Interactions of Vanadocene(IV)-Chelated Complexes with Artificial Membranes

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The membrane interactions of five vanadocene(IV)-chelated complexes, which are very effective spermicidal agents, have been studied using zwitterionic and negative unilamellar liposomes. In permeability studies, bis(cyclopentadienyl)vanadium(IV) (2,2′-bipyridine)trifluoromethanesulfonate (**1**) and bis(cyclopentadienyl)-vanadium(IV) (phenyl benzohydroxamato)trifluoromethanesulfonate (**2**) cause the release of about 35% and 20% encapsulated carboxyfluorescein, respectively, in both types of liposomes, whereas the congeneric vanadocene derivatives of diethyl dithiocarbamate (**3**), acetyl acetonate (**4**), and catecholate (**5**) have little or no effect on the permeability. Of the five compounds, only **4** and **1** initiate peroxidation of the lipids. None of the vanadocene-chelated complexes cause appreciable liposome aggregation, fusion, or changes in packing order of the liposomes as observed from UV/vis spectroscopy, fluorescence energy resonance transfer, and fluorescence polarization studies. The ability of the two vanadocene derivatives (**1** and **2**) to cause the liposomes to become permeable is therefore not related to the extent of peroxidation of the lipids or to complete disruption of the membrane. We propose that these vanadocene-chelated complexes have unique configurational preferences which alter the membrane by intercalation, creating "leaky patches" in the liposomal membrane.

Introduction

Biological membranes are of fundamental importance to living cells by serving as selective barriers for transport and boundaries for energy and information.[1a] Observations of simple model membrane systems, like those composed of vesicles or liposomes, have proven experimentally useful in offering insights into the fundamental mechanisms of biological membrane functions.[1b]

Permeation of liposomal membranes has been effected by peptides, polyether compounds, and surfactants. Mechanisms proposed for the permeation include pore formation and development of localized inverted micelle structures within the lipid bilayer. Polypeptides,[2] macrocyclic ionophores,[3] and polymeric crown ethers[4] form artificial channels by spanning the bilayer. It has been shown that to form such a channel, it is optimal for the compound to have hydrophilic end groups, lipophilic portions in the channel, and appropriate infrastructural size to span the bilayer, which is approximately 40 Å.[4a,5] Low levels of surfactants and polymers have been found to increase the permeability of liposomes without destroying the membrane.[6−8] Triton X-100 is postulated to form inverted micelle structures within the bilayer, promoting both permeability and membrane fusion.[6] The model proposed by Regen et al. for surfactants, bolaphiles, and polymers implies that the leakage of dye from the vesicle is due to aggregates of surfactant causing membrane rupture.[7c] In addition, surfactants designed with rigid, wedge-shaped hydrophobic units show increased ability to release the dye encapsulated in osmotically stressed vesicles.[7d] Recently, Scrimin et al. reported that the addition of lipophilic amines affects the permeability of vesicular membranes by forming "leaky patches" in the membrane.[8] Addition of the long-chain amines promotes the concentration-dependent leakage of a fluorescent dye without the destruction of the vesicles.

Several metal salts also have been shown to perturb the bilayer structures of liposomal membranes. Cu^{2+} and Al^{3+} induce changes in the permeability of membranes and cause damage due to Fe^{2+}-initiated lipid peroxidation.[9,10] Studies with Sc^{3+}, Ga^{3+}, In^{3+}, Y^{3+}, and Be^{2+} demonstrate their ability to promote aggregation, fusion, permeabilization, and membrane rigidification.[11] These effects correlate with their capacity to induce Fe^{2+}-initiated lipid peroxidation, prompting the hypothesis that the metal ions alter the membrane by creating rigid clusters where the hydrocarbon chains are closer together, thus increasing the susceptibility of the lipids to peroxidation.[11b]

Inorganic V(IV) salts are very effective as modulators of cellular redox potentials and are known to exert pleiotropic effects.[12] Most recently, we discovered that some organometallic vanadium(IV) complexes have spermicidal and apoptosis-inducing properties.[13] In these reports, it was shown that unlike other metallocenes, such as Ti(IV), Zn(IV), Mo(IV), and Hf-(IV) complexes, only V(IV) derivatives cause the cessation of sperm motility in a concentration- as well as time-dependent fashion without affecting the sperm plasma and acrosomal membrane integrity.[13a,b] These compounds lead to the depolarization of mitochondrial membranes, which is a critical event in apoptotic cell death.[13] Such effects lead to leakage of cytochrome C and proteolytic enzymes, which could be the cause of vanadocene-induced apoptosis.[14] The lack of membrane disruption of these complexes is quite different than the detergent type of commercially available spermicides (e.g., nonoxynol-9, octoxynol-9). These results prompted us to undertake a thorough investigation of the physicochemical properties of the vanadocene(IV) derivatives with unilamellar liposomes to better understand the effects of vanadocene-chelated complexes on membranes.

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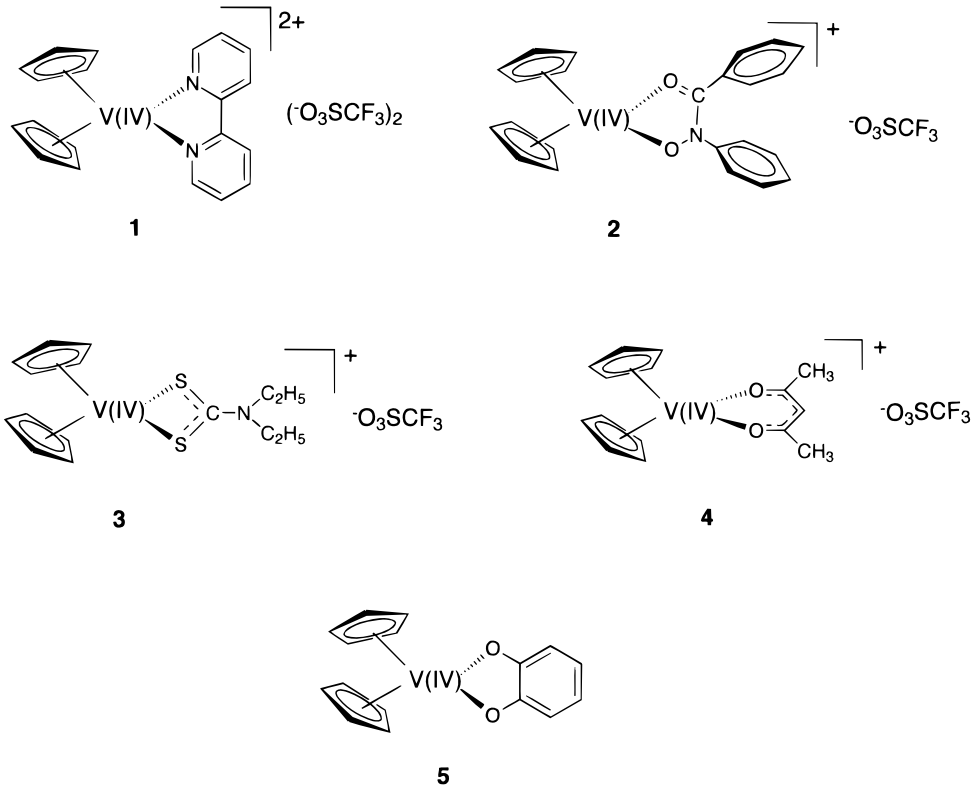


Figure 1. Chemical structures of the bis(cyclopentadienyl)vanadium(IV) chelate complexes.

In this paper, we report experimental evidence that some vanadocene-chelated complexes cause changes in the permeability of liposomal membranes. These permeability changes effected by organometallic V(IV) do not, however, correspond to lipid peroxidation nor are they promoting aggregation, fusion, or rigidity of the liposomes. The experimental results lead us to believe that the alteration in liposomal membrane permeability is due to the formation of leaky patches in the vesicular membrane, thus leaving the membrane integrity intact.

Materials and Methods

Materials. Egg yolk phosphatidylcholine (PC) and egg yolk phosphatidylglycerol (PG) were purchased from Avanti Polar Lipids (Alabaster, AL). Cholesterol, 1,6-diphenyl-1,3,5-hexatriene, and Triton X-100 were obtained from Aldrich Chemical Co. (Milwaukee, WI). 5(6)-Carboxyfluorescein, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)dipalmitoyl-L-α-phosphatidylethanolamine (N-NBD-PE) and *N*-(lissamine rhodamine B sulfonyl)-dipalmitoyl-L-α-phosphatidylethanolamine (N-Rh-PE) were from Molecular Probes, Inc. (Eugene, OR).

Instrumentation. Electron paramagnetic resonance (EPR) spectra were recorded in PBS (phosphate buffer saline, 0.015 M $NaHPO_4$, 0.10 M NaCl, pH 7.2) on a Bruker ESP 300 EPR spectrometer (9.64 GHz). The *g* values were calibrated with a Varian strong pitch (0.1% in KCl) standard (*g* value of 2.0028). The samples for the EPR spectral analysis were studied in a Willmad WG-814 standard TE102 aqueous cell cavity (0.3-mm inner path length) to minimize the dielectric loss.[15]

Cyclic voltammograms were obtained both in PBS and PC−cholesterol liposome solutions using a Bioanalytical Systems B/W 100 electrochemical analyzer with internal resistance (IR) compensation. Measurements were made in a cell fitted with a glassy carbon working electrode and a Ag/AgCl reference electrode (3 M NaCl in water). No corrections were made for junction potential effects. The reference for each of the applied systems was calibrated with a ferrocene/ferrocenium couple ($E_{1/2}$ = 0.185 V vs Ag/AgCl in PBS). Solutions were purged with nitrogen and scanned at 200 mV/s.

All fluorescence measurements were made using a Shimadzu spectrofluorophotometer (Model RF-5301PC). A UV/vis polarizer attachment was used for the polarization experiments. UV/vis measurements were made using a Beckman spectrophotometer (Model DU7400).

Synthesis of Bis(π-cyclopentadienyl)vanadium(IV)(L−L)$^{n+}$ (n = 0, 1+, or 2+) Complexes. The vanadocene-chelated complexes used in this study are shown in Figure 1. The ligands and their abbreviations are as follows: acac, acetyl acetonate; bpy, 2,2′-bipyridine; cat, catecholate; Cp, cyclopentadiene; Et_2-(dtc), *N*,*N*-diethyldithiocarbamate; PH, *N*-phenylbenzohydroxamate. $[VCp_2(Et_2(dtc))](O_3SCF_3)$ (**3**) and $[VCp_2(acac)](O_3SCF_3)$ **(4)** were prepared following literature procedures.[16] $[VCp_2(bpy)](O_3SCF_3)_2$ (**1**) and $[VCp_2(cat)]$ (**5**) were prepared and characterized as will be communicated elsewhere.[13c] The procedure for preparing the other new complex, $[VCp_2(PH)](O_3SCF_3)$ (**2**), and its chemical characterization is described as follows.

$[VCp_2(PH)](O_3SCF_3)$ (2). The reaction mixture composed of VCp_2Cl_2 (0.2 g, 0.79 mmol) and $AgCF_3SO_3$ (0.43 g, 1.7 mmol) in 10 mL of H_2O was stirred for 2 h and then filtered through a fine glass frit with Celite in air. A solution of *N*-phenylbenzohydroxamic acid (0.17 g, 0.79 mmol) in 5 mL of ethanol was added to the filtrate with stirring, and the resulting solution was kept for 4 h to complete the precipitation of a dark compound. The precipitate was collected by filtration, thoroughly washed with hexane, and dried overnight under vacuum to give 265 mg (62%) of the title compound, mp 160 °C. Anal. Calcd for $VC_{24}H_{20}NF_3O_5S$ (542.429): C, 53.10; H, 3.69; N, 2.58; S, 5.90. Found: C, 52.48; H, 3.72; N, 2.51; S, 5.73. UV/vis (CH_2Cl_2) λ_{max}: 680, 501 (d−d), 377 (LMCT), 314 (π−π* of hydroxamic moiety), 261, 233 (π−π* of Cp ring) nm. IR: 3345 (sb), 3117 (s), 1651 (mb), 1600 (m), 1539 (vs), 1495 (m),

1450 (m), 1300 (m), 1281 (s), 1244 (vs), 1173 (s), 999 (m), 758 (m), 694 (m), 638 (s), 578 (w), 515 (m) cm^{-1}.

Carboxyfluorescein Leakage Experiments. Liposomes with PC:cholesterol (3:1) or PG:cholesterol (3:1) were formed in 0.1 M phosphate buffer, pH 7.2, using the ethanol injection method[17] to a 1 mM total lipid concentration with carboxyfluorescein encapsulated under self-quenching conditions (0.1 M carboxyfluorescein). The liposomes encapsulated with carboxyfluorescein were separated from free carboxyfluorescein by passage over a Sephadex 25 column (Pharmacia Biotech) to give a final lipid concentration of 0.7 mM. **1**−**4** were each dissolved in methanol and added to the liposome solutions to a final vanadacene complex concentration of 50-400 μM.[18] **5** was unstable in methanol and so was dissolved in distilled water and added to the liposome solutions in the same way. The increase in fluorescence was monitored at $\lambda_{exc} = 550$ nm and $\lambda_{em} = 490$ nm for 6 min at 20 °C.[10c,11a,19] Complete liposome disruption was achieved by the addition of excess Triton X-100 (10 μL of a 10% aqueous solution). Carboxyfluorescein release was calculated as

$$\text{CF}\ (\%) = (F - F_0)/(F_t - F_0)100$$

where CF = carboxyfluorescein release, F_0 = fluorescence intensity of the intact liposome, F = fluorescence intensity at time = 6 min, and F_t = fluorescence intensity with Triton X.

Lipid Peroxidation. Aliquots of 0.5 mL of liposomes (0.7 mM total lipid) were incubated with the vanadocene-chelated complexes (50-400 μM) at 37 °C for 90 min. The incubation was stopped by the addition of 0.1 mL 4% butylated hydroxytoluene in EtOH. Sodium dodecyl sulfate (3%; 0.25 mL) was added to destroy the liposomes followed by the addition of 0.5 mL of 1% 2-thiobarbituric acid in 0.05 M NaOH and 0.5 mL of 25% HCl. The samples were mixed and heated in boiling water for 15 min. The 2-thiobarbituric acid reactive substances were extracted into 3 mL of 1-butanol, and the fluorescence of the butanol layer was measured at $\lambda_{exc} = 515$ nm and $\lambda_{em} = 555$ nm.[9a,10,11] The 2-thiobarbituric acid reactive substances are reported as malonaldehyde equivalents.[20]

Lipid Packing Order. Liposomes (0.7 mM) containing 1 mol % of 1,6-diphenyl-1,3,5-hexatriene were prepared as described.[17] Only liposomes with zwitterionic lipids could be used because the presence of diphenylhexatriene caused the negative liposomes to precipitate out immediately. The vanadocene-chelated complexes were added to the liposome solutions in increments of 50 μM. The solutions were incubated for 5 min at 20 °C prior to measurement of fluorescence intensities at $\lambda_{exc} = 360$ nm and $\lambda_{em} = 450$ nm. The extent of polarization was calculated according to the method described by Jahnig.[21]

Liposome Aggregation. The vanadocene-chelated complexes were added to 0.5 mL of liposome solution (0.7 mM total lipid) to yield a final concentration of 50−400 μM vanadium. The aggregation of liposomes was measured as the increase in absorbance at 300 nm in a UV/vis spectrophotometer over 15 min at 20 °C.[11a]

Liposomes Fusion. A 1:4 ratio of liposomes (0.7 mM total lipid) containing 2% N-NBD−PE, N-Rh−PE, and liposomes with no labeled lipids was prepared in phosphate buffer, pH 7.2. Fusion was measured after the addition of the vanadocene-chelated complexes by changes in the fluorescence intensity at $\lambda_{exc} = 530$ nm and $\lambda_{em} = 470$ nm at 20 °C.[11a,22] Complete liposome disruption was achieved by the addition of excess Triton X-100 (10 μL of a 10% solution). When liposomes containing N-NBD−PE and N-Rh−PE fuse with liposomes not containing probes, the surface density decreases, resulting in a decreased efficiency of resonance energy transfer from N-NBD−PE to N-Rh−PE.[22]

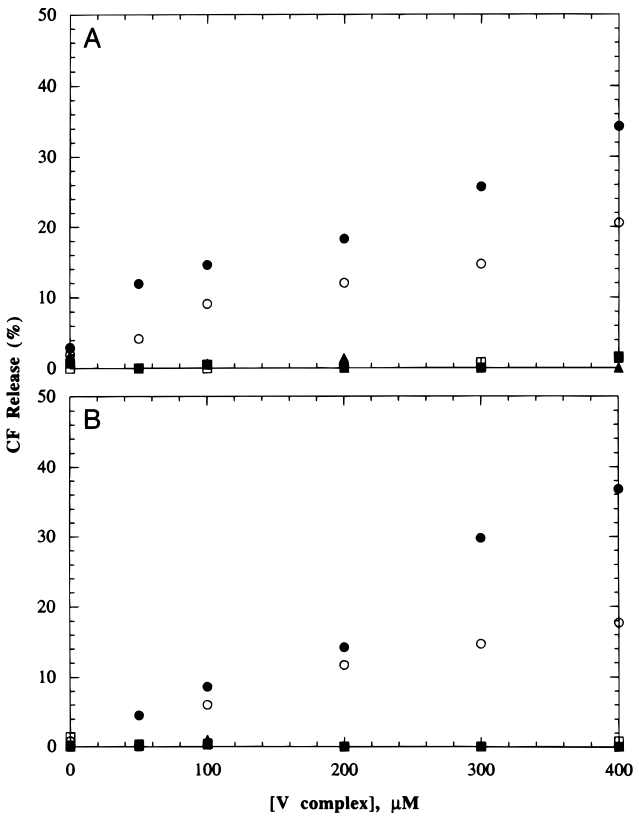


Figure 2. Relative amount of released CF after 6 min from (A) CF-loaded PC liposomes and (B) CF-loaded PG liposomes as a function of the concentration of added vanadocene-chelated complexes: (●) **1**, (○) **2**, (□) **3**, (■) **4**, (▲) **5**.

Results

Effects of Vanadocene-Chelated Complexes on Liposome Leakage. The concentration dependence for vanadocene-chelated complex-induced permeability of liposomes, as measured by the release of 5(6)-carboxyfluorescein, is shown in Figure 2 for zwitterionic (PC) and negative (PG) liposomes. The vanadocene-chelated complexes themselves do not interfere with the fluorescence of the carboxyfluorescein probe. The kinetics of **1**-induced liposome leakage shows that the process has reached a plateau after 2 min of incubation (Figure 3). Similar kinetics are observed for **2**. The vanadocene derivatives **1** and **2** cause liposome permeability in a concentration-dependent manner. **1** induces the release of approximately 35% of the entrapped carboxyfluorescein at a concentration of 400 μM **1**, while **2** causes a 20% release at the same concentration. The vanadocene-chelated complexes **3**−**5** have no effect on carboxyfluorescein leakage. Simultaneous control experiments with all of the chelating ligands confirmed that the ligands had no effect on the permeation (data not shown).

Effects of Vanadocene-Chelated Complexes on Lipid Peroxidation. The amount of lipid peroxidation caused by the incubation of PC and PG liposomes with the vanadocene-chelated complexes is shown in Figure 4 for PC and PG liposomes. The vanadocene complexes **4** and **1** induce lipid peroxidation, as measured by the production of 2-thiobarbituric acid reactive substances. However, initiation of lipid peroxidation by **1** is significantly less (Figure 4). The other complexes, **2**, **3**, and **5**, have no effect on lipid peroxidation.

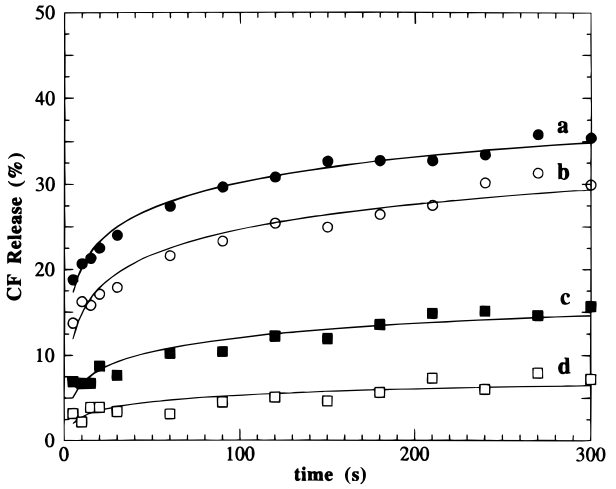


Figure 3. Time course of the fluorescence increase when CF-loaded PG liposomes are exposed to different concentrations of added **1**. [**1**] = 1×10^{-6} M (a), 2×10^{-6} M (b), 3×10^{-6} M (c), 4×10^{-6} M (d).

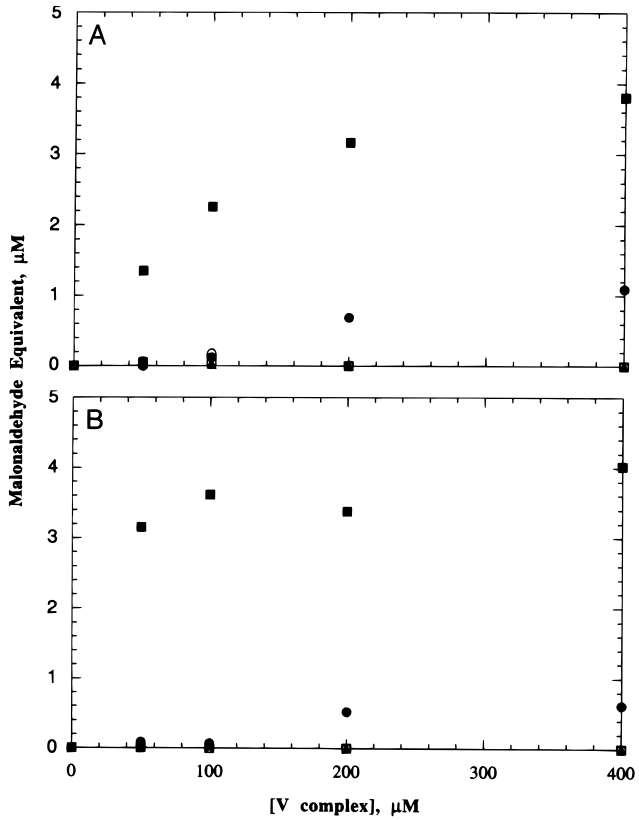


Figure 4. Effects of vandocene-chelated complexes on lipid peroxidation. PC liposomes (A) or PG liposomes (B) were incubated in the presence of vanadocene-chelated complexes: (●) **1**, (○) **2**, (□) **3**, (■) **4**, (▲) **5**. Lipid peroxidation was evaluated as the production of 2-thiobarbituric acid reactive substances and is reported in malonaldehyde equivalents.

Effects of Vanadocene-Chelated Complexes on Lipid Packing Order. Since the PC:cholesterol liposome is already in the liquid phase, an increase in fluorescence polarization would indicate membrane rigidification. No such increase occurs after the addition of any of the vanadocene-chelated complexes at 20 °C (data available as supporting information).

Effects of Vanadocene-Chelated Complexes on Liposomal Aggregation and Fusion. No appreciable turbidity, resulting from aggregation of the liposomes, is caused by the addition of any of the vanadocene-chelated complexes up to a 400 μM concentration as measured by an increase in absorbance at 300 nm for PC or PG liposomes (data available as supporting information). Similarly, no fusion of the liposomes is seen with the addition of any of the studied vanadocene-chelated complexes as indicated by the constant efficiency of energy transfer between fluorescent liposomes (data available as supporting information).

TABLE 1: Cyclic Voltametry and EPR Studies of the Vanadocene-Chelated Complexes

	cyclic voltametry V^{VI}/V^{III} redox couple, $E_{1/2}$, mV		EPR g	
compd	PBS[a]	liposomes[b]	PBS	liposomes
1	−441	−434	1.985	1.985
2	−448[c]	−449[c]	1.983	1.984
3	−668	−665	1.987	1.981
4	−772	−770	1.980	1.980
5	−856[c]	854[c]	1.981	1.981

[a] Data collected in PBS solution, with Ag/AgCl as a reference electrode, a glassy carbon working electrode, and platinum wire as an auxillary electrode. Solutions were purged with nitrogen and scanned at 200 mV/s. [b] Electrochemical data as collected in PC liposome solution (0.7 mM lipids) under identical conditions as described for PBS. [c] E_{pc} value, cathodic peak potential.

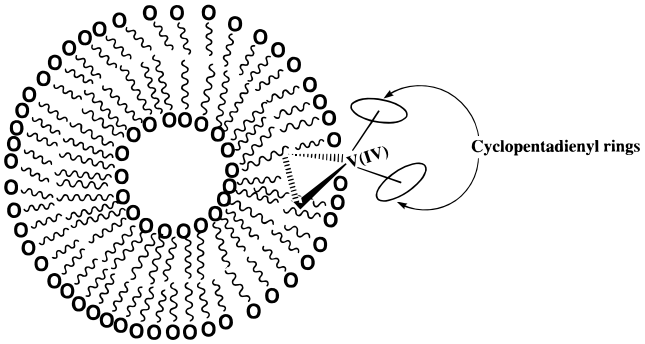
Cyclic Voltammetry and EPR Studies of the Vanadocene-Chelated Complexes in the Presence of Liposomes. Table 1 shows the electrochemical as well as the EPR results of these vanadocene-chelated complexes in the presence of liposomes. The data without liposomes in PBS buffer are provided for comparison.

Discussion

Vanadocene complexes **1** and **2** cause the leakage of carboxyfluorescein from both zwitterionic and negatively charged liposomes as seen in Figure 2. The vanadocene-chelated complexes **3**−**5,** on the other hand, have little or no effect on the permeability of liposomes, indicating special effects imparted by **1** and **2**. None of the vanadocene-chelated complexes cause appreciable liposome aggregation or fusion in either type of liposome. If the complexes were acting in such a way as to create localized areas of inverted micelles, fusion and aggregation would have been expected, as areas of hydrophobic patches were exposed to the aqueous solution.[6] Experimental evidence herein indicates that this is not the case. In addition, based on the cyclic voltammetric measurements as well as from the characteristic sharp eight line spectral features in the EPR of the complexes, all of the vanadocene-chelated complexes studied retain their pseudotetrahedral-like structure in the liposomal matrices with respect to the central metal V(IV) ion.[23]

The type of permeation that we observe caused by the two vanadocene-chelated complexes **1** and **2** is similar to what was seen by Scrimin et al. with the addition of lipophilic amines.[8] The amine additives caused concentration-dependent permeation of probe molecules without altering the gross features of the vesicles. Previous studies with low concentrations of surfactants also reported an increase in membrane permeability but did not systematically measure changes in the liposome or vesicle morphology.[6,7] In the present studies, however, the compounds are not surfactants nor do they contain long hydrocarbon chains. More importantly, the vanadocene complexes are much shorter than the 30−40 Å required to span the bilayer and form channels.[5] Therefore, the mechanism of activity of these organometallic complexes to cause such effects is likely to be quite different from that of the surfactants or ionophores.

SCHEME 1. Pictoral Representation of the Intersertion of 1 in a Liposome



The permeation effect caused by **1** and **2** could be explained in terms of the difference in their overall structural configurations compared to the three other chelated vanadocene derivatives. Both **1** and **2** possess two aromatic rings besides their core VCp_2^{2+} unit in the inner coordination sphere of the V(IV) ion. In the former complex, the two heterocyclic aromatic rings are fused in a plane constituting the V, N, N atoms of the ancillary positions of a pseudotetrahedral geometry, while in the latter complex, the two planar phenyl rings are covalently linked to the carbon and nitrogen atoms of the hydroxamate moiety, thus conferring flexibility along the C−C and C−N axis, respectively. It is reasonable that both configurations are more able to insert inside the hydrophobic portion of the membrane compared to the other three chelated complexes. Cationic complexes of several octahedral Ru(II), Rh(III), and Co(III) coordination complexes using 2,2′-bipyridyl, 1,10-phenanthroline, and 9,10-phenanthrenequinonediimine-type planar fused ring systems as ligands have been shown to bind to micelles in the Stem layer with the ligands inserted into the hydrophobic portion of the micelle.[24] This interaction is related to the intercalative stacking phenomenon documented in the literature by Barton's group using coordinated complexes in the DNA duplex.[25]

The vanadocene-chelated complexes described here are tetrahedral in geometry in which the two Cp rings are positioned in a bent sandwich conformation with respect to the V(IV) central metal ion. Presumably, this geometry is necessary for leakage to occur because the same effect could not be detected for the free ligand in control experiments using 2,2′-bipyridine or phenylbenzohydroxamic acid instead of their respective vanadocene-chelated complexes. The effect of the vanadocene-chelated complexes wedged in localized patches of the membrane may render it temporarily "leaky" without affecting the overall integrity of the liposome. A schematic representation of the intercalation of **1** in the liposome is depicted in Scheme 1.

The difference in the level of permeation, viz. 35% vs 20%, between **1** and **2** at 400 μM concentration could be attributed to their relative difference in perturbation once intercalated. The two fused heterocyclic rings in the bipyridine ligand, by virtue of its rigidity, may cause relatively wider patches of perturbation in the packing of the hydrocarbon chains than the two separate rotatable phenyl rings present in the phenylbenzohydroxamate ligand. Although the vanadocene derivative **5** also contains a planar, aromatic ring as a chelating ligand, it is possible that one ring may not be large enough to create the required patch in the liposome through which the carboxyfluorescein can permeate. These results strengthen our hypothesis that it is the preferential configuration of these two compounds which is responsible for interacting with the membrane to cause permeation of encapsulated dye molecules.

Although **4** does not cause any permeation of the membrane, it does cause significant lipid peroxidation. Among the four other complexes, only **1** shows lipid peroxidation, albeit to a lesser extent (Figure 4). It has been well documented[26] that reactive $OH^{\bullet}$ radicals have to be formed initially through a Fenton-type reaction to propagate lipid peroxidation in order to achieve such a significant amount of lipid peroxidation. In view of this experimental evidence related to the lipid peroxidation of the PC and PG liposomes in the presence of vanadocene-chelated complexes, we believe that **4** or **1**, through an as yet undefined reaction pathway, is capable of generating the reactive oxygen intermediates required for initiation of lipid peroxidation. In this context, we tried to correlate the V(IV)/(V) redox potential with the observed vanadium-mediated reactivity difference in lipid peroxidation without success (see Table 1).[27] The V(IV)/(V) redox potential was not observed for any of the complexes either in PBS or in liposomal solutions over the potential range of +1.0 to −1.0 V with a glassy carbon electrode, although the V(IV)/V(III) couple is readily observed. Detailed mechanistic studies on this particular issue are in progress. The present results lead us to believe that peroxidation is not the cause of the formation of leaky patches since **4** exhibits the strongest peroxidation of the lipids but does not increase the permeability of a dye molecule through the membrane.

Furthermore, since rigidification of membranes correlates with metal-ion-stimulated propagation of lipid peroxidation, it has been suggested that changes in the membrane fluidity could facilitate the lipid peroxidation process.[6] However, since none of the vanadocene-chelated complexes have an effect on the overall packing order of the hydrophobic chains of the zwitterionic liposomes at the temperature the experiments were conducted, it is highly unlikely that the differences in lipid peroxidation can be explained by rigidification of the hydrocarbon portion of the liposome. It is also not likely that charge effects play an important role with respect to the vanadocene-chelated complex−liposome interaction because there is very little difference between zwitterionic and negatively charged liposomes for any of the properties studied. No correlation has been found between the charge on the vanadocene derivative and its ability to cause permeation or peroxidation effects, which leads us to conclude that hydrophobic interactions between the chelated ligands and the hydrocarbon portion of the liposome appear to be more important than electrostatic interactions between the complexes and the charged headgroups. This observation is similar to studies in which comparisons between the equilibrium constants for the association of cationic complexes with anionic micelles revealed that hydrophobic effects dominate the binding properties.[25]

Aggregation, measured by turbidity with UV/vis spectroscopy, fusion properties, by fluorescence energy resonance transfer measurements, and rigidification studies, through fluorescence polarization, clearly reveal the fact that none of the added complexes change the structural integrity of the liposomes. These results closely resemble the observations made by D'Cruz et al. from biological assays, which indicated that the spermicidal vanadocene-chelated complexes do not disrupt the sperm plasma membrane.[13a,b]

Conclusions

In summary, we have explored the interaction between membranes using PC and PG liposomes, with five vanadocene-(IV)-chelated complexes under physiological conditions as

model studies. We found that two of these compounds (**1** and **2**) cause the liposomes to become permeable, and this effect is *not* related to the extent of peroxidation of the lipids by the vanadium-chelated complexes or to a tendency to cause aggregation or fusion or alter the membrane packing order. We believe that in order to observe such properties, these two compounds must have a unique structural requirement, particularly the hydrophobicity, planarity, and rigidity of the coordinated ancillary ligands, which could alter the membrane by intercalation. Although these results do not explain the cause of the sperm immobilization and apoptosis by vanadocene complexes, we can concur with the findings of D'Cruz et al.[13a,b] that these complexes certainly do not cause disruption of membranes, as evidenced from the above physicochemical studies of the model liposomal membranes.

Supporting Information Available: Figures depicting the vanadocene-chelated complex concentration-dependent liposomal aggregation, fusion, and fluorescence polarization experiments with different liposomes (PC and PG) (3 pages). See any current masthead page for ordering and Internet access instructions.

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