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TRANSFER PROPERTIES OF THE BOVINE BRAIN PHOSPHOLIPID TRANSFER PROTEIN

EFFECT OF CHARGED PHOSPHOLIPIDS AND OF PHOSPHATIDYLCHOLINE FATTY ACID COMPOSITION

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The monolayer technique has been used to study the transfer of [^{14}C]phosphatidylinositol from the monolayer to phosphatidylcholine vesicles. An equivalent transfer rate was found for egg phosphatidylcholine, dioleoylphosphatidylcholine, dielaidoylphosphatidylcholine and dipalmitoylphosphatidylcholine. A reduced transfer rate was found for a shorter-chain derivative, dimyristoylphosphatidylcholine, and for species with two polyunsaturated fatty acid chains such as dilinoleoylphosphatidylcholine, diheptadecadienoylphosphatidylcholine, dilinolenoylphosphatidylcholine and diether and dialkyl derivatives. No activity was found for 1,3-dipalmitoylphosphatidylcholine. The presence of up to 5 mol% phosphatidylinositol in egg phosphatidylcholine vesicles had no effect on the transfer rate. Introduction of more than 5 mol% phosphatidylinositol or phosphatidic acid into the phosphatidylcholine vesicles gradually decreased the rate of phosphatidylinositol transfer from the monolayer. 20 mol% acidic phospholipid was nearly completely inhibitory. Transfer experiments between separate monolayers of phosphatidylcholine and phosphatidylinositol showed that the protein-bound phosphatidylcholine is readily exchanged for phosphatidylinositol, but the protein-bound phosphatidylinositol exchange for phosphatidylcholine occurs at a 20-times lower rate. The release of phosphatidylinositol is dependent on the lipid composition and the concentration of charged lipid in the acceptor membrane, but also on the ratio between donor and acceptor membranes. The main transfer protein from bovine brain which transfers phosphatidylinositol and phosphatidylcholine transfers also phosphatidylglycerol, but not phosphatidylserine or phosphatidic acid. The absence of significant changes in the surface pressure indicate that the phosphatidylinositol and phosphatidylcholine transfer is not accompanied by net mass transfer.

Introduction

The major phospholipid exchange protein from bovine brain shows the highest affinity for phosphatidylinositol and, to a lesser extent, for phosphatidylcholine. Net transfer of phosphatidylinositol from the monolayer to phosphatidylcholine vesicles has been demonstrated [1]. In a previous paper [2] it has been shown that the phosphatidylinositol loss from the interface is compensated by a simultaneous transfer of phosphatidylcholine to the interface. Under these conditions there is a 100-fold excess of phosphatidylcholine in the subphase as compared to phosphatidylinositol in the interface. It has to be shown

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now that there is also an exchange of phosphatidylinositol and phosphatidylcholine if both pools are equal. This can be studied using two separate monolayers connected by a subphase containing the transfer protein. It was proposed by Helmkamp [3] that the fatty acid composition, and particularly the hydrocarbon fluidity, strongly affects the transfer activity of the bovine brain protein. A low transfer activity was found for dimyristoylphosphatidylcholine [2,3]. However, dipalmitoylphosphatidylcholine is transferred well, even below the transition temperature [2]. Systematic variation of the phosphatidylcholine fatty acid residues should give more information about the hydrophobic interaction of lipid and protein. The bovine brain protein can transfer phosphatidylinositol and phosphatidylcholine as well. With respect to the transfer of the neutral lipid it was demonstrated, using different analogs, that the protein has a rather low specificity for phosphatidylcholine – it is, however, inhibited by sphingomyelin. With respect to the transfer of the charged lipid it is not known whether it is specific for phosphatidylinositol. Transfer reactions were found to be inhibited by pure phosphatidylinositol vesicles [1]. The results of Harvey et al. [4] showed that increasing the phosphatidylinositol content in liposomes decreases the phosphatidylinositol transfer from microsomes to liposomes but increases the transfer from liposomes to microsomes. The results of Helmkamp [5] suggest that the transfer of phosphatidylinositol was not affected by the incorporation of 5–20 mol% phosphatidic acid, phosphatidylserine or phosphatidylglycerol. The higher levels of acidic phospholipids in the liposomes can lead, however, to association of liposomes with microsomes [5]. The measurement of lipid transfer from monolayer to vesicles does not require separation of donor and acceptor membrane. In the present paper the effect of phosphatidylcholine fatty acid composition and the effect of negatively charged lipids and their possible transfer are studied.

Materials and Methods

The phospholipid exchange protein was purified from fresh bovine cerebral cortex using published procedures [1,6]. The phospholipids used were purified by high-pressure liquid chromatography [7]. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phospho[*methyl*-^{14}C]choline (spec. act. 40 Ci · mol^{-1}) was obtained by the method of Stoffel et al. [8]. Phosphatidyl[^{14}C]inositol (spec. act. 1 Ci · mol^{-1}) was prepared by *myo*-[^{14}C]inositol exchange with microsomal phosphatidylinositol and incorporation of *myo*-inositol in de novo synthesized phosphatidylinositol [1,9]. Phosphatidylinositol was isolated from rat liver microsomes. Phosphatidylserine was isolated from pig brain. 1,2-Dioleoyl-*sn*-glycero-3-phosphate and 1,2-dielaidoyl-*sn*-glycero-3-phosphatidylglycerol were derived from the phosphatidylcholines by phospholipase D-catalyzed base exchange [10,11]. Diether phosphatidylcholine (1-hexadecyl-2-octadec-9-enyl-*sn*-glycero-3-phosphocholine) and dialkyl phosphatidylcholine (2-hexadecyl-2-(hexadec-9-enyl)-ethoxy-1-phosphocholine) were a gift of Dr. H. Brockerhoff (New York). *rac*-1-Octadec-9-enyl-2-hexadecyl-*sn*-glycero-3-phosphocholine was obtained from Calbiochem (Lucerne). 1,2-[^{14}C]Dipalmitoyl-*sn*-glycero-3-phosphoglycerol (spec. act. 10 Ci · mol^{-1}) was a gift of Dr. M. Post (Utrecht).

A clear vesicle suspension is formed by a 1 min sonication of handshaken liposomes [12]. The lipid concentration was 10 mM.

The measurements of surface pressure and surface radioactivity [13] and collection of the film have been described before [2,14]. The initial surface pressure was 20 mN · m^{-1}; the temperature, 37°C.

Results

The effect of fatty acid composition of phosphatidylcholine on the transfer activity of the bovine brain protein is demonstrated in Table I. The transfer of [^{14}C]phosphatidylinositol from the interface to phosphatidylcholine vesicles in the subphase is measured by the change in surface radioactivity as described before [1]. The transfer rate of phosphatidylinositol to egg phosphatidylcholine is comparable to that of *cis* or *trans* monounsaturated phosphatidylcholine (Table I, Expts. 1–3). A 25% decrease in activity is observed for phosphatidylcholines with two polyunsaturated fatty acid chains (Expts. 4–6). Changes in the fatty acid chain length show a decrease of 60% for

TABLE I

EFFECT OF FATTY ACID COMPOSITION OF PHOSPHATIDYLCHOLINE (PtdCho) VESICLES IN THE SUBPHASE ON THE RATE OF [^{14}C]PHOSPHATIDYLINOSITOL TRANSFER FROM THE MONOLAYER

The subphase contained 1 μmol phosphatidylcholine vesicles and 1.5 nmol transfer protein in 10 mM Tris-HCl buffer (pH 7.4). The initial rate of surface radioactivity decrease is expressed relative to the rate measured in the presence of egg phosphatidylcholine.

Experiment	Vesicles	Transfer rate
1	egg PtdCho	100
2	1,2-di$C_{18:1_c}$ PtdCho	81
3	1,2-di$C_{18:1_t}$ PtdCho	97
4	1,2-di$C_{18:2_c}$ PtdCho	65
5	1,2-di$C_{17:2_c}$ PtdCho	75
6	1,2-di$C_{18:3_c}$ PtdCho	74
7	1,2-di$C_{14:0}$ PtdCho	42
8	1,2-di$C_{16:0}$ PtdCho (32°C)	99
9	1,2-di$C_{18:0}$ PtdCho (37°C)	52
10	16:0/18:1_c diether PtdCho	54
11	16:0/18:1_c dialkyl PtdCho	58
12	18:1_c/16:0 diether PtdCho (*rac*)	26
13	1,3-di$C_{16:0}$ PtdCho	0

dimyristoylphosphatidylcholine (Expt. 7). The experiments are performed at 37°C. This is above the phase-transition temperature of dimyristoylphosphatidylcholine. However, fluidity of the phosphatidylcholine acyl chains is not strictly required. This is demonstrated by dipalmitoylphosphatidylcholine. Even at 32°C, which is well below the phase-transition temperature of this lipid, the transfer rate is identical to that of egg phosphatidylcholine (Expt. 8). Also, distearoylphosphatidylcholine still shows activity, although reduced by about 50% (Expt. 9). The binding and position of the phosphatidylcholine acyl chain strongly affects the transfer activity. The diether and dialkyl phosphatidylcholine derivatives show 40–50% reduction in transfer activity (Expts. 10, 11). However, the *rac*-phosphatidylcholine (1-octadec-9-enyl-2-hexadecyl ether) shows only 26% of the egg phosphatidylcholine activity (Expt. 12). For the 1,3-isomer of dipalmitoylphosphatidylcholine no transfer activity is found at all (Expt. 13).

It is known that addition of pure phosphatidylinositol vesicles inhibits any transfer by the protein from bovine brain. It is now shown that, in addition, the injection of 0.5 μmol phosphatidic acid, diphosphatidylglycerol or phosphatidylserine vesicles underneath a [^{14}C]phosphatidylinositol monolayer inhibits the transfer reaction completely. The question arises as to whether in mixed vesicles with phosphatidylcholine the inhibition is specific for phosphatidylinositol or not. Fig. 1 shows that the phosphatidylinositol transfer is not affected by the incorporation of up to 5 mol% phosphatidylinositol in the phosphatidylcholine vesicles. However, higher concentrations of phosphatidylinositol cause a gradual decrease in transfer activity and a nearly complete inhibition at 20 mol%. Essentially the same effects are found when phosphatidic acid is incorporated in the phosphatidylcholine vesicles.

The specificity of the bovine brain protein with respect to the polar part of phosphatidylcholine has been studied in a previous paper [2]. It has not yet been established whether the bovine brain protein is specific to phosphatidylinositol or whether other negatively charged lipids can be transferred as well. Therefore, monolyers are formed of negatively charged lipids, after which

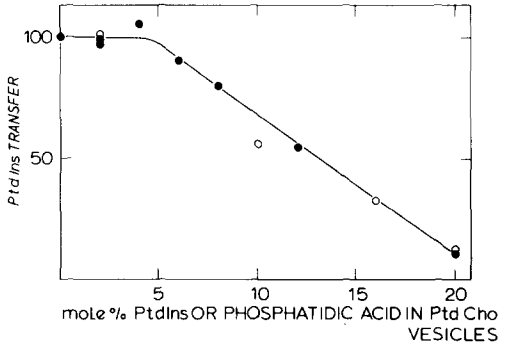

Fig. 1. Variation of the rate of [^{14}C]phosphatidylinositol transfer from the monolayer as a function of the vesicle composition in the subphase. Mixtures of phosphatidylcholine (PtdCho) and phosphatidylinositol (PtdIns) (●); mixtures of phosphatidylcholine and phosphatidic acid (○). The subphase contained 1 μmol vesicles and 1.5 nmol transfer protein in 10 mM Tris-HCl buffer (pH 7.4).

[^{14}C]phosphatidylcholine vesicles are injected underneath in the presence or absence of transfer protein. After an incubation of 1.5 h the monolayer is transferred to a clean subphase and collected as described before [2,14]. The [^{14}C]phosphatidylcholine, transferred to the interface is determined. If a monolayer of phosphatidylinositol is used, about 90% of the lipid can be transferred to the subphase [1]. At the same time, 7.4 nmol phosphatidylcholine are transferred to the monolayer (Table II, Expts. 1,2). If phosphatidylserine or phosphatidic acid monolayers are used, there is no transfer of phosphatidylcholine to the monolayer (Expts. 3–6). However, in case of phosphatidylglycerol (Expts. 7–8) essentially the same phosphatidylcholine transfer is found as with phosphatidylinositol. This means that phosphatidylinositol as well as phosphatidylglycerol can be transferred by the bovine brain protein. The transfer of phosphatidylglycerol could be confirmed using a monolayer of [^{14}C]dipalmitoylphosphatidylglycerol and egg phosphatidylcholine vesicles and measuring the decrease in surface radioactivity. The same rate was found as for phosphatidylinositol.

Under the conditions as described above there is a 30-fold excess of phosphatidylcholine in the subphase as compared to the phosphatidylinositol at the interface. The transfer rate of phosphatidylinositol is about 19.4 pmol/nmol protein per min. The phosphatidylinositol-phosphatidylcholine exchange between equal pools can be studied using two separate monolayers. 7.5 nmol dioleoylphosphatidylcholine and 7.5 nmol [^{14}C] phosphatidylinositol were spread. The subphase contained 4.5 nmol bovine brain transfer protein. After 5 h, 25.5% of the radioactivity was still present in the phosphatidylinositol monolayer, 70.7% was present in the subphase and only 3.7% was present in the phosphatidylcholine monolayer (Fig. 2).

The bovine brain protein can bind phosphatidylinositol and/or phosphatidylcholine [1]. The labeled phosphatidylinositol present in the subphase got there primarily by exchange of native protein-bound phosphatidylcholine for [^{14}C]phosphatidylinositol at the interface. The exchange of native protein-bound phosphatidylinositol for interfacial phosphatidylinositol is a much slower process. This is apparent from the following experiment. After injection of the protein underneath a [^{14}C]phosphatidylinositol monolayer, the protein is loaded with labeled phosphatidylinositol. Then the monolayer is removed and a monolayer of unlabeled phosphatidylinositol is spread. The occurrence of label at the interface can be measured after collecting the monolayer. The calculated phosphatidylinositol-phosphatidylinositol exchange was only 1 pmol/nmol protein per min. The exchange of native protein-bound phosphatidylcholine for phosphatidylinositol at the interface can be defined in a similar way. Phos-

TABLE II

THE SPECIFICITY OF ACIDIC LIPID TRANSFER AS CATALYZED BY BOVINE BRAIN TRANSFER PROTEIN

The transfer of [^{14}C]phosphatidylcholine (PtdCho) from vesicles to monolayer in the presence or absence of protein is measured. The monolayer consisted of 8.4 nmol phosphatidylinositol (PtdIns); pig brain phosphatidylserine (PtdSer); dioleoylphosphatidic acid (PA) and dielaidoylphosphatidylglycerol (PtdGro). The subphase contained 1 μmol [^{14}C]phosphatidylcholine vesicles and 1.5 nmol transfer protein in 10 mM Tris-HCl buffer (pH 7.4). The incubation time was 1.5 h.

Experiment	Monolayer	Vesicles	Transfer protein	nmol [^{14}C]PtdCho in monolayer
1	PtdIns	[^{14}C]PtdCho	+	10.3
2	PtdIns	[^{14}C]PtdCho	−	2.9
3	PtdSer	[^{14}C]PtdCho	+	3.2
4	PtdSer	[^{14}C]PtdCho	−	3.0
5	PA	[^{14}C]PtdCho	+	3.6
6	PA	[^{14}C]PtdCho	−	2.9
7	PtdGro	[^{14}C]PtdCho	+	9.1
8	PtdGro	[^{14}C]PtdCho	−	2.8

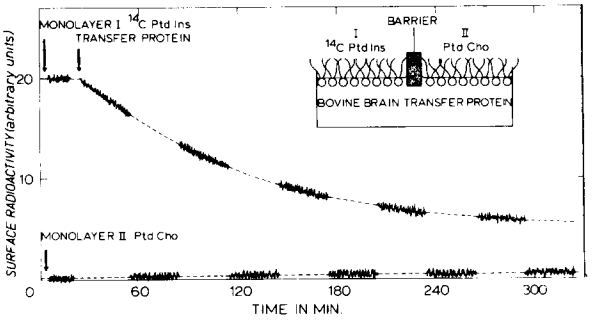

Fig. 2. Transfer of [^{14}C]phosphatidylinositol (PtdIns) between two separate monolayers. Monolayer I consisted of 7.5 nmol [^{14}C]phosphatidylinositol (PtdIns); monolayer II consisted of dioleoylphosphatidylcholine (PtdCho). The subphase contained 4.5 nmol transfer protein in 10 mM Tris-HCl buffer (pH 7.4).

phatidylcholine-labeled protein is obtained after injection of the protein underneath a [^{14}C]phosphatidylcholine monolayer. This monolayer is removed and replaced by unlabeled phosphatidylinositol. The rate of exchange of protein-bound phosphatidylcholine for phosphatidylinositol was 7.5 pmol/nmol protein per min. The phosphatidylcholine-phosphatidylcholine exchange measured by injection of protein-bound [^{14}C]phosphatidylcholine underneath a phosphatidylcholine monolayer was only 0.8 pmol/nmol protein per min. Injection of protein-bound [^{14}C]phosphatidylinositol underneath a phosphatidylcholine monolayer gave a rate of 0.4 pmol/nmol per min.

Discussion

The bovine brain protein binds and transfers phosphatidylinositol and phosphatidylcholine. However, the affinity is approximately 10-times higher for phosphatidylinositol [1]. In a previous paper [2] it was shown that the binding of phosphatidylcholine occurs with a rather slow specificity. 1,2-Dipalmitoyl-*sn*-glycero-3-phospho-(*N*,*N*,*N*-trimethylamine)-hexanolamine, where the distance between phosphorus and nitrogen is increased to six carbon atoms and 1,2-diacyl-*sn*-glycero-3-phospho-(*N*,*N*-dimethyl)-ethanolamine are still transferred. However, it was noticed that 1,2-dimyristoylphosphatidylcholine was transferred at a much lower rate than egg phosphatidylcholine. It was concluded by Helmkamp [3] that hydrocarbon fluidity and particularly *cis* unsaturated species would favour the transfer, whereas saturated species would decrease it. We showed, however, that the transfer of dipalmitoylphosphatidylcholine (even below the phase transition) is not reduced compared to egg phosphatidylcholine. Highly liquid phosphatidylcholine species with two polyunsaturated fatty acids showed a 25% reduction in the transfer rate. It is likely that the occupation of the hydrophobic cleft is favoured by a certain chain length. It is known that hydrophobic interactions are reduced by reduction of chain length and polyunsaturation of both chains [13]. Additionally, the phosphatidylcholine ester linkage seems to be involved in the transfer protein-phosphatidylcholine interaction, possibly by hydrogen bridges. Dialkyl and diether derivatives showed reduced activity. The formation of additional hydrogen bridges could explain the inhibitory effect of sphingomyelin [2,5,15]. The reduced activity of racemic phosphatidylcholine and the inactivity of the 1,3-stereoisomer indicate the importance of the orientation of the hydrophobic site with regard to the polar binding site.

Vesicles of phosphatidylinositol, phosphatidic acid, phosphatidylserine and cardiolipin inhibit the transfer reaction. That this inhibition is not due to a specific interacion with phosphatidylinositol but is due to the surface charge density is clear from Fig. 1. Concentrations of more than 5 mol% phosphatidylinositol or phosphatidic acid in the phosphatidylcholine vesicles reduce the transfer reaction, while 20% is completely inhibitory. These results are at variance with those published by Helmkamp [5]. This might be due to the low liposome recovery and association with microsomes in the liposome-microsome system he used. That the transfer reaction is not inhibited by a pure phosphatidylinositol monolayer must be due to the ratio of monolayer lipid (8 nmol) to transfer protein (1.5–6 nmol). The present study shows that the bovine brain protein is not specific to phosphatidylinositol, but that phosphatidylglycerol can be transferred at a similar rate. However, phosphatidic acid and phosphatidylserine are not transported. Although phosphatidylinositol, phosphatidylserine, phosphatidic acid and phosphatidylglycerol have one net negative charge at physiologic pH, only phosphatidylinositol and

phosphatidylglycerol have the same charge distribution. The inability to transfer phosphatidylserine could not yet be concluded from liposome-microsome exchange experiments [15] due to the inhibitory effects of surface charge. The presence of a specific phosphatidylglycerol transfer protein in the rat lung was described by Van Golde et al. [16].

Due to the difference in affinity for phosphatidylinositol and phosphatidylcholine, it is likely that the binding depends on the ratio and concentration of the lipids in the membrane. Therefore a high value is found for the exchange of protein-bound phosphatidylcholine for phosphatidylinositol at the interface, namely 7.5 pmol/nmol protein per min. The exchange of protein-bound phosphatidylinositol for phosphatidylcholine at the interface is, however, very low, namely 0.4 pmol/nmol protein per min. Also, the exchange of phosphatidylcholine for phosphatidylcholine and phosphatidylinositol for phosphatidylinositol is rather slow and about 1 pmol/nmol protein per min. Therefore low concentrations of phosphatidylinositol in the vesicles did not increase the release of radioactive phosphatidylinositol from the interface. A high phosphatidylinositol transfer from the monolayer is noticed only when there is a high excess of phosphatidylcholine vesicles. The rate was about 20 pmol/nmol protein per min. When the phosphatidylinositol transfer from a [^{14}C]phosphatidylinositol monolayer to a phosphatidylcholine monolayer is studied, there is a rather fast decrease in the radioactivity of the [^{14}C]phosphatidylinositol monolayer due to the binding of phosphatidylinositol by the protein. The release of phosphatidylinositol from the protein to the phosphatidylcholine monolayer, however, is slow. This transfer of phosphatidylinositol will increase the charge density of the phosphatidylcholine monolayer and this will finally reduce also the phosphatidylinositol release. Even after 5 h, no equilibrium distribution of phosphatidylinositol was found. We have shown that there was a net transfer of phosphatidylinositol from the monolayer to phosphatidylcholine vesicles and a transfer of phosphatidylcholine from the vesicles to the phosphatidylinositol monolayer but there was no net mass transfer. This conclusion was recently reached also by Kasper and Helmkamp [17]. These authors, however, used for some experiments liposomes containing 20 mol% phosphatidylinositol—a concentration which we found inhibitory. In an earlier paper, Harvey et al. [4] described the inhibitory effect of higher phosphatidylinositol concentrations for the transfer of phosphatidylinositol and phosphatidylcholine from microsomes to liposomes. At the same time the phosphatidylcholine transfer from liposomes to mocrosomes is decreased, but the phosphatidylinositol transfer increased. This is difficult to reconcile with the present observations. It is clear that many factors such as charge density, ions, lipid composition, the presence of sphingomyelin and the ratio of donor and acceptor membrane will affect the final lipid transfer as catalyzed by the bovine brain protein. Before a more detailed description of the transfer mechanism can be given, we have to establish how many lipid molecules are bound by the protein and whether both phosphatidylinositol and phosphatidylcholine are competing for the same binding site.

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