Biochimica et Biophysica Acta, 986 (1989) 301–309
Elsevier

BBAMEM 74651

Membrane properties modulate the activity of a phosphatidylinositol transfer protein from the yeast, *Saccharomyces cerevisiae*

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(Received 8 June 1989)

Key words: Phospholipid transfer protein; Phosphatidylcholine; Phosphatidylinositol; Fluorescent phospholipid; Membrane fluidity; Membrane charge; (Yeast)

A phospholipid transfer protein from yeast (Daum, G. and Paltauf, F. (1984) Biochim. Biophys. Acta 794, 385–391) was 2800-fold enriched by an improved procedure. The specificity of this transfer protein and the influence of membrane properties of acceptor vesicles (lipid composition, charge, fluidity) on the transfer activity were determined in vitro using pyrene-labeled phospholipids. The yeast transfer protein forms a complex with phosphatidylinositol or phosphatidylcholine, respectively, and transfers these two phospholipids between biological and/or artificial membranes. The transfer rate for phosphatidylinositol is 19-fold higher than for phosphatidylcholine as determined with 1:8 mixtures of phosphatidylinositol and phosphatidylcholine in donor and acceptor membrane vesicles. If acceptor membranes consist only of non-transferable phospholipids, e.g., phosphatidylethanolamine, a moderate but significant net transfer of phosphatidylcholine occurs. Phosphatidylcholine transfer is inhibited to a variable extent by negatively charged phospholipids and by fatty acids. Differences in the accessibility of the charged groups of lipids to the transfer protein might account for the different inhibitory effects, which occur in the order phosphatidylserine > phosphatidylglycerol > phosphatidylinositol > cardiolipin > phosphatidic acid > fatty acids. Although mitochondrial membranes contain high amounts of negatively charged phospholipids, they serve effectively as acceptor membranes, whereas transfer to vesicles prepared from total mitochondrial lipids is essentially zero. Ergosterol reduces the transfer rate, probably by decreasing membrane fluidity. This notion is supported by data obtained with dipalmitoyl phosphatidylcholine as acceptor vesicle component; in this case the transfer rate is significantly reduced below the phase transition temperature of the phospholipid.

Introduction

Phospholipid transfer proteins are ubiquitous in most organisms and cells, and have been isolated from mammalian and plant tissues as well as from prokaryotic and eukaryotic microorganisms (for recent reviews, see Refs. 1–4). Transfer proteins with different substrate specificities and catalytic properties have been described.

The occurrence of phospholipid transfer protein(s) in the yeast, *Saccharomyces cerevisiae*, has been reported by several authors [5–7]. In our laboratory [6] a protein specific for phosphatidylinositol and phosphatidylcholine has been purified to homogeneity from yeast cytosol. A phospholipid transfer protein with apparently distinct properties has been described by Bozzato and Tinker [7]. It is not clear if the two proteins are identical or not; differences in the yeast strains, isolation procedures, and in assay conditions might explain the discrepancies.

Phospholipid transfer proteins facilitate the transport of lipid molecules between artificial and/or isolated biological membranes in vitro. They are thought to contribute to the biogenesis of cellular membranes in vivo by modification and/or maintenance of the membrane lipid composition and asymmetry. Vice versa the lipid composition of membranes modulates the activity of phospholipid transfer proteins (for reviews, see Refs. 1 and 2). Especially phospholipids with a net negative charge, e.g., phosphatidic acid, phosphatidylserine, phosphatidylglycerol, cardiolipin, but also phosphatidylinositol, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate [8,9] have been described to inhibit the activity of mammalian phospho-

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lipid transfer proteins. Phospholipids with saturated fatty acyl chains, and sterols as components of membranes decrease the rate of phospholipid transfer in vitro, indicating that protein catalyzed phospholipid transfer is sensitive to membrane fluidity [1].

In the present paper we describe a modified and largely improved procedure for the isolation of the yeast phosphatidylinositol transfer protein. Fluorescent pyrene-labeled phospholipids were used to determine substrate specificity of the protein with respect to transfer and binding, and to study the influence of membrane properties (lipid composition, charge, fluidity) on the transfer. The properties of the yeast phosphatidylinositol transfer protein are compared to those of mammalian phosphatidylinositol transfer proteins.

Materials and Methods

Yeast strain and culture conditions

Saccharomyces cerevisiae D 273-10B was grown aerobically at 30 °C on a semisynthetic medium with 2% lactate as a carbon source [10]. Cells were harvested in the mid-logarithmic phase with a yield of about 5 g wet weight per liter.

Procedure for the isolation of the phosphatidylinositol transfer protein from yeast cytosol

The procedure for the purification of the phosphatidylinositol transfer protein from yeast described here is a modification of the method published previously by Daum and Paltauf [6].

Step 1: Preparation of yeast cytosol. Spheroplasts from yeast cells were prepared as described by Daum et al. [10] and homogenized in the presence of 0.6 M mannitol, 10 mM Tris-HCl (pH 7.4), 1 mM phenylmethylsulfonyl fluoride, using a Dounce homogenizer. The homogenate was cleared of membranous particles by sequential centrifugation at $1000 \times g$ (5 min), $10\,000 \times g$ (10 min) and $100\,000 \times g$ (60 min). Centrifugations as well as all the following steps were carried out at 0–4 °C.

Step 2: Fractionated ammoniumsulfate precipitation (50–90% saturation). Solid ammoniumsulfate (29.1 g/100 ml) was added in small portions with gentle stirring to yeast cytosol to give a 50% saturation. After 5–10 h on ice precipitated proteins were removed by centrifugation at $23\,000 \times g$ for 12 min. The resulting supernatant was saved, and additional ammoniumsulfate (26.8 g/100 ml) was added for 90% saturation. Precipitated proteins were collected by centrifugation as described above and suspended in 10 mM Tris-HCl (pH 7.4), 0.02% NaN_3 (in the following referred to as standard buffer). Insoluble material was removed by centrifugation ($23\,000 \times g$, 12 min) and discarded.

Step 3: Sephadex G-75 chromatography. Portions of 20–25 ml of the sample obtained in step 2 were applied to a Sephadex G-75 column (2.6 × 75 cm). Fractions of 5 ml were collected after elution with the standard buffer at a flow rate of 25 ml/h. Phosphatidylcholine transfer activity was found in fractions 29 to 38.

Step 4: DEAE-Sephacel chromatography. Pooled active fractions of the previous step were adjusted to 70 mM NaCl and applied to a DEAE-Sephacel column (1.6 × 32 cm) equilibrated with 70 mM NaCl in standard buffer. Unbound proteins were eluted with 150 ml of the same buffer at a flow rate of 25 ml/h. Elution was continued with 250 ml of a linear salt gradient (70–120 mM NaCl), and fractions of 4.2 ml were collected. Active transfer protein was eluted with 100–110 mM NaCl.

Step 5: Second Sephadex G-75 chromatography. Portions (20–25 ml) of pooled active fractions from the previous step were applied to a Sephadex G-75 column and eluted as described for step 3. Transfer activity was found in fractions 29 to 39 with a maximum at fractions 34–35, corresponding to an apparent molecular mass of 34–37 kDa.

Step 6: Phenyl-Sepharose chromatography. Pooled active fractions from step 5 were dialyzed against 1 mM NaP_i (pH 7.2), 0.02% NaN_3 and adjusted to 0.1 M NaCl. The sample (50–100 ml) was applied to a Phenyl-Sepharose CL-4B column (1 × 11 cm) equilibrated with 1 mM NaP_i (pH 7.2), 0.02% NaN_3, 0.1 M NaCl. Unbound proteins were eluted with the starting buffer at a flow rate of 20 ml/h. Elution was continued with a step gradient using 30 ml 1 mM NaP_i (pH 7.2), 0.02% NaN_3, 50 mM NaCl; 30 ml 20 mM NaCl in NaP_i buffer and 100 ml of buffer without NaCl. Fractions of 6.2 ml were collected; the active transfer protein was eluted between fractions 2 and 19.

For some experiments the protein solution was concentrated after the last step of purification using Centricon 30 (Amicon). During this step 10–20% of the protein were lost probably due to irreversible binding to the filter material. The purified protein was stored at 4 °C in the presence of 10% glycerol at protein concentrations of 5–100 μg/ml. Within 12 months approximately 10% of the activity were lost.

Preparation of phospholipid vesicles for transfer assays

Vesicles for transfer assays were routinely prepared by the ethanol injection method [11]. Final concentrations of 0.2–0.4% ethanol in the incubation mixtures for transfer and binding assays have only a marginal influence on membrane properties [12], and do not affect the activity of the transfer protein. Donor vesicles were prepared by injecting 10 μl of a 0.1 mM ethanolic solution of 1-palmitoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphocholine into 300 μl 10 mM Tris-HCl (pH 7.1), 0.02% NaN_3 with stirring at 37 °C. The buffer was filtered (Sartorius membrane filters, 0.45 μm) prior to use. After an equilibration period of at least 10 min

portions of 15 μl of the vesicle suspension were used in 0.5 ml Tris-HCl (pH 7.1), 0.02% NaN_3 to give a final concentration of 0.1 μM fluorescently labeled donor phospholipid. For comparative studies of phosphatidylcholine and phosphatidylinositol transfer donor vesicles containing 10 mol% of fluorescently labeled phospholipids were prepared. Phosphatidylcholine donor vesicles contained 10% 1-stearoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphocholine, 10% *N*-trinitrophenyl phosphatidylethanolamine as an internal quencher [13], 70% phosphatidylcholine and 10% phosphatidylinositol; phosphatidylinositol donor vesicles consisted of 10% 1-stearoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphoinositol, 10% *N*-trinitrophenyl phosphatidylethanolamine and 80% phosphatidylcholine.

Acceptor vesicles were prepared by mixing egg phosphatidylcholine with the appropriate amount of other lipids. Mixtures were dried under a stream of nitrogen and dissolved either in DMSO/ethanol (1 : 3, v/v) or in ethanol to a final concentration of 5 mM. This solution (24 μl) was injected into 480 μl Tris-HCl (pH 7.1), 0.02% NaN_3 with stirring at 37°C. For transfer assays 10 μl of this vesicle suspension were used in a total volume of 0.5 ml giving a final concentration of 5 μM acceptor vesicle lipid. In cases where the donor lipid concentration was increased, e.g., during comparative measurements of phosphatidylcholine and phosphatidylinositol transfer, the amount of acceptor vesicles was increased correspondingly to maintain the 50-fold excess. As an alternative to the ethanol injection method acceptor membrane vesicles (e.g., consisting of 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine or 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine) were prepared by sonication. In this case lipids were dried under a stream of nitrogen, suspended in standard buffer and sonicated at 30–40°C for 5 min with 70 W under a stream of nitrogen using a Braun Labsonic 2000 sonicator. Acceptor vesicles were used within 1–5 days after preparation.

Mitochondria used as acceptor membranes in some cases were isolated by the method described by Daum et al. [10].

Fluorescence assay of phospholipid transfer

The phospholipid transfer assay in vitro using fluorescently labeled phospholipids was similar to those described by Somerharju et al. [13] and Van Paridon et al. [8]. The excitation wavelength was 342 nm (2 nm slit), and the emission wavelength was 380 nm (10 nm slit). Pyrene-labeled phospholipids (50 pmol) were incubated with a 50-fold excess of acceptor membrane vesicles in a total volume of 0.5 ml 10 mM Tris-HCl (pH 7.1), 0.02% NaN_3 at 40°C. The spontaneous transfer of fluorescently labeled phospholipids from donor to unlabeled acceptor membranes was followed continously by the increase of pyrene monomer fluorescence intensity using a Shimadzu RF-540 spectrofluorimeter or a fluorimeter from I.S.S., La Spezia, Italy. Protein-catalyzed transfer was measured in the presence of 5–100 ng transfer protein and corrected for spontaneous transfer. In order to correlate the increase of pyrene monomer fluorescence intensity and transfer rates (nmol/mg per min) the pyrene monomer fluorescence intensity of vesicles consisting of a given amount of pyrene-labeled and egg phosphatidylcholine (1 : 1000) was determined.

Binding assay

Complex formation of the yeast phosphatidylinositol transfer protein with fluorescent phospholipids was measured essentially as described by Somerharju et al. [13] and Van Paridon et al. [9,14,15].

Two types of vesicle were used for binding experiments. Type 1 consisted of 90% of the respective fluorescently labeled phospholipid and 10% *N*-trinitrophenyl phosphatidylethanolamine. Type 2 consisted of 10% fluorescent phosphatidylcholine and 10% unlabeled phosphatidylinositol, or 10% fluorescent phosphatidylinositol and 10% unlabeled phosphatidylcholine, respectively, 10% *N*-trinitrophenyl phosphatidylethanolamine and 70% phosphatidylethanolamine. An ethanolic solution of the lipids (10 μl, 25 μM labeled phospholipid) was injected into 500 μl 10 mM Tris-HCl (pH 7.1), 0.02% NaN_3 with stirring at 37°C.

Fluorescently labeled phospholipid vesicles (100 μl) were added to 0.4 ml 10 mM Tris-HCl (pH 7.2), 0.02% NaN_3, and incubated at 30°C. The final concentration of labeled phospholipid was 0.1 μM. Aliquots of the transfer protein (60–180 ng) were added, and the resulting increase of the pyrene monomer fluorescence intensity was recorded. Fluorescence was measured as described for transfer assays. The stoichiometry of the transfer protein-phosphatidylinositol complex was calculated from data obtained with pyrene-labeled phosphatidylinositol vesicles of type 1 (see above) essentially as described by Van Paridon et al. [15].

Materials

Egg yolk phosphatidylcholine and phosphatidylglycerol were from Lipid Products (Nutfield, U.K.), bovine brain phosphatidylserine and ergosterol were from Sigma, yeast phosphatidylethanolamine and phosphatidic acid [16], bovine heart cardiolipin and bovine heart sphingomyelin [17] were isolated from natural sources and purified by preparative thin-layer chromatography. Soybean phosphatidylinositol was a gift of R. Franzmair, Chemie Linz Pharma. Fatty acids were from NuChek Prep (Elysian, U.S.A.) and lauric acid was from Fluka (Buchs, Switzerland).

1-Stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine [18], 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine [19], *N*-trinitrophenyl phosphatidylethanolamine [20]

and 1-palmitoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphocholine [21] were synthesized by published procedures. 1-Stearoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphoinositol and 1-stearoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphocholine were gifts of P.J. Somerharju (Helsinki).

Analytical procedures

Thin-layer chromatography of lipids [22], SDS-PAGE of proteins [23], isoelectric focusing [24], and quantitation of proteins [25] and lipid phosphorus [26] were carried out by published procedures. Pyrene-labeled phospholipids were quantitated by measuring the absorbance at 342 nm in ethanolic solution; the molar absorption coefficient is 42000 $mol^{-1} \cdot cm^{-1}$ [13].

Results

Improved preparation of the phosphatidylinositol transfer protein from yeast

Following the protocol for the purification of the phospholipid transfer protein from yeast as described previously [6] a 320-fold enrichment of this protein over the 100000 × *g* supernatant with a recovery of 4.5% could be obtained. Employing a radioassay the specific transfer activity for phosphatidylcholine was distinctly below the specific activity of mammalian phospholipid transfer proteins [2]. In the course of further investigations [27] we observed a marked pH-lability of the yeast transfer protein. This led us to replace the last step of the original purification protocol, chromatofocusing, by another chromatographic method, because the transfer protein was unstable at the pH of its isoelectric point. The new step introduced is hydrophobic interaction chromatography employing Phenyl-Sepharose CL-4B. This effective purification step allows also the omission of hydroxyapatite chromatography. The new preparation scheme (Table I) leads to a purification factor of 2760 with a yield of 19% based on the activity of crude cytosol. The specific transfer activity of the final product was 18.5 nmol phosphatidylcholine transferred per min per mg as determined by the fluorescence assay described in Materials and Methods, which is a considerable improvement over the procedure described previously [6]; using a similar assay Van Paridon et al. [8] found a value of approximately 30 $nmol \cdot min^{-1} \cdot mg^{-1}$ for the bovine brain phosphatidylinositol transfer protein. The purified yeast phosphatidylinositol transfer protein was detected as a single band on SDS-PAGE with an apparent molecular mass of 37 kDa. The isoelectric point was 5.2 as determined by isoelectric focusing; in contrast to mammalian phosphatidylinositol transfer proteins [2] only one form of the yeast transfer protein was detectable. Another phosphatidylinositol transfer protein, different from that described here, has not been detected in yeast so far.

TABLE I

Purification of the phosphatidylinositol transfer protein from yeast

Preparation of cytosol from the yeast *Saccharomyces cerevisiae* D 273-10B, chromatographic and analytical methods are described in Materials and Methods.

Purification step	Volume (ml)	Protein (mg)	Spec. act. (nmol PC/min per mg)	Recovery (%)	Purification factor
100000 × *g* supernatant	3200	7840	0.0067	100	1.0
Ammoniumsulfate 50–90% saturation	95	2990	0.0134	76	2.0
Sephadex G-75	200	1550	0.0214	63	3.2
DEAE-Sephacel	45	75	0.307	44	46
Sephadex G-75	110	12.3	1.27	30	190
Phenyl-Sepharose CL-4B	110	0.54	18.5	19	2760

Transfer assays and substrate specificity of the transfer protein

In the fluorescence assay employed for measuring phospholipid transfer donor vesicles containing pyrene-labeled phospholipids were incubated with unlabeled acceptor phospholipid vesicles in the presence of the transfer protein. Increase of pyrene monomer fluorescence intensity reflects the migration of fluorescent phospholipids to the acceptor membrane. The assay is linear with respect to the amount of transfer protein within a range of 10–200 ng protein per ml, and the time (approximately 10 min for a protein concentration of 20 ng/ml). Temperature and pH dependence of the transfer assay are shown in Figs. 1 and 2. The transfer rate reaches its maximum at 40–45 °C (Fig. 1); the decrease at higher temperature is obviously due to the instability of the protein. The influence of temperature

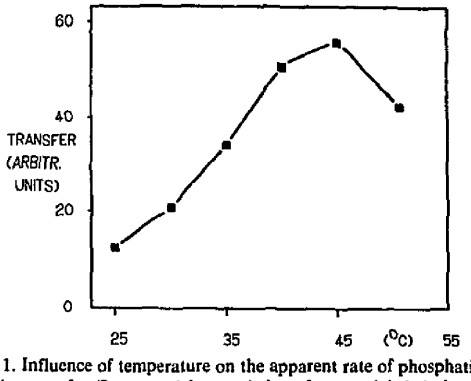

Fig. 1. Influence of temperature on the apparent rate of phosphatidylcholine transfer. Donor vesicles consisting of pyrene-labeled phosphatidylcholine (50 pmol), egg phosphatidylcholine acceptor vesicles (2.5 nmol) and yeast phosphatidylinositol transfer protein (20 ng) were incubated in a total volume of 0.5 ml at the temperatures indicated. The increase of pyrene monomer fluorescence intensity during transfer is expressed in arbitrary units.

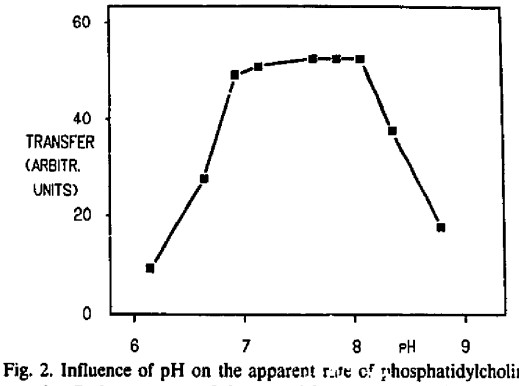

Fig. 2. Influence of pH on the apparent rate of phosphatidylcholine transfer. Both acceptor and donor vesicles were prepared in 10 mM Tris-HCl buffer at the pH values indicated. Transfer of phosphatidylcholine was measured at 40 °C. All other conditions were the same as described in the legend to Fig. 1.

on the fluorescence yield has been taken into account for the calculation of transfer rates at different temperatures. The pH optimum lies between pH 7 and 8 (Fig. 2), which is also the range of optimal stability of the protein [27].

In order to compare the rates of transfer of the two substrates, phosphatidylinositol and phosphatidylcholine, transfer was measured in a system where the amount of phosphatidylinositol was kept constant and at a low concentration in order to avoid inhibition of transfer activity by the negatively charged phospholipid (see below). Under these conditions the rate of phosphatidylinositol transfer was about 19-times higher than the rate of phosphatidylcholine transfer despite an 8-fold excess of phosphatidylcholine in the assay system. Independent experiments showed that pyrene-labeled phospholipids are transferred at similar rates as unlabeled phospholipids (data not shown).

It is a standing question whether or not phospholipid transfer proteins are able to catalyze net phospholipid transport. This problem was addressed in an experiment using acceptor vesicles consisting only of stearoyloleoyl glycerophosphoethanolamine. In several control experiments pyrene-labeled phosphatidylethanolamine had been shown to be a non-transferable phospholipid, even in the presence of high amounts of the phosphatidylinositol transfer protein. The transfer rate of fluorescent phosphatidylcholine observed with acceptor membranes consisting of stearoyloleoylglycerophosphoethanolamine was 10% of the control value measured for stearoyloleoylglycerophosphocholine acceptor vesicles. This result indicates that a moderate, but significant net transfer of phosphatidylcholine had occurred.

Binding of phospholipids to the yeast phosphatidylinositol transfer protein

Complex formation of transfer proteins with single phospholipid molecules is thought to be the initial step in the transfer process. One possible explanation for differences in transfer rates of distinct phospholipids would be a difference in their binding affinities to the transfer protein. As can be seen from Fig. 3 the binding affinity of phosphatidylinositol to the yeast transfer protein is 12–15-times higher than that of phosphatidylcholine, assuming similar quantum yields for protein bound pyrene-labeled phosphatidylinositol and phosphatidylcholine. Data shown in Fig. 3 were obtained with phospholipid vesicles consisting of pyrene-labeled phosphatidylinositol or phosphatidylcholine, respectively, and 10% *N*-trinitrophenyl phosphatidylethanolamine. The results were essentially the same when vesicles consisting of labeled phosphatidylinositol and unlabeled phosphatidylcholine, or unlabeled phosphatidylinositol and labeled phosphatidylcholine (1 : 1 mixtures. 10% each) in a matrix of 70% phosphatidylethanolamine and 10% *N*-trinitrophenyl phosphatidylethanolamine were used (data not shown).

The stoichiometry of the complex of the transfer protein with phosphatidylinositol was calculated as described by Van Paridon et al. [15] from data obtained in binding experiments (Fig. 3), using the purified phosphatidylinositol transfer protein and vesicles consisting of pyrene-labeled phosphatidylinositol; a protein to phospholipid ratio of 1 : 0.36 was determined. Several

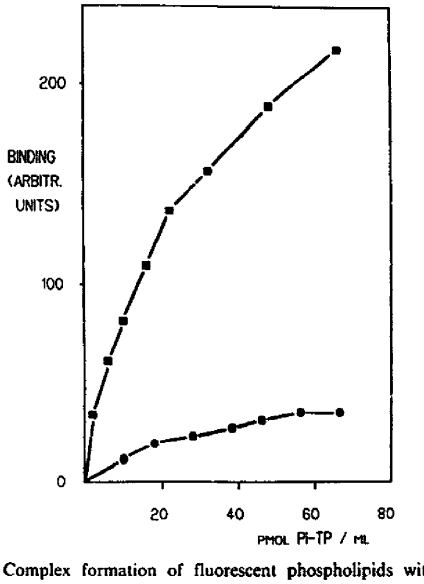

Fig. 3. Complex formation of fluorescent phospholipids with the phosphatidylinositol transfer protein from yeast. Vesicles consisting of 50 pmol fluorescent phosphatidylinositol or phosphatidylcholine, respectively, and 5.6 pmol *N*-trinitrophenyl phosphatidylethanolamine were incubated in 0.5 ml 10 mM Tris-HCl (pH 7.1), 0.02% NaN_3. The increase of pyrene monomer fluorescence intensity resulting from binding of fluorescent phospholipids to the phosphatidylinositol transfer protein added is expressed in arbitrary units. ■——■, Stearoylpyrenedecanoylglycerophosphoinositol; ●——●, stearoylpyrenedecanoylglycerophosphocholine.

TABLE II

Influence of the acceptor membrane lipid composition on the rate of phosphatidylcholine transfer catalyzed by the yeast phosphatidylinositol transfer protein

The transfer assay using fluorescently labeled phosphatidylcholine is described under Materials and Methods. Values are expressed as mol% of individual phospholipids which lead to a 50% inhibition of the transfer activity when present in egg phosphatidylcholine acceptor vesicles. Data shown in the table are mean values of at least three experiments.

Lipid component added to acceptor vesicles	% of lipid in acceptor vesicles leading to a 50% transfer inhibition
Phosphatidylinositol	10
Phosphatidylserine	7
Phosphatidylglycerol	8
Cardiolipin	16
Phosphatidic acid	50
Phosphatidylethanolamine	n.i. 0–50% [a]
Sphingomyelin	n.i. 0–50% [a]
Ergosterol	> 50

[a] No inhibition between 0 and 50%.

effects have to be taken into account which decrease the apparent phospholipid to protein ratio: higher binding affinity of the endogenous phospholipid versus the exogenous pyrene-labeled substrate employed due to differences in the fatty acid composition (e.g., 1-palmitoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphocholine is bound with an approximately 2-fold higher affinity as compared to the 1-stearoyl analog); inactivation of the transfer protein during preparation and storage. Considering these effects it is very likely that the yeast phosphatidylinositol transfer protein forms 1 : 1 complexes with its substrates.

Acceptor membrane properties influence the transfer of phosphatidylcholine

To test the influence of membrane properties on the phospholipid transfer catalyzed by the yeast phosphatidylinositol transfer protein acceptor vesicles with various lipid compositions were employed. The matrix of these vesicles was egg phosphatidylcholine; 0–50% of phosphatidylcholine were replaced by various lipids as indicated in Table II. That amount (mol%) of the additive which led to a 50% decrease of the apparent phosphatidylcholine transfer rate was taken as a measure for its inhibitory effect on transfer activity. Among the lipids tested (Table II) phosphatidylinositol is the only one that is also a substrate for the transfer protein. The apparent inhibition of phosphatidylcholine transfer by phosphatidylinositol is therefore at least partially due to a competitive effect. The other negatively charged, but non-transferable phospholipids showed also marked inhibitory effects which were most pronounced with phosphatidylserine followed by phosphatidylglycerol, cardiolipin and phosphatidic acid (Table II). The influence of various fatty acids on the transfer of phosphatidylcholine was studied with acceptor vesicles containing 15 mol% fatty acids. When donor and acceptor vesicles are mixed, most certainly a rapid redistribution of fatty acids between the two populations occurs. Due to the large excess of acceptor vesicles present in transfer assays this redistribution leads only to a marginal decrease of the concentration of fatty acids in acceptor membranes, but also to a final concentration of approximately 15 mol% free fatty acids in donor vesicles. Only minor inhibition of the phosphatidylcholine transfer rate due to the presence of fatty acids was observed; chain length and degree of unsaturation were of marginal influence (data not shown). When part of the acceptor vesicle phosphatidylcholine was replaced by the zwitterionic phospholipids phosphatidylethanolamine or sphingomyelin the rate of phosphatidylcholine transfer catalyzed by the yeast phosphatidylinositol transfer protein was not affected (Table II).

Bozzato et al. [28] reported that mixed vesicles consisting of phosphatidylcholine/phosphatidylethanolamine/phosphatidylinositol/phosphatidylserine (4 : 3 : 2 : 1) were the optimal acceptor membranes for phosphatidylcholine transfer catalyzed by their preparation of a yeast phospholipid transfer protein. This result does not conform to our findings (Table III). In accordance with results shown in Table II the presence of 10% phosphatidylserine and 20% phosphatidylinositol in the acceptor vesicles led to a complete inhibition of the transfer of phosphatidylcholine. The same result was obtained when acceptor membranes consisted of lipids extracted from yeast mitochondria, which have a phospholipid composition [29] similar to that found to be optimal by Bozzato et al. [28]. Mitochondrial membranes, on the other hand, could be used rather efficiently as acceptor; the rate of phosphatidylcholine transfer was decreased only by about 30% as compared

TABLE III

Phosphatidylcholine transfer to complex acceptor membranes

Transfer of fluorescent donor phosphatidylcholine to various acceptor membranes was measured under standard conditions as described in Materials and Methods. Data are mean values of at least three experiments. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; CL, cardiolipin.

Acceptor membrane components	Relative transfer rate (%)
Egg phosphatidylcholine	100
PC/PE/PI/PS (4 : 3 : 2 : 1)	9
Yeast mitochondria	71 [a]
Lipid extract from yeast mitochondria	0 [a]

[a] Yeast mitochondria contain PC/PE/PI/PS/PG/CL/ergosterol at an approximate molar ratio of 4 : 3 : 1 : 0.5 : 0.6 : 0.6 : 1.2.

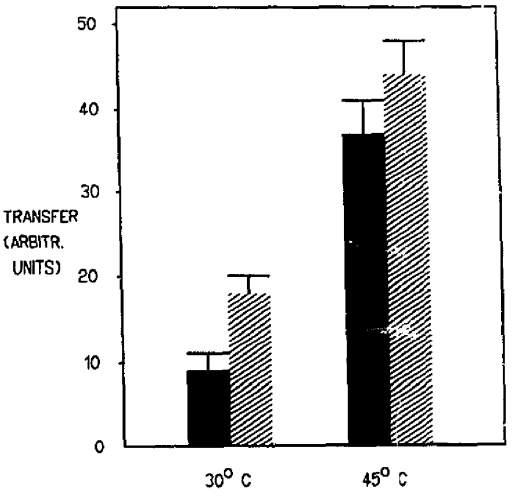

Fig. 4. Transfer of fluorescent phosphatidylcholine to acceptor membrane vesicles with different fatty acyl chain unsaturation. Transfer of fluorescent phosphatidylcholine to egg phosphatidylcholine (hatched bars) or dipalmitoyl phosphatidylcholine (full bars) acceptor membrane vesicles was measured above (45°C) and below (30°C) the phase transition temperature of dipalmitoylphosphatidylcholine. Assay conditions were the same as described in the legend to Fig. 1.

to egg phosphatidylcholine vesicles (see Table III). This result suggests that in biological membranes negative charges of phospholipids are masked by proteins.

The presence of increasing amounts of ergosterol in acceptor vesicles resulted in a proportional decrease of the phosphatidylcholine transfer rate, probably due to the membrane rigidifying effect of the sterol; at equimolar ratios of phosphatidylcholine and ergosterol the transfer rate was reduced to 60% of the control (see Table II). As a measure for the membrane fluidity of these vesicles the fluorescence anisotropy using diphenylhexatriene-labeled phosphatidylcholine as a probe was determined [30]. Vesicles consisting of an equimolar mixture of phosphatidylcholine and ergosterol showed a 1.6-fold higher fluorescence anisotropy than the control without ergosterol, indicating a decrease of membrane fluidity.

The notion that the fluidity state of the membrane can modulate the activity of the yeast phosphatidylinositol transfer protein is supported by the fact that the transfer rate was significantly reduced when acceptor vesicles consisted of dipalmitoyl phosphatidylcholine instead of egg phosphatidylcholine. This effect was most pronounced below the phase transition temperature of dipalmitoyl phosphatidylcholine (Fig. 4). The ratio of transfer rates using egg phosphatidylcholine or dipalmitoyl phosphatidylcholine, respectively, as acceptor vesicle component was 1.2 at 45°C but 2.1 at 30°C.

Discussion

The properties of the phosphatidylinositol transfer protein from yeast described previously [6] and in this communication clearly resemble those reported for phosphatidylinositol transfer proteins isolated from mammalian tissues (for a review, see Ref. 2). Molecular size, isoelectric point, substrate specificity and the mode of action of the yeast and mammalian proteins are essentially the same (Table IV). However, antibodies raised against the bovine brain phosphatidylinositol transfer protein cross-react with other mammalian phosphatidylinositol transfer proteins [31], but not with the yeast protein (personal communication, Snoek, G.T., Utrecht). This result indicates that in spite of similar overall properties and function the structural similarity between mammalian and yeast phosphatidylinositol transfer proteins seems to be rather limited. Another major difference between the yeast and mammalian proteins concerns susceptibility to sulfhydryl-group specific reagents: all mammalian transfer proteins tested are inhibited by these reagents [2], whereas the yeast phosphatidylinositol transfer protein is insensitive [6].

The yeast transfer protein forms complexes either with phosphatidylinositol or with phosphatidylcholine. We assume, that, similar to the bovine brain phosphatidylinositol transfer protein [15], the phospholipid to protein ratio of the complex is 1:1. Phospholipids bound to the yeast phosphatidylinositol transfer protein

TABLE IV

Properties of the yeast phosphatidylinositol transfer protein as compared to mammalian phosphatidylinositol transfer proteins

PITP, phosphatidylinositol transfer protein; PLTP, phospholipid transfer protein; Sph, sphingomyelin.

Transfer protein	Molecular mass (kDa)	Isoelectric point	Specificity	Ref.
PITP, yeast	37	5.2	PI > PC	this paper
PLTP, yeast	33.4	6.3	PC > PI > PE > PS	7
PITP, bovine brain	36	5.3, 5.6 [a]	PI > PC > PG = Sph	2, 31
PITP, bovine heart	33.5	5.3, 5.6 [a]	PI > PC > Sph > PE	2, 37
PITP, rat tissues	36	4.9, 5.3 [a]	PI > PC	31
PITP, human platelets	29	5.6, 5.9 [a]	PI > PC > PG	2, 38

[a] Dual forms with different isoelectric points due to the binding of phosphatidylinositol or phosphatidylcholine, respectively.

are most likely embedded in the same binding site, phosphatidylinositol being the preferred substrate. Higher binding affinity for phosphatidylinositol as compared to phosphatidylcholine (Fig. 3) correlates with the preferred transfer of the former phospholipid. A similar correlation between transfer rate and binding affinity has been described for the phosphatidylinositol transfer protein from bovine brain [32], although exceptions have been observed, especially with phospholipid species containing shorter acyl chains [14].

In the transfer assay with unilamellar vesicles as donor and acceptor membranes negatively charged phospholipids inhibit the activity of the yeast transfer protein (see Table II). Decreased transfer rates in the presence of negatively charged phospholipids have also been observed for mammalian phosphatidylinositol transfer proteins [8,9], although significant quantitative differences exist. For example, the bovine brain phosphatidylinositol transfer protein is less sensitive to inhibition by phosphatidylinositol and phosphatidylserine, but tolerates less phosphatidic acid. Obviously the affinities of the putative positive charged membrane interaction sites of the yeast and mammalian phosphatidylinositol transfer proteins differ in their affinities for the various negatively charged phospholipids. The zwitterionic phospholipids, phosphatidylethanolamine and sphingomyelin, incorporated into acceptor vesicle membranes in concentrations up to 50 mol% (see Table II) had no effect on the activity of the yeast phosphatidylinositol transfer protein. Interestingly, with mammalian phosphatidylinositol transfer proteins sphingomyelin is inhibitory [2]. The reason for this distinct behavior of yeast and mammalian proteins is not clear, but it should be mentioned that in its natural environment the yeast protein does not encounter sphingomyelin, since yeast membranes are devoid of this phospholipid.

The substrate specificity and susceptibility to inhibition by negatively charged phospholipids of the yeast phosphatidylinositol transfer protein described here are at variance with results reported by Bozzato et al. [28] who investigated a 530-fold enriched transfer protein from *Saccharomyces cerevisiae* cytosol. These authors found that the transfer activity strongly depends on the membrane phospholipid composition. Optimum transfer rates were observed with a mixture of phosphatidylcholine/phosphatidylethanolamine/phosphatidylinositol/phosphatidylserine (4:3:2:1) as components of unilamellar donor and multilamellar acceptor vesicles. The order of transfer rates for these four phospholipids was phosphatidylcholine > phosphatidylinositol > phosphatidylethanolamine > phosphatidylserine. The transfer protein described here does not transfer phosphatidylethanolamine and phosphatidylserine at all, irrespective of the membrane phospholipid composition, including that described by Bozzato et al. [28]. When vesicles with the phospholipid composition mentioned above were used as acceptor the activity of the phosphatidylinositol transfer protein described here was reduced to zero (see Table III). The reason for the discrepancies between the results of this study and those reported by Bozzato et al. [28] is unclear. Possible explanations might be different yeast strains and/or assay conditions. Alternatively, these authors might have isolated a different type of transfer protein.

A mixture of phospholipids isolated from yeast mitochondria and used for the preparation of acceptor vesicles completely inhibited the phosphatidylcholine transfer catalyzed by the phosphatidylinositol transfer protein (see Table III). Mitochondrial membranes, on the other hand, were effective as acceptors in an otherwise analogous system. This observation demonstrates quite clearly that results obtained with model membranes need not reflect the situation in a biological system. Obviously the presence of proteins in mitochondrial membranes reduces the inhibitory effect of negatively charged phospholipids, either by neutralizing the charge or by burying it in the surface of the membrane. This modulating effect of membrane proteins might be relevant for the regulation of protein mediated phospholipid transfer in vivo.

Using acceptor membranes consisting only of nontransferable phosphatidylethanolamine the yeast phosphatidylinositol transfer protein catalyzed a net transfer of phosphatidylcholine from donor to acceptor membranes. This observation shows that the phosphatidylinositol/phosphatidylcholine specific transfer protein can return from the acceptor to the donor membrane in a phospholipid-free form. It is questionable, however, if such a situation is ever realized in vivo where any eukaryotic membrane will always contain phosphatidylcholine and/or phosphatidylinositol. Nevertheless it should be mentioned that the bovine brain phosphatidylinositol transfer protein does not catalyze a similar net transport (without exchange) of phosphatidylinositol and phosphatidylcholine [33], whereas phosphatidylcholine transfer protein from bovine liver can catalyze a net transport of phosphatidylcholine [34].

The conservation of phosphatidylinositol transfer proteins during evolution points to an essential role of these proteins in eukaryotic cells. It has been suggested that phosphatidylinositol transfer proteins are involved in the maintenance of the signal transducing phosphatidylinositol cycle by supplying membranes active in phosphatidylinositol turnover with sufficient quantities of substrate originating from the endoplasmic reticulum [2,32]. Since the phosphatidylinositol cycle is also operating in yeast [35,36], a similar role for the phosphatidylinositol transfer protein can be envisaged for this unicellular organism. In order to function in the manner proposed, i.e. by transporting phosphatidylinositol to the plasma membrane in exchange for phosphatidylcholine, the phosphatidylinositol transfer protein must

be assisted by an independent translocation vehicle which carries phosphatidylcholine to the plasma membrane. In higher eukaryotic cells, phosphatidylcholine-specific or non-specific lipid transfer proteins might have this function. In yeast, however, no such proteins have as yet been identified. They might either have escaped detection, or they do not exist at all, and other transport systems such as membrane vesicle migration from the endoplasmic reticulum to the plasma membrane might be involved in phosphatidylcholine translocation.

Evidently phosphatidylinositol transfer proteins from yeast and higher eukaryotic cells are remarkably similar in their function, substrate specificity and response to alterations of membrane lipid composition, although protein structure and details of the catalytic mechanism might be different. The close functional relationship between the yeast and mammalian phosphatidylinositol transfer proteins justifies further investigation of the less complex yeast system, which offers ample possibilities for the physiological and genetic manipulation of membrane biogenesis, structure and phospholipid metabolism.

Acknowledgements

The authors appreciate the excellent technical assistence of H. Stütz and M. Zeiler, who prepared most of the fluorescent phospholipids. The precious gifts of two fluorescent substrates by P.J. Somerharju, Helsinki, and of soybean phosphatidylinositol by R. Franzmair, Linz, are gratefully acknowledged. This work was financially supported by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (projects 5746 and 6958).

References

1 Helmkamp, G.M., Jr. (1986) J. Bioenerg. Biomembr. 18, 71–91.
2 Helmkamp, G.M., Jr. (1985) Chem. Phys. Lipids 38, 3–16.
3 Kader, J.-C. (1985) Chem. Phys. Lipids 38, 51–62.
4 Paltauf, F. and Daum, G. (1989) in Subcellular Biochemistry, Vol. 16 (Hilderson, H., ed.), Plenum Publishing Corporation, New York, London, in press.
5 Cobon, G.S., Crowfoot, P.D., Murphy, M. and Linnane, A.W. (1976) Biochim. Biophys. Acta 441, 255–259.
6 Daum, G. and Paltauf, F. (1984) Biochim. Biophys. Acta 794, 385–391.
7 Bozzato, R.P. and Tinker, D.O. (1987) Biochem. Cell Biol. 65, 195–202.
8 Van Paridon, P.A., Gadella, T.W.J., Jr. and Wirtz, K.W.A. (1988) Biochim. Biophys. Acta 943, 76–85.
9 Somerharju, P., Van Paridon, P. and Wirtz, K.W.A. (1983) Biochim. Biophys. Acta 731, 186–195.
10 Daum, G., Böhni, P.C. and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033.
11 Kremer, J.M.H., Van de Esker, M.W.J., Pathmamanoharan, C. and Wiersema, P.H. (1977) Biochemistry 16, 3932–3935.
12 Rowe, E.S. (1983) Biochemistry 22, 3299–3305.
13 Somerharju, P.J., Van Loon, D. and Wirtz, K.W.A. (1987) Biochemistry 26, 7193–7199.
14 Van Paridon, P.A., Gadella, T.W.J., Jr., Somerharju, P.J. and Wirtz, K.W.A. (1988) Biochemistry 27, 6208–6214.
15 Van Paridon, P.A., Visser, A.J.W.G. and Wirtz, K.W.A. (1987) Biochim. Biophys. Acta 898, 172–180.
16 Paltauf, F., Zinser, E. and Daum, G. (1985) Biochim. Biophys. Acta 835, 322–330.
17 Hermetter, A., Stütz, H. and Paltauf, F. (1983) Chem. Phys. Lipids 32, 145–152.
18 Hermetter, A., Stütz, H., Franzmair, R. and Paltauf, F. (1989) Chem. Phys. Lipids 50, 57–62.
19 Comfurius, P. and Zwaal, R.F.A. (1977) Biochim. Biophys. Acta 488, 36–42.
20 Higgins, J.A. and Pigott, C.A. (1982) Biochim. Biophys. Acta 93, 151–158.
21 Gupta, C.M., Radhakrishnan, R. and Khorana, H.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4315–4319.
22 Daum, G., Glatz, H. and Paltauf, F. (1977) Biochim. Biophys. Acta 488, 484–492.
23 Laemmli, U.K. (1970) Nature 227, 680–685.
24 O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.
25 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
26 Broekhuyse, R.M. (1968) Biochim. Biophys. Acta 152, 307–315.
27 Daum, G., Schwelberger, H.G. and Paltauf, F. (1986) Biochim. Biophys. Acta 879, 240–246.
28 Bozzato, R.P., Woolley, D. and Tinker, D.O. (1987) Biochem. Cell Biol. 65, 203–210.
29 Daum, G. (1985) Biochim. Biophys. Acta 822, 1–42.
30 Parente, R.A. and Lentz, B.R. (1985) Biochemistry 24, 6178–6185.
31 Venuti, S.E. and Helmkamp, G.M., Jr. (1988) Biochim. Biophys. Acta 946, 119–128.
32 Van Paridon, P.A., Gadella, T.W.J., Jr., Somerharju, P.J. and Wirtz, K.W.A. (1987) Biochim. Biophys. Acta 903, 68–77.
33 Kasper, A.M. and Helmkamp, G.M., Jr. (1981) Biochim. Biophys. Acta 664, 22–32.
34 Berkhout, T.A., Van den Bergh, C., Mos, H., De Kruijff, B. and Wirtz, K.W.A. (1984) Biochemistry 23, 6894–6900.
35 Kaibuchi, K., Miyajima, A., Arai, K.-I. and Matsumoto, K. (1986) Proc. Natl. Acad. Sci. USA 83, 8172–8176.
36 Uno, I., Fukami, K., Kato, H., Tanekawa, T. and Ishikawa, T. (1988) Nature 333, 188–190.
37 DiCorletto, P.E., Warach, J.B. and Zilversmit, D.B. (1979) J. Biol. Chem. 254, 7795–7802.
38 George, P.Y. and Helmkamp, G.M., Jr. (1985) Biochim. Biophys. Acta 836, 176–184.