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Characterization of a non-specific lipid transfer protein associated with the peroxisomal membrane of the yeast, *Saccharomyces cerevisiae*

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Abstract

A lipid transfer protein with a broad substrate specificity is associated with the peroxisomal membrane of the yeast *Saccharomyces cerevisiae*. The protein catalyzes in vitro the transfer of various phospholipids, phosphatidylinositol and phosphatidylserine being translocated at the highest rates. The transfer protein can be released from peroxisomal membranes by treatment with 0.25 M KCl and highly enriched using conventional chromatographic techniques. It is inactivated by heat, detergents, divalent cations and proteinases. During various steps of purification this lipid transfer protein co-fractionated with peroxisomal acyl-CoA oxidase (Pox1p). In a *pox1* disruptant peroxisomal lipid transfer activity was still present, although at a reduced level. The peroxisomal lipid transfer protein from the *pox1* mutant exhibited different chromatographic properties as compared to the wild-type strain suggesting that acyl-CoA oxidase and the peroxisomal lipid transfer protein may form a complex.

Keywords: Phospholipid transfer; Phosphatidylinositol; Phosphatidylserine; Peroxisome; Acyl-CoA oxidase; *pox1* mutant; Yeast; (*S. cerevisiae*)

1. Introduction

Soluble proteins catalyzing transfer of lipids between artificial and biological membranes in vitro have been detected in higher eukaryotes [1], yeast [2,3] and prokaryotes [4]. According to their substrate specificity three types of transfer proteins can be distinguished, namely phosphatidylcholine transfer protein (PCTP), phosphatidylinositol transfer protein (PITP) and non-specific lipid transfer protein (nsLTP) [5,6]. PITP and PCTP exhibit a high substrate specificity, whereas nsLTP is characterized by its ability to transfer a large variety of phospholipids and sterols between donor and acceptor membranes in vitro. In mammalian cells, nsLTP is identical to sterol carrier protein 2 (SCP-2) [7].

PITP isolated from the cytosol of the yeast *Saccharomyces cerevisiae* was characterized in our laboratory [8,9] and by others [10,11]. Bankaitis and co-workers [12,13] demonstrated that yeast PITP is identical to the Sec14 gene product, which is a component of the protein secretory pathway. Association of this protein with Golgi membranes was demonstrated [14], but a considerable amount was also found in the cytosol. Recently, Sec14p/PITP was shown to modulate the phosphatidylcholine to

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phosphatidylinositol ratio in Golgi membranes by effecting ligand-dependent inhibition of choline phosphate cytidylyltransferase activity [15].

A second lipid transfer protein of *Saccharomyces cerevisiae* with a broad substrate specificity was named phosphatidylserine transfer protein (PSTP) because of its preference for transferring this phospholipid in vitro [16]. The physiological role of this protein, which resembles mammalian nsLTP regarding its substrate specificity, is not understood at present.

In mammalian cells a higher molecular weight form (58 kDa) of the cytosolic nsLTP/SCP-2 (13 kDa) has been detected in peroxisomes [17,18]. There is only indirect proof for the involvement of nsLTP/SCP-2 in sterol trafficking by correlation of the protein level with sterol production [19,20] and sterol distribution [21,22]. There is no evidence that organelle-bound nsLTP/SCP-2 analogs are involved in lipid binding and/or lipid transport.

A microbial counterpart of the mammalian nsLTP/SCP-2 has been described by Tan et al. [23,24] who isolated a protein named PXP-18 from the peroxisomal matrix of the yeast *Candida tropicalis*. PXP-18 shows high amino acid sequence homology to mammalian nsLTP/SCP-2 and exhibits a similar broad substrate specificity. In a previous study from our laboratory [25] an antibody against mammalian nsLTP was used to search for homologous proteins of the yeast *Saccharomyces cerevisiae*. Cross-reacting yeast proteins were detected, but they were found to be unrelated to lipid transfer processes (Tahotna et al., manuscript in preparation). In continuation of the search for nsLTP-related proteins in *Saccharomyces cerevisiae*, we screened membranes of this microorganism for lipid transfer activity. These studies were also motivated by the possibility that, in analogy to the mammalian system, a relationship between membrane-bound forms of lipid transfer proteins and the cytosolic PSTP may exist in yeast.

2. Materials and methods

2.1. Cultivation of yeast cells

Saccharomyces cerevisiae X2180-1A (*MATa SUC2 mal gal2 CUP1*) was grown on YPD medium (1% yeast extract, 2% peptone, 2% glucose), and *Saccharomyces cerevisiae* D237-10B (ATCC 25657; *MATa*) on YPLac (2% lactate) [26] in 2-liter flasks containing 500 ml media at 30°C with vigorous aeration. To induce proliferation of peroxisomes the wild-type yeast strain D273-10 B was grown in the presence of 0.1% oleic acid and 0.2% Tween 80 [27]. The culture medium of the disruptant A232-4A-23 (*MATα ura3 trp1 pox1::leu2*) [28] (kindly provided by D.Y. Thomas, Montreal, Canada), which is deficient in the peroxisomal acyl-CoA oxidase, was supplemented with 0.1% oleic acid, 0.2% Tween 80, and 0.1% glucose.

2.2. Isolation and characterization of yeast subcellular fractions

Plasma membrane [29], mitochondria [26], microsomes [27], and vacuoles [27] were isolated by published procedures.

Peroxisomes were purified essentially as described by Tahotna et al. [25]. In brief, a $30000 \times g$ organelle pellet was prepared from spheroplasts [26] by differential centrifugation in a pH 6.0 breaking buffer [27,30] and fractionated on an Accudenz step gradient consisting of equal volumes of 17, 24 and 35% Accudenz in 5 mM MES, 1 mM KCl, 0.24 M sucrose (pH 6) [30]. After centrifugation for 90 min in a SW-28 rotor (Beckman) at $100000 \times g$, peroxisomes were collected from the 24–35% Accudenz interface, diluted with breaking buffer and isolated by centrifugation at $34500 \times g$ for 30 min. To separate peroxisomal membranes from the matrix, intact peroxisomes were hypotonically shocked for 1 h on ice in 10 mM Tris-HCl (pH 7.4) and frozen overnight [25]. After thawing, membranes were separated from the soluble fraction by centrifugation at $100000 \times g$ in a T-865 rotor (Sorvall) for 1 h. The resulting membrane pellet was washed once with 10 mM Tris-HCl (pH 7.4).

Integral and peripheral membrane proteins were separated by treatment of peroxisomal membranes with 0.25 M KCl [25] for 20 min at 4°C. Insoluble membrane components were sedimented by centrifugation at $100000 \times g$ for 1 h, whereas solubilized proteins were collected from the supernatant.

Subcellular fractions were characterized by Western blot analysis as summarized by Zinser and Daum

[31]. Proteins of subcellular fractions were quantified by the method of Lowry et al. [32]. SDS-polyacrylamide gel electrophoresis using 12.5% gels was carried out by the method of Laemmli [33]. Western blot analysis using nitrocellulose filters (HyboadTM-C, Amersham) was performed according to standard protocols [34]. Proteins were detected by ELISA using rabbit antibodies against the respective antigens and peroxidase-conjugated goat-anti-rabbit secondary antibodies following the manufacturer's instructions. Antisera against yeast plasma membrane ATPase, porin (outer mitochondrial membrane marker), Sec61p (endoplasmic reticulum marker; provided by R. Schekman, Berkeley, USA), carboxypeptidase Y (vacuolar marker; provided by D. Wolf, Stuttgart, Germany), and acyl-CoA oxidase and 3-oxoacyl-CoA thiolase (peroxisomal markers; provided by W.D. Kunau, Bochum, Germany) were used throughout this study. The quality of organellar preparations was the same as described by Zinser et al. [27] and Zinser and Daum [31].

2.3. Purification of the peroxisomal lipid transfer protein

Isolated peroxisomes were subjected to a hypotonic shock and a freeze-thaw treatment, and membranes were separated from the soluble components by centrifugation as described above. Peroxisomal membrane proteins soluble in 0.25 M KCl were used as starting material for the purification of the lipid transfer protein.

2.3.1. Step 1: Ammonium sulfate precipitation.

Solid ammonium sulfate was added to 40% saturation in small portions to the solution containing peroxisomal membrane proteins soluble in 0.25 M KCl. After 2 h on ice, precipitated proteins were collected by centrifugation in a table top centrifuge at 12000 rpm for 5 min, and resuspended in 0.5 M $(NH_4)_2SO_4$, 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4). Insoluble material was removed by centrifugation as described above and discarded.

2.3.2. Step 2: Phenyl-Sepharose chromatography.

Proteins contained in the supernatant of the last step were applied to a HiTrap Phenyl-Sepharose fast flow (low sub) column (0.7 × 2.5 cm) equilibrated with 0.5 M $(NH_4)_2SO_4$, 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4). Unbound proteins were eluted with 6 ml of the same buffer at a flow rate of 1 ml/min. After washing with 5 ml 0.25 M KCl, 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4), bound proteins were eluted with 20 ml of a linear gradient formed from 10 ml 0.25 M KCl, 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4) and 10 ml ethyleneglycol. Fractions of 2 ml were collected. Active lipid transfer protein was eluted with 33–38% ethyleneglycol.

2.3.3. Step 3: Molecular sieve chromatography.

Pooled active fractions from the previous step were loaded onto a Superdex 200 column (prep. grade; Pharmacia; 57 cm × 1.6 cm). Fractions of 2.5 ml were collected during elution of proteins with 0.25 M KCl, 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4) at a flow rate of 0.4 ml/min. Lipid transfer activity was detected in fractions 26, 27 and 28.

2.4. Butyl-Sepharose chromatography

As an alternative method to obtain enriched preparations of the peroxisomal lipid transfer protein Butyl-Sepharose chromatography was employed. Peroxisomal membrane proteins soluble in 0.25 M KCl (1 ml) were diluted with 1 ml 0.5 M $(NH_4)_2SO_4$, 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4). After centrifugation the supernatant was applied to a 1 ml Butyl-Sepharose column (Pharmacia). Unbound proteins were eluted with 10 ml of the same buffer. After extensive washing with 0.5 M $(NH_4)_2SO_4$, 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4), elution of bound proteins was performed with 20 ml of a linear ethyleneglycol gradient (see Section 2.3.2). Fractions of 2 ml were collected. Lipid transfer activity was eluted with 45–50% ethyleneglycol.

2.5. Determination of lipid transfer activity

The rate of protein-catalyzed phospholipid transfer from fluorescently labeled small unilamellar donor vesicles to unlabeled unilamellar acceptor membranes was measured as described previously [35]. In brief, donor vesicles were prepared by injection of an ethanolic solution [36] containing 5 mol% pyrene-labeled phospholipid, 85 mol% egg phosphatidyl-

choline, and 10 mol% *N*-trinitrophenyl phosphatidylethanolamine, into 10 mM Tris-HCl, 1 mM EDTA, 0.02% NaN_3 (pH 7.4) [37]. Acceptor vesicles consisting of phosphatidylcholine were prepared by the same technique. Donor vesicles (25 pmol of lipid) were incubated with a 50-fold excess of acceptor vesicles in 0.5 ml 10 mM Tris-HCl, 1 mM EDTA, 0,02% NaN_3 (pH 7.4). Lipid transfer between the quenched donor vesicles and unlabeled acceptor vesicles was determined by monitoring the increase of pyrene monomer fluorescence intensity upon addition of the transfer protein. The phospholipid transfer activity was measured using a Shimadzu RF-540 spectrofluorimeter. The excitation wavelength was set at 342 nm (2 nm slit) and the emission wavelength was set at 380 nm (10 nm slit). All measurements were carried out at 35°C. Values for the spontaneous exchange between donor and acceptor vesicles were subtracted from total transfer rates. The assay was performed for 5 min with measurements taken every 0.1 min. The lipid transfer activity was determined from the initial slope of the kinetic curve. In order to correlate the increase of pyrene monomer fluorescence intensity and transfer rate (nmol/mg per min) the maximum monomer fluorescence intensity of the respective samples was determined in the presence of 5 μl of 10% Triton X-100.

Fluorescently labeled phosphatidylcholine [38], phosphatidylethanolamine and phosphatidylserine [39], phosphatidylinositol [40], and *N*-trinitrophenyl phosphatidylethanolamine [41] were synthesized by published procedures.

3. Results

3.1. Lipid transfer activity associated with yeast subcellular membranes

Subcellular fractions isolated from the yeast, *Saccharomyces cerevisiae*, and used in this study can be obtained at high purity as shown by Tahotna et al. [25] and Zinser and Daum [31]. Membranes of all yeast organelles contain proteins soluble in 0.25 M KCl that exhibit lipid transfer activity (Table 1). Cross-contamination between these fractions as a source of lipid transfer activities can be excluded because of the high purity of organellar preparations.

Table 1
Lipid transfer activities associated with yeast subcellular membranes

	Specific lipid transfer activity (nmol/min per mg) [a]			
	PS	PE	PI	PC
Peroxisomes	1.38	0.92	2.44	0.46
Mitochondria	0.67	0.34	0.55	0.51
Microsomes (30000× *g* pellet)	0.66	0.25	0.54	0.09
Microsomes (40000× *g* pellet)	0.42	n.d.	0.28	0.16
Microsomes (100000× *g* pellet)	0.09	n.d.	0.08	0.01
Vacuoles	0.62	0.98	1.13	0.37
Plasma membrane	1.06	0.25	0.63	1.24

[a] Isolated organellar membranes were washed with 0.25 M KCl. Samples of solubilized proteins were tested for lipid transfer activity in a fluorescence assay as described in Section 2. Data shown in the table are mean values from at least three independent experiments with a mean deviation of ±15%. n.d.: not determined. PS: phosphatidylserine; PE: phosphatidyletnanolamine; PI: phosphatidylinositol; PC: phosphatidylcholine.

Cytosolic proteins including the previously described PITP [8,9] were absent from isolated organelles (data not shown). The specificity of phospholipid transfer varies in different membranes. As can be seen from Table 1, peroxisomes are the organelle that contains the highest specific lipid transfer activities for phosphatidylserine and phosphatidylinositol. The specific transfer activity for phosphatidylethanolamine is similar in peroxisomes and vacuoles. Phosphatidylcholine transfer activity is highest in the plasma membrane.

3.2. Isolation and characterization of the yeast peroxisomal non-specific lipid transfer protein

Using KCl soluble peroxisomal membrane proteins as a starting material we isolated a protein fraction that was highly enriched in phospholipid transfer activity (Table 2). The enrichment factor of the transfer protein over the 0.25 M KCl soluble peroxisomal membrane protein fraction was 62, and the yield of lipid transfer activity was 19%. Taking into account that KCl soluble peroxisomal membrane proteins are 32-fold enriched over the homogenate (see Ref. [25]) a total enrichment factor of approximately 1800 can be calculated for the peroxisomal lipid transfer protein. However, preparations were not homogenous as

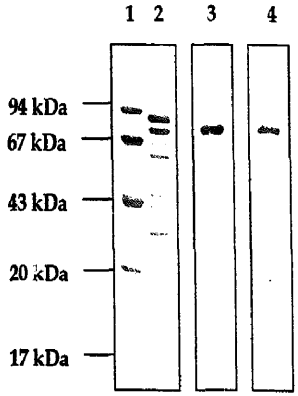

Fig. 1. Protein pattern of fractions containing the highly enriched and functionally active peroxisomal lipid transfer protein SDS-polyacrylamide gel electrophoresis of proteins was carried out as described in Section 2 with 20 μg protein applied to each lane. 1: protein standards; 2: KCl-soluble peroxisomal membrane proteins. Purified sample after ammonium sulfate precipitation and Phenyl-Sepharose chromatography (3), and Superdex 200 chromatography (4).

judged by SDS-PAGE even after the last step of purification. Fractions of highest purity contained one prominent polypeptide band at 70 kDa, which was later identified as the Pox1p (see below), and smaller bands in the molecular weight range of 60–80 kDa and 30 kDa (Fig. 1). Further attempts to purify the lipid transfer protein failed, because they resulted in a complete loss of activity.

Peroxisomal lipid transfer activity was eluted from a molecular sieve column (Superdex 200, Pharmacia) in an apparent molecular weight range of 70 ± 10 kDa. This is much higher than the molecular weight of PXP-18 from *Candida tropicalis* [23,24] and nsLTP from mammalian cells [5,6]. Accompanying proteins, especially Pox1p, seem to affect the chromatographic behavior of the yeast peroxisomal lipid transfer protein (see below).

The substrate specificity of the highly enriched peroxisomal lipid transfer protein was similar to that of the total 0.25 M KCl soluble peroxisomal membrane protein fraction (see Table 1) indicating that only one protein is responsible for lipid transfer activity in this organelle. The lipid transfer protein was slightly stabilized by 5 mM mercaptoethanol and 1 mM PMSF. Increase of temperature as well as freezing and thawing reduced the transfer activity of the purified protein. Incubation of the protein for 10 min at 56°C completely abolished lipid transfer activity, and freezing at −20°C for 24 h with or without addition of PMSF resulted in an 80% inactivation.

Among possible activators and inhibitors tested, Ca^{2+} had the most pronounced effect (Table 3) leading to a markedly reduced lipid transfer activity. EDTA, on the other hand, was slightly stimulating. The activity of the peroxisomal lipid transfer protein was neither significantly influenced by the SH-reagents β-mercaptoethanol and dithiothreitol nor by treatment with NEM. Treatment with proteinase K and trypsin led to loss of lipid transfer activity.

3.3. Co-isolation of the peroxisomal lipid transfer protein with acyl-CoA oxidase

During purification of the yeast peroxisomal lipid transfer protein we realized a coincidence with the

Table 2
Isolation of the yeast peroxisomal lipid transfer protein

	Protein (mg)	Specific activity (nmol/min per mg)	Total activity (nmol/min)	Yield (%)	Enrichment (factor)
0.25 M KCl soluble peroxisomal membrane fraction	3.85	1.38	5.31	100	1
Ammonium sulfate precipitation (40% sat.)	0.44	7.03	3.34	63	5.2
HIC Phenyl-Sepharose	0.15	14.04	2.04	46	10.3
Superdex 200	0.012	85.61	1.02	19	62

Data shown in the table are mean values from at least five independent experiments with a mean deviation of ± 15%. For calculation of the yield the transfer activity of 0.25 M KCl soluble peroxisomal membrane proteins was set at 100%. The enrichment factor of the 0.25 M KCl soluble peroxisomal membrane fraction over the homogenate is 32 according to marker enzyme measurements. In standard lipid transfer assays fluorescently labeled phosphatidylserine was used as a substrate.

appearance of acyl-CoA oxidase (Pox1p) in all chromatographic steps. As an example, lipid transfer activity and acyl-CoA oxidase were found in the same fractions during Butyl-Sepharose chromatography (Fig. 2). The striking analogy of this observation with former findings by Niki et al. [42], who described formation of a 1:1 molar complex of the non-specific lipid transfer protein PXP-18 of *Candida tropicalis* with acyl-CoA oxidase led us to investigate a possible association of the peroxisomal lipid transfer protein from *Saccharomyces cerevisiae* with Pox1p in more detail. For this purpose we employed a *pox1* disruptant that is deficient in the peroxisomal acyl-CoA oxidase. The absence of Pox1p from this strain was confirmed by Western blot analysis (data not shown). However, peroxisomes isolated from this mutant still exhibited 65% of the lipid transfer activity of the wild-type strain. Lipid transfer activity isolated from *pox1* peroxisomes was eluted from a molecular sieve column at an apparent molecular weight similar to that of wild-type cells. This puzzling finding may be due to the fact that the transfer protein interacts with Pox1p in the wild-type strain, but with other protein(s) in the *pox1* disruptant. A marked alteration of the chromatographic behavior of the peroxisomal lipid transfer protein from the *pox1* mutant was observed using Butyl-Sepharose chromatography. The lipid transfer protein of wild-type cells was eluted with 30–40% ethyleneglycol concomitantly with acyl-CoA oxidase (Fig. 2), whereas the protein isolated from the *pox1* mutant was found exclusively in the flow through (fraction 11) of the column (data not shown). Although this is not a direct proof for the interaction of the peroxisomal lipid transfer protein with acyl-CoA oxidase, these results argue in favor of an association of these two proteins. Niki et al. [42] localized acyl-CoA oxidase as well as PXP-18 of *Candida tropicalis* in the peroxisomal matrix. In contrast acyl-CoA oxidase of *Saccharomyces cerevisiae* cofractionates at least in part with the peroxisomal membrane (data not shown). Thus, interaction of this enzyme with the membrane-bound peroxisomal lipid transfer protein should be possible.

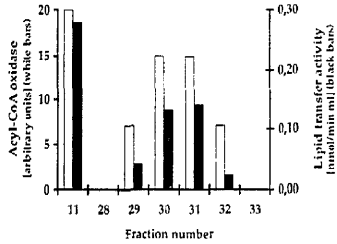

Fig. 2. Cofractionation of peroxisomal lipid transfer activity with acyl-CoA oxidase (Pox1p). Peroxisomal membrane proteins soluble in 0.25 M KCl were applied to a Butyl-Sepharose column and eluted as described in Section 2.4. Relative amounts of Pox1p (white bars) as detected by Western blot analysis are expressed as arbitrary units. Lipid transfer activities (black bars) are expressed as nmol phosphatidylserine transferred per min and ml. Fraction 11 represents the flow through and the pool of unbound proteins. The ethyleneglycol gradient elution was started at fraction 21. For details of the procedure see Section 2.

Table 3
Modulation of phospholipid transfer activity in vitro

	Relative lipid transfer activity (%)
Control	100
EDTA (5 mM)	127
Ca^{2+} (0.1 M)	24
Mg^{2+} (0.1 M)	73
N-Ethylmaleimide (5 mM)	96
β-Mercaptoethanol (5 mM)	90
Dithiothreitol (5 mM)	63
Proteinase K (1:200; 35°C; 10 min)	29
Trypsin (1:200; 35°C; 10 min)	0

A highly purified fraction of the peroxisomal lipid transfer protein was pretreated for 5 min with the respective reagents. Fluorescently labeled phosphatidylserine was used as substrate in routine assays. Data shown in the table are mean values from at least three independent experiments with a mean deviation of ±15%.

4. Discussion

Two lipid transfer proteins of the yeast *Saccharomyces cerevisiae* have been characterized and purified, namely PITP/Sec14p and PSTP. PITP/Sec14p is a functional, but not a structural homologue of mammalian PITP [43]. PSTP resembles mammalian nsLTP with respect to its broad substrate specificity. The major location of both transfer proteins is the cytosol, although association of the PITP/SEC14p with Golgi membranes has been reported [14]. In

contrast to mammalian cells, which harbor an nsLTP analog in peroxisomes, and *Candida tropicalis*, which also contains a peroxisomal nsLTP analog named PXP-18 [23,24], such membrane-bound forms of lipid transfer proteins or related proteins have so far not been detected in the yeast *Saccharomyces cerevisiae*.

In this paper we present evidence that besides PITP/Sec14p and PSTP membrane-bound polypeptides with lipid transfer activity in vitro exist in the yeast *Saccharomyces cerevisiae*. Four reasons motivated us to purify the lipid transfer protein from peroxisomes of the yeast *Saccharomyces cerevisiae*: (i) the analogy to mammalian peroxisomes, which harbor an nsLTP related protein [17,18]; (ii) the occurrence of an nsLTP analog in peroxisomes of the yeast *Candida tropicalis* [23,24]; (iii) the high specific lipid transfer activity in peroxisomes of the yeast *Saccharomyces cerevisiae* (see Table 1); and (iv) the relatively simple pattern of yeast peroxisomal proteins that are soluble in 0.25 M KCl [25].

The peroxisomal lipid transfer protein of *Saccharomyces cerevisiae* is structurally different from PITP/Sec14p, because an antibody raised against PITP/Sec14p failed to recognize any of the peroxisomal polypeptides in Western blot analysis (data not shown). Furthermore, the two transfer proteins can be easily distinguished by their substrate specificity. On the other side, the peroxisomal lipid transfer protein resembles the cytosolic PSTP with respect to its broad substrate specificity. The quantitative rates of transfer activities for individual phospholipids vary, but this could be result of conformational changes, accompanying factors, or other environmental conditions. The insufficient degree of purity of both proteins in our preparations prevented us so far from raising monospecific antibodies and obtaining partial amino acid sequences. Therefore, the question as to a possible structural relationship between the cytosolic PSTP and the peroxisomal lipid transfer protein remains unanswered. Furthermore, the relationship between transfer protein(s) associated with different yeast organelles is unclear. These proteins may belong to a family of proteins encoded by individual, probably related genes with different targeting signals for each organelle. Alternatively, organelle-bound lipid transfer proteins may be product of a single gene, and targeting to organelles may be regulated by mechanisms not understood as yet. Differences in the substrate specificities of organelle-bound lipid transfer proteins (see Table 1) may be an argument against the latter hypothesis.

Regarding the physiological function of membrane-bound lipid transfer proteins one has to take into account that proteins with lipid transfer activity in vitro may fulfill quite different roles in vivo. The lesson we learned from investigations with PITP/Sec14p was that this so-called lipid transfer protein affects processes that are distinct from lipid transport between a donor and an acceptor membrane. The Golgi bound portion of yeast PITP/Sec14p is a component of the protein secretory pathway and functions as a modulator of choline phosphate cytidylyltransferase [15], affecting budding of protein secretory vesicles by regulating the phosphatidylcholine to phosphatidylinositol ratio. Organelle-bound LTPs may fulfill a role different from lipid transport as well as suggested for PXP-18 from *Candida tropicalis* [43]. PXP-18 was shown to protect acyl-CoA oxidase against thermal inactivation. Our results obtained with *Saccharomyces cerevisiae* indicate that the peroxisomal lipid transfer protein of this yeast may also interact with acyl-CoA oxidase. Coisolation of the transfer protein with acyl-CoA oxidase (Pox1p) in wild-type cells, and altered chromatographic behavior of the transfer protein in a *pox1* disruptant support this view. In the *pox1* mutant the peroxisomal lipid transfer protein may interact with other peroxisomal proteins. We are aware of the fact that our data are not a definite proof for the association of these two proteins. Experiments with isolated proteins will be required to test this hypothesis in more detail.

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