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Potentialities of magnetoliposomes in studying symmetric and asymmetric phospholipid transfer processes

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Using high-gradient magnetophoresis, the non-protein-mediated transfer and exchange of phosphatidylglycerol (PG) molecules between sonicated phospholipid dispersions and magnetoliposomes is studied. The latter structures consist of nanometer-sized magnetite (Fe_3O_4) cores which are enwrapped by a phospholipid bilayer. Their dimensions are similar to those of small unilamellar vesicles (De Cuyper and Joniau (1988) Eur. J. Biophys. 15, 311–319). Using these particles, spontaneous lipid movements were studied in three different cases. In a first setup, symmetric exchange between dimyristoylphosphatidylglycerol (DMPG) magnetoliposomes, labelled with [^{3}H]DMPG, and DMPG vesicles was followed. Within the time scale of the experiment (1 day) both the lipid molecules residing in the inner and outer leaflet of the magnetoliposomes participate in the exchange process, although 'flip-flop' movements have a retarding effect. In the second approach a unidirectional flux of DMPG from DMPG magnetoliposomes to distearoylphosphatidylglycerol (DSPG) acceptors is noted. In this case, the outer phospholipid leaflet of the magnetoliposomes (in contrast to the inner one) can be largely stripped off; the extent of depletion is determined by the relative amount of the DSPG receiving structures. Furthermore, it is found that with a 15-fold molar excess of receptors, the whole depletion course can be described by a single first-order rate expression. The reluctancy of the inner shell phospholipids to migrate is further illustrated by the virtual lack of transfer, observed with monolayer-coated Fe_3O_4 colloids. In the third case, asymmetric bidirectional PG transfer is followed between equimolar amounts of DMPG magnetoliposomes and dipentadecanoylphosphatidylglycerol vesicles. In the initial stage of the incubation period, the mmol PG/g Fe_3O_4 ratio decreases, but progressively restores later on. By quantitatively measuring the transfer rate of each of the individual components, this complex behavior could be unravelled.

Introduction

Phospholipid molecules are able to transfer spontaneously both between biological and artificial membranes [1,2]. For unravelling the mechanistic aspects of this process, artificial phospholipid membranes in particular have proven to be valuable tools. These structures indeed have the advantage that their composition and physical characteristics can be varied at will within a broad range. The consensus emerging from these studies now is that, at least in dilute systems [3], the kinetics of spontaneous lipid transfer agree with a mechanism involving a rate-limiting escape of monomers from the donor surface, followed by rapid diffusion through the intervening water phase and uptake by an acceptor particle [4–10].

Broadly speaking, the assay systems designed to monitor lipid transfer processes can be classified into two major categories: (i) those in which the time course of modifications occurring in donors and/or acceptors is followed in situ, either in a continuous (e.g., by fluorescence measurements [11–13]) or a non-continuous way (e.g., by differential scanning calorimetry [5,14]); and (ii) those which require an actual physical separation of the two vesicle populations. In the latter case, differences in size [15], density [16] or charge [4,7,17] have been exploited to fractionate donors and acceptors. Also immunoprecipitation [18] and agglutination [19–21] techniques have been employed.

As part of our ongoing interest in intervesicular transfer processes we developed a new vesicle type which consists of a nanometer-sized magnetizable iron

Abbreviations: DMPG, dimyristoylphosphatidylglycerol; $DC_{15}PG$, dipentadecanoylphosphatidylglycerol; DSPG, distearoylphosphatidylglycerol; Tes, 2-([tris(hydroxymethyl)methyl]amino)ethanesulfonic acid; Fe_3O_4, magnetite, $t_{1/2}$, half-time.

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oxide core which is envelopped with a phospholipid bilayer [22]. Evidence for this architecture comes both from theoretical calculations (based on the geometry and dimensions of the iron oxide cores and the DMPG molecules) as well as experimental data (electron micrographs, degree of lipid depletion after detergent treatment, shape of the adsorption isotherms and kinetic profiles). By high-gradient magnetophoresis, the so-called 'magnetoliposomes' can be very easily and efficiently captured and separated from non-magnetic vesicles, even if they are equally-sized and equally-charged. In the present work, the unique possibilities that magnetoliposomes have in monitoring lipid transfer kinetics are illustrated in three different setups: (i) in which magnetoliposomes and vesicles are composed of only one phospholipid type so that a symmetric bidirectional exchange occurs; (ii) in which donors and acceptors are constructed with transferable and non-transferable phospholipids respectively, so that a unidirectional transport is operative; and (iii) in which the phospholipid molecules of both vesicle populations transfer at a different rate so that an asymmetric bidirectional lipid flux occurs. In the latter case, the overall transfer event is unravelled by measuring the intrinsic transfer capacities of the individual components.

Experimental Procedures

Materials

DMPG, $DC_{15}PG$ and DSPG were purchased from Avanti Biochemical Corp. (Birmingham, AL). They were stored at $-20\,^{\circ}C$ and periodically monitored for purity by analytical thin-layer chromatography. [^{3}H]DMPG was made by enzymatic exchange of the choline headgroup of dimyristoylphosphatidylcholine (Sigma, St. Louis, MO) for [^{3}H]glycerol (Amersham, U.K.), catalyzed by phospholipase D [23]. $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ were obtained from UCB (Belgium). All other chemicals (pro analysi) were Merck products (Darmstad, F.R.G.). Throughout this paper a 5 mM Tes buffer (Sigma), pH 7.0 was used.

Preparation of phospholipid dispersions and magnetoliposomes

Lipid dispersions were prepared by ultrasonication at $37\,^{\circ}C$ as described previously [4]. [^{3}H]DMPG, used in a few experiments to generate doped magnetoliposomes (see below), was incorporated at a concentration of 0.1 μCi/mg of vesicle lipid. It was added before the organic solvent evaporation step. At the end of sonication, the DMPG and $DC_{15}PG$ samples appeared clear to visual inspection and their turbidity, measured at 400 nm ($37\,^{\circ}C$) didn't change within the time frame of two days. In contrast, the DSPG dispersion was opaque and demonstrated a further increase in turbidity. These behaviors point to the presence of stable, small unilamellar vesicles in the case of DMPG and $DC_{15}PG$, whereas the DSPG entities were continuously converted into larger structures.

The protocol for the construction of DMPG magnetoliposomes is outlined in our previous report [22]. Briefly, small magnetite (Fe_3O_4) particles with a diameter of approx. 14 nm were prepared by co-precipitation of $FeCl_2$ and $FeCl_3$ in the presence of an excess of ammonia and subsequently coated by lauric acid to obtain a stable dispersion. Upon co-incubation and dialysis for 2–3 days of this so-called water-adapted magnetic fluid in the presence of a sufficient amount of DMPG vesicles, the laurate coat is replaced by phospholipid molecules. Typically, for routinely preparing DMPG magnetoliposomes, i.e., structures with a phospholipid *bilayer*, 2.04 ml of the Fe_3O_4-laurate stock solution (22.90 mg Fe_3O_4/ml) was added to 17.00 ml of DMPG vesicles (11.26 μmol DMPG/ml). After removal of non-adsorbed DMPG molecules by high-gradient magnetophoresis (see below), the resulting DMPG-Fe_3O_4 complex was characterized by a DMPG/Fe_3O_4 ratio (mmol/g) of approx. 0.80. Occasionally, *monolayered* DMPG-coated Fe_3O_4 colloids were also constructed. In this case 4.08 ml of the magnetic fluid and only 3.06 ml of the DMPG stock solution were mixed and diluted with buffer to a final volume of 19.04 ml. In this case the final DMPG/Fe_3O_4 ratio in the magnetizable phospholipid structure equalled 0.30, which is approximately one third of the value found for the bilayered structure.

High-gradient magnetophoresis

Magnetic fields were generated with a water-cooled Bruker electromagnet (Type BE15) (Karlsruhe, F.R.G.), equipped with plane 15-cm diameter pole pieces put at a distance of 3 mm from one another. The instrument was operated at 80 V and 30 A and produced under these conditions a magnetic field intensity of approx. 1.5 Tesla. Local, high-gradient magnetic fields, needed to attract the small subdomain Fe_3O_4 grains, were created by putting a plug of magnetic stainless steel fibers (approx. 60 mg) (Bekaert, Belgium) in the conduit tubings through which the sample (0.5 ml) was pumped at a rate of 12 ml/h. A further washing of the retentate with an additional 0.5 ml of buffer was found to be necessary to remove iron oxide-free vesicles, which remained in the magnetic filter device by capillarity. Then, in the absence of the external magnetic field, the retentate was flushed out of the filter by a buffer stream (0.5 ml) at high speed (0.5 l/h) and collected as a stable dispersion. The recovery, expressed in terms of Fe_3O_4, was 95–99%.

Transfer experiments

Transfer of phospholipids between DMPG-Fe_3O_4 complexes (0.5 μmol phospholipid/ml) and the PG

dispersions (0.5 μmol phospholipid/ml for 1 : 1 mixtures and 7.5 μmol phospholipid/ml for 1 : 15 mixtures) was followed kinetically. At different times, 0.5 ml aliquots of the incubation mixture were subjected to high-gradient magnetophoresis, and the phospholipid content of the magnetizable population was measured. However, in the case where radioactivity was followed, we preferred to analyze the eluate in order to avoid considerable quenching effects, which are brought about by the dark color of magnetite.

The kinetics were expressed by the first-order rate equation

$$\log \frac{|X_t - X_{eq}|}{|X_o - X_{eq}|} = -\frac{k_1 \cdot t}{2.303}$$

where X refers to the amount of phospholipid associated with the magnetic structure (or to the amount of radioactivity found in the pure vesicle population) before transfer starts (X_o), at any time during the transfer process (X_t), and at equilibrium (X_{eq}). Half-times ($t_{1/2}$) were calculated from the pseudo first-order rate constant (k_1) by the usual relationship for first-order reactions.

$$t_{1/2} = \frac{0.69}{k_1}$$

Routine analyses

Spectrophotometric phosphate determinations were done after perchloric acid digestion of the phospholipids according to Vaskovsky et al. [24]. Iron determinations were done by atomic absorption spectroscopy (Varian AAG) at 372.2 nm. The magnetite samples were first dissolved in 0.1 M oxalic acid and, in case needed, diluted so that the iron concentration was in the range of 0–100 μg Fe/ml. ^{3}H Radioactivity was measured in a Packard Tricarb 2425 liquid scintillation counter and corrected for quenching. Fatty acid composition of the phospholipids was checked by gas chromatography (Packard-Model 419), using a GP 5% DEGS-PS stationary phase on 100/120 Supelcoport (Supelco Inc., Bellefonte). This column packing allows a direct injection of non-esterified, free fatty acids, obtained after phospholipid hydrolysis (45 min in 5 M HCl at 90 °C) and extraction with petroleum ether (boiling range: 60–80 °C; Merck). Phase transition temperatures were detected at 400 nm by light scattering measurements on an Aminco Bowman spectrophotofluorometer. Dimensions of the magnetoliposomes were checked by Sepharose CL-2B column chromatography and electron microscopy (Zeiss EM10C). They were characterized as particles with a diameter of approx. 30 nm.

Results and Discussion

1. Transfer between DMPG-Fe_3O_4 donors and DMPG acceptor vesicles

Experimental setups in which a perfectly symmetric, spontaneous exchange of phospholipids between two vesicle populations can be monitored are hard to design. Indeed, strictly speaking, both the polar headgroup and apolar part of the transferring phospholipids must be identical. In addition, symmetric exchange conditions presume that the physico-chemical properties of the donor and acceptor bilayers must be very much alike. As a result, the problem of distinguishing between both vesicle types becomes almost insurmountable. Recently, Bayerl et al. [14] approached the problem in an elegant way by investigating lipid transfer between vesicles made of dimyristoylphosphatidylcholine, the acyl chains of which were either protonated or deuteriated. Deuteration of the apolar moiety, however, resulted in an increase in the 'order ↔ disorder' phase transition temperature.

In the present work, we took up the challenge by utilizing DMPG magnetoliposomes which were doped with DMPG, radiolabeled with ^{3}H in its polar headgroup to most closely mimic the structure of the unmodified molecule. Transfer of [^{3}H]DMPG from DMPG magnetoliposomes donors (0.5 μmol/ml) to equal-sized DMPG acceptor vesicles (0.5 or 7.5 μmol/ml) was studied at pH 7.0 in 5 mM Tes buffer at 37 °C. At this temperature, DMPG vesicles are supposed to be in a 'fluidized' state since (in agreement with Small [25]) a gel-to-liquid crystalline phase transition of 23 °C was detected.

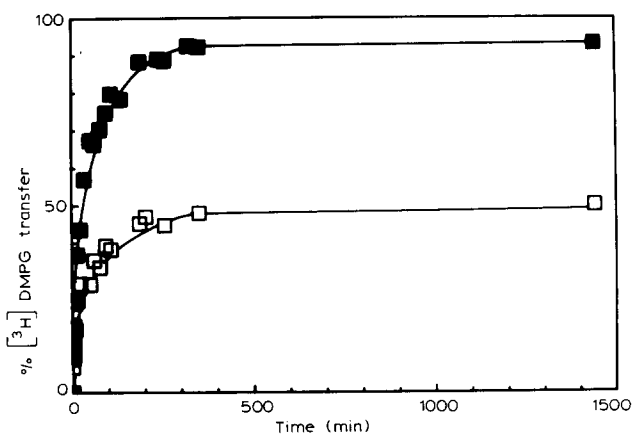

Fig. 1. Symmetric transfer of [^{3}H]DMPG between DMPG magnetoliposomes and DMPG small unilamellar vesicles. Vesicles (0.5 μmol DMPG/ml) were incubated with DMPG magnetoliposomes in equimolar conditions (□) and in a 15-fold excess (■) at 37 °C in 5 mM Tes (pH 7.0). The magnetoliposomes population was prelabeled with [^{3}H]DMPG. At stated times 0.5 ml aliquots of the incubation mixture were treated by high-gradient magnetophoresis and the eluate was analyzed for radioactivity. The fractional uptake of [^{3}H]DMPG (expressed as %) by the non-magnetic vesicles is plotted against time.

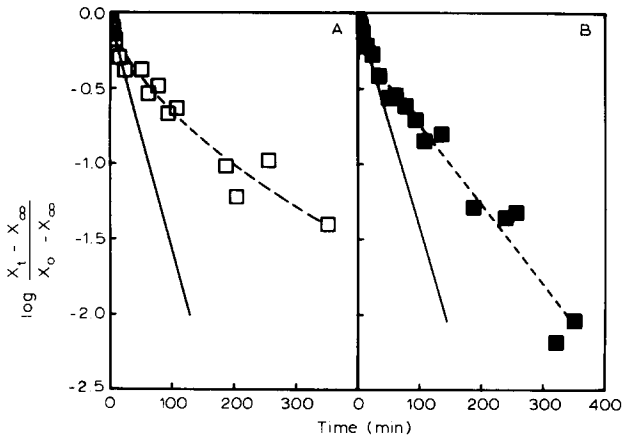

Fig. 2. First-order kinetic plots for [^{3}H]DMPG transport from DMPG magnetoliposomes to DMPG vesicles as shown in Fig. 1. Kinetics are followed by measuring the appearance of [^{3}H]DMPG into the DMPG vesicles. The fractional transfer is defined as $X_t - X_\infty / X_o - X_\infty$ (see Experimental Procedures). The acceptor/donor ratios equal 1 (A) and 15 (B). For calculating $t_{1/2}$ values for the initial transfer process, the time points covering less than 20% transfer were taken. The dashed line in B represents a linear regression plot through the time points, starting at $t = 51$ min and covering the last 30% of [^{3}H]DMPG transfer.

As shown in Fig. 1, the radioactive label gradually appears in the DMPG vesicle population as a function of time. At equilibrium, for donor/acceptor ratios of 1:1 and 1:15, it is found that, respectively, approx. 50% and 90% of the label is present in the acceptors. This indicates that on the time scale of the experiment the DMPG molecules, residing both in the outer and inner leaflet of the magnetoliposomes, participate in the exchange process.

In the initial stage of the transfer event, during which approx. 20% of total transfer occurred, the first-order reaction plots (Fig. 2) can be fitted by a straight line. From the slope, similar $t_{1/2}$ values for DMPG equilibration are calculated: 20 min in equimolar donor-acceptor conditions, and 22 min with a 15-fold acceptor excess. This independence of the first-order rate constant on the donor/acceptor ratio rules out the collision route as a mechanism to explain the observed lipid movement, and also suggests that transfer is limited by the rate at which molecules can leave the donor bilayer [7,9,26].

Inspection of Fig. 2 further shows that the whole transfer process does not proceed entirely in a mono-exponential way. In the case of equimolar donor and acceptor amounts, this biphasic character can be explained (i) by back exchange of [^{3}H]DMPG, and (ii) by a transbilayer movement of [^{3}H]DMPG which is slow relative to its transfer rate. Significant back exchange effects, however, are circumvented in the set-up in which a 15-fold excess of acceptors is used [10]. Therefore, the biphasic pattern observed in this case attests that [^{3}H]DMPG, present in the outer and inner leaflet of the magnetoliposomes, does not behave as a single kinetic pool, i.e., that transbilayer migration of the labeled phospholipids becomes rate-limiting after the initial transfer phase. For this slower decay component, which gives an indication of the rate of 'flip-flop' movements [27], a $t_{1/2}$ of 57 min is computed. The difference in transbilayer migration and interbilayer transfer rate measured in other model systems is usually much greater and has even been exploited to generate asymmetric membranes [28,29], although exceptions on this rule have been reported [14,30,31]. In this respect, it is worth mentioning that besides intrinsic properties of the lipid molecules, also some membrane features such as curvature [30,32], charge quality [33] and degree of fluidity [8] have been shown to be of paramount relevance. The precise interdependence of these parameters as well as the impact of the iron oxide-phospholipid association forces in our magnetoliposome system are unknown.

2. Transfer of DMPG from DMPG-Fe_3O_4 complexes to DSPG structures

A different transfer pattern is observed if 'solidified' DSPG membranes, demonstrating a phase transition temperature of 54°C (see Ref. 25), are chosen as acceptors. At 37°C and in equimolar conditions of DMPG magnetoliposomes and DSPG structures (each at a concentration of 0.5 mM), the phospholipid content of the magnetoliposomes gradually decreases to approx. 79% of the original value (Fig. 3). The $t_{1/2}$ for the initial process equals 31 min, which agrees with that found for [^{3}H]DMPG transfer in the above-mentioned cases. Al-

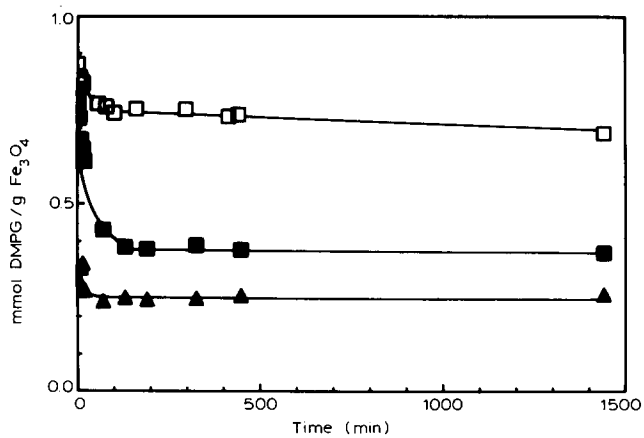

Fig. 3. Unidirectional transfer of DMPG from DMPG-Fe_3O_4 colloids to DSPG structures at 37°C in 5 mM Tes (pH 7.0). The DMPG-Fe_3O_4 complexes were prepared as described under Experimental Procedures. □, Bilayered DMPG magnetoliposomes in the presence of an equimolar amount (expressed in terms of phospholipid content) of DSPG vesicles; ■, bilayered DMPG magnetoliposomes in the presence of a 15-fold excess of DSPG structures; ▲, monolayered DMPG-Fe_3O_4 particles in the presence of a 15-fold excess of DSPG structures. In the different setups, the magnetic population was present in the incubation mixtures at a phospholipid concentration of 0.5 μmol/ml.

ternatively, with a 15-fold excess of DSPG, the lipid-depleting effect is more pronounced; at steady-state conditions approx. 54% of the lipids are withdrawn (Fig. 3). Interestingly, the first-order conditions ($t_{1/2} = 27$ min) are maintained throughout the entire lipid-depleting process. Fatty acid analysis further reveals that, within the time scale of both experiments, the decrease in phospholipid content of the magnetoliposome population is solely due to a depletion of DMPG molecules which is not accompanied by a (partial) compensatory flux of DSPG molecules. Most probably, this virtual inability of DSPG to transfer is due to its longer chain length [6,8,17].

An intriguing problem in studying such net transfer phenomena concerns the fate of the donating structures. For instance, as mass imbalance between outer and inner monolayer of the donors grows, transbilayer migration may become the rate-limiting parameter for the overall transfer rate. Moreover, if the donors are small sonicated vesicles, a concurrent increase in surface energy may mitigate any further shrinking. Small vesicles, which are continuously deprived of phospholipids to a threshold necessary for vesicle formation, must indeed either burst and/or fuse with other membrane fragments before ultimately disappearing. These processes, too, can slow down the transfer kinetics [5,14,34]. Conversely, with magnetoliposomes, the iron oxide core apparently exerts a stabilizing influence and allows a first-order unidirectional transfer (for a time period which covers at least 6-times the $t_{1/2}$ interval (not shown)) until the outer layer is largely stripped off as represented in Fig. 3, ■. Therefore, based on this kinetic analysis, we assume that a considerable change in surface energy during the efflux of outer leaflet DMPG molecules is very unlikely. This conclusion is further supported by the uniform binding behavior of the outer leaflet phospholipids on monolayered DMPG-Fe_3O_4 colloids, as we recently reported [22]. We indeed found that their adherence can be accurately described by a single Langmuir adsorption expression which, most interestingly, covers the entire free DMPG concentration regime in which the outer layer is built up.

Upon comparing the equilibrium transfer states shown in Figs. 1 and 3, at first sight, some contradiction may arise as to what extent the magnetoliposome DMPG molecules are involved in the overall transfer process. For instance, the observations made with DMPG acceptors (Fig. 1) indicate that both inner and outer leaflet molecules of the donors participate, whereas the final DMPG distribution observed with DSPG acceptors suggests that the inner shell DMPG molecules of the donors are reluctant to jump out from the magnetizable colloid. To check this latter point in more detail, we constructed Fe_3O_4 cores which were enrobed by only one layer of DMPG molecules (mmol DMPG/g $Fe_3O_4 \approx 0.30$, see Experimental Procedures). In these structures the lipid molecules are supposed to adopt a similar configuration as in the inner layer of magnetoliposomes, i.e., they presumably bind with their phosphate group to the iron oxide surface, thereby orienting their fatty acyl chain residues to the outside [22] *. To accentuate possible lipid depletion effects we confronted these monolayer-coated Fe_3O_4 structures (0.5 μmol DMPG/ml) with a 15-fold molar excess of DSPG vesicles. However, as shown in Fig. 3, ▲, the DMPG monolayer remains intact. This reluctancy to transfer cannot be entirely explained, either by the putative avid chemisorption of phosphate groups onto iron oxide (which in fact is also operative in the system with DMPG acceptor vesicles, see Fig. 1) [35,36], or by a low on-rate of lipid molecules in gel-state membranes (as observed by Nichols and Pagano [9] and Bayerl et al. [14]), since, as deduced from Fig. 3, □ and ■, outer layer DMPG molecules are readily accepted. Rather it appears that a prerequisite for the inner DMPG molecules to participate in the overall transfer event is that they have to be simultaneously replaced by outer shell phospholipids. In our system, the latter molecules are either continuously exchanged with the surrounding vesicles if these are constructed with transferable molecules (as in Fig. 1), or gradually withdrawn in the case where the surrounding membrane structures are built up of 'immobile' lipids (as in Fig. 3).

3. Transfer between DMPG magnetoliposomes and $DC_{15}PG$ vesicles

Next, we further investigated the transfer kinetics between donors and acceptors, both of which are built up of a different type of PG. This time, however, a net flow of the longer chain length homolog was measurable within the time course of the experiment. As an example, such a constellation was created in a mixture of DMPG magnetoliposomes and $DC_{15}PG$ vesicles. In vesicle structures, we found for both PG homologs a gel-to-liquid crystalline phase transition temperature at 23 and 31°C, respectively (not shown), which corresponds to the values (23.7 and 33.5°C, respectively) listed by Small [25].

At 37°C, the interaction course, followed in a 1:1 mixture of both structures (each present at a phospho-

* The possibility exists that on top of the monolayer-coated particles some additional DMPG molecules are attached (e.g., in a flat orientation or by interdigitation) with their polar headgroups protruding into the water phase, thereby improving electrostatic stabilization. The (partial) hydrophobic character of the colloids, however, can be deduced from the easiness by which additional PG molecules are adsorbed, the binding strength of which increases upon lengthening the hydrophobic tails (see Ref. 22). Also, the firm adhesion of monolayered particles (in contrast to bilayered ones) on the dextran matrix of Sephadex G-25 is illustrative for their (partial) apolar nature (unpublished observation).

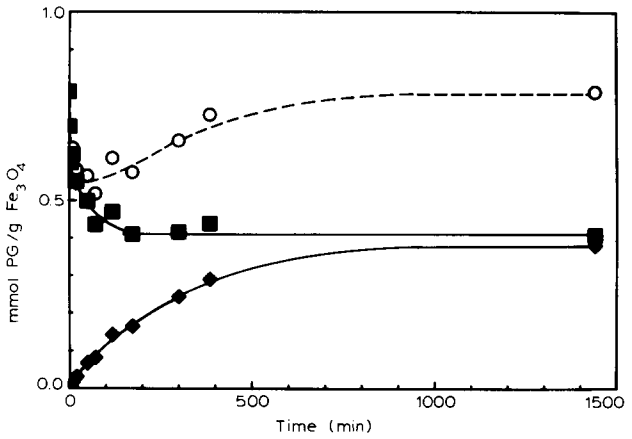

Fig. 4. Asymmetric, bidirectional phosphatidylglycerol transfer between DMPG magnetoliposomes and $DC_{15}PG$ small sonicated vesicles at 37°C in 5 mM Tes (pH 7.0). Equimolar amounts of donors and acceptors (each at a concentration of 0.5 μmol/ml) were used. After high-gradient magnetophoresis at different times, the magnetic structures were analyzed for total phospholipid content (○- - -○) and for the fatty acyl chain composition of the adsorbed phospholipids. ■, Represents the time-dependent decrease in DMPG content; ◆, corresponds to the time-dependent increase in $DC_{15}PG$ content.

lipid concentration of 0.5 μmol/ml), manifests a complex behavior. As shown in Fig. 4 (○), the total PG content of the magnetizable structure first rapidly decreases and subsequently increases to its original value of 0.80 mmol PG/g Fe_3O_4. A further inspection of the time-dependent variation in the lipid quality of the magnetoliposomes shows that the DMPG molecules escape from the magnetoliposomes (Fig. 4, ■) at a faster rate than $DC_{15}PG$ is able to desorb from the vesicles (Fig. 4, ◆). From the initial parts of the first-order profiles (not shown), halftimes of 20 and 214 min, respectively, are calculated. The higher $t_{1/2}$ value for $DC_{15}PG$ as compared with the value for DMPG is in accordance with the general observation that within a homologous series of phospholipids the transfer capacity decreases by a factor of 5–10 for each $-CH_2$-group which is added to a single fatty acyl chain [6,17,37].

To the best of our knowledge, only Duckwitz-Peterlein et al. [5] have studied similar asymmetric, bidirectional, spontaneous lipid fluxes. These authors used *trans*-Δ_9-hexadecenoate and *trans*-Δ_9-octadecenoate enriched phospholipid vesicles which were generated from total *E. coli* phospholipid extracts. By mathematically treating time-dependent changes in light scattering scans, it was concluded (in agreement with our observations) that the shorter chain length phospholipids had the highest transfer rate. The $t_{1/2}$ value they found was on the order of days. With magnetoliposomes, however, the lower limit in the detection of $t_{1/2}$ values can be reduced to a few minutes. Furthermore, since apparently energy barriers are not operative in our system (see above), we could calculate pseudo first-order rate constants of both the faster and slower moving lipid component; in the setup of Duckwitz-Peterlein et al. [5] only a $t_{1/2}$ value for the palmitoleoyl-containing lipids could be graphically detected. Moreover, the $t_{1/2}$ value they report (42 h in a 1 : 1 mixture) has to be considered as an average since the predominant phospholipid classes present in their starting vesicle populations consist of PG, phosphatidylethanolamine and cardiolipin. Undoubtedly, even with the same apolar tails, these phospholipid classes will transfer according to different rates [9,38]. By contrast, using well-defined phospholipids, the $t_{1/2}$ values which we calculated most probably reflect intrinsic transferabilities of DMPG and $DC_{15}PG$. The reasonably good agreement in $t_{1/2}$ values which we found for the initial DMPG transfer with the very different DMPG (Fig. 1), DSPG (Fig. 3) and $DC_{15}PG$ acceptor vesicles (Fig. 4) supports this statement.

Conclusions and physiological relevance

In the present work we have described the use of high-gradient magnetophoresis to fractionate magnetizable and non-magnetizable phospholipid structures. To highlight the unique potentialities of the technique in studying spontaneous lipid transfer processes, we purposively investigated in a quantitative way very extreme cases, i.e., symmetric and asymmetric bidirectional transport processes as well as unidirectional lipid fluxes.

Our hope is that the unique features of the newly designed magnetoliposomes may contribute to the understanding of lipid dynamics between biological membranes. Magnetoliposomes can indeed can be prepared with a lot of other 'fluidized' phospholipids (unpublished observations), including some naturally occurring ones. In this respect, it should also be possible to include magnetoliposomes with equal success into systems designed to monitor protein-stimulated phospholipid movements [39,40]. Finally, our magnetite-lipid complexes may be of relevance in other biological domains. More specifically, they can act as a model in the study of both structural and dynamic aspects of the envelope around magnetite particles which are encountered in a lot of magnetically responsive animals [41].

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References

1 Barańska, J. and Wojtczak, L. (1988) Arch. Biochem. Biophys. 260, 301–308.

2 Papahadjopoulos, D., Hui, S., Vail, W.J. and Poste, G. (1976) Biochim. Biophys. Acta 448, 245–264.
3 Jones, J.D. and Thompson, T.E. (1989) Biochemistry 28, 129–134.
4 De Cuyper, M., Joniau, M. and Dangreau, H. (1983) Biochemistry 22, 415–420.
5 Duckwitz-Peterlein, G., Eilenberger, G. and Overath, P. (1977) Biochim. Biophys. Acta 469, 311–325.
6 Ferrell, J.E., Jr., Lee, K.-J. and Huestis, W.H. (1985) Biochemistry 24, 2857–2864.
7 McLean, L.R. and Phillips, M.C. (1981) Biochemistry 20, 2893–2900.
8 Nichols, J.W. and Pagano, R.E. (1981) Biochemistry 20, 2783–2789.
9 Nichols, J.W. and Pagano, R.E. (1982) Biochemistry 21, 1720–1726.
10 Phillips, M.C., Johnson, W.J. and Rothblat, G.H. (1987) Biochim. Biophys. Acta 906, 223–276.
11 Massey, J.B., Gotto, A.M. and Pownall, H.J. (1982) Biochemistry 21, 3630–3636.
12 Nichols, J.W. (1985) Biochemistry 24, 6390–6398.
13 Xü, Y.-H., Gietzen, K., Galla, H.-J. and Sackmann, E. (1983) Biochem. J. 213, 21–24.
14 Bayerl, T.M., Schmidt, C.F. and Sackmann, E. (1988) Biochemistry 27, 6078–6085.
15 Crain, R.C. and Zilversmit, D.B. (1980) Biochemistry 19, 1433–1439.
16 Dawidowicz, E.A. and Rothman, J.E. (1976) Biochim. Biophys. Acta 455, 621–630.
17 McLean, L.R. and Phillips, M.C. (1984) Biochemistry 23, 4624–4630.
18 Ehnholm, C. and Zilversmit, D.B. (1972) Biochim. Biophys. Acta 274, 652–657.
19 Backer, J.M. and Dawidowicz, E.A. (1981) Biochemistry 20, 3805–3810.
20 Hoekstra, D. and Düzgüneş, N. (1986) Biochemistry 25, 1321–1330.
21 Kasper, A.M. and Helmkamp, G.M., Jr. (1981) Biochemistry 20, 146–151.
22 De Cuyper, M. and Joniau, M. (1988) Eur. Biophys. J. 15, 311–319.
23 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348.
24 Vaskovsky, V.E., Kostetsky, E.Y. and Vasendin, I.M. (1975) J. Chromatogr. 114, 129–141.
25 Small, D.M. (1986) in Handbook of Lipid Research, Vol. 4, The Physical Chemistry of Lipids: From Alkanes to Phospholipids, p. 631, Plenum Press, New York, London.
26 Frank, A., Barenholz, Y., Lichtenberg, D. and Thompson, T.E. (1983) Biochemistry 22, 5647–5651.
27 Homan, R. and Pownall, H.J. (1988) Biochim. Biophys. Acta 938, 155–166.
28 Pagano, R.E., Martin, O.C., Schroit, A.J. and Struck, D.K. (1981) Biochemistry 20, 4920–4927.
29 Scherphof, G., Van Leeuwen, B., Wilschut, J. and Damen, J. (1983) Biochim. Biophys. Acta 732, 595–599.
30 Wimley, W.C. and Thompson, T.E. (1989) Biophys. J. 55, 114a.
31 De Kruijff, B. and Baken, P. (1978) Biochim. Biophys. Acta 507, 38–47.
32 De Cuyper, M. and Joniau, M. (1986) in Liposomes as Drug Carriers (Schmidt, K.H., ed.), pp. 94–99, Thieme Verlag, Stuttgart, New York.
33 De Cuyper, M., Joniau, M., Engberts, J.B.F.N. and Sudhölter, E.J.R. (1984) Coll. Surfaces 10, 313–319.
34 Lichtenberg, D., Freire, E., Schmidt, C.F., Barenholz, Y., Felgner, P.L. and Thompson, T.E. (1981) Biochemistry 20, 3462–3467.
35 Sczekan, S.R. and Joshi, J.G. (1989) Biochim. Biophys. Acta 990, 8–14.
36 Van der Meeren, P., Vanderdeelen, J. and Baert, L. (1989) Coll. Surfaces 42, 9–22.
37 Massey, J.B., Hickson, D., She, H.S., Sparrow, J.T., Via, D.P., Gotto, A.M., Jr. and Pownall, H.J. (1984) Biochim. Biophys. Acta 794, 274–280.
38 De Cuyper, M. and Joniau, M. (1985) Biochim. Biophys. Acta 814, 374–380.
39 Helmkamp, G.M. (1986) J. Bioenerg. Biomembr. 18, 71–91.
40 Barsukov, L.I., Hauser, H., Hasselbach, H.-J. and Semenza, G. (1980) FEBS Lett. 115, 189–192.
41 Gorby, Y.A., Beveridge, T.J. and Blakemore, R.P. (1988) J. Bacteriol. 170, 834–841.