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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

of protein has been isolated from a fast-growing rat hepatoma. This protein, in contrast to the liver nsL-TPs, gives a pronounced stimulation of sphingomyelin transfer[16]. So far, all phospholipid transfer proteins purified from plants belong to the nsL-TP type[23]. Similarly, despite a preference for phosphatidylglycerol, the phospholipid transfer protein isolated from *Rhodopseudomonas sphaeroides* is also non-specific[45]. Very interestingly, the protein purified to homogeneity from yeast has a dual specificity closely resembling that of mammalian PI-TP[44]. Although it is likely that transfer proteins with other phospholipid specificities exist, up to now none have been purified to homogeneity.

The outstanding characteristic of phospholipid transfer proteins is to extract phospholipid molecules from one membrane so that those molecules can then be inserted into another membrane. This property raises a whole series of fundamental questions. How are lipids accommodated in the transfer proteins? Do they have specific lipid binding sites? How do these proteins interact with membranes? What interfacial recognition sites determine the lipid specificity? What makes a lipid molecule move from a membrane which is, after all, a minimum-energy structure, to a transfer protein? How should we understand the energetics that govern this transfer process? Another interesting point is that these proteins restrict their action the phospholipids of the outer membrane leaflets. Apparently, their interaction with membranes does not perturb the phospholipid bilayer organization. This property has made these proteins very useful tools in membrane research[6, 59]. As for their physiological function, it is still an open question whether these transfer proteins actually move phospholipids (cholesterol) around within the cell or whether they are involved in other, as yet unknown and unrecognized, intracellular processes[60]. The aim of this review is to deal with some of these questions by focusing on what we know about rat and bovine PC-TP, PI-TP and nsL-TP. Additional information can be found in several recent reviews[20, 42, 58].

Molecular facts and figures

PC-TP from bovine liver (mol. wt 24,681; isoelectric point of 5.8) consists of a single polypeptide chain of 213 amino acid residues[1]. This chain contains some very hydrophobic peptide segments of which the most prominent are Val^{98}-Val-Tyr-Trp-Gln-Val^{103}, Val^{171}-Phe-Met-Tyr-Tyr-Phe^{176} and Trp^{186}-Val-Ile-Asn-Trp-Ala-Ala^{192}. There is good evidence that the peptide Val^{171}-Phe^{176} is part of the lipid binding site that accommodates PC that is to be transported (see below). Recently the cDNA clone encoding rat brain PI-TP has been isolated and sequenced[14]. The amino acid sequence deduced from the DNA sequence is a polypeptide chain of 271 amino acid residues with a total mass of 31,911 Da. The N-terminal sequence shows an extensive homology with the N-terminal sequences (determined up to residue 21) of both bovine brain PI-TP[14] and rat lung PI-TP[18]. In view of their similar mode of action (see below) it is striking that, in contrast to what is seen with bovine liver PC-TP, rat brain PI-TP lacks distinctly hydrophobic peptide segments[14]. In fact, computer searches have failed to reveal any significant sequence homologies between PC-TP and PI-TP or between these transfer proteins and any other protein analyzed to date[2, 14].

The amino acid sequence of bovine liver nsL-TP (mol. wt 14,500; isoelectric point 9.55) consists of a single chain of 121 residues[54]. Rat liver nsL-TP is very similar (> 90 % homologous) to bovine nsL-TP, with the exception that it has an extension of 2 residues at the C-terminus to give -Gly-Lys-Ala-Lys-Leu[32, 37]. It has been pointed out that this C-terminal sequence may be a peroxisomal targeting signal[32]. Rat and bovine nsL-TP have a very poor homology with other lipid transfer proteins[37].

In addition to the very characteristic primary structure, phospholipid transfers proteins have another striking feature: the presence of a non-covalently bound phospholipid molecule. So it was found that upon purification from bovine liver, PC-TP contains one molecule of PC[11]. Similarly, in agreement with its dual specificity, PI-TP from bovine brain contains one molecule of either PI or PC[51]. Due to the one negative charge difference between PI and PC, PI-TP may have an isoelectric point of 5.5 (PI-TP I) or an isoelectric point of 5.7 (PI-TP II)[22]. This one charge difference is sufficient to separate PI-TP I from PI-TP II during purification. Accordingly it was observed that in the membrane-free cytosol from bovine brain there is twice as much PI-TP I as PI-TP II. This ratio may possibly differ for other cells, as it is determined by the relative affinity of PI-TP for PI and PC, and by the membrane PI and PC pools accessible to this protein[50]. In contrast to analyses of PC-TP and PI-TP, analysis of bovine liver nsL-TP has failed to detect any bound phospholipid[8]. However, it has been reported that rat liver nsL-TP can bind cholesterol in a 1:1 molar ratio from adrenal lipid droplets[42].

Independent binding sites for the sn-1- and sn-2-acyl chains

As mentioned above, PC-TP and PI-TP carry an endogenous phospholipid molecule. It is normally observed that incubation of these proteins with membrane interfaces results in the binding of a membrane phospholipid molecule in exchange for their own lipid. This kind of exchange reaction has been shown to occur with phospholipid monolayers spread at the air-water interface and carrying radiolabeled PI or PC[11, 12], as well as with vesicles containing radiolabeled[26], spin-labeled[13] or fluorescent-labeled PC and PI analogues[3, 43, 51, 52]. In all these instances the labeled phospholipids were incorporated in the transfer proteins.

In view of their distinct specificity we presume that PC-TP and PI-TP have recognition sites for the phosphoryl-

choline and phosphorylinositol head groups. In addition, these proteins must have sites that accommodate the acyl chains. The acyl binding site(s) of PC-TP has been probed with PC analogues that carry a photolabile moiety on the methyl-terminal end of 2-acyl chains of different length. Covalent coupling by photoactivation followed by peptide analysis of the PC/PC-TP adduct, has indicated that the 2-acyl chain may be aligned along the hydrophobic peptide Val^{171}-Phe^{176} [53]. This acyl binding site has been further investigated with PC containing fluorescent parinaric acid at either the *sn*-1 position (1-PnA-PC) or *sn*-2 position (2-PnA-PC). Time-resolved fluorescence measurements on the 1-PnA-PC/PC-TP and 2-PnA-PC/PC-TP complexes have yielded rotational correlation times of 26 ns for the 1-parinaroyl chain and of 11 ns for the 2-parinaroyl chain [3]. This difference in correlation times must mean that PC-TP has two hydrophobic binding sites: one for the 1-acyl chain and the other for the 2-acyl chain. Furthermore, the length of the correlation times indicates that both acyl chains are completely immobilized in their respective binding sites. From theoretical considerations it has been argued that PC-TP is an ellipsoid (axial ratio of 2.50) with the chromophore of the 1-acyl chain parallel to the long symmetry axis, making an angle of 60–90° with the chromophore of the 2-acyl chain (fig. 1).

In order to explore the properties of these two binding sites in more detail, positional isomers of fluorescent pyrene-labeled PC species were used [43]. These species carried pyrenylacyl and saturated acyl chains of different lengths in either the *sn*-1 or *sn*-2 position. From measuring binding and transfer it became clear that PC-TP discriminated between positional isomers with a distinct preference for those species that carried the pyrenylacyl chain in the *sn*-2 position. Binding affinity and rates of transfer were greatly reduced when the pyrenylacyl chain was present in the *sn*-1 position. Moreover, the binding data indicated that the palmitoyl chain was optimal for the 1-acyl binding site and the pyrenyl-decanoyl and -dodecanoyl chains (length equivalent to an acyl chain of 16 and 18 C-atoms) were optimal for the 2-acyl binding site. In general, it appears that the 1-acyl binding site prefers saturated acyl chains while the 2-acyl binding site is more flexible in being able to accommodate the more bulky pyrenylacyl chains.

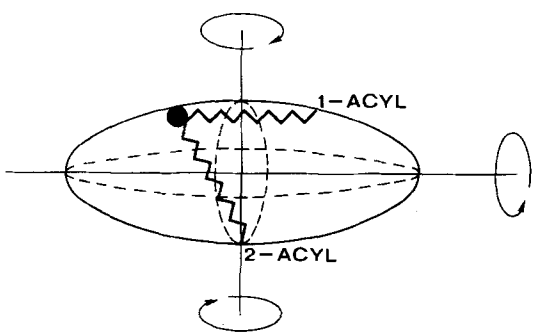

Figure 1. Tentative orientation of the *sn*-1- and *sn*-2-acyl chains of phosphatidylcholine bound to PC-TP. For details, see Berkhout et al. [3].

As estimated from competition binding experiments, bovine brain PI-TP has an approximately 16-fold higher affinity for PI than for PC [50]. This preference for PI is most probably dictated by a high-affinity binding site for the phosphorylinositol head group. At the same time, the observed dual specificity also raises questions about where the fatty acyl chains of PI and PC are accommodated in PI-TP. So far, this question has only been partly answered for the *sn*-2-acyl chain. Time-resolved fluorescence measurements using the 2-parinaroyl derivatives of PI and PC have provided evidence that the 2-acyl chains of both phospholipids are immobilized in the protein, probably occupying the same hydrophobic binding site [51]. This acyl binding site was further explored with PI and PC species carrying pyrenylacyl chains of different lengths in the *sn*-2 position [52]. Despite the much higher affinity for pyrene-labeled PI, the binding affinity as a function of chain length was quite similar for both sets of PI and PC species. This supports the above conclusion that the *sn*-2 acyl chains of PI and PC share a common binding site in PI-TP. Similarly to PC-TP, PI-TP was found to discriminate between positional isomers of pyrene-labeled PC species, with the major difference that the latter protein prefers species with the pyrenylacyl chain in the *sn*-1 position [52]. This strongly suggests that PI-TP has separate binding sites for the *sn*-1- and *sn*-2-acyl chains.

At present the chemical characteristics of the 1- and 2-acyl binding sites in PC-TP and PI-TP are very poorly understood. In view of the strong immobilization of the acyl chains it is most likely that these binding sites are very hydrophobic. By becoming exposed to the membrane interface, these sites may locally perturb the lipid bilayer in such a way that the 1- and 2-acyl chains can interact with their respective binding sites. In this respect, it is of great interest that the 1- and 2-acyl chains of the PC molecule bound to PC-TP may make an angle of 60–90°. This strongly suggests that these acyl chains come apart, when PC-TP 'lifts' PC from the membrane. In fact, it may be inherent in the mode of action of PC-TP that PC goes through the motion of its acyl chains being aligned in the bilayer, these chains coming apart when bound to the protein. This model would predict that a PC analogue which has its 1- and 2-acyl chains linked together towards the methyl terminal end will not be transported by PC-TP. Recently it was shown that a dimeric form of PC, with a disulfide bond linking the distal ends of the 1-acyl chains, was indeed not a substrate for PC-TP [41].

In what way do these transfer proteins act?

As a general rule, lipids with a high critical micelle concentration (CMC $> 10^{-7}$ M) may redistribute spontaneously between membrane interfaces through the aqueous phase by way of monomer equilibrium. This

spontaneous transfer tends to be very slow (half-time of the order of hours up to days) for natural phospholipids which have a very low CMC [29]. Apparently, owing to the relatively high hydrophobicity-hydrophilicity balance, the off-rate in the membrane-monomer equilibrium is negligible [39]. It is typical for the action of phospholipid transfer proteins that the off-rate of the phospholipid monomer is increased, giving rise to a greatly enhanced transfer of phospholipids between membranes. In support of this notion, kinetic analyses of PC-TP-mediated transfer of PC have indicated that the rate at which PC dissociates from the membrane is the rate-limiting step [7]. However, desorption of phospholipids by PC-TP and PI-TP is different from the spontaneous desorption of lipids in the monomer-membrane equilibrium, because the former involves straight binding to these proteins in exchange for an endogenous phospholipid molecule. This exchange reaction is the fundamental step in the process by which PC-TP and PI-TP act as freely diffusable carriers of phospholipids between membranes through an aqueous phase. This mode of action implies that PC-TP and PI-TP can effectively compete with membrane bilayers for a phospholipid molecule. Apparently, the energetics governing the phospholipid-transfer protein interactions are similar to the forces that keep phospholipids in the bilayer (hydrophobic effect, van der Waals energy).

As mentioned above, nsL-TP is non-specific in that it mediates the transfer of a wide range of phospholipids, cholesterol and glycolipids between membranes. By measuring the transfer of fluorescent phospholipids with different polar head groups in a continuous spectrofluorometric assay, it was observed that the rate of nsL-TP-mediated transfer was highly correlated with the rate of the spontaneous intermembrane transfer of these phospholipids [35]. This was confirmed for a series of pyrene-labeled PC species carrying acyl chains of different lengths at either the *sn*-1 or the *sn*-2 position [49]. A high correlation between nsL-TP-mediated and spontaneous transfer was also observed for a series of oxysterol derivatives using the monolayer-vesicle assay [49]. The questions that now arise are: What mode of action is compatible with this observed correlation? Does nsL-TP act as a carrier of lipids just like PC-TP and PI-TP, or should another mechanism be considered? In this regard it is of importance that in contrast to PC-TP and PI-TP, nsL-TP is not able to bind a lipid molecule from a monomolecular lipid film spread at the air-water interface. It was shown that injection of nsL-TP under a phospholipid monolayer containing highly radiolabeled cholesterol (10 mol %) or 25-hydroxycholesterol (5 mol %) did not lead to a decrease of surface radioactivity. This indicates that under these conditions there was no significant binding of these sterols to nsL-TP [49]. However, nsL-TP stimulated the transfer of these same sterols when vesicles were present in the subphase (fig. 2).

These observations, therefore, suggest that nsL-TP does not act as a freely diffusable carrier of lipids; rather, they support the model that by interacting with the membrane interface, nsL-TP lowers the energy barrier to lipid monomer-interface dissociation, and thus enhances intermembrane lipid transfer (model II, see fig. 3) [35]. On the other hand, titration of vesicles carrying fluorescent-labeled PC with nsL-TP has provided some evidence that this protein may have a low affinity binding site for lipids [33]. Taking this property into account, kinetic analysis has supported a model for intermembrane lipid transfer in which lipid monomer diffusion by dissociation of the free PC-nsL-TP complex, and lipid monomer insertion by collision of the complex with a membrane, occur simultaneously (model I, fig. 3) [34]. Model I may hold for membranes which are in close approximation during the nsL-TP-mediated transfer reaction as, for ex-

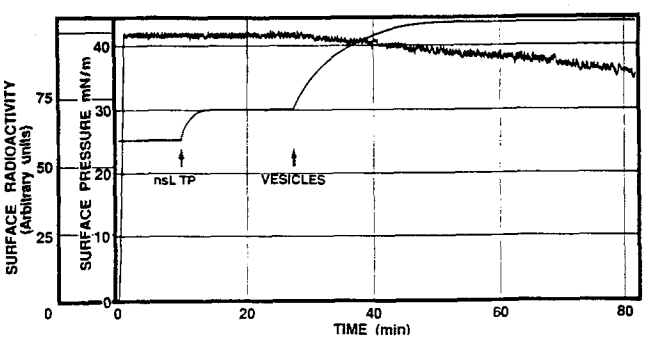

Figure 2. The nsL-TP mediated transfer of [^{14}C]cholesterol from monolayer to vesicles. The monolayer at the air-water interface consisted of [^{14}C]cholesterol-phosphatidylcholine-phosphatidic acid (45:45:10 mol %, 10 nmol lipid) and the vesicles in the subphase of phosphatidylcholine-phosphatidic acid (98:2 mol %, 1 μmol lipid). Transfer was initiated by the injection of bovine nsL-TP (10 nmol) in the subphase. For further details, see Van Amerongen et al. [49].

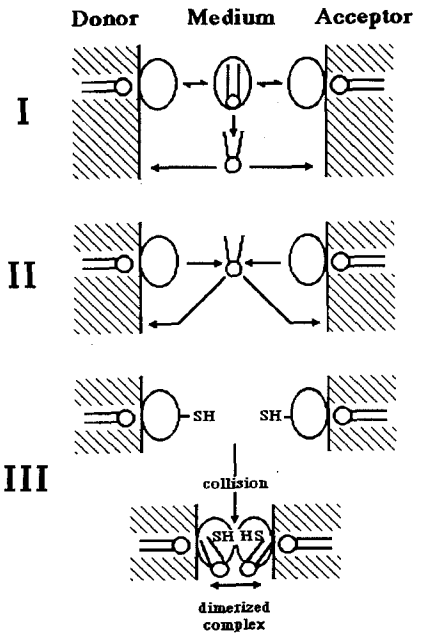

Figure 3. Possible models for the mode of action of nsL-TP.

ample, is the case in donor/acceptor vesicle assay. However, in contrast to PC-TP and PI-TP, nsL-TP was not able to mediate the transfer of lipids between two physically separted monolayers connected by a common subphase[49]. In general, one cannot distinguish between a) nsL-TP directly enhancing the rate at which the lipid monomer comes off the interface into the aqueous phase (model II), and b) nsL-TP first binding a lipid molecule from the interface, which is followed by release of the complex into the aqueous phase and then dissociation (model I).

In the monolayer-vesicle assay, PC-TP and PI-TP shuttle phospholipids between the monomolecular phospholipid film at the air-water interface and phospholipid vesicles in the subphase without affecting the surface pressure of the monolayer, initially set at approximately 25 mN/m[11, 12]. This implies that, under these conditions of transfer, PC-TP and PI-TP catalyze a one-for-one molecular exchange reaction at the monolayer. When nsL-TP is tested in a similar assay, quite a different response is observed (fig. 2). First, injection of nsL-TP under the monolayer increases the surface pressure up to 30 mN/m, showing that nsL-TP is very surface active and able to penetrate a tightly packed lipid film. Secondly, the subsequent injection of vesicles under the monolayer increases the pressure further to 43 mN/m, which is in the range of the collapse pressure of the monolayer. Since injection of vesicles in the absence of nsL-TP has no effect on the surface pressure, the above observation indicates that nsL-TP facilitates a net flow of lipids to the monolayer. Concomitantly with the pressure increase, one observes a decrease of surface radioactivity showing that nsL-TP also mediates the transfer of [^{14}C]cholesterol from monolayer to vesicles (fig. 2).

This simultaneous occurrence of net transfer and exchange reactions is compatible with both models I and II, in which lipid monomers redistribute between two interfaces with an overall net flow of lipids to the interface with the lower surface pressure (in this instance the monolayer). However, in view of the tendency of nsL-TP to interact strongly with interfaces, we have proposed yet another mode of action (model III) by which nsL-TP effectuates transfer of lipids by being instrumental in juxtaposing two membrane interfaces[49]. Since nsL-TP easily forms dimers[48], it may even be possible that such dimers are essential parts of the collisional complex between membranes, enabling lipids to redistribute subsequently at these collision sites. Again, the rate at which this redistribution occurs may be highly correlated with the rate of spontaneous transfer. It is of great interest to note that modification of the single cysteine residue (Cys^{71}) in nsL-TP by mersalyl and N-ethylmaleimide inhibited both the binding and the transfer activity[31, 33, 38]. At this point it is not clear whether blockage of this essential sulfhydryl group alters the conformation of nsL-TP or interferes with dimer formation. Replacement of Cys^{71} by site-directed mutagenesis may provide an answer to how the transfer activity depends on this residue. Based on our current understanding, it is still an open question which of the models, I, II or III, correctly describes the mode of action of nsL-TP.

Net mass transfer versus exchange

As observed in the monolayer-vesicle assay, nsL-TP may catalyze a net mass transfer of lipids from the vesicles to the monolayer (fig. 2). Similarly, nsL-TP was shown to mediate a net mass transfer of phospholipids from PC/PI multilamellar vesicles to intact or delipidated human high density lipoprotein, as well as from PC unilamellar vesicles to rat liver mitoplasts[10]. Further, nsL-TP induced drastic changes in the lipid content and cholesterol/phospholipid ratio of synaptic plasma membranes depending on whether these membranes were incubated with PC vesicles or PC/cholesterol vesicles[36]. Similar observations were made when nsL-TP was incubated with human erythrocytes[17]. Apparently, under conditions where donor and acceptor membrane differ in lipid composition and content, the activity of nsL-TP is directed towards eliminating this "chemical gradient" by mediating both a net mass transfer and a random exchange of lipids. The ability to transfer cholesterol to membranes of a low cholesterol content may explain why in vitro nsL-TP could stimulate the conversion of cholesterol into cholesterol esters, bile acids and steroid hormones (see Scallen et al.[42]). On the other hand, nsL-TP was found to catalyze a strict exchange of phospholipids between intact erythrocytes and acceptor vesicles which had a lipid composition equivalent to that of the erythrocyte outer leaflet[9]. It appears that under carefully selected conditions of incubation, nsL-TP can be used as a non-perturbing membrane probe, thereby providing information on membrane lipid asymmetry and transbilayer movement.

In transferring PC between donor and acceptor membranes, bovine liver PC-TP does not affect the PC content of either membrane, which indicates that it catalyzes a genuine exchange process[11, 19]. By incubating PC-TP with acceptor membranes devoid of PC, it could be shown that this protein catalyzed a net mass transfer of PC to these membranes[4, 35, 40, 57]. This implies that on the insertion of PC into a membrane. PC-TP may leave that membrane without a lipid molecule being bound. It should, however, be noted that the initial rate of PC transfer to acceptor membranes devoid of PC is considerably lower than the rate of PC transfer to membranes containing PC. It has been argued that PC in acceptor membranes enhances the catalytic activity of PC-TP[40].

In transferring phospholipids between membranes, bovine brain PI-TP displays a dual specificity with a preference for PI over PC[12, 32]. All experiments carried out so far indicate that the PI-TP-mediated transfer of PI and PC is based on a one-for-one molecular exchange reaction at the membrane[12, 27]. There is, as yet, no evidence

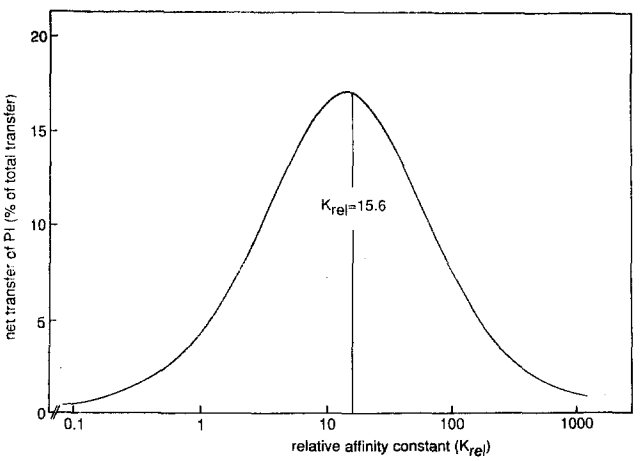

Figure 4. Dependence of the net transfer of phosphatidylinositol (PI) to a membrane deficient in PI, on the affinity constant of PI-TP for PI relative to PC (K_{rel}). The net transfer of PI is equal to the transfer of PI from donor to acceptor membrane *minus* the transfer of PI from acceptor to donor membrane. The net transfer is expressed as the percentage of PI transferred to the acceptor membrane in total. K_{rel}, equal to 15.6, is experimentally determined. For details, see Van Paridon et al.[50].

that PI-TP can mediate a net mass transfer of phospholipids. On the other hand, PI-TP can transfer PI to membranes initially deficient in this phospholipid in exchange for PC being transferred in the opposite direction. In principle, net transfer of PI will continue until the PI/PC ratios of the receiving and donating membranes have become equal. For a given situation, where the PI/PC ratio of the donor membrane is 0.1 (e.g., endoplasmic reticulum of liver) and that of the acceptor membrane is 0.05 (e.g., plasma membrane after stimulus-induced PI breakdown) we have calculated the value of K_{rel} (i.e., the affinity of PI-TP for PI relative to PC) at which net PI transfer to the acceptor membrane is optimal. As shown in figure 4, the efficacy of this net transfer process varies greatly with K_{rel}, with an estimated optimum very close to the experimentally determined K_{rel} value of 15.6[50]. In addition, it has been established that at the membrane interface, PI-TP exchanges its bound PC for PI 15–30 times faster than its bound PI for PC. These properties support the idea that PI-TP may be ideally suited for maintaining PI levels in intracellular membranes[50]. However, it still remains to be established whether in the cell PI-TP is actually involved in such a fine-tuning mechanism.

Concluding remarks

The phospholipid transfer proteins described here have some unique properties in terms of lipid-protein and membrane-protein interactions. At the molecular level, their mode of action is still poorly understood. One reason for this lack of understanding is that, so far, none of these transfer proteins has been successfully crystallized. Another reason is that these proteins have a limited solubility; if no way can be found to overcome this problem, it will not be possible to use powerful techniques like nuclear magnetic resonance spectroscopy for their investigation. It is, however, established that these proteins move phospholipids between membranes – in some instances very specifically. Under conditions of one-for-one molecular exchange they do not seem to perturb the bilayer organization. Since their avidity is only directed towards phospholipids present in the medium-exposed half of the lipid bilayer, these proteins have proved to be powerful tools in probing the lipid organization of membranes.

Despite an extensive body of literature, it still remains to be unequivocally established what physiological function these phospholipid transfer proteins fulfill in the cell. Pending further investigation, it is most reasonable to assume that they are involved in intracellular lipid transport phenomena. It may be necessary to select mutant cells deficient in, or with restricted levels of, these lipid transfer proteins to reveal their actual role in intracellular processes.

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Intracellular sterol trafficking

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Summary. Sterols are acquired by cells either biosynthetically by the interaction of cytoplasmic and endoplasmic reticulum elements, or by endocytosis. The subcellular distribution of sterols, however, argues that sterols are trafficked quickly from sites of acquisition to target membranes, particularly the plasma membrane. The mechanisms mediating this movement might include aqueous diffusion, vesicles of either a unique pathway or of the protein secretory pathway, or carrier proteins. These mechanisms are discussed and the limited data concerning each are presented. Finally, a theory is proposed which describes how sterols and other membrane reinforcing molecules might have driven the evolution of intracellular membranes, thus establishing the dynamic membrane system of modern eukaryotes.

Key words. Sterol synthesis; cholesterol; plasma membrane; endoplasmic reticulum; sterol carrier proteins; bacteriohopanes.

Introduction

The connection between cholesterol and the appearance and progression of atherosclerosis has fostered a voluminous amount of sterol-related research. This research has led to our detailed understanding of the extracellular trafficking of sterols by lipoproteins and of lipoprotein uptake by receptor-mediated endocytosis[16]. In addition, the major regulatory enzyme of sterol biosynthesis in higher animals has been identified as 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) and this enzyme has been examined in detail. Still, our understanding of the cell biology of sterols remains in its infancy. For example, something as fundamental as the subcellular distribution of sterols remains in dispute[40, 119] as does the exact site of sterol synthesis[55, 84]. Further, little research has been directed toward the elucidation of the mechanisms which mediate the intracellular movement of sterols, although such movement would be expected to be important in fueling retrotransport of cholesterol to the liver for excretion which in turn could have favorable impact on atherosclerotic regression[81].

In this paper, it will first be documented that sterol trafficking does occur by demonstrating that sterol synthesis proceeds in a membrane which is not the major repository of cellular cholesterol. Several mechanisms by which the sterol might be trafficked between the site of synthesis and target membranes will then be described and the limited data available for each will be discussed. Finally, attention will turn from the question of 'how' sterol is trafficked to an equally interesting topic: why? This discussion will culminate with the presentation of a theory which suggests that the evolutionary development of sterols necessitated the co-appearance of sterol-poor intracellular membranes in order to preserve a site compatible with protein translocation.

Subcellular site of sterol biosynthesis

Chesterton[21] originally demonstrated that when radiolabeled mevalonic acid (a water-soluble intermediate in sterol biosynthesis) is injected into rats, radiolabeled sterol can be detected in liver tissue within 2 min. Analysis of the distribution of the label at early times after mevalonate administration revealed that radiolabeled squalene, lanosterol and cholesterol are all found in both the granular and agranular microsomal elements (rough and smooth endoplasmic reticulum (RER and SER)). These membranes were concluded to be the site of sterol biosynthesis.

Lange and Steck[57] however, questioned this conclusion, since the microsomal fraction of liver tissue contains organelle membranes other than RER and SER[108]. These include plasma, Golgi, and perhaps other membranes. In a series of experiments involving double label incorporation in order to distinguish old versus newly synthesized cholesterol, they demonstrated that newly synthesized sterols are not found in the ER fraction. In an alternative approach, we[84] and others[42, 66] analyzed the location of the microsomal enzymes involved in sterolgenesis rather