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MINI-REVIEW

Phospholipid Transfer Proteins: Mechanism of Action

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Abstract

Phospholipid transfer proteins are generally localized in the cytosolic fraction of cells and are capable of catalyzing the flux of phospholipid molecules among membranes. Artificial membranes also participate in protein-catalyzed phospholipid movements. In this review the major phospholipid transfer proteins are discussed with respect to their phospholipid substrate specificity and the contributions of membrane physical properties to this process. The phenomenon of net transfer of phospholipids is described. The use of various kinetic approaches to the study of these catalysts is reviewed. A detailed consideration of the distinct phospholipid binding and membrane interaction domains of one phospholipid transfer protein is presented. Finally, some recent applications of phospholipid transfer proteins to the examination of membrane structure and function and further directions for the continued research activity with this class of proteins are summarized.

Key words: Phospholipid transfer protein; phosphatidylcholine; phosphatidyllinositol; exchange; net transfer; lipid–protein interactions; kinetics.

Introduction

Protein-catalyzed transport of phospholipids between membranes was first described in rat liver preparations (Wirtz and Zilversmit, 1968; Akiyama and Sakagami, 1969; McMurray and Dawson, 1969). Soluble cytosolic proteins have been shown to accelerate the rate of phospholipid transfer in a variety of natural and artificial membrane systems. Phospholipid transfer proteins have been purified to homogeneity from bovine liver, brain, and heart, rat liver and lung, human platelets, maize, yeast, and a photoheterotrophic

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Table I. Phospholipid transfer proteins purified to homogeneity

Protein	Principal source	Substrate specificity[a]	Reference
Phosphatidylcholine	Liver, bovine	PC	Kamp *et al.* (1973)
Phosphatidylinositol	Brain, bovine; heart, bovine	PI > PC ≫ PG, SM	Helmkamp *et al.* (1974); DiCorleto *et al.* (1979)
Nonspecific	Liver, bovine	PA, PC, PE, PG, PI, PS, SM, cholesterol	Crain and Zilversmit (1980)
Phosphatidylcholine	Liver, rat	PC	Lumb *et al.* (1976); Poorthuis *et al.* (1980)
Nonspecific	Liver, rat	PC, PE, PI, PS, SM, cholesterol	Bloj and Zilversmit (1977)
Nonspecific	Hepatoma, rat	PC, PI, SM	Dyatlovitskaya *et al.* (1978)
Nonspecific	Lung, rat	PC, PE, PI, PS	Read and Funkhouser (1983)
Phosphatidylinositol	Platelets, human	PI > PC > PG	George and Helmkamp (1985)
Nonspecific	Maize (seeds)	PC, PE, PI	Douady *et al.* (1982)
Phosphatidylinositol	*Saccharomyces cerevisiae*	PI > PC	Daum and Paltauf (1984)
Phospholipid	*Rhodopseudomonas sphaeroides*	PG > PC > PE	Tai and Kaplan (1984)

[a]PA, phosphatidate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

bacterium. These proteins differ not only in their physical and chemical properties but more dramatically in their catalytic activities.

All mammalian tissues thus far examined contain measurable phospholipid transfer activity that can be attributed to one or more proteins; this generalization also holds for plant tissues and selected prokaryotic cells (Wirtz, 1982; Kader *et al.*, 1982). Some of the major phospholipid transfer proteins are listed in Table I. For example, bovine liver contains at least three phospholipid transfer proteins: (1) a protein which is specific for phosphatidylcholine (Wirtz *et al.*, 1972), (2) a protein which transports both phosphatidylinositol and phosphatidylcholine (Helmkamp *et al.*, 1976a), and (3) a nonspecific lipid transfer protein which facilitates the movement of most phospholipids and other amphiphilic lipids such as cholesterol and glycolipids (Crain and Zilversmit, 1980). Rat tissues, particularly liver and small intestine, also contain a nonspecific lipid transfer protein which exhibits a broad spectrum of substrate recognition (Bloj and Zilversmit, 1977; Poorthuis *et al.*, 1981). Lipids which are not transferred by such nonspecific transfer proteins include bis-phosphatidylglycerol, cholesteryl esters, and triacylglycerols. The unequivocal identity between nonspecific lipid transfer

protein and sterol carrier protein II has finally been established by Scallen *et al.* (1985). The recent observation by Crain and Clark (1985) that this nonspecific lipid transfer protein is secreted by rat hepatoma (HTC-TR) cells maintained in culture provides additional speculation concerning the physiological role of phospholipid transfer proteins. In addition, several rat hepatoma and carcinoma cell lines as well as fetal rat liver express a nonspecific lipid transfer protein which differs in isoelectric point from the nonspecific lipid transfer protein in adult rat liver and has a pronounced specificity for sphingomyelin (Dyatlovitskaya *et al.*, 1978, 1982).

In addition to transfer proteins which primarily recognize phospholipid substrates, other lipid transfer proteins have been described. Glycolipid transfer proteins have been isolated from porcine, rat, and bovine brain and bovine spleen; they display no activity toward phospholipids (Metz and Radin, 1982; Abe *et al.*, 1982; Wong *et al.*, 1984). Transfer of the extremely apolar cholesteryl ester and triacylglycerol molecules is catalyzed by proteins in human plasma and bovine liver microsomes (Brewster *et al.*, 1978; Ihm *et al.*, 1982; Morton and Zilversmit, 1982; Wetterau and Zilversmit, 1984b). Involvement of these proteins in plasma lipoprotein metabolism has been suggested by Morton and Zilversmit (1983).

In this review special attention has been devoted to the role of phospholipid transfer proteins as true biological catalysts. This is appropriate because transfer proteins impart to the process of intermembrane phospholipid transport the characteristics of rate enhancement, substrate and membrane specificity, and inhibition. An appreciation of these characteristics has been strengthened by recent advances in elucidating the mechanism of spontaneous intermembrane transfer of phosphatidylcholine and anionic phospholipids (Duckwitz-Peterlein *et al.*, 1977; McLean and Phillips, 1981; De Cuyper and Joniau, 1985). Different sections of the review deal with the functional domains of transfer proteins, substrate requirements, membrane physical properties, kinetic analysis, and net transfer as a special event. Future directions of research on phospholipid transfer proteins are also discussed.

The reader is directed to other reviews on phospholipid transfer proteins. Wirtz (1982) and Kader *et al.* (1982) have detailed the general properties of phospholipid transfer proteins and their participation in cellular phospholipid metabolism. The structure, catalytic properties, and research applications of nonspecific lipid transfer proteins have been reviewed by Crain (1982). Helmkamp (1985) has focused more specifically on the family of phosphatidylinositol transfer proteins in a description of their chemical properties, catalytic activity, and physiological function. An excellent summary of the quantitation of phospholipid transfer activity has been written by Wetterau and Zilversmit (1984a). The effects of membrane lipid

composition and bilayer fluidity on phospholipid transfer protein activity have been reviewed (Helmkamp, 1983). The application of transfer proteins to investigations of lipid organization and membrane structure and function in biological and artificial systems has been addressed by Zilversmit (1978) and Bloj and Zilversmit (1981).

Substrate Specificity

Among the lipid transfer proteins bovine liver phosphatidylcholine exhibits the most dramatic substrate specificity. Kamp *et al.* (1977) demonstrated that modifications to the phosphorylcholine moiety produced analogues which were poorly transferred. These changes included additional methylenes between the phosphorus and nitrogen atoms and removal or elongation of the methyls on the quaternary nitrogen. A threefold preference for the natural stereoisomer, *sn*-glycero-3-phosphocholine, was also noted. Replacement of the ester linkage at the 1 or 2 positions of the glycerol backbone by an ether link or by a carbon–carbon bond to an alkyl moiety resulted in some reduction, but not complete loss in transfer activity. Neither lysophosphatidylcholine nor sphingomyelin was transferred by bovine phosphatidylcholine transfer protein. Thus, the affinity of this protein for its substrate is marked by extremely narrow tolerances in the spatial features of the polar head group and the apolar fatty acyl chains.

Similar studies have defined the phospholipid binding domain of bovine phosphatidylinositol transfer protein. Phosphatidylinositol is the preferred substrate; any modification to the inositol ring is detrimental to transfer activity. Periodate oxidation and subsequent reduction with sodium borohydride (Somerharju *et al.*, 1983) or phosphorylation at the 4-position (Schermoly and Helmkamp, 1983) render phosphatidylinositol inactive. Bovine phosphatidylinositol transfer protein also shows significant activity toward phosphatidylcholine (Helmkamp *et al.*, 1974; Johnson and Zilversmit, 1975). Changes to the phosphorylcholine moiety, similar to those discussed above for the liver protein, lead to reductions in transfer (Demel *et al.*, 1982). Interestingly, the brain and heart proteins are slightly active toward phosphatidylglycerol and sphingomyelin, both of which share structural features with the more actively transferred phosphatidylinositol and phosphatidylcholine (DiCorleto *et al.*, 1979; Helmkamp, 1985). No transfer of phosphatidylethanolamine, phosphatidylserine, or phosphatidate was detected (Helmkamp *et al.*, 1974; Zborowski and Demel, 1982).

Several approaches have been taken to establish the acyl chain specificity of bovine phosphatidylcholine transfer protein. Kamp *et al.* (1977) recognized that dipalmitoylphosphatidylcholine was transferred at approximately

one-third the rate of 1-palmitoyl-2-oleoylphosphatidylcholine between single bilayer vesicles. However, the saturated lipid was present at a level of 1 mol.% or less in egg phosphatidylcholine vesicles. A recent investigation by Child *et al.* (1985) utilized intact human erythrocytes as donor membranes and single bilayer acceptor vesicles of cholesterol and chemically defined phosphatidylcholines. Unsaturated phosphatidylcholines were transferred in marked preference to dipalmitoylphosphatidylcholine. This specificity was verified by a 10-fold more rapid transfer of ^{14}C-labeled soybean phosphatidylcholine, added exogenously to the erythrocytes, compared to ^{14}C-labeled dipalmitoylphosphatidylcholine. Among the unsaturated species, the rates of efflux generally paralleled the initial distribution of phosphatidylcholines in the erythrocyte membrane.

Welti and Helmkamp (1984) prepared an extensive series of phosphatidylcholine donor and acceptor vesicles. The general design of the vesicle–vesicle assay system, described in detail by Kasper and Helmkamp (1981a), is outlined in Fig. 1. By measuring transfer between homologous vesicles at 37°C in the presence of bovine phosphatidylcholine transfer protein, it was shown that long-chain, unsaturated, liquid crystalline phase molecular species were readily transferred, whereas long-chain, saturated species which were in a gel phase or short-chain, saturated species which were in a liquid crystalline phase were completely inactive. If such vesicles were added as a third population of membranes to an egg phosphatidylcholine donor-acceptor vesicle assay system, each inhibited protein-mediated transfer to an extent which was consistent with its activity as a substrate. That is, the third vesicle population competed with the other two for the transfer protein. However, dimyristoylphosphatidylcholine presented a surprising anomaly: although it was an extremely poor substrate, from either single species or mixed species donor vesicles, it was one of the most potent inhibitors of transfer when added as a third vesicle. This suggests a truly unique interaction between bovine phosphatidylcholine transfer protein and a molecule or membrane of dimyristoylphosphatidylcholine, such that catalytic activity is lost. In summary, the important factors in substrate specificity for this transfer protein are an acyl chain of greater than 14 carbons and a liquid crystalline lipid phase; unsaturation further enhances the transfer of long-chain, liquid crystalline phase molecular species.

An unusual acyl-chain specificity has been described for a phospholipid transfer protein purified from adult rat lung. Read and Funkhouser (1984) noted a preference for dipalmitoylphosphatidylcholine, relative to dioleoylphosphatidylcholine or 1-palmitoyl-2-arachidonoylphosphatidylcholine. These three lipids were compared at a level of 0.5 mol.% in egg phosphatidylcholine donor vesicles. Yet transfer activity was dramatically reduced as the proportion of dipalmitoylphosphatidylcholine in the donor vesicle

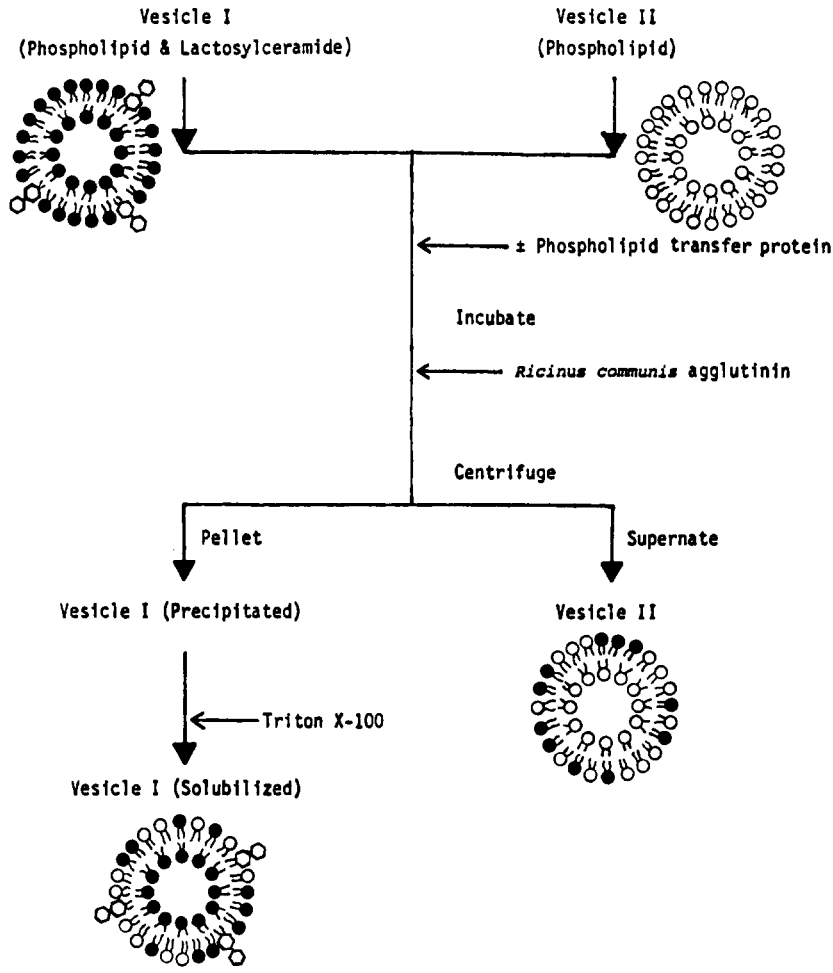

Figure 1. Protocol for a typical vesicle–vesicle assay of phospholipid transfer protein activity. Small unilamellar vesicles are prepared from pure or mixed phospholipids by sonication or ethanol injection. Into the Vesicle I population is incorporated 6–8 mol.% lactosylceramide. The phospholipid whose transfer is being measured is usually also incorporated into Vesicle I, which therefore becomes the donor membrane. Vesicle I and Vesicle II are mixed and incubated in the presence or absence (blank) of phospholipid transfer protein. Transfer is arrested by adding *Ricinus communis* agglutinin, which reacts with the lactosylceramide-containing membranes and allows them to be precipitated quantitatively. Vesicle II, the acceptor membrane, is analyzed for the extent of recovery and the amount of phospholipid transferred from Vesicle I. Such analysis may utilize liquid scintillation spectrometry, gas–liquid chromatography, or fluorescence spectroscopy.

population approached 100 mol.%. Again, the bulk phase of the membrane lipids exercises a profound influence on protein-catalyzed phospholipid transfer.

Net Transfer of Phospholipids

As the catalytic mechanism of phospholipid transfer proteins became the subject of investigation, one question of paramount importance was whether these proteins facilitated the exchange or the net transfer of lipid molecules between membranes. This distinction applies to the total membrane phospholipid, on the one hand, and the individual phospholipid classes, on the other. The processes of exchange and net transfer are illustrated in Fig. 2. These two modes of intermembrane phospholipid movement occur spontaneously or are catalyzed by various transfer proteins. In the case of

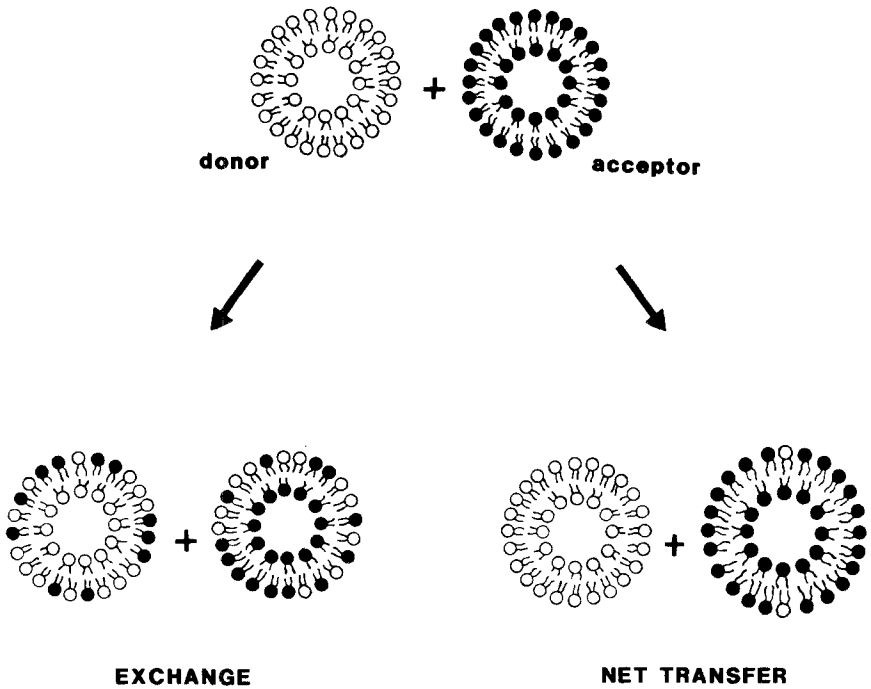

Figure 2. Modes of intermembrane phospholipid transfer. When donor and acceptor membranes are mixed, phospholipid transfer may occur spontaneously or be protein-catalyzed. Exchange requires equivalent bidirectional fluxes, *i.e.*, as many donor phospholipid molecules are transferred to the acceptor membrane as acceptor phospholipids to the donor membrane. Net transfer, on the other hand, may result in only a flux of donor phospholipids to the acceptor membranes.

exchange, there are obviously equivalent bidirectional fluxes of phospholipid molecules; for net transfer, however, the unidirectonal fluxes may differ in magnitude so as to result in a net gain by one membrane population at the expense of the other. Irrespective of the mode of transfer, it has been established that only the outer monolayer of phospholipids participates in protein-catalyzed transfers (Barsukov *et al.*, 1974; Johnson *et al.*, 1975; Rothman and Dawidowicz, 1975; de Kruijff and Wirtz, 1977).

The absolute polar head group specificity of bovine phosphatidylcholine transfer protein makes the analysis of net transfer a straightforward exercise. Indeed, using acceptor vesicles prepared from phosphatidylethanolamine and phosphatidate, a net transfer of phosphatidylcholine can be observed (Wirtz *et al.*, 1980a; Nichols and Pagano, 1983). In both cases, however, the transferred phosphatidylcholines contained relatively bulky ESR or fluorescence spectroscopic labels and high concentrations of protein (0.2–0.4 mg ml^{-1}) had to be employed. Berkhout *et al.* (1984a) re-examined net transfer and demonstrated a unidirectional flux of *cis*-parinaric acid-labeled phosphatidylcholine into vesicles prepared from phosphatidate. Using 1.4 $\mu g\, ml^{-1}$ phosphatidylcholine transfer protein, the rate of transfer was significantly slower for net transfer than for the presumed exchange which occurred into vesicles prepared from phosphatidylcholine and phosphatidate. When even lower concentrations of this protein (0.2–0.7 $\mu g\, ml^{-1}$) were used and transfer rates were measured in both directions between two populations of single bilayer vesicles, Helmkamp (1980b) found no evidence for net transfer. Transfer between two monolayers through a common aqueous subphase also proceeded as a true exchange process, as indicated by no change in the surface film pressures (Demel *et al.*, 1973).

This preferred exchange mechanism was also described for bovine phosphatidylinositol transfer protein in a vesicle–vesicle assay system by Kasper and Helmkamp (1981b). A significant consequence of the mixed polar head group specificity exhibited by this transfer protein is the possibility of a net transfer of certain phospholipid classes. Net transfer of phosphatidylinositol has been recognized from vesicles or monolayers containing the lipid to vesicles or liposomes which were initially devoid of phosphatidylinositol (Demel *et al.*, 1977; Kasper and Helmkamp, 1981b). Net transfer of phosphatidylcholine also occurs to vesicles initially lacking that phospholipid. In the vesicle–vesicle systems a compensatory flux of equimolar magnitude took place in the opposing direction. For example, the combined movement of phosphatidylinositol and phosphatidylcholine molecules in the "forward" direction was balanced by an equivalent movement of phosphatidylcholine molecules in the "reverse" direction. The phenomenon of net transfer of specific lipid classes may be an intrinsic characteristic of these proteins' biological activity, particularly when relatively rare phospholipids such as

phosphatidylinositol are exchanged for relatively abundant classes such as phosphatidylcholine.

Several groups have prepared delipidated lipoprotein fractions and used the apoprotein-rich complexes as potential acceptors of protein-transferred phospholipids. Both phosphatidylcholine and phosphatidylinositol move to high-density lipoprotein apoproteins in the presence of bovine nonspecific lipid transfer protein (Crain and Zilversmit, 1980). Similarly, rat liver inner mitochondrial membranes accumulated considerable phosphatidylcholine (50–60%) from vesicles, but lost a significant quantity of phosphatidylethanolamine (10–20%) when bovine nonspecific transfer protein was added to this membrane mixture. An overall gain of phospholipid was realized by the mitoplasts. In another series of experiments, Jackson *et al.* (1978) demonstrated clearly that phosphatidylcholine and phosphatidylinositol were readily transferred between rat liver microsomes and all major classes of human plasma lipoproteins upon incubation with bovine phosphatidylcholine and phosphatidylinositol transfer proteins; however, the possibility of net transfer was not considered.

It is obvious that both net transfer and exchanges are possible with many lipid transfer proteins and that the actual catalytic mode is critically dependent on the selected experimental conditions. From a physiological perspective the net transfer of phospholipids of specific polar head group or fatty acyl composition or total lipid is more reasonable than simple exchange. Several instances where phospholipid transfer proteins could be significant are (1) in the generation and maintenance of specific membrane lipid environments, particularly as a result of stimulated phosphoinositide turnover, and (2) in the assembly of lipoproteins by liver and small intestine. In the former instance, the broad tissue distribution of phosphatidylinositol transfer proteins and dual specificity toward phosphatidylinositol and phosphatidylcholine are compatible with a unidirectional transport of phosphatidylinositol to the plasma membrane of stimulus-responsive cells. In the latter instance, both phosphatidylcholine transfer protein and the nonspecific lipid transfer protein are found primarily in those tissues which synthesize, assemble, and secrete lipoproteins.

It should be stressed that there are many difficulties inherent to the design and interpretation of phospholipid transfer assay systems which mimic conditions in living systems. In comparing phospholipid transfer from microsomes to mitochondria in intact, cultured cells and *in vitro*, Yaffe and Kennedy (1983) concluded that a partially purified phosphatidylcholine transfer protein from rat liver served an essential *in vivo* role, but a partially purified phosphatidylcholine transfer protein from baby hamster kidney (BHK-21) cells did not. However, little consideration was given to other lipid transfer proteins which are known to be present in rat liver and, probably, hamster kidney.

Kinetic Analysis

With the purification of transfer proteins from bovine liver, brain, and heart, the catalytic nature of the transfer process became apparent. The number of phospholipid molecules transported between membranes in a given time exceeded by several orders of magnitude the number of protein molecules in the assay system (Van den Besselaar *et al.*, 1975; Helmkamp *et al.*, 1976b). It must be noted that a given assay system generally underestimates the catalytic efficiency of a phospholipid transfer protein, since only one of multiple transfer routes can be conveniently quantitated. Another important early observation was the dependence on membrane concentration of the rate of protein-catalyzed phospholipid transfer. Plots of transfer activity versus increasing membrane concentration were hyperbolic or, more frequently, increased to a maximum and then decreased. Curves with the former shape could be analyzed graphically by assuming saturation kinetics, while curves with the latter shape appeared to follow a classical pattern of substrate inhibition. A summary of these investigations is presented in Table II.

Using a kinetic model of two reactants, representing the two populations of membranes usually employed in an assay system, several mathematical expressions have been derived for phospholipid transfers catalyzed by bovine phosphatidylcholine transfer protein and bovine phosphatidylinositol transfer

Table II. Kinetic Analysis of Bovine Phospholipid Transfer Proteins

Transfer protein	Membrane[a]		Variables	Reference
	Donor, D	Acceptor, A		
Phosphatidylcholine	SUV	SUV	[D], [A], mol.% phosphatidate in D	[b]
Phosphatidylcholine	SUV	MLV	[D], pH 3.5–8.5, mol.% phosphatidate in D	[c]
Phosphatidylcholine	SLV	MLV	[D], [A], temperature, fatty acyl composition of phosphatidylcholine	[d]
Phosphatidylcholine	SUV	SUV	[A], vesicle diameter of A, mol.% phosphatidate in D and A	[e]
Phosphatidylinositol	RLM	SUV	[D], [A], mol.% phosphatidylinositol in A	[f]

[a] SUV, small unilamellar vesicles; MLV, multilamellar vesicles; RLM, rat liver microsomes.
[b] Van den Besselaar *et al.* (1975).
[c] Wirtz *et al.* (1979).
[d] Bozzato and Tinker (1982).
[e] Berkhout *et al.* (1984a).
[f] Helmkamp *et al.* (1976b).

protein. These expressions and the resulting graphical interpretations have yielded estimations of protein–membrane association and dissociation constants and association and dissociation rate constants. Essential to the generation of a kinetic model for protein-catalyzed phospholipid transfer were the observations that protein–phospholipid complexes could be isolated (Demel *et al.*, 1973; Helmkamp *et al.*, 1976b). Such complexes were assumed to be the functional intermediates in the catalytic mechanism, that is, the protein-bound, water soluble, freely diffusible form of a phospholipid molecule.

Relationships between the kinetic parameters and rate constants and such variables as vesicle size, curvature, and phospholipid composition have been established. The protein–membrane dissociation event was found to be rate-limiting; the rate constant for this step tended to decrease as the molar proportion of phosphatidate increased in vesicles (Wirtz *et al.*, 1979) and to increase as much as 5-fold as vesicles underwent a phase transition from gel to liquid crystalline (Bozzato and Tinker, 1982). The activity of bovine phosphatidylcholine transfer protein is markedly affected by membrane curvature. Machida and Ohnishi (1980) observed that vesicle–vesicle transfer was 100 times more rapid than vesicle–liposome transfer and that transfer from vesicles to spiculated erythrocyte ghosts was 4 times faster than to cup-shaped erythrocyte ghosts; in both comparisons, the more highly curved acceptor membrane was more active. Recently, Berkhout *et al.* (1984a) determined that the apparent association constant for complex formation between phosphatidylcholine transfer protein and vesicles decreased sharply with increasing vesicle diameter, over the range 22–40 nm.

As stated earlier, kinetic treatments to date have dealt with only the donor-to-acceptor route of phospholipid transfer. It is critical to develop a more general model which would take into account the acceptor-to-donor route, as well as donor-to-donor and acceptor-to-acceptor routes. Indeed, a transfer protein must be expected to partition its activity among these four routes in any given experimental system.

Contributions of Membrane Physical Properties

A clearly different approach to the influence of phase transition on protein-mediated phospholipid transfer was taken by Xu *et al.* (1982, 1983). Vesicles were prepared from dimyristoyl-, dipalmitoyl-, or distearoylphosphatidylcholine. Phase properties were monitored by freeze-fracture electron microscopy or fluorescence polarization of diphenylhexatriene. Each vesicle exhibited a rippled structure and a characteristic phase transition temperature, both of which were used to identify the initial state of mixtures of two

vesicle populations. After mixing in the presence of bovine phosphatidylcholine transfer protein a new lipid phase was observed, intermediate between the initial pure phases. The new phase resulted from a mixing of phosphatidylcholines. For example, as dimyristoylphosphatidylcholine was transferred into dipalmitoylphosphatidylcholine vesicles, the rippled pattern of pure dipalmitoylphosphatidylcholine was replaced by a mosaic of smooth and rippled zones or even by a uniformly smooth texture on the freeze-fracture faces. Investigating protein-mediated transfer in the dipalmitoylphosphatidylcholine vesicle–distearoylphosphatidylcholine vesicle system as a function of temperature, they noted that transfer activity was highest at the phase transition temperatures (42 and 52°C) of the two pure phospholipids. These results suggest that bovine phosphatidylcholine transfer protein is more active toward membranes at the phase transition temperature of their component phospholipids, where regions of gel and liquid crystalline phases coexist. This conclusion, however, must be made with some caution for several reasons. The concentration of protein was unusually high (13–20 $\mu g\,ml^{-1}$); initial rates of transfer were not determined; and the transfer activity was not expressed quantitatively.

A detailed kinetic analysis of phosphatidylcholine transfer protein activity with dipalmitoylphosphatidylcholine multilamellar acceptor vesicles was undertaken by Bozzato and Tinker (1982). With donor vesicles of egg phosphatidylcholine the maximal rate of transfer increased fivefold as the temperature increased from below the phase transition (37°C) to above (45°C); the dissociation rate constant increased in a proportionate manner. The comparable parameters for egg phosphatidylcholine multilamellar vesicles were 6 to 30 times larger, but remained unchanged at the two temperatures. Despite the lower activity toward the saturated phosphatidylcholine membranes, phosphatidylcholine transfer protein displayed a marked preference for the more fluid environment of the liquid crystalline phase above the transition temperature.

Bovine phosphatidylinositol transfer protein is also sensitive to the fluidity of the membranes with which it interacts. Using rat liver microsomes donor membranes and acceptor vesicles composed of chemically defined phosphatidylcholines, Helmkamp (1980a) found that donor-to-acceptor transfer activity varied inversely with the microviscosity of the acceptor vesicle. In these studies microviscosity was measured by the relative fluorescence polarization of diphenylhexatriene, a technique which can describe subtle differences in the liquid crystalline phase behavior of these membranes. Included in the comparison were *cis*- and *trans*-unsaturated, saturated, and mixed acyl chain phosphatidylcholines.

The effect of the transition between gel and liquid crystalline phases of phospholipid bilayers was examined in a vesicle–vesicle assay system (Kasper

and Helmkamp, 1981a). Bovine phosphatidylcholine transfer protein was less active toward dimyristoylphosphatidylcholine acceptor vesicles than toward egg phosphatidylcholine vesicles between 11 and 45°C. Moreover, the Arrhenius plots of phosphatidylcholine transfer with these two membranes yielded a biphasic function for the saturated phosphatidylcholine, with a discontinuity at 31°C, and a smooth function for egg phosphatidylcholine. The discontinuity corresponded to the upper end of the temperature range over which the phase transition occurs for small unilamellar vesicles. Significantly, the discontinuities in transfer activity and fluorescence polarization were abolished when the dimyristoylphosphatidylcholine vesicles were prepared with 33 mol.% cholesterol. These results are a further indication that the physical organization of lipids in participating membranes is an important aspect of protein-catalyzed phospholipid transfer.

Mapping the Active Site

At the level of protein structure, bovine phosphatidylcholine transfer protein is the most extensively studied of all lipid transfer proteins. The complete primary structure of this 213-residue polypeptide was determined by proteolytic digestion, chemical cleavage, and Edman degradation (Akeroyd *et al.*, 1981b). The molecule is a single polypeptide and contains two disulfide bridges and an acetylated methionyl residue at the N terminus. The proportion of alpha-helix is approximately 30%, as determined by circular dichroism and predicated by statistical techniques (Akeroyd *et al.*, 1982). A region of the protein molecule containing two antiparallel, amphiphilic beta-strands has been proposed as the putative membrane interaction domain. This region is rich in aromatic amino acid residues as well as a residue each of lysine and arginine. These strategically oriented cationic amino acids could provide an explanation for the enhanced association of phosphatidylcholine transfer protein with phospholipid vesicles containing significant quantities of phosphatidate or other anionic phospholipids (Wirtz *et al.*, 1976, 1979).

Detailed information on the phospholipid binding domain has accrued from a series of elegant chemical and spectroscopic studies. Evidence for a specific arginyl residue in the active site is based upon inactivation by 2,3-butanedione and phenylglyoxal (Akeroyd *et al.*, 1981a) and partial protection of this residue in the presence of negatively charged vesicles. The guanidinium function of this amino acid could readily accommodate the phosphodiester moiety of a protein-bound phosphatidylcholine molecule.

Using a number of synthetic photoactivable phosphatidylcholine analogues, Moonen *et al.* (1979), Wirtz *et al.* (1980b), and Westerman *et al.* (1983) were able to localize the binding of phosphatidylcholine on

phosphatidylcholine transfer protein. Both nitrene- and carbene-generating fatty acid derivatives were linked to the *sn*-2 position of the glycerol backbone. Upon photolysis and subsequent structural analysis, the peptide segment val^{171}-phe-met-tyr-tyr-phe-asp^{177} was found to be coupled preferentially to the phospholipid. Crosslinks between the *sn*-1 and *sn*-2 fatty acyl groups were not produced. The excellent agreement between the two different classes of photoactivable phospholipids suggests that this hydrophobic peptide, if folded into a beta-strand, could be positioned along the length of the *sn*-2 fatty acyl chain.

That phosphatidylcholine binds to a relatively inaccessible domain of the protein is further demonstrated by electron spin resonance spectroscopy. Nitroxide-labeled fatty acids were esterified to the *sn*-2 position; the probe phosphatidylcholines were then bound to phosphatidylcholine transfer protein. Strongly immobilized spectra were recorded for nitroxide probes near the methyl end as well as the ester link of the fatty acyl chain (Devaux *et al.*, 1977; Machida and Ohnishi, 1978). These data were interpreted to indicate a hydrophobic cavity within the protein to which a lipid molecule could bind. Significantly, ascorbate was unable to reduce the protein-bound, nitroxide-labeled phosphatidylcholines (Devaux *et al.*, 1977). It should be pointed out that phosphatidylcholines containing spin-labeled fatty acids were readily transferred to and from phospholipid vesicles by phosphatidylcholine transfer protein, but those phosphatidylcholines containing spin-labeled choline analogues were not (Devaux *et al.*, 1977; Machida and Ohnishi, 1978).

Most recently, the phospholipid binding domain of phosphatidylcholine transfer protein has been investigated by fluorescence spectroscopy, using *cis*-parinaric acid as a reporter group (Berkhout *et al.*, 1984b). This conjugated polyene fatty acid was incorporated into phosphatidylcholine at the *sn*-1, *sn*-2, or both the *sn*-1 and *sn*-2 positions. All parinaroyl derivatives formed complexes with the transfer protein, but the rate of formation for diparinaroylphosphatidylcholine was markedly slower than either monoparinaroyl species. From the decay kinetics of fluorescence anisotropy, different rotational correlation times were calculated for 1-parinaroyl- and 2-parinaroylphosphatidylcholines. However, for the decay of fluorescence, the same short lifetime component predominated for both species, in contrast to a longer lifetime characteristic of parinaroylphosphatidylcholine vesicles. These results are consistent with a tight binding of the phospholipid to the protein such that the rotational mobility of the reporter group assumes that of the protein itself. Furthermore, the two fatty acyl chains sense protein environments and geometries which are apparently nonparallel and spatially distinct. Again, essentially no interaction between the two acyl binding sites was detected, supporting the results of the photoactivation experiments described above.

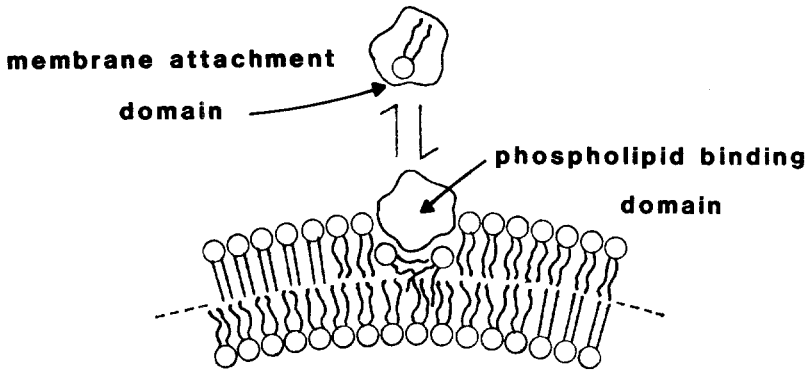

Fig. 3. Functional domains of a phospholipid transfer protein. A phospholipid binding domain accommodates a single phospholipid molecule through concerted electrostatic, hydrogen, and hydrophobic bonding. A membrane interaction domain assures a transient and reversible association/dissociation between a membrane surface and the phospholipid–phospholipid transfer protein complex. Penetration of the membrane by this complex may be required for efficient and selective phospholipid transfers.

These studies support the concept of well-defined regions formed by the secondary and tertiary conformations of bovine phosphatidylcholine transfer protein. It is likely that similar domains exist in all phospholipid transfer proteins (Fig. 3). The phospholipid binding domain provides the necessary electrostatic interactions for the phosphorylcholine polar head group and sufficient hydrophobic interactions for the fatty acyl chains. Surprisingly, occupation of the phospholipid binding domain by a phosphatidylcholine leads to the physical separation of the two fatty acyl chains. The membrane interaction domain, which appears to comprise a physically distinct region of the protein, assures the appropriate association and dissociation between the transfer protein and a membrane surface. This interaction is characterized by a significant electrostatic component.

Future Research Directions

In the preceding sections a large body of literature on phospholipid transfer proteins has been summarized. A basic understanding of the catalytic properties of many of these proteins is now possible. Consideration has been given to the detailed substrate specificity, membrane interactions, and kinetic analysis of two bovine catalysts: phosphatidylcholine transfer protein prepared from liver and phosphatidylinositol transfer protein isolated from brain and heart. Knowledge of the primary and secondary structures of bovine phosphatidylcholine transfer protein has led to a tentative model of

the molecular organization of the stoichiometric complex of this protein and a bound, transferable phosphatidylcholine.

Yet much remains to be elucidated on the nature of the complexes which result upon the binding of transfer proteins to membranes. For those proteins which function as true carriers of phospholipid molecules through the aqueous phase between membranes, it is necessary that the protein–membrane complex be transient and reversible. To what extent is a monolayer or bilayer of lipid molecules perturbed by a transfer protein, and conversely, what is the impact of the membrane lipid organization on the structural properties of a transfer protein? These and similar questions will most likely be answered using spectroscopic experimental approaches. Some progress has already been made with electron spin resonance and fluorescence methods (Wirtz and Moonen, 1977; Devaux *et al.*, 1977; Machida and Ohnishi, 1978). But the possibility of the transfer protein's dissociating from the surface of the membrane before reliable and representative measurements have been made is quite real. Studies in this area would be facilitated by more stable, long-lived complexes between phospholipid transfer proteins and membranes. The usually long-chain, potentially membrane-spanning *bis*-phosphatidylcholines, recently described by Runquist and Helmkamp (1985), could provide a surface to which phospholipid transfer proteins could bind and, because the phospholipids themselves are not transferable, with which they would remain associated.

The appreciation of the *in vitro* activities and capabilities of phospholipid transfer proteins has led to the widespread use of these proteins in the investigation or manipulation of phospholipid organization in biological and artificial membranes. Many of the early applications of phospholipid transfer proteins to this field focused on transbilayer distributions of specific classes of phospholipids and the rates of transbilayer mobility (Bloj and Zilversmit, 1981). More recently, the systematic replacement, extraction, or insertion of a phospholipid class or molecular species in a membrane has been accomplished with various phospholipid transfer proteins. The consequences of the controlled alteration of membrane phospholipid structure on functional parameters can be assessed directly. In Table III several recent examples of specific applications are listed. These approaches represent a means of structural modification with minimal perturbation. The increased availability and continued characterization of phospholipid transfer proteins will permit greater progress in this exciting area of protein–lipid and lipid–lipid interactions in membranes.

Despite the wealth of information on the structure and activity of various phospholipid transfer proteins, there is a large void in understanding the physiological functions of these catalysts. The proteins are ubiquitously distributed among plants, animals, other eukaryotic cells, and several

Table III. Applications of Phospholipid Transfer Proteins to the Investigation of Membrane Structure and Function

Membrane modified	Transfer protein	Lipid(s) transferred[a]	Experimental purpose
Murine neuroblastoma cells[b]	Nonspecific (rat)	PC, SM	Monitor metabolic turnover and subcellular distribution of exogenous phospholipids
Turkey erythrocytes[c]	Nonspecific (bovine)	PI	Modify catecholamine-stimulated adenylate cyclase
Electroplax plasma membranes[d]	Nonspecific (bovine)	PE, PS, sterol	Incorporate fluorescent lipid probes to study Na^+, K^+-ATPase and acetylcholinesterase
Rat liver microsomes[e]	Nonspecific (rat)	Cholesterol	Alter activity of 3-hydroxy-3-methylglutaryl-CoA reductase
Human erythrocytes[f]	Phosphatidylcholine (bovine)	PC	Determine cell shape as a function of fatty acid content of phosphatidylcholine

[a] Abbreviations as in Table I.
[b] D'Souza *et al.* (1983).
[c] McOsker *et al.* (1983).
[d] Muczynski and Stahl (1983).
[e] Van Heusden and Wirtz (1984).
[f] Kuypers *et al.* (1984).

prokaryotic cells. Correlations between transfer protein activity and phospholipid metabolic rates have been suggested from studies conducted in developing brain (Ruenwongsa *et al.*, 1979; Brophy and Aitken, 1979; Carey and Foster, 1984), maturing lung (Engle *et al.*, 1978), normal and transformed liver (Teerlink *et al.*, 1984), and yeast grown in the presence and absence of glucose (Daum and Paltauf, 1984). In studies where activity measurements were supplemented by immunoprecipitation techniques, changes in protein concentration were evident (Poorthuis *et al.*, 1980; Teerlink *et al.*, 1984). Discrepancies between the amount of phosphatidylcholine transfer protein present in rat liver calculated from specific activity measurements and the immunoassayable levels of this transfer protein amounted to about 2-fold. Interestingly, such discrepancies were not observed in several slow- and fast-growing hepatoma cell lines.

Another recent application of immunochemical analysis has been reported by Van der Krift *et al.* (1985). Antibodies to the nonspecific lipid transfer protein were coupled to protein A–colloidal gold and applied to fixed thin sections of rat liver. Approximately 70% of the nonspecific lipid transfer protein was localized in the cytosol. This distribution compared favorably with a subcellar fractionation which indicated that 60% of phosphatidylcholine transfer protein activity was in the cytosol and the remainder very

loosely bound to various membranous organelles (Teerlink *et al.*, 1982). Quite unexpectedly, another immunoreactive polypeptide with a molecular mass considerably greater than that of the nonspecific lipid transfer protein was localized in the peroxisome compartment of rat liver. The ability to raise specific antibodies to a number of rat phospholipid transfer proteins should be viewed as the first step of a long-term effort to decipher the intracellular activity of these protein catalysts. In addition to the continued use of immunochemical and immunoassay techniques, attempts to introduce the purified antibodies, or better still, monoclonal antibodies into permeabilized cells will undoubtedly be made. Disruption of the time-dependent flux of phospholipid among membranes, following pulse-labeling with a suitable synthetic precursor, would be evaluated. Indeed, early experimental observations by Jungalwala and Dawson (1970) of phospholipid metabolism in liver firmly established a movement of phospholipid molecules from the endoplasmic reticulum, where the major synthetic enzymes residue, to the nucleus, the plasma membrane, and mitochondria.

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