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The Effect of Steric Constraints on the Adsorption of Phenyltin onto the Dipalmitoylphosphatidylcholine Bilayer

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Abstract. In this paper, we present studies concerning phenyltin adsorption onto the dipalmitoylphosphatidylcholine bilayer. Phenyltin compounds are known to be biologically active, and their molecular geometry makes it possible to study the effect of steric constraints on their ability to penetrate the model lipid membrane. Using a fluorescence probe as a reporter of the amount of adsorbed compound, we evaluated their affinity to the membrane as a function of the membrane state. The amount of the adsorbed compound was found to depend on the adsorbing molecule's geometry and lipid bilayer organization. The fluorescence measurements were supported by the density functional theory (DFT) method of quantum mechanical computations. The penetrant location was correlated with the possible relative positions of its polar and hydrophobic moieties to determine if it could adopt structural requirements of the local membrane environment. Molecules were deformed by a model force, mimicking interactions within the membrane interfacial region. Computations show that the diphenyltin molecule can be deformed to such an extent that it can adopt an amphiphilic conformation. Triphenyltin is different, as its bending requires more energy. Born repulsion energies from hydrophobic fluid into water for phenyltins were also computed in an isodensity-polarized continua model of DFT computation. Our results indicate that the phenyltin compounds incorporate into the interface of the lipid membrane, although diphenyltin integrates more deeply than triphenyltin, which locates on the double layer's surface, and this is due to the fact that the main role is played by steric and not electrostatic interactions.

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Key words: Amphiphilicity — Fluorescence — Tributyltin — Lipid membrane — Molecular potential energy surface — DFT computations

Introduction

Organotin compounds are widely applied in various industries. For example, they are used as stabilizers for polyvinyl chloride plastics and other materials containing chlorine, in agriculture and as antifouling paints (Smith & Smith, 1975; Craig, 1982; Crowe, 1987). All of them are known to be environmentally hazardous, as they are biologically active via many ways (Röderer, 1987; Boyer, 1989; Colosio et al., 1991; Ogino et al., 1996; Fargasova, 1996; Kuczera et al., 1997; Ohhira & Matsui, 1997; Kleszczyńska, Pruchnik & Przestalski, 1998; Ohhira, Matsui & Watanabe, 1999; Stramac & Braunbeck, 1999; Lascourreges, Caumette & Donard, 2000; Sroka et al., 2001). For these reasons organotin compounds are also interesting from the biological point of view. In our previous paper (Langner et al., 1998), we studied the influence of phenyltins on egg-yolk phosphatidylcholine membranes at room temperature. The main results of the paper were obtained by the ^{1}H-NMR method, praseodymium ion release NMR detection and the fluorescence method using NBD-PE, TMA-DPH and fluorescein-PE probes. The main experimental issues of our previous paper show that Phe_3Sn^+ is adsorbed on the surface of the lipid bilayer, while Phe_2Sn^{++} interacts more weakly with the ammonium groups. Moreover, Phe_2Sn^{2+} competes efficiently with Pr^{3+} in binding to the phosphate group of the phosphatidylcholine, and the release of Pr^{3+} ions is much greater than the release caused by

Phe_3Sn^+. This proves that diphenyltin cations enter the region of the phosphate groups. Our fluorescence results (NBD-PE probe used) show that the perturbation of the headgroup packing is affected by Phe_2Sn^{++} much more greatly than by Phe_3Sn^+. Also the hydrophobic core perturbation caused by Phe_2Sn^{++} is greater than that observed when Phe_3Sn^+ acts on the PC membranes. The latter result follows from the fluorescence studies with the usage of the TMA-DPH probe. The membrane surface charge perturbation was detected by the fluorescein-PE probe, and we showed that Phe_3Sn^+ introduces a greater charge at the surface than Phe_2Sn^{++} does. The overall macroscopic bilayer organization is not affected by organotins in the opinion of other authors (Chicano et al., 2001), but the carbonyl groups of the phospholipid become less accessible to the water. However, our investigations on the influence of triphenyltin and diphenyltin on cell membranes and model lipid membranes indicate that they affect (disorganize) both the living and lipid model membranes (Langner et al., 1998; Gabrielska et al., 2000). Moreover, they showed that triphenyltin is more membrane-toxic than diphenyltin is. It is not easy to explain those results if we assume that 1) the disorganization of a membrane is the greater the more deeply a toxic substance penetrates the bilayer and 2) triphenyltin penetrates the bilayer more deeply than diphenyltin because the triphenyltin molecule with its three hydrophobic groups is more hydrophobic than the diphenyltin molecule. The aim of the present work was to explain that apparent contradiction on the molecular level. To study it more carefully, we performed experiments in which the membrane state changes with the temperature. Penetration of molecules and their adsorption on the lipid membrane is not an easy and well-understood phenomenon. However, small amphiphilic compounds, bearing a simple structure, may provide valuable information regarding various types of interactions when adsorbed onto the lipid bilayer surface (Przestalski et al., 1996). This has been clearly shown with tryptophan (Yau et al., 1998). Due to the bulky molecular structure, it is preferentially located within the lipid bilayer interfacial region, even if it is associated with peptides or proteins (Persson et al., 1998; Yau et al., 1998). Phenyltins in some respects are similar to tryptophan. They have rigid hydrophobic phenyl rings that restrict the location of these molecules in the membrane to its interfacial region (Chicano, 2001, 2002). The central part of the phenyltin ion is a positively charged tin atom, which can be used as an indicator of the compound's presence on the lipid bilayer surface; and we did so in our fluorescence experiments using fluorescein-PE probe. Phenyltins are to a certain degree flexible along their C-Sn bonds. This feature allows them to adopt to a limited degree to the membrane environment.

In the present paper we measure the effect of the lipid bilayer organization (state) on the adsorption of two phenyltins (diphenyltin and triphenyltin) with the fluorescence method using the fluorescein-PE probe. Mechanical deformations are likely to be imposed on these compounds by the lipid bilayer. We have calculated the energy needed to deform the molecules to learn their adaptability to the membrane. We also studied the Born repulsion energy of phenyltins from the hydrophobic fluid into water in a model of isodensity polarized continua. After careful consideration we are convinced our experimental data show that the molecular mechanical aspect of the adsorption phenomenon of phenyltins in DPPC membranes is more important and significant than the commonly believed electrostatic explanations state.

Materials and Methods

Materials

Egg phosphatidylcholine (PC) and dipalmitoylphosphatidylcholine (DPPC) were obtained from Avanti Polar Lipids (Alabaster, AL). Organotin compounds, i.e., $(C_6H_5)_3SnCl$–triphenyltin chloride (TPhT). $(C_6H_5)_2SnCl_2$–diphenyltin dichloride (DPhT), and $(C_4H_5)_3SnCl$–tributyltin chloride (TBT) were purchased from Alfa Johnson Matthey (Karlsruhe, Germany), and the fluorescent probe *N*-(5-fluorescein-thio-carbamoyl)-dipalmitoyl-L-alpha-phosphatidylethanolamine (Fluorescein-PE) from Molecular Probes (Eugene, OR). All other reagents were of analytical grade.

Liposome Preparation

Multilamellar liposomes were formed from DPPC at a temperature above that of the main phase transition, and from egg PC at room temperature. In short, a lipid with an appropriate amount of Fluorescein-PE (0.05%) was dissolved and mixed in chloroform (Gabrielska et al., 2000).

The solvent was then evaporated under vacuum, after which a phosphate buffer (in mM: 140 NaCl, 1.79 KCl, 0.86 $MgCl_2$, 11.79 $Na_2HPO_4\,2H_2O$, 1.8 $Na_2H_2PO_4\,H_2O$, pH 7.4) was added. The next sample was vortexed (at 60°C) until a milky liposome suspension was obtained. Lipid concentration in the vesicle stock solution was 1.3 mM.

Fluorescence Measurements

Each time before fluorescence was measured, the diluted egg PC or DPPC vesicle suspension (0.26 mM) was thermally equilibrated in a cuvette holder until the required temperature was reached (about 5 min). Organotins were then added from a concentrated ethanol stock solution (the final concentration of ethanol never exceeded 1% (vol/vol)). The concentration of the organotin compound varied from 0 μM to 52 μM. Fluorescence intensity was measured both before (F_0) and after (F_i) each addition of the organotin compound. Fluorescence data presented throughout this paper are expressed as relative fluorescence change, calculated according to the formula $(F_i - F_0)/F_0$. Excitation and emission wavelengths were 495 nm and 520 nm, respectively. All fluorescence measurements were carried out using a fluorimeter with a built-in thermoregulated cuvette holder (Kontron Instruments, Switzerland). Fluorescence intensities were corrected for the inner filter and dilution effects.

Quantum Mechanical Computations

Closed-shell cations Phe_2Sn^{++} and Phe_3Sn^{+} were built with the help of the molecular modeling package Sybyl v. 6.4 (Sybyl v. 6.4, 1991–97). Their geometries were optimized using Gaussian 94 Rev. E.2 (Gaussian 94, 1995) with the methods *ab initio* Hartree-Fock and B3LYP potential[1] (Becke, 1993) of the density functional theory (DFT). A generalized basis set and relativistic effective core potentials were used for tin (LaJohn et al., 1987) and carbon (Pacios & Christiansen, 1985). For hydrogen atoms the generalized basis set was applied alone (van Duijneveldt, 1971). The total energy of each cation was determined with the B3LYP potential (including electron correlation and exchange effects). In order to account for possible steric adoption to constraints exerted by the surrounding medium, the total energy excesses of specific molecule conformations, as obtained by altering their previously optimized geometry, were computed using the B3LYP density functional method with the same basis sets and core potentials. In the case of Phe_2Sn^{++}, molecular potential energy surface scans of the molecule were calculated for the C-Sn-C bond angle (perpendicular and parallel to the phenyl rings, from 180° to 100°, every 5°). For Phe_3Sn^{+}, umbrella-like deformations of C_{3v} symmetry were evaluated. The C-Sn-C bond angle was from 120° through 90°, every 3°. Scans were relaxed with respect to all other internal degrees of freedom; hence, the minimal total energy under a given constraint was determined after each scan step. For Phe_2Sn^{++}, scanning procedures were performed starting from two initial conformations (parallel and orthogonal mutual orientation of the phenyl rings), their total energy differing insignificantly. The optimized geometries of diphenyltin and triphenyltin cations were taken for SCRF (selfconsistent reactions field) computations in Gaussian. We used the Density Functional Theory model of quantum mechanical computations with B3LYP potential for the selfconsistent reaction field (SCRF) corresponding to the Isodensity Polarized Continum Model (IPCM) at the isosurface of phenyltin's electronic density equal to 10^{-4}. The functional basis set and pseudopotentials used in these Gaussian computations are the same as described above in the Materials and Methods section of this paper.

Results and Discussion

The effect of the lipid bilayer state on compound adsorption can be studied only if the lipid molecule organization remains globally unperturbed. As thermographs obtained with Differential Scanning Calorimetry were not significantly changed (Różycka-Roszak, Pruchnik, Kamiński, 2000), we assume that the lipid bilayer is not affected by the adsorbing compound at the concentrations applied. In order to detect such low adsorbent surface concentrations, we have chosen the surface electrostatic potential to quantitate the amount of phenyltin present at the lipid bilayer surface (Cevc, 1990; Langner, Isac & Hui, 1995). The phenyltin ion carries a positive charge associated with its tin atom; therefore the surface electrostatic potential will change upon its adsorption (Langner et al., 1995; Langner & Kleszczyńska, 1997). In order to measure this change in the surface potential, the fluorescent dye fluorescein (covalently attached to the phosphatidylethanolamine head-group) has been used. The hydrophilic character of fluorescein substantiates the assumption that the fluorophore is located within a short distance from the membrane surface, and that its position does not change even when the lipid bilayer topology is altered (Langner et al., 1995, 1998; Langner & Kleszczyńska, 1997; Langner, Pruchnik & Kubica, 2000). The fixed dye position with respect to the membrane surface and the dye's sensitivity to changes in the local electrostatic potential ensure that the amount of surface charge can be detected and quantitated.

The dipalmitoylphosphatidylcholine (DPPC) bilayer has been chosen as the model membrane, because its phase behavior is well characterized and a number of various lipid lamellar conformations can be obtained by temperature alone. There are three (well characterized) phases possible for a lipid bilayer formed from DPPC: the gel phase; the "ripple phase" (with hydrocarbon chains well organized, but bearing a complex surface topology); and the liquid-crystal phase (Marsh, 1990; Meyer, Dobner & Semmler, 1996). A membrane transformation from one phase to another is a cooperative process that takes place simultaneously throughout the whole sample at a specific phase transition temperature. It has been shown that the three phases differ not only with respect to their lipid molecules' organization but to other parameters as well. The permeability of the DPPC bilayer to ions is drastically enhanced at temperatures around the main phase transition (Langner et al., 1990, 1995). Adsorption of selected molecules depends on the state of the lipid and is more efficient in the liquid-crystal state.

Figure 1*A* shows the relative fluorescence intensity change induced by triphenyltin as a function of its concentration in the DPPC vesicle suspension and of temperature. Relative fluorescence intensity increases sharply at 36°C (pretransition temperature), whereas it remains constant throughout the main phase transition (around 41°C). One can see that adsorption of Phe_3Sn^{+} does not change significantly at the pretransition temperature of DPPC or at the main phase transition temperature, as evidenced from the data shown in Fig. 1*A*. Triphenyltin adsorption occurs even if the lipid bilayer is in the gel phase (but to a much lesser extent), which means that the compound is able to adsorb onto the membrane surface regardless of the membrane state. Figure 1*B* shows how the relative fluorescence intensity is affected by diphenyltin as a function of its concentration in the DPPC vesicle suspension and temperature. There is no detectable diphenyltin adsorption when the bilayer is in the gel state, but it increases abruptly when the temperature approaches the main phase transition temperature. Adsorption reaches its maxi-

[1]B3LYP = Becke 3 parameter electron exchange and Lee, Yang, Parr electron correlation functional

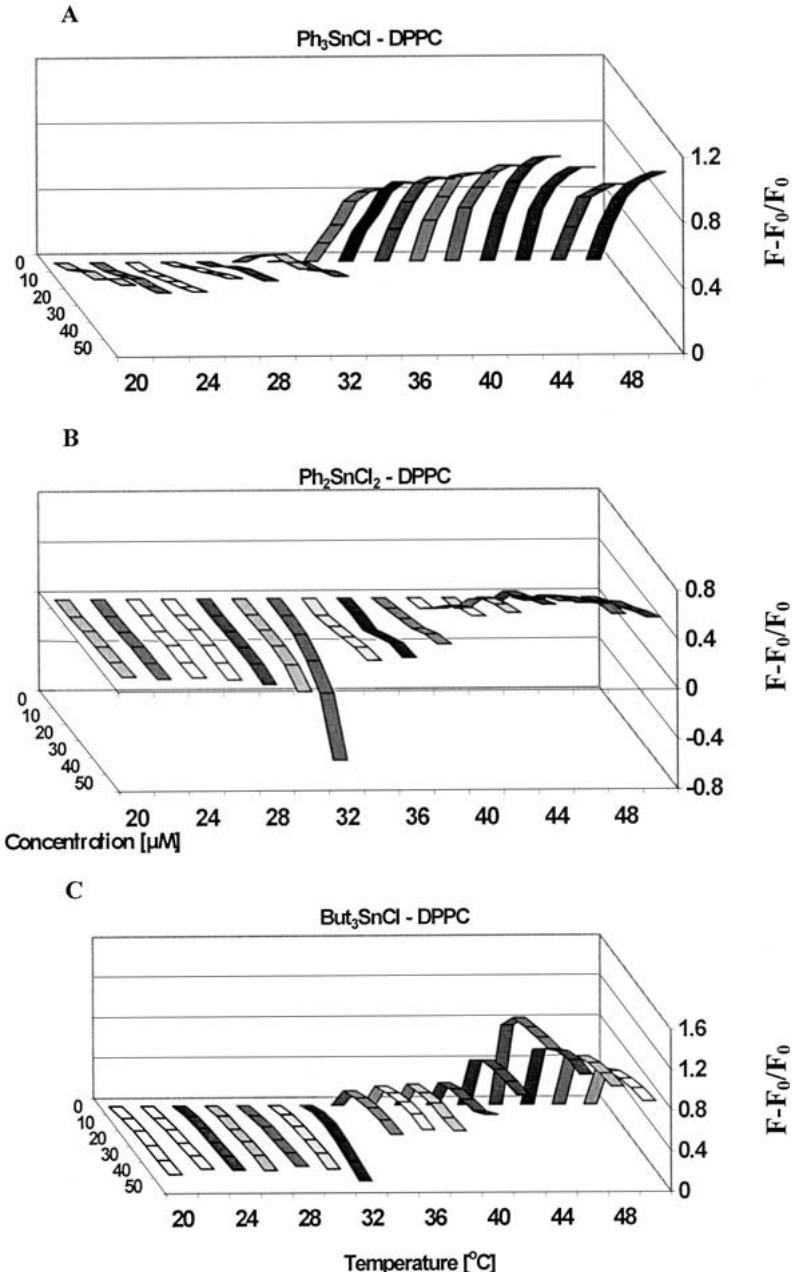

Fig. 1. Relative change in Fluorescein-PE fluorescence intensity as a function of temperature and triphenyltin chloride (*A*), diphenyltin dichloride (*B*) and tributyltin chloride (*C*) concentration in a DPPC membrane. Concentrations are the totals for each phenyltin compound in the sample. DPPC vesicles were suspended in a 140 mM NaCl phosphate buffer, pH 7.4. Lipid and fluorescent probe concentrations in the sample were 0.26 mM and 0.05 mol%, respectively. Presented fluorescence intensities are expressed as relative changes, calculated according to the formula $(F-F_0)/F_0$, where F_0 is fluorescence intensity at a given temperature for unmodified vesicles, and F is the intensity after adding a certain amount of organometallic compound.

mum when the membrane is in the fluid state, when the conformation of its hydrocarbon chains alters. We conclude from these facts that Phe_3Sn^+ does not perturb the region of the alkyl chains of DPPC to such an extent as Phe_2Sn^{++} does.

Diphenyltin adsorption depends strongly on membrane organization, which correlates with its steric compatibility to the membrane in a liquid crystalline state. Do ordered lipid hydrocarbon chains prevent diphenyltin adsorption when the lipid bilayer is in the gel phase? When the chains "melt", the diphenyltin phenyl rings could be accommodated within the fluid hydrocarbon chain region, and that may be why the effective adsorption rises. However, the problem of adsorption is not only a problem of the steric forces—usually called the cavity-formation forces. Such forces act on the adsorbing molecule and arise from the separation of lipids to create a cavity. They arise also from the change of the conformation of the lipid molecules, which is necessary for the adsorbing molecules to be able to enter the cavity. One can also think about the deformations of the adsorbing molecules to adapt to the medium. There is also a dipole potential arising from the zwitter-ionic character of phosphatidylcholines. It acts on ions and polar molecules. The dipole potential forces are repulsive to cations entering the PC membrane and can be attractive to water molecules. There is also an

electrostatic repulsion of charged bodies from the dielectric medium toward the regions of higher dielectric permittivity, and the potential energy of this force creates the energetic barrier for ions, which is often called Born repulsion energy. There are adsorption phenomena controlled by different forces or combinations of them. The greater the volume of the adsorbing molecule, the greater the work of cavity formation. It is worth noting that the volume of Phe_3Sn^+ is greater than the volume of Phe_2Sn^{++}. The dipole potential acting on the greater positive charge produces a higher energetic barrier. Also, the Born energy of the diphenyltin cation seems to be greater than it is for the triphenyltin cation. If the adsorption of the molecules is controlled by cavity formation, Born energy, and dipole potential terms, then the deformation energy of the adsorbing molecules can be neglected. It follows from our experimental data, that the steric forces control the process of adsorption of phenyltin cations on the DPPC membrane, but this statement seems to be unclear as the electrostatic repulsion exerted on diphenyltin cation must be considered prohibitive for such a simple model. We shall discuss it later after having calculated the Born repulsion energy. The question also arises why a more hydrophobic and less charged triphenyltin cation adsorbs to a much lesser extent than diphenyltin.

The tributyltin adsorption was measured (Fig. 1*C*) using the same method in order to examine how the adsorption is affected by substituting three rigid phenyl rings with flexible butyl residues. In this case fluorescence changes indicate that tributyltin penetrates the membrane hydrophobic core more easily, as revealed by the dependence of the adsorption on temperature. The obtained adsorption pattern falls somewhere between those of di- and tri-phenyltins. Its temperature characteristic shows a step-like increase in fluorescence intensity at the pretransition and the main phase transition temperature.

To study how the phenyltin adsorption efficiency depends on temperature alone (i.e., when the membrane state does not change), measurements were carried out on a model lipid membrane formed from egg PC (it is known that this lipid does not have any conformational transition in the relevant temperature range). Figure 2 shows the relative fluorescence intensity as a function of temperature and the compound concentration during the adsorption of triphenyltin, diphenyltin and tributyltin (parts A, B, and C, respectively) onto the egg-PC lipid bilayer. The adsorption patterns of all three compounds are different. The diphenyltin adsorption does not change with rising temperature, whereas the triphenyltin and tributyltin adsorptions decrease with temperature. An increase in temperature causes larger surface fluctuations (surface undulations, headgroup mobility etc.), which may influence the adsorption of amphiphilic molecules. Thermal fluctuations are likely to affect most the compounds located within the interfacial region, and those least that penetrate the hydrocarbon chain region. Data presented in Fig. 2 support our previous findings regarding phenyltin location in the lipid bilayer. The adsorption pattern exemplifies the importance of steric compatibility between molecule structure and lipid bilayer organization. Both molecules have rigid phenyl rings attached via Sn-C bonds to a charged central tin ion. These flexible bonds can bend in response to forces originating from the interfacial region.

There have been numerous attempts to identify the parameters that would allow the prediction of a compound's ability to penetrate the biological membrane (Ketterer, Neumcke & Läuger, 1971; Andersen & Fuchs, 1975; Andersen et al., 1978). The accessible molecular hydrophobic surface, the hydrophobic moment and the ability to form hydrogen bonds are only selected examples of such parameters (Matsuzaki, 1999). Most of them, however, are only rough estimates of an arbitrarily chosen parameter, calculated on the basis of the molecule's chemical structure alone (without considering the surroundings). Consequently, such an approach possesses a very limited power of prediction concerning whether the molecules can adsorb onto the interface region of the lipid membrane. This is so mainly due to mutual structural adjustments of a molecule and the lipid bilayer, which do not allow for such parameterization.

Diphenyl- and triphenyltin both have hydrophobic (phenyl rings) and polar (tin atom) moieties. Due to Sn-C bond flexibility, they can change their conformation in response to steric restrictions imposed by the lipid bilayer. In order to test the abilities of such molecules to reach various conformations, we performed quantum-mechanical DFT computations. These calculations were performed with the aim of determining the most probable molecule conformations when the molecule is located at the lipid bilayer interface, and subsequently of correlating these with the measured adsorption patterns and other available experimental data. The presented computations need to be appended with the following remarks.

(1) The incorporation of a molecule into the lipid bilayer can be limited by interfacial constraints, which results in molecular geometry deformations from the geometry of the lowest energy (in a vacuum). Such deformations result in an accumulation of excess energy. They are likely to occur if the membrane can compensate the excess energy by intermolecular interactions.

(2) Lipid-lipid interaction energy in a hydrated membrane is the measure of the membrane's ability to compensate such excess energy if the membrane can be considered thermodynamically unperturbed by the intruder molecules. For the DPPC membrane

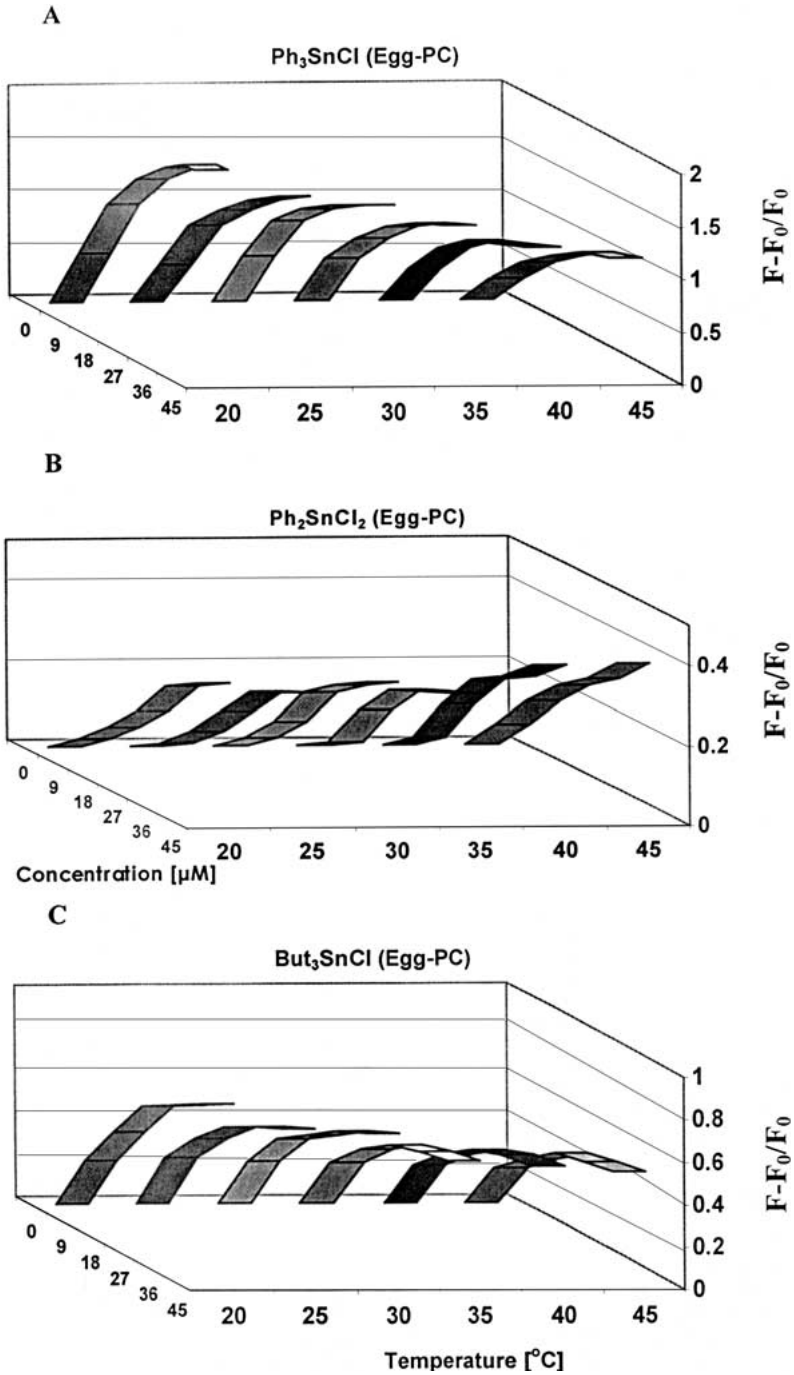

Fig. 2. Relative change in Fluorescein-PE fluorescence intensity as a function of temperature and triphenyltin chloride (*A*), diphenyltin dichloride (*B*) and tributyltin chloride (*C*) concentration in an egg-PC membrane. Concentrations are the total amounts of a phenyltin compound added to the sample. Small lamellar PC vesicles were suspended in a 140 mM NaCl phosphate buffer pH 7.4. Lipid and fluorescent probe concentrations in the sample were 0.26 mM and 0.05 mol%, respectively. Presented fluorescence intensities are expressed as relative changes, calculated as $(F-F_0)/F_0$, where F_0 is fluorescence intensity at a given temperature for unmodified vesicles, and F the intensity after adding a certain amount of organometallic compound.

the steric adoption of lipid molecules results in 8.7 kcal/mole (Boggs, 1987) of molar enthalpy of the main phase transition. For this reason, we estimate the ability of the DPPC membrane's compensation of the excess energy of the deformed penetrant to be 17.4 kcal/mole, taking into account fluctuations of the membrane and entropic effects. Any greater energetic perturbation of the DPPC membrane we consider unlikely if the integrity of the membrane is to be preserved.

When phenyltin is in the interfacial region, it has to adopt its geometry in order to satisfy the requirement of the sharp polarity gradient. This can be achieved by increasing its hydrophobic moment; in other words, the charged tin ion needs to move away from the hydrophobic phenyl rings as far as the energetics of the system will allow.

The extent of phenyltin deformation is limited only by the interaction between the guest molecule and lipid bilayer. In the following section we present

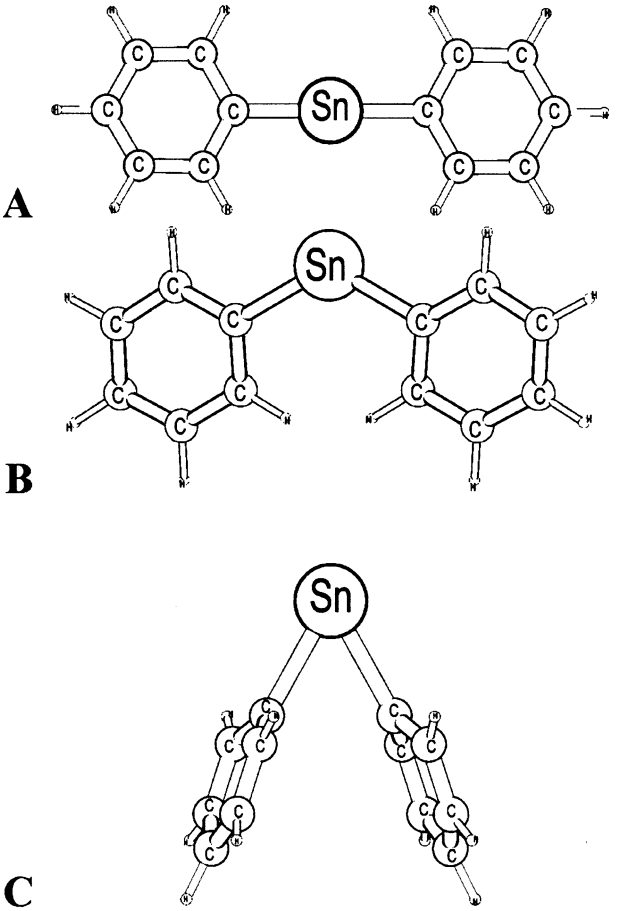

Fig. 3. (*A*) Diphenyltin conformation of lowest energy, according to density functional theory (DFT) quantum mechanical computations. (*B*) A deformed diphenyltin molecule when C-Sn-C bonds are bent along the phenyl ring plane. (*C*) A deformed diphenyltin molecule with rotated phenyl ring planes.

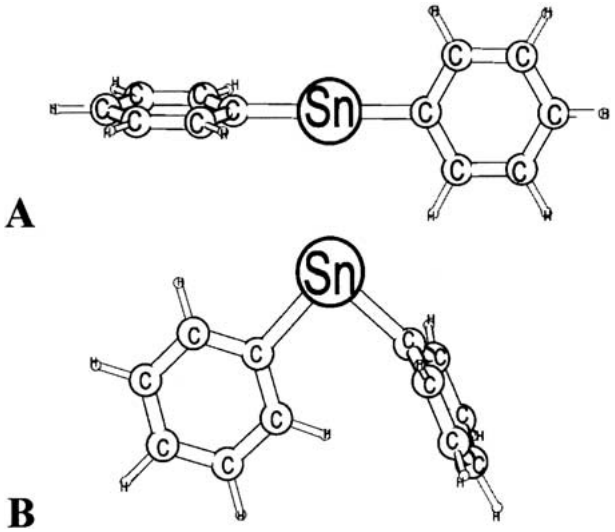

Fig. 4. (*A*) A deformed diphenyltin molecule with one of the phenyl rings rotated to a position perpendicular with respect to the other. (*B*) Further deformation of a diphenyltin molecule by bending C-Sn-C bonds when the phenyl rings are perpendicular.

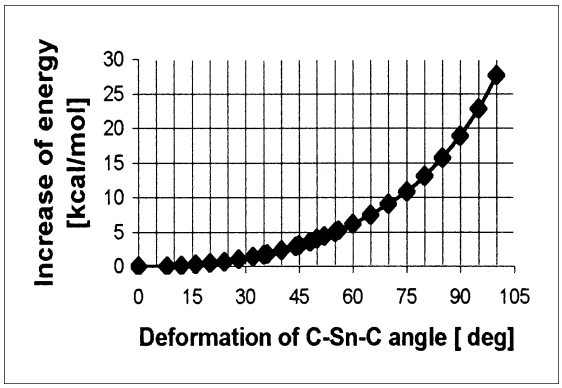

Fig. 5. Influence of a bending angle on the energy needed to bend the diphenyltin C-Sn-C bond from its conformation of lowest energy. The molecule was deformed in the direction shown in Fig. 3*C*. Energy computations for distorted structures were relaxed with respect to all the other degrees of freedom and calculated using the same DFT method as was used for the fully relaxed structure.

estimations of the energy required to bend the C-Sn-C bond into two phenyltin molecules.

Figure 3 shows diphenyltin conformation prior to (Fig. 3*A*) and after (Figs. 3*B* and 3*C*) two selected deformations. Deformation of the phenyl ring itself is not likely (as being energetically too expensive); therefore any deformation is exclusively due to bending the C-Sn-C bonds and/or phenyl ring rotation around the Sn-C axis. When the two phenyl rings are coplanar, a deformation occurs along the phenyl ring plane (Fig. 3*B*) and 5 kcal/mole is needed to bend the C-Sn-C bond by 60 degrees.

Figure 4 shows a diphenyltin molecule with its phenyl rings perpendicular to one another (Fig. 4*A*) and deformed to a final conformation (Fig. 4*B*). To enforce such deformation, only 10 kcal/mole is needed. A third possible molecule deformation is shown in Fig. 3*C*. Here, the diphenyltin molecule is bent from its initial position (Fig. 3*A*) to one which is perpendicular to the phenyl ring plane. The required energy as a function of the C-Sn-C bonding angle is presented in Fig. 5. In order to bend the molecule by 60 degrees, 7 kcal/mole only is required.

According to the presented analysis, when diphenyltin is exposed to external unidirectional forces that imitate lipid bilayer interface constraints, it will likely adopt a conformation that requires the smallest excess energy, in other words, the one presented in Fig. 3*C*. When the molecule has this conformation within the membrane interface, the charged tin atom remains in the polar environment, whereas its hydrophobic phenyl rings penetrate the membrane hydrophobic core. The dimensions of a diphenyltin molecule allow the distance between tin and the last phenyl ring carbon atom to be estimated at about 4.89 Å. This means that diphenyltin pene-

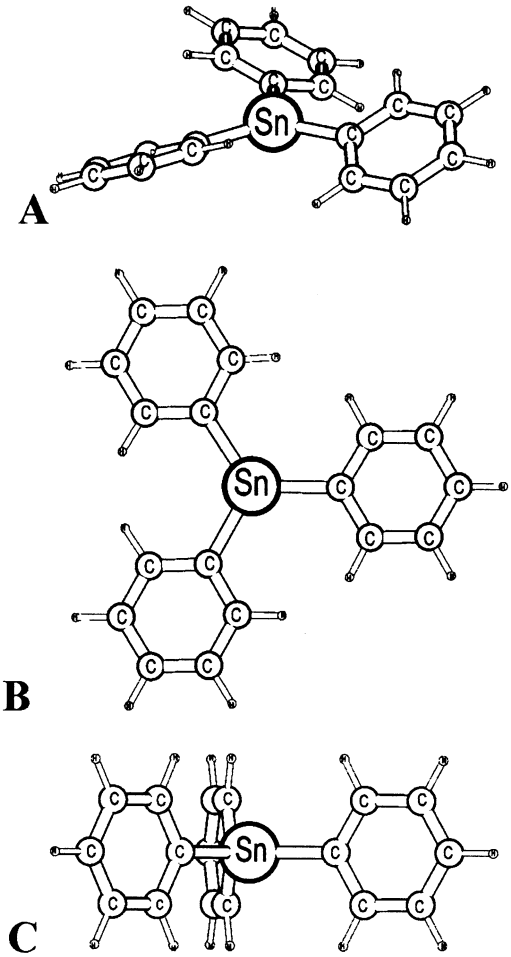

Fig. 6. (*A*) Triphenyltin conformation of lowest energy, according to DFT quantum mechanical computations. (*B*) The D_{3H} symmetrical and completely flat structure of triphenyltin. (*C*) The D_{3H} symmetrical structure of triphenyltin, with its rings orthogonal to the plane of Sn and its nearest neighboring carbon atoms.

trates only the upper, highly ordered hydrocarbon chain region. Such a location in the lipid bilayer agrees with the adsorption pattern observed when the membrane state is altered. If the lipid bilayer is highly ordered (the gel phase), phenyl rings do not penetrate the upper hydrocarbon chain region, thus preventing diphenyltin adsorption. The incorporation of diphenyltin is possible when the membrane "melts", in other words, at temperatures above the main phase transition (Fig. 1*B*).

We have previously shown that triphenyltin and diphenyltin interact with the lipid bilayer differently. Fluorescence and ^{1}H NMR experiments show triphenyltin adsorbing onto the membrane surface and causing limited disturbances within the hydrophobic membrane core (Langner et al., 1998). Such experimental results can be qualitatively correlated with spatial arrangements of triphenyltin polar and hydrophobic residues. Triphenyltin (Fig. 6*A*), in order to become amphiphilic, has to change its conformation perpendicularly to the plane of the bonds between the tin atom and phenyl rings. The charged tin ion's central position makes the triphenyltin molecule qualitatively different from diphenyltin. Even when deformed, the former has an effective hydrophobic cross-section much larger than that of diphenyltin. Three phenyl rings make the molecule very rigid; in other words, any significant deformation requires a substantial amount of energy. For example, a change in the phenyl ring orientation only from that shown in Fig. 6*A* to Fig. 6*B* requires 4.1 kcal/mole. Altering the conformation from Fig. 6*A* to Fig. 6*C* needs 18.07 kcal/mole. None of the conformations shown in Fig. 6 makes the molecule amphiphilic, being the result of its symmetry only (the tin ion still occupies the central position). In order to make the molecule amphiphilic, further deformations are needed. For example, in order to bend the triphenyltin molecule from the one shown in Fig. 7*A* to that in Fig. 7*B*, an additional 13.73 kcal/mole are needed, giving an overall energetic increase of 31.8 kcal/mole. Figure 8*B* shows the calculated energy required to bend the Sn-C bonds in an umbrella-like fashion (Fig. 8*A*). The energy needed to obtain triphenyltin with the 110-degree angle between the adjacent Sn-C bonds is only 20 kcal/mole.

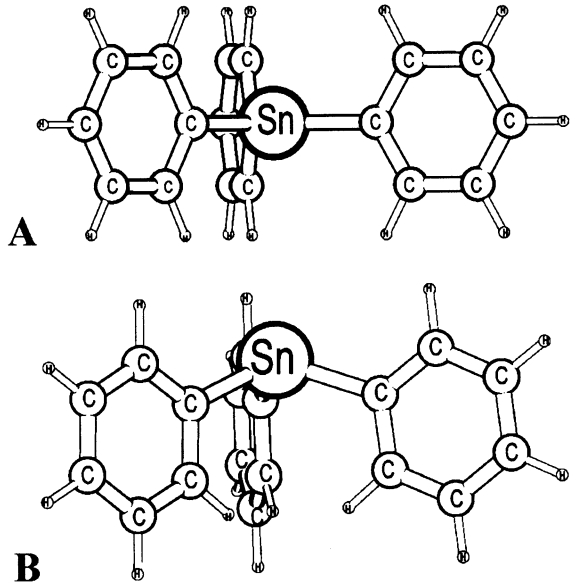

Fig. 7. (*A*) The D_{3H} symmetrical structure of triphenyltin, with its rings orthogonal to the plane of Sn and its nearest neighbouring carbon atoms. (*B*) Deformation of a triphenyltin molecule, beginning from the structure in Fig. 7*A*. The tin atom is pulled up from the plane of its neighbouring carbon atoms. The C-Sn-C angle is fixed and equals 109.2° — all other degrees of freedom are fully relaxed.

One can doubt the above presented sophistication based on the mechanical properties of the adsorbing molecules, recalling that the phenyltins are charged and perhaps the electrostatic effects have to be studied first as being more important. For this

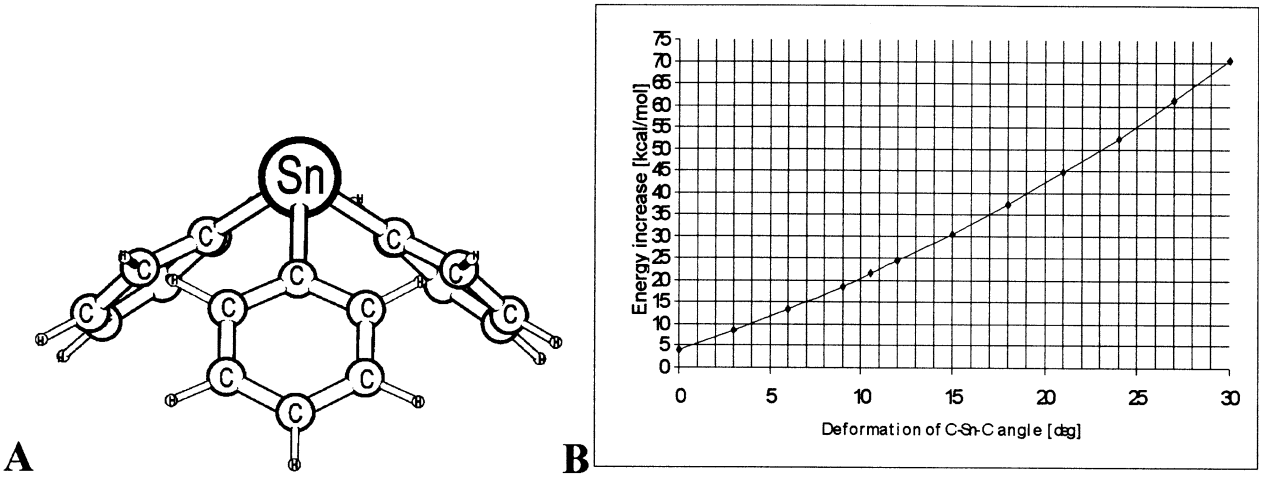

Fig. 8. (*A*) Deformation of a triphenyltin molecule from its completely flat D_{3h} symmetrical structure to that of C_{3V} symmetry in an umbrella-like manner, performed by changing the C-Sn-C angle. The remaining degrees of freedom are fully relaxed. (*B*) Energy increase corresponding to a C-Sn-C angle deformation in the triphenyltin structure in Fig. 8*A*, computed with the DFT method. Even slight deformation of the C-Sn-C bond (by 3°) causes an increase in energy from that of optimized geometry (Fig. 6*A*).

reason, we also decided to perform isodensity polarized continua computations for phenyltin cations. Unfortunately, the interphase region of the DPPC membrane cannot be characterized by a single value of permittivity (it varies from 2.00 for the hydrocarbon chain region to about 80 for bulk water); therefore, the calculated values of the Born energy for constant permittivity can be considered only as estimations. However, we have calculated the Born repulsion energy for phenyltin transfer from bulk water (ϵ = 78.39) into the hydrocarbon fluid (ϵ = 2.00), treating them as limiting values of the real Born energy for phenyltin transfer from bulk water into the interphase medium. Such model values of Born energy are 87.0 kcal/mol for diphenyltin and 17.9 kcal/mol for triphenyltin. From comparison of the model values of Born energies one could expect that triphenyltin adsorbs more easily and to a greater extent than diphenyltin does due to the lower electrostatic repulsion from the hydrophobic core of the membrane. However, it follows from our experiments that this is not the case. It was indirectly proved by experimental data that it is not the barrier of electrostatic repulsion from the hydrophobic core of the membrane that limits and controls the adsorption of phenyltin cations, but that molecular mechanical properties of the penetrants and molecular mechanical properties of the interphase region do the limiting and controlling, the last ones changing significantly at phase transition temperature. It follows from the presented empirical data that the adsorption of phenyltins increases drastically at phase transition temperature, at which the geometric relations of all the interacting molecules (DPPC, water, diphenyltin) change and the dielectric permittivity does not. This experimental fact proves that, to a first approximation, the changed adsorption efficiency is predominantly due to the altered membrane lipid organization, whereas the electrostatic interaction can be safely neglected. However, the fact of no penetration of phenyltins into the region of hydrophobic chains can be understood as an electrostatic, in other words, a Born effect.

By theoretical methods we thus showed that the two molecules at hand differ in their properties, namely in their hydrophobic moment, hydrophobic region size and energetically feasible deformations. Diphenyltin bends easily along the C-Sn-C bonds, which enables the molecule to penetrate the fluid upper hydrocarbon chain region. When bent, its hydrophobic moment is large and the hydrophobic region cross-section small. Consequently, diphenyltin adsorption strongly depends on the lipid bilayer hydrocarbon chain organization; in other words, adsorption is prevented when the membrane is in the gel phase. Triphenyltin is different; even when bent (within feasible energetic limitations) it has a very small hydrophobic moment and a large hydrophobic-region cross-section. Such molecular geometry causes it to adsorb within the upper part of the interface and prevents its penetration into the hydrocarbon chain region. That consequent location makes the molecule's adsorption less sensitive to the hydrocarbon chain organization.

Both the phenyltins bear hydrophobic phenyl rings; therefore both should associate with model lipid bilayers as well as biological membranes. As shown by ourselves and others, the two phenyltins differ in their affinity towards model membranes, taking up different positions in the lipid bilayer as well (Langner et al., 1998; Chicano et al., 2002). The calculations and experimental data presented in this

paper prove that the adsorption of amphiphilic molecules depends on their molecular makeup and structural compatibility with the organized lipid bilayer matrix. The lipid bilayer adsorption patterns of di- and triphenyltin are driven by a combination of factors, such as electrostatics, hydrophobicity, steric compatibility with the lipid bilayer, and relative hydrophobic and polar moiety positions (which depend on molecule flexibility). The obtained qualitative correlations between the calculated values and experimental observations are by no means a complete analysis of phenyltin interaction with the lipid bilayer interface. To achieve this, a whole-scale molecular dynamic simulation is required (such studies are in progress in our laboratory). On the other hand, an entirely classical analysis of the adsorption process will not provide reliable data, since medium continuity cannot be assumed and approximating phenyltin by a point charge is not valid for obvious reasons (Tieleman & Marrink, Berendsen, 1997; Damodaran, 1998). We believe that, in the case of phenyltins, steric constraints are a predominant factor that determines a compound's location within the lipid bilayer. Other factors, such as electrostatic interaction and the overall compound's hydrophobicity, are of minor importance. This conclusion is the consequence of the experimental results interpreted in this paper. It can reasonably be assumed that at phase transitions neither the adsorbing compound (its electrostatic charge or phenyl rings hydrophobicity) nor membrane dielectric properties are significantly altered. This means that the abrupt increase in the phenyltin's adsorption closely follows the conformational changes occurring within the lipid bilayer. Thus, the changing steric compatibility of phenyltin towards the lipid bilayer is a major factor governing the adsorption process, whereas other interactions have only a minor effect.

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