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INTERACTION OF BOVINE BRAIN PHOSPHOLIPID EXCHANGE PROTEIN WITH LIPOSOMES OF DIFFERENT LIPID COMPOSITION

GEORGE M. HELMKAMP, Jr.

Department of Biochemistry, University of Kansas Medical Center, Kansas City, KS 66103 (U.S.A.)

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Summary

The major phospholipid exchange protein from bovine brain catalyzes the transfer of phosphatidylinositol and phosphatidylcholine between rat liver microsomes and sonicated liposomes. The effect of liposomal lipid composition on the transfer of these phospholipids has been investigated. Standard liposomes contained phosphatidylcholine-phosphatidic acid (98 : 2, mol%); in general, phosphatidylcholine was substituted by various positively charged, negatively charged, or zwitterionic lipids. The transfer of phosphatidylinositol was essentially unaffected by the incorporation into liposomes of phosphatidic acid, phosphatidylserine, or phosphatidylglycerol (5—20 mol%) but strongly depressed by the incorporation of stearylamine (10—40 mol%). Marked stimulation (2—4-fold) of transfer activity was observed into liposomes containing phosphatidylethanolamine (2—40 mol%). The inclusion of sphingomyelin in the acceptor liposomes gave mixed results: stimulation at low levels (2—10 mol%) and inhibition at higher levels (up to 40 mol%). Cholesterol slightly diminished transfer activity at a liposome cholesterol/phospholipid molar ratio of 0.81. Similar effects were noted for the transfer to phosphatidylcholine from microsomes to these various liposomes. Compared to standard liposomes, the magnitude of K_m tended to increase for liposomes which depressed phospholipid transfer and to decrease for those which stimulated; little change was observed in the values of V. Single phospholipid liposomes of phosphatidylinositol were inhibitory when added to standard liposomes.

Abbreviations: PtdIns, phosphatidylinositol; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine; PtdGro, phosphatidylglycerol. These abbreviations are in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature (1977) Hoppe-Seyler's Z. Physiol. Chem. 358, 617—631.

Because bovine brain phospholipid exchange protein is able to distinguish among a wide spectrum of membrane interfaces, taking into account variations in the polar head groups as well as the fatty acyl moieties of the liposomal phospholipids, it may be considered a reasonable model system for protein-lipid and protein-membrane interactions.

Introduction

The major phospholipid exchange protein from bovine brain catalyzes the transfer of phosphatidylinositol and, to a lesser extent, phosphatidylcholine between a variety of membrane surfaces [1]. Membranes participating in the transfer of phospholipids include rat liver mitochondria and microsomes [2], sonicated phospholipid liposomes [1], and, more recently, phospholipid monolayers [3]. Systematic changes in the concentration of two membrane surfaces, microsomes and liposomes, and the isolation of a stoichiometric phospholipid-protein complex yield results which can be interpreted in terms of a ping-pong kinetic mechanism for the protein-catalyzed, intermembrane transfer of phospholipid molecules [4]. The exchange protein is isolated from the cytosol fraction of cerebral cortex, purified to homogeneity, and has a molecular weight of 29 000 [1].

Previous experiments suggested that the rate of phospholipid transfer was also sensitive to the composition of the membrane surface. For example, increasing proportions of PtdIns in mixed PtdCho-PtdIns liposomes resulted in decreased transfer of PtdIns and PtdCho from microsomes to liposomes [5]. The inhibition was associated with decreased apparent Michaelis constants for the interaction between phospholipid exchange protein and liposomes [4]. A net transfer of PtdIns from microsomes or monolayers into liposomes which initially lack this phospholipid has also been demonstrated [3,5]. The sensitivity of phospholipid transfer toward liposomal lipid composition has been reported for several other purified exchange proteins, including the PtdCho exchange protein from bovine liver [6—8] and the phospholipid exchange protein from bovine heart [8,9].

The purpose of the present study is to extend these initial observations on the bovine brain exchange protein by incorporating into PtdCho liposomes a variety of positively and negatively charged, zwitterionic, and neutral lipids. Using these different liposome species, the rates of PtdIns and PtdCho transfer are measured and values of K_m and V are calculated.

Materials and Methods

Lipids. PtdCho was isolated from egg yolk [10] and served as the precursor of phosphatidic acid using a partially purified phospholipase D from Savoy cabbage [11]. A Folch extract of bovine liver was chromatographed on TEAE-cellulose to yield PtdEtn and PtdIns [12,13]. PtdIns and PtdGro, purchased from Serdary Research Laboratories, were further purified on CM-cellulose [14]. From bovine spinal cord were isolated PtdSer [15] and sphingomyelin [16]. All phospholipids were chromatographically pure when analyzed on thin

layers of silica gel HR developed in chloroform/methanol/acetic acid/water (50 : 25 : 7 : 3, by vol.) [17]. Acidic methanolysis of phospholipid samples (0.5 μmol) was performed as described by Ways et al. [18]; the resulting fatty acid methyl esters were analyzed by gas-liquid chromatography. Identification of the various fatty acids was made by comparison with commercially available standards (Nu Chek Prep., Inc.). The fatty acid compositions of the phospholipids were in reasonable agreement with previously reported analyses [19,28]. Cholesterol was purified as the dibromide derivative and recrystallized twice from diethyl ester/methanol (1 : 1, by vol.) [20]. Stearylamine was a product of P-L Biochemicals. *Myo*-[2-^{3}H]Inositol and [*methyl*-^{3}H]choline chloride were purchased from Amersham Corporation. Cholesteryl [1-^{14}C]oleate was purchased from New England Nuclear. Acidic phospholipids were converted to sodium salts [21]. Lipids were stored in chloroform/methanol (2 : 1, by vol.) under nitrogen at −20°C.

Phospholipid exchange protein. Using published procedures phospholipid exchange protein was purified to homogeneity from fresh bovine cerebral cortex [1]. A minor modification was the use of preparative, flat-bed isoelectric focussing (LKB Multiphor) during which a power of 10 W was maintained for 16 h at 5°C. The active fraction used in the present experiments resolved at a pH of 5.40—5.45. The protein was stored in 20 mM Tris-HCl (pH 7.4) containing 50% glycerol at −20°C.

Membrane preparations. Details of the preparation of rat liver microsomes labelled specifically with phosphatidyl-*myo*-[2-^{3}H]inositol or phosphatidyl-[*methyl*-^{3}H]choline have been described [1,3]. These microsomes contained, on the average, 830 nmol phospholipid/mg protein in the following molar distribution: 64.6% PtdCho, 23.4% PtdEtn, 4.7% PtdIns, 4.6% sphingomyelin, and 2.4% PtdSer. Liposomes were prepared in 50 mM Tris-HCl, 1 mM Na_2EDTA (pH 7.3) according to the method of de Gier et al. [22] and sonicated (Heat Systems model W185-F Sonicator) under nitrogen and in an ice bath at 60—80 W for 8—10 min. Following sonication the liposomes were centrifuged at 48 000 × *g* for 30 min to yield, in the supernate, a suspension of single bilayer vesicles. Verification of single bilayer vesicle formation was provided by chromatography and retention of the membranes on a 2.5 × 50 cm column of Sepharose 4B (Pharmacia Fine Chemicals), as described by Huang [23]. The inclusion of cholesteryl [1-^{14}C]oleate (less than 0.5 mol%) in each liposome preparation permitted an estimation of liposome recovery, 55—80%, following incubation and extraction.

Assay of phospholipid transfer activity. Phospholipid transfer activity was determined using rat liver microsomes as the donor membrane and liposomes as the acceptor membrane [1]. Briefly, 1.25 mg of microsomal protein and a variable amount of liposomal phospholipids were incubated in the presence or absence of exchange protein (1—3 μg) for 30 min at 37°C in 2.5 ml 50 mM Tris-HCl, 1 mM Na_2EDTA (pH 7.2). To terminate transfer, the reaction medium was acidified to pH 5.2, causing the microsomes to aggregate. Microsomes were pelleted at 20 000 × *g* for 10 min, and the liposomal lipids extracted from the supernate. Activity is expressed as nmol phospholipid transferred/h. In experiments in which the concentration of liposomes was varied, the data were expressed in double-reciprocal plots and fitted to a linear curve

by method of least squares. Regression coefficients ranged from 0.88 to 0.98.

Protein was determined by the method of Lowry et al. [24] using bovine plasma albumin as standard. Microsomal and liposomal phospholipids were quantitated according to Rouser et al. [25].

Results

Transfer to negatively charged liposomes

A reasonable point for the continuation of the previous observations on the effects of PtdIns on phospholipid transfer would be the consideration of other acidic phospholipids. Phosphatidic acid, PtdSer and PtdGro, like PtdIns, possess a net charge of −1 under the pH conditions of phospholipid exchange [26]. Liposomes were prepared from mixtures of phosphatidic acid and PtdCho in different molar proportions and tested for their ability to accept PtdIns or PtdCho transferred from rat liver microsomes. As shown in Fig. 1, incorporation of phosphatidic acid up to 15 mol% had no significant effect on the transfer of either PtdIns and PtdCho. Similar results were obtained for mixed liposomes containing up to 20 mol% PtdSer or PtdGro. It should be pointed out that liposomes containing greater levels of these acidic phospholipids could not be recovered after exchange assays in sufficient quantities to permit meaningful analyses of phospholipids transfer, presumably as a result of an undefined association with microsomes.

The absence of either stimulation or inhibition by liposomes containing phosphatidic acid, PtdSer or PtdGro is in sharp contrast to the effect of PtdIns, which has been shown to abolish all phospholipid transfer to liposomes containing 12 mol% and present at concentration greater than 0.8 mM [5]. These observations, coupled with the fact that neither phosphatidic acid nor PtdSer is a substrate for bovine brain phospholipid exchange protein, are consistent with an association between exchange protein and negatively charged liposomes based primarily on the recognition of transferable phospholipid molecules rather than surface charge.

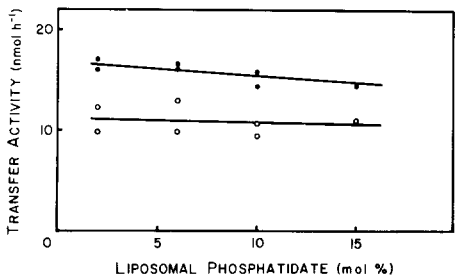

Fig. 1. Effect of phosphatidic acid on the phosphatidylinositol and phosphatidylcholine transfer activities of bovine brain phospholipid exchange protein. The indicated molar proportions of phosphatidic acid (from egg PtdCho) are incorporated into egg PtdCho liposomes. After sonication and centrifugation, the liposomes (1 μmol lipid phosphorus) are tested as acceptors of PtdIns or PtdCho transferred from rat liver microsome donor membranes, labelled with phosphatidyl[2-^{3}H]inositol or phosphatidyl[*methyl*-^{3}H]-choline, respectively. Incubations are carried out in the presence or absence of 2.2 μg of phospholipid exchange protein. Other details of the assay are given in Materials and Methods. •———•, PtdIns transfer activity; ———, PtdCho transfer activity.

Transfer to positively charged liposomes

Stearylamine may be used to generate liposomes with positively charged surfaces. When increasing molar proportions of stearylamine were incorporated into PtdCho lipsomes, the liposomes became less competent in accepting PtdIns transferred from rat liver microsomes (Fig. 2). As the concentration of stearylamine increased to 40 mol%, the transfer activity decreased continuously to less than 10% of the rate in the absence of this lipid. These results suggest that favorable interactions between the exchange protein and liposomes become impaired as the liposomal surface acquires a positive electrical character. While this is an unlikely consequence of naturally occurring lipids in biological membranes, localized regions of positively charged surface may arise from the presence of certain membrane proteins.

Effect of zwitterionic liposomal phospholipids on phospholipid transfer

To examine the effects of altered surface chemistry while leaving unchanged the surface electrical properties, two zwitterionic phospholipids were substituted for PtdCho in liposomes containing a constant 2 mol% phosphatidic acid. Upon addition of PtdEtn to liposomes significant increases in transfer activity were observed for both PtdIns and PtdCho (Fig. 3). This stimulation was apparent at levels as low as 2 mol%, became maximal between 10 and 20 mol%, and continued at levels as high as 40 mol%. It was also observed from the data shown in Fig. 3 that in the range of PtdEtn incorporation between 15 and 30 mol% the specificity of the bovine brain exchange protein was modified, in that the more actively transferred phospholipid became PtdCho rather than PtdIns.

Increases in the transfer of PtdIns and PtdCho were also demonstrated for liposomes containing low concentrations of sphingomyelin, another zwitterionic phospholipid. The maximal enhancement occurred at approximately 4 mol% (Fig. 4). Beyond 10 mol% and up to 40 mol%, however, the inclusion of sphingomyelin in the liposome structure was inhibitory toward all phospholipid transfers.

In comparing the effects of PtdEtn and sphingomyelin, both were able to

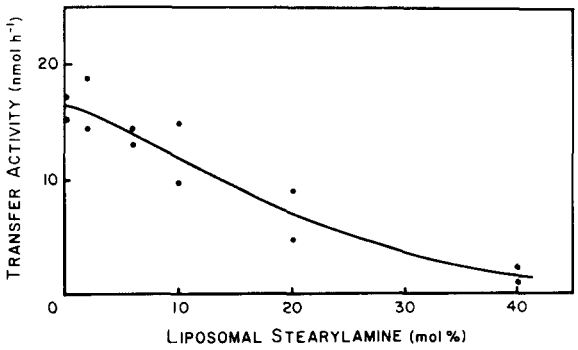

Fig. 2. Effect of stearylamine on phosphatidylinositol transfer activity. The indicated molar proportions of stearylamine are incorporated into egg PtdCho liposomes which contain a constant level of 2 mol% phosphatidic acid. After sonication and centrifugation, the liposomes (1 μmol total lipid), are tested as acceptors of PtdIns transferred from rat liver microsomes. See Fig. 1 and Materials and Methods for other details.

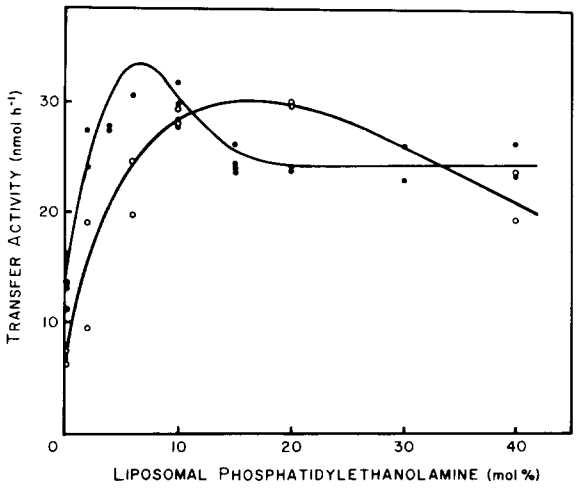

Fig. 3. Effect of phosphatidylethanolamine on the transfer activities of bovine brain phospholipid exchange protein. The indicated molar proportions of bovine liver PtdEtn are incorporated into egg PtdCho liposomes which contain a constant level of 2 mol% phosphatidic acid. See Fig. 1 and Materials and Methods for other details. ●———●, PtdIns transfer activity; ○———○, PtdCho transfer activity.

modify the structure of the liposome acceptor membranes in a manner that facilitated protein-catalyzed transfer of phospholipids into these membranes. Further, enhancement of transfer activity was apparent at levels of PtdEtn and sphingomyelin similar to those associated with the cytosolic surfaces of a number of representative intracellular membranes [17]. Finally, increased transfer activities were measured for both substrates of the bovine brain phospholipid exchange protein. It should be noted that the rates of transfer observed in the absence of exchange protein were normally less than 1—2

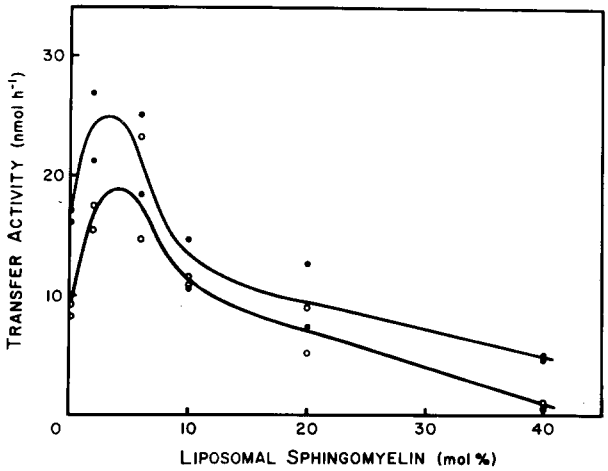

Fig. 4. Effect of sphingomyelin on the transfer activities of bovine brain phospholipid exchange protein. The indicated molar proportions of bovine spinal cord sphingomyelin are incorporated into egg PtdCho liposomes which contain a constant level of 2 mol% phosphatidic acid. See Fig. 1 and Materials and Methods for other details. ●———●, PtdIns transfer activity; ○———○, PtdCho transfer activity.

nmol · h^{-1} and were essentially independent of liposome composition and concentration. A possible explanation for the diverse actions of PtdEtn and sphingomyelin at higher concentrations may be the dramatic differences in the fatty acid composition of the liposomal phospholipids. By gas-liquid chromatographic analysis, PtdEtn contains more than 20% fatty acids of 20- and 22-carbon polyunsaturated varieties while sphingomyelin contains only saturated or very long chain mono-unsaturated fatty acids in amide linkage. The orientation of the sphingomyelin fatty acyl residues in a lipid bilayer would presumably be more rigid and more closely packed than those of PtdCho or PtdEtn. This prediction has been verified experimentally by Shinitzky and Barenholz who reported a thermal transition at 32 ± 2°C and an intrinsic viscosity at 37°C of 3.75 P for bovine spinal cord sphingomyelin [28]. Comparable values for egg PtdCho are a thermal transition at −15 to −5°C [29] and an intrinsic viscosity at 37°C of 0.36 P [28].

Activity of cholesterol-containing liposomes

The amphiphilic physicochemical properties of cholesterol permit its incorporation into phospholipid bilayers, up to a maximum of about 1 mol cholesterol/mol phospholipid. Since cholesterol is a common constituent of many biological membranes, it was of interest to examine its effect on the ability of liposomes to accept transferred phospholipid molecules. The transfer activity of bovine brain phospholipid exchange protein was unaltered with PtdCho liposomes containing cholesterol in amounts up to a molar ratio of 0.43 and was decreased 20% at a molar ratio of 0.81. Thus, the well-known ability of cholesterol to fluidize or rigidify phospholipid hydrocarbon chains [30,31] or simply the mere presence of the sterol in the lipid bilayer has only a minimal effect on intermembrane phospholipid transfer.

Kinetic parameters for mixed phospholipid liposomes

The preceding experiments demonstrated clearly that protein-catalyzed phospholipid transfer was sensitive to the chemical composition of the liposome into which lipid molecules were inserted. However, the interpretation of the results must be made with some caution. Obviously, in those situations of enhanced transfer rates, there must have been an increased affinity of the exchange protein for the liposomal surface which promoted a greater deposition of microsomal phospholipids in the acceptor membrane. But in those situations of diminished transfer rates, one cannot readily distinguish between (a) an increased affinity which could lead to an unusually strong association between exchange protein and liposomes, such as that observed between PtdIns and the brain protein [4] or between phosphatidic acid and the liver protein [32], or (b) a decreased affinity which would be counterproductive to microsome-liposome transfers.

To describe more completely the nature of exchange protein-liposome interactions, the dependence of PtdIns transfer activity on the concentration of a number of mixed phospholipid liposomes was examined. Based on the data shown in Figs. 1—4, liposome compositions were selected so that reasonable rates of phospholipid transfer could be anticipated over a wide range of liposome concentrations, generally 0.05—1 mM. The results of these experiments

were expressed as double-reciprocal, Lineweaver-Burk plots for each liposome (Fig. 5). The linearity of the curves suggests saturable associations between exchange protein and the various membrane surfaces, those surfaces reflecting to some extent the chemical properties of the lipids which comprise them. Only in case of sphingomyelin was a deviation from linearity seen (Fig. 5A), and this had the characteristic of 'substrate inhibition'. That is, at higher concentrations of liposomes containing 20 mol% sphingomyelin, transfer activity declined. Not unexpectedly, at the higher levels of sphingomyelin incorporation into liposomes (Fig. 4), transfer activity also declined.

From the Lineweaver-Burk plots were calculated values of K_m and V (Table I). While the magnitude of K_m for the different liposome acceptor membranes varied about seven-fold, from 0.14 mM for liposomes containing 5 mol%

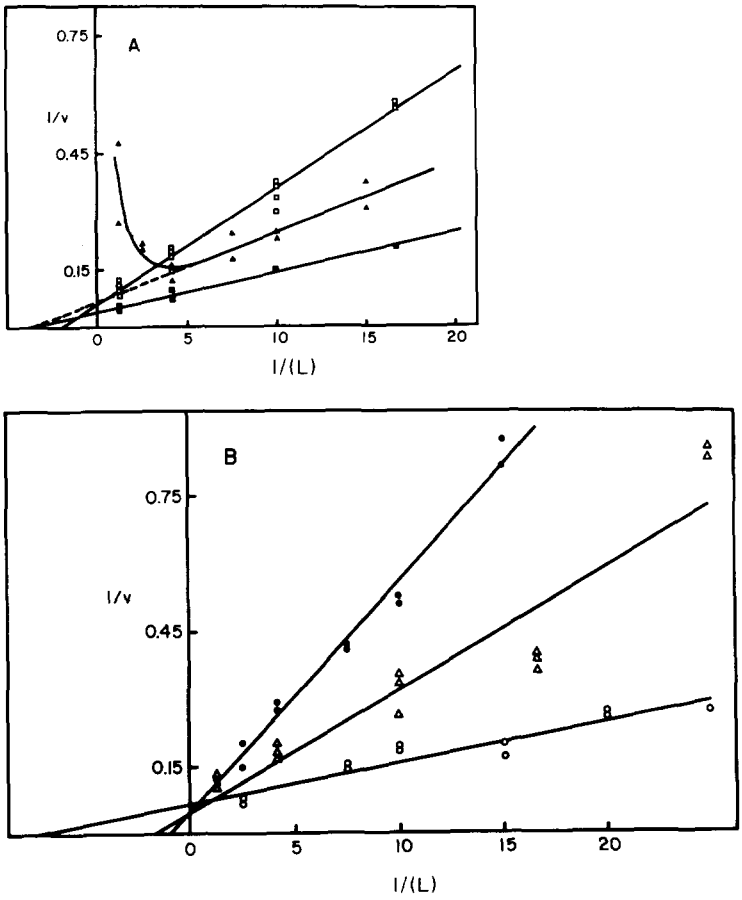

Fig. 5. Phosphatidylinositol transfer activity as functions of liposome composition and concentration. The transfer of PtdIns is measured from rat liver microsomes (1.25 mg protein) to various concentrations of liposomes with the indicated composition. The data are expressed as double-reciprocal, Lineweaver-Burk plots for each liposome species, where (L) is mM liposomal lipid phosphorus (or total lipid for stearylamine-containing liposomes) and v is nmol PtdIns transferred/h. Linear curves are generated by the method of least squares. For these experiments 3.3 μg phospholipid exchange protein is used. In addition to egg PtdCho, the liposomes contain (A) 2 mol% phosphatidic acid (□); 10 mol% phosphatidylethanolamine (■); 20 mol% sphingomyelin (▲); or (B) 5 mol% phosphatidylinositol (○); 10 mol% stearylamine (●); 30 mol% cholesterol (△).

TABLE I

KINETIC PARAMETERS FOR PHOSPHATIDYLINOSITOL TRANSFER

The phospholipids are added at the indicated proportions to a known quantity of PtdCho. After thorough removal of the organic solvents, sonicated liposomes are prepared and used as acceptor membranes for the transfer of PtdIns from rat liver microsomes. Other details are found in Materials and Methods.

Liposome composition (mol%)	Apparent K_m (mM phospholipid)	V (nmol · h^{-1})
Phosphatidylinositol (5)	0.14	14.9
Phosphatidic acid (2)	0.51	17.0
+ Phosphatidylethanolamine (10)	0.27	25.2
+ Sphingomyelin (20)	0.30	16.2
+ Stearylamine (10)	1.02	19.8
+ Cholesterol (30)	0.60	24.3

PtdIns to 1.02 mM for liposomes containing 10 mol% stearylamine, the value of V differed by less than two-fold. Clearly then, the differences among the rates of phospholipid transfer arise from the relative efficiencies of association and dissociation between exchange protein and liposome.

The apparent Michaelis constant of 0.14 mM found for liposomes containing 5 mol% PtdIns is in good agreement with values of 0.07 mM and 0.10 mM for liposomes containing 8 mol% and 2 mol% PtdIns, respectively [4]. It is also lower than the K_m values obtained for all other liposomes examined in the present study. Thus, bovine brain phospholipid exchange protein exhibits the strongest binding to those membrane surfaces which contain the phospholipid most actively transferred.

Egg PtdCho liposomes containing 2 mol% phosphatidic acid serve as the basis of comparison for the other K_m values listed in Table I. The addition to such liposomes of either 10 mol% PtdEtn or 20 mol% sphingomyelin caused a significant decrease in K_m. The enhanced activities of these membranes in phospholipid transfers is most likely due to increased affinities between exchange protein and liposomes. The addition of 10 mol% stearylamine led to a dramatic increase in K_m suggesting that the observed decreased activity of this membrane resulted from a less efficient association between exchange protein and liposome. For liposomes containing 30 mol% cholesterol, there was essentially no change in the apparent K_m, in keeping with the absence of any effect by cholesterol on the rate of PtdIns transfer between microsomes and liposomes.

Effects of single phospholipid liposomes

An alternative approach to describing the interaction of phospholipid exchange protein with membrane surfaces was to examine the effect of liposomes prepared from a single phospholipid on the transfer of PtdIns. Unlabelled, sonicated dispersions of PtdIns were, therefore, added to a system of rat liver microsomes and PtdCho-phosphatidic acid (98 : 2, mol%) liposomes. At concentrations less than 0.2 mM, PtdIns liposomes completely abolished transfer activity (Table II). The recovery of the radiolabelled, mixed phospholipid liposomes was unaltered under these conditions. Based on the observation of an increased affinity of exchange protein for mixed PtdCho-PtdIns lipo-

TABLE II

EFFECT OF SINGLE PHOSPHOLIPID LIPOSOMES ON THE TRANSFER OF PHOSPHATIDYLINOSITOL

Sonicated liposomes are prepared from the indicated phospholipids and chromatographed on Sepharose 4B to yield a preparation of single bilayer vesicles. They are added in the indicated concentrations to an assay system for PtdIns transfer, consisting of 1.25 mg rat liver microsomes, 1 μmol PtdCho-phosphatidic acid (98:2, mol%) liposomes, and 2.1 μg phospholipid exchange protein. Other details are found in Materials and Methods.

Liposomal phospholipid	Concentration (mM)	Transfer activity (% of control) (nmol · h^{-1})
Phosphatidylinositol	0	9.9 (100)
	0.11	1.9 (19)
	0.22	0 (0)
Phosphatidylglycerol	0	12.6 (100)
	0.20	12.2 (97)
	0.40	9.5 (75)
Phosphatidylserine	0	9.0 (100)
	0.20	9.1 (101)
	0.40	8.9 (99)

somes, it is likely that the protein interacts strongly with and remains tightly bound to the PtdIns liposomes during the incubation period. The exchange protein is thus unavailable to catalyze microsome-liposome phospholipid transfers. In contrast, two other acidic phospholipids, PtdGro and PtdSer, had very little effect on transfer activity when added separately as liposomes (Table II), thereby confirming the absence of any effect by these phospholipids in mixed liposomal acceptor membranes. Bovine brain phospholipid exchange protein exhibits virtually no affinity toward PtdGro or PtdSer, either as a transferable substrate or as a component of a membrane surface.

Discussion

The unique properties of phospholipid exchange proteins permit their use in the detailed study of protein-lipid interactions at several organizational levels. On the one hand, the structural features of those phospholipids which are transferred may be described, and the information derived may be used to construct an 'active site' for the exchange protein and to predict the forces involved in protein-phospholipid binding. This has recently been accomplished for bovine liver PtdCho exchange protein with an extensive series of radiochemical and spin-labelled analogues of PtdCho [33,34]. On the other hand, the freely diffusable exchange protein must interact with lipid-containing membranes during its catalytic cycle, and it is likely that an entirely different set of forces becomes significant. Rather than the simple bimolecular complex of exchange protein and phospholipid, what occurs at this point involves the adsorption and possible penetration of the protein into the three-dimensional surface generated by the membrane constituents. The present results confirm and extend earlier observations that membrane lipid composition has a profound effect on the transfer of phospholipids to and from such membranes,

catalyzed by exchange proteins isolated from bovine liver, brain and heart [4—9].

What emerges from the current investigation is a description of those structural properties of membrane lipids which influence protein-catalyzed phospholipid movement into and out of model membranes. Since two critical parameters of phospholipid transfer, liposome recovery and background transfer in the absence of exchange protein, were essentially unaffected by changes in liposomal lipid composition and a third parameter, the concentration of rat liver microsomes, remained constant, it can be expected that alterations in phospholipid transfer are most readily explained in terms of exchange protein-liposome interactions. This interaction is dramatically enhanced by some rather subtle manipulations of liposome structure, such as the incorporation of low levels of PtdEtn or sphingomyelin. One identifiable effect of these zwitterionic lipids in phospholipid transfer systems is to decrease the K_m for liposome acceptor membranes. A decreased Michaelis constant is suggestive of an increased affinity of the exchange protein for these liposomes and could account for the increased rates of phospholipid transfer. While the molecular basis of this effect has not been established, recent studies which have investigated the orientation and mobility of the zwitterionic polar head groups of PtdCho, PtdEtn and sphingomyelin at the surface of phospholipid bilayers may provide some insight. X-ray crystallography and nuclear magnetic resonance spectroscopy have shown that the phosphorylethanolamine and phosphorylcholine groups of the respective phospholipid molecules are oriented parallel to the membrane surface [35—37]. Such an orientation would favor electrostatic interactions between the phosphate ion of one molecule and the primary or quaternary ammonium ion of a neighboring molecule. Furthermore, hydrogen bonding between the amide and hydroxyl functions in sphingomyelin could provide an additional locus for intermolecular interaction [38]. Despite similar polar head group conformation, differences among the three phospholipids in the strength of their intermolecular interactions have been demonstrated and are most often characterized by the extent of hydration. Thus, with tightly associated polar head groups, the ionic residues are less accessible to the solvent and bind fewer water molecules. Compared to PtdCho, it has been concluded that both sphingomyelin [38] and PtdEtn [37] exhibit less surface hydration. Liposomes containing PtdEtn or sphingomyelin would possess relatively less polar surfaces than those containing only PtdCho and could therefore participate more efficiently in protein-catalyzed phospholipid exchange.

The current results present a striking contrast to the membrane lipid specificity of a similar protein, the PtdCho exchange protein of bovine liver. PtdEtn (22 mol%) or sphingomyelin (30 mol%) were slightly inhibitory to the transfer of PtdCho; phosphatidic acid, PtdSer, PtdIns, or PtdGro (20—23 mol%) were strongly inhibitory, and stearylamine (20 mol%) was stimulatory [6,7]. The general inhibition exerted by the acidic phospholipids was independently shown by kinetic analysis [32] and fluorometric titration [39] to arise from decreased dissociation constants for the exchange protein-liposome complexes. It has also been demonstrated that PtdCho transfer could be inhibited by the addition of PtdSer liposomes to the assay system [40]. Thus, it is clear that the major exchange proteins isolated from bovine liver and brain differ not only in

molecular properties and substrate specificity [41] but also in their interaction with various membrane surfaces.

The remarkable ability of the bovine brain phospholipid exchange protein to distinguish among a variety of lipid interfaces, as one stage in the formation of a productive protein-membrane complex, is another example of the specificity exhibited between proteins and membrane lipids. It also suggests a mechanism by which proteins may adsorb to specific regions of the lipid bilayer. Such a mechanism takes into account not only the polar head groups but the fatty acyl moieties of the phospholipids and depends upon both electrostatic and non-ionic interactions.

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References

1 Helmkamp, G.M., Jr., Harvey, M.S., Wirtz, K.W.A. and van Deenen, L.L.M. (1974) J. Biol. Chem. 249, 6382–6389
2 Harvey, M.S., Wirtz, K.W.A., Kamp, H.H., Zegers, B.J.M. and van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 323, 234–239
3 Demel, R.A., Kalsbeek, R., Wirtz, K.W.A. and van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 466, 10–22
4 Helmkamp, G.M., Jr., Wirtz, K.W.A. and van Deenen, L.L.M. (1976) Arch. Biochem. Biophys. 174, 592–602
5 Harvey, M.S., Helmkamp, G.M., Jr., Wirtz, K.W.A. and van Deenen, L.L.M. (1974) FEBS Lett. 46, 260–262
6 Hellings, J.A., Kamp, H.H., Wirtz, K.W.A. and van Deenen, L.L.M. (1974) Eur. J. Biochem. 47, 601–605
7 Wirtz, K.W.A., Geurts van Kessel, W.S.M., Kamp, H.H. and Demel, R.A. (1976) Eur. J. Biochem. 61, 515–523
8 DiCorleto, P.E. and Zilversmit, D.B. (1977) Biochemistry 16, 2145–2150
9 Johnson, L.W. and Zilversmit, D.B. (1975) Biochim. Biophys. Acta 375, 165–175
10 Bangham, A.D., Hill, M.W. and Miller, N.G.A. (1974) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. 1, pp. 1–68, Plenum Press, New York
11 Davidson, F.M. and Long, C. (1958) Biochem. J. 69, 458–466
12 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
13 Rouser, G., Kritchevsky, G., Yamamoto, A., Simon, G., Galli, C. and Bauman, A.J. (1969) Methods Enzymol. 14, 272–317
14 Comfurius, P. and Zwaal, R.F.A. (1977) Biochim. Biophys. Acta 488, 36–42
15 Sanders, H. (1967) Biochim. Biophys. Acta 144, 485–487
16 Kates, M. (1972) Techniques of Lipidology, p. 572, North-Holland Publishing Co., Amsterdam
17 Skipski, V.P., Peterson, R.F., Sanders, J. and Barclay, M. (1963) J. Lipid Res. 4, 227–228
18 Ways, P., Reed, C.F. and Hanahan, D.J. (1963) J. Clin. Invest. 42, 1248–1260
19 White, D.A. (1973) in Form and Function of Phospholipids (Ansell, G.B., Dawson, R.M.C. and Hawthorne, J.N., eds.), 2nd edn., pp. 441–482, Elsevier Scientific Publishing Co., Amsterdam
20 Fieser, L.F. (1963) J. Am. Chem. Soc. 75, 5421–5422
21 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–637
22 De Gier, J., Mandersloot, J.G. and van Deenen, L.L.M. (1968) Biochim. Biophys. Acta 150, 666–675
23 Huang, C. (1969) Biochemistry 8, 344–352
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
25 Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494–496
26 Abramson, M.B., Katzman, R., Wilson, C.E. and Gregor, H.P. (1964) J. Biol. Chem. 239, 4066–4072

27 DePierre, J.W. and Ernster, L. (1977) Annu. Rev. Biochem. 46, 201—262
28 Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652—2657
29 Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304—367
30 Oldfield, E. and Chapman, D. (1972) FEBS Lett. 23, 285--297
31 Kawato, S., Kinosita, K., Jr. and Ikegami, A. (1978) Biochemistry 17, 5026—5031
32 Van den Besselaar, A.M.H.P., Helmkamp, G.M., Jr. and Wirtz, K.W.A. (1975) Biochemistry 14, 1852—1858
33 Kamp, H.H., Wirtz, K.W.A., Baer, P.R., Slotboom, A.J., Rosenthal, A.F., Paltauf, F. and van Deenen, L.L.M. (1977) Biochemistry 16, 1310—1316
34 Devaux, P.F., Moonen, P., Bienvenue, A. and Wirtz, K.W.A. (1977) Proc. Natl. Acad. Sci. U.S. 74, 1807—1810
35 Hitchcock, P.B., Mason, R., Thomas, K.M. and Shipley, G.G. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3036—3040
36 Seelig, J., Gally, H.-U. and Wohlgemuth, R. (1977) Biochim. Biophys. Acta 467, 109—119
37 Yeagle, P.L., Hutton, W.C., Huang, C. and Martin, R.B. (1977) Biochemistry 16, 4344—4349
38 Hertz, R. and Barenholz, Y. (1975) Chem. Phys. Lipids 15, 138—156
39 Wirtz, K.W.A. and Moonen, P. (1977) Eur. J. Biochem. 77, 437—443
40 Machida, K. and Ohnishi, S. (1978) Biochim. Biophys. Acta 507, 156—164
41 Helmkamp, G.M., Jr., Nelemans, S.A. and Wirtz, K.W.A. (1976) Biochim. Biophys. Acta 424, 168—182