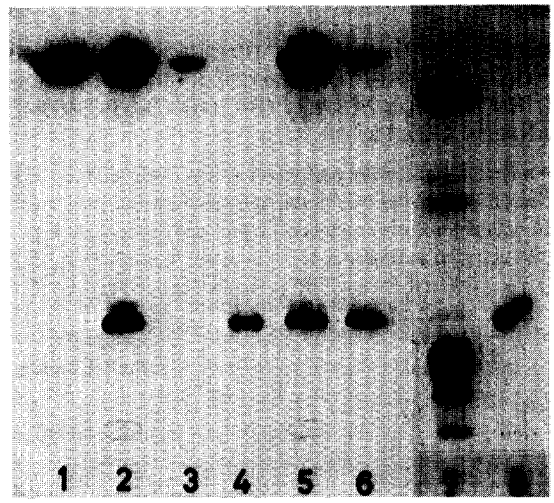

Fig. 5. Thin-layer chromatogram of lipid extracts from the donor and acceptor liposome fractions which were obtained by the concanavalin A-mediated separation after the lactosylceramide transfer reaction. The incubations were performed in the presence (lanes 5, 6 and 7) or absence (lanes 2 and 3) of the glycosphingolipid transfer protein as described in Materials and Methods. Lanes 2 and 5, lipids extracted from the donor liposome fractions; lanes 3, 6 and 7, lipids extracted from the acceptor liposome fractions; lane 1, [^{14}C]cholesteryloleate; lane 4, [^{3}H]lactosylceramide; lane 8, lactosylceramide. Lanes 1–6 show fluorography patterns. Lanes 7 and 8 show lipids located by charring with sulfuric acid.

preparation obtained by combining fractions No. 60 through No. 64 of the Sephadex G-75 chromatography. The glycosphingolipid transfers facilitated by the Sephadex G-75 fraction are shown in Fig. 2 (time courses) and in Figs. 3 and 4 (dependences on the amount of protein). In these measurements of the activities, glycosphingolipid transfers between cholesterol-free, unilamellar liposomes were determined and concanavalin A-Sepharose 2B was used instead of concanavalin A to attain a rapid separation of the donor liposomes from the acceptor liposomes; the rapid separation is important in the time course experiments. For some unknown reason, the lactosylceramide transfer we measured by the filter method scaled off at a low (about 7%) transfer value under the conditions of the assay shown in Fig. 2. Therefore, the dependence of the lactosylceramide transfer on the amount of the Sephadex G-75 fraction was measured by the use of cholesterol-containing liposomes, the donor and acceptor components of which were separated by the precipitation method (Fig. 4). The galactosylceramide transfer activity was inactivated by trypsin treatment of the Sephadex G-75 fraction: the inactivation was 57% after 20 min of the treatment and 74% after 60 min of the treatment under the conditions described in Materials and Methods.

The results shown in Fig. 5 and Table I prove that the transfer of [^{3}H]glycosphingolipids from the donor to acceptor liposomes proceeds without measurable breakdown of the [^{3}H]glycosphingolipids. In each glycosphingolipid transfer reaction, a single ^{3}H-labeled component, cochromatographing with the glycosphingolipid used in the reaction, was detected in the acceptor liposomes: a result obtained in the lactosylceramide transfer reaction is shown in Fig. 5. The translocation of [^{3}H]galactosylceramide, [^{3}H]lactosylceramide, and [^{3}H]glucosylceramide from the donor to acceptor liposomes was strongly dependent on the addition of the Sephadex G-75 fraction to the incubation mixture (Table I and Fig. 5). [^{14}C]Cholesteryloleate, a nonexchangeable marker added as a constituent of the donor liposomes, was found in small amount in the acceptor liposome fractions

TABLE I

DISTRIBUTION OF RADIOACTIVITIES OF [^{14}C]CHOLESTERYLOLEATE AND [^{3}H]GLYCOSPHINGOLIPIDS IN THE DONOR AND ACCEPTOR LIPOSOME FRACTIONS WHICH WERE OBTAINED BY THE CONCANAVALIN A-MEDIATED SEPARATION AFTER THE LACTOSYLCERAMIDE, GALACTOSYLCERAMIDE AND GLUCOSYLCERAMIDE TRANSFER REACTIONS

Experiments 1 and 2 were performed as described in Figs. 5 and 7, respectively. The [^{14}C]cholesteryloleate and [^{3}H]glycosphingolipids located on TLC plates by fluorography as shown in Figs. 5 and 7 were eluted from the silica gel, which was scraped from the located areas, with chloroform/methanol/acetic acid/water (50 : 39 : 1 : 10, v/v). The labeled lipids were counted in 5 ml Triton X-100-toluene scintillation medium containing 0.6 ml water with a Beckman LS-250 liquid scintillation spectrometer. [^{14}C]C.O., [^{14}C]cholesteryloleate; [^{3}H]G.L., [^{3}H]lactosylceramide, [^{3}H]galactosylceramide, or [^{3}H]glucosylceramide.

Expt.	Assays	Addition of transfer protein	Distribution of radioactivities (dpm)			
			Donor liposomes		Acceptor liposomes	
			[^{14}C]C.O.	[^{3}H]G.L.	[^{14}C]C.O.	[^{3}H]G.L.
1	Lactosylceramide transfer	+	64782	248747	921	72841
		−	64162	297182	766	4214
	Galactosylceramide transfer	+	62133	323456	1140	63812
		−	62643	363440	1704	8738
	Glucosylceramide transfer	+	73076	270121	1433	111441
		−	69476	350197	633	5225
2	Lactosylceramide transfer	+	71083	231151	2678	89836
		−	73874	305454	3433	15867
	Galactosylceramide transfer	+	88010	242685	3949	94422
		−	86410	287866	4046	14292
	Glucosylceramide transfer	+	70683	229022	3156	156510
		−	71372	357055	4046	18446

and the amount was not affected by the addition of the Sephadex G-75 fraction to the incubation mixture (Table I and Fig. 5). As shown by the chromatogram in which the lipids was located by charring with sulfuric acid (lanes 7 in Fig. 5), no marked change in the lipid composition of the acceptor liposomes was found after incubation for 60 min with the Sephadex G-75 fraction. Table I shows that the decrease in the [^{3}H]glycosphingolipid radioactivity in the donor liposomes balances well with the increase in the [^{3}H]glycosphingolipid radioactivity in the acceptor liposomes; the results indicate that a loss of ^{3}H-radioactivity from the donor liposomes by glycosidase reactions is negligible.

Fig. 6 shows the effects of various glycosphingolipids added as a constituent of the acceptor liposomes on the transfer rates of either [^{3}H]galactosylceramide, [^{3}H]lactosylceramide, or [^{3}H]glucosylceramide. In all three glycosphingolipid transfers, it was found that the transfer rates were highest when the acceptor liposomes contained no glycosphingolipid. Addition of one mol% of either galactosylceramide, glucosylceramide, or lactosylceramide to the acceptor liposomes as a constituent caused a decrease in the measured rates of all three glycosphingolipid transfers: the effect was strongest with galactosylceramide, intermediate with glucosylceramide, and weak with lactosylceramide; the decrease was most prominent in the lactosylceramide transfer, appreciable in the glucosylceramide transfer, and small in the galactosylceramide transfer. As shown in Fig. 7 and Table I, each of the three [^{3}H]glycosphingolipids was truly translocated from the donor liposomes to the acceptor liposomes containing no glycosphingolipid. In this experiment, the acceptor liposomes instead of the donor liposomes were made reactive to concanavalin A. Therefore the results shown in Fig. 7 and Table I unambiguously

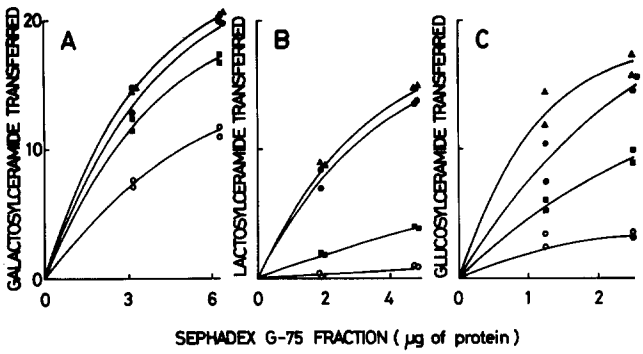

Fig. 6. Effects of various glycosphingolipids in acceptor liposomes on the transfer rates of [^{3}H]galactosylceramide, [^{3}H]lactosylceramide, and [^{3}H]glucosylceramide from the donor to acceptor liposomes. The transfer activities were determined in duplicate under the standard conditions by the assay procedure A except that acceptor liposomes containing the indicated glycosphingolipid at 1 mol% were used in each assay. Incubations were at 25°C for 60 min. Results are shown as percentage of a [^{3}H]glycosphingolipid transferred to the acceptor liposomes per 60 min. ○, galactosylceramide in the acceptor liposomes; ●, lactosylceramide in the acceptor liposomes; ■, glucosylceramide in the acceptor liposomes; △, no glycosphingolipid in the acceptor liposomes. A, galactosylceramide transfer reaction; B, lactosylceramide transfer reaction; C, glucosylceramide transfer reaction.

TABLE II

EFFECT OF GLYCOSPHINGOLIPID CONTENT OF LIPOSOMES ON THE ACTIVITY OF THE GLYCOSPHINGOLIPID TRANSFER PROTEIN

The glycosphingolipid transfer activities were determined in duplicate under the standard conditions by the assay procedure A except that the donor and acceptor liposomes containing the indicated concentrations of one of the glycosphingolipids indicated were used in each assay. Incubations were at 25°C for 60 min. Each transfer activity was measured at two different concentrations of protein: each incubation mixture of galactosylceramide, lactosylceramide, and glucosylceramide transfer assays contained 3.0 μg or 5.9 μg protein, 1.8 μg or 4.4 μg protein, and 1.5 μg or 3.0 μg protein, respectively, of the Sephadex G-75 fraction. Results are shown as mean±S.D. of the four determinations.

Glycosphingolipids	Glycosphingolipid exchange activities (nmol/mg per min)	
Glycosphingolipid content of liposomes	1 mol%	10 mol%
Galactosylceramide	0.50±0.05	2.86±0.48
Lactosylceramide	1.04±0.01	2.35±0.25
Glucosylceramide	2.89±0.61	3.80±0.58

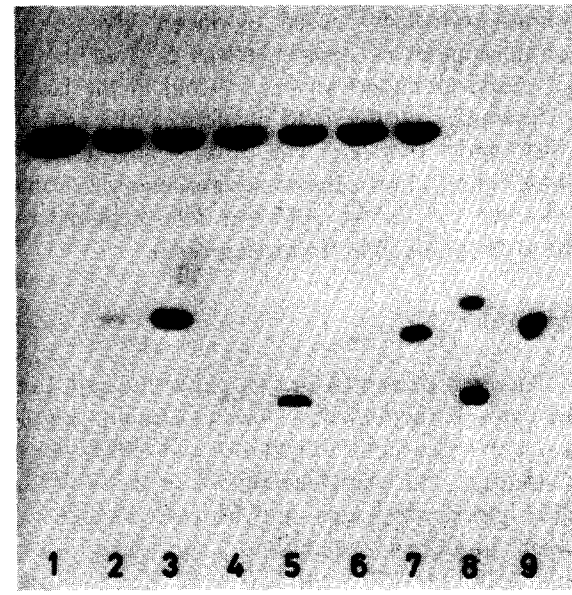

Fig. 7. Thin-layer chromatogram of lipid extracts from the acceptor liposome fractions obtained after the galactosylceramide, lactosylceramide, and glucosylceramide transfer reactions, where liposomes lacking in glycosphingolipids were used as an acceptor. The experiments were performed in the same way as described in Fig. 5 except that acceptor liposomes contained no glycosphingolipid and the acceptor liposomes instead of the donor liposomes contained dimannosyldiacylglycerol and therefore were made precipitable by concanavalin A. Lanes 2, 4 and 6, incubations performed without addition of the glycosphingolipid transfer protein; lanes 3, 5 and 7, incubations performed in the presence of 29.6 μg protein of the Sephadex G-75 fraction. Lanes 2 and 3, glucosylceramide transfer reaction; lanes 4 and 5, lactosylceramide transfer reaction; lanes 6 and 7, galactosylceramide transfer reaction; lane 1, [^{14}C]cholesteryloleate; lane 8, [^{3}H]glucosylceramide (upper spot) and [^{3}H]lactosylceramide (lower spot); lane 9, [^{3}H]galactosylceramide. A fluorography pattern was shown.

proved the translocation of [^{3}H]glycosphingolipids from the donor liposomes to the concanavalin A-precipitable structure, liposomes, in the glycosphingolipid transfer reactions.

Table II shows the effect of glycosphingolipid content of the donor and acceptor liposomes on the rates of the three glycosphingolipid transfers. In all three glycosphingolipid transfer assays, larger amounts of glycosphingolipids were exchanged between liposomes when the assay was performed using donor and acceptor liposomes containing 10 mol% of a glycosphingolipid instead of using liposomes containing one mol% of the glycosphingolipid: the increase in the rates of glycosphin-

golipid exchanges caused by larger glycosphingolipid content of liposomes was 5.7-fold in the galactosylceramide transfer, 2.3-fold in the lactosylceramide transfer, and 1.3-fold in the glucosylceramide transfer. The results suggest that phospholipids in the liposomes participate in some unknown way in the lipid transfers mediated by the glycosphingolipid transfer protein.

Discussion

The data presented in this paper indicate that the cytosol fraction of rat brains contains a protein with a molecular weight of about 18000 which accelerates the translocation of galactosylceramide, glucosylceramide, and lactosylceramide between liposomal membranes. The three glycosphingolipid transfer activities of rat brain cytosol were about 0.3–0.6 nmol of the glycosphingolipid exchanges between liposomes/30 min per mg protein when the activities were assayed using liposomes containing 1 mol% of a glycosphingolipid. Under the conditions of our liposome-liposome assay of lipid exchange activities, the cytosol fraction of rat liver contained 23.4 nmol/30 min per mg protein of phosphatidylcholine exchange activity and 7.5 nmol/30 min per mg protein of phosphatidylinositol exchange activity [23]. According to Helmkamp et al. [2] the membrane-free supernatant of bovine brain cortical tissue contained 6.9 nmol/30 min per mg protein of phosphatidylinositol exchange activity when the activity was assayed by the transfer of phosphatidyl[^{3}H]inositol from microsomes to liposomes. Therefore the glycosphingolipid transfer activities amount to less than one twentieth of the phospholipid exchange activities. However, a few-fold higher activities of the glycosphingolipid transfers than those described above will be found if the activities are measured by the use of liposomes containing larger amount of the glycosphingolipid being assayed, since results shown in Table II indicate that the rates of the glycosphingolipid transfers are dependent on the glycosphingolipid content of the liposomes used in the assay.

The galactosylceramide transfer activity found in the cytosol fraction of suckling rat brains was about 60% of the activity in the cytosol fraction of adult rat brain. The activity in suckling rat brains was eluted from a Sephadex G-75 column at the same position as the activity in adult rat brain. However, the activity in the Sephadex G-75 fraction of suckling rat brains gradually increased on storage at 4°C: in a preparation 9-fold activation was found after 2 months. The galactosylceramide transfer activity was also found in rat liver (Yamada, K. and Sasaki, T., unpublished data), which does not contain any significant level of galactosylceramide [24]. It is totally unknown whether the galactosylceramide transfer activity we found in the brain cytosol is involved in the metabolism of galactosylceramide in myelin.

As shown in Fig. 6, the three glycosphingolipid transfer reactions proceed most efficiently when the acceptor liposomes contained no glycosphingolipid. This results imply either of the two possibilities: the first one is that the transfer protein acts in an unidirectional transfer of glycosphingolipids which will cause a small increase in the mass of each acceptor liposome particle as a result of the transfer; the second one is that the transfer protein accelerates an exchange between glycosphingolipid molecules in the donor liposomes and phospholipid molecules in the acceptor liposomes. The results shown in Fig. 6 suggest that the three glycosphingolipid transfers studied in this paper are mediated by the activity of a single protein because the inhibitory effects of galactosylceramide and glucosylceramide in the acceptor liposomes on the glycosphingolipid transfer activities were commonly found in all three glycosphingolipid transfer reactions. A clear answer to these questions concerning the lipid specificity of the glycosphingolipid transfer protein can only be obtained after a purification of the protein, which is now in progress in our laboratory.

Bloj and Zilversmit [25] showed that bovine liver lipid transfer protein with broad lipid specificity, which accelerated the transfer of various glycerophospholipids, sphingomyelin, and cholesterol [3,4], also facilitated the transfer of globoside and GM_1 ganglioside from liposomes to erythrocyte ghosts. It seems that the glycosphingolipid transfer protein described in this paper is different from the nonspecific lipid transfer protein purified by Bloj and Zilversmit [3] for the two reasons: firstly the Sephadex G-75 fraction of our glycosphingolipid transfer protein did not accel-

erate the transfer of phosphatidylethanolamine; secondly our glycosphingolipid transfer protein was eluted from a Sephadex G-75 column at the position of a protein with a molecular weight of about 18000, whereas the molecular weight of the nonspecific lipid transfer protein purified by Bloj and Zilversmit [3] from rat liver had been estimated to be 13500 by a Sephadex G-50 gel filtration.

It has been shown that glycosphingolipids are exposed on cell surfaces [9–11]. Specific biological functions as ligand molecules on cell surfaces have been implicated to glycosphingolipids [26–29]. Although the plasma membrane has been shown to possess several glycosyltransferases possibly involved in the glycosphingolipid biosynthesis [30–32], the major site of the glycosphingolipid biosynthesis has been shown to be the Golgi complex [12–14]. Therefore, there should exist some mechanism of the intracellular translocation of glycosphingolipids. This mechanism has not been studied until quite recently [15,25]. The protein described in this paper may possibly function in the intracellular translocation of glycosphingolipids. Now, it seems important to find out proteins which facilitate the intermembranous translocation of such major glycosphingolipids as globoside, hematoside, and GD_{1a} ganglioside.

Acknowledgement

We wish to thank Professor Toshio Sakagami for discussions and encouragement.

References

1 Kamp, H.H., Wirtz, K.W.A. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 318, 313–325
2 Helmkamp, G.M., Jr., Harvey, M.S., Wirtz, K.W.A. and Van Deenen, L.L.M. (1974) J. Biol. Chem. 249, 6382–6389
3 Bloj, B. and Zilversmit, D.B. (1977) J. Biol. Chem. 252, 1613–1619
4 Crain, R.C. and Zilversmit, D.B. (1980) Biochemistry 19, 1433–1439
5 DiCorleto, P.E., Warach, J.B. and Zilversmit, D.B. (1979) J. Biol. Chem. 254, 7795–7802
6 Van Golde, L.M.G., Oldenborg, V., Post, M., Batenburg, J.J., Poorthuis, B.J.H.M. and Wirtz, K.W.A. (1980) J. Biol. Chem. 255, 6011–6013
7 Lumb, R.H., Kloosterman, A.D., Wirtz, K.W.A. and Van Deenen, L.L.M. (1976) Eur. J. Biochem. 69, 15–22
8 Yamada, K., Sasaki, T. and Sakagami, T. (1978) J. Biochem. 84, 855–863
9 Gahmberg, C.G. and Hakomori, S. (1975) J. Biol. Chem. 250, 2438–2446
10 Moss, J., Manganiello, V.C. and Fishman, P.H. (1977) Biochemistry 16, 1876–1881
11 Willison, K.R. and Stern, P.L. (1978) Cell 14, 785–793
12 Richardson, C.L., Keenan, T.W. and Morré, D.J. (1977) Biochim. Biophys. Acta 488, 88–96
13 Keenan, T.W., Morré, D.J. and Basu, S. (1974) J. Biol. Chem. 249, 310–315
14 Pacuszka, T., Duffard, R.O., Nishimura, R.N., Brady, R.O. and Fishman, P.H. (1978) J. Biol. Chem. 253, 5839–5846
15 Metz, R.J. and Radin, N.S. (1980) J. Biol. Chem. 255, 4463–4467
16 Sasaki, T. and Sakagami, T. (1978) Biochim. Biophys. Acta 512, 461–471
17 Radin, N.S. (1972) in Methods in Enzymology (Ginsburg, V., ed.), Vol. 28, pp. 300–306, Academic Press, New York
18 Takagi, R. and Sasaki, T. (1979) J. Biochem. 85, 29–39
19 Sasaki, T. and Sakagami, T. (1979) Tohoku J. Exp. Med. 128, 139–149
20 Sugita, M., Shirai, S., Itasaka, O. and Hori, T. (1975) J. Biochem. 77, 125–130
21 Hellings, J.A., Kamp, H.H., Wirtz, K.W.A. and Van Deenen, L.L.M. (1974) Eur. J. Biochem. 47, 601–605
22 Laskey, R.A. and Mills, A.D. (1975) Eur. J. Biochem. 56, 335–341
23 Sasaki, T., Yamada, K. and Sakagami, T. (1979) Tohoku J. Exp. Med. 128, 367–376
24 Handa, S., Ueno, K., Kushi, Y. and Rokukawa, C. (1981) in Glycoconjugates (Yamakawa, T., Osawa, T. and Handa, S., eds.), (Proc. 6th Int. Symp. Glycoconjugates), pp. 173–174, Japan Scientific Societies Press, Tokyo
25 Bloj, B. and Zilversmit, D.B. (1981) J. Biol. Chem. 256, 5988–5991
26 Kasai, M., Iwamori, M., Nagai, Y., Okumura, K. and Tada, T. (1980) Eur. J. Immun. 10, 175–180
27 Hakomori, S. (1975) Biochim. Biophys. Acta 417, 55–89
28 Hakomori, S. (1980) in Tumor Cell Surfaces and Malignancy (Hynes, R.O. and Fox, C.F., eds.), pp. 873–886, Alan R. Liss, Inc., New York
29 Mullin, B.R., Fishman, P.H., Lee, G., Aloj, S.M., Ledley, F.D., Winand, R.J., Kohn, L.D. and Brady, R.O. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 842–864
30 Yogeeswaran, G., Laine, R.A. and Hakomori, S. (1974) Biochem. Biophys. Res. Commun. 59, 591–599
31 Cummings, R.D., Cebula, T.A. and Roth, S. (1979) J. Biol. Chem. 254, 1233–1240
32 Sasaki, T. (1981) Biochim. Biophys. Acta 666, 418–425