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Adsorption of Phenyltin Compounds onto Phosphatidylcholine / Cholesterol Bilayers

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Phenyltin compounds are known to be biologically active and, whan widely spread, are potentially hazardous. As their chemical structure suggests, they interact with the lipid fraction of the cell membrane. Their effect on the model phosphatidylcholine/cholesterol bilayer has been studied using fluorescence and ^{1}H NMR techniques. The change in the fluorescein-PE fluorescence intensity indicates the amount of charge added by phenyltin compounds to the membrane surface. Although the presence of cholesterol alone does not alter membrane interface properties measured with fluorescein-PE, ^{1}H NMR measurements show that lipid mobility is altered throughout the hydrophobic core of the membrane. Cholesterol in the phosphatidylcholine bilayer does not alter tetraphenyltin interaction with the membrane, though the effect of diphenyltin dichloride, penetrating deeply into the hydrophobic core of the membrane, is reduced when the amount of cholesterol in the membrane is increased, suggesting decreased compound adsorption. Triphenyltin chloride has a qualitatively different effect on the lipid bilayer, when observed using this fluorescence technique. The adsorption of triphenyltin onto the phosphatidylcholine/cholesterol membrane induces a lateral phase separation of membrane components. Since triphenyltin chloride is known to be adsorbed onto the interface of the lipid bilayer, this separation mechanism must originate in this region and does not seem to be electrostatic in origin. ^{1}H NMR measurements have confirmed the observation that these two active phenyltin compounds interact with the phosphatidylcholine/cholesterol membrane differently, disrupting different regions of the bilayer to a different degree. **Copyright © 2000 John Wiley & Sons, Ltd.**

Keywords: phenyltin; cholesterol; lipid bilayer; domains; interface

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INTRODUCTION

Phenyltins are industrial pollutants,[1–3] and their presence in the environment is widely spread, although (due to their hydrophobicity) their concentrations are low and do not usually exceed the micromolar range.[4,5] The hydrophobic core of the lipid bilayer is an environment in which hydrophobic metalorganics are likely to accumulate,[6] interfering with the cell's vital functions.[5,7,8] When approaching the cell from the extracellular environment, phenyltins encounter the plasma membrane, the natural barrier for harmful environmental pollutants, and so the interaction of these compounds with the membrane is the initial process to be described in order to understand their biological activity. The outer plasma membrane layer is composed mainly of phosphatidylcholines and cholesterol.[9,10] Cholesterol is one of the most abundant mammalian cell membrane components and, depending on the cell line, it constitutes 10–50% of the total amount of lipids in the plasma membrane.[10–12] A cholesterol molecule contains three well-distinguished regions: a small polar hydroxyl group, a rigid plate-like steroid ring, and an alkyl chain 'tail'. Due to this molecular structure, cholesterol is located within the hydrophobic core of the lipid bilayer, with its polar hydroxyl group in the vicinity of the interface.[11] The presence of cholesterol in the membrane results in decreased membrane permeability for water and water-soluble molecules,[13–18] causing the so-called 'condensing' effect, i.e. its presence reduces the membrane's ability to accommodate bulky hydro-

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phobic molecules.[15,19] We have shown previously that diphenyltin chloride penetrates into the bilayer interior, whereas triphenyltin chloride is adsorbed onto the membrane surface.[20,21] In this paper, we determine the manner in which cholesterol influences the interaction of phenyltins with the lipid bilayer (formed from a variety of phosphatidylcholine/cholesterol mixtures).

We expect that the presence of cholesterol in the membrane should most affect the adsorption of bulky compounds which penetrate the hydrophobic core of the lipid bilayer; and that it should affect those adsorbed onto the bilayer surface the least. From the data presented in this paper we compare the interactions of surface-active triphenyltin chloride (Phe_3SnCl) and diphenyltin chloride (Phe_2SnCl_2) with a mixed phosphatidylcholine/cholesterol lipid bilayer. A combination of steady-state ^1H NMR and steady-state fluorescence spectroscopy has been used to measure the effect of phenyltin derivative interaction with the liposome membrane.

MATERIALS AND METHODS

Materials

Phosphatidylcholine was extracted from egg yolk, as described elsewhere.[22] Cholesterol was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All organotin compounds, namely $(C_6H_5)_4Sn$— tetraphenyltin (Phe_4Sn), $(C_6H_5)_3SnCl$—triphenyltin chloride (Phe_3SnCl) and $(C_6H_5)_2SnCl_2$—diphenyltin chloride (Phe_2SnCl_2) were purchased from Alfa Products. Aldrich Chemical Co. and Merck. Praseodymium (Pr^{3+}) chloride ($PrCl_3{\cdot}6H_2O$) and heavy water (D_2O) were obtained from Aldrich Chemical Co. and Swierk (Poland), respectively. The fluorescence probe *N*-(5-fluoresceinthiocarbamoyl)dipalmitoyl-L-α-phospatidylethanolamine (fluorescein-PE) was obtained from Molecular Probes (Eugene, OR, USA).

Fluorescence measurements

The lipid, with an appropriate amount of cholesterol and fluorescent probe (0.1 mol%), was dissolved and mixed in chloroform. The chloroform was removed under vacuum, a buffer was added and the sample was vortexed to obtain a milky suspension of multilamellar vesicles. The final concentration of lipid in the vesicle stock suspension was 1 mg ml^{-1}. Such a suspension was then sonicated for 15 min with a 20 kHz sonicator (equipped with a titanium probe) to obtain a transparent suspension of small unilamellar vesicles (SUVs). The vesicle suspension was then centrifuged to remove titanium particles. Each sample was prepared shortly before measurements and kept on ice during the experiment. Prior to each fluorescence experiment, the diluted vesicle suspension (0.2 mg ml^{-1}) was thermally equilibrated in a cuvette holder so as to obtain stable fluorescence (about 5 min). The organotin compound was then added from a concentrated ethanol solution (2×10^{-3} M) to the stirred sample of suspended vesicles. The final concentration of ethanol never exceeded 2% (v/v). The effect of such quantities of ethanol on fluorescence was smaller than the normal fluctuations in sample emission intensity; hence it was not accounted for in relative fluorescence change calculations (data not shown). Fluorescence values presented throughout the paper were measured when fluorescence intensity stabilized after the addition of phenyltin (usually fluorescence was measured 10 min after compound addition). Fluorescence intensity was measured prior to (F_o) and after each addition of organotin compound (F_i). Excitation (λ_{EX}) and emission (λ_{EM}) wavelengths for fluorescein-PE were: $\lambda_{EX} = 485$ nm, $\lambda_{EM} = 520$ nm. Throughout this paper, fluorescence data are presented as relative change in fluorescence intensity, according to the following formula: $(F_i - F_o)/F_o$ Steady-state fluorescence measurements were carried out on a Kontron Instruments fluorimeter (Switzerland). Fluorescence intensities were corrected for the inner filter and dilution effects.[23,24]

^1H NMR spectroscopy

Lecithin and an appropriate amount of cholesterol were dissolved in chloroform, dried in a nitrogen atmosphere and dispersed in D_2O. The final concentration of lecithin was 25 mg ml^{-1}. SUVs were prepared as described above. The dispersion of vesicles was divided into samples, into which praseodymium ions and phenyltin compounds, dissolved in deuterated methanol, were added. All data were corrected for the effect of deuterated methanol.

Praseodymium ions serve as shift reagents, i.e. their addition to the vesicle suspension causes an initial single peak in $N^+(CH_3)_3$ resonance, splitting into the resolved downfield and upfield components. The downfield signal comes from the

extravesicular and the upfield resonance from the intravesicular (unmodified) choline headgroups. Signal splitting is proportional to the amount of praseodymium ions present at the membrane interface. Hence, any additional sorption or desorption of the shifting reagent can be easily detected on the ^{1}H NMR spectrum.

Praseodymium ions were added to the vesicle suspension from a stock solution in D_2O. The final concentration of praseodymium calculated as a molar fraction of lipid in the sample was 1:3.38 (4.06 mM). The ^{1}H NMR spectra were recorded on a Bruker Avance DRX 300 MHz spectrometer at 30 °C when the spectral window was 6173 Hz; digital resolution 145.2145 Hz cm^{-1} or 0.48348 ppm cm^{-1}; pulse width 10.7 ms, and acquisition and delay times 2.65 s and 1 s, respectively.[25,26]

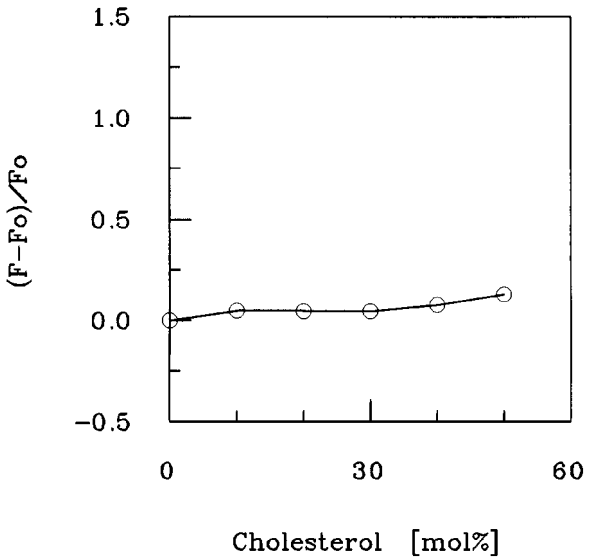

Figure 1 Changes in fluorescence intensity in the presence of various amounts of cholesterol in the phosphatidylcholine bilayer. In all samples the same amount of fluorescein-PE was incorporated into the membrane. The fluorescence intensity of the probe in the bilayer containing cholesterol (F) is compared with that when the probe was in a pure phosphatidylcholine membrane (F_0).

RESULTS

Fluorescence spectroscopy measurements

The amount of charge on the membrane surface was measured with the pH-sensitive fluorescent probe, fluorescein-PE.[21,27] Fluorescein dye is covalently attached to the headgroup of a phosphatidylethanolamine molecule, which ensures that the hydrophilic dye is located in the aqueous phase adjacent to the membrane surface. This localization of the dye allows the measurement of changes occuring at the membrane–water interface.[27–29]

First, we measured the effect of cholesterol on fluorescein-PE fluorescence intensity. As shown in Fig. 1, there is little change in fluorescence intensity when the amount of cholesterol in the phosphatidylcholine bilayer is raised to 50 mol%. This result allows us to assume that changes in fluorescence intensity are predominantly a result of phenyltin compound adsorption onto the membrane surface.

Figure 2 presents changes in fluorescein-PE fluorescence intensity caused by the addition of phenyltins to vesicle suspensions, as a function of the amount of cholesterol present in the phosphatidylcholine bilayer. Adsorption of a charged phenyltin onto the membrane surface introduces a positive charge at the interface, which causes probe fluorescence to rise.

As expected,[20] Phe_4Sn does not affect probe fluorescence intensity at the membrane interface. This compound does not interact with a pure phosphatidylcholine membrane and the presence of cholesterol does not alter the lipid bilayer structure to such an extent as to enable it to penetrate the interface (Fig. 2C). When Phe_2SnCl_2 was added to the vesicles labeled with fluorescein-PE, the presence of cholesterol reduced changes in fluorescence caused by the adsorption of phenyltin compounds (Fig. 2A. This result implies that the amount of charge added to the membrane surface by Phe_2SnCl_2 is reduced when cholesterol is present in the membrane. It may be considered, therefore, that Phe_2SnCl_2 partitions weakly into the lipid phase. The effect of cholesterol on surface-active Phe_3SnCl was qualitatively different (Fig. 2B). When the concentration of Phe_3SnCl was lower than 10 μM, the rise in fluorescence was reduced with the increasing content of cholesterol in the membrane. However, at higher Phe_3SnCl concentrations fluorescein-PE fluorescence intensity is no longer a monotonic function of the amount of the phenyltin in the sample, as it first reaches a maximum and then begins to fall. The concentration of Phe_3SnCl which causes the maximum of fluorescence intensity depends on membrane cholesterol content: the larger the amount of cholesterol, the lower the concentration of Phe_3SnCl required to reach a fluorescence

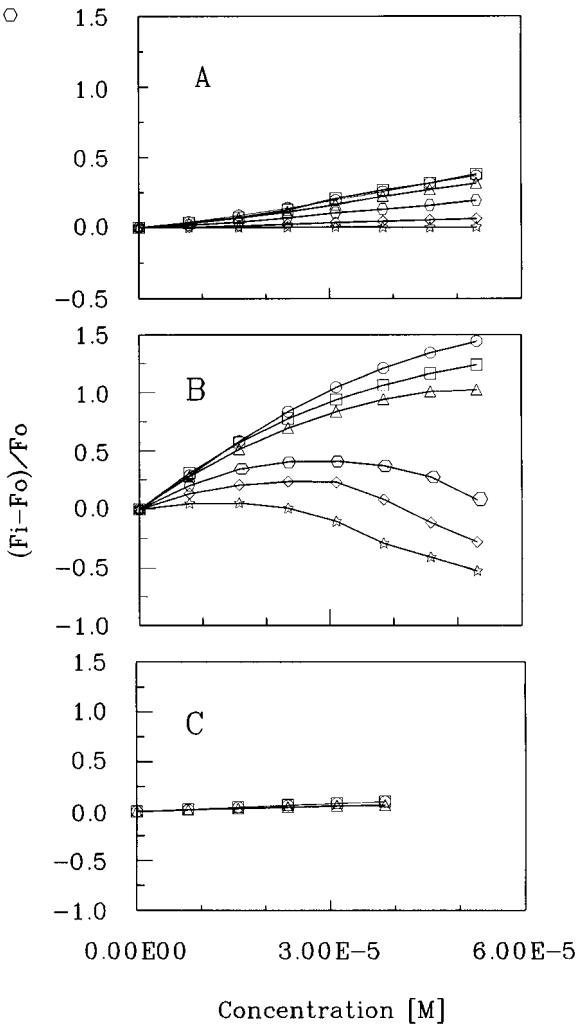

Figure 2 Relative changes in the fluorescein-PE fluorescence intensity induced by addition of phenyltin compounds. Small unilamellar vesicles were suspended in 140 mM NaCl/phosphate buffer pH 7.4. The amount of phenyltin compound added to the sample is shown as total concentration in the sample. The lipid concentration was 0.26 mM. (A) Phe_2SnCl_2; (B) Phe_3SnCl; (C) Phe_4Sn compounds □, Phenyltins were added to pure phosphatydylcholine liposomes; the other data were obtained when the relative cholesterol contents in the bilayer were: (○) 10 mol%; (△) 20 mol%; (⬡) 30 mol%; (◇) 40 mol%; (☆) 50 mol%.

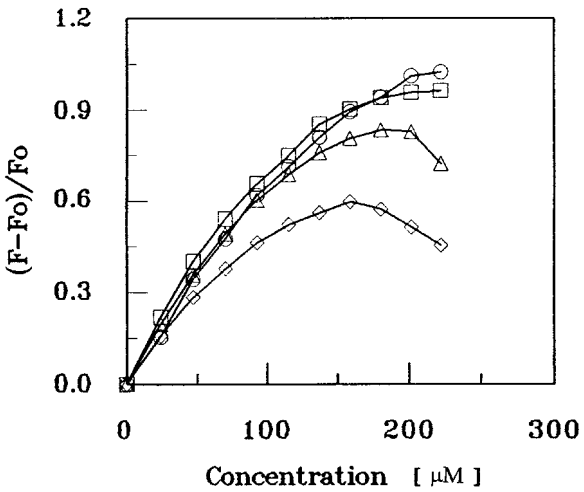

Figure 3 The relative change in fluorescence induced by the addition of Phe_3SnCl to a liposome suspension in the presence of different amounts of fluorescein-PE in the membrane. Vesicles were formed from phosphatidylcholine with 30 mol% of cholesterol. The amounts of fluorescein-PE in the lipid bilayer were: (○) 0.05 mol%; (□) 0.1 mol%; (△) 0.18 mol%; and (◇) 0.37 mol%.

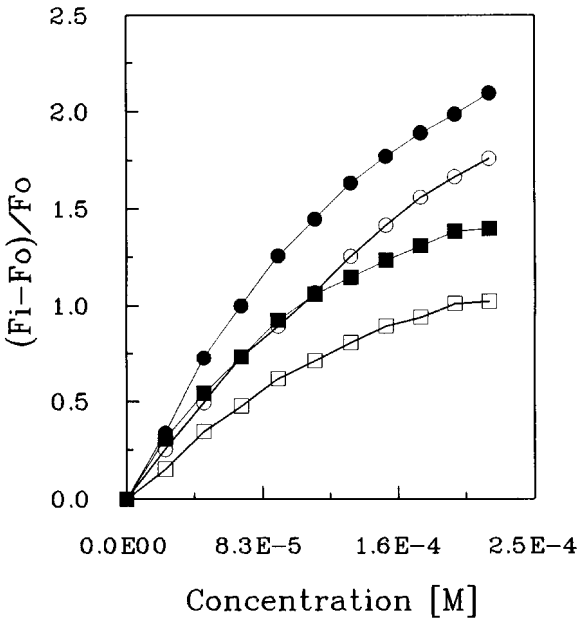

Figure 4 The relative change in fluorescence intensity induced by the adsorption of Phe_3SnCl as a function of salt concentration in the bulk buffer solution. Circles are for pure phosphatidylcholine, and squares denote phosphatidylcholine with 30 mol% cholesterol in the phosphate buffer containing 14 mM; NaCl (filled symbols) and 140 mM NaCl (open symbols).

maximum. At around 30 mol% of cholesterol, the effect is altered and the fluorescence intensity falls below the initial level. The Phe_3SnCl charge is not likely to change, so such a drop in fluorescence intensity may be a result of changes in local fluorophore concentration. When local concentra-

tion of the probe is sufficiently high, then it will quench itself.[23] To test this possibility, we have varied the amount of the dye in the membrane and measured the concentration of Phe_3SnCl, at which the maximum occurred. As expected, when the dye concentration in the membrane was varied from 0.05 mol% to 0.37 mol%, the fluorescence maximum shifted toward lower Phe_3SnCl concentrations (Fig. 3).

Figure 4 shows how change in fluorescein-PE fluorescence depends on the salt concentration in the aqueous solution. Fluorescence intensity was measured when the probe was in phosphatidylcholine, and in phosphatidylcholine with 30 mol% cholesterol at two salt concentrations: 14 mM and 140 mM. It is well established that an elevated salt concentration results in a screening of surface charges, which in our experiment should reduce changes in fluorescence caused by Phe_3SnCl.[30,31] In both cases, i.e. in the presence and absence of cholesterol, an elevated salt concentration depressed changes in fluorescence induced by the addition of Phe_3SnCl, proving the electrostatic nature of the effect (regardless of membrane composition). At both salt concentrations, relative changes in fluorescence in the presence of cholesterol, when compared with the sample with vesicles formed from pure phosphatidylcholine, were similar.

^{1}H NMR experiments

Examples of ^{1}H NMR spectra obtained from suspensions containing vesicles with various amounts of cholesterol in the presence of 4.03 mM Pr^{3+} are presented in Fig. 5. The presence of cholesterol broadens all peaks, with limited shifts in position. Significant changes in the membrane interior caused by the presence of cholesterol are reflected by an increased bandwidth of signals originating from the $—(CH_2)_n$ groups of hydrophobic chains (Fig. 5) and confirm the effect of cholesterol on membrane hydrophobic core organization.[11,17,32–35] Signal splitting from $—N^+(CH_3)_3$ groups of both inner and outer monolayers caused by Pr^{3+} adsorption in the presence of various amounts of cholesterol are shown in Fig 6(a). Figure 6(b) shows the percentage of Pr^{3+} bound against the amount of cholesterol content. When 50 mol% of cholesterol is present, the amount of bound Pr^{3+} to decreased by about 25%, indicating limited changes occurring at the membrane interface.

Phenyltin addition changes signal splitting from $—N^+(CH_3)_3$ groups located at the inner and outer lipid layers. Such signal splitting is proportional to the amount of Pr^{3+} ions on the outer layer of the membrane. Figure 7(a) shows selected examples of ^{1}H NMR spectra in the presence of 0.98 m**M** Phe_2SnCl_2, whereas Fig. 8(a) was obtained in the

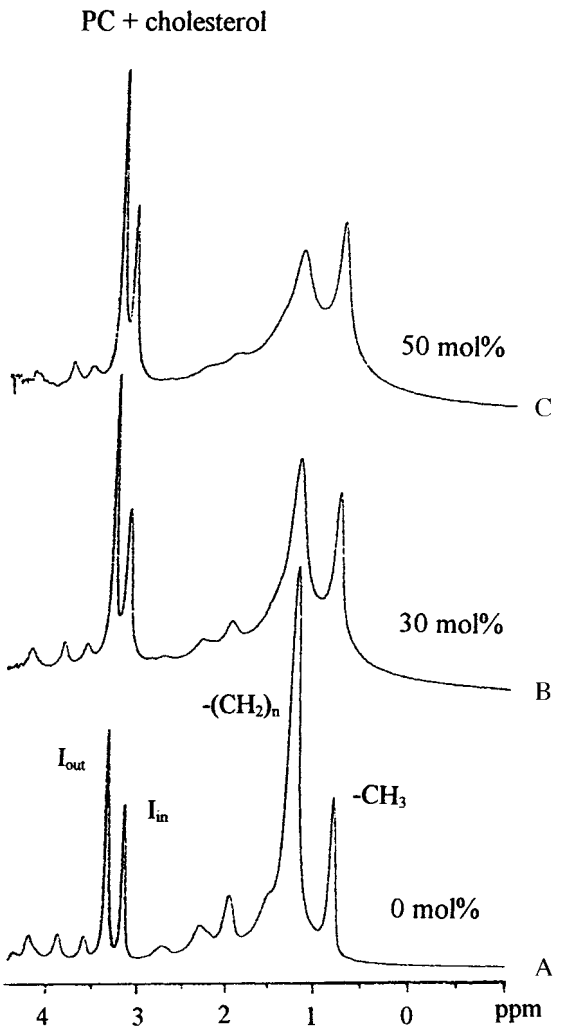

Figure 5 Examples of typical 300 MHz ^{1}H NMR spectra of sonicated vesicles formed from phosphatidylcholine/cholesterol mixtures (33 mM) pretreated with Pr^{3+} in D_2O at 30 °C. The extravesicular concentration of Pr^{3+} was 4.06 mM. The water peak at 4.700 ppm was used as the reference signal (not shown here). (A) The main signals come from extravesicular (I_{out}) and intravesicular (I_{in}) choline headgroups, acyl chain methylenes $=(CH_2)_n$ and the terminal methyl groups $—CH_3$. Spectra B and C were obtained for praseodymium-pretreated phosphatidylcholine/cholesterol liposomes at relative cholesterol/phosphatydylcholine molar concentrations of 30 mol% and 50 mol%, respectively.

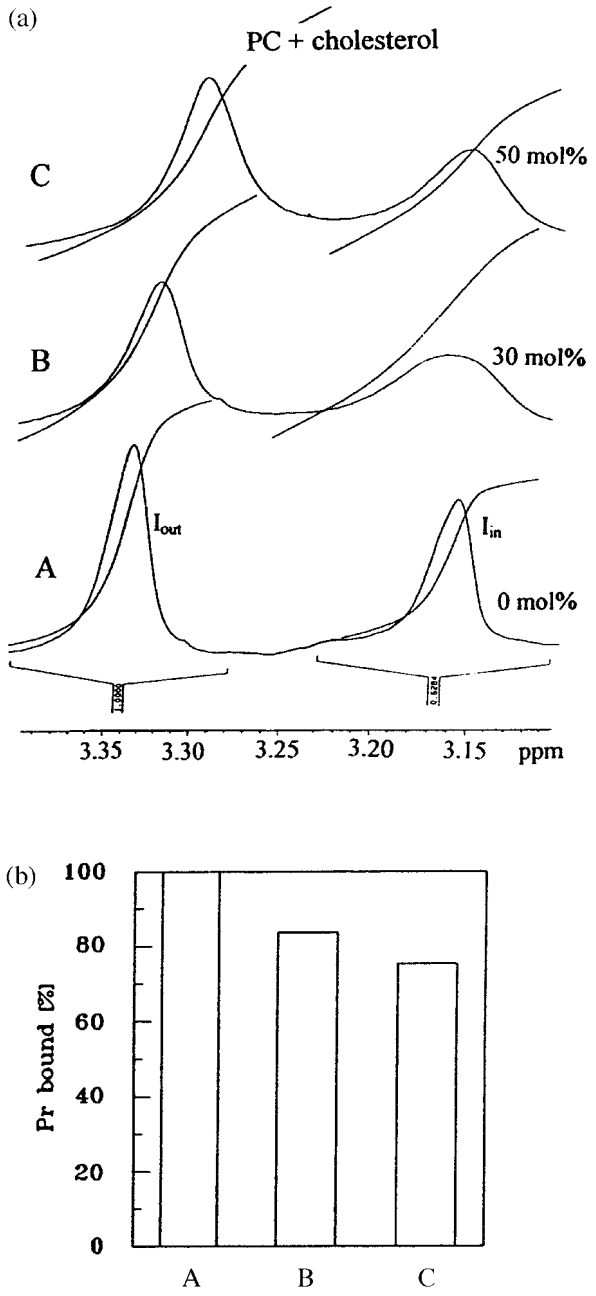

Figure 6 (a) Changes of the $—N^+(CH_3)_3$ signals caused by the presence of various amounts of cholesterol (b) Percentage of Pr^{3+} bound to phosphatidylcholine/cholesterol liposomes at an extravesicular $PrCl_3$ concentration of 4.06 mM. The lipid concentration in the samples was 33 mM. Spectrum A is for pure phosphatydylcholine liposomes; spectra B and C are for phosphatydylcholine/cholesterol mixtures at 30 mol% and 50 mol% relative cholesterol/phosphatydylcholine molar concentrations, respectively.

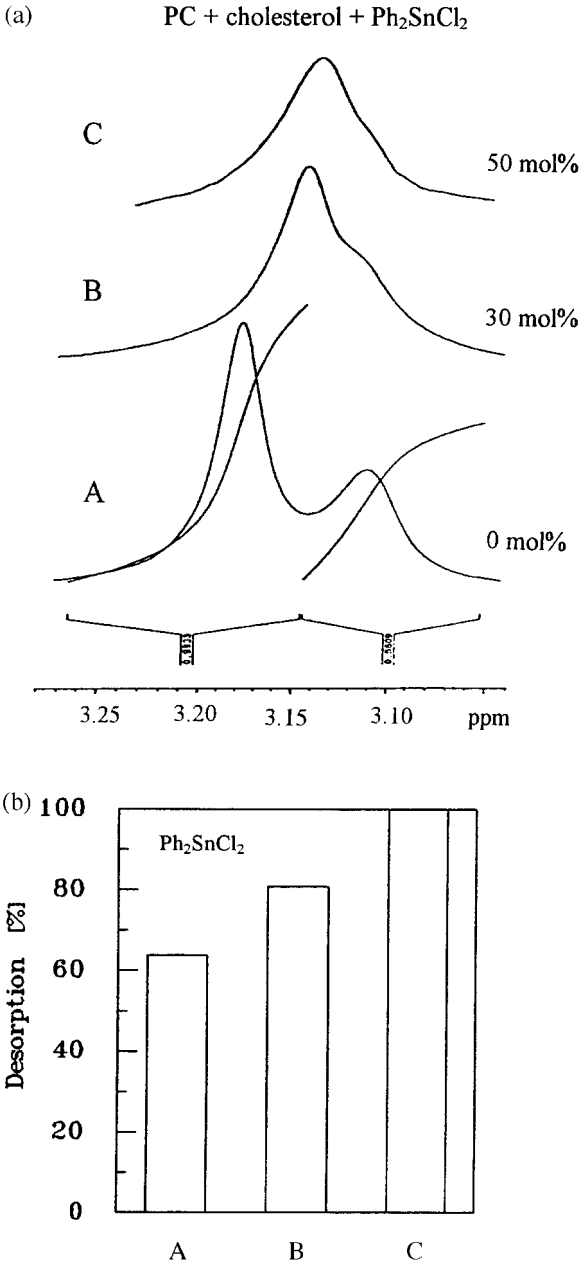

Figure 7 The effect of Phe_2SnCl_2, added to a phosphatydylcholine/cholesterol vesicle suspension at a concentration 0.98 mM, on signals of $—N^+(CH_3)_3$ groups when Pr^{3+} and Phe_2SnCl_2 were added to the suspension of vesicles formed from phosphatidylcholine mixed with various amount of cholesterol. The lipid vesicles were exposed to Pr^{3+} prior to addition of the phenyltin compounds. Spectra A, B and C are for pure phosphatidylcholine and phosphatidylcholine with 30 mol% and 50 mol% of cholesterol, respectively. The lower panel shows the fraction of desorbed Pr^{3+} caused by adsorption of the phenyltin compound.

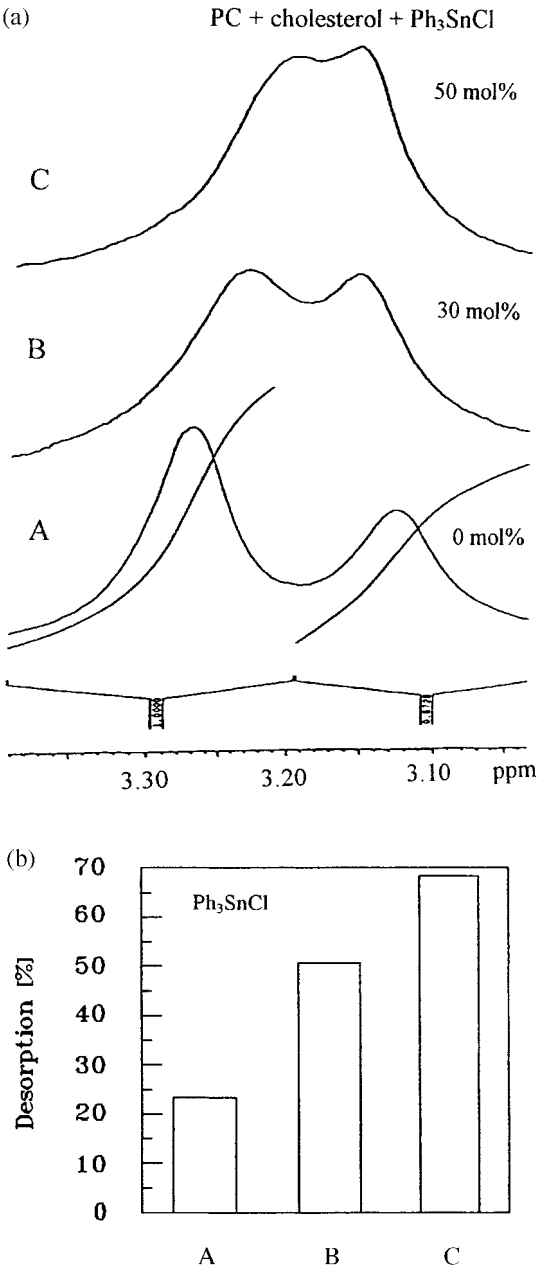

Figure 8 The effect of Phe_3SnCl, added to a phosphatydylcholine/cholesterol mixed vesicle suspension at concentration 0.98 mM, on the ^{1}H-NMR lineshape of $—N^+(CH_3)_3$ groups when Pr^{3+} and Phe_2SnCl_2 were added to the suspension of vesicles formed from phosphatidylcholine mixed with various amounts of cholesterol. Lipid vesicles were exposed to Pr^{3+} prior to the addition of a phenyltin compound. Spectrum A, B and C are for pure phosphatidylcholine and phosphatidylcholine with 30 mol% and 50 mol% of cholesterol, respectively. The lower panel shows the fraction of desorped Pr^{3+} ions caused by the phenyltin compound.

presence of 0.98 mM Phe_3SnCl. Phenyltin adsorption onto the lipid bilayer causes the desorption of praseodymium ions, which is reflected in changes in the magnitude of signal splitting.[26] Figures 7(b) and 8(b) summarize the effect of Phe_2SnCl_2 and Phe_3SnCl on praseodymium desorption. Pr^{3+} desorption caused by Phe_2SnCl_2 is more intensive than that caused by Phe_3SnCl (even without cholesterol).

DISCUSSION

The presence of cholesterol in the lipid bilayer greatly affects its properties. Lateral membrane organization is altered[36–39] and the organization of its lipid hydrocarbon chains is modified,[33,40,41] the result of which is a changed environment for the membrane processes.[42–45] Adsorption, transport and partition phenomena are affected as well.[17,18,35,40,43,44,46]

In earlier studies we have shown that there are qualitative differences between the effect of various phenyltins on the lipid bilayer. Phe_4Sn does not interact with the phosphatidylcholine bilayer, although it is the most hydrophobic compound studied.[20] The least hydrophobic, Phe_2SnCl_2, when bound to the lipid bilayer, caused substantial changes in hydrophobic chain organization within the membrane. On the other hand, Phe_3SnCl is adsorbed onto the lipid bilayer surface, causing a limited disturbance in the hydrophobic interior but dramatically affecting interface properties. It causes a substantial increase in surface charge density and alters the organization of lipid headgroups.[20] On the basis of an experimental data, we have concluded that the two molecules are located in the membrane in different regions of the lipid bilayer. In addition, it has been observed that the surface-active compound Phe_3SnCl has the highest hemolytic activity.[21] These observations show the importance of surface-associated phenomena in compound biological activity.

Data presented in this paper show that the effect of cholesterol on the lipid bilayer is limited predominantly to its hydrophobic core. The same conclusion can be reached on the basis of a large body of literature.[9,11,12,17,18,33,34,39,45] ^{1}H NMR results show that the presence of cholesterol in the membrane broadens signals originating from all the carbonyl and methyl groups within the hydrophobic interior of the membrane, whereas at the interface, where the effect is only limited, choles-

terol broadens signals coming from choline groups at the inner monolayer of small unilamellar vesicles. This effect is likely to be the result of high lipid bilayer curvature in such liposomes, and is caused by additional tension introduced by cholesterol molecules. Despite all these changes, signal splitting caused by Pr^{3+} adsorption onto the outer monolayer of phosphatidylcholine vesicles is not greatly affected. ^{1}H NMR data correlate with fluorescence measurements, which show that there are no changes in charge distribution at the interface in the presence of cholesterol.

As stated above, the various phenyltins are located in different regions of the membrane and therefore their adsorption should be affected differently by the presence of cholesterol. Phe_2SnCl_2 is located within the hydrophobic region in a phosphatidylcholine membrane, and is competing for the same free volume as the cholesterol. Consequently, cholesterol is expected to reduce the amount of Phe_2SnCl_2 able to accumulate in the lipid bilayer. ^{1}H NMR data show that Phe_2SnCl_2 affects the lipid bilayer in a somewhat similar manner to cholesterol. The signal coming from the inner monolayer of vesicles is affected the most, with the simultaneous decrease in signal splitting reflecting the Pr^{3+} desorption. The effect of Phe_3SnCl is different: the —$N(CH_3)$ of the inner monolayer does not broaden so much, which shows that inner monolayer organization is not greatly affected. The presence of cholesterol affects the extent of signal-splitting reduction, caused by Phe_3SnCl addition. This observation shows that cholesterol modifies the steric balance at the interface, influencing the Phe_3SnCl interaction with the membrane surface. The effect of phenyltins on the lipid bilayer interior is difficult to estimate, since cholesterol itself modifies the ^{1}H NMR spectra to such an extent that any additional changes are difficult to measure.

No measurable changes are noticed in charge distribution at the cholesterol/phosphatidylcholine surface. In the experiments presented, cholesterol is within the concentration range at which it does not mix well with phosphatidylcholine. It is well established that above 30 mol% of cholesterol there are two distinct (cholesterol-rich and cholesterol-poor) domains on the membrane surface.[11,17,32,35–38,41,42,47]

The presence of cholesterol in the bilayer enhances Phe_2SnCl_2 and Phe_3SnCl Pr^{3+} desorption, which indicates changes in membrane organization, resulting further in the enhanced accessibility of phosphate groups to phenyltins. Relative changes in Pr^{3+} desorption measured using the ^{1}H NMR technique are greater when Phe_3SnCl is added, which indicates changes in surface steric balance. Fluorescence data indicate a decrease in the amount of charge introduced onto the membrane surface by phenyltins in the presence of cholesterol. At 50 mol% of cholesterol, Phe_2SnCl_2 practically does not affect fluoresceine-PE fluorescence. Changes in Phe_3SnCl adsorption caused by cholesterol are more complex. The data presented suggest that the effect of Phe_2SnCl_2 on surface properties is reduced, whereas Phe_3SnCl causes structural changes at the membrane surface, inducing phase separation in phosphatidylcholine bilayers containing high amounts of cholesterol (above 30 mol%). The presence of cholesterol and Phe_3SnCl in the bilayer results in nonmonotonic fluorescein-PE fluorescence intensity changes. A possible explanation of the fluorescence maximum and the decrease in fluorescence intensity below its initial value in membranes containing 50 mol% of cholesterol is the fluorescent probe self-quenching. As the amount of the dye in the lipid bilayer is constant, such self-quenching results only from increased local probe concentration. This means that the entire membrane surface is not accessible to the dye in the presence of Phe_3SnCl, which is confirmed further by the dependence of the Phe_3SnCl concentration at which the fluorescence maximum is reached on the amount of dye in the membrane. An increased molar fraction of fluorophore in the bilayer lowers the Phe_3SnCl concentration required to reach a local dye concentration at which self-quenching occurs. Salt concentration changes in the aqueous phase only as a consequence of quantitative changes in measured fluorescence. Therefore, cholesterol does not modify the electrostatic effect of phenyltins on the membrane interface, and the concentration-generated fluorescence maximum is not affected by changes in salt concentration. All this suggests that the effect of cholesterol on the adsorption of Phe_3SnCl is steric in nature.

It has been shown that many cell processes are located at the lipid bilayer interface of the biological membrane. The drastic effects of Phe_3SnCl on membrane lipid organization, such as changes in membrane interface properties, should be a potent mechanism affecting metabolic processes involving membrane-associated proteins.[1,48,49] Accordingly, erythrocyte hemolysis caused by surface-active Phe_3SnCl was the highest of all induced by the phenyltin compounds that were studied.[20]

In summary, the presence of cholesterol in the phosphatidycholine bilayer reduces the amount of

charge introduced onto the membrane surface by Phe_2SnCl_2. This may be associated with the reduced adsorption of this compound, resulting from the reduction of free volume in the membrane by cholesterol. Hence, both cholesterol and Phe_2SnCl_2 are located in the hydrophobic interior of the bilayer. Surface-active Phe_3SnCl behaves differently, as its adsorption onto the phosphatidylcholine/cholesterol membrane is likely to cause lateral phase separation.

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