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Trapping of dextran-coated colloids in liposomes by transient binding to aminophospholipid: preparation of ferrosomes

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Abstract

A procedure is described that allows to increase the efficiency of the loading of liposomes with dextran-stabilized iron oxides (MION). The method produces a preparation of liposomes (REVs) with high iron oxide content as a result of transient binding of oxidized dextran with aminogroups of aminophospholipids. Phosphatidylethanolamine (PE)-containing lipid mixtures (PC/DOPE/CH or SM/DOPE/CH, 9:2:9 molar ratio) in organic phase were combined with oxidized MION at pH 8. Liposomes then were obtained by reversed-phase evaporation. Liposomes, 263 ± 89 nm in diameter, contained up to 11.8 mol Fe/mol phospholipid (encapsulation yield 49%). 10.2% of liposome-associated iron was dissociated from liposomes upon changing the pH to 4.5. When lipid compositions of extracts prepared from liposomes incubated at pH 4.5 and pH 8.0 were compared, an increase of relative PE-content in extracts of liposomes incubated at lowered pH was detected. This indicates a dissociation of imine bonds between aldehydes on the MION surface and PE. The accessibility of liposomal PE for acylation was demonstrated by modification with an activated ester of methoxy poly(ethylene glycol) succinate. Control liposomes, containing no aminophospholipid, or PE-containing liposomes obtained in the presence of non-oxidized MION, were 3.5–5-fold less effective for MION encapsulation and showed extensive aggregation.

Key words: Liposome; Phosphatidylethanolamine; Iron oxide; Magnetite; Dextran

1. Introduction

Liposomes with encapsulated superparamagnetic colloids (ferrosomes) were initially described as tools for cell sorting by the application of the external magnetic field [1]. More recently a variety of paramagnetic molecules (usually chelates of lanthanides or transitional metals) have been trapped in liposomes and resultant magnetoliposomes have been used as contrast agents for magnetic resonance (MR) imaging (reviewed in Ref. [2]). In the majority of applications, liposomes loaded with (super)paramagnetic materials were used for delivery of encapsulated [3,4] or lipid-bound [5] contrast material into tissues of either the reticulo-endothelial system or blood. Lipid vesicles have also been used to deliver iron oxide colloids into immunocompetent cells in order to trace homing sites (e.g., lymph nodes) [6].

Ferrosomes may be produced by adsorption of lipids directly onto the surface of iron oxide crystals [7]. In this case the first monolayer of lipids is tightly bound to the iron oxide with the lipid headgroups while the outer monolayer assembles presumably by interaction of hydrophobic fatty acid chains [8]. These lipid-coated particles have very little water surrounding the iron oxide core. They were shown to assist visualization of focal inflammation sites after being taken up by neutrophils [9].

Encapsulation of solutes into true liposomes, however, implies their entrapment into the internal water compartment of a liposome. Two methods for such a true encapsulation have been suggested so far.

Abbreviations: PE, 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; PC, 1,2-diacyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; PS, 1,2-diacyl-*sn*-glycero-3-phospho-L-serine; SM, *N*-acylsphingosylphosphocholine; CH-cholesterol; MION, monocrystalline iron oxide nanocompound; REV, reversed-phase evaporation vesicle(s).

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The first method is based on the ability of lipid bilayers to trap water and solutes during spontaneous liposome formation as a result of lipid hydration, [10,11] or evaporation of organic phase from lipid-stabilized emulsions of water in organic phase (reversed-phase evaporation vesicles, REV) [12,13].

The second approach utilizes transmembrane pH gradients that may be created to achieve transmembrane transport of Fe (III) and Ba(II) cations into liposomal lumen with the aid of ionophores [14,15].

Unfortunately, none of these experimental approaches have proven to be efficient enough to obtain homogenous non-aggregated population of highly superparamagnetic liposomes. We hypothesized that the reversible covalent attachment of iron oxide/dextran nanoparticles (MION [16]) to aminophospholipids, during the preparation of the water/lipid emulsion, would increase the entrapment of colloids in REVs. After the formation of liposomes, the iron/dextran attached to external monolayer of the bilayer may be detached by lowering the pH. This detachment is a result of the instability of a Schiff bond at acidic pH values [17]. The remaining aminogroups may be used either for attachment of targeting ligands or for acylation.

2. Materials and methods

Materials. Lipids (dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dioleoylphosphatidylethanolamine) were obtained from Avanti Polar Lipids (Alabaster, AL). Egg-yolk sphingomyelin (primarily *N*-hexadecanoyl-D-sphingosine-1-phosphocholine) and cholesterol were from Sigma (St. Louis, MO). Buffer components and other reagents were of HPLC or Enzyme grade (Fisher).

Dextran-stabilized iron oxide nanoparticles (MION, [16]) were prepared as in [18]. Properties of MION are reported elsewhere [16].

Oxidation of MION. 2 ml of solution of MION containing 31 mg of Fe/ml was treated with 15 mg of sodium metaperiodate at pH 6.0 for 30 min. The reaction was stopped by the addition of ethyleneglycol (20 mM). The solution was extensively dialyzed against 0.15 M NaCl.

Liposome preparation. Chloroform solutions of lipids (PC/DOPE/CH or SM/DOPE/CH were mixed at a molar ratio of 9:2:9 (20 μmol of lipid) and evaporated from chloroform to dryness in a borosilicate glass tube. 200 μl of 0.1 M sodium borate, pH 9 and 0.5 ml of oxidized and purified MION (30 mg Fe/ml) were added, vortexed and mixed with 2 ml of diethyl ether. In some preparations Sulforhodamine B (Molecular Probes, Eugene, OR) was added at 0.01 M in the water phase to monitor liposome leakage. A stable emulsion formed upon sonication for 30 s at RT (Laboratory Supplies, bath-type, 80 W output). Diethyl ether was removed by slow flow of nitrogen through the emulsion at 25°C. The remaining traces of diethyl ether were removed at high vacuum.

Control liposome preparations contained no DOPE, i.e., DOPC/CH were used at a molar ratio of 11:9. Alternatively, in some control preparations non-oxidized MION or oxidized MION, reduced with an excess of sodium borohydride, was used for encapsulation in PE-containing liposomes.

Liposomes were dialyzed against three changes of 10 mM sodium citrate, 0.15 M NaCl, pH 4.5 for 1h. An aliquot of dialyzed iron-liposomes was used for iron and phosphate determinations. 0.5 ml of iron-liposome suspension was passed through a polycarbonate filter with 0.4 μm pores (Poretics, Livermore, CA) [19] and applied on Bio-Gel A15m column (1 × 30 cm). The column was eluted with 10 mM sodium citrate, 0.15 M NaCl (pH 6). Liposomes incubated at pH 8 were eluted with 5 mM sodium tetraborate, 0.15 M NaCl (pH 8). The first peak was collected, the pH was adjusted to 8 by addition of 1 M Hepes. Liposomes were then used for phosphate and iron determinations as well as for relaxivity measurements.

Alternatively, liposomes were purified from unincorporated MION by sedimentation at 11 000 × *g* for 5 min and washing with an appropriate buffer. This procedure was repeated three times. Resuspended liposomes were used for iron, phospholipid and fluorescence analysis.

Liposome surface modification. Liposomes eluted from the column (0.5 ml, 350 nmol of phospholipid) were treated with 14 mg of methoxy poly(ethylene glycol)2000 *N*-sulfosuccinyl succinate prepared as in [20] and semi-purified by gel-filtration through G-25 spun column and lyophilization. Methoxy poly(ethylene glycol)2000 *N*-sulfosuccinyl succinate (purity approx. 80%) was added to liposomes drop-wise from a concentrated water solution (100 μl total) with immediate vortexing. The reaction proceeded overnight at room temperature. The completeness of modification of aminogroups was tested by TNBS titration [21] and by thin-layer chromatography.

Relaxivity measurements. Relaxation times were measured at 0.47 T at 37°C (PC-20 Minispec, IBM, Danbury, CT and Bruker Instruments, Canada). T_1 (longitudinal relaxation time) was measured from eight data points generated by an inversion-recovery pulse sequence. T_2 (transverse relaxation time) was measured from 10 data points using a Carr-Purcell-Meiboom-Gill pulse sequence with τ of 1 ms. Inverse relaxation times were plotted against the iron concentration values.

Analytical procedures. Iron concentrations were determined in 20 μl samples combined with 20 μl of 3% hydrogen peroxide and dissolved in 2 ml of concen-

trated HCl. Absorbance at 430 nm was measured and iron concentration was determined using a calibration curve.

Phospholipid concentrations in liposomes were determined using a method of phosphate determination [22]. Samples were extracted with chloroform/methanol (2:1, by vol.) in polypropylene microfuge tubes, centrifuged at 11000 × *g* for 5 min and phospholipids were determined in organic phase.

Thin-layer chromatography (TLC) was performed using Kieselgel 60-coated plates (EM Science), developed by chloroform/methanol/water (65:25:4, by vol.). Plates were stained for phospholipid [23]. Aminogroups were separately determined by ninhydrin spray. For phospholipid determination in individual lipid spots, TLC plates were stained by iodine vapor, spots corresponding to individual lipid standards were scraped off the backing and phospholipid was determined as in Ref. [22].

Size analysis of liposomes was performed by photon correlation spectroscopy using a 90°-angle scattering mode (N4MD, Coulter Electronics, Hialeah, FL).

Fluorescence measurements were performed using Hitachi F-4500 fluorescence spectrophotometer. Fluorescence of sulforhodamine B was excited at 554 nm and detected at 590 nm using a stirred cuvette holder. Fluorescence intensity was measured at 50 μM of total liposomal lipid. Time-course of fluorescence was recorded at pH 4.5 or 9 for 1 h and then NP-40 was added to achieve final concentration of 1%. A molar extinction coefficient of 87000 was used for calculations of sulforhodamine B concentration.

Electron microscopy. A formvar/carbon coated copper grid (EM Sciences) was floated on a top of a drop of liposome suspension (0.5 mg of lipid/ml) for 5 min. The grid was then transferred to a drop of 2% ammonium molybdate (pH 6.5). A grid was dried at room temperature and examined using JEOL 200cx instrument.

3. Results

Dextran-stabilized iron oxide nanoparticles (monocrystalline iron oxide nanocompound, MION) were prepared in our laboratory [18]. The fraction of colloids with a diameter of 20 ± 4 nm (MION-46) was used in a given study. MION was treated with periodate in order to generate aldehyde groups on the surface of nanoparticles. These groups are known to react with aminogroups of aminophospholipids with the formation of covalent Schiff bonds [24]. When periodate was added at weight ratios from 1:10 to 1:1 no aggregation of MION was detected. We routinely used the oxidizer/MION ratio of approx. 1 mol sodium periodate per 6 mol of dextrose since it allowed sufficiently high encapsulation yields.

Upon mixing and a very brief irradiation with ultrasound, oxidized MION solutions formed stable emulsions with solutions of lipids (PC/PE/CH or SM/PE/CH, 9:2:9, by mol) in diethyl ether. The same was observed for control samples: (1) non-oxidized MION in the presence of PE-containing lipid solution; (2) oxidized and then reduced MION in the presence of PE-containing lipid mixture; (3) oxidized MION mixed with PE-free lipid mixture. After the removal of the organic solvent, a suspension of liposomes (REVs) was sized by passing through a 0.4 μm filter. Vesicles with a diameter of 263 ± 89 nm were obtained after filtration according to laser light scattering measurements. However, all types of control REVs re-aggregated to sizes of 402 ± 100 nm. Dialysis of liposomes at pH 8.0 or pH 4.5 did not result in detectable changes of liposome diameter (either in experiment or in control).

In order to purify liposomes from non-trapped MION the suspension was subjected to gel-filtration on Bio-Gel A15m. Only PE-containing REVs obtained in the presence of oxidized MION gave a substantial yield of lipid which eluted close to void volume of a column. The major fraction of control REVs which were prepared in the presence of oxidized or non-oxidized MION was retained on a column presumably because of extensive aggregation.

Size measurements were confirmed by transmission electron microscopy. The majority of liposomes represented oligolamellar multivesicular structures (Fig. 1). Electron-dense iron oxide particles were confined to the inner compartment of liposomes and appear to be surrounded with lipid bilayer(s).

The results of lipid and iron determinations are presented in Table 1. The encapsulation yield of MION into control REVs did not exceed 9% in the case of PE-free REVs and oxidized MION, and 15% for non-oxidized MION. However, at the same initial iron/phospholipid ratio oxidized-MION was loaded in PE-containing REVs with a high yield of 49%. At an alkaline pH, liposomal MION is both entrapped in, and associated with the outer and inner surfaces of bilayer(s). In order to remove surface-bound iron nanoparticles, liposomes were dialyzed at pH 4.5 overnight. No detectable solubilization of iron oxide colloid was detected at this pH value. After separation of a dialyzed sample from unbound iron oxide on a Bio-Gel A15m column we found that approximately 10.2% of the total liposome-associated iron was dissociated from liposomes and thus can be regarded as associated with outer liposome surface (Table 1). It should be noted that the calculation of encapsulation yields were performed assuming that iron/lipid ratio in liposomes retained on the column is the same as for

Table 1
Iron and lipid content of liposomes before and after separation of non-trapped iron oxide

Liposome composition	Initial concentrations (mM)		Molar ratio Fe/P_i	Final concentrations (μM)		Molar ratio Fe/P_i	%Fe, trapped	Phospholipid yield (%)
	Phospholipid	Iron		Phospholipid	Iron			
DOPC/CHOL (11:9) [a]	9.9	272	27.5	107.5	270	2.5	9	11
DOPC/PE/CHOL (9:2:9) [b]	10.7	240	22.4	136.5	480	3.5	14.1	13
DOPC/PE/CHOL (9:2:9) [a]	9.7	233	24	685	7350	10.7	44.6	71
DOPC/PE/CHOL (9:2:9) [a,*]	9.7	233	24	628	7430	11.8	49.3	65

[a] REV were prepared using oxidized MION and incubated at pH 4.5.
[b] REV were prepared using non-oxidized MION and incubated at pH 4.5.
* REV were incubated at pH 8.

eluted liposomes. To prove this we used an alternative method of liposome separation from unincorporated MION by centrifugation at $11000 \times g$. REV (DOPC/DOPE/CH; 9:2:9) prepared in the presence of oxidized MION contained 7.1 ± 3.5 M Fe/M phospholipid (average encapsulation yield 45% of MION, phospholipid yield 92%). Control liposomes of the same lipid composition contained 1.1 ± 0.4 M Fe/M phospholipid (average encapsulation yield – 5%, phospholipid yield 97%). Therefore, control liposomes retained on a Bio-Gel A15m column did not contain higher loads of MION than those which eluted from the column.

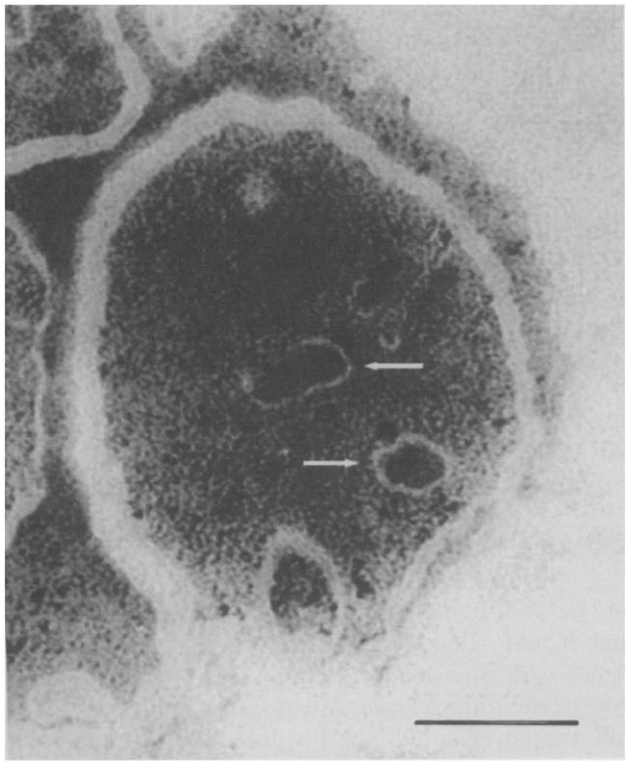

Fig. 1. Electron microscopy micrograph of a MION-46- containing REV obtained after negative staining with ammonium molybdate. Magnification: ×185000. Bar = 100 nm. Arrows indicate MION particles.

We additionally performed TLC analysis of liposome lipids to determine whether our observation with iron dissociation from liposomes may reflect actual changes in free PE-content in MION-REVs after lowering the pH. Chromatography of lipids from all samples have shown the satisfactory level of lipid stability in the presence of high concentration of iron oxide at both alkaline and acidic pH values (Fig. 2). No formation of lysoPC or lysoPE was observed for at least 3 days of storage. Extraction of lipids from REVs before separation of non-trapped MION (pH 8.0) revealed a slightly reduced extractability of PE into the organic

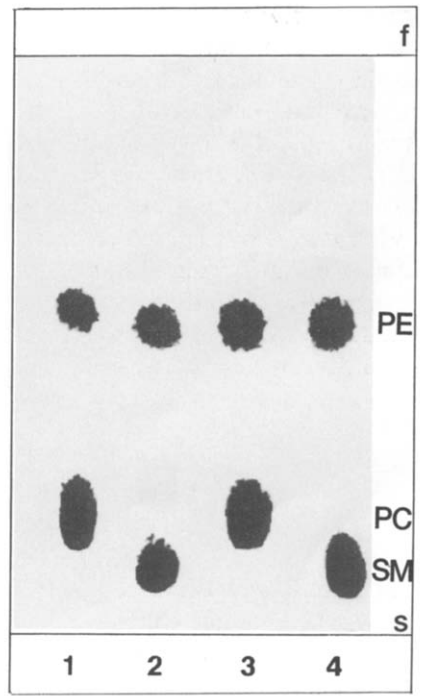

Fig. 2. Thin-layer chromatography of lipid extracts. Liposomes were incubated at pH 8.0 (lanes 1 and 2) and pH 4.5 (lanes 3 and 4). 200 μl of liposomes (REV) obtained from DOPC/DOPE/CH (9:2:9) (lanes 1 and 3), SM/DOPE/CH (9:2:9) (lanes 2 and 4) in the presence of MION (30 mg Fe/ml) were incubated overnight at pH values indicated overnight, extracted with chloroform/methanol (1:1, by vol). Lipids were resolved by TLC and stained for phospholipid as described in Materials and methods. Position of standards migration is shown; s, start; f, front of the solvent.

phase (Fig. 2, lanes 1,2). By TLC analysis of individual phospholipids we found that after dialysis at pH 4.5, average PE/PC molar ratio was 1:5 (20%, molar of PE in a phospholipid mixture), while at pH 8.0 this ratio was only to 9:70 (13%, molar of PE). In a separate experiment with MION-free liposomes we determined that lipid extract initially contained about 19%, molar of PE.

To demonstrate the availability of PE aminogroups for covalent modification with acylating agents, we attempted to modify them with PEGsuccinate activated ester. As determined from TLC analysis performed by staining of free aminogroups with ninhydrin, modifications performed in the presence of 5-fold molar excess of acylating agent per mol of aminogroups resulted in complete derivatization of PE (Fig. 3, lanes 2 and 4). The same result was obtained using TNBS titration (not shown). Since sulfosuccinimide esters are known to be membrane-impermeable [25], addition of MPEG-succinyl sulfosuccinate to liposomes should not lead to modification of 100% of all available PE in oligolamellar liposomes. To explore the possibility of membrane rupture or increased permeability for solutes caused by MION incorporation we compared trapping efficiency

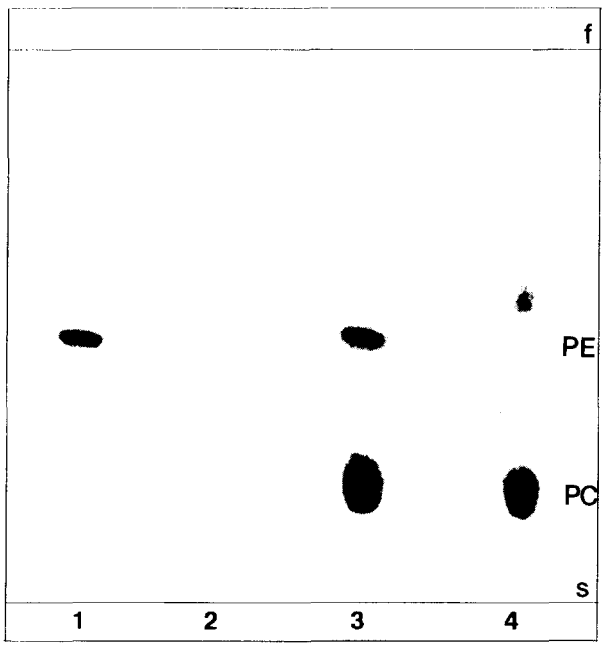

Fig. 3. Thin-layer chromatography of liposomes modified with MPEG-*N*-sulfosuccinyl-succinate. Liposomes (REV, DOPC/DOPE/CH, 9:2:9) were obtained in the presence of MION (30 mg Fe/ml) at pH 8.0, separated from non-trapped MION on Bio-Gel A15m column at pH 6.5 and derivatized by MPEG(2000)-*N*-((sulfo)succinyl)succinate; lipids were extracted and analyzed by TLC as described in Materials and methods. Lanes 1,3: REVs prior to modification; lanes 2,4: REVs after modification. Lanes 1,2: staining for aminogroup presence by ninhydrin; lanes 3,4: staining for phospholipid. Position of standards migration is shown: s, start; f, front of the solvent.

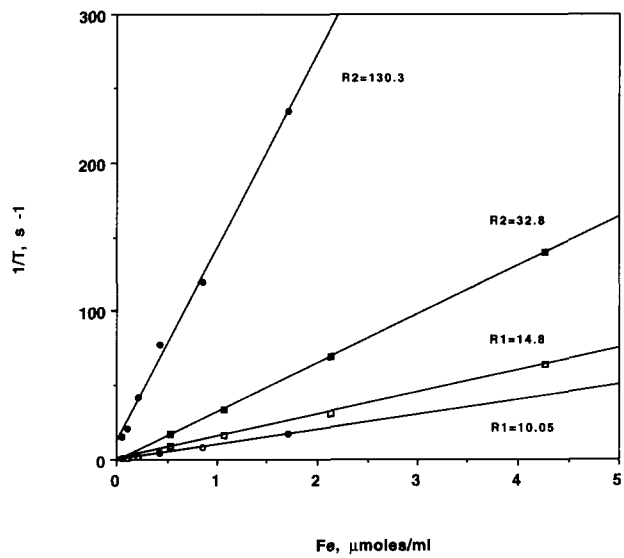

Fig. 4. Relaxivity measurements of free MION (squares) and MION-containing REVs (DOPC/DOPE/CH, 9:2:9, by mol) (circles). Open symbols, T_1 measurements; closed symbols, T_2 measurements. Corresponding relaxivity values (in $s^{-1}\,mmol^{-1}\,l^{-1}$), obtained by linear fitting of experimental data ($r = 0.99$) are indicated above each plotted set of data.

for MION-free liposomes and liposomes prepared in the presence of oxidized MION. Both types of liposomes at identical lipid concentrations had trapping efficiencies in the range of $3.75 \pm 0.75\%$ for sulphorhodamine B. Leakage of fluorescent marker from liposomes amounted for 0.05% of total encapsulated sulforhodamine B per 1 h.

Measurements of longitudinal (T_1) and transverse (T_2) relaxation times of water protons were performed for MION-loaded REVs or plain MION used for liposome preparation (Fig. 4). We used relaxivity values (R_2), expressed per atom of Fe, to measure the effectiveness of iron in a form of crystalline iron oxide in shortening proton relaxation times. MION-REVs ($R_2/Fe = 130.3\ (mM\,s)^{-1}$ were approx. 4-times more effective than MION itself ($R_2/Fe = 32.8\ (mM\,s)^{-1}$) in decreasing T of water protons (Fig. 4). R_1/Fe of MION-REVs was 1.4-fold smaller than that of MION.

4. Discussion

The goal of this study was to develop a procedure that would allow us to obtain non-aggregated liposomes with high loading of iron oxide. Non-stabilized iron oxide cores induce an extensive lipid aggregation and therefore could not be used for entrapment in liposomes. However, the majority of currently available stabilized iron oxide preparations contain polysaccharides adsorbed on the surface of iron oxide core. The one used in our study (MION) consists predominantly

of iron oxide monocrystals of 4.6 ± 1.2 nm in diameter, stabilized with 25 ± 6 molecules of dextran (B-512F, molecular mass 10 kDa) and has a size of 20 ± 4 nm [16]. This makes the preparation suitable for entrapping in mid-size liposomes. We originally found that dextran-stabilized iron oxide was entrapped into liposomes by reversed phase evaporation to a small extent (with a yield less than 15%) with concomitant extensive aggregation of liposomes. The aggregation observed is likely caused by dextran coating of MION. Dextran is known to aggregate and fuse liposomes [26,27].

We attempted to improve the ability of dextran-stabilized iron oxide to be trapped in REVs by a temporal increase of lipophilicity of MION by covalent binding to aminophospholipid headgroups during the liposome preparation. We anticipated that this would promote association with lipid bilayer. The reversibility of this interaction would allow us to remove iron oxide colloids positioned on the outer surface of liposomal membrane.

To facilitate the entrapment of MION in liposomes we generated aldehyde groups on the surface of dextran-stabilized iron oxide by oxidation with periodic acid in order to facilitate binding of MION to aminogroups of PE via imine (Schiff) bonds. Aldehyde-aminophospholipid binding was utilized for the first time by Heath et al. [24] for covalent attachment of proteins to liposomes. However, this reaction is thought to proceed in natural membranes as well. For example, retinal is believed to form Schiff bases with aminophospholipids in disk membranes [28].

We found that relatively low amounts of oxidizer used to modify the dextran coating (1 mol periodate per 6 mol of dextrose) did not induce any aggregation of iron oxide colloid. When mixed with an organic lipid solution containing PE, oxidized MION forms stable emulsions at alkaline pH which collapse with a formation of vesicles upon the removal of the organic solvent. These vesicles (REVs) contain high amounts of associated and encapsulated iron colloids (8–12 mol Fe/mol phospholipid, for different lipid compositions used) and have a size of 263 ± 89 nm.

Control vesicles prepared from lipid mixtures lacking PE in the presence of oxidized MION, or those PE-containing but obtained in the presence of non-oxidized MION, entrapped at least 6-fold less iron per mol of phospholipid. They showed a tendency to aggregate even after repetitious extrusion through polycarbonate filters.

To induce dissociation of MION bound to lipid bilayer we used known tendency of Schiff bonds to dissociate upon protonation of imines in acidic media [17]. After dialysis of liposomes against a buffer solution with pH 4.5, we usually observed a 10.2% loss of liposome-associated iron after separation of REVs by gel-chromatography. This may indicate that iron oxide initially bound to liposomal surface is at least partially dissociated. To test whether PE dissociates from MION at low pH, we performed extraction of liposomes incubated at different pH values with chloroform/methanol and analyzed lipid extracts obtained. MION itself does not favor chloroform-enriched organic phase either before or after incubation with lipids and remains in water-enriched phase. By quantitation of PE content in lipid extracts before and after lowering pH to 4.5, we found that at pH 8.0 PE tend to associate with MION. This resulted in diminished amounts of PE extractable into organic phase at alkaline pH (13%, molar of PE at pH 8.0 vs. 20%, molar at pH 4.5). Thus PE is indeed tightly associated with MION at alkaline pH and this association could be reversed by protonation.

The exposure of aminophospholipids onto the liposomal surface may greatly accelerate their removal from circulation by RES [29] and thus may limit the use of aminophospholipid-containing liposomes, e.g., as blood pool contrast agents. Though PE is much less effective in this respect than PS, elevated levels of this lipid tentatively may influence circulation times of liposomes. Therefore, we used covalent derivatization of aminophospholipids by modification with MPEG which prevents rapid removal of liposomes by liver and spleen. PEG-bearing liposomes are known to escape the uptake into liver and spleen and to circulate in the bloodstream for extended periods of time [30,31]. The modification resulted in efficient derivatization of aminogroups as shown by TLC analysis liposomal lipids. We observed almost 100% of liposomal PE derivatization by MPEGsuccinyl sulfosuccinate. Since MPEG-(2000)succinyl sulfosuccinate is unlikely to cross the intact lipid membrane to react with PE of inner bilayers of oligolamellar REV, the result obtained may indicate that MION induces formation of defects in the membrane which facilitate penetration of MPEG activated ester into the liposomal interior. However, trapping efficiencies of hydrophilic marker sulforhodamine B in liposomes obtained in the absence or in the presence of MION were similar, so were kinetics of sulforhodamine leakage. This indicates that membrane permeability of MION-liposomes is similar to control (MION-free) REV. Thus the effect observed may be attributed, firstly, to insufficient sensitivity of PE detection procedure used. Secondly, peculiarities of PE extraction into organic phase in the presence of MPEG may effect final recovery of lipid which was used for TLC analysis. Both possibilities are currently under study.

Relaxivity measurements by proton NMR spectrometry enabled us to determine the effect of entrapment of large amounts of iron oxide on its ability to shorten proton relaxation times (Fig. 4). 1.5-decrease in R_1/Fe, i.e., the effect of liposomal iron on T_1 (longitudinal) relaxation times of water protons may indicate smaller

number of accessible (coordinated) water molecules around encapsulated iron oxide. However, MION-loaded liposomes are 4-fold more effective than MION itself in generating microscopic magnetic field gradients which results in more rapid proton spin dephasing. This potentially may be important for use of MION-REVs as MR contrast agents.

In conclusion, we have demonstrated that generating aldehyde groups on the surface of colloids (e.g., carbohydrate-stabilized iron oxides) can be used to facilitate their encapsulation into PE-containing REVs. The technique is versatile since it is feasible for other glycosylated macromolecules as well as for other detachable covalent bonds, such as S-S bonds. Liposomes described in our study may be used as a model system that allows us to investigate magnetic properties of iron oxides which concentrate in the phagosomes of RES cells as a result of clearance form the blood in vivo.

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