Interaction of Manganese−Mesoporphyrin with Oleic Acid Vesicles†

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ABSTRACT: We investigated the interaction between manganese(III)mesoporphyrin (MnMeso), a metalloporphyrin, and liposome membranes containing oleic acid (OA; *cis*-9-octadecenoic acid). MnMeso associates preferentially with OA but minimally with egg phosphatidylcholine (EPC). Using small unilamellar vesicles, we characterized the MnMeso−OA binding at neutral pH. Our data suggest that MnMeso binds to the OA bilayer with $K_d = 6.8 \times 10^{-4}$ M; the binding stoichiometry of MnMeso−OA was 1:3.4. This OA−MnMeso interaction was analyzed further for changes in the $T1$ relaxation property of MnMeso. OA increased the $T1$ of MnMeso significantly more than did EPC, suggesting that the OA−MnMeso interaction was stronger than that of PC−MnMeso. The side-chain specificity of the OA interaction with this porphyrin derivative was further supported in an experiment with manganese meso-tetra(4-sulfonatophenyl)porphine, which lacks hydrophobic side chains for OA interaction. The association of MnMeso with the OA membrane was proposed according to the structure of MnMeso and OA and further verified using electron microscopy. A strong association of MnMeso with OA, an absorption enhancer of the gastrointestinal tract, may be useful for delivery of MnMeso as an oral contrast agent for magnetic resonance imaging.

Manganese(III)mesoporphyrin (MnMeso) is a metalloporphyrin that exhibits paramagnetic properties and has been shown to be potentially useful as a contrast agent for detecting liver tumors by magnetic resonance (MR) imaging (Schmiedl et al., 1992). Because MnMeso preferentially accumulates in normal liver parenchyma but not focal liver lesions, it could improve the sensitivity of MR imaging in the diagnosis of liver tumors. So far, clinically available paramagnetic compounds such as Gd-DTPA are not specific for liver imaging. Some hydrophilic metalloporphyrin derivatives such as manganese meso-tetra(4-sulfonatophenyl)porphine (Mn-TPPS4) are cleared rapidly through renal excretion (Ogan et al., 1987). Other metalloporphyrin derivatives such as Mn-hematoporphyrin, though exhibiting the same contrast-enhancing effect as MnMeso, display extended retention time in the liver that may produce significant toxicity. Therefore, MnMeso, which associates mainly with the liver and clears through biliary secretion, may minimize potential accumulation in other tissues (Ogan et al., 1987).

However, MnMeso must be given systemically via intravenous injection in order to provide the liver contrast enhancement. This route of administration may be problematic because of the potential systemic toxicity of lipophilic agents. Systemic delivery of MnMeso may be circumvented by choosing the oral route of administration. MnMeso absorbed through the gastrointestinal (GI) tract reduces the MnMeso level in the blood because of the rapid and preferential accumulation of MnMeso in the liver during its first passage via the portal vein. MnMeso accumulated in the liver is eventually eliminated by biliary secretion. As a result, high systemic levels of MnMeso can be avoided. If this strategy is chosen, the absorption efficiency of MnMeso in the GI tract becomes important. However, free MnMeso is poorly absorbed by the GI tract (R. J. Y. Ho, unpublished results). Therefore, we used oleic acid (OA, *cis*-9-octadecenoic acid) to enhance MnMeso absorption across epithelial cells lining the GI tract. OA is an unsaturated fatty acid that can form a stable membrane bilayer at pH 8. At a slightly acidic pH (5.0), OA membrane aggregates, exhibits pH-dependent content leakage, and promotes membrane fusion. Therefore, liposomes containing OA are described as pH sensitive (Connor et al., 1986; Collins et al., 1990). In addition, liposomes constructed with pure OA demonstrate absorption enhancement characteristics for GI tract absorption (Muranishi, 1990).

In our attempt to develop an OA−MnMeso mixture to enhance MnMeso absorption through the GI tract, we found an unexpected, preferential molecular interaction between MnMeso and OA at neutral pH, prompting further investigation. In this report, we characterize the OA−MnMeso molecular interaction. Our results show that MnMeso binds to the OA bilayer with a much stronger affinity than it does to the phosphatidylcholine bilayer.

EXPERIMENTAL PROCEDURES

Materials. Oleic acid was purchased from Sigma Chemical Co. (St. Louis, MO); egg phosphatidylcholine (EPC) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Mn(III)mesoporphyrin and Mn(III)-$TPPS_4$ were the products of Porphyrin Products (Logan, UT). Bio-Gel A-0.5 m used for size-exclusion chromatography was obtained from Bio-Rad (Hercules, CA).

Determining Metalloporphyrin Association to Lipid Membrane. Routinely, either empty or porphyrin-containing liposomes were prepared by sonication (New, 1992). Thus, 265 μmol of lipid—OA, EPC/OA, or EPC—in $CHCl_3$ was dried under N_2 for solvent removal and subsequently

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desiccated. To prepare metalloporphyrin-containing liposomes, 10 mL of phosphate−bicarbonate buffer (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 0.1 mM Na_2HPO_4, 35 mM $NaHCO_3$, and 15 mM Na_2CO_3) containing metalloporphyrin was added to the desiccated OA, EPC, or OA/EPC mixture to reach a final lipid concentration of 26.5 mM, pH 8, yielding a desirable ratio of metalloporphyrin to lipid. Empty liposomes were prepared in the same buffer without metalloporphyrin. The mixture was sonicated in a bath-type sonicator for about 20 min until a translucent suspension formed.

Liposomes with only their outer leaflet accessible to metalloporphyrin binding were prepared by incubating a mixture of varying amounts of metalloporphyrin at 37 °C for 1 h in phosphate−bicarbonate buffer and empty lipid vesicles, with the final lipid concentration of 26.5 mM. In our preliminary experiments, we found that this 1-h incubation at 37 °C was the optimal condition required to reach binding equilibrium.

The degree of metalloporphyrin−lipid membrane association was determined by size-exclusion chromatography. We used Bio-Gel A-0.5 m (10 × 1 cm) as a gel matrix and phosphate−bicarbonate buffer (pH 8) as the elution buffer to separate free metalloporphyrins from their liposome-associated counterparts. The MnMeso and $MnTPPS_4$ associated with lipid vesicles were eluted in the excluded volume, while the unassociated metalloporphyrins were in the included volume. The elution profile was monitered with a UV detector at 364 nm. The percentage association is calculated as

$$\text{percent association} = S_a/(S_a + S_f) \times 100$$

where S_a represents the peak area of the associated porphyrin and S_f stands for that of the free porphyrin. Experiments of two to three batches of liposomes were tested in triplicate, and the results were presented as mean ± SD.

Scatchard Plot Analysis. Data from the size-exclusion chromatography was further analyzed by Scatchard plot analysis. This analysis was performed according to the single binding site mechanism model. The equation describing this process is

$$[\text{MnMeso}]_{\text{bound}}/[\text{MnMeso}]_{\text{free}} = -K_d[\text{MnMeso}]_{\text{bound}} + NK_d$$

$[\text{MnMeso}]_{\text{bound}}$ and $[\text{MnMeso}]_{\text{free}}$ represent the concentration of the bound or free MnMeso, respectively. K_d is the dissociation constant, and N is the number of MnMeso molecules bound at a given lipid concentration. The data for each set of MnMeso−liposome binding experiments was fitted by the linear regression method, and NK_d and $-K_d$ were determined from the intercept and the slope of the curve, respectively. Since the MnMeso−lipid association assays were conducted at a lipid concentration of 26.5 mM, the stoichiometry of MnMeso to lipid is $N/26.5$, which is the inverse of the stoichiometry of lipid-to-MnMeso association, n.

Determination of T1 Relaxation Time. The relative effects of paramagnetic MnMeso on proton relaxation times of H_2O were determined at room temperature using an MR spectrometer operating at 0.47 T (20 MHz) (Bruker Minispec, PC-20; Bruker Instruments, Rheinstetten, Germany). The measurement was performed at room temperature because the $T1$ relaxations of paramagnetic compounds are shown to be independent of temperatures between 20 and 45 °C (Koenig & Schillinger, 1969). Spin−lattice ($T1$) relaxation times were determined for each concentration by an inversion−recovery pulse sequence, with two-parameter fittings applied to eight data points. Each sample was assayed at least three times, and the machine error is less than 7.5%.

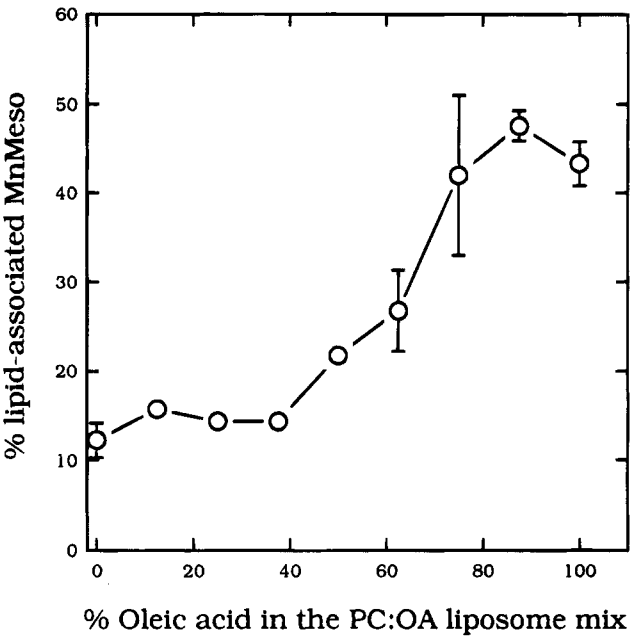

FIGURE 1: Percentage association of MnMeso with lipid vesicles of varied PC−OA composition. Liposomes of various PC−OA compositions were made in phosphate−bicarbonate buffer at pH 8 by sonication in the presence of MnMeso. Liposome-associated and free MnMeso were separated on a size-exclusion column, and the proportions of entrapped and free MnMeso were determined by absorbance reading of the eluate at 364 nm. The overall lipid:MnMeso molar ratio is 1.75:1.

Electron Microscopy. Liposome samples were loaded onto the carbon-coated copper grids for 2−3 min, and then any liquid excess was removed. Subsequent staining of liposomes with phosphatungstate (PTA) was performed by adding PTA at one end of the grid and removing it at the distal end. Grids were air-dried completely before being loaded into the sample chamber of the electron microscope. Samples were visualized at magnifications of 8000 and 15 000. The size distribution of each type of vesicle is determined from measurements of at least 200 vesicles on electron micrographs.

RESULTS

Preferential Association of Manganese Mesoporphyrin with Oleic Acid in Phosphatidylcholine Lipid Bilayer. To determine whether MnMeso preferentially associates with OA in the lipid membrane, we prepared lipid vesicles composed of varying OA-to-EPC ratios in the bilayer while fixing the molar ratio of total lipid (EPC + OA) to MnMeso at 1.75. The amount of the MnMeso-associated lipid vesicle was assessed with size-exclusion column chromatography using Bio-Gel A-0.5 m matrix. If there were no MnMeso−OA interactions, an increasing fraction of OA to EPC in the bilayer should not have had any effect on the percentage association of MnMeso to the bilayer. However, the results in Figure 1 show a significant increase in association of MnMeso to lipid vesicles with an increasing proportion of

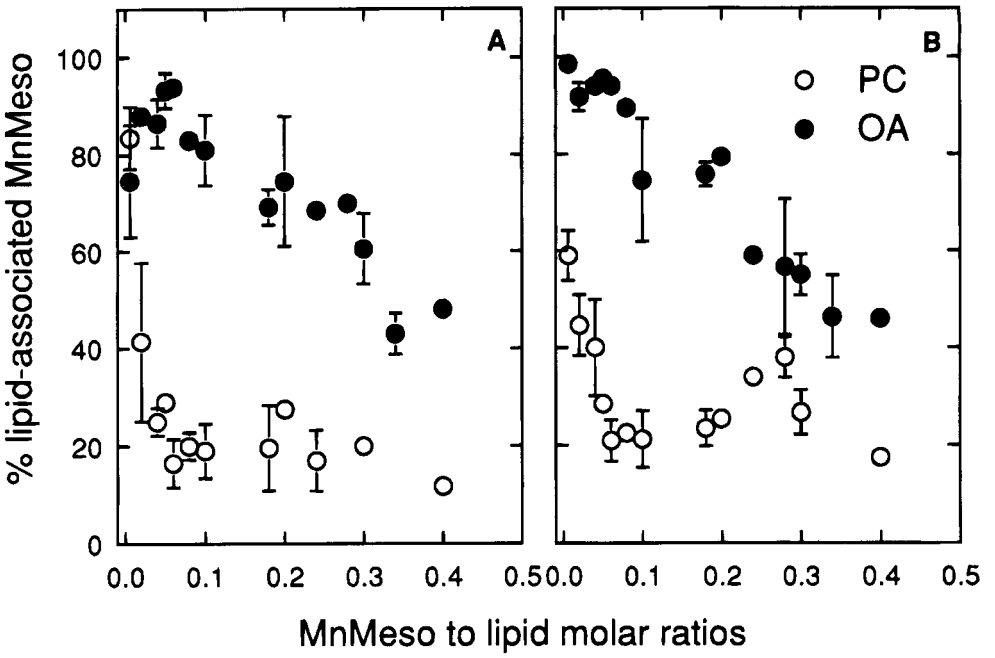

FIGURE 2: Percentage association of MnMeso with lipid bilayers at various MnMeso:lipid molar ratios. Panel A displays the association of MnMeso with lipid as the result of a 1-h incubation of the MnMeso and lipid mixture; panel B exhibits the association as the result of sonication of the mixture. The lipid concentration was kept constant at 26.5 mM for EPC (○) or OA (●) vesicles. The proportions of liposome-associated and free MnMeso were determined by use of a size-exclusion column.

Table 1: Scatchard Binding Analysis of Manganese Mesoporphyrin to Lipid Vesicles

	sonication			incubation		
	K_d[a] (mM)	*n* (lipid/MnMeso)	r^2 [b]	K_d (mM)	*n* (lipid/MnMeso)	r^2
			OA Vesicles			
trial A	0.63	3.8	0.63	0.24	5.7	0.81
B	0.59	4.4	0.73	0.20	5.8	0.84
C	0.73	2.8	0.83	0.26	6.3	0.82
D	0.78	3.6	0.73	0.29	5.8	0.97
E	0.65	2.5	0.86			
mean ± SD	0.68 ± 0.08	3.4 ± 0.8	0.76 ± 0.09	0.25 ± 0.04	5.9 ± 0.3	0.86 ± 0.07
			EPC Vesicles			
trial A	*c*	*c*	0.42	*c*	*c*	0.92
B	*c*	*c*	0.41	*c*	*c*	0.82
C	77	*c*	0.60	6.25	*c*	0.53
D	2.2	*c*	0.81			
mean ± SD			0.56 ± 0.19			0.76 ± 0.2

[a] K_d is the dissociation constant. [b] r^2 is the regression coefficient of the Scatchard plot fitted by the linear regression method. [c] The slope of the Scatchard plot ($-1/K_d$) is a positive value; therefore K_d and *n* cannot be calculated.

OA in the OA/EPC lipid bilayer. About 10−15% MnMeso associated with pure EPC vesicles, and this value remained constant up to 40% OA in the mixed OA/EPC bilayer (Figure 1). A significant increase in the MnMeso association to lipid vesicles was observed for lipid vesicles containing 50−85% OA (under these conditions, the percentage of MnMeso association jumped from 22 to 48%; Figure 1). No additional increase in MnMeso association was apparent beyond 85% OA in the lipid bilayer at the same lipid-to-MnMeso ratio. Collectively, these data indicate that MnMeso binds to the lipid bilayers in an OA-dependent manner.

Characterization of MnMeso Association to Oleic Acid Vesicles. To determine the interaction between MnMeso and OA, we first prepared lipid vesicles in the presence of varying amounts of MnMeso while fixing OA or EPC concentrations at 26.5 mM. With varying MnMeso-to-lipid molar ratios, we determined the degree of MnMeso association to lipid vesicles (Figure 2A). Practically all MnMeso (90−100%) was associated with OA vesicles at a MnMeso-to-OA molar ratio less than 0.1. When the MnMeso-to-OA molar ratio increased, the MnMeso-binding sites of OA in the vesicles became saturated. As a result, a decrease in the percentage of MnMeso associated with OA vesicles was observed. The percentage of vesicle-associated MnMeso reached a plateau of about 40% at MnMeso:OA = 0.4 (Figure 2B). In contrast, the association of EPC with MnMeso was much weaker under identical conditions (Figure 2B). MnMeso association with EPC vesicles was only observed at excess EPC. Scatchard analysis provided the kinetic information of the lipid-MnMeso association (Table 1). The dissociation constant between OA and MnMeso is about 0.25 mM, and the stoichiometry of the interaction is about 3.4:1 (OA:MnMeso). Similar analysis failed to provide a reliable estimation of the binding constant between EPC and MnMeso because the interaction is near-background level (Table 1).

Probing the MnMeso Binding Site in the Lipid Bilayer. To further characterize the association between OA and

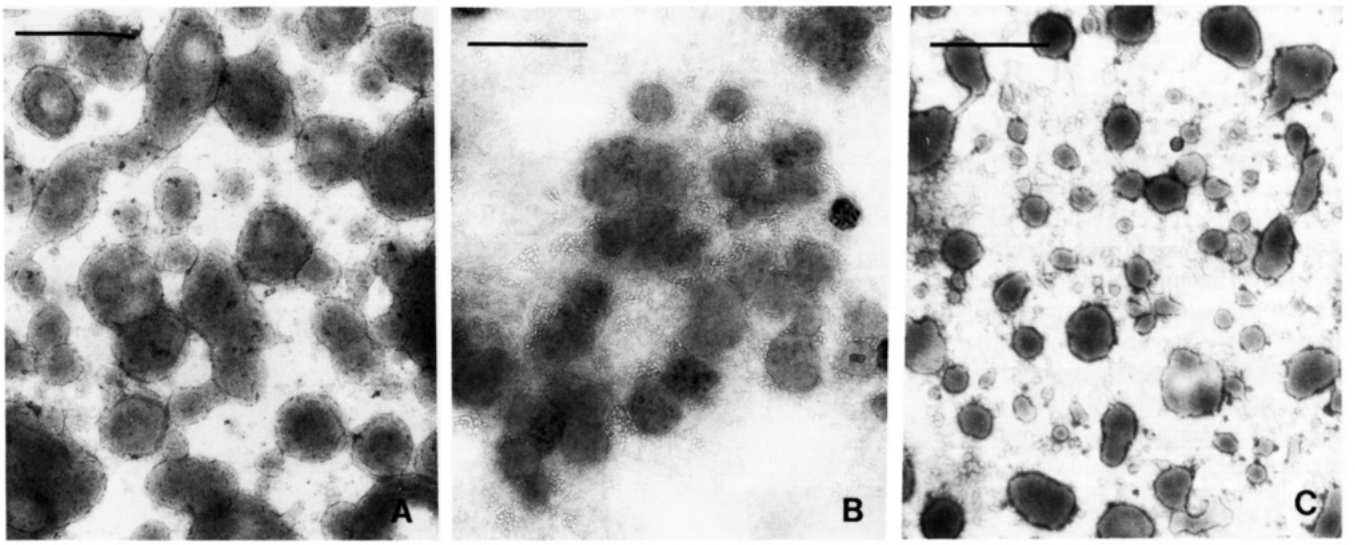

FIGURE 3: Electron microscopic images of OA (A, B) and PC (C) liposomes. All of the pictures are enlargements from negatives taken at 8000× or 15 000× magnification. Liposomes were stained with 0.5% PTA (A and C) and MnMeso (B). No OA or PC liposomes were visualized when stained with $MnTPPS_4$ (data not shown). The sizes of the liposomes in panels A and B are comparable, indicating that the association of MnMeso with OA bilayer does not introduce additional restraints on the membrane. The size bars represent 500 nm in each picture.

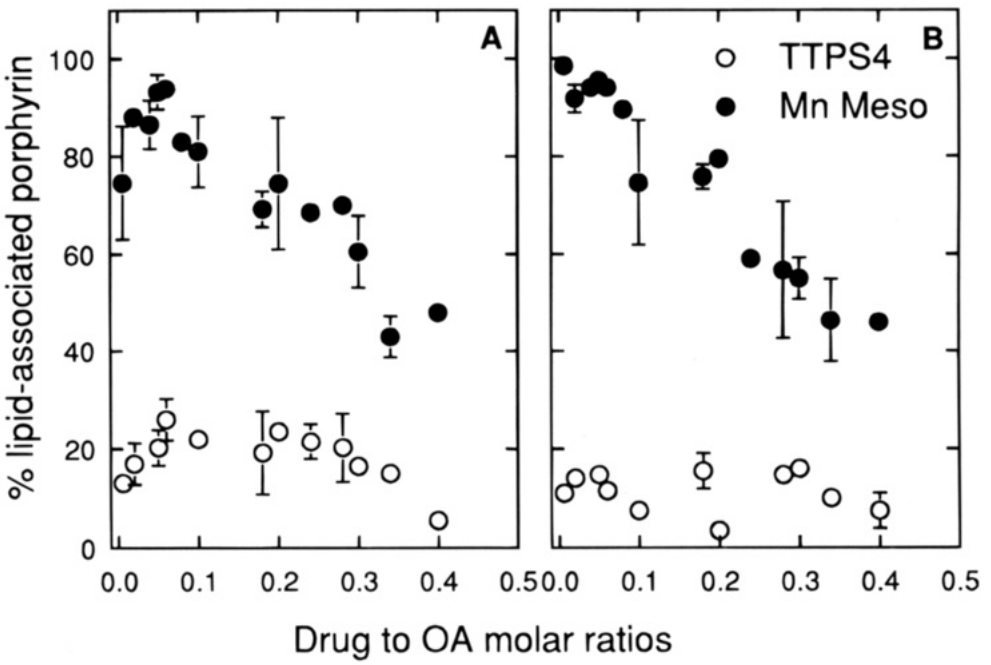

FIGURE 4: Comparison of percentage association with OA bilayers between $MnTPPS_4$ and MnMeso at various Mn-porphyrin:OA ratios. OA liposomes were either sonicated (A) or incubated (B) with $MnTPPS_4$ (○) or MnMeso (●) in phosphate−bicarbonate buffer at pH 8. The proportions of Mn-porphyrin associated with OA bilayers were determined by size-exclusion chromatography.

MnMeso, we incubated (rather than sonicated) preformed empty lipid vesicles with MnMeso solution for 1 h at 37 °C. We found that the percentage of membrane-associated MnMeso after a 1-h incubation is the same as that after 24 h. The percentage of membrane-associated MnMeso was determined at a fixed OA concentration of 26.5 mM while varying the concentration of MnMeso. The rationale behind this experiment was as follows. When OA vesicles were formed in the presence of MnMeso, the vesicle-associated MnMeso was composed of three fractions: that trapped inside the aqueous compartment of the vesicle, that bound to the inner monolayer, and that bound to the outer monolayer. When MnMeso was incubated together with preformed empty OA vesicles, only the outer monolayer was accessible to porphyrins. Therefore, the difference in the percentage of associated porphyrin between two preparation procedures—MnMeso encapsulation and incubation—should be ascribed to the additional MnMeso bound to the inner monolayer and that entrapped within the aqueous compartment of the vesicles. We found a significant difference in the percentage of MnMeso association between the sonication and incubation procedures at a constant lipid concentration of 26.5 mM (Figure 2). Scatchard plot analysis was performed on data obtained from three to five trials of different MnMeso−OA preparation procedures, and the results are summarized in Table 1. When mixtures were incubated or sonicated, the stoichiometry of the OA−MnMeso interaction was estimated to be 5.9 or 3.4,

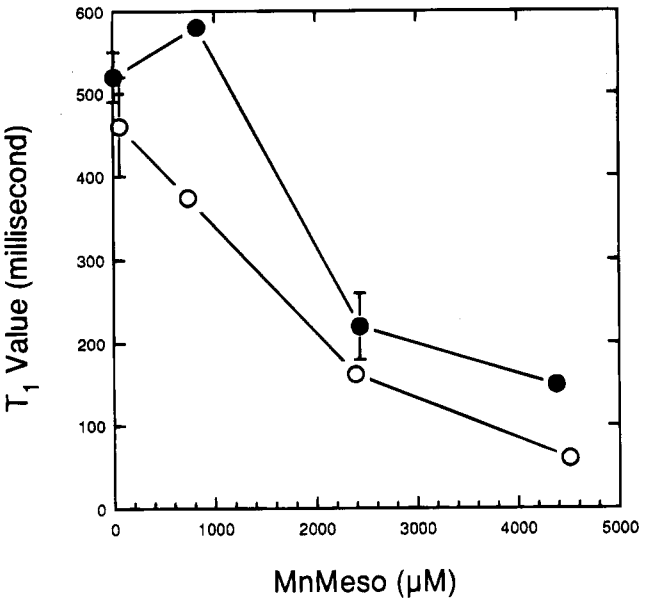

FIGURE 5: Change in $T1$ relaxation times in the presence of OA. MnMeso alone (○) and the MnMeso−OA mixture (●) were assayed for their $T1$ relaxation times. The OA:MnMeso molar ratio was 2.7 in all MnMeso−OA mixtures.

respectively (Table 1). The increase in the amount of MnMeso bound to OA in the sonicated mixture can be attributed to the fact that both inner and outer monolayers are accessible to MnMeso binding. Therefore, we conclude that MnMeso is likely to bind to both the inner and the outer leaflet of the OA bilayer.

Analysis of MnMeso Association with OA Vesicles with Electron Microscopy. To visualize the morphology of OA lipsomes and the binding of MnMeso to them, both empty OA liposomes and MnMeso-containing OA liposomes were analyzed using electron microscopy. OA liposomes, having no electron-dense material and hence being barely visible by electron microscopy, should become positively stained upon binding to MnMeso. As shown in Figure 3, OA liposomes (3A) but not EPC liposomes (3C) become positively stained with MnMeso (3B) and not with $MnTPPS_4$ (data not shown). The average size of an OA liposome is 510 ± 160 nm, regardless of whether it is negatively stained with PTA or MnMeso; the size of PC liposomes is 125 ± 133 nm.

Comparison of Interactions of MnMeso and $MnTPPS_4$ with OA Vesicles. To determine whether OA−MnMeso association is due to the porphyrin ring, we compared these results with those from another porphyrin derivative, $MnTPPS_4$, also used as an experimental contrast agent. Results in Figure 4 indicate no association between OA and the more water-soluable metalloporphyrin $MnTPPS_4$. In contrast to the MnMeso−OA interaction, increasing the porphyrin TPPS4-to-OA ratio has no effect on the percentage of $MnTPPS_4$ associated with OA vesicles, regardless of the preparation procedure (i.e., sonication or incubation). These results indicate that $MnTPPS_4$ has a minimal affinity for OA vesicles and that association of OA to MnMeso is unique to the more lipophilic porphyrin.

T1 Relaxation Studies of Molecular Interaction between OA and MnMeso. To demonstrate the molecular interaction between MnMeso and OA vesicles, we compared OA and MnMeso interaction to that of OA and $MnTPPS_4$ using NMR

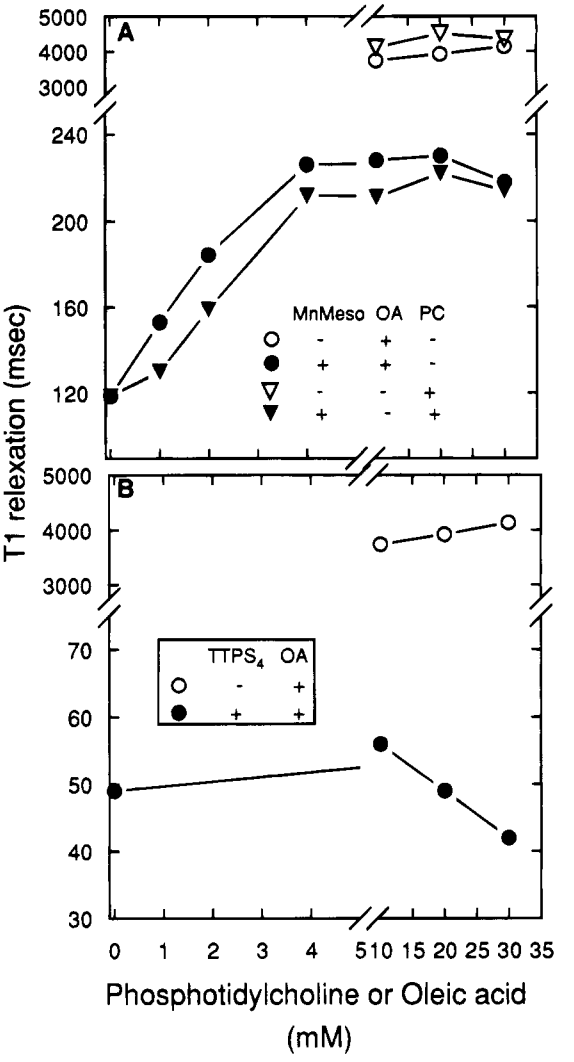

FIGURE 6: Change in $T1$ relaxation time resulting from the addition of PC or OA. Panel A is a comparison of the effects of OA and PC on the $T1$ relaxation time of MnMeso solution. Open symbols represent the $T1$ readings of pure PC or OA; closed symbols represent the $T1$ readings of mixtures of OA or PC and MnMeso. Panel B is a comparison of the $T1$ relaxation times between MnMeso and $MnTPPS_4$ solutions in the presence of OA. Open symbols represent the $T1$ readings of pure OA; closed symbols represent the $T1$ readings of mixtures of OA and $MnTPPS_4$. The Mn-porphyrin concentration was constant at 2 mM in any individual mixtures.

spectroscopy (Schmiedl et al., 1992; Ogan et al., 1987). The $T1$ relaxation time for protons in water molecules decreases when the concentration of metalloporphyrin, a paramagnetic compound, increases. In addition, the $T1$ relaxation time of the same protons will increase when metalloporphyrin-bound water molecules interact locally with adjacent molecules (i.e., OA). Figure 5 shows that the $T1$ relaxation time decreased from about 500 to 50 ms with an increase from 0 to 4.5 mM in MnMeso concentration. In particular, the decrease in $T1$ was linear against the increase in MnMeso concentration over the range 0−2.5 mM (Figure 5). In the presence of OA vesicles, the $T1$ relaxation time of MnMeso increased at each given MnMeso concentration, suggesting that the interaction of MnMeso with OA replaced that between MnMeso and water molecules.

To further verify the difference in association with MnMeso between EPC and OA, we determined the $T1$ relaxation time of a series of lipid−MnMeso emulsions with

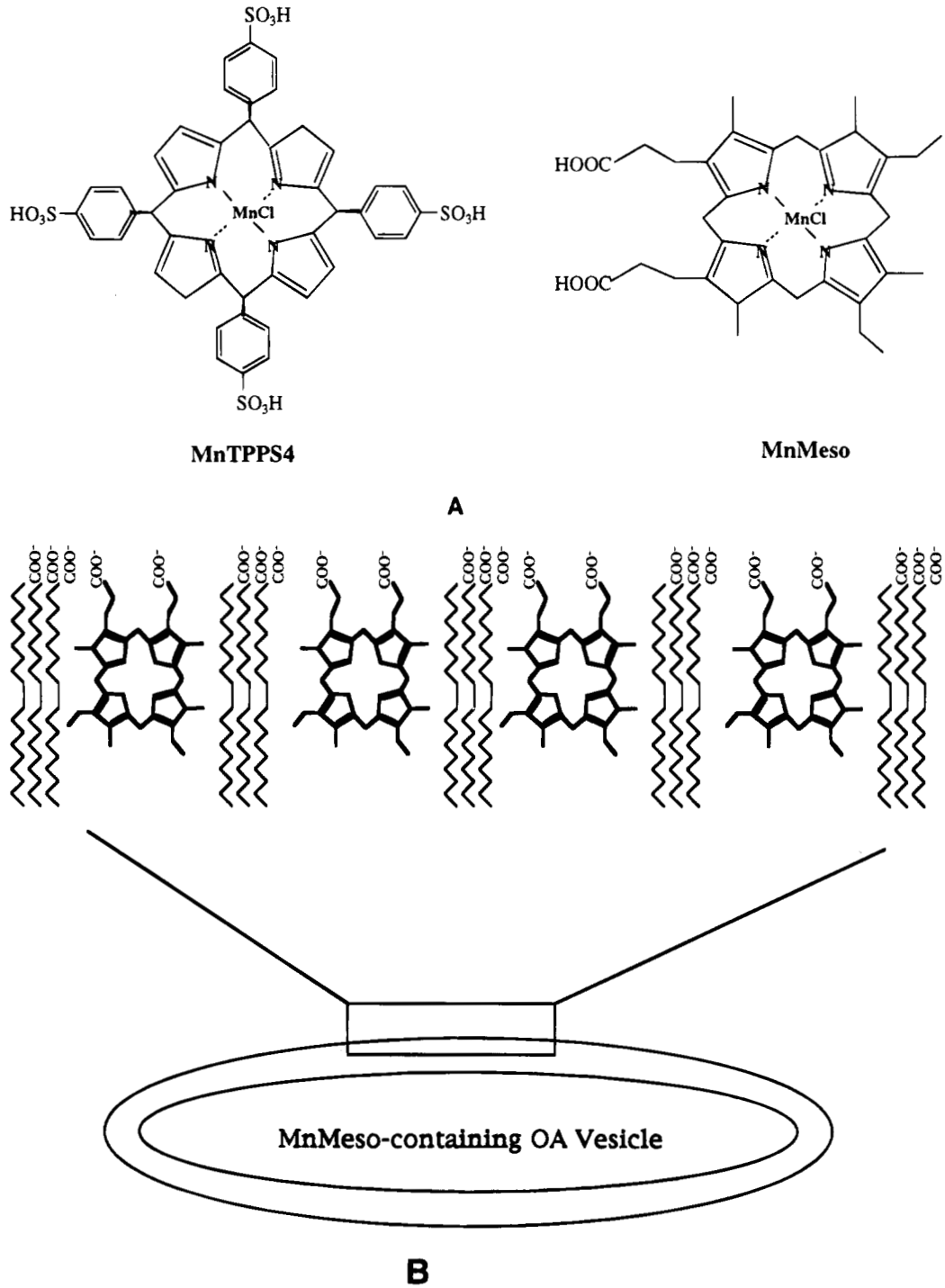

FIGURE 7: Schematic representation of MnMeso interactions with OA bilayer. Panel A shows that MnMeso is an amphiphilic molecule, while $MnTPPS_4$ is a highly hydrophilic one. Panel B shows the possible orientation of MnMeso in the OA bilayer and where the interaction may occur between OA and MnMeso.

varying lipid concentrations and a constant MnMeso concentration of 2 mM. Figure 6A shows that a 2 mM MnMeso solution gives a $T1$ of about 120 ms. Mixing 4 mM OA with 2 mM MnMeso is sufficient to increase the relaxation time to 220 ms (Figure 6A). At least 10 mM EPC was essential to produce similar changes in $T1$ relaxation times (Figure 6A). These results indicate that, compared with OA, more EPC molecules are necessary to affect the $T1$ value of MnMeso molecules, suggesting that the OA−MnMeso interaction is stronger than that of EPC−MnMeso. Parallel experiments using $MnTPPS_4$ (2 mM) have provided a constant $T1$ value (about 50 ms), regardless of the quantities or the types (OA or EPC) of lipid vesicles (Figure 6B), indicating the lack of association between OA or EPC and $MnTPPS_4$.

DISCUSSION

The interactions of hydrophobic porphyrins such as hemin, protoporphyrin, and hematoporphyrin with lipid membranes have been well described. Hemin binds to EPC bilayers with the dissociation constant 2×10^{-6} M (Cannon et al., 1984; Rose et al., 1985). Hematoporphyrin and deuteroporphyrin displayed association constants of 2×10^5 and 6×10^6 M^{-1} with EPC liposomes, respectively (Brault et al., 1986).

Protoporphyrin or deuteroporphyrin binds to PC/cholesterol liposomes (molar ratio 3:2) witha K_a around 2.3×10^4 M^{-1}. In this study, we demonstrated that the water-soluable, less hydrophobic MnMeso does not bind well to the EPC liposome bilayer at neutral pH. However, MnMeso binds selectively to OA in the liposome membranes with an association constant of 1.4×10^3 M^{-1}.

The MnMeso−OA interaction is specific in that experiments with OA and another water-soluble metalloporphyrin, $MnTPPS_4$, showed minimal association with OA. The structural difference between MnMeso and $MnTPPS_4$ (Figure 7A) may explain these observations. $MnTPPS_4$ has four sulfonatophenyl groups located between the pyrrole rings. Since both the porphyrin core and the phenyl goups of $MnTPPS_4$ are extremely electrophilic, the carboxylic groups on the $MnTPPS_4$ side chains are highly hydrophilic and easily deprotonated in an aqueous solution. These strong hydrophilic side chains attract H_2O molecules, thus preventing $MnTPPS_4$ from binding with the hydrophobic domain of the OA molecule. On the other hand, the carboxylic groups of MnMeso are less acidic than those of $MnTPPS_4$, therefore carrying less negative charge. In addition, MnMeso appears to be an amphiphilic molecule. The two hydrophilic propyl carboxylate groups are positioned at one side, while the hydrophobic ethyl groups are located at the distal end of the molecule. The length of the molecule—from the carboxylic end to the ethyl end—is similar to that of a molecule with a linear 14-carbon backbone. The entire molecule can fit nicely into the OA lipid bilayer with the carboxylic groups staying with the hydrophilic portion of the monolayer and the ethyl groups pointing toward the hydrophobic core of the bilayer (Figure 7B).

It is possible that the plane of MnMeso is likely to be positioned perpendicular to the lipid bilayer as schematically presented in Figure 7B, similar to the previous projection proposing that heme and hematoporphyrin bind evenly to both the inner and outer PC monolayers (Cannon et al., 1984) and that the plane of the porphyrin ring is likely to be perpendicular to the lipid bilayer. Tetraphenylporphyrin derivatives entrapped in DMPC liposomes have also been suggested to be embedded between the monolayers, far away from the hydrophilic surface of the bilayer (Yuasa et al., 1986).

Due to the amphipathic properties of OA and MnMeso, the OA−MnMeso association may be pH dependent. Cannon et al. (1993) have shown that EPC vesicles bind strongly to Sn-mesoporphyrin (SnMeso) at pH 5 but weakly at pH 7. With a decrease in pH, mesoporphyrin bears fewer negative charges on its carboxylate groups and becomes less polar, and may therefore may favor the association of SnMeso to EPC.

In our study, we probed the site of MnMeso association to OA monolayers at neutral pH. The stoichiometry of MnMeso binding to OA in the outer leaflet of the bilayer is estimated to be 5.9 OA per MnMeso molecule, and the stoichiometry is decreased to 3.4 for binding to both of the leaflets. Therefore, it is likely that MnMeso binds to both monolayers. The decrease in the latter case, slightly more than 50%, could be due to the curvature of the inner membrane leaflet. As a result, the overall binding of MnMeso to both leaflets is less than 2 times the binding to the outer leaflet.

*T*1 relaxation measurements can be used to demonstrate intermolecular interactions between porphyrins and lipids. Paramagnetic porphyrins decrease the *T*1 relaxation time of aqueous solutions significantly. This effect is based on a rapid exchange of protons between the "free" proton pool and the pool that is associated with the paramagnetic center (Figure 7A). In an OA−MnMeso mixture (Figure 7B), the interaction between OA and MnMeso makes the paramagnetic center of the porphyrin no longer easily accessible to H_2O molecules. $MnTPPS_4$, on the other hand, remains accessible for protons because it is not associated with OA or EPC bilayers. Therefore, no change in *T*1 is observed.

Fatty acids and fatty acid derivatives, such as OA, have been used to increase the absorption efficiency of orally administered drugs. Oba et al. (1992) reported that OA liposome-entrapped carboxyfluorescein (CF), when administered through the enteral route, was absorbed more efficiently by the rat small intestine than was free CF. Our preliminary studies indicated that OA-formulated MnMeso is more efficiently absorbed by rat intestine and that the MnMeso level in both liver and bile is higher than that of the free MnMeso (Ho and Dong, unpublished results). The understanding of the interaction between MnMeso and OA described in this paper will enable us to design an effective carrier for MnMeso delivery through the enteral route. Systematic studies are under way to optimize intestinal absorption of lipophilic paramagnetic porphyrins.

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