Lipid transport in microorganisms

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Summary. Microorganisms are useful model systems for the study of intracellular transport of lipids. Eukaryotic microorganisms, such as the yeast *Saccharomyces cerevisiae,* are similar to higher eukaryotes with respect to organelle structure and membrane assembly. Experiments in vivo showed that transport of phosphatidylcholine between yeast microsomes and mitochondria is energy independent; transfer of phosphatidylinositol to the plasma membrane and the flux of secretory vesicles take place by different mechanisms. Linkage of transfer and biosynthesis of phospholipids was demonstrated in the case of intramitochondrial phospholipid transfer. A yeast phosphatidylinositol/phosphatidylcholine transfer protein, which is essential for cell viability, was isolated and characterized. Another phospholipid transfer protein present in yeast cytosol, which has a different specificity, is currently under investigation. Transfer of phospholipids between cellular membranes was also demonstrated with prokaryotes. The cytoplasm and the periplasma of the gram-negative facultative photosynthetic bacterium *Rhodopseudomonas sphaeroides* contain phospholipid transfer proteins; these seem to be involved in the biosynthesis of prokaryotic membranes.
Key words. Phospholipids; bacterial membranes; yeast membranes; phospholipid transfer proteins; mitochondria.

Introduction

The existence of various mechanisms of intracellular lipid transfer (spontaneous transport, protein catalyzed transfer, vesicle flow, membrane fusion) is conceivable, but cannot easily be verified in complex biological systems such as experimental animals. As a simpler alternative, microorganisms can be used as model systems to study problems of lipid migration in vivo and in vitro [35, 46].

Among the eukaryotic microorganisms yeast has become the organism of choice; it is well characterized with respect to the biochemistry of lipids and membrane biogenesis. Application of genetic methods and variation of culture conditions allow the manipulation of physiological processes.

Prokaryotes (bacteria), with their paucity of cellular membranes, offer an even less complex model system. Gram-positive bacteria possess only one membrane; gram-negative bacteria have two, the cytoplasmic membrane and the outer membrane. Facultative phototrophic bacteria, e.g. *Rhodopseudomonas sphaeroides,* contain in addition an intracytoplasmic membrane, which is inducible by light and which harbors the photosynthetic machinery.

Lipid transfer in eukaryotes

Eukaryotic microorganisms are similar to higher eukaryotes with respect to intracellular compartmentalization and organization of subcellular membranes. These facts make them useful for studies of the localization of lipid synthesizing enzymes and of lipid transfer routes.

Sites of phospholipid biosynthesis in yeast

Both microsomes and mitochondria of the yeast, *Saccharomyces cerevisiae,* harbor enzymes of phospholipid metabolism [12, 28]. Phosphatidylserine decarboxylase and enzymes of cardiolipin synthesis are located in the inner mitochondrial membrane, whereas phosphatidylethanolamine methyltransferases, the terminal enzymes of de novo phosphatidylcholine synthesis, are restricted to microsomes. Phosphatidylserine synthase and phosphatidylinositol synthase also seem to be microsomal enzymes (Sperka-Gottlieb et al., unpublished), although an association with the outer mitochondrial membrane has also been reported [28]. Other subcellular membranes of yeast, e.g. vacuoles or the plasma membrane, are apparently devoid of lipid synthesizing enzymes.

Phospholipid transport in yeast in vivo

The spatial separation of enzymes involved in phospholipid biosynthesis necessitates an efficient transport of phospholipids between yeast organelle membranes. In particular, intermediates of phosphatidylcholine synthesis (phosphatidylserine, phosphatidylethanolamine) and phosphatidylcholine itself have to migrate between microsomes and mitochondria, or to cellular membranes uncapable of synthesizing their own phospholipids.

Spontaneous, energy-independent transfer of lipid molecules (diffusion or protein-catalyzed transfer), energy-dependent flux of vesicles, or processes of membrane fusion are all possible mechanisms for phospholipid transport in vivo. Experiments were designed in our laboratory [16] (Gnamusch et al., unpublished results) to investigate which of these mechanisms is operating in growing yeast cells. Pulse-chase experiments using ^{3}H-serine or ^{3}H-methyl methionine as precursors, followed by subcellular fractionation demonstrated that phosphatidylserine and phosphatidylcholine are transported by an energy-independent process from microsomes, where they are synthesized, to mitochondria. In contrast,

intracellular translocation of phosphatidylethanolamine did not occur when cells were depleted of energy.

To circumvent problems of subcellular fractionation (cross-contamination of fractions) metabolic modification of phospholipids, which depends on their appearance in certain cellular fractions, was employed to study phospholipid transfer in vivo. For example, the appearance of ^{3}H-phosphatidylethanolamine after labeling cells with ^{3}H-serine is a measure of the translocation of phosphatidylserine to the inner mitochondrial membrane, where decarboxylation of phosphatidylserine occurs. In the same experiment, ^{3}H-phosphatidylcholine will only appear when newly synthesized phosphatidylethanolamine is transferred from mitochondria to microsomes. By such experiments it was demonstrated (Gnamusch et al., unpublished results) that the energy inhibitors N_3^- and F^- block the transfer of phosphatidylethanolamine and its conversion to phosphatidylcholine; CN^- inhibits the phosphatidylserine decarboxylase and probably the transfer of phosphatidylserine, too. Neither the uncoupler CCCP, nor the ionophore valinomycin, nor the disruption of the cytoskeleton by using nocodazole, had any effect on the transfer of phosphatidylserine and phosphatidylethanolamine between microsomes and mitochondria.

Vesicles involved in the secretion of proteins [24, 51] migrate from internal membranes to the plasma membrane. They could also be regarded as transport vehicles for lipids to the cellular surface. We tested this hypothesis with the aid of temperature-sensitive secretory mutants [39], which are defective in the secretory pathway for proteins. If protein secretion and phospholipid migration to the plasma membrane were linked processes, increase of the incubation temperature from 24 °C (permissive) to 37 °C (restrictive temperature) should not only result in an interruption of protein secretion, but also stop the supplying of the plasma membrane with phospholipids. That this is not the case was shown by the unchanged release of water-soluble deacylation products of phosphatidylinositol and phosphatidylcholine into the growth medium at 37 °C [16]. Deacylation of phospholipids transported to the cell surface is catalyzed by a phospholipase B attached to the outer side of the plasma membrane [53, 54] and can be used to measure transport of phospholipids from internal membranes to the plasma membrane. It is still possible that vesicle populations different from secretory vesicles are involved in intracellular phospholipid migration in yeast [1a]. It remains to be demonstrated whether phospholipid transfer proteins (see below) present in yeast cytosol play a role in this process.

The presence of the energy inhibitors N_3^- and F^-, which largely inhibits the synthesis of aminophospholipids, also stops the migration of phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine to the plasma membrane. In contrast to the migration of phosphatidylserine and phosphatidylcholine from the endoplasmic reticulum to the mitochondria, which is energy independent [16], flux of phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine to the plasma membrane appears to be inhibited in the presence of N_3^- and F^-, i.e. under conditions where the synthesis of these phospholipids is inhibited.

Intramitochondrial transfer of phospholipids in yeast

Localization of phosphatidylserine decarboxylase in the inner mitochondrial membrane necessitates translocation of phosphatidylserine, the substrate for this enzyme, from the outer to the inner membrane. In addition phosphatidylcholine and phosphatidylinositol are not synthesized in the inner mitochondrial membrane and must be imported, too. On the other hand phosphatidylethanolamine, formed in the inner mitochondrial membrane, has to reach the mitochondrial surface in order to be transported to the endoplasmic reticulum, where it is needed as a substrate for phosphatidylcholine synthesis. In mitochondria isolated from spheroplasts by a very gentle procedure [15], part of the cellular enzyme activities of phosphatidylserine synthase and phosphatidylinositol synthase cofractionate with the outer mitochondrial membrane [28]. In these preparations phosphatidylserine or phosphatidylinositol are labeled in situ with ^{3}H-serine or ^{3}H-inositol, respectively. Transfer of phospholipids to the inner membrane can subsequently be followed either by subfractionation of mitochondria or, in the case of phosphatidylserine, by metabolic conversion to phosphatidylethanolamine [41]. Experiments using ^{3}H-inositol as a precursor revealed that intramitochondrial translocation of phosphatidylinositol is linked to the biosynthesis of the phospholipid; therefore transport of phosphatidylinositol to the inner membrane is a vectorial process. Consequently phosphatidylinositol, once integrated into the inner mitochondrial membrane, is not transferred back to the mitochondrial surface. Using ^{3}H-serine as a phospholipid precursor additional characteristics of the intramitochondrial translocation of phosphatidylserine and phosphatidylethanolamine were clarified. The importing of phosphatidylserine into the inner mitochondrial membrane is independent of an electrochemical gradient across this membrane. Phosphatidylserine that reaches the inner membrane is rapidly converted to phosphatidylethanolamine, which is preferentially transferred back to the mitochondrial surface without mixing with the pool of inner membrane phosphatidylethanolamine.

Selective removal of the mitochondrial intermembrane space by mild hypotonic treatment leads to the isolation of 'mitoplasts', which have, in the case of yeast, the outer membrane still adherent to the inner mitochondrial membrane, most likely through original contact sites [36, 40]. These contact sites, which are known to be involved in the translocation of proteins across mitochondrial membranes, are most likely also zones of intramitochondrial phospholipid translocation. Support-

ing evidence comes from experiments, where 'mitoplasts'[41] as well as isolated contact sites (Simbeni et al., unpublished results) were shown to be capable of intermembrane phosphatidylserine transfer. Import of ^{3}H-phosphatidylserine from artificial phosphatidylserine/phosphatidylcholine vesicles into mitochondria demonstrated that, in contrast to the import of proteins[2], the translocation of phosphatidylserine to the inner mitochondrial membrane does not require a membrane potential across the inner membrane.

Phospholipid transfer proteins of eukaryotic microorganisms

Eukaryotic microorganisms, analogously to higher eukaryotes[22], contain lipid transfer proteins, which are thought to participate in biogenesis and maintenance of membranes in vivo. Cobon et al.[13] detected phospholipid transfer activity in yeast cytosol. The phospholipid classes transferred by this crude preparation were phosphatidylcholine, phosphatidylinositol and, to a lesser extent, phosphatidylethanolamine and phosphatidylserine. In more detailed studies a transfer protein specific for phosphatidylinositol and phosphatidylcholine was isolated and characterized in our laboratory[17, 42, 43]. Bozzato and Tinker[7] described the isolation of this yeast phospholipid transfer protein by a different isolation protocol. Another yeast phospholipid transfer protein distinct from the phosphatidylinositol/phosphatidylcholine specific protein is currently under investigation in our laboratory (Lafer et al., unpublished results). This protein displays specificity for phosphatidylserine, phosphatidylethanolamine and, to a lesser extent, for phosphatidic acid and cardiolipin.

Chavant and Kader[11] presented evidence for the existence of phospholipid transfer protein(s) in the filamentous fungus, *Mucor mucedo*. Very recent data (J.-C. Kader, unpublished results) showed that transfer activity can be attributed to a 24 kDa protein, which exhibits specificity for phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine in decreasing order.

Characterization of the phosphatidylinositol/phosphatidylcholine transfer protein from yeast

By a combination of standard chromatographic techniques a phosphatidylinositol/phosphatidylcholine transfer protein from yeast cytosol was purified to homogeneity[17, 42]. This protein has a molecular weight of 37 kDa as determined by SDS-PAGE and an isoelectric point of 5.2. It is sensitive to protease and detergent treatment[18] and is inactivated by heat. The maximal phosphatidylcholine transfer activity in the cytosol is expressed during logarithmic growth which suggests an involvement of the transfer protein in membrane biosynthesis.

Employing pyrene-labeled phosphatidylcholine and phosphatidylinositol, fluorescence assays for transfer and complex formation were carried out[42, 43]. The transfer rate for phosphatidylinositol was found to be 19-fold higher than for phosphatidylcholine. Phosphatidylethanolamine or phosphatidylserine were not transferred at all, which is at variance with results reported by Bozzato and Tinker[7, 8]. If the acceptor membrane consisted only of a non-transferable phospholipid, e.g. phosphatidylethanolamine, a moderate but significant net mass transfer of phosphatidylcholine was observed[42]. The yeast transfer protein forms complexes with its substrates (very probably in a 1:1 stoichiometry), displaying a marked preference for phosphatidylinositol, which correlates very well with the higher rate of transfer of this phospholipid.

Membrane lipid composition has marked effects on the activity of the yeast phosphatidylinositol/phosphatidylcholine transfer protein[42, 43]. Negatively charged phospholipids in acceptor membrane vesicles decrease the rate of phosphatidylcholine transfer. Differences in the accessibility of the charged groups of the lipids to the transfer protein might account for the difference in inhibitory effects, which occur in the order phosphatidylserine > phosphatidylglycerol > phosphatidylinositol > cardiolipin > phosphatidic acid. When mitochondrial membranes, which contain high quantities of negatively charged phospholipids, are used as acceptor membranes the inhibitory effect is less pronounced. This compensation of the negative charge of phospholipids by membrane proteins might be important for the regulation of phospholipid transfer processes in vivo. Bozzato and Tinker[8] found that the presence of 30 % negatively charged phospholipids (phosphatidylinositol and phosphatidylserine) in acceptor vesicles provided the ideal lipid composition for their transfer assays. We explain this discrepancy from our results[42] by differences in the assay systems employed.

The fluidity state of the acceptor membrane also has a pronounced effect on phosphatidylcholine transfer catalyzed by the yeast transfer protein[42, 43]. The transfer rate was markedly reduced when dipalmitoyl phosphatidylcholine was used as an acceptor vesicle component instead of egg yolk phosphatidylcholine (containing approximately 40 % palmitoyl oleoyl phosphatidylcholine). Addition of the yeast sterol, ergosterol, to acceptor membrane vesicles also led to a reduction of the phosphatidylcholine transfer rate; 50 mol% ergosterol present in vesicles consisting otherwise of egg phosphatidylcholine resulted in a 50 % decrease of the transfer rate.

Possible physiological functions of the yeast phospholipid transfer protein

It has always been a matter of dispute whether lipid transfer proteins, which are characterized by their mode of action in vitro, play the same role in the living cell.

Using model substrates for a comparison of protein-catalyzed phospholipid transfer in vivo and in vitro, Yaffe and Kennedy[55] raised some arguments against such a hypothesis.

Very recent experiments presented by Aitken et al.[1] showed that the phosphatidylinositol/phosphatidylcholine transfer protein is essential for the viability of yeast cells, since disruption of the PIT-gene led to a lethal mutation. It remains to be established precisely which cellular events are affected by a defect in protein-catalyzed transport of phosphatidylinositol and/or phosphatidylcholine. The effect might be on the phosphatidylinositol cycle, which has been demonstrated to exist also in yeast[27,49]; or on the recycling of phospholipids from the Golgi to the endoplasmic reticulum, leading to an increased sterol-to-phospholipid ratio in Golgi-derived vesicles[52]; or on the supply of phospholipids to membranes which have only a limited or no capacity to synthesize them.

Sterol transport in yeast

Very little is known about the intracellular transport of sterols in yeast or in other eukaryotic microorganisms. Even the terminal steps in the synthesis of the yeast sterol, ergosterol, have not been localized with certainty. Whereas Osumi et al.[34] reported that these steps of ergosterol synthesis in *Saccharomyces cerevisiae* occur in the endoplasmic reticulum, McCammon et al.[33] reported that sterol methylation was localized in mitochondria, microsomes and a floating lipid layer. Intracellular transport of sterols in yeast is probably governed by vesicle flux in a similar way to that in higher eukaryotes (for recent reviews see refs 3 and 50). Henschke et al.[23] and Youings et al.[55] described populations of vesicles which could be involved in the supply of the plasma membrane with ergosterol. Lipid analysis of various membranous fractions of yeast carried out in our laboratory (Zinser et al., unpublished results) provided evidence that the plasma membrane contains high quantities of ergosterol; secretory vesicles[24,51] are also enriched in ergosterol, which might be an indication of the linkage between protein secretion and sterol transport. Sterol uptake mutants decribed very recently by Lewis et al.[31] will probably provide more insight into the process of intracellular sterol transport in yeast.

Lipid transport in prokaryotes

In contrast to eukaryotic cells prokaryotes (bacteria) are characterized by their simple cellular compartmentalization. This fact, and the well-known advantages of microorganisms (ease of growth, possibility of nutritional and genetic manipulations) are good reasons for using prokaryotes as a model system. The relatively simple pattern of phospholipids in eubacterial membranes, which contain mainly phosphatidylethanolamine, phosphatidylglycerol and cardiolipin, makes studies of phospholipid transfer easier than in the complex eukaryotic system.

Site of phospholipid biosynthesis in prokaryotes

The major site of phospholipid synthesis in prokaryotes is the cytoplasmic membrane. CDP-diacylglycerol synthase, phosphatidylglycerol phosphate synthase and also phosphatidylserine decarboxylase were found to cofractionate with the cytoplasmic membrane of *Rhodopseudomonas sphaeroides*[10] during heterotrophic growth. Substantial quantities of phosphatidylserine decarboxylase were also found in the intracytoplasmic membrane of *Rhodopseudomonas sphaeroides*, although artificial distribution to this membrane by self-aggregation of the enzyme[21] could not be excluded. Phosphatidylserine synthase and phosphatidylethanolamine methyltransferase seem to be very loosely bound to membranes, since they were detected in the soluble fraction after cell disruption. Phosphatidylserine synthase of *Escherichia coli* was found to be associated with ribosomes[37] although in this case, too, artificial redistribution of the enzyme during cell disruption could not be ruled out. The bacterial outer membrane has never been shown to be a site of phospholipid biosynthesis, and the capacity of the intracytoplasmic membrane to synthesize lipids seems to be rather limited. As a result of this distribution these two membranes rely to a large extent on being supplied with phospholipids from the cytoplasmic membrane.

Phospholipid transfer proteins in microorganisms

Organism	Subcellular localization	Molecular weight (kDa)	Isoelectric point	Specificity	Reference
Yeast	Cytosol	37	5.2	PI > PC	17, 42
	Cytosol	33.4	6.3; 6.1*	PC > PE > PI > PS	7
	Cytosol	35–40	–	PS > PE > PA > CL	[a]
M. mucedo	Cytosol	24	5.05	PI > PC > PE	11, [b]
B. subtilis					
PT I	Soluble	18	–	PE > PG > CL	30
PT II	Soluble	15	–	PG > CL > PE	30
R. sphaeroides	Cytoplasm	27	5.2	PG > PE > PC	44
	Periplasma	56	–	PE > PG > PC	45

* The pI 6.1 protein is probably a degradation product of the pI 6.3 protein. [a] Lafer et al., unpublished results; [b] J.-C. Kader, unpublished results.

Phospholipid transport in bacteria in vivo

Two mechanisms of phospholipid transfer between the inner (cytoplasmic) and the outer membrane are discussed in the literature: diffusional flow across membrane contact sites and protein-catalyzed transfer.

Jones and Osborn[25, 26] studied the transport of extracellular phospholipids to the inner membrane of *Salmonella typhimurium*. Very recently Tomlinson et al.[48] presented a similar system for the import of phospholipids into membranes of *Salmonella minnesota* and *Escherichia coli*. Phospholipids were inserted into the outer membrane by direct fusion, which was highly dependent on the incubation conditions. Subsequently phospholipids were translocated to the inner membrane, irrespective of the type of phospholipid[26, 48]. This intermembrane transport was found to be bidirectional, which led to an equilibrium of phospholipids between the two membranes. The authors[26] argued that lipid migration across membrane contact sites could be the mechanism of translocation. This notion was supported by Langley et al.[29] who observed that migration of phosphatidylserine from the cytoplasmic to the outer membrane of *Escherichia coli* occurred with a half-time of 12–13 min. Lloyd[32] studied the transport of phospholipids from the inner to the outer membrane of *Micrococcus cryophilus* and concluded that a rather non-specific process, probably migration through zones of adhesion between the two membranes, governed this translocation. Bogdanov et al.[4–6] described in a series of papers the linkage between phospholipid transport to the outer membrane and protein secretion in *Escherichia coli*. The authors suggested an interaction between secreted enzymes and phospholipids and an involvement of membrane translocation sites in this process. A linkage between protein export and lipid metabolism in *Escherichia coli* was demonstrated by De Vrije et al.[20] and De Cock et al.[19].

Evidence for protein-catalyzed transfer between bacterial membranes comes mainly from experiments with *Rhodopseudomonas sphaeroides*[9, 14, 44, 45, 47]. Two types of transfer proteins were isolated from this bacterium, one being present in the periplasma, the other in the cytoplasm (for details see the following section). The periplasmic protein is thought to govern the phospholipid translocation between the inner and the outer membrane, whereas the cytoplasmic transfer protein could play a role in the supply of the intracytoplasmic membrane of the facultative photosynthetic bacterium *Rhodopseudomonas sphaeroides*.

Transmembrane movement of newly synthesized phosphatidylethanolamine across the plasma membrane of *Bacillus megaterium* was studied by Rothman and Kennedy[38]. Translocation from the cytoplasmic side to the external leaflet of the membrane occurs rapidly, which suggests the involvement of protein catalysis.

Phospholipid transfer proteins of prokaryotes

Rhodopseudomonas sphaeroides, a gram-negative facultative photosynthetic bacterium, has been studied extensively with respect to the occurrence of phospholipid transfer proteins by the group of Kaplan[44, 45, 47]. Two phospholipid transfer proteins were isolated and characterized from *Rhodopseudomonas sphaeroides;* one transfer protein is present in the cytoplasm, the other in the periplasmic space. The cytoplasmic transfer potein has a molecular weight of 27 kDa, an isoelectric point of 5.2 and a substrate specificity for phosphatidylglycerol, phosphatidylcholine and phosphatidylethanolamine in decreasing order. Environmental factors influence its phospholipid transfer activity: Tai and Kaplan[45] observed that a high level of illumination increased the cellular level of transfer activity as compared to low-light conditions or chemoheterotrophic growth. Light conditions also influence the transient membrane binding state of the transfer protein. Under low-light conditions, when the content of intracytoplasmic membrane in *Rhodopseudomonas sphaeroides* is highest, membrane association of the transfer protein is maximal, suggesting its involvement in the biogenesis of this membrane.

During the course of preparation of the cytoplasmic transfer protein of *Rhodopseudomonas sphaeroides*, a second transfer protein with a different isoelectric point was detected[44], but not further characterized. The phospholipid transfer protein from the periplasma is distinct from the cytoplasmic transfer protein(s) in several respects. It has a molecular weight of 56 kDa, and it transfers phospholipid classes corresponding to the bulk composition of the donor membrane.

The soluble fraction from *Bacillus subtilis* contains proteins which accelerate the transfer of phospholipids between isolated mesosomes and protoplasts[30]. Two proteins with different molecular weights were observed: one of 18 kDa, which showed a substrate preference for phosphatidylethanolamine, and one 15 kDa protein, which was more specific for phosphatidylglycerol and cardiolipin.

Conclusions and future perspectives

Unraveling the processes and mechanisms of intracellular lipid migration is an important step towards the understanding of membrane biogenesis. Most of the evidence concerning lipid transport comes from experiments in vitro. The value of microorganisms for studying lipid traffic lies in the ease of manipulation of physiological conditions in vivo. All mechanisms of lipid transfer detected so far in higher cells, namely vesicle flux, membrane fusion and spontaneous or protein-catalyzed transfer, can also be studied in microorganisms. Prokaryotic as well as eukaryotic systems provide the additional advantage of accessibility to genetic manipulations; yeast seems to be especially suited for this type of

investigation. In vitro mutagenesis will provide the possibility of constructing mutants whose phenotype can be studied by biochemical means, which will help to elucidate the physiology of intracellular lipid transport.

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Properties and modes of action of specific and non-specific phospholipid transfer proteins

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Summary. We have described the mode of action of the phosphatidylcholine transfer protein (PC-TP), the phosphatidylinositol transfer protein (PI-TP) and the non-specific lipid transfer protein (nsL-TP) isolated from bovine and rat tissues. PC-TP and PI-TP specifically bind one phospholipid molecule to be carried between membranes. PC-TP, and most likely PI-TP as well, have independent binding sites for the *sn*-1- and *sn*-2-fatty acyl chains. These sites have different properties, which may explain the ability of PC-TP and PI-TP to discriminate between positional phospholipid isomers. nsL-TP, which is identical to sterol carrier protein 2, transfers all common phospholipids, cholesterol and oxysterol derivatives between membranes. This protein is very efficient in mediating a net mass transfer of lipids to lipid-deficient membranes. Models for its mode of action, which is clearly different from that of PC-TP and PI-TP, are presented.
Key words. Phosphatidylcholine; phosphatidylinositol; cholesterol; phospholipid transfer protein; sterol carrier protein 2; lipid monolayer; lipid binding site.

Introduction

The first evidence for the occurrence of phospholipid transfer proteins followed from the observation that in vitro the membrane-free cytosol from rat liver stimulated the redistribution of radiolabeled phospholipids between mitochondria and microsomes[30, 56]. Subsequently, phospholipid transfer activity was detected in all mammalian tissues tested[55], in plants[24], and in yeast and other microorganisms[46]. From these sources a number of phospholipid transfer proteins have been purified and, in some instances, extensively characterized. To date, we know that mammalian tissues contain at least three different transfer proteins. One protein, purified from bovine and rat liver[25, 28], is highly specific for phosphatidylcholine (PC) and, therefore, designated PC transfer protein (PC-TP). The second protein, originally isolated from bovine brain[21] and heart[15], has a distinct preference for phosphatidylinositol (PI) and is designated PI transfer protein (PI-TP). However, PI-TP has a dual specificity in that it is also able to transfer PC. The third protein, first purified from rat and bovine liver[5, 8], transfers a great variety of phospholipids including cholesterol, hence it is called non-specific lipid transfer protein (nsL-TP). It is to be noted that nsL-TP is identical to sterol carrier protein 2, a protein known to have a non-enzymatic stimulatory effect on various aspects of cholesterol metabolism[42, 47]. In addition, a nsl-TP type