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A NEW ASSAY SYSTEM OF PHOSPHOLIPID EXCHANGE ACTIVITIES USING CONCANAVALIN A IN THE SEPARATION OF DONOR AND ACCEPTOR LIPOSOMES

TERUKATSU SASAKI and TOSHIO SAKAGAMI

Department of Biochemistry, Sapporo Medical College, Sapporo (Japan)

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Summary

A new assay system of phospholipid exchange activities is described. The exchange activities were quantitated by measuring the stimulation of phospholipid transfer between two separate populations of liposomes, which contained, as the major constituents, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, and cholesterol in molar ratios of 6 : 2 : 1 : 1 : 5. One population of the liposomes was made reactive to concanavalin A by the incorporation of 1.8 mol% α-D-mannosyl-(1 → 3)-α-D-mannosyl-*sn*-1,2-diglyceride from *Micrococcus lysodeikticus*. The concanavalin A-reactive liposomes, a phospholipid donor, were doubly labelled with [6-^{3}H]galactosyl-glucosyl ceramide and that class of ^{32}P-labelled phospholipids whose exchange was being measured. The ^{3}H-labelled glycolipid served as a non-exchangeable reference marker. The other population of the liposomes, a phospholipid acceptor, was concanavalin A nonreactive. These two populations of liposomes were incubated with the cytosol protein of rat liver in a total volume of 0.2 ml.

After the incubation, two different procedures were used to separate the two liposomal populations. In one procedure concanavalin A was added to agglutinate the reactive liposomes; the flocculated lectin · liposome complex was separated from the non-reactive liposomes by brief centrifugation. In the other procedure the reactive liposomes were trapped by binding to concanavalin A covalently coupled to Sepharose 2B; the complex was separated from the non-reactive liposomes by filtration through a filter paper under suction. In both assay procedures the amount of phospholipid transferred from the donor to the acceptor liposomes was calculated from the decrease of ^{32}P/^{3}H ratio of the concanavalin A-reactive liposomes during the incubation. By the assay system it is possible to determine phosphatidylcholine and phosphatidylinositol exchange activities in 100 μg of rat liver cytosol protein.

Introduction

Since the intracellular exchange of phospholipids between microsomes and mitochondria was found [1—3], phospholipid exchange proteins have been isolated from bovine liver, heart and brain, and from rat liver and intestine [4—11]. In these purifications and characterizations of phospholipid exchange proteins, their activities were quantitated by measuring the stimulation of phospholipid transfer either between liposomes and microsomes or between liposomes and mitochondria. At the end of the exchange reaction in these assays, mitochondria or aggregated microsomes were separated from liposomes by centrifuging 2—4 ml incubation mixture at high speed.

In addition to the two widely used assay systems, two liposome-liposome assay systems have been used for the quantitation of phospholipid exchange activities. Ehnholm and Zilversmit [5,12] used the immunoprecipitation by anti-sheep erythrocyte antibody to separate Forssman glycolipid-sensitized liposomes from non-sensitized liposomes. Hellings et al. [13] employed a DEAE-cellulose column to separate liposomes containing different amounts of negatively charged phospholipids. These assay systems, in which phospholipid exchange activities are measured between similar types of liposomes, are simple in assay conditions and allow kinetic analyses of the exchange reaction [13, 14]. However, certain disadvantages can be pointed out for each procedure. The separation of two liposomal populations by the immunoprecipitation requires a relatively long time. Thus, for a fast exchange, the endpoint becomes rather uncertain. The use of an anion-exchange column in the separation of liposomes requires the incorporation of either 7 mol% phosphatidic acid or 9 mol% phosphatidylinositol in one liposomal population [13]. Since the negative charge on liposomes inhibits the activity of phospholipid exchange proteins [13,14], one is not able to study the exchange activity in the absence of inhibitor molecules.

In the first part of this paper we describe a liposome-liposome assay system of phospholipid exchange activities. Concanavalin A, a lectin from Jack beans, was used as a reagent to precipitate one population of liposomes, which was made reactive to the lectin by the incorporation of dimannosyl diglyceride from *Micrococcus lysodeikticus*. The glycolipid has the structure of 3-(*O*-α-D-mannopyranosyl-(1 → 3)-*O*-α-D-mannopyranosyl)-*sn*-1,2-diglyceride [15]. Above pH 7 concanavalin A consists largely of tetramers of identical subunits of molecular weight 25 500 [16—18]. Each subunit has a single carbohydrate binding site [19,20]. These properties of concanavalin A prompted us to use the lectin in the precipitation of one population of liposomes, which was made reactive to the lectin. In the second part of the paper, concanavalin A covalently coupled to Sepharose 2B was used to separate the two liposomal populations in a variant of the assay system.

Materials and Methods

Materials. Concanavalin A used in the precipitation reaction was obtained from Pharmacia Fine Chemicals (Uppsala). Concanavalin A used in the prepara-

tion of concanavalin A-Sepharose 2B was a product of Sigma Chemical Company (St. Louis).

Thin-layer chromatography. The chromatography was carried out on Silica gel H plates (Merck, Darmstadt), which were prepared from a slurry in 1 mM Na_2CO_3. Solvents used were: (A) chloroform/methanol/acetic acid/water (75 : 45 : 11 : 5, by vol.) [21]; (B) chloroform/methanol/water (65 : 25 : 4, by vol.); (C) tetrahydrofuran/methylal/methanol/water (10 : 6 : 4 : 1, by vol.) [22]. Lipids were located with 2',7'-dichlorofluorescein, and eluted from the gel according to the method of Arvidson [23].

Preparation of lipids. Lipids were extracted from homogenized rat liver with chloroform/methanol (2 : 1, by vol.). The extract was washed according to the procedure of Folch et al. [24]. The lipids were separated into groups by DEAE-cellulose column chromatography according to the method of Rouser et al. [25]. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and sphingomyelin were purified from DEAE-cellulose fractions by the thin-layer chromatography in solvent A.

^{32}P-labelled phospholipids were prepared from the liver of a rat, which was injected intraperitoneally with 15 mCi [^{32}P]phosphate (Japan Isotope Association, Tokyo) 18 h before the excision of the liver. The liver was diced and lipids were extracted as described above. Phospholipid classes in the extract were separated by DEAE-cellulose column chromatography and thin-layer chromatography as described above.

Dimannosyl diglyceride was isolated in the following way. Spray-dried *M. lysodeikticus* cells (Miles Laboratories, Elkhart) were suspended in water and extracted with chloroform/methanol (2 : 1, by vol.). The extract was washed by the method of Folch et al. [24]. Dimannosyl diglyceride, the major glycolipid of the extract [15], was purified by the thin-layer chromatography in solvent B and in solvent C.

[6-^{3}H]Galactosylglucosyl ceramide (70.3 μCi/μmol) was prepared according to the method of Radin [26] by the sodium boro[^{3}H]hydride (New England Nuclear, Boston) reduction of the aldehyde, which was derived from lactosyl ceramide by the oxidation with galactose oxidase (Worthington Biochemical Corp., Freehold). Lactosyl ceramide was prepared from hematoside by hydrolysis in 0.1 M H_2SO_4 at 80°C for 2 h to remove the *N*-glycolylneuramic acid residue. Hematoside was isolated from equine erythrocyte stroma. Lactosyl ceramide, the aldehyde, and [^{3}H]lactosyl ceramide were purified by the thin-layer chromatography in solvent B.

Preparation of liposomes. 1 ml of concanavalin A-reactive liposome preparation contained 0.9 μmol of a mixture of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and sphingomyelin in molar ratios of 6 : 2 : 1 : 1, 0.45 μmol cholesterol, 7.48 nmol of [^{3}H]lactosyl ceramide, and 24.3 nmol of dimannosyl diglyceride in 10 mM sodium phosphate (pH 7.0) containing 0.15 M NaCl (hereafter referred to as phosphate-buffered saline). In each experiment, that class of phospholipid in the reactive liposomes whose exchange was being measured contained ^{32}P label at a specific activity of about 0.5 μCi/μmol. 1 ml of concanavalin A-non-reactive liposome preparation contained in phosphate-buffered saline 2.25 μmol of a mixture of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and sphingomyelin in

molar ratios of 6 : 2 : 1 : 1, and 1.13 μmol of cholesterol.

Liposomes were prepared in a volume of 1.0—1.5 ml in the following way. Constituent lipids in chloroform were mixed and dried under a stream of nitrogen. An appropriate volume of phosphate-buffered saline was added to the dried lipids. After 30 min incubation at 25°C, the lipids were dispersed into the buffer by sonication with a Branson Sonifier (model W 185) at a 35 W output for 30 min in an ice-water-bath under a stream of nitrogen. The liposome preparation was centrifuged at 1500 × *g* for 30 min to remove any undispersed lipid and titanium fragments released from the sonication probe.

Preparation of rat liver cytosol fraction. Male rats of the Wistar strain were starved overnight and decapitated before the perfusion of the liver via the portal vein with ice-cold 0.9% NaCl containing 1 mM Na—EDTA (pH 7.4). The livers were removed and minced. A 30% homogenate was made in phosphate-buffered saline with four strokes of a Teflon pestle in a glass homogenizer. The homogenate was centrifuged at 15 000 × *g* for 20 min. The supernatant fraction was centrifuged at $10^5 \times g$ for 60 min. The $10^5 \times g$ supernatant fraction was kept frozen until use. In this paper protein in the $10^5 \times g$ supernatant fraction is called cytosol protein.

Preparation of concanavalin A-Sepharose 2B. 100 ml (sedimented volume) of Sepharose 2B were activated by 2 g of cyanogen bromide according to the method of Porath and Kristiansen [27]. The activated gel was coupled to 1 g concanvalin A in 1 M NaCl/0.07 M $NaHCO_3$/0.05 M methyl-α-D-glucopyranoside/1 mM $MnCl_2$/1 mM $CaCl_2$. After completion of the coupling reaction, excess activated groups were blocked by glycine.

Assay of phospholipid exchange activity by precipitating concanavalin A-reactive liposomes with concanavalin A (assay procedure A). Phospholipid exchange activities were measured by determining the transfer of a class of ^{32}P-labelled phospholipids from the reactive liposomes to the non-reactive liposomes. The standard incubation mixture contained 45 nmol lipid-phosphorus of the reactive liposomes as a phospholipid donor, 112.5 nmol lipid-phosphorus of the non-reactive liposomes as a phospholipid acceptor, and at most, 250 μg of the cytosol protein in 0.2 ml of phosphate-buffered saline. The donor liposomes were doubly labelled with [^{3}H]lactosyl ceramide and that class of ^{32}P-labelled phospholipids whose exchange was being measured in the particular experiment. [^{3}H]Lactosyl ceramide was added as a non-exchangeable marker. The mixture was incubated at 25°C for 30 min or the indicated time with gentle shaking. At the end of the incubation, the mixture was chilled on ice and 20 μl of 10 mM *N*-ethylmaleimide in phosphate-buffered saline were added, since the SH-binding reagent has been reported to inhibit some phospholipid exchange proteins [6,28]. 30 μl concanavalin A in phosphate-buffered saline (20 mg/ml), were mixed with the incubation mixture. The mixture was allowed to stand overnight at 4°C. The floccules formed were precipitated by centrifugation at 1200 × *g* for 20 min. The supernatant was decanted. The inside above the bottom of the tube was wiped with a piece of paper. The pellet was dispersed in 0.6 ml of phosphate-buffered saline containing 0.1 M methyl-α-D-glucopyranoside, a 'haptenic' inhibitor of the concanavalin A binding. The dispersed material was decanted into a vial. After being mixed with 5 ml of Patterson and Greene's scintillation medium containing 33% Triton

X-100 [29], the sample was counted in a Beckman LS 250 liquid scintillation spectrometer with an autoquench compensator. ^{3}H radioactivity was corrected to 100% relative efficiency by external standard. The extent of the phospholipid transfer from the donor liposomes to the acceptor liposomes was measured from the $^{32}P/^{3}H$ ratio (X) of the reactive liposomes precipitated from the incubation mixture. Percentage of ^{32}P-labelled phospholipid transferred is calculated as $(1 - XY) \times 100\%$. Where Y is the $^{3}H/^{32}P$ ratio of the reactive liposomes precipitated from the reaction mixture that was incubated in the absence of phospholipid exchange proteins. In the assay, the [^{3}H]lactosyl ceramide served as a reference marker to correct for incomplete recovery of the donor liposomes.

Assay of phospholipid exchange activity by binding of concanavalin A-reactive liposomes to concanavalin A-Sepharose 2B (assay procedure B). In this procedure the incubation conditions were the same as described in assay procedure A. At the end of the incubation, the mixture was chilled on ice and 0.3 ml of concanavalin A-Sepharose 2B suspension was added; the suspension was prepared by adding 1 vol. of phosphate-buffered saline to one sedimented volume of concanavalin A-Sepharose 2B. The mixture was allowed to stand for 10 min in ice and then filtered by suction through Toyo No. 2 filter paper (diameter, 2.5 cm, Toyo Roshi, Tokyo). Concanavalin A-Sepharose 2B retained on the filter was washed with nine 5-ml portions of ice-cold phosphate-buffered saline. Washed concanavalin A-Sepharose 2B on the filter was placed in a vial with 0.5 ml water and 5 ml Triton-toluene scintillation medium, and counted in a liquid scintillation spectrometer. Percentage of ^{32}P-labelled phospholipid that was transferred from the donor liposomes to the acceptor liposomes was calculated as described above from the $^{32}P/^{3}H$ ratio of the reactive liposomes bound to concanavalin A-Sepharose 2B.

Analytical methods. Protein was determined by the method of Lowry et al. [30] with bovine serum albumin as a standard. Total lipid phosphorus was determined according to the method of Bartlett [31]. Carbohydrate concentration was determined by the phenol-sulfuric acid reaction [32].

Results

Separation of two populations of liposomes by concanavalin A. When dimannosyl diglyceride from *M. lysodeikticus* was incorporated into liposomes made from phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, and cholesterol in molar ratios of 6 : 2 : 1 : 1 : 5, concanavalin A agglutinated the liposomes; after several hours at 4° C the complex grew to floccules easily precipitable on brief centrifugation. We found that at least one molecule of dimannosyl diglyceride was required to be incorporated in 52 molecules of phospholipids to obtain good precipitation. In all the experiments presented below, concanavalin A-reactive liposomes were prepared at a molar ratio of dimannosyl diglyceride to phospholipid of 1 : 37. Concanavalin A-non-reactive liposomes and the reactive liposomes were used as two populations of liposomes separable by the lectin-mediated precipitation.

Fig. 1 shows the separation, by the precipitation method, of the two populations of liposomes under the standard assay conditions of phospholipid

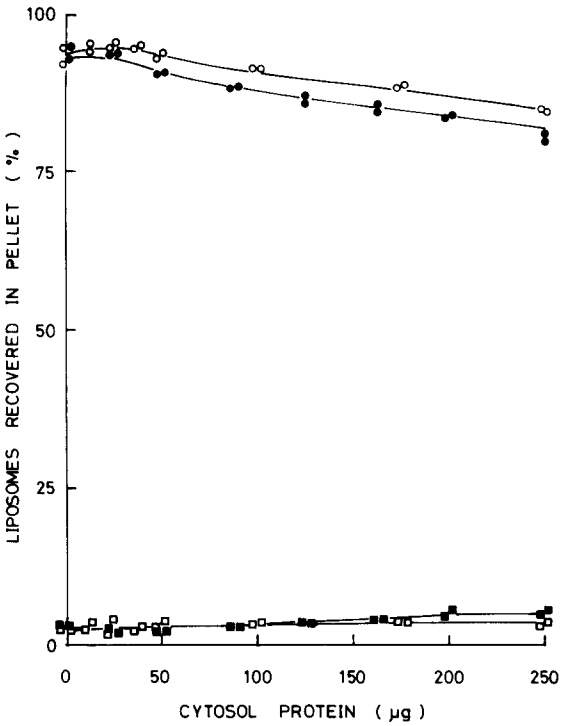

Fig. 1. Separation of concanavalin A-reactive liposomes from concanavalin A-non-reactive liposomes by the precipitation method under the assay conditions of phospholipid exchange activity. In the reaction mixture described in assay procedure A, [^{3}H]lactosyl ceramide served as the tracer to measure the recovery of the reactive liposomes in the pellet. The recovery of the non-reactive liposomes in the supernatant was measured in a modified incubation mixture, in which a trace of [^{3}H]lactosyl ceramide was added as a constituent of the non-reactive liposomes instead of the reactive liposomes. After precipitation of aggregated liposomes, the supernatant was decanted into a vial. Radioactivity in the supernatant was counted in 5 ml Triton-toluene scintillation medium and an additional 0.4 ml of water. Results from duplicate incubations were shown as percentage of ^{3}H radioactivity in the reactive liposomes or in the non-reactive liposomes that was recovered in the pellet as compared with the total ^{3}H radioactivity present both in the supernatant and in the pellet. ○, the reactive liposomes after 30 min or (●) 60 min of incubation; □, the non-reactive liposomes after 30 min or (■) 60 min of incubation.

exchange activity. The two populations of liposomes were mixed at a lipid-phosphorus ratio of 1 : 2.5 and incubated at 25°C for 30 or 60 min in the presence of various amounts of rat liver cytosol protein. As shown, a good separation of the two populations of liposomes was obtained by the method in the presence of up to 250 μg of the cytosol protein, an amount which was enough to measure phospholipid exchange activities. With an increase in the amount of the cytosol protein, the recovery of the reactive liposomes in the pellet and that of the non-reactive liposomes in the supernatant decreased to 80% and 95%, respectively, after 60 min of incubation.

Assay of phospholipid exchange activity by the concanavalin A-mediated precipitation of concanavalin A-reactive liposomes. Phospholipid exchange activities in rat liver cytosol were quantitated by measuring the stimulation of phospholipid transfer from the reactive liposomes to the non-reactive liposomes. Fig. 2, A and B shows the results of assays of phosphatidylcholine and

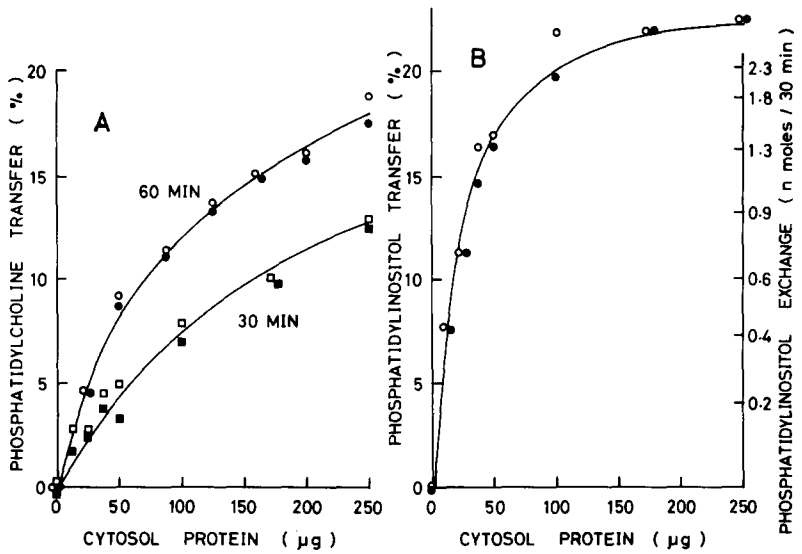

Fig. 2. Effect of various amounts of the cytosol protein on the exchanges of phosphatidylcholine and phosphatidylinositol between the two populations of liposomes assayed by assay procedure A. A. The phosphatidylcholine exchange. The exchange activity was assayed under the standard conditions except that each mixture was incubated for 60 or 30 min as indicated. B. The phosphatidylinositol exchange. The exchange activity was assayed under the standard conditions. Each incubation mixture in A and B contained the indicated amount of the cytosol protein. Open and closed symbols represent results from duplicate incubations. Where superimposition of values occurs, the symbols are inserted side by side for clarity.

phosphatidylinositol exchange activities by the precipitation method. As shown in Fig. 2A, the transfer of [^{32}P]phosphatidylcholine from the donor to the acceptor liposomes increased with increase in the amount of the cytosol protein at least up to 250 μg per incubation mixture. Fig. 2A shows that the percentage of the [^{32}P]phosphatidylcholine transfer, which was brought about by a certain amount of cytosol protein on incubation for 60 min, was also brought about by about twice that amount on incubation for 30 min. As shown in Fig. 2B, along with the addition of increasing amounts of the cytosol protein up to 175 μg per incubation, the [^{32}P]phosphatidylinositol transfer between the two liposomal populations gradually approached the equilibrium point, where about 22.6% of [^{32}P]phosphatidylinositol were found to be transferred from the donor to the acceptor liposomes under the conditions.

Gel filtration on a Sepharose 4B column and electron microscopic examination by negative staining showed that the liposomes used were heterogeneous with respect to both size and shape. Therefore, the fraction of the liposomal phospholipid available for the exchange reaction cannot be estimated by a simple assumption. However, from the percentage of the ^{32}P-labelled phospholipid that is transferred to the acceptor liposomes at the equilibrium of the ^{32}P-labelled phospholipid transfer, it is possible to estimate the fraction of the liposomal phospholipid that can participate in the exchange reaction. 31.6% of the liposomal phosphatidylinositol was estimated to have participated in the exchange reaction shown in Fig. 2B. By the use of the estimated value, the quantity of the phospholipid exchanged between the two liposomal populations

can be calculated as described by Hellings et al. [13] from the percent value of the ^{32}P-labelled phospholipid that is transferred to the acceptor liposomes. The calculated values for the phosphatidylinositol exchange are given in nmol per incubation on the right ordinate of Fig. 2B.

We found that the major portion of [^{32}P]phosphatidylcholine and [^{32}P]phosphatidylinositol was transferred intact under the conditions of the phospholipid exchange reaction described above.

Assay of phospholipid exchange activity by binding of concanavalin A-reactive liposomes to concanavalin A-Sepharose 2B. In the precipitation method assay, the formation of macroscopic floccules, an aggregate formed by concanavalin A and the reactive liposomes, is required in the separation of the reactive liposomes from the non-reactive liposomes. After addition of concanavalin A into the incubation mixture, overnight standing at 4° C is necessary to obtain a good floccule formation. In order to separate the two populations of liposomes promptly after the exchange reaction, concanavalin A coupled to cyanogen bromide-activated Sepharose 2B was used in a procedure for the separation of these liposomes. In this procedure, the complex formed by the reactive liposomes and concanavalin A-Sepharose 2B was trapped on filter paper and, thus, separated from the non-reactive liposomes by filtration.

Fig. 3 and 4 show the results of assays of phosphatidylcholine and phosphatidylinositol exchange activities. In these assays, where each incubation mixture either contained various amounts of the cytosol protein or were incubated for different times, about 40% of the reactive liposomes and about 6% of the non-reactive liposomes were trapped by concanavalin A-Sepharose 2B. Results of experiments on time courses shown in Fig. 4 indicate that by the procedure the

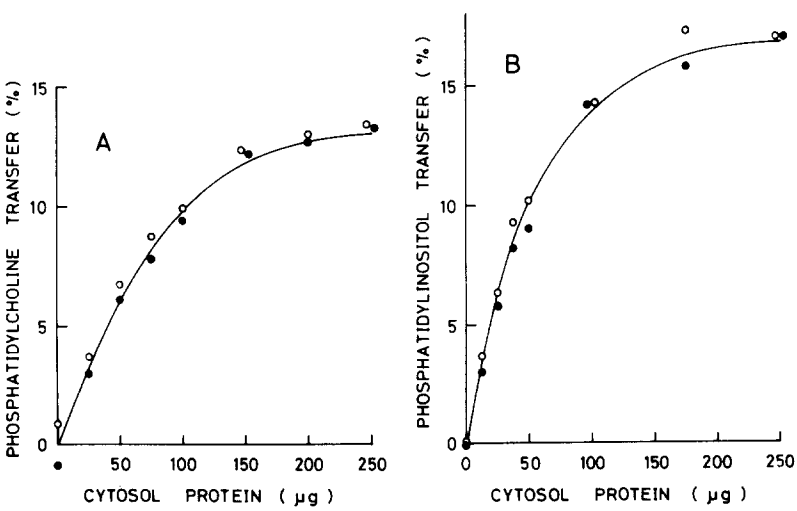

Fig. 3. Effect of various amounts of the cytosol protein on the exchanges of phosphatidylcholine and phosphatidylinositol between the two populations of liposomes assayed by assay procedure B. A. The phosphatidylcholine exchange. The exchange activity was assayed under the standard conditions except that the mixtures were incubated for 60 min. B. The phosphatidylinositol exchange. The exchange activity was assayed under the standard conditions. Each incubation mixture in A and B contained the indicated amount of the cytosol protein. ○ and ● represent results from duplicate incubations.

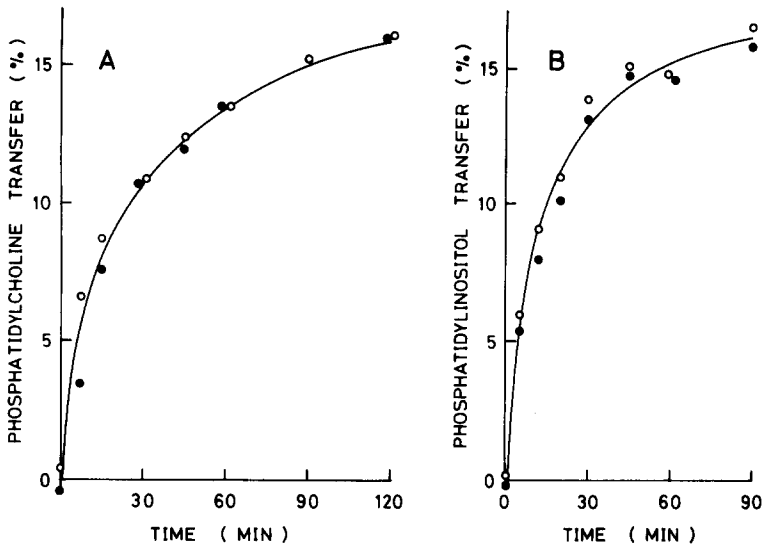

Fig. 4. Time courses of phosphatidylcholine and phosphatidylinositol exchange reactions catalyzed by the cytosol protein. The exchange activities were assayed under the standard conditions of assay procedure B except that each mixture was incubated for the indicated time at 25°C. A. The phosphatidylcholine exchange. Each incubation mixture contained 200 μg of the cytosol protein. B. The phosphatidylinositol exchange. Each incubation mixture contained 75 μg of the cytosol protein. ○ and ● represent results from duplicate incubations.

exchange reaction is stopped rapidly. Thus, the method can be used in the measurement of the rate of phospholipid exchange reaction.

We found that the reactive and the non-reactive populations of single bilayer phospholipid vesicles could be separated by the use of concanavalin A-Sepharose 2B but not by the concanavalin A-mediated precipitation. Phosphatidylcholine and phosphatidylinositol exchange activities of partially purified phospholipid exchange proteins, but not of the crude cytosol protein, can be measured by determining the transfer of these phospholipids between the two populations of liposomes, which contained, as the major constituents, phosphatidylcholine and phosphatidylinositol in a molar ratio of 9 : 1. More than 70% of the reactive liposomes of this composition were trapped by concanavalin A-Sepharose 2B under the conditions described in Materials and Methods.

Discussion

The assay system of phospholipid exchange activities described in this paper used concanavalin A to trap, either by precipitation or by binding to concanavalin A-Sepharose 2B, one population of liposomes, which contained a glycolipid with terminal α-(1 → 3)-linked mannose. Concanavalin A is convenient as a reagent in the assay because the lectin can be purified by a simple procedure and pure concanavalin A is stable on storage as a freeze-dried powder [33]. The binding specificity of concanavalin A has been extensively studied [34,35]; the lectin binds to saccharides with terminal non-reducing α-D-mannopyranosyl and α-D-glucopyranosyl residues, and to certain non-terminal mannose residues. In mammalian tissues no sphingoglycolipid containing either mannose or α-

linked glucose is known. Thus, liposomes made of the lipids from mammalian tissues presumably do not interact with concanavalin A by its saccharide-binding sites.

The assay system described in this paper, especially the precipitation method, is suitable for use in the purification of phospholipid exchange proteins. In the measurement of their activities throughout the steps of the purification, we found it convenient to use liposomes instead of microsomes or mitochondria as an acceptor and a donor of phospholipids. Assay of the exchange activities of different classes of phospholipids can be designed by replacing the labelled phospholipid in the concanavalin A-reactive liposomes from one class to another class. Moreover, by the present method exchange activities are assayed on a microscale.

A hydrophobic interaction between concanavalin A and phosphatidylcholine liposomes and monolayer has been reported [36,37]. At a surface pressure of 15 dynes/cm, but not at 25 dynes/cm or higher, concanavalin A binds to and penetrates dipalmitoyl phosphatidylcholine monolayers, which contain no saccharide to interact with the saccharide-binding sites of the lectin. Although these properties of concanavalin A raise some question about the usefulness of the lectin in the separation of two liposomal populations, the results shown in this paper prove that the lectin can be used for the purpose at least under the conditions used in this paper. When our procedure is compared with the two previously reported assay procedures of phospholipid exchanges between liposomes, certain advantages of our procedure can be pointed out. The separation of two liposomal populations by immunoprecipitation according to the procedure of Ehnholm and Zilversmit [12] requires relatively long time, which makes the endpoint of the exchange reaction uncertain. We have solved this problem by the use of concanavalin A-Sepharose 2B. When the method of Hellings et al. [13] is used in the assay of the phosphatidylinositol and other acidic phospholipid exchange activities, the liposomal content of one of these acidic phospholipids, which is being studied in a particular exchange reaction, is required to be as low as a few mol% of the total liposomal phospholipids to obtain a good separation of the two liposomal populations. Our method has no such limitation and is used successfully to quantitate the phosphatidylinositol exchange activity.

As non-exchangeable marker lipids, [^{3}H]cholesteryloleate, cholesteryl[^{14}C]-oleate, and [^{14}C]triolein have been used in the assay of phospholipid exchange activities [4—14]. In this paper [^{3}H]lactosyl ceramide was used for the purpose. The glycolipid was found to be non-exchangeable and only a small portion of the ^{3}H label was hydrolyzed during the incubation. Thus, the glycolipid serves as a good non-exchangeable reference marker.

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