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The rate of uptake and efflux of phosphatidylcholine from human erythrocytes depends on the fatty acyl composition of the exchanging species

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The rate of uptake of radioactive phosphatidylcholine molecules of different fatty acid composition in intact erythrocytes as facilitated by a phosphatidylcholine-specific transfer protein has been studied. When trace amounts of radiolabeled phosphatidylcholine molecules are present in donor vesicles consisting of egg phosphatidylcholine and cholesterol, the transfer of the radiolabeled species depends strongly on their fatty acyl composition: dipalmitoylphosphatidylcholine is transferred at the lowest rate, 1-saturated-2-unsaturated species are transferred faster and the highest rate is observed for dioleoyl phosphatidylcholine. Transfer of the various phosphatidylcholine molecules was measured furthermore using donor systems in which the bulk phosphatidylcholine was varied in its fatty acyl composition. Also in this type of experiment, the transfer protein preferentially stimulated transfer of unsaturated phosphatidylcholine molecules, especially from an environment containing more saturated molecules. Finally, the efflux of labeled phosphatidylcholine from intact erythrocytes to plasma in the absence of the phosphatidylcholine-specific transfer protein was studied and it became clear that in this case the nature of the effused molecules itself, rather than the composition of the bulk lipids, determined the effuse rates. An important conclusion to be drawn from these experiments is that radiolabeled phosphatidylcholine molecules, when used as markers for phospholipid exchange or transfer, should resemble in their fatty acid composition the composition of the bulk lipid in order to provide reliable data on rates and extents of the process studied.

Introduction

The facilitation of phospholipid transfer between two membrane systems by phospholipid-transfer protein has made these proteins very useful tools in membrane research [1]. In addition, the red blood cell is a favorite model of biological membrane structure and the use of transfer proteins could further clarify the role of the phospholipids in these membranes. Whereas short-chain phosphatidylcholine (PC) molecules can be spontaneously transferred between a liposome and the erythrocyte [2], the longer, naturally occurring, PC molecules can be transferred only by using phospholipid-transfer protein. Bovine liver PC-specific transfer protein is a cytosolic protein which has been characterized in great deal [3]. The protein is highly specific for PC and does not transfer

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Abbreviation: PC, phosphatidylcholine.

phospholipids of other polar head-group classes [4,5]. It has been shown to facilitate the transfer of PC between intact erythrocytes and a variety of donor membrane systems [6]. The protein can be used to measure the exchangeable pool and transbilayer movement of PC in intact erythrocytes [7], but also to retailor the molecular species of PC in the membrane without altering the overall phospholipid and cholesterol content [8]. Rates of protein-facilitated intermembrane transfer of PC in vesicle systems appeared to be sensitive to the chemical and physical characteristics of the membranes [9–13]. The PC-specific protein from bovine liver seems to have the ability to bind to any fluid phase of PC [11], but preferentially extracts and transfers long-chain fluid-phase PCs [14]. It was shown that PC transferred by the protein from erythrocyte membranes to liposomes is enriched in unsaturated species [15]. There also seems to be a correlation between hydrophobicity of a phospholipid and its rate of exchange between plasma and erythrocytes [16]. It was shown that unsaturated ^{14}C-labeled soy PC introduced into erythrocytes effused faster to plasma than the saturated ^{14}C-labeled 1,2-dipalmitoyl species [15].

The exchange reaction is routinely monitored by the use of radioactively labeled PC molecules as markers. It is of highest importance to know the fate of the markers in these systems to be able to relate the results to that of the bulk. In this study, we describe the uptake of different radioactive PC species in intact erythrocytes from vesicles, as facilitated by PC transfer protein from bovine liver, as well as the efflux of these molecules from the red cell to plasma.

Materials and Methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine, egg phosphatidylcholine and phosphatidic acid prepared from egg phosphatidylcholine were purchased from Sigma (St. Louis, MO, U.S.A.); 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine was obtained from Avanti Polar Lipids (Birmingham, AL, U.S.A.) and soybean phosphatidylcholine was kindly donated by Natterman (Köln, F.R.G.). Egg phosphatidyl[*N-methyl*-^{14}C]-choline, soybean phosphatidyl[*N-methyl*-^{14}C]choline and 1-palmitoyl-2-linoleoylphosphatidyl[*N-methyl*-^{14}C]choline were synthesized according to Stoffel et al. [17]. 1-Palmitoyl-2-[^{14}C]oleoylphosphatidylcholine, 1,2-[^{14}C]dipalmitoylphosphatidylcholine, 1,2-[^{14}C]dioleoylphosphatidylcholine and glycerol tri[^{3}H]oleate were purchased from Amersham International (Amersham, U.K.). All other chemicals and solvents used, were of analytical grade.

Preparation of erythrocytes

Fresh human erythrocytes were obtained from healthy volunteers by venipuncture, using acid-citrate-dextrose as anticoagulant. Cells were packed for 10 min at $2500 \times g$ and washed three times with a 5-fold volume of buffer comprising 150 mM NaCl/25 mM glucose/1 mM EDTA/100 IU $\cdot$ ml^{-1} penicillin/100 μg $\cdot$ ml^{-1} streptomycin/10 mM Tris (pH 7.4) (referred to as 'buffer' throughout).

Preparation of vesicles

Vesicles were prepared from the various phosphatidylcholine species, mixed with an equimolar amount of cholesterol, 6 mol% of phosphatidic acid, trace amounts of [^{14}C]phosphatidylcholine and glycerol tri[^{3}H]oleate (0.1 mol% of total vesicle phosphatidylcholine). The lipid mixture was dried under nitrogen from a chloroform/methanol solution (2 : 1, v/v). The dried mixture was dispersed in buffer to give a final concentration of 5 μmol PC/ml. The lipid dispersion was sonicated under nitrogen for 5–6 min at 70 W and subsequently centrifuged at $100\,000 \times g$ for 45 min. The pellet was discarded and the supernatant was used in the experiments described. Depending on the composition of the vesicles, the recovery varied from 50 to 85% as determined by radioactivity measurements in emulsifier scintillator solution 299 TM from Packard, using a Packard-PRIAS-Tricarb scintillation counter. Vesicles showed a ^{3}H/^{14}C ratio identical to that in the starting lipid mixture. Further analysis of the vesicles was performed by making a lipid extract according to the procedure of Rose and Oklander [18] and measuring the specific radioactivity of PC and the lipid

composition of the extract. This composition was virtually identical to the starting lipid composition.

Preparation of transfer protein

The phosphatidylcholine specific transfer protein was purified from bovine liver [19] and stored at a concentration of 75 μg/ml, determined according to Lowry et al. [20], in 50% glycerol at −20°C. Before use, glycerol was removed by overnight dialysis of the protein solution against a 1000-fold volume of buffer at 4°C. The protein solution was concentrated against flake poly(ethylene glycol) (Acquacide III, Calbiochem, San Diego, CA, U.S.A.).

Incubation conditions

Incubations were carried out at 37°C under gentle shaking. The incubation mixture for the uptake reaction contained 2 μM transfer protein and the ratio of vesicle PC to erythrocyte PC was 1. Aliquots were taken at timed intervals and the cells were pelleted by centrifugation. The supernatant containing the vesicles was taken and hemolysis was determined by measuring the absorbance at 408 nm. The cells were washed three times with a 100-fold excess of buffer. Lipids were extracted from erythrocytes according to the procedure of Rose and Oklander [18] and the increase in specific radioactivity of PC was determined using established procedures for determination of phosphorus [21] and radioactivity (see above). An aliquot of the vesicle containing supernatant was placed directly in a liquid scintillation vial and radioactivity was measured in Instagel Liquid scintillation emulsifier cocktail from Packard, using a Packard-PRIAS-Tricarb scintillation counter. From the $^{3}H/^{14}C$ ratio and the known specific radioactivity at the start of the incubation, the amount of vesicle PC and the relative decrease of [^{14}C]PC in the donor system could be determined. The data are expressed as the ^{14}C dpm remaining in the vesicles or appearing in the erythrocytes, relative to the total ^{14}C dpm present in the sample. The efflux of [^{14}C]PC from prelabeled cells to plasma was measured using a modification of the technique described by Lange et al. [22] for the analysis of cholesterol exchange. The cells were first incubated with donor vesicles as described above, resulting in the introduction of vesicle PC and [^{14}C]PC into the cells. After this incubation, the cells were centrifuged and washed well with buffer to remove adherent vesicles. Incubation solutions were prepared in triplicate, and contained 100 μl of the prelabeled cells (0.15 μmol PC), 450 μl of plasma (1.0 μmol PC) and 450 μl of buffer. Samples of 100 μl were taken at various time points, dispersed in 0.5 ml of buffer and centrifuged (0.5 min, 8000 × g). The supernatant was placed directly in a liquid scintillation vial. The cell pellet was washed twice with 0.5 ml buffer and lysed with 20 μl distilled water. An extract of the cell lysate was made with 0.5 ml isopropranol and following extensive vortexing and centrifugation, the extract was transferred into a scintillation vial. The recovery of the labeled PC from the cells by this technique was virtually identical to that of control cells extracted with chloroform/methanol using standard procedures [23]. The radioactivity of the supernatant and pellet extracts was determined in 4.5 ml Instagel Liquid scintillation emulsifier cocktail from Packard in the Packard-PRIAS-Tricarb liquid scintillation counter. The data are expressed as the ratio of ^{14}C dpm remaining in the cells against the total dpm in the sample at each time point.

Results

Phosphatidylcholine exchange experiments, using donor and acceptor systems of different composition, are often difficult to interpret. Incorporation of specific PC species from the donor into the acceptor membrane will consequently lead to incorporation of acceptor PC into the donor system. This results in a continuous change in composition of the bulk PC in both the donor as well as acceptor system, which may change their properties. Moreover, it is shown that the membranes of intact human erythrocytes tolerate only limited changes in the fatty acyl chains of their PC [8]. Therefore, we started to investigate the transfer of various radioactive PC species from vesicles of which the bulk PC was composed of species similar to those in the erythrocyte membrane and which had been shown not to change the integrity of the red cell membranes to any appreciable extent. The protein-mediated transfer of various

^{14}C-radiolabeled PC species from donor vesicles composed of egg PC, cholesterol and phosphatidic acid to human erythrocytes is shown in Fig. 1. In absence of transfer protein, very low transfer of [^{14}C]PC species from vesicles to erythrocytes was observed (not shown). After correction for contamination by vesicles, the fraction of label incorporated into the cellular membrane usually did not exceed 4%. The extent of contamination of erythrocytes with vesicles after washing could be monitored by including the nonexchangeable marker glycerol tri[^{3}H]oleate in the vesicles. The contamination of erythrocyte PC with bulk vesicle PC, based on the ^{3}H counts in the erythrocytes after washing, usually did not exceed 5% of the total erythrocyte PC. The increasing fraction of ^{14}C label found in the erythrocytes was accompanied by a decrease of ^{14}C in the vesicles. Recovery of the total ^{14}C radioactivity was 90–100%. Hemolysis of the erythrocytes was lower than 3% after 8 h of incubation and did not exceed 5% after 24 h of incubation. From the results shown in Fig. 1, it can be concluded that the best-transferred substrates are the unsaturated species 1,2-dioleoyl-PC and soy PC. Small differences are observed in the transfer of 1-palmitoyl-2-linoleoyl-PC, 1-palmitoyl-2-oleoyl-PC or egg PC from egg PC vesicles. The disaturated 1,2-dipalmitoyl-PC appears to be a relatively poor substrate for transfer from egg PC vesicles to erythrocytes. This observed preference was investigated in more detail by changing the bulk PC composition of the vesicle. In Fig. 2 the flow of three different ^{14}C-

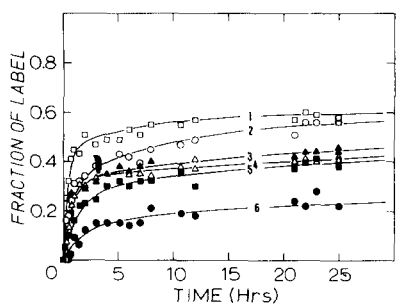

Fig. 1. Protein-mediated transfer of various radiolabeled PC species from vesicles composed of egg PC, cholesterol and phosphatidic acid, as described in Materials and Methods, to intact human erythrocytes. (1, □) 1,2-[^{14}C]dioleoyl-PC; (2, ○) soy [^{14}C]PC; (3, ▲) 1-palmitoyl-2-linoleoyl-[^{14}C]PC; (4, △) egg [^{14}C]PC; (5, ■) 1-palmitoyl-2-[^{14}C]oleoyl-PC; (6, ●) 1,2-[^{14}C]dipalmitoyl-PC. The molar ratio of donor vesicle PC to erythrocyte PC was 1. The extent of label found at various time points in the erythrocyte is expressed as the fraction of total label present. The curves 1 to 6 are computed as described in the Addendum and based on the constants given in Table I.

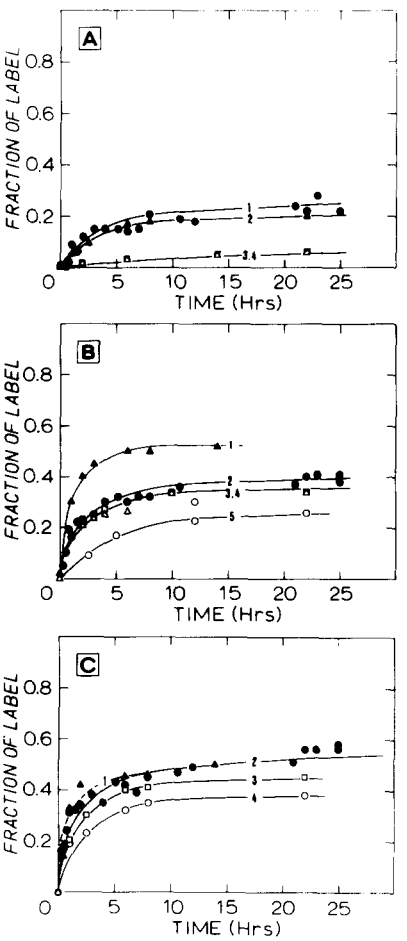

Fig. 2. Transfer of radiolabeled PC species from various PC vesicles composed of PC, cholesterol and phosphatidic acid, as described in Materials and Methods, to intact human erythrocytes. (A) Transfer of 1,2-[^{14}C]dipalmitoyl-PC from vesicles composed of egg PC (1, ●); 1,2-dipalmitoyl-PC (2, ▲); 1-palmitoyl-2-oleoyl-PC (3, □); and 1-palmitoyl-2-linoleoyl-PC (4, △). (B) Transfer of 1-palmitoyl-2-[^{14}C]oleoyl-PC from vesicles in which the PC component is 1,2-dipalmitoyl-PC (1, ▲); egg PC (2, ●); 1-palmitoyl-2-oleoyl-PC (3, □); 1-palmitoyl-2-linoleoyl-PC (4, △) and 1-palmitoyl-2-arachidonoyl-PC (5, ○). (C) Transfer of soy [^{14}C]PC from vesicles in which the PC component is 1,2-dipalmitoyl-PC (1, ▲); egg PC (2, ●); 1-palmitoyl-2-oleoyl-PC (3, □) and dilinoleoyl-PC (4, ○). The molar ratio of donor vesicle PC to erythrocyte PC was 1. The extent of label found at various time points in the erythrocyte is expressed as the fraction of total label present.

labeled PC molecules, namely 1,2-dipalmitoyl-PC (A), 1-palmitoyl-2-oleoyl-PC (B) and soy PC (C) from vesicles with varying bulk PC composition is shown. Transfer of 1,2-[^{14}C]dipalmitoyl-PC from vesicles composed of 1-palmitoyl-2-oleoyl-PC or 1-palmitoyl-2-linoleoyl-PC is extremely slow (Fig. 2A, curves 3 and 4). There was no significant difference between the slow transfer of 1,2-^{14}C]dipalmitoyl PC from vesicles containing either 1,2-dipalmitoyl- or egg PC as bulk PC (Fig. 2A, curves 2 and 1, respectively). Transfer of 1-palmitoyl-2-oleoyl-PC is virtually identical from vesicles composed of 1-palmitoyl-2-oleoyl-PC, 1-palmitoyl-2-linoleoyl-PC or egg PC (Fig. 2B, curves 3, 4 and 2, respectively). This label however, seems to be preferentially transferred from vesicles containing 1,2-dipalmitoyl-PC as bulk PC (Fig. 2B, curve 1) and is only slowly transferred from vesicles composed of 1-palmitoyl-2-arachidonoyl-PC (Fig. 2B, curve 5). The unsaturated ^{14}C-labeled soy PC label is transferred rapidly from several vesicle systems (Fig. 2C). Although the differences are small, the label seems to be transferred more slowly from more unsaturated PC vesicles (Fig. 2C, curves 3 and 4) than from vesicles in which the bulk PC is of a less unsaturated type (Fig. 2C, curves 1 and 2). In general, the data depicted in Fig. 2 clearly indicate a preference of transfer of the more unsaturated PC species, especially from an environment containing more saturated PCs.

In order to see whether this preference in transfer is a particular and specific feature of the PC-transfer protein of bovine liver, or whether the differences in transfer can be ascribed to the nature of intermolecular interactions of the PC molecules in the lipid bilayer, prelabeled erythrocytes were incubated in the absence of PC-transfer protein, with plasma and the efflux of label monitored. Fig. 3 shows the flow of various ^{14}C-labeled PC species from erythrocytes to plasma. Cells, in which native PC had been partly replaced by 1,2-dipalmitoyl-PC loose the ^{14}C-labeled soy PC they contained in trace amounts rapidly to plasma (Fig. 3A, curve 4), whereas 1,2-[^{14}C]dipalmitoyl PC and 1-palmitoyl-2-[^{14}C]oleoyl-PC effused much slower. The extent of replacement of native PC by 1,2-dipalmitoyl-PC did not significantly influence the efflux of 1,2-[^{14}C]dipalmitoyl-PC (compare Fig. 3A, curves 1 and 2). The same pattern of efflux is found from cells in which 40% of the native PC had been replaced by 1-palmitoyl-2-oleoyl-PC (Fig. 3B).

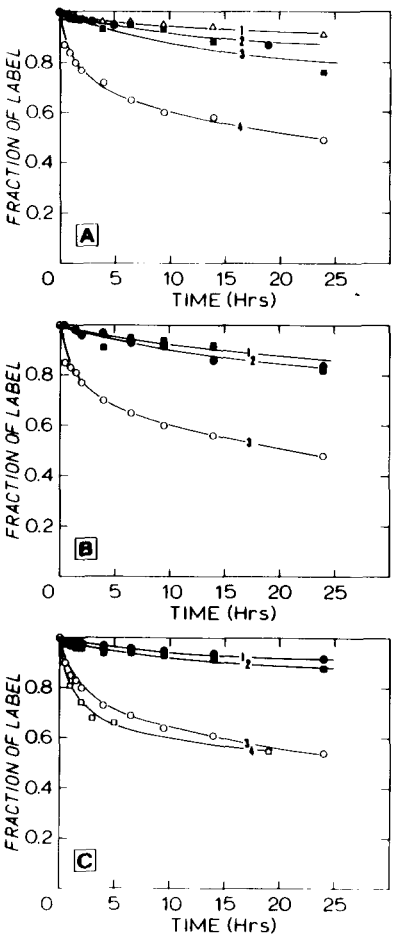

Fig. 3. Efflux of various radiolabeled PC species from prelabeled human erythrocytes. Cells were prelabeled during an incubation with transfer protein and vesicles composed of either 1,2-dipalmitoyl-PC (A), 1-palmitoyl-2-oleoyl-PC (B), 1-palmitoyl-2-linoleoyl-PC (C, curves 1–3) or dilinoleoyl-PC (C, curve 4) as described in the Materials and Methods. The vesicles contained trace amounts of 1,2-[^{14}C]dipalmitoyl-PC (●, △), 1-palmitoyl-2-[^{14}C]oleoyl-PC (■) or soy *[N-methyl-*^{14}C]PC (○, □). After the replacement reaction, native PC had been replaced by approx. 25% 1,2-dipalmitoyl-PC (A, 1, 3, 4) or 15% 1,2-dipalmitoyl-PC (A, 2), 40% 1-palmitoyl-2-oleoyl-PC (B), 40% 1-palmitoyl-2-linoleoyl-PC (C, 1–3) or 25% dilinoleoyl-PC (C, 4), respectively. Efflux was subsequently measured during incubation of the cells with heat-inactivated human plasma. Efflux was determined as outlined in the Material and Methods section, and the data are expressed as the fraction of the total radioactivity that is present in the erythrocyte at each time point and are the means of values derived from triplicate reaction mixtures.

As before, ^{14}C-labeled soy PC (Fig. 3B, curve 3) was transferred much more rapidly than 1,2-[^{14}C]dipalmitoyl-PC or 1-palmitoyl-2-[^{14}C]oleoyl-PC (Fig. 3B, curves 1 and 2). ^{14}C-labeled soy PC effused much faster than either 1,2-[^{14}C]dipalmitoyl-PC or 1-palmitoyl-2-[^{14}C]oleoyl-PC from cells in which the native PC had been replaced by either 1-palmitoyl-2-linoleoyl-PC (40%) or 1,2-dilinoleyol-PC (25%). In general, it can be concluded from the data in Fig. 3 that the efflux of label from erythrocytes depends on the characteristics of the label itself and is not greatly dependent on the composition of the bulk PC.

Discussion

The phosphatidylcholine-transfer protein from bovine liver has been used to exchange PC species between vesicles and intact human erythrocytes. The transfer protein is responding to differences in species composition of the interface and exhibits a clear preference for the transport of molecules that are in the liquid state. Even within this class of PC species, increase in unsaturation will lead to a higher rate of transfer. Before discussing in detail this observed characteristic of the transfer reaction, it is important to underline the following.

The PC-transfer protein from bovine liver is a very powerful tool to retailor PC in natural membranes in a subtle way. Our studies, however, demonstrate very clearly that caution must be exercised in using a single radioactively labeled PC species to monitor the transfer of other types of PC. Although the approach is very convenient, it is valid only if this labeled PC molecule is identical to the bulk PC species. Only when this prerequisite is fulfilled can the exact amount of donor PC introduced into the acceptor membrane be determined conclusively. A good approximation of such data will be derived by using a labeled PC molecule of which the fatty acid composition is comparable to that of the bulk PC, e.g., 1-palmitoyl-2-[^{14}C]oleoyl-PC to monitor the transfer of egg PC. An other choice will lead to an over- or underestimation of the amount of donor bulk PC transferred into the acceptor membrane. The movement of PC from donor to acceptor membrane is to be offset by a movement of equivalent magnitude in the opposite direction. In our vesicle/erythrocyte system, this transfer appeared to take place in a non-random fashion, as was shown by gas-chromatographic analysis [15,24]. Unsaturated species are preferably transferred from the cell to the vesicles. It was argued, therefore, that the best way to monitor the exact effect that the replacement reaction has on the PC composition of the acceptor membrane is by means of gas-chromatography. A quantitative analysis of PC species, rather than a quantitation of fatty acyl chains, is the safest way to monitor a retailoring process. All other approaches can only give approximate figures on the changes that are induced in the PC composition of the membrane.

The radioactive PC molecule is a minor component of the bulk phase of the lipid vesicle and is assumed to have a negligible effect on the physical properties of the membrane. In our experimental arrangement, a completely random distribution of PC species in the vesicles is to be expected. In the presence of 50 mol% of cholesterol, even small amounts of unsaturated PC species will mix completely in a disaturated PC bulk [25]. No phase transitions are expected [26], although local packing irregularities cannot be ruled out.

The marked difference therefore, in the ability of the transfer protein to transfer various radiolabeled PC molecules from vesicles composed of egg PC (Fig. 1) is apparently due to a preference of the protein for the transfer of labeled species itself, which is not, in this case, influenced by the physical properties of the bulk. This preference is further underlined by the experimental data shown in Fig. 2. The protein discriminates between two PC species, bulk PC and labeled PC, and obviously the more unsaturated species are transferred preferentially. It has to be taken into account, however, that during the exchange process the composition of the vesicles will be changed, and this may affect the rate of transfer. This is quite obvious in case of the transfer of 1,2-[^{14}C]dipalmitoyl-PC from 1,2-dipalmitoyl-PC vesicles. As a consequence of the exchange reaction, unsaturated erythrocyte PC species will preferentially enter the vesicle [15]. The transfer protein obviously prefers to transfer these species above the fully saturated bulk and label. The extent of transfer of 1,2-dipalmitoyl-PC to erythrocytes can be increased by

introducing a fresh population of 1,2-dipalmitoyl-PC vesicles [8]. These observations undoubtedly lead to the conclusion that the preference of the transfer protein will lead not only to a difference in transfer rate, but also to a net flow of certain PC species. Complete equilibration is not therefore reached in the time-scale of our experiments (30 h).

Also, characteristics other than PC species composition of the interface seem to play an important role in determining the rate of the protein-mediated PC transfer. This is clearly illustrated by the following observations: (i) the cholesterol content of a membrane affects the exchange rates of both its PC and cholesterol [33]; (ii) PC species which are comparable in fatty acyl constituents, such as microsomal PC and egg PC, are transferred at different rates when the donor systems used are either rat liver microsomes or egg PC vesicles [6]; (iii) PC transfer proceeds more easily and at higher rates when using red cell ghosts instead of intact cells [6]. Such differences are even found in one particular cell type from different origin, e.g., PC in rat erythrocytes is transferred more rapidly than that in human red cells [6].

The rate at which a PC molecule enters or leaves a bilayer is dependent upon both its molecular structure and the nature of its environment, but the mechanism by which the protein senses the fatty acyl chains of PC in a bilayer is still unclear. Monolayer studies showed that the protein is able to penetrate a layer composed of 1-palmitoyl-2-oleoyl-PC up to an initial pressure of 20 dyn/cm. Above this pressure, however, it still facilitates transfer of PC from that monolayer [28,29], and recently we found that, even at pressures near the collapse pressure, the protein is able to transfer PC from monolayers of lipid composition similar to that of the outer monolayer of the human erythrocyte (not shown). This clearly indicates that penetration may not be an absolute requirement. It has been suggested earlier [15] that the intermolecular interactions between the membrane constituents could underlie the specificity of the transfer process.

Species with a higher degree of unsaturation are expected to be transferred at a higher rate as a result of weaker intermolecular interactions [15,30]. These intermolecular interactions depend both on the molecule itself and its environment, whether this is a simple lipid vesicle or a complex biological membrane composed of lipid and non-lipid molecules. The suggestion was made that the major factor determining the rate of the exchange reaction is the facility of extraction of a lipid from the membrane interface [31]. An increase in the intermolecular interactions between the membrane constituents will increase the effort it takes for a transfer protein to extract a molecule from such an interface and will therefore decrease the rate of transfer.

The ability of a protein to choose between different PC species is not restricted exclusively to the PC-transfer protein from bovine liver. Discrimination between PC species has also been reported for the transfer proteins from bovine brain [32], rat liver [33] and rat lung [34]. It is of interest to note that such differences are not confined to transfer processes which are catalyzed by these lipid-transfer proteins. Marked higher rates are also found for the efflux of more unsaturated PC species form the intact erythrocyte to plasma. (Fig. 3).

Whether the acyl-chain specificity of phospholipid-transfer proteins can be related to their physiological function in vivo remains a matter of speculation. It has been shown that the PC-transfer protein from bovine liver differs markedly from the other transfer proteins which have been recently characterized [35]. It is highly specific for PC and a net transfer of phospholipid is observed only under very special conditions [36]. In our system, the protein facilitates a real exchange of PC and it is therefore very tempting to speculate that the observed specificity points to a role for this transfer protein in the maintenance of a distinct membrane lipid composition with respect to its fatty acyl chains.

Addendum

We tried to fit our data to a rigorous mathematical model in order to explain the observations. A mechanism was proposed for protein-facilitated PC transfer between membranes [9]. Implicit in the mechanism is the assumption that exchange, rather than net transfer, occurs and that

the protein functions as a freely diffusable carrier of phospholipid. The rate of transfer in a vesicle/liposome system was shown to be dependent on the association constants of the protein for donor and acceptor membranes, the concentration of the negatively charged phospholipids they contained, pH value and the pool-sizes of acceptor and donor [12]. In our experiments, the protein concentration, pH and pool-sizes of donor and acceptor bulk PC are constant, as is the composition of the donor system, except for the ^{14}C-label. We tried to fit our data to a three-pool closed system model as will be described below. Large excess of donor PC could simplify the system to a two-pool model as described previously [37,38], but it will also lead to a massive replacement of native PC in the erythrocyte membrane. Furthermore, it will provide information on the transbilayer movement in the erythrocytes, rather than on the overall transfer process. In our experiments, we chose for equal amounts of donor and acceptor PC, which implies that the return of labeled phospholipid from the acceptor to the donor has to be taken into account.

The mathematical model we used is visualized in Fig. 4. Label is transferred from the available vesicle pool (A) to the outer monolayer of the erythrocyte membrane (B) and is then translocated to the inner monolayer (C) of the membrane. The transbilayer movement in the fesicles is neglected. It was shown [39] that the equilibration over the two halves of these vesicles will proceed with a half-time that exceeds 10 days and that only 70% of the total PC is readily available for exchange. The flow of label in such a system can be described by the differential equations:

$$\frac{dq_a}{dt} = K_{ab} \cdot q_b - K_{ab} \cdot q_a \quad (1)$$

$$\frac{dq_b}{dt} = K_{ba} \cdot q_a + K_{bc} \cdot q_c - (K_{ab} + K_{cb}) \cdot q_b \quad (2)$$

$$\frac{dq_c}{dt} = K_{cb} \cdot q_b - K_{bc} \cdot q_c \quad (3)$$

where q_i is the [^{14}C]PC in compartment i at time t. K_{ab} and K_{ba} are the rate constants for movement of label out and into the red cell outer monolayer and K_{bc} and K_{cb} are the rate constants for movement of label to and from the inner monolayer, respectively. Solving the differential equation, thereby assuming the conservation of radioactivity, gives mathematical equations for the flow of label from the vesicle pool into the erythrocyte. The fractional decrease of label from the vesicle pool can be described by:

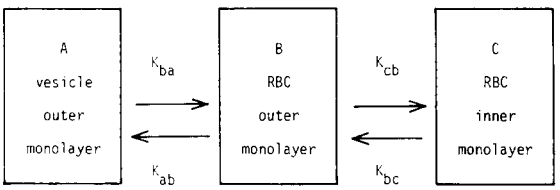

Fig. 4. Three-pool closed system as a model for [^{14}C]PC transfer between vesicles and intact erythrocytes (RBC) through the aqueous medium facilitated by phospholipid-transfer protein.

$$\frac{q_{at}}{q_{a0}} = H_1 \cdot e^{-q_1 t} + H_2 \cdot e^{-q_2 t} + H_3$$

where q_{at} is the [^{14}C] PC in the vesicle pool at time t and q_{a0} represents the amount of label in the vesicles at time zero, i.e. the total amount of label present in the system. From the semilogarithmic plot derived from the experimental data, the constants H_i and q_i can be determined by curve peeling and the rate constants can be calculated [40]. It was evident from a search within the optimal range of the constants derived from curve peeling that several combinations of rate constants

TABLE I

RATE CONSTANTS OF TRANSFER OF VARIOUS PC SPECIES ASSUMING A THREE-POOL MODEL AS DEPICTED IN FIG. 4

Donor vesicles contained [^{14}C]PC, while the accepting human erythrocytes were initially unlabeled. Rate constants were derived from the measured data shown in Fig. 1, as described in the text. Abbreviations: DPPC, 1,2-dipalmitoyl-PC; POPC, 1-palmitoyl-2-oleoyl-PC; PLPC, 1-palmitoyl-2-linoleoyl-PC; DOPC, 1,2-dioleoyl-PC.

Donor PC composition		Rate constant			
Bulk	Label	K_{ba}	K_{ab}	K_{cb}	K_{bc}
Egg PC	DPPC	0.08	0.43	0.018	0.054
Egg PC	POPC	0.20	0.50	0.015	0.045
Egg PC	egg PC	0.40	0.80	0.008	0.024
Egg PC	PLPC	0.40	0.80	0.015	0.045
Egg PC	soy PC	0.40	0.80	0.030	0.090
Egg PC	DOPC	1.00	1.17	0.040	0.120

gave equally good fits of the data. Since PC is asymmetrically distributed over the bilayer of the erythrocyte membrane, and the existence of a steady-state situation can be assumed, we are able to limit the permutations. Of the total PC present in the red cell membrane, 75% is found in the outer monolayer, while the remaining 25% resides in the inner leaflet [41]. The species of PC are known to be randomly distributed over the inner and outer monolayer [42]. This taken into account and the fact that in the steady state the flow of PC from inner to outer monolayer equals the flow of PC in the opposite direction, we can assign $K_{bc} = 3 \times K_{cb}$. In Fig. 1 the lines represent the theoretical curves based on the rate constants given in Table I.

In a model of free diffusion, specific radioactivity in donor and acceptor is expected to reach identical values at infinite time. With equal pool sizes, this will lead to an equal amount of label in donor and acceptor system. From our data, however, it is clear that only PC vesicles with egg [^{14}C]PC, 1-palmitoyl-2-[^{14}C]oleoyl-PC or 1-palmitoyl-2-linoleoyl-[^{14}C]PC as label fit to this expectation in the time-scale of our experiments. The data found with more unsaturated or saturated species can only be interpreted as a preference of the transfer reaction for certain species.

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