Biochimica et Biophysica Acta, 1063 (1991) 197–202

ADONIS 000527369100152E

BBAMEM 75180

Protein-catalyzed transport of ether phospholipids

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(Received 14 August 1990)
(Revised manuscript received 10 December 1990)

Key words: Phospholipid transfer protein; Transfer protein; Plasmalogen; Pyrene-labeled glycerophospholipid; Fluorescence; Yeast

The protein-catalyzed transfer of alkenylacyl-, alkylacyl-, or diacyl-glycerophosphocholines, carrying a pyrenedecanoyl residue as a fluorogenic acyl chain, was studied using unilamellar bilayer vesicles as donor and acceptor membranes in a fluorescence assay. Specific phospholipid transfer proteins, such as phosphatidylinositol transfer protein from yeast and phosphatidylcholine transfer protein from bovine liver showed higher transfer rates with ether lipid substrates. Transfer rates for alkylacyl- and alkenylacyl-glycerophosphocholine as compared to the diacyl analog were rather similar in the presence of non-specific lipid transfer proteins from maize or from bovine liver, respectively. When vesicles of fluorogenic compounds were titrated with the yeast phosphatidylinositol transfer protein, only a 15–20% higher binding affinity for alkenylacyl- and alkylacyl-glycerophosphocholine than for diacyl-glycerophosphocholine was observed. Thus the marked differences of transfer rates measured with this transfer protein cannot be attributed to different binding affinities for the respective phospholipid subclasses. A possible explanation for differences in transfer rates could be differences in the organization of the phospholipid subclasses at the hydrophobic/hydrophilic interface of bilayer membranes.

Introduction

Phospholipid transfer proteins from the cytosol of eukaryotic cells catalyze the transport of phospholipids between membranes through the aqueous phase in vitro. Specific phospholipid transfer proteins form stoichiometric complexes with the phospholipid molecules to be transferred. Some of these proteins are highly specific for only one phospholipid class, e.g. the phosphatidylcholine transfer protein from liver [1]. Other transfer proteins show preference for one phospholipid, but catalyze in addition the transport of other phospholipids. For example, the phosphatidylinositol transfer proteins from brain [1] and yeast [2,3] are specific for phosphatidylinositol and phosphatidylcholine. On the other hand, non-specific lipid transfer proteins exert transfer activities with a variety of different lipids including sterols. Specific and non-specific lipid transfer proteins apparently act by different mechanisms. In contrast to specific lipid transfer proteins [3,4] non-specific lipid transfer proteins do not form stable stoichiometric complexes with lipids [5]. Only with 7-nitrobenz-2-oxa-1,3-diazol-4-yl-labeled phosphatidylcholine formation of rather unstable complexes was observed [6].

Protein-catalyzed phospholipid transfer can be observed in vitro between biological and/or artificial membranes. Transport rates depend on the physicochemical properties of the constituent phospholipids, such as structure and charge of polar head groups, hydrophobic side chain composition, and side chain mobility ('fluidity') as influenced by the presence of sterols and the phase state of the bilayer [1]. Less attention has been paid to the influence of the type of chemical bonding (ether or ester) between glycerol and the aliphatic chain(s). Considering the proposed mechanism of transfer by specific phospholipid transfer proteins, which includes head group recognition and phospholipid binding after extraction from the membrane, it is plausible to assume that transfer protein–membrane interactions depend on the phospholipid arrangement at the site of initial interaction at the hydrophobic/hydrophilic membrane interface. Differences in conformation, polarity and segment mobility arising from the type of chemical bonding between glycerol and the aliphatic moieties might therefore have an effect on protein-catalyzed phospholipid transport. The phospha-

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tidylcholine transfer protein from bovine liver was reported to catalyze the transport of diacyl, di-*O*-alkyl- and 1-*O*-alkyl-2-acyl-glycerophospholipids at similar rates [7]. However, the lipid subclasses used in these experiments differed not only in the type of bonding between glycerol and the hydrophobic residues, but also in the length of the side chains.

In the present study we determined the rates of protein-mediated transfer of fluorescent alkenylacyl-, alkylacyl- and diacyl-glycerophosphocholines bearing a pyrenedecanoyl residue in position *sn*-2 of glycerol. The respective model substrates differed only with respect to the chemical linkage between glycerol and the hydrophobic chains (enolether, alkylether or carboxylester) in position *sn*-1 of the glycerol backbone. Specific lipid transfer proteins, namely the phosphatidylinositol transfer protein from yeast and the phosphatidylcholine transfer protein from bovine liver as well as the non-specific lipid transfer proteins from bovine liver and from maize, respectively, were tested for their ability to catalyze intermembrane transport of the three phospholipid subclasses.

Materials and methods

Synthesis of 1-O-(1'-Z-alkenyl)-2-pyrenedecanoyl-sn-glycero-3-phosphocholine

The one pot-acylation procedure is based on the protocol described by Gupta et al. [8]. Lysoplasmalogen was prepared from bovine heart total choline glycerophospholipids by mild alkaline hydrolysis as described [9] and acylated with pyrenedecanoic acid as follows. A solution of the lysophospholipid (20 mg, 41.5 μmol), pyrenedecanoic acid (20 mg, 53.7 μmol), dimethylaminopyridine (20 mg, 163.9 μmol), and dicyclohexylcarbodiimide (17 mg, 82.5 μmol) was incubated in 1 ml chloroform at 40 °C over night. After addition of 0.5 ml methanol and 2 ml chloroform/methanol (2:1, v/v), the reaction mixture was washed twice with 0.7 ml methanol/water (1:1, v/v), and the solvent was removed under reduced pressure. The crude product was purified by MPLC on a Silica gel column (1 × 10 cm) using a chloroform/methanol gradient for elution. The purified compound (16 mg, 47% yield) was eluted at a chloroform to methanol ratio of 7:3 (v/v) and showed a single spot on thin-layer chromatography ($R_f \approx 0.3$) using chloroform/methanol/water (65:25:5, by vol.) or chloroform/methanol/25% aqueous ammonia (65:35:5, by vol.) as solvents. The ratio of pyrenedecanoic acid (derived from the absorbance at 342 nm) to phosphorus (from elemental analysis) was 0.9. Label degradation during chemical synthesis and partial micellization of phospholipids leading to high local chromophore concentrations may account for this deviation from the theoretical ratio of 1.0. Pyrenedecanoic acid in different organic solvents (chloroform, dimethyl formamide, methanol) served as a reference for measurements of absorbance at 342 nm (see also Ref. 10).

Synthesis of 1-O-hexadecyl-2-pyrenedecanoyl-sn-glycero-3-phosphocholine

1-*O*-Alkylglycerophosphocholine was prepared according to established methods [11] and acylated with pyrenedecanoic acid as described for the alkenyl analog. After MPLC purification, the product (18 mg, 50% yield) showed a single spot on thin-layer chromatography ($R_f \approx 0.3$) using chloroform/methanol/water (65:25:5, by vol.) or chloroform/methanol/25% aqueous ammonia (65:35:5, by vol.) as solvents. The ratio of pyrenedecanoic acid to phosphorus was 0.9.

Synthesis of 1-palmitoyl-2-pyrenedecanoyl-sn-glycero-3-phosphocholine

Palmitoylglycerophosphocholine was prepared according to Mason et al. [11] and acylated with pyrenedecanoic acid as described for the ether analogs. The spectral properties of the product obtained were identical to those of the fluorescent lipid described by Somerharju et al. [10].

Phospholipid transfer proteins

The yeast phosphatidylinositol transfer protein was isolated from the cytosol of *Saccharomyces cerevisiae* as described by Szolderits et al. [3]. Enriched bovine liver phosphatidylcholine transfer protein [13] and bovine liver non-specific lipid transfer protein [14] were prepared by published procedures. Maize phospholipid transfer protein was a gift from J.-C. Kader, Paris.

Phospholipid transfer assays

Phospholipid transfer measurements [3] are based on an assay using fluorescent phospholipids similar to that described before by Somerharju et al. [15] and Van Paridon et al. [16]. In brief the increase of pyrene monomer fluorescence intensity resulting from transfer of fluorescent phospholipid molecules from donor vesicles containing high concentrations of pyrene-labeled lipid to acceptor vesicles consisting of unlabeled phospholipids was recorded.

Donor vesicles were prepared by injecting 10 μl of a 0.1 mM ethanolic solution of the fluorescent phospholipid into 300 μl 10 mM Tris-HCl (pH 7.1), 0.02% NaN_3 with stirring at 37 °C [17]. The buffer was filtered through a Sartorius membrane filter, 0.45 μm, prior to use. For some experiments donor vesicles consisted of 10% labeled phosphatidylcholine, 80% unlabeled phosphatidylcholine and 10% *N*-trinitrophenylphosphatidylethanolamine as an internal quencher [15]. Lipids were mixed in ethanol, and 14 μl of the solution (0.13 mM lipid) were injected into 0.3 ml Tris-HCl (pH 7.1), 0.02% NaN_3 with stirring at 37°C. For transfer assays 87 μl of the resulting vesicle suspension were used in a

total volume of 0.5 ml yielding a final concentration of 0.1 μM pyrene-labeled phospholipid. Vesicles were equilibrated for at least 10 min prior to use.

Acceptor vesicles were prepared by injecting 24 μl of an ethanolic solution of unlabeled phosphatidylcholine (5 mM egg phosphatidylcholine, choline plasmalogen or alkylacylglycerophosphocholine, respectively) into 480 μl Tris-HCl (pH 7.1), 0.02% NaN_3 with stirring at 37°C. The final concentration in the assay was 5 μM lipid.

Transfer measurements were carried out at 40°C as described before [3]. A standard assay mixture contained 50 μmol of pyrene-labeled phospholipid (donor vesicles), a 50-fold excess of unlabeled phospholipid (acceptor vesicles) and 5–100 ng transfer protein in a total volume of 0.5 ml 10 mM Tris-HCl (pH 7.1), 0.02% NaN_3. The rate of spontaneous lipid transfer was recorded prior to addition of the transfer protein; rates of protein catalyzed lipid transfer were corrected for spontaneous transfer. Fluorescence measurements were carried out on a Shimadzu RF-540 spectrofluorimeter, or on a GREG 200 fluorimeter from I.S.S., Ceparana, Italy. The excitation wavelength was 342 nm (2 nm slit), and the emission wavelength was 380 nm (10 nm slit).

Complex formation of the yeast phosphatidylinositol transfer protein with fluorescent phospholipids

Binding of fluorescent pyrene-phospholipids to the yeast phosphatidylinositol transfer protein was measured as described before [3] by a method similar to that introduced by Somerharju et al. [15] and Van Paridon et al. [16]. Vesicles used for these experiments consisted of 90% of the respective fluorescent phospholipid and 10% *N*-trinitrophenylphosphatidylethanolamine. A typical assay mixture contained a final concentration of 0.1 μM pyrene-labeled phospholipid in 0.5 ml 10 mM Tris-HCl (pH 7.2), 0.02% NaN_3. Aliquots of the transfer protein were added and the resulting increase of pyrene-monomer fluorescence intensity due to binding of the labeled phospholipid to the transfer protein was recorded.

Analytical procedures

Published procedures were used for the quantitation of proteins [18] and phospholipids [19].

Results

Spectral properties of the fluorescent ether phospholipids

Vesicles of alkenyl- and alkyl-pyrenedecanoylglycerophosphocholines exhibit very intense excimer fluorescence at 480 nm and very low monomer emission at 380 and 400 nm as already described for the 1-acyl analog [10]. Dilution of the pyrene-labeled lipids with unlabeled phospholipids leads to an increase of monomer fluorescence intensity and a decrease of the excimer fluorescence intensity. The respective fluorescence intensity changes were similar for ether and diacyl lipids. Upon further dilution of the labeled lipids with a large excess of detergent (Triton X-100) or unlabeled phospholipid (1 : 1000) only the pyrene-monomer fluorescence is observed. The maximum monomer fluorescence intensities obtained after dilution with a 1000-fold excess of egg phosphatidylcholine are the same for 1-*O*-alkenyl-, 1-*O*-alkyl-, and 1-acyl-2-pyrenedecanoylglycerophosphocholine (data not shown).

Table I

Transfer of different subclasses of choline glycerophospholipids catalyzed by specific and non-specific lipid transfer proteins.

Phospholipid transfer assays were carried out as described in Methods. The final concentration of the transfer proteins were 0.1 μg/ml for the yeast phosphatidylinositol transfer protein (PITP), 0.63 μg/ml for the phosphatidylcholine transfer protein from bovine liver (PCTP), 2,94 μg/ml for the non-specific lipid transfer protein from bovine liver (nsLTP), and 2.1 μg/ml for the maize phospholipid transfer protein (PLTP). The final concentrations of fluorescent diacyl-, alkenylacyl- and alkylacylglycerophosphocholine (GPC) were 0.1 μM, respectively. For each transfer protein the rate of transfer of pyrene-labeled diacylglycerophosphocholine to acceptor vesicles consisting of egg phosphatidylcholine was set 100%. Data are expressed as means ± S.D. ($n = 4$–6). n.d., not determined.

Donor phospholipid	Relative transfer rate (%)			
	yeast PITP	bovine PCTP	bovine nsLTP	maize PLTP
Diacyl-GPC	100 ± 4	100 ± 6	100 ± 3	100 ± 6
Alkenylacyl-GPC	250 ± 18	175 ± 13	93 ± 4	115 ± 8
Alkylacyl-GPC	295 ± 12	n.d.	85 ± 3	n.d.

Protein-catalyzed transfer of choline glycerophospholipid subclasses

The transfer of different subclasses of fluorescently labeled choline glycerophospholipids between unilamellar donor and acceptor vesicles was investigated. As described earlier [3] this assay was linear with respect to the amount of transfer protein (up to 200 ng phosphatidylinositol transfer protein/ml) employed. The spontaneous transfer of phospholipid molecules between donor and acceptor membrane vesicles was very slow.

When donor vesicles contained only the pyrene-labeled phospholipids (Table I), the yeast phosphatidylinositol transfer protein showed a marked preference for choline plasmalogen and alkylacylglycerophosphocholine as compared to the diacyl analog. A similar effect was demonstrated with the bovine liver phosphatidylcholine transfer protein. In contrast the non-specific lipid transfer proteins from maize and bovine liver showed similar transfer rates for the different choline glycerophospholipid subclasses. Differences in transfer rates for ether and diacylglycerophosphocholine, respectively, in the presence of yeast phosphatidylinositol transfer protein became significantly smaller when the pyrene-labeled analogs in the donor membrane were

Table II

The influence of matrix lipid components of donor vesicles on the transfer of different subclasses of choline glycerophospholipids catalyzed by the yeast phosphatidylinositol transfer protein.

Phospholipid transfer from donor vesicles consisting of 10% fluorescently labeled phospholipid in a matrix of 10% *N*-trinitrophenylphosphatidylethanolamine and 80% egg phosphatidylcholine, alkenylacyl- or alkylacyl-glyerophosphocholine, respectively, was measured as described in Methods. Acceptor membranes consisted of egg phosphatidylcholine. The transfer rate of labeled diacylglycerophosphocholine out of an egg phosphatidylcholine (egg PC) matrix was set 100%. Data are expressed as means ± S.D. ($n = 4$–6). n.d., not determined; GPC, glycerophosphocholine.

Pyrene-labeled phospholipid (10%) in donor vesicles	Relative transfer rate (%); matrix component (80%) of donor vesicles		
	egg PC	alkenylacyl-GPC	alkylacyl-GPC
Diacyl-GPC	100 ± 5	120 ± 7	n.d.
Alkenylacyl-GPC	127 ± 7	152 ± 10	n.d.
Alkylacyl-GPC	139 ± 7	n.d.	209 ± 14

diluted with unlabeled lipid (Table II). Nevertheless alkenyl- and alkylacyl-glycerophosphocholine were transferred with higher rates than the diacyl analog. The highest transfer rates in this assay system were found with donor membranes consisting of 10% pyrene-labeled alkylacylglycerophosphocholine in a matrix of unlabeled alkylacylglyerophosphocholine.

From data presented in Tables I and II it becomes evident that transfer rates depend on the chemical structure of the glycerol region of the phospholipid molecule (ether or ester bonds), whose transfer is observed, and on the bulk membrane properties of donor vesicles. Pyrene-labeled ether lipids embedded in a diacylglycerophosphocholine donor membrane are transferred better than pyrene-labeled diacylglycerophosphocholine in the same lipid matrix (Table II). Transfer rates are generally higher when ether phospholipids form the donor matrix (Table II). The type of acceptor vesicle phospholipid (Table III) had only a marginal effect on the transfer of individual subclasses of choline glycerophospholipids catalyzed by the yeast phosphatidylinositol transfer protein.

Complex formation of different subclasses of choline glycerophospholipids with the yeast phosphatidylinositol transfer protein

Formation of complexes of the phosphatidylinositol transfer protein from yeast with fluorescent alkenylacyl-, alkylacyl- or diacyl-glycerophosphocholines, respectively, was determined by measuring the increase in pyrene-monomer fluorescence intensity after adding increasing amounts of transfer protein to vesicles containing the fluorescent substrates and a small amount of *N*-trinitrophenylphosphatidylethanolamine as an internal quencher. The affinity of the protein for alkylacyl-

Table III

Transfer of different subclasses of pyrene-labeled choline glycerophospholipids to acceptor membrane vesicles consisting of various subclasses of choline glycerophospholipids catalyzed by the yeast phosphatidylinositol transfer protein

Donor vesicles consisted only of the respective fluorescently labeled choline glycerophospholipids. Data are expressed as means ± S.D. ($n = 4$–6). n.d., not determined; GPC, glycerophosphocholine.

Donor phospholipid	Transfer rate (nmol/mg per min); acceptor vesicles consisting of		
	egg PC	alkenylacyl-GPC	alkylacyl-GPC
Diacyl-GPC	18.5 ± 0.9	18.1 ± 1.1	19.8 ± 1.1
Alkenylacyl-GPC	46.3 ± 3.3	42.6 ± 4.2	n.d.
Alkylacyl-GPC	54.6 ± 2.2	n.d.	50.7 ± 3.4

and alkenylacyl-glycerophosphocholine is almost the same, and only 15–20% lower for diacylglycerophosphocholine (Fig. 1). This interpretation is, of course, only correct if the quantum yields of the protein-bound ether and diacylglycerophospholipids are identical. It can be anticipated that the *sn*-2 pyrenedecanoyl chains of alkylacyl-, alkenylacyl- and diacyl-glycerophosphocholine, respectively, are accomodated in the same binding site of the protein and should, therefore, exhibit a similar fluorescence yield in the protein-lipid complex. Relative fluorescence intensities of the respective pyrene-labeled phospholipids are identical in vesicles containing a 1000-fold excess of egg phosphatidylcholine.

If differences existed in binding between ether and ester phospholipids, these titration experiments should have reflected such effects as already reported in an

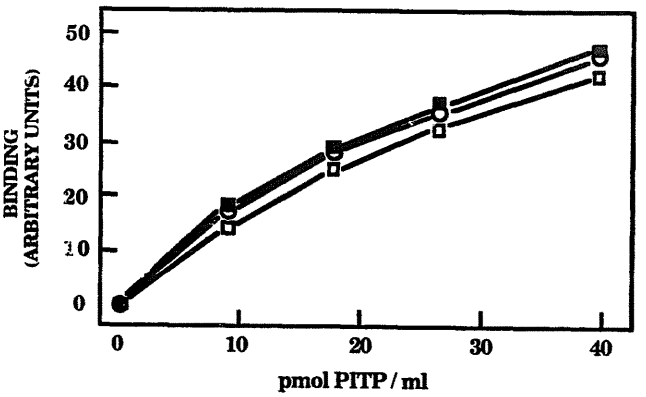

Fig. 1. Complex formation of fluorescently labeled subclasses of choline glycerophospholipids with the phosphatidylinositol transfer protein from yeast. Incubations of the yeast phosphatidylinositol transfer protein with pyrene-labeled phospholipids were carried out as described in Methods. The increase of pyrene-monomer fluorescence intensity resulting from binding of fluorescent phospholipids to the transfer protein is expressed as arbitrary units. □——□, acylpyrenedecanoylglycerophosphocholine; ■——■, alkylpyrenedecanoylglycerophosphocholine; ○——○, alkenylpyrenedecanoylglycerophosphocholine.

analogous manner for the different binding of phosphatidylinositol and phosphatidylcholine to phosphatidylinositol transfer proteins [20,21]. The protein used in the present study was not delipidated and still contained its endogenously bound phospholipid (presumably a large excess of phosphatidylinositol over phosphatidylcholine). Thus, the titration experiment was a competition binding assay as the endogenous phosphatidylinositol had to be replaced by exogenous (ether or ester) phosphatidylcholine. Under the experimental conditions employed saturation of the phosphatidylinositol transfer protein with a fluorescent choline phospholipid can be excluded, as the label-to-protein ratio was rather low. More specifically, 100 μmol pyrene-phospholipid were titrated with 10 to 40 pmol protein. According to data on phosphatidylcholine binding to phosphatidylinositol transfer proteins [20,21] quantitative replacement of the protein-bound phosphatidylinositol followed by 100% binding of phosphatidylcholines to the phosphatidylinositol transfer protein would require extremely high lipid-to-protein ratios. Thus, we should observe pyrene-lipid binding under equilibrium conditions, and from the results obtained it can be concluded that ether and ester choline glycerophospholipids compete with the endogenous phosphatidylinositol of the phosphatidylinositol transfer protein to a very similar extent.

Discussion

Transport of phospholipids between membranes is facilitated in vitro by soluble proteins which are universally distributed throughout eukaryotic cells. Several such proteins have been isolated and characterized, yet their mechanisms of action are largely unknown. Phospholipid transfer proteins with phospholipid class specificity, such as phosphatidylcholine or phosphatidylinositol transfer proteins, form 1 : 1 complexes with phospholipids, which can then be transferred between membranes. It is conceivable that head group recognition and penetration of the protein into the membrane are initial steps in the transfer process catalyzed by specific phospholipid transfer proteins. Such penetration could indeed be demonstrated by measuring the increase in surface pressure after injection of phosphatidylcholine or phosphatidylinositol transfer proteins, respectively, into the subphase of phospholipid monolayers [22,23]. Non-specific lipid transfer proteins, on the other hand, mediate the transport of phospholipids most likely by the formation of membrane contact [5]. They do not form stable complexes with phospholipids, nor do they catalyze phospholipid transport through an aqueous phase separating donor and acceptor membranes.

The three phospholipid subclasses, diacyl-, alkylacyl-, and alkenylacyl-glycerophosphocholine differ in the type of bonding between the aliphatic chain and the oxygen in the *sn*-1 position of glycerol. In a bilayer membrane this region of a phospholipid molecule is part of the hydrophobic/hydrophilic interface into which phospholipid transfer proteins supposedly penetrate during the transfer event. Therefore, differences in the physicochemical properties in this membrane region can be expected to affect rates of protein-catalyzed phospholipid transport. Such differences could actually be observed when rates of intervesicular transport of the three phospholipid subclasses were measured in the presence of the phosphatidylinositol transfer protein from yeast, or the phosphatidylcholine transfer protein from bovine liver (see Table I).

When the fluorescently labeled phospholipids were embedded into a matrix of egg phosphatidylcholine, ether phospholipids were transferred at higher rates than the diacyl analog (see Table II). On the other hand, the transfer rate of each choline glycerophospholipid subclass was increased when donor vesicles contained an ether phospholipid matrix instead of an egg phosphatidylcholine matrix (see Table II). Thus, the molecular properties of a phospholipid molecule affect the rate of its transfer to some extent, but the bulk properties of the vesicle bilayer are also a determining factor.

Why then are ether phospholipids (alkylacyl- and alkenylacyl-glycerophospholipids) transferred at higher rates than diacyl-glycerophospholipids? The slightly higher (15–20%) binding affinities for ether phospholipids as compared to the diacyl analogs (Fig. 1) should not be responsible for the much higher (2–3-fold) rate of ether lipid transfer (see Table I). Ether lipid membranes have a significantly lower surface dipolar potential than membranes consisting of diacylglycerophospholipids [24]. If positive charges on the surface of phospholipid transfer proteins play a role in the initial step of phospholipid transfer, as can be inferred from the essential role of arginine and lysine residues in the case of the phosphatidylcholine transfer protein from bovine liver [25,26], then the potential barrier at the hydrophobic/hydrophilic interface would be much lower in the case of ether lipids.

Conformational differences in the head group [27] and interface region of choline [27, 28] and ethanolamine plasmalogens [28] in comparison to the diacyl analogs have been observed by NMR methods. According to the ^{2}H-NMR studies of Malthaner et al. [28] the mobility is enhanced at the C-2 segment of the *sn*-2 acyl chain of choline plasmalogens as compared to the diacyl analog. The replacement of a carboxylester by an (enol)ether bond reduces polarity and might lead to a less sharply defined hydrophobic-hydrophilic boundary of the bilayer membrane. These distinct dipolar and motional properties might effect water penetration into deeper regions of ether phospholipid membranes. Sup-

port for this assumption came from fluorescence studies. 1-*O*-Radyl-2-diphenylhexa-1,3,5-trienepropionylglycerophosphocholine showed a much broader lifetime distribution when embedded in plasmalogen membranes as compared to diacylglycerophosphocholine membranes. Such differences were ascribed to a more inhomogenous environment around the fluorophor due to a broader polarity gradient caused by solvent penetration into the membrane [29]. Differences in interface polarity and mobility became apparent also from much faster relaxation times for the solvent-sensitive fluorescent probe PRODAN in ether phospholipid as compared to diacylphospholipid membranes [30]. It is concluded that any of these criteria – conformation, motion and polarity at the membrane boundary – or their combination might be relevant for the interaction of specific phospholipid transfer proteins with membranes.

Non-specific lipid transfer proteins act obviously by a different mechanism, which is largely independent of phospholipid head group recognition and binding. In this case transfer rates are independent of the type of the choline glycerophospholipid subclass and thus of the chemical structure of the phospholipid glycerol region.

Rates of protein-catalyzed transport of plasmalogens have not been reported before. Kamp et al. [7] reported that the transfer rate of alkylacylglyerophosphocholine was slightly lower than that of the diacyl analog in the presence of the phosphatidylcholine transfer protein from bovine liver. These authors, however, compared 1-*O*-octadecyl-2-oleoylglycerophosphocholine to 1-palmitoyl-2-oleoylglycerophosphocholine. Since aliphatic chain-length is known to affect the rate of protein-catalyzed phospholipid transfer [15,16], results presented by Kamp et al. [7] are not completely conclusive.

Enhanced rates of ether phospholipid transfer have also been observed between unilamellar donor vesicles and natural membranes, such as cultured human skin fibroblasts [31], erythrocyte ghosts [32] or thrombocytes (Hermetter, A., unpublished results). Also in these systems specific phospholipid transfer proteins seem to be involved. It will be of considerable interest to further study the phenomenon of facilitated intermembrane transport of ether phospholipids and to evaluate its physiological significance.

Acknowledgements

The authors gratefully acknowledge the expert technical assistance of H. Stütz and M. Zeiler. We are indebted to J.-C. Kader, Paris, for the precious gift of maize PLTP. This work was financially supported by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (project 5746B to A.H., and project 6958 to G.D.).

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