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Rapid Report

Interaction of the yeast phosphatidylserine transfer protein with artificial and biological membranes

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Transfer of pyrene-labeled phosphatidylserine catalyzed by the yeast phosphatidylserine transfer protein in vitro largely depends on the membrane lipid composition of artificial unilamellar acceptor vesicles. Negatively charged phospholipids markedly decrease the rate of protein-catalyzed phosphatidylserine transfer. Although biological membranes contain a significant proportion of negatively charged phospholipids they serve more effectively as acceptors than artificial membranes with a similar phospholipid composition, but without proteins. This result indicates that proteins present in biological membranes mask negative charges of phospholipids on the surface of acceptor membrane vesicles. When proteins of the membrane surface are removed by proteinase treatment this protective effect is partially lost. A correlation between the activity of the phosphatidylserine transfer protein in yeast cytosol and the extent of membrane biogenesis during growth could not be observed.

Lipid transfer proteins have been isolated from various types of cells including mammalian and plant cells, and eukaryotic and prokaryotic microorganisms (for reviews see Refs. 1–4). According to their substrate specificity most of the lipid transfer proteins characterized so far can be grouped into three categories, namely phosphatidylinositol transfer protein, phosphatidylcholine transfer protein and the so-called non-specific lipid transfer protein, which is identical to sterol carrier protein-2 [5]. Two types of phospholipid transfer proteins have been isolated from yeast cells. The yeast phosphatidylinositol transfer protein [6,7], which was shown to be identical to the SEC14 gene product [8,9], closely resembles mammalian phosphatidylinositol transfer proteins. The yeast phosphatidylserine transfer protein [10] exhibits several characteristics similar to non-specific lipid transfer proteins from higher eukaryotes, but lacks the ability to transfer phosphatidylinositol and phosphatidylcholine.

The observation that the yeast phosphatidylinositol transfer protein is a component of the protein secretory machinery [9,11] raised new questions as to the possible function(s) of such proteins. An important feature of the phosphatidylinositol transfer protein seems to be its ability to interact with organelles, especially with Golgi membranes [12], where it supports protein secretion. The role of the phosphatidylinositol transfer protein as a probe for a balanced membrane phospholipid composition is discussed, but its function on the molecular level is still obscure. Even less clear is the physiological function of non-specific lipid transfer proteins. Their ability to form bridges between donor and acceptor membranes [5], which might be zones of the membrane flow, is discussed as an alternative mechanism of lipid translocation. However, more detailed knowledge of the mode of interaction of lipid transfer proteins with membranes seems to be a key for the understanding of their function.

In order to study the interaction of the yeast phosphatidylserine transfer protein with membranes we measured the rate of protein-catalyzed phosphatidylserine transfer from small unilamellar vesicles to various acceptor membranes. Donor vesicles consisted of 10 mol% 1-palmitoyl-2-pyrenedecanoyl-*sn*-glycero-3-phosphoserine, 80 mol% egg phosphatidylcholine and 10 mol% *N*-trinitrophenyl phosphatidylethanolamine [13]. Donor vesicles containing 50 pmol of lipid were incubated with a 50-fold excess of acceptor membrane lipid in 0.5 ml 10 mM Tris-HCl (pH 7.1), 0.02% NaN_3. The rate of transfer of fluorescently labeled phospholipid was followed continuously by the increase of monomer fluorescence intensity using a Shimadzu RF-540 spectrofluorimeter [10]. For all assays phospha-

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TABLE I

The acceptor membrane lipid composition modulates the rate of phosphatidylserine transfer catalyzed by the yeast phosphatidylserine transfer protein

Pyrene-labeled phosphatidylserine was used as a substrate in a standard lipid transfer assay [10]. Values are expressed as mol% of individual lipids which led to a 50% inhibition of the transfer rate when present in egg phosphatidylcholine acceptor vesicles. Data are mean values of at least three experiments.

Lipid component added to acceptor membrane vesicles	% of lipid in acceptor vesicles leading to a 50% inhibition of phosphatidylserine transfer
Phosphatidylserine	17
Phosphatidylethanolamine	n.i. 0–50% [a]
Phosphatidic acid	23
Phosphatidylinositol	14
Phosphatidylglycerol	16
Sphingomyelin	n.i. 0–50%
Ergosterol	50

[a] n.i. 0–50%, no inhibition between 0 and 50 mol%.

tidylserine transfer protein approx. 100-fold enriched from yeast cytosol was used.

Acceptor vesicles with various lipid compositions were employed to test the influence of acceptor membrane lipids on the protein-catalyzed phosphatidylserine transfer. The matrix of acceptor vesicles was egg phosphatidylcholine; up to 50% of phosphatidylcholine were replaced by various other lipids as indicated in Table I. The amount (mol%) of the additive which led to a 50% decrease of the phosphatidylserine transfer rate was taken as a measure for the inhibitory effect. Among the lipids tested phosphatidylserine is by far the preferential substrate for the transfer protein; also phosphatidylethanolamine, phosphatidic acid and ergosterol are transferred by the yeast phosphatidylserine transfer protein in vitro, although to a lesser extent [10]. The apparent inhibition of the transfer of fluorescently labeled phosphatidylserine by unlabeled phosphatidylserine, phosphatidic acid and ergosterol present in acceptor membrane vesicles can therefore at least in part be regarded as a competitive effect. However, since the non-transferable phospholipids, phosphatidylinositol and phosphatidylglycerol, are also inhibitory this effect has to be ascribed to the negative charges of these phospholipids in acceptor membranes. Ergosterol at a concentration of 50 mol% in acceptor membrane vesicles led to a 50% inhibition of phosphatidylserine transfer activity. This effect of ergosterol is most likely due to reduced membrane fluidity, but at this high concentration sterol domain formation cannot be excluded. The zwitterionic phospholipid phosphatidylethanolamine, which is also a substrate for the yeast phosphatidylserine transfer protein, and sphingomyelin had no inhibitory effect on phosphatidylserine transfer. These results parallel those obtained with phosphatidylinositol transfer proteins from yeast [7] and from mammalian cells [14,15].

Negative charges of lipids in acceptor membranes have a less pronounced effect when they are masked by proteins in biological membranes (Table II). Although there is some variation in the phospholipid composition all the different yeast subcellular membranes tested contain negatively charged phospholipids (phos-

TABLE II

Phosphatidylserine transfer in vitro to complex acceptor membranes

Transfer of phosphatidylserine to various artificial and biological acceptor membranes was measured under standard conditions [10] with 2.5 nmol total lipid present in all acceptor membranes. Biological membranes were isolated from yeast cells by published procedures (see Ref. 16). Protease treatment was carried out at room temperature for 30 min using proteinase K with a protein/proteinase ratio of 40:1 (w/w). Proteolysis was stopped by the addition of a 20-fold (w/w) excess of PMSF. Results shown are mean values from three experiments. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

Acceptor membrane composition	% of negatively charged phospholipids	Relative transfer rate (%)	
		untreated membranes	proteinase treated membranes
PC	– [a]	100	–
PC/PE (1:1)	–	100	–
PC/PE/PI/PS (4:3:2:1) [b]	30	15	–
Mitochondria	18	68	21
30000 × *g* microsomes	14	170	90
40000 × *g* microsomes	19	110	43
100000 × *g* microsomes	17	37	7
Plasma membrane	51	49	33
Peroxisomes	20	25	12
Nucleus	21	25	–
Vacuoles	23	31	–
Secretory vesicles	32	80	–

[a] –, not determined.

[b] Phospholipid composition corresponding to yeast bulk membrane.

phatidylinositol, phosphatidylserine) [16]. Nevertheless, not all yeast organellar membranes are equally well suitable as acceptor for protein-catalyzed phosphatidylserine transfer. A correlation between the phosphatidyl-serine content of membranes and their acceptor capacity could not be observed. The plasma membrane with its high concentration of phosphatidylserine (30% of total phospholipids) exhibits a transfer capacity similar to that of mitochondria (3% phosphatidylserine) or vacuoles (4% phosphatidylserine). Microsomes with a low phosphatidylserine content (5–7% of total phospholipids) are the best acceptor membranes followed by secretory vesicles (13% phosphatidylserine). Obviously proteins of acceptor membranes, especially those which are exposed at the membrane surface, contribute markedly to the acceptor capacity. This notion was confirmed by the observation, that proteinase treatment of biological membranes led in all cases to a marked reduction of the acceptor capacity (see Table II). These findings confirm previous results from our laboratory, which demonstrated that limited digestion of proteins of the mitochondrial surface led to a decrease of the rate of protein-catalyzed import of phosphatidylserine into mitochondria [17]. One explanation for this observation could be that negative charges of phospholipids on the membrane surface, which are masked by proteins in native membranes, become accessible upon degradation of the proteins. Another possibility would be that specific 'docking proteins' for the phosphatidylserine transfer protein exist on the surface of cellular membranes, but there is no proof for this hypothesis. Finally we are tempted to speculate about another explanation. Very recently we observed (Flekl, W., personal communication) that phosphatidylserine transfer activity is associated with all yeast subcellular membranes, and that the factor (protein) responsible is solubilized during incubations with a buffer of high ionic strength. If this transfer activity is due to a membrane bound form of the phosphatidylserine transfer protein, efficient dimer formation of the transfer protein leading to bridges between acceptor and donor similar to that described for non-specific transfer proteins [5] would only occur with native membranes.

The question as to the function of lipid transfer proteins in general and specifically of the yeast phosphatidylserine transfer protein is still open. An involvement of these proteins in the assembly of lipids into proliferating membranes is rather unlikely. We were able to demonstrate that in the temperature-sensitive *pit1/sec14*$^-$ yeast mutant strain [9,11] the transport of phosphatidylinositol and phosphatidylcholine to the plasma membrane is not negatively affected under non-permissive conditions [18]; also the supply of these phospholipids to mitochondria seems to be normal under conditions where the phosphatidylinositol transfer protein is inactive (Leber, A., personal communication). In previous studies [19] we demonstrated that the specific activity of the phosphatidylinositol transfer protein in yeast cytosol increased in the logarithmic growth phase, but remained constant in the stationary phase, when proliferation of cellular membranes does not occur. Comparable results were obtained with the phosphatidylserine transfer protein (Fig. 1). Phosphatidylserine as well as phosphatidylethanolamine transfer activity in the cytosol of yeast cells increased in the logarithmic growth phase and remained constant even in the late stationary phase. This observation indicates that there is no correlation between the biogenesis of membranes and the cellular level of these transfer activities. Lipid transfer proteins might rather be involved in the maintenance of membrane integrity, which is important for many cellular processes including protein secretion. The interaction of transfer proteins with membranes, which was originally regarded as an important initial step in the translocation process of lipids, might reflect functions of these proteins that are hitherto unknown.

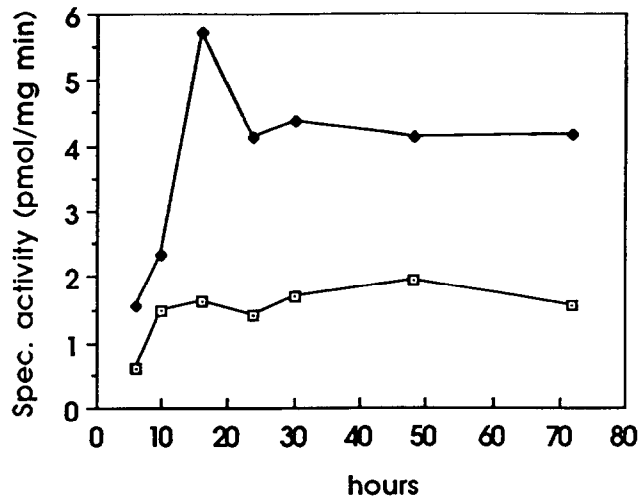

Fig. 1. The phosphatidylserine (♦) and phosphatidylethanolamine (□) transfer activity in yeast cytosol prepared from cells at different growth stages was determined in vitro. Cells were grown in the presence of 2% lactate as a carbon source [20], and cytosol was prepared by standard fractionation techniques [10].

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References

1 Helmkamp, G., Jr. (1986) J. Bioenerg. Biomembr. 18, 71–91.
2 Arondel, V. and Kader, J.-C. (1990) Experientia 46, 579–585.
3 Daum, G. and Paltauf, F. (1990) Experientia 46, 586–592.

4 Paltauf, F. and Daum, G. (1990) in Subcellular Biochemistry, Vol. 16 (Hilderson, H., ed.), pp. 279–299, Plenum Press, New York and London.
5 Wirtz, K.W.A. and Gadella, T.W.J., Jr. (1990) Experientia 46, 592–599.
6 Daum, G. and Paltauf, F. (1984) Biochim. Biophys. Acta 794, 385–391.
7 Szolderits, G., Hermetter, A., Paltauf, F. and Daum, G. (1989) Biochim. Biophys. Acta 986, 301–309.
8 Aitken, J.F., Van Heusden, G.P.H., Temkin, M. and Dowhan, W. (1990) J. Biol. Chem. 265, 4711–4717.
9 Bankaitis, V.A., Aitken, J.R., Cleves, A.E. and Dowhan, W. (1990) Nature 347, 561–562.
10 Lafer, G., Szolderits, G., Paltauf, F. and Daum, G. (1991) Biochim. Biophys. Acta, 1069, 139–144.
11 Cleves, A.E., McGee, T. and Bankaitis, V. (1991) Trends Cell Biol. 1, 30–34.
12 Cleves, A.E., McGee, T.P., Whitters, E.A., Champion, K.M., Aitken, J.R., Dowhan, W., Goebl, M. and Bankaitis, V.A. (1991) Cell 64, 789–800.
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. and Wirtz, K.W.A. (1988) Biochim. Biophys. Acta 943, 76–85.
15 Somerharju, P., Van Paridon, P. and Wirtz, K.W.A. (1983) Biochim. Biophys. Acta 731, 186–195.
16 Zinser, E., Sperka-Gottlieb, C.D.M., Fasch, E.-V., Kohlwein, S.D., Paltauf, F. and Daum, G. (1991) J. Bacteriol. 173, 2026–2034.
17 Simbeni, R., Tangemann, K., Schmidt, M., Ceolotto, C., Paltauf, F. and Daum, G. (1993) Biochim. Biophys. Acta 1145, 1–7.
18 Gnamusch, E., Kalaus, C., Hrastnik, C., Paltauf, F. and Daum, G. (1992) Biochim. Biophys. Acta 1111, 120–126.
19 Daum, G., Schwelberger, H.G. and Paltauf, F. (1986) Biochim. Biophys. Acta 879, 240–246.
20 Daum, G., Boehni, P.C. and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033.