Eur Biophys J (1988) 15: 311–319

European Biophysics Journal

Magnetoliposomes

Formation and structural characterization

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Received April 13, 1987/Accepted October 15, 1987

Abstract. The adsorption of different types of phosphatidylglycerols onto magnetizable solid particles is studied. The super-paramagnetic magnetite spheres used have an average diameter of only 14 nm and are stabilized by lauric acid to keep them in solution. During incubation and dialysis of this water-based magnetic fluid in the presence of preformed sonicated phospholipid vesicles, magnetoliposomes are formed which are captured from solution with high efficiency by high-gradient magnetophoresis. Support for the bilayer character of the phospholipid coat is derived from both theoretical calculations and experimental data. Phospholipids which form the *inner* monolayer are adsorbed very quickly with their charged headgroup orientated towards the iron oxide surface. The high-affinity character of the binding is reflected in the adsorption isotherms and is further illustrated by their non-extractability with high concentrations of Tween 20. The *outer* layer assembles through interaction with the exposed hydrocarbon chains. As compared to the inner layer, the phospholipids adsorb at a much slower rate and are displaced by Tween 20 concentrations which usually disrupt conventional membranes. The adsorption isotherms for this layer obey the Langmuir expression. The affinity constants, derived from them, progressively increase as the hydrophobic nature of the phosphatidylglycerols is more pronounced.

Key words: Phospholipid vesicles, phospholipid adsorption, magnetoliposomes, high-gradient magnetophoresis, membrane models

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Abbreviations: DXPG, di-fatty acyl form of phosphatdylglycerol where X = L, Lauroyl; *M*, myristoyl; $C_{15:0}$, pentadecanoyl; *O*, oleoyl. TES, 2-((*tris*(hydroxymethyl)methyl)amino)ethanesulfonic acid

Introduction

Important contributions to the present knowledge of the structure and dynamics of biological membranes have been achieved by observing the behaviour of artificial phospholipid membranes in external fields such as gravitation, hydrodynamic velocity gradients and electric and magnetic fields. For instance, by free-flow electrophoresis we were able to assess the impact of the membrane surface charge on intervesicular phospholipid transfer processes (De Cuyper et al. 1984). Also, NMR spectroscopy has proven to be a powerful technique for structural analysis of membranes, for instance to study membrane asymmetry in the presence of interacting paramagnetic shift reagents (Düzgünes et al. 1983) or to monitor the transition of lamellar-to-inverted micelle structures (Gruner et al. 1985).

In contrast to forces operating in centrifugation and electrophoresis, magnetic ones have not been explored for separation purposes. Obviously, the reason for not using magnetic techniques is that phospholipids demonstrate only weak diamagnetic properties which are attributable to the fatty acyl chains and which – at first sight – should expel them from high magnetic field zones. However, even in lipid clusters (as membranes are) in which the diamagnetic anisotropy is considerably enhanced (Maret and Dransfeld 1985), the effect of the magnetic field remains limited to an elastic deformation of the vesicles into elongated ellipsoids. Optical birefringence (Helfrich 1973; Maret and Dransfeld 1985), optical turbidity (Braganza et al. 1984) and NMR measurements (Seelig et al. 1985) indeed reveal that the phospholipids tend to orient with their hydrocarbon chains perpendicular to the field vector (and hence the plane of the lipid bilayer aligns parallel to the field). A magnetically induced translation force however has not been observed.

Since ferromagnetic attraction forces exceed diamagnetic repelling forces by several orders of magnitude (Hirschbein et al. 1982), we thought that a valu-

able alternative for removal of bilayer structures from a suspension with the use of magnetic forces could be achieved by forming a complex with ferromagnetic particles. As a magnetic material we used a so-called magnetic fluid, which in our experiments consists of ultramicroscopic (nm range) stabilized magnetite (Fe_3O_4, ferrous-ferric oxide) colloids, which we mixed with sonicated vesicles. During this incubation step, the phospholipids arrange around the iron core in a bilayer configuration, thereby producing magnetoliposomes. In this paper, we present detailed information concerning the generation and biophysical characterization of the magnetite-covering phospholipid membranes, as well as the conditions in which they can be selectively captured in a high-gradient magnetic field. As a model phospholipid we have chosen dimyristoylphosphatidylglycerol (DMPG) since its biophysical behaviour is well documented in the literature (Findlay and Barton 1978; De Cuyper et al. 1983). A few analogues have also been studied in a comparative way.

Experimental

Materials

DMPG and DPPG were synthetized from the corresponding phosphatidylcholines by a single step transphosphatidylation catalyzed by phospholipase D according to Papahadjopoulos et al. (1973). High purity DOPG, $DC_{15:0}PG$ and DLPG were purchased from Avanti Polar Lipids Inc (Birmingham, AL, USA) in powder form as the sodium salt. TES, lauric acid and lyso-PG were from Sigma (St. Louis, MO, USA) and tridecanoic acid was from Merck (Darmstadt, FRG). $FeCl_2$.4aq and $FeCl_3$.6aq (pro analysis grade) were obtained from UCB (Belgium).

Methods

Preparation of magnetoliposomes and fractionation by high-gradient magnetophoresis. Stabilized colloidal magnetite was generated according to Reimers and Khalafalla (1976). The iron core was prepared by coprecipitation of 3 g $FeCl_2$.4aq and 6 g $FeCl_3$.6aq (dissolved in 25 ml water) with an excess (12.5 ml) of concentrated ammonia. The resultant precipitate was washed two times with 50 ml ammonia/water (5/95) and heated to 90 °C for 4 min, meanwhile adding 1 g of lauric acid (instead of oleic acid as reported by Reimers and Khalafalla 1976) as a dispersing agent. The resulting coated particles were diluted with water and stored at a concentration of 28 mg Fe_3O_4/ml. Incubation and dialysis (molecular weight cut-off: 10,000) of this water-adapted magnetic fluid with preformed vesicles results in Fe_3O_4 particles covered with phospholipids (see below). Non-adsorbed phospholipids were separated from the Fe_3O_4-phospholipid colloids by high-gradient magnetophoresis. Portions (1 ml) were pumped (6 ml/h) through the tubing plugged with magnetic steel wool (~ 60 mg) (Bekaert, Belgium), placed in the 3.1 mm gap between the two conical poles (smallest diameter 25 mm) of an electromagnet (Type LMM 50, Le Matériel Magnétique, Brest, France) operating at full power (6 A, 20 V) unless otherwise specified. In these conditions, but in the absence of the magnetic filter, the magnetic field at the centre of the poles equals 2 Tesla as monitored by a Hall-effect field probe (Yokohama Gauss Meter Type 3251). After separation, the retentate was thoroughly washed with buffer (0.5 ml) to remove non-adsorbed phospholipids remaining in the liquid surrounding the iron fibres. Then the magnetic field was turned off and the retentate was washed through with buffer at high speed (500 ml/h) and collected.

Construction of adsorption isotherms. Binding of phospholipids to Fe_3O_4 was studied by adding increasing volumes of sonicated phospholipid dispersions to a constant volume of the lauric acid-coated magnetite stock solution (0.12 ml). Buffer (5 m*M* TES, pH 7.0) was added to bring the final volume to 2.54 ml. The incubation mixtures were then dialyzed separately at 37 °C for 48 h against 500 ml of buffer liquid with four changes at regular time intervals. After high-gradient magnetophoresis both the retentate and the effluent were analyzed for their iron and phosphate content. The true phospholipid equilibrium concentration in the effluent was calculated by correcting for the phospholipids which were associated with the small amount of magnetite which occasionally was not captured (max. 2% of the original Fe_3O_4 amount).

Other methods. Phospholipid vesicles were prepared by sonication (150 W MSE disintegrator) at a concentration of 5–10 mg/ml in the TES buffer and centrifuged at 37 °C for 10 min at 20,000 × *g* to remove metal particles derived from the sonication tip. Phosphate analysis was done according to the method of Vaskovsky et al. (1975) and iron concentrations were measured by atomic absorption at 372.2 nm (Varian AA6). Fatty acid composition of the phospholipids and magnetic colloids was checked by gas-liquid chromatography (Packard Model 419). Electron micrographs were taken on a Zeiss EM10C apparatus.

Results

I. Characterization of the Fe_3O_4-lauric acid complexes

As deduced from the electron micrograph (Fig. 1), the iron cores of the Fe_3O_4-lauric acid colloids have a quasi-uniform diameter of only 14 nm. Even in high magnetic fields (up to more than 2 Tesla) these small particles are not captured (Fig. 2). The presence of the magnetic filter in the separation tube does, however, greatly increase the attraction efficiency (98% – 99%).

In the Fe_3O_4 stock solution, the iron oxide spheres are surrounded by approximately 1.56 mmol lauric acid per gram Fe_3O_4 as judged by gas-liquid chromatography using tridecanoic acid as an internal standard (not shown). In these conditions the magnetic particles remain in solution for at least one year. The stability however is abrogated upon depleting the iron oxide from its lauric acid coat, for instance during dialysis or as a result of a single magnetic separation cycle (Table 1 A).

Table 1. Lipid depletion of (A) lauric acid-coated Fe_3O_4 particles and (B) DMPG-covered magnetoliposomes during consecutive magnetophoresis sequences in 5 m*M* TES buffer, pH 7.0. The starting iron oxide suspension (Fe_3O_4 concentration: 1.32 mg/ml) contained either (A) 1.56 mmol lauric acid/g Fe_3O_4 or (B) 5.20 mmol DMPG/g Fe_3O_4

Number of magnetic separation cycles	Lauric acid/Fe_3O_4 [mmol/g]	DMPG/Fe_3O_4 [mmol/g]
	A	B
1	0.37[a]	0.95
2	–	0.88
3	–	0.82
4	–	0.83

[a] At this stage, the Fe_3O_4-lauric acid colloids are no longer stabilized and precipitate on the magnetic filter

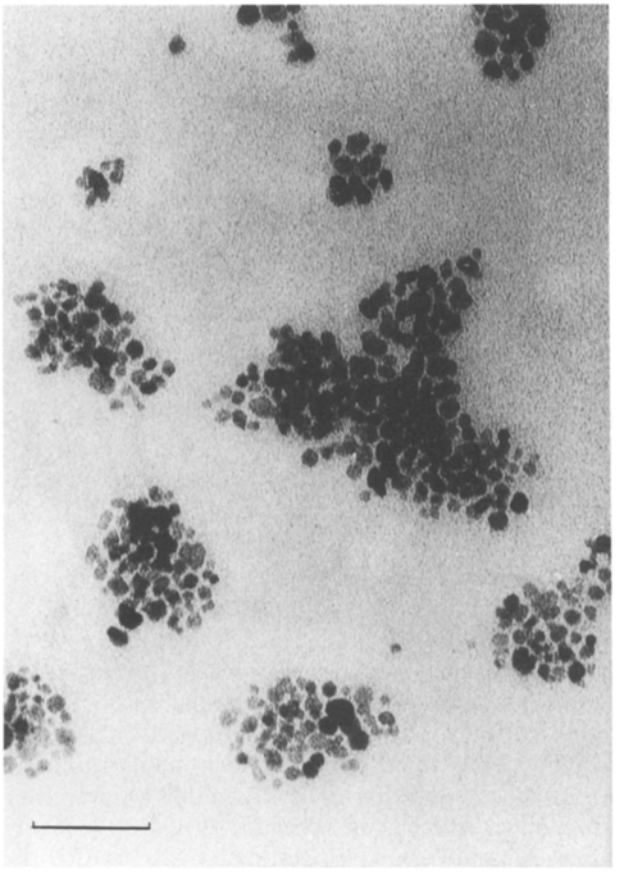

Fig. 1. Transmission electron micrograph of lauric acid-coated Fe_3O_4 particles. *Bar:* 100 nm

II. Kinetics of Fe_3O_4-DMPG association

Precipitation of magnetite during dialysis of the above described magnetic fluid is prevented by including a sufficient amount of DMPG vesicles ($\geq$ 0.3 mmol DMPG/g Fe_3O_4), suggesting that DMPG takes over the stabilizing role. To examine the rate of DMPG adsorption the following experiment was done: 1.7 ml of the magnetic fluid (48 mg Fe_3O_4) was incubated with 28 ml DMPG vesicles (185 µmol DMPG) and dialyzed against 5 m*M* TES buffer at 37 °C for 2 days with four changes of buffer of 500 ml each at regular time intervals. At suitable times, 1 ml of the mixture was subjected to a magnetophoresis cycle and the retentate was analyzed for its fatty acid composition (and also for its iron content to take into account small changes in magnetite concentration during dialysis). The time-dependent increase in DMPG/Fe_3O_4 ratio

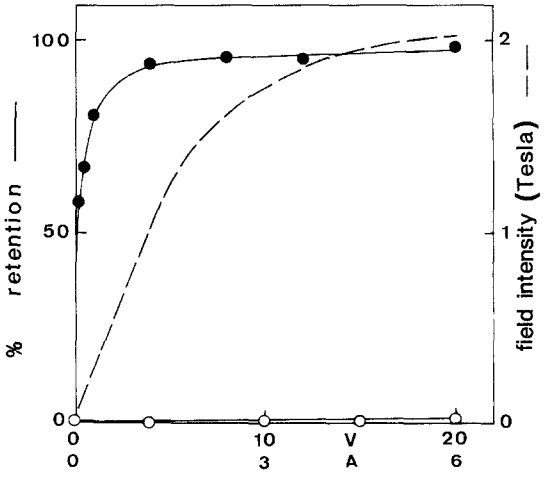

Fig. 2. Magnetically induced retention of 14 nm-sized, lauric acid-coated magnetic particles in the absence (○) and presence (●) of a magnetic filter. The magnetic field intensity (*dashed line*) in the different electrical conditions is measured in the absence of the magnetic filter. The distance between the two magnet poles equals 3.1 mm

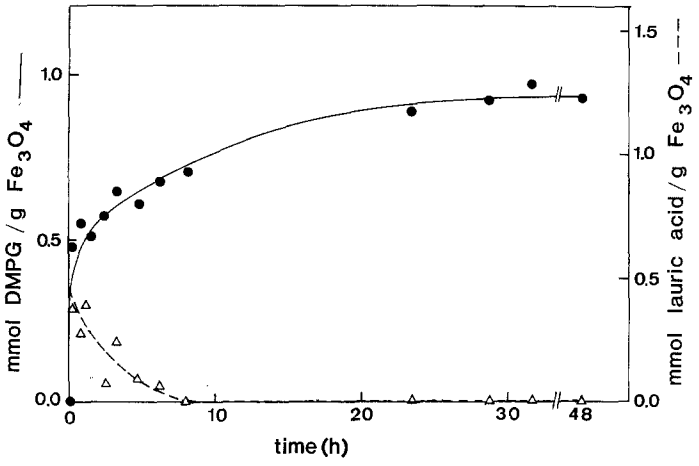

Fig. 3. Displacement of lauric acid from the magnetite surface by DMPG-vesicles at pH 7.0 and 37 °C. The *solid curve* (•) represents the time-dependent DMPG association; the *dashed line* (△) illustrates lauric acid depletion

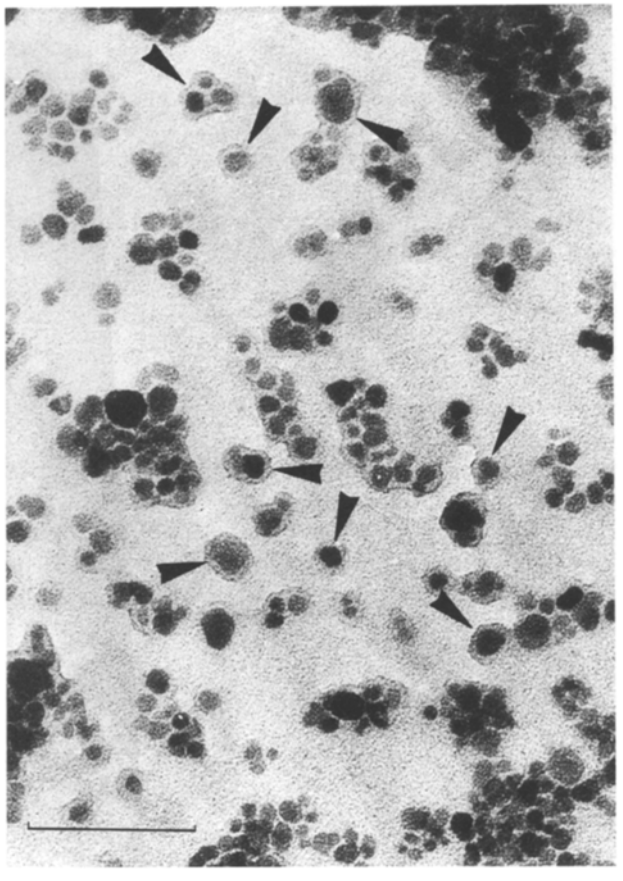

Fig. 4. Electron micrograph of a representative sample of Fe_3O_4-DMPG colloids, negatively stained with a 2% solution of phosphotungstate. The *arrows* clearly indicate the typical phospholipid bilayer architecture (see Discussion). *Scale bar:* 100 nm

is illustrated in Fig. 3. 30% – 35% of the phospholipid adsorption occurs within the first minute (or even faster). The next sequence occurs at a slower rate and a steady state ratio of 0.90 – 0.95 mmol/g Fe_3O_4 is reached in the ensuing 24 h. Concomitantly, lauric acid is displaced and removed completely after about 8 h. In transmission electron microscopy, the phospholipid envelop appears as a thin electron-dense layer which is separated from the magnetite surface by an electron translucent sheet of uniform width (Fig. 4).

III. Adsorption isotherm of Fe_3O_4-DMPG association

The adsorption of DMPG onto Fe_3O_4 is further studied by constructing the adsorption isotherm (Fig. 5, curve •) which expresses the amount of DMPG adsorbed per gram Fe_3O_4 as a function of the equilibrium concentration of DMPG (i.e. the amount of DMPG which remains free in solution after adsorption has reached a steady-state). Based on the kinetic data of Fig. 3 an incubation period of 48 h was considered to be appropriate before starting the magnetic separation. At low total phospholipid concentration (less than $\approx$ 0.3 mmol DMPG per g Fe_3O_4), DMPG is completely adsorbed, or at least there is no measurable amount remaining in solution. The initial part of the isotherm is therefore vertical. At higher phospholipid concentrations the adsorption graph is concave towards the concentration axis until a saturation level of 0.95 mmol/g Fe_3O_4 is reached. At this saturation level, the integrity of the DMPG coating is preserved even after five subsequent magnetic separation cycles, in contrast to the lauric acid complexes (Table 1).

IV. Adsorption isotherms with phosphatidylglycerols differing in the nature of their hydrophobic moiety

Sorption measurements were also carried out on systems containing phosphatidylglycerols which have different fatty acyl side chains (DOPG, $DC_{15:0}PG$, DMPG, DLPG and lysoPG, which mainly contains C_{16} or C_{18} chains) (Fig. 5). It is seen that at saturation the number of molecules per unit weight of Fe_3O_4 is quite similar for all PG's investigated. Also, in all cases the initial high-affinity zone ends at approx. 1/3 of the saturation level. A most remarkable difference howev-

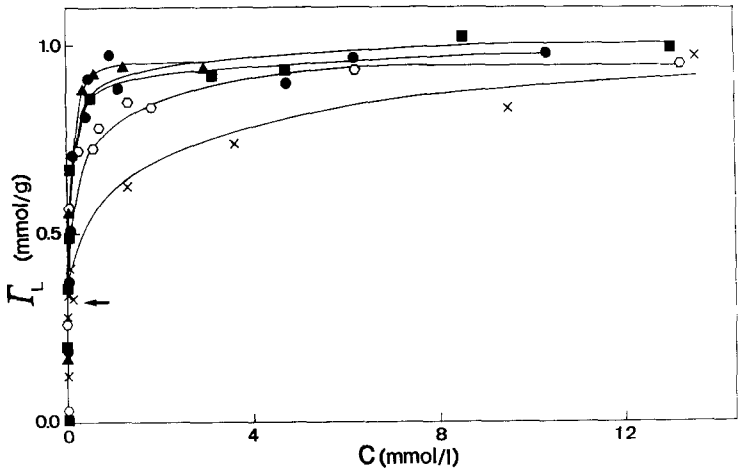

Fig. 5. Adsorption isotherms for different types of phosphatidylglycerols on magnetite (▲) DOPG, (■) $DC_{15:0}PG$, (●) DMPG, (○) DLPG, (×) lysoPG. Incubation and dialysis occurred at 37 °C for 2 days in 5 m*M* TES buffer, pH 7.0. The arrow marks the change from the high-affinity to the L-shaped adsorption profile

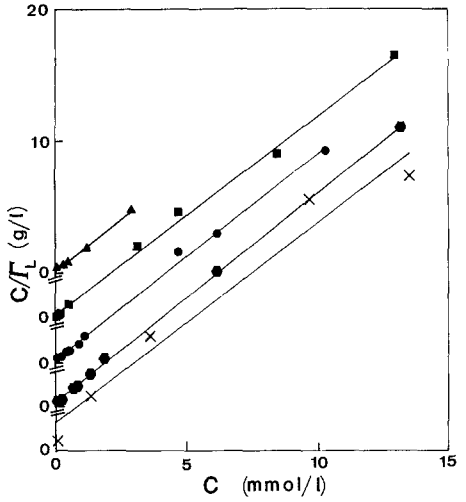

Fig. 6. Linearised form of the Langmuir adsorption isotherms, derived from the data in Fig. 4. (▲) DOPG, (■) $DC_{15:0}PG$, (●) DMPG, (●) DLPG and (×) lysoPG. Note the shift on the y-axis

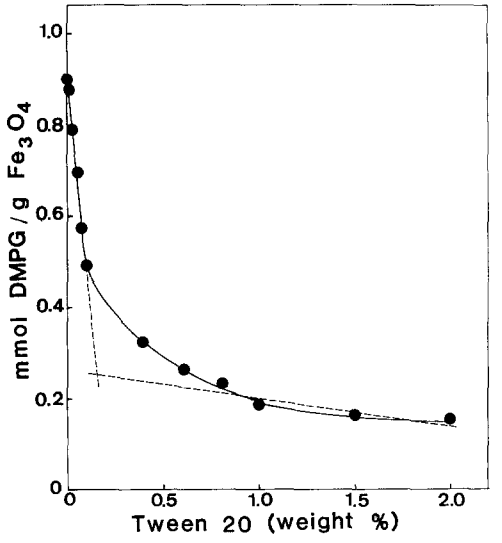

Fig. 7. Extraction of DMPG-saturated Fe_3O_4 magnetoliposomes by Tween 20. The dashed lines are linear regression approximations of the extraction profile of the two subpopulations of phospholipids involved: the outer shell phospholipids at high DMPG/Fe_3O_4 ratios and the inner leaflet phospholipids at lower DMPG/Fe_3O_4 ratios

er is that the smaller the apolar part, the higher is the concentration necessary to reach the saturation level. In the case of the diacylphospholipids the isotherms beyond the high-affinity zone apparently obey the Langmuir type equation (Fig. 6):

$$\frac{c}{\Gamma_L} = \frac{1}{\Gamma_L^\circ}\left(c + \frac{1}{K_L}\right), \tag{1}$$

where Γ_L is the number of moles of lipid adsorbed per gram of Fe_3O_4 in the Langmuir zone, Γ_L° is the value at saturation, c is the phospholipid equilibrium concentration (in mmol/l) and K_L is the association constant. For DOPG, $DC_{15:0}PG$, DMPG and DLPG the K_L values were calculated to be 12.2, 7.5, 6.4 and 4.3 mM^{-1} respectively. In the case of the single chain lysoPG a mean K_L value of 0.7 mM^{-1} was found.

V. Detergent treatment

Treatment of DMPG saturated Fe_3O_4 colloidal particles with increasing amounts of the non-ionic detergent Tween 20 (from 0 to 2 weight %) decreases their phospholipid content (Fig. 7). The data points are fitted using linear regression analysis by two straight lines: one between 0% and 0.1% Tween 20 (regression coefficient 0.99_1) and the other between 0.8% and 2% (regression coefficient 0.88_6). The intersection of the two lines falls at an abscissa value of 0.15% Tween 20 and a DMPG/Fe_3O_4 ratio of approx. 0.27 mmol/g.

Discussion

The interest in liposomes as carrier system for water-soluble drugs has resulted in an explosion of papers dealing with the enveloping of hydrophilic substances by the phospholipid bilayer structure (for a review see Hauser 1982). A common characteristic of the various protocols is that the encapsulation occurs simultaneously with the formation of the membrane. The trapping efficiency however largely depends on the method used. By sonication only 0.5% – 1% is built in

whereas by reversed organic solvent evaporation or detergent removal the efficiency mounts to 60% – 70% (Szoka and Papahadjopoulos 1978). The above-mentioned procedures fundamentally differ from our method not only in the way in which the bilayer is generated (see below) but also because in our case the inner space is completely stacked with the (magnetic) material.

For Fe_3O_4-phospholipid colloids with a diameter similar to that of small unilamellar vesicles, a complete packing of the interior with magnetite is an absolute requirement since attraction in a magnetic field varies greatly with Fe_3O_4 grain size. Indeed, the force, F, by which a particle is attracted equals

$$F = V\chi_v H\frac{dH}{dx} \tag{2}$$

with V: the volume of the particle; χ_v: the magnetic susceptibility per unit volume; H: the magnetic field strength and dH/dx: the magnetic field gradient (Hirschbein et al. 1982). In practice, Fe_3O_4 particles with diameters below 50 nm (so-called subdomain entities) are only captured in high-gradient magnetic fields. In our set-up (with 14 nm diameter colloids) such fields are generated by magnetizable steel fibres which distort and concentrate the magnetic field lines in their vicinity and which we preferred to be corrosion-free to avoid any interference in subsequent iron determinations. The main advantages (Hirschbein et al. 1982) of the use of subdomain sized magnetite are (1) that gravity forces are overshadowed by Brownian thermal agitation and (2) that, after exposure to a magnetic field, the coated colloids have no magnetic remnant so that they can be very easily released from the magnetic filter without the need for demagnetization or mechanical stress (e.g. sonication).

The orientation of lauric acid on the Fe_3O_4 surface is not studied in detail here, but indirectly we have some evidence that the COOH groups face the magnetite surface with the hydrophobic chain protruding into the aqueous medium. Indeed, covering of the iron oxide colloid with the longer chains of oleic acid no longer produces water-based but organic-adapted magnetic fluids (unpublished observations; Reimers and Khalafalla 1976).

Lauric acid molecules, which prevent coagulation of Fe_3O_4 particles, are easily displaced by phospholipids (Fig. 3), pointing to a strong binding of the phosphate-containing lipids with the iron oxide. In fact, this high-affinity behaviour is not unexpected since the "phosphatizing" process in which iron (or iron oxides) are immersed in dilute phosphoric acid solutions to produce a corrosion-resistant phosphate layer, is widely used in industry (Puderbach and Grosse-Böwing 1984). In addition, the phospholipid-Fe_3O_4 association is probably further improved by the lateral hydrophobic interchain interactions which are supposed to be more pronounced in the case of phospholipids than with lauric acid. The water solubility of ionic surfactants, which gives an idea about their hydrophobic character (Tanford 1980) indeed is much higher for lauric acid (critical micellar concentration $\approx 2.8 \cdot 10^{-2} M$; Lindman 1984) than for the phospholipids we used here (critical bilayer concentration typically $> 10^{-6} M$, Tanford 1980; see also De Cuyper et al. 1983). In line with this consideration we also found that, in contrast to the lauric acid-coated colloids, DMPG covered ones withstand successive magnetic fractioniation cycles (Table 1). Consequently, phospholipid-coated, nanometer-sized magnetic particles can be envisaged as dilution-insensitive magnetic fluids which may be useful in some selected industrial applications (Rosensweig 1966).

A lot of confusion still exists in the literature concerning the configuration adopted by phospholipids when they adsorb onto hydrophilic solid supports from aqueous solutions. For instance by a sequential transfer of two monolayers by the Langmuir-Blodgett technique, Albrecht et al. (1982) and Tamm and McConnell (1985) formed phospholipid bilayers on glass and quartz surfaces. From Tamm's diffusion experiments however, it was concluded that there must be a water-filled space between the bilayer and the solid support. If phospholipid vesicles instead of lipid monolayers are used as starting structures, the situation is even more obscure. Thus, from the elegant work of Margolis et al. (1983) it appears that the integrity of dipalmitoyl- and distearoylphosphatidylcholine vesicles is not affected by mixing them with ferrite particles. On the other hand, the thorough investigation of Jackson et al. (1986) reveals that during interaction of dipalmitoylphosphatidylcholine vesicles with curved glass surfaces, an expanded phospholipid monolayer is formed. By contrast, Horn (1984) claimed that a bilayer spontaneously forms by immersing mica surfaces in a solution of sonicated vesicles, but Marra (1985) questioned the reproducibility of the method.

In the present work, both theoretical and experimental evidence is gathered to prove that, at saturation, we really are dealing with a bilayer architecture. Using a diameter of 14 nm for the Fe_3O_4 particles (decuded from Fig. 1; see also Hough and Rendall 1983), a cross-sectional area of 0.62 nm^2 for a PG polar headgroup (Toko and Yamafuji 1980) and a bilayer thickness of 3.5 nm for a DMPG membrane (Lewis and Engelman 1983; Marra and Israelachvili 1985), the calculated number of phospholipids for an inner monolayer surface coverage is 992 molecules and 2,233 molecules for the outer layer. This figure of 3,225 molecules agrees with the number of phospholipid molecules found in small unilamellar vesicles (Huang

1969). Assuming a density of 5.1 g/cm^3 for Fe_3O_4 (Handbook of chemistry and physics 1975–1976), these calculated values correspond to a ratio of 0.73 mmol DMPG/g Fe_3O_4 [1] which approaches the saturation value in the adsorption isotherm (Fig. 5, curve •).

This theoretical indication for the existence of a bilayer is further supported by experimental evidence. For instance, the sequence in electron density (dark/bright/dark) found for the confines of our biocolloids (Fig. 4) strongly mimics that of other membrane structures. In our view however, another most conclusive indication is obtained from the shape of the adsorption isotherms, which moreover shed some light on the different phases of the adsorption process: the first step involves the so-called high-affinity behaviour which at higher PG concentrations is followed by a Langmuirian type of adsorption.

Irrespective of the type of PG studied, the high-affinity zone stops at approximately 1/3 of the PG/Fe_3O_4 ratio found at saturation. Thus, in view of the above mentioned calculations, we believe that this zone corresponds to the build-up of the *inner* monolayer. Interestingly, in agreement with this picture is the empirical observation that the stability of the colloidal suspension (except in the case of DOPG and lysoPG) considerably increases as this value is reached. Different orientational positions of the phospholipids with respect to the iron oxide surface can be imagined. Both a flat and perpendicular (with the polar headgroup facing the medium) orientation must be excluded: the first possibility because in a flat position the different PG species clearly would occupy different areas, and the second because the DOPG and lysoPG complexes should be equally well soluble as the colloids with the shorter chain length PG's. More likely, as with lauric acid, the phospholipids are adsorbed head-on to the metal oxide surface thereby presenting the apolar tails to the aqueous phase [2]. As already discussed above in considering the lauric acid-phospholipid competition, also the very fast, initial binding of DMPG to Fe_3O_4-lauric acid colloids (Fig. 3), and the resistance of the inner layer to an extraction with high concentrations of Tween 20 (Fig. 7), points to a very strong binding character (see also De Cuyper and Joniau 1987).

As compared to the inner layer, the *outer* one is generated with some difficulty since now mainly hydrophobic Van der Waals forces are involved. The choice of the correct isotherm to describe the overall adsorption behaviour is not always straightforward. For instance, in theory the Langmuir expression is only valid in an ideal situation where there are no solute-solute or solute-solvent interactions. However, as mentioned by Attwood and Florence (1983), in reality it is often found that Langmuirian mathematics adequately describe the adsorption behaviour of ionic surfactants onto hydrophobic surfaces, e.g. the binding of sodium dodecylsulphate or dodecyltrimethylammonium bromide onto (hydrophobic) Graphon. Similarly, our experimental data are also well fitted by Langmuir isotherms. Most probably, during adsorption the lipids gradually change from a flat to a perpendicular mode of orientation. The affinity constants in these two (extreme) conditions probably do not differ significantly for the various diacylphosphatidylglycerols since the c/Γ_L versus c plots are linear over the entire concentration range (Fig. 6). Moreover, in this context we note Hough and Rendall's (1983) considerations concerning the deposition of amphiphilic surfactants on surfaces with a hydrocarbon character, as our single layer-coated Fe_3O_4 particles have. These authors proposed that the increment in free energy change per methylene group due to cohesive chain-surface interactions is of the same order of magnitude as the incremental change in free energy brought about by chain-chain associations. In view of this reasoning it remains difficult to explain why lysoPG does not follow rigorously Langmuir mathematics beyond the high-affinity zone. Possibly, the deviating behaviour reflects an assembly structure which differs from a bilayer. Single chain amphiphiles are indeed excellent candidates to create interdigitated phases (Jain 1983).

From the Tween 20 extraction experiments on DMPG magnetoliposomes (Fig. 7) it further appears that the Fe_3O_4-inner layer binding is much stronger than the inner-outer layer association. A relatively weak interlayer association was also theoretically calculated by Georgallas et al. (1984). The fact that both the outer layer of our magnetoliposomes and that of classical small unilamellar vesicles are destroyed within a similar concentration range of non-ionic detergents (Lichtenberg et al. 1983; Sweet et al. 1985), provides a first indication that the new type of lipo-

1 If it is assumed that the number of phospholipids in the outer monolayer is determined by the geometrical constraint associated with the densely packed fatty acyl chains in the middle of the bilayer (radius 8.75 nm) the number of molecules in the outer layer (cross sectional area 0.46 nm^2; Huang and Mason 1978) equals 2,090, resulting in a DMPG/Fe_3O_4 ratio of 0.70.
One can also estimate the number of lipid molecules by dividing the volume of the bilayer (inner radius 7 nm, outer radius 10.5 nm) by the volume of a DMPG molecule. According to the procedure used by Marra (1986) the latter one equals 1.054 nm^3 (0.300 nm^3 for the PG polar headgroup and 0.754 nm^3 for the hydrophobic chain). In this case a ratio of 0.73 is calculated.

2 Within the first minutes of magnetoliposome formation, during which the inner monolayer is built up, we never observed particle flocculation, not even with the longer chain length DOPG. Most probably the single layer coated Fe_3O_4 spheres remain stabilized by some residual laurate molecules (see Fig. 3).

some we introduced mimics conventional membrane types.

Conclusions

The principal aim of this work was to demonstrate that phospholipids spontaneously arrange around a magnetite colloid in a bilayer configuration. In this way, we not only designed a new type of liposome but we also introduced a new separation method with remarkable potentialities which cannot be achieved by other fractionation methods. Indeed, high-gradient magnetophoresis allows the separation of magnetic and non-magnetic membranes which have the same dimensions and charge and which moreover are composed of identical phospholipid components.

Acknowledgements. We wish to express our appreciation for the valuable assistance of Ms. K. Vancaillie who carried out part of the experimental work. Thanks are also due to Dr. D. Horn and Dr. J. Eisenlauer (BASF, Ludwigshafen, Federal Republic of Germany) for providing us with some samples of magnetic fluids during the initial stage of this work and to Dr. Ir. I. Lefever, Ir. J. Saelens and Ir. J. Samyn (Bekaert Steel Corporation, Zwevegem, Belgium) for the magnetic stainless steel fibres. This work was supported by the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Contract nr. 3.0063.86).

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