Specificity of the Phosphatidylcholine Exchange Protein from Bovine Liver†

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ABSTRACT: The phosphatidylcholine exchange protein from bovine liver stimulates the specific transfer of phosphatidylcholine (PC) from rat liver microsomes to mitochondria or phospholipid vesicles (Wirtz, K. W. A., Kamp, H. H., and van Deenen, L. L. M. (1972), *Biochim. Biophys. Acta 274,* 606). In the present study, it has been established which components of the PC molecule are essential to the specific interaction with the protein. Radiochemically labeled analogues of PC have been synthesized with modifications in the polar and apolar moiety, and their transfer was measured between donor and acceptor vesicles. Relative to 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine (egg yolk PC), transfer is inhibited or abolished when (a) the distance between phosphorus and nitrogen is decreased or increased and (b) a methyl group on the quaternary nitrogen is removed or substituted by an ethyl or propyl group. Transfer is much less affected when (a) the ester bonds are replaced by ether or carbon–carbon bonds, (b) the PC molecule contains two saturated fatty acids, and (c) the D stereoisomer is used. It is concluded that the protein has a binding site which interacts specifically with the phosphorylcholine head group and which cannot accommodate substantial configurational changes. Interaction with the apolar moiety of PC is less specific. However, lyso-PC is not transferred, suggesting that two hydrocarbon chains are required to stabilize the exchange protein–phospholipid complex. Interaction of [^{14}C]PC-labeled exchange protein with vesicles of different phospholipid composition has been analyzed by measuring the release of [^{14}C]PC into these vesicles. Vesicles of egg PC or dimethylphosphatidylethanolamine function as acceptors, in contrast to vesicles of sphingomyelin or phosphatidylethanolamine.

A phospholipid exchange protein has been isolated from bovine liver which catalyzes specifically the transfer of PC[1] between membrane interfaces. This specificity has been established by using ^{32}P-labeled rat liver microsomes (Wirtz et al., 1972; Kamp et al., 1973), monolayers consisting of [^{14}C]PC, [^{32}P]P, or [^{32}P]PE (Demel et al., 1973), and ^{32}P-labeled influenza virus (Rothman et al., 1976) as donor membranes of labeled phospholipids. This preference for PC suggests that the protein possesses a site which interacts with the phosphorylcholine head group oriented at the membrane–water interface. The fact that cations inhibit the transfer of PC alludes to the electrostatic nature of the interaction (Johnson and Zilversmit, 1975; Wirtz et al., 1976). As regards the recognition of the polar head group, the exchange protein may resemble β-hydroxybutyrate dehydrogenase which depends specifically on PC for a maximal expression of its enzymatic activity (Grover et al., 1975; Gazzotti et al., 1975).

The phosphatidylcholine exchange protein from bovine liver contains one molecule of noncovalently bound PC (Kamp et al., 1973). In order to function as a carrier of PC between membranes, we have proposed the following sequence of events: (1) the protein releases its endogenous PC upon formation of a collision complex with the membrane; (2) a specific binding site on the protein interacts with a PC molecule from the membrane, followed by the release of the protein–PC complex into the medium (van den Besselaar et al., 1975). In the present study, we have investigated the binding site by determining what structural requirements have to be met for PC or a structurally related compound to be transferred by the exchange protein. To this end, phospholipids of high specific radioactivity with variations in the polar and apolar moiety have been synthesized, and their rate of transfer was measured in the donor–acceptor vesicle system (van den Besselaar et al., 1975). Measurements are performed under conditions where the interaction of the protein with the vesicles is not affected by the particular phospholipid, the transfer of which is under investigation. Rates of transfer, therefore, can be related directly to the affinity of the phospholipid for the exchange protein at the interface. In this study, we have circumvented the general problem that the physical character of the membrane itself could be a factor in controlling the affinity of phospholipids for proteins acting at interfaces (Dawson, 1973).

In this paper, it will be shown that any change in the polar moiety of PC adversely affects the interaction with the exchange protein, whereas changes in the apolar moiety have less effect. Removal, however, of one acyl chain makes the ensuing lyso-PC unfit for transfer. It will be discussed how both electrostatic and hydrophobic interactions have to be satisfied for the exchange protein to extract a phospholipid molecule from the membrane.

Materials and Methods

Lipids. A total lipid extract was prepared from egg yolk following the method of Singleton (1965) and from bovine red blood cell ghosts according to Bligh and Dyer (1959). PC, PE,

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[1] Abbreviations used are: PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; SPM, sphingomyelin; PA, phosphatidic acid; DMPE, dimethylphosphatidylethanolamine; DEAE, diethylaminoethyl; Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride; TLC, thin-layer chromatography.

and SPM were isolated from these extracts by column chromatography on Kieselgel 60 reinst (E. Merck, Darmstadt, Germany). PA was prepared from egg yolk PC with phospholipase D extracted from Savoy cabbage (Davidson and Long, 1958). PC, PE, SPM, and the sodium salt of PA were stored at −20 °C under nitrogen in chloroform–methanol (1:1, v/v). The purity of these lipids was established by TLC in solvent system A (chloroform–methanol–acetic acid–water, 25:15:4:2, v/v) and solvent system B (chloroform–methanol–ammonia–water, 90:54:5.5:5.5, v/v).

Egg yolk PC and erythrocyte SPM were demethylated by refluxing under nitrogen with diazabicyclooctane (E. Merck, Germany) in dimethylformamide (person communication of Dr. W. Stoffel to K.W.A.W.). The dimethyl derivatives were purified by TLC in solvent system C (chloroform–methanol–water, 65:25:4, v/v) and remethylated with [^{14}C]methyl iodide (The Radiochemical Centre, Amersham, England) as described by Stoffel et al. (1971). The reaction products 1,2-[*methyl*-^{14}C]diacyl-*sn*-glycero-3-phosphorylcholine (I) and [*methyl*-^{14}C]ceramide phosphorylcholine (XVII) were purified by TLC in solvent system C. Compounds I and XVII (specific radioactivity 55–60 μCi μmol^{-1}) cochromatographed with PC and SPM, respectively, in solvent systems A and B. 1-[*methyl*-^{14}C]Monoacyl-*sn*-glycero-3-phosphorylcholine was prepared from compound I by incubation with phospholipase A_2 from *Crotalus adamanteus* (van den Bosch and van Deenen, 1965).

Phospholipase D from Savoye cabbage was used to catalyze the transphosphatidylation reaction between egg PC and dimethylaminopropanol (E. Merck, Germany) or dimethylaminobutanol (ICN) as described by Jezyk and Hughes (1973). After incubation, the reaction mixture which contained the dimethyl derivatives of the "base-exchange" reaction and PA was evaporated in vacuo. The dimethyl derivatives were extracted from the residue with methanol and converted into 1,2-[*methyl*-^{14}C]diacyl-*sn*-glycero-3-phosphoryltrimethylpropanolamine (II) and 1,2-[*methyl*-^{14}C]diacyl-*sn*-glycero-3-phosphoryltrimethylbutanolamine (III) by methylation with [^{14}C]methyl iodide. Compounds II and III (specific radioactivity 55–60 μCi/μmol) were purified by TLC in solvent system B.

1,2-Dipalmitoyl-*sn*-glyceroaminoethylphosphonate was synthesized and characterized by physical and chemical means as described by Rosenthal and Pousada (1965). This compound was converted into the ^{14}C-labeled dimethyl derivative by a reductive methylation with [^{14}C]paraformaldehyde (The Radiochemical Centre, Amersham) according to Lekim and Bentzing (1973). The ^{14}C-labeled dimethyl compound, which cochromatographed with DMPE derived from egg yolk PC, was purified by TLC in solvent system C. After conversion into 1,2-[*methyl*-^{14}C]dipalmitoyl-*sn*-glycero-3-cholinephosphonate (IV) by methylation with methyl iodide, the chromatographical behavior of compound IV resembled that of egg yolk PC. 1,2-[*methyl*-^{14}C]Diacyl-*sn*-glycero-3-phosphoryl-*N*,*N*-dimethylethanolamine (V) was prepared by reductive methylation of egg yolk PE with [^{14}C]paraformaldehyde. After purification by TLC in solvent system C, compound V was realkylated with ethyl iodide and propyl iodide (E. Merck, Germany) to form 1,2-[*methyl*-^{14}C]diacyl-*sn*-glycero-3-phosphoryl-*N*-ethyl-*N*,*N*-dimethylethanolamine (VI) and 1,2-[*methyl*-^{14}C]diacyl-*sn*-glycero-3-phosphoryl-*N*-propyl-*N*,*N*-dimethylethanolamine (VII). Compound VI and VII were purified from compound V by TLC in solvent system B and A, respectively. The specific radioactivities of compounds IV to VII varied between 6 and 14 μCi/μmol.

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine (VIII) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (X) were prepared as described by van Deenen and de Haas (1964). *rac*-1-Palmitoyl-2-oleoylglycero-3-phosphorylcholine was prepared according to de Haas and van Deenen (1961). After stereospecific degradation with phospholipase A_2 of porcine pancreas, the unreacted 3-palmitoyl-2-oleoyl-*sn*-glycero-1-phosphorylcholine (IX) was isolated by thin-layer chromatography (Bonsen et al., 1972). *rac*-1-Oleoyl-2-hexadecylglycero-3-phosphorylcholine (XV) and *rac*-1-oleoyl-2-hexadecyl-2-deoxyglycero-3-phosphorylcholine (XVI) were obtained by the method of Slotboom et al. (1970). The identity of compounds VIII, IX, X, XV, and XVI has been established by TLC, fatty acid analysis, stereospecific degradation with phospholipase A_2, analysis of C, H, N, and P content, determination of melting point, and optical rotation in the corresponding references. The ^{14}C-labeled analogues (specific radioactivity 55–60 μCi/μmol) were formed by demethylation and remethylation with [^{14}C]methyl iodide as described above for compound I.

1-[^{3}H]Octadecyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine (XI, specific radioactivity 1.1 μCi/μmol) and 1-[^{3}H]octadecyl-2-oleoyl-*sn*-glycero-3-phosphorylethanolamine (XIII, specific radioactivity 12 μCi/μmol) were synthesized by the method of Paltauf (1972); *rac*-1-[^{3}H]octadecyl-2-oleoylglycero-3-phosphoryl-*N*,*N*-dimethylethanolamine (XII, specific radioactivity 65 μCi/μmol) was synthesized as described by Paltauf and Holasek (1973); *rac*-1,2-di[^{3}H]octadecylglycero-3-phosphorylcholine (XIV, specific radioactivity 0.6 μCi/μmol) was synthesized as described by Paltauf (1969). Compounds XI to XIV have been characterized by TLC and chemical analysis in the corresponding references. [7α-^{3}H]-Cholesterol oleate was prepared as described previously (van den Besselaar et al., 1975). Lipid phosphorus was determined by the method of Chen et al. (1956) after destruction of the sample according to the procedure of Ames and Dubin (1960).

Exchange Protein. Phosphatidylcholine exchange protein from bovine liver was purified to homogeneity according to the procedure of Kamp et al. (1973). [^{14}C]PC (I) was introduced into the exchange protein as previously described (Kamp et al., 1975).

Assay of Phospholipid Transfer. The affinity of the exchange protein for a particular phospholipid was determined by measuring the transfer of the labeled analogue from donor to acceptor vesicles. The preparation of the vesicles and the measurement of the transfer were essentially as described by van den Besselaar et al. (1975). The acceptor vesicles consisted of egg PC, 2 mol % PA, and a trace of [^{3}H]cholesterol oleate (0.01%, w/w). The donor vesicles contained egg PC, 9 mol % PA, and minor quantities of the labeled phospholipids (0.1–1.0%, w/w). We assume that in all instances the labeled phospholipids are homogeneously distributed in the bilayer of the vesicles, and that in view of the small quantities involved the "quality" of the donor interface is not affected by the presence of the labeled phospholipids (Verger et al., 1973).

The incubation mixture contained acceptor vesicles (0.625 μmol of PC), donor vesicles (0.25 μmol of PC), and exchange protein (1 μg) in a total volume of 1.5 mL of 12.5 mM sodium phosphate buffer (pH 7.0). For further experimental details, see legends to figures and tables. Donor vesicles were separated from the acceptor vesicles by applying the mixture to a DEAE-cellulose column (0.2 mL of bed volume). After the column was washed with 4.5 mL of phosphate buffer, the recovery of the acceptor vesicles in the column eluent amounted

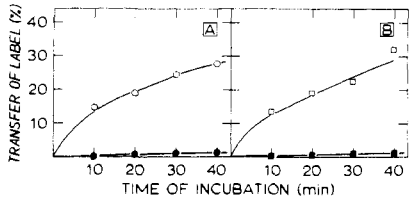

FIGURE 1: Transfer of egg [^{14}C]PC and [^{14}C]DMPE from donor vesicles, the bulk of which consisted of egg PC (A) or egg DMPE (B), to acceptor vesicles. Transfer was determined as described under Materials and Methods. (A) [^{14}C]PC (○); [^{14}C]DMPE (●). (B) [^{14}C]PC (□); [^{14}C]-DMPE (■).

to approximately 50%. Losses were due to a nonspecific adsorption to the DEAE-cellulose. In all instances, control experiments without exchange protein were run in parallel. The percentage of the label transferred to the acceptor vesicles by the exchange protein was determined as described previously (van den Besselaar et al., 1975).

To measure the transfer of lyso-PC to the above ^{3}H-labeled acceptor vesicles, rat liver microsomes have been used as donor membranes of [^{14}C]lyso-PC. [^{14}C]lyso-PC was introduced into the microsomes by a degradation of 20% of the endogenous [^{14}C]PC with phospholipase A_2 from porcine pancreas (van den Besselaar et al., 1976). The incubation of the ^{14}C-labeled microsomes (2.4 mg of protein) with the ^{3}H-labeled vesicles (1 μmol of PC, 0.02 μmol of PA, 0.01%, w/w, [^{3}H]cholesteryl oleate) and the recovery of the vesicles after incubation were performed as described (Kamp et al., 1973). The lipids were extracted from the vesicles according to Bligh and Dyer (1959). The extract was dissolved in 6 mL of chloroform–methanol (1:2, v/v) and 1.5 mL were used to determine the ^{14}C/^{3}H ratio. From this ratio and the amount of ^{3}H label in the vesicles before incubation, the total transfer of ^{14}C label to the vesicles was calculated. The remaining 4.5 mL of extract was used for thin-layer chromatography to determine the distribution of ^{14}C label transferred among lyso-PC and PC. From these values and the ^{14}C label in microsomal lyso-PC and PC before incubation, the percentage of transfer was calculated (see Table III).

Interaction of Exchange Protein with Vesicles. The interaction of exchange protein with vesicles of different phospholipid composition has been determined by measuring the release of [^{14}C]PC bound to the protein into these vesicles. Vesicles were prepared by sonication of a phospholipid suspension to clarity in a Branson sonifier. [^{14}C]PC-labeled exchange protein (1 nmol) and vesicles (2–30 nmol of phospholipid) were incubated at 37 °C for 30 min in a total volume of 0.36 mL of 25% sucrose (w/v)–0.02 M Tris-HCl (pH 7.4). At the end of incubation, protein and vesicles were separated by polyacrylamide gel electrophoresis according to the method of Davis (1964). Duplicate aliquots of 0.150 mL of the incubation mixture were applied to the gels. Electrophoresis was carried out for 1 h with 4 mA/tube; the buffer front was indicated by bromophenol blue. One gel was stained with Coomassie brillant blue to indicate the protein; the other gel was divided in 2-mm segments to determine the distribution of ^{14}C label over the gel. Procedures for staining and counting of the gel have been described previously (Kamp et al., 1975).

Results

Assay of Phospholipid Transfer. In a previous study, it was shown that the exchange protein catalyzes the transfer of [^{14}C]PC from donor to acceptor vesicles (van den Besselaar et al., 1975). In the following experiments, evidence is provided for the use of this donor–acceptor vesicle system as a means to establish the specificity of the exchange protein. Minor quantities of egg yolk [^{14}C]PC (I) or egg yolk [^{14}C]DMPE (V) are incorporated into donor vesicles consisting of egg yolk PC (see legend to Figure 1A). From measuring the protein-mediated transfer of label to the acceptor vesicles, it may be seen that the transfer of [^{14}C]DMPE is very low as compared to [^{14}C]PC (Figure 1A). This shows that upon interaction with the donor vesicle the exchange protein discriminates between PC and DMPE. Since the bulk of the donor phospholipid consists of egg yolk PC, it could be argued that the donor interface is unfavorable for the protein to interact with [^{14}C]-DMPE. Therefore, the same experiment is repeated with donor vesicles composed of DMPE (see legend to Figure 1B). As before, transfer of [^{14}C]DMPE is very low, whereas [^{14}C]PC is transferred at a rate similar to that observed with the donor PC vesicles (Figure 1B). This is interpreted to mean that the exchange protein interacts equally well with donor vesicles composed of PC and DMPE, in both instances demonstrating a low affinity for DMPE. Based on the percentage of transfer after, for example, 10 min of incubation, the protein has a 150-fold lower afffinity for DMPE (V) than for egg PC (see Table I). In addition, we conclude from Figure 1A, 1B that measuring the transfer of labeled phospholipids incorporated in minor quantities into donor vesicle of egg PC provides a valid approach to establishing the specificity of the exchange protein.

TABLE I: Transfer of Analogues of PC Modified in the Polar Head Group.[a]

	[*methyl*-^{14}C]Phospholipid	Transfer (%)[b]
I	1,2-diacyl-*sn*-glycero-3-phosphorylcholine[c]	14.8
II	1,2-diacyl-*sn*-glycero-3-phosphoryltrimethylpropanolamine[c]	8.0
III	1,2-diacyl-*sn*-glycero-3-phosphoryltrimethylbutanolamine[c]	0.0
IV	1,2-dipalmitoyl-*sn*-glycero-3-cholinephosphonate	0.3
V	1,2-diacyl-*sn*-glycero-3-phosphoryl-*N,N*-dimethylethanolamine[c,d]	0.1
VI	1,2-diacyl-*sn*-glycero-3-phosphoryl-*N*-ethyl-*N,N*-dimethylethanolamine[d]	8.6
VII	1,2-diacyl-*sn*-glycero-3-phosphoryl-*N*-propyl-*N,N*-dimethylethanolamine[d]	0.0

[a] Transfer has been measured as described under Materials and Methods. Incubations are performed at 37 °C for 5, 10, 20, and 30 min. [b] Percentage of ^{14}C label transferred after 10 min. [c] Phospholipids derived from egg PC. [d] Phospholipids derived from egg PE.

Specificity of the Exchange Protein. In order to explore in detail how a chemical modification of the polar head group of PC affects the interaction with the exchange protein, a series of analogues of high specific radioactivity were synthesized (compounds II to VII, Table I). Incorporation of these analogues into the donor vesicles did not exceed 1% of the total donor phospholipid pool. The transfer of each labeled analogue was followed with time for up to 30 min (see legend to Table I); only the percentage of transfer at 10 min is indicated in Tables I and II. The transfer of egg yolk [^{14}C]PC (I) amounted to 14.8% (Table I). The introduction of an additional methylene group between phosphorus and nitrogen in the phosphorylcholine moiety (compound II) resulted in about half the transfer. Upon introduction of a further methylene group (compound III), transfer was absent. A reduction of the distance between phosphorus and nitrogen by elimination of the

oxygen leads to the phosphonate analogue of PC (IV), which is barely transferred. This suggests that the charge distribution in the phosphorylcholine moiety of PC provides for an optimal interaction with the exchange protein.

DMPE (V), which is obtained from PC by removal of one methyl group from the polar head, is transferred at a very low rate, indicating that a quaternary nitrogen is required (Figure 1). Realkylation of DMPE with ethyl iodide gives compound VI, which is transferred at a rate 60% of that of egg PC (Table I). An increase of the bulkiness of the polar head by alkylation of DMPE with propyl iodide inhibits the transfer of the resulting compound VII completely. From Table I it may be inferred that the binding site on the protein has a close fit with the phosphorylcholine head group not allowing for a substantial configurational change. It is of relatively little consequence whether the phosphorylcholine group is part of the L or the D stereoisomer of PC. Both stereoisomers of palmitoyloleoyl-PC (VIII and IX) are transferred, although the protein has a threefold higher affinity for the natural L isomer (Table II). This raises the question of how molecular changes in the diglyceride part of the PC-molecule affect the interaction (see Table II).

1-Palmitoyl-2-oleoyl-PC is the major molecular species of egg PC. Incorporation of a trace (0.1%, w/w) of [^{14}C]dipalmitoyl-PC (X) into the egg PC donor vesicle shows that the protein transfers this saturated species at one-third of the rate of 1-[^{14}C]palmitoyl-2-oleoyl PC (VIII). Although dipalmitoyl-PC has a transition temperature of 42 °C, the small quantity present in the donor vesicle allows a complete mixing with egg PC at the temperature of incubation, i.e., 37 °C (Grant et al., 1974). This implies that the protein at the donor interface discriminates against dipalmitoyl-PC. This discrimination may be based on a hydrophobic interaction between the protein and the apolar moiety of PC being less effective when two palmitoyl chains are attached to the glycerol backbone. Replacing the ester bond at the 1 position for an ether bond gives an alkyl analogue of PC (XI), which is transferred at a slightly reduced rate. In agreement with the data of Table I, the corresponding alkyl analogue of DMPE (XII) is not transferred. An identical alkyl analogue of PE (XIII) fails also to be transferred, providing additional evidence for the critical role of the polar head group in governing the interaction with the exchange protein. Transfer, however, is observed with the dialkyl analogue of PC (XIV), where both ester bonds are replaced by ether bonds, with the alkyl analogue of PC (XV), where an ether bond is present at the 2 position, and with a PC analogue (XV), where the alkyl chain is attached to the 2 position of the glycerol backbone through a carbon–carbon bond. The reduced transfer of the D stereoisomer of PC (IX) may, in part, explain why the transfer of compounds XIV to XVI, which are racemic mixtures, is also lower. From Table II it appears that all phospholipids with a phosphorylcholine group are transferred, and that ester bonds and the L stereoisomer are not essential for a phospholipid molecule to be transferred by the exchange protein. Sphingomyelin (XVII) from bovine red blood cells and egg yolk, however, provides an exception in that this phospholipid, in spite of its phosphorylcholine group, is not transferred. Increasing the incubation temperature to 45 °C failed to have any effect. It remains to be determined whether the free hydroxyl group, the amide bond, and/or the high content of long-chain fatty acids make this molecule unsuitable for transfer.

Transfer of 1-Acyllysophosphatidylcholine. Since hydrophobic interactions between exchange protein and phospholipid may play a role in the transfer process, it is of interest to determine whether 1-acyllyso-PC is transferred. The donor–acceptor vesicle system, as used above, cannot be used as [^{14}C]lyso-PC (0.05%, w/w) redistributes spontaneously and very rapidly between the donor and acceptor vesicles (experiment A, Table III). The exchange protein does not have an effect on this redistribution, which has attained equilibrium already within 5 min. The transfer of [^{14}C]PC in the absence and presence of the exchange protein is also included to show the completely different behavior of PC under these conditions.

The spontaneous transfer of lyso-PC to the acceptor vesicles is greatly reduced when the donor membranes are ^{14}C labeled rat liver microsomes of which part of the endogenous [^{14}C]PC pool, i.e., 20%, is converted into 1-[^{14}C]acyllyso-PC by a controlled degradation with phospholipase A_2 (experiment B, Table III). Addition of the exchange protein stimulates the transfer of microsomal [^{14}C]PC to the vesicles but fails to have an effect on [^{14}C]lyso-PC. This could mean that, in spite of the presence of the phosphorylcholine group, one acyl chain is not sufficient to stabilize a hydrophobic interaction between lyso-PC and exchange protein.

Interaction with Phospholipid Interfaces. For the exchange protein to transfer PC between membranes it has to interact with the membrane interface. The influence of the interface on this interaction has been studied by measuring the release of [^{14}C]PC bound to the protein into vesicles of different phospholipid composition. Protein and vesicles are separated by polyacrylamide gel electrophoresis where the vesicles collect in the top of the separation gel without affecting the electrophoretic behavior of the protein. The redistribution of [^{14}C]PC between protein and vesicles is determined as described under Materials and Methods. The electrophoretic behavior of [^{14}C]PC-labeled exchange protein is seen in Figure 2A where the ^{14}C label and protein are found to coincide. Upon incu-

TABLE II: Transfer of Analogues of PC Modified in the Apolar Moiety.[a]

	Radiolabeled Phospholipid	Transfer (%)[b]
VIII	[*methyl*-^{14}C]1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine	14.5
IX	[*methyl*-^{14}C]3-palmitoyl-2-oleoyl-*sn*-glycero-1-phosphorylcholine	5.0
X	[*methyl*-^{14}C]1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine	5.4
XI	1-[^{3}H]octadecyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine	8.6
XII	*rac*-1-[^{3}H]octadecyl-2-oleoylglycero-3-phosphoryl-*N,N*-dimethylethanolamine	0.0
XIII	1-[^{3}H]octadecyl-2-oleoyl-*sn*-glycero-3-phosphorylethanolamine	0.0
XIV	*rac*-1, 2-[^{3}H]dioctadecenylglycero-3-phosphorylcholine	9.0
XV	*rac*-[*methyl*-^{14}C]1-oleoyl-2-hexadecylglycero-3-phosphorylcholine	4.8
XVI	*rac*-[*methyl*-^{14}C]1-oleoyl-2-hexadecyl-2-deoxyglycero-3-phosphorylcholine	7.5
XVII	[*methyl*-^{14}C]ceramide phosphorylcholine (sphingomyelin)	0.0

[a] Transfer has been measured as described under Materials and Methods. [^{14}C]Cholesteryl oleate replaces [^{3}H]cholesteryl oleate in the acceptor vesicles when the donor vesicles contain [^{3}H]phospholipid. Incubations are performed at 37 °C for 5, 10, 20, and 30 min. [b] Percentage of label transferred after 10 min.

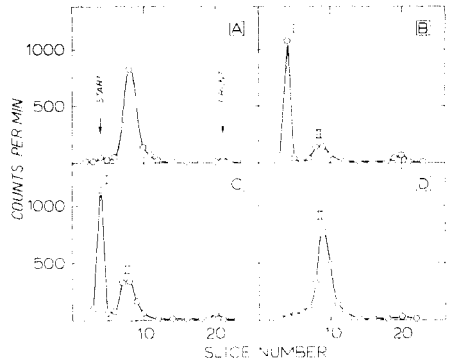

FIGURE 2: Polyacrylamide gel electrophoresis of [^{14}C]phosphatidylcholine exchange protein (A) after incubation with vesicles consisting of 10 nmol of egg PC (B), 10 nmol of egg DMPE (C), and 7.5 nmol of egg PE (D). Electrophoresis and determination of radioactivity were performed as described under Materials and Methods. Position of vesicles and exchange protein on the gel are indicated by I and II, respectively.

bation of the ^{14}C-labeled protein (1 nmol) with egg PC vesicles (10 nmol of PC), the ^{14}C label is recovered from the vesicles (Figure 2B, peak I) with a small percentage of the label remaining with the protein (peak II). An increase of the vesicle concentration to 20 nmol of PC results in a complete release of [^{14}C]PC from the protein. From previous studies, we know that the redistribution of ^{14}C label between protein and vesicles is governed by a process where the protein exchanges its endogenous PC molecule for one from the interface (Demel et al., 1973; Kamp et al., 1975). Repeating the above experiment with vesicles of DMPE (10 nmol) gives rise to an insertion of [^{14}C]PC into these vesicles (Figure 2C). This agrees with a previous experiment (Figure 1B) where it was shown that the protein interacts with DMPE vesicles to extract [^{14}C]PC from these vesicles. Since the protein was found to transfer [^{14}C]-DMPE at a very low rate, it remains to be elucidated whether the exchange protein releases [^{14}C]PC into DMPE vesicles without binding DMPE in return. From Figure 2C, it is seen that a substantial amount of [^{14}C]PC remains with the protein (peak II). This could mean that the protein holds on to its endogenous [^{14}C]PC more tightly with DMPE as compared to PC vesicles. Incubation of the ^{14}C-labeled protein with dispersions of egg yolk PE does not result in any release of [^{14}C]PC (Figure 2D). A similar absence of PC insertion is found with vesicles of sphingomyelin. We assume that PE and sphingomyelin, in contrast to PC and DMPE vesicles, lack the interfacial characteristics necessary for an interaction with the exchange protein.

Discussion

The physical state of the lipid–water interface could be an important factor in controlling the specificity of proteins that have an affinity towards the lipids making up this interface (Dawson, 1973; Verger and de Haas, 1976). In attempts to deal with this complicating aspect in the interpretation of the kinetics of lipolytic enzymes, phospholipid monolayers (Verger et al., 1973; Lagocki et al., 1973), micelle-forming synthetic lipids (Entressangles and Desnuelle, 1968; de Haas et al., 1971; Wells 1974), and lipids dispersed in nonionic surfactants (Desnuelle, 1971; Deems et al., 1975) have been used. In the present study on the substrate specificity of the phosphatidylcholine exchange protein, we have adopted another approach to neutralize the effect of the lipid–water interface changing with different phospholipids. The specificity of the protein is determined by measuring the rate at which a radiolabeled phospholipid is transported from a donor to an acceptor vesicle. The bulk of the donor vesicle consists of egg yolk PC mixed with a small quantity (0.1–1%, w/w) of the labeled phospholipid. In view of this minor amount, we presume that the interaction of the exchange protein with the donor interface is not affected by the labeled phospholipid present, and that the label is homogeneously distributed throughout the PC bilayer. Under these conditions, the rate of transfer, which is expressed as the percentage of the label recovered in the acceptor vesicles, reflects the affinity of the protein at the interface for the labeled phospholipid, i.e., the substrate specificity. It should be realized, however, that the protein interacts at the interface also with the PC which forms the bulk of the donor vesicles. Therefore, the affinity for the various labeled phospholipids may be relative to egg yolk PC and could be different when the bulk of the donor vesicle consists of another phospholipid. Although this possibility requires further investigation, it is worth noting that the protein transfers egg yolk [^{14}C]PC at virtually the same rate whether the bulk consists of egg yolk PC or DMPE; under both conditions, the transfer of [^{14}C]DMPE is negligible (Figure 1A,B). From this experiment, it may be inferred that the protein interacts with both kind of donor vesicles equally well, demonstrating a high affinity for [^{14}C]PC and a low affinity for [^{14}C]DMPE independent of the composition of the donor vesicle.

It is seen from Table I that the transfer is optimal when the polar moiety of the phospholipid consists of phosphorylcholine. The failure of the protein to transfer DMPE suggests very strongly that the molecular configuration of the polar head should match the steric requirements of a specific binding site on the protein. Thus, replacement of a methyl by an ethyl group on the quaternary nitrogen reduces the transfer by half, while the presence of a propyl group inhibits the transfer completely. Similarly, an increase or decrease of the distance between phosphorus and nitrogen reduces the interaction with the protein. Transfer is abolished completely when a total of four methylene groups are present. Apart from a close conformational fit, the interaction between the binding site and the polar head of PC may also be electrostatically stabilized. An interference with this electrostatic interaction would explain the inhibitory effect of cations on the transfer of PC (Wirtz et al., 1976). Johnson and Zilversmit (1975) have also provided evidence for electrostatic interactions being involved in the transfer of PC by the phospholipid exchange protein from bovine heart.

Electrostatic interactions have been demonstrated to exist between a PC monolayer and basic peptides (Miller and Bach, 1974). These investigators presume that the charged groups of the polypeptides interact with the phosphate groups which form a fixed layer of negative charge at the PC–water interface. In general, electrostatic interactions between charged phospholipid vesicles and oppositely charged proteins and polypeptides result in stable complexes where subsequent hydrophobic interactions direct the rearrangement of protein and lipid in these complexes (Hammes and Schullery, 1970; Kimelberg and Papahadjopulos, 1971; London and Vossenberg, 1973). Certainly as a carrier of PC, the exchange protein does not form a stable complex with the interface. An additional argument against a strong hydrophobic interaction derives from the observation that the protein has no effect on the surface pressure of a PC monolayer (Demel et al., 1973). The nonperturbing nature of the exchange proteins follows also from the fact that only phospholipids from the outer half of the membrane are transferred (Johnson et al., 1975; Rothman et al., 1976). However, the apparent dissociation constant of the

TABLE III: Transfer of [^{14}C]PC and 1-[^{14}C]Acyllyso-PC.

Expt	Donor Membrane	Time (min)	Transfer of [^{14}C]Label to Acceptor Vesicles (%) Without Protein PC	Without Protein Lyso-PC	With Protein PC	With Protein Lyso-PC
A[a]	Egg PC Vesicles	5	1.8	38.7	7.7	34.0
		20		37.0		36.2
		30	2.4	40.2	17.9	37.0
B[b]	Rat liver Microsomes	0	0.6	5.5	0.7	5.4
		10	1.1	6.6	7.7	6.6
		20	1.2	7.6	10.0	5.6
		40	1.3	6.7	14.1	7.6

[a] In experiment A, the transfer of 1-[*methyl*-^{14}C]acyllyso-PC has been determined in the donor–acceptor vesicle assay as described under Materials and Methods. The donor vesicles contained 0.05%, w/w, of [^{14}C]lyso-PC. Transfer of [*methyl*-^{14}C]PC has been determined in a separate experiment. [b] In experiment B, the transfer of 1-[*methyl*-^{14}C]acyllyso-PC and [*methyl*-^{14}C]PC has been determined simultaneously in the assay consisting of ^{14}C-labeled microsomes and acceptor vesicles, as described under Materials and Methods.

exchange protein–vesicle complex decreases with an increasing content of negatively charged phospholipids in these vesicles (van den Besselaar et al., 1975; Helmkamp et al., 1976). This indicates that exchange proteins are sensitive to changes in the physical properties of the interface.

The above results strongly suggest that a specific binding site on the exchange protein interacts at the interface with the phosphorylcholine moiety of PC. This interaction may involve a zwitterionic amino acid pair, as has been proposed for the apoproteins from high-density lipoproteins forming a complex with PC vesicles (Assmann and Brewer, 1974; Segrest et al., 1974). It is of interest to note that glycerophosphorylcholine does not inhibit the transfer activity of the exchange protein (Wirtz, K. W.A., unpublished observation). This suggests that the binding site is only accessible to the phosphorylcholine moiety in the more hydrophobic environment of the polar region of the interface. Salem (1962) has calculated that the dielectric constant is about 15 in the polar region of a membrane and about 2 in the apolar lipid core. This implies that electrostatic interactions between protein and PC will be reenforced at the interface (Chapman, 1969). The exchange protein probably also contains a hydrophobic site to accommodate the hydrocarbon chains of PC. It is seen from Table II that the D stereoisomer of PC and PC analogues with ether bonds or carbon–carbon bonds, instead of ester bonds, are transferred. This implies that, in contrast to the binding site for phosphorylcholine, the hydrophobic site on the protein is less sensitive to changes in the conformation and configuration of the apolar part of PC. Lyso-PC, however, that lacks one acyl chain, is not transferred (Table III). This suggests that two apolar chains are required to stabilize the interaction of the exchange protein with the lipid molecule.

In the transfer of PC between membranes, we presume that the exchange proteins forms a collision complex with the membrane (van den Besselaar et al., 1975). Within this complex, then, PC bound to the protein is released into the interface, whereupon the protein interacts with a phospholipid molecule from that interface. The specificity of the protein, discussed so far, relates to this latter interaction. The interaction of the protein with the membrane itself, i.e., the formation of the collision complex, has been studied by measuring the release of [^{14}C]PC bound to the protein into the membrane (Figure 2). Although DMPE is barely transferred by the exchange protein, the protein releases [^{14}C]PC into vesicles composed of DMPE. This suggests that the protein can catalyze the net transfer of PC to an interface provided it can form the proper collision complex. Vesicles composed of PE and spingomyelin do not function as acceptors of [^{14}C]PC. Interaction of the exchange protein may be impaired because PE and sphingomyelin vesicles are in the crystalline state at the temperature of incubation, i.e., 37 °C (Ladbrooke and Chapman, 1969; Shinitzky and Barenholz, 1974). The release of [^{14}C]PC from the protein into the vesicles is currently under investigation to see whether it provides a method to study the physical properties of membranes.

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