Tin Compounds Interaction with Membranes of Egg Lecithin Liposomes

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This work is a continuation of earlier research concerning the influence of tin compounds on the dynamic properties of liposome membranes produced with lecithin hen egg yolks (EYL). The experiments were carried out at room temperature (about 25 °C). Four tin compounds were chosen, including three organic ones, $(CH_3)_4Sn$, $(C_2H_5)_4Sn$ and $(C_3H_7)_3SnCl$, and one inorganic, $SnCl_2$. The investigated compounds were admixed to water dispersions of liposomes. The content of the admixture changed within the range 0 mol-% to 11mol-% in proportion to EYL. Two spin probes were used in the experiment: 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and 2-ethyl-2-(15-methoxy-15-oxopentadecyl)-4,4-dimethyl-3-oxazolidinyloxyl (16-DOXYL-stearic acid), which penetrated through different areas of the membrane. It was found that tin compounds containing chlorine were the most active in interaction with liposome membranes. In the case of $(C_3H_7)_3SnCl$, after exceeding 4% admixture content, an additional line appeared in the spectrum of the TEMPO probe which can be a result of formation of domain structures in the membranes of the studied liposomes. Compounds containing chlorine are of ionized form in water solution. The obtained results can thus mean that the activity of admixtures can be seriously influenced by their ionic character. In case of an admixture of non-ionic compounds the compound with a longer hydrocarbon chain displayed a slightly stronger effect on the spectroscopic parameters of the probes.

Key words: EYL Liposomes, ESR Method, Tin Compounds

Introduction

The technical civilization, apart from a good number of benefits, brings also threats connected mainly with poisoning of natural environment. A regular subject of research conducted frequently by biologists, biochemists and biophysicists these days is the effect of heavy metal compounds on living organisms, which are waste products of various technological processes (Verity, 1990; Vijverberg *et al.*, 1994; Cooper *et al.*, 1984; Moebus *et al.*, 1997; Syng-ai *et al.*, 2002). The toxic properties of lead and mercury compounds are mentioned most often in relation to this, although there also exists a number of other metals which are burdensome to the environment. Tin, which most often is treated as a metal of low toxicity due to its popular occurrence in plenty of products, as well as because of its ability to accumulate in the organism, can be a source of serious poisonings, which can conduce to neurological disorders. Alkyl compounds of tin are particularly toxic and their toxicity rises along with the number of alkyl groups contained in them. Organic tin compounds inhibit, among others, oxidative phosporylation and accelerate hemolysis of erythrocytes (Falcioni *et al.*, 1996; Gray *et al.*, 1987; Kleszczyńska *et al.*, 1997; Hamasaki *et al.*, 1995), as well as change the physical properties of model lipid membranes. Ionized forms of tin compounds (*e.g.* chlorines) display a special activity in influencing lipid membranes (Gabrielska *et al.*, 1997; Radecka *et al.*, 1999; Kleszczyńska *et al.*, 1999). It was concluded by Man *et al.* (2006) that the influence of tin chlorides on spectroscopic parameters of the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) spin probe placed in the membranes of liposomes formed of synthetic DPPC lecithin is considerable. In the case of a 2-ethyl-2-(15-methoxy-15-oxopentadecyl)-4,4-dimethyl-3-oxazolidinyloxyl (16-DOXYL-stearic acid) probe penetrating deeper regions of the lipid bilayer (the neighbourhood of the center of the bilayer), the obtained results were not too explicit, the effect having been caused – as it may be supposed – by relatively high stiffness of the lipid bilayer of the liposomes. The DPPC lecithin applied by Man *et al.* (2006) at 40.5 °C (Shimshick and McConnell, 1973) passes from the gel phase to the liquid-crystalline phase, still the experi-

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ments were carried out at 25 °C (gel phase). It is possible that, according to Man *et al.* (2006), the relatively high stiffness of the liposome membrane at this phase made it difficult for tin compounds to penetrate the bilayer. In the experiment conducted in the present work it was lecithin obtained from yolks of hen eggs (EYL) that was used. It remains in its liquid-crystalline phase at 25 °C. EYL lecithin does not constitute a uniform structure (particles of phospholipids differ in the length of hydrocarbon chains), as a result the temperature range of its main phase transition (gel-liquid crystal) is broadened, while the mean value of this temperature amounts to about −5 °C (Mason and Huang, 1978). The conditions of investigations carried out in this work, such as procedures of preparation of samples, type of admixtures, their concentrations, were maintained exactly the same as in Man *et al.* (2006).

Materials and Methods

Liposomes were formed from EYL lecithin in distilled water in the process of sonication by means of a ultrasonic disintegrator (TECHPAN UD-20). The total sonication time for a single sample of 1.5 ml volume amounted to 5 min and was carried out in alternating cycles of 60 s of sonication followed by 60 s of cooling. The concentration of EYL in the sample was 0.04 M, while that of the probe in relation to lecithin was −0.01 M. There were four tin compounds chosen for the investigation, which – due to their chemical structure – could be divided into two classes. The first (class I) included two chlorides: the organic $(C_3H_7)_3SnCl$ and the inorganic $SnCl_2$, while the other two organic compounds [$(CH_3)_4Sn$ and $(C_2H_5)_4Sn$], devoid of chlorine and differing in the number of hydrocarbon groups surrounding the tin atom, belonged to the other class (class II). The compounds of the first class dissociate in an aqueous solution into negatively charged chlorine ions and bi-positively charged inorganic tin ions or uni-positively charged organic ions. The structural formulas of the studied compounds are shown in Fig. 1.

Admixtures of the studied compounds were introduced into the samples containing liposomes formed earlier in water. The contents of the admixtures changed within the range 0 to 11 mol-% to the EYL. Measurements were taken at room temperature (about 25 °C).

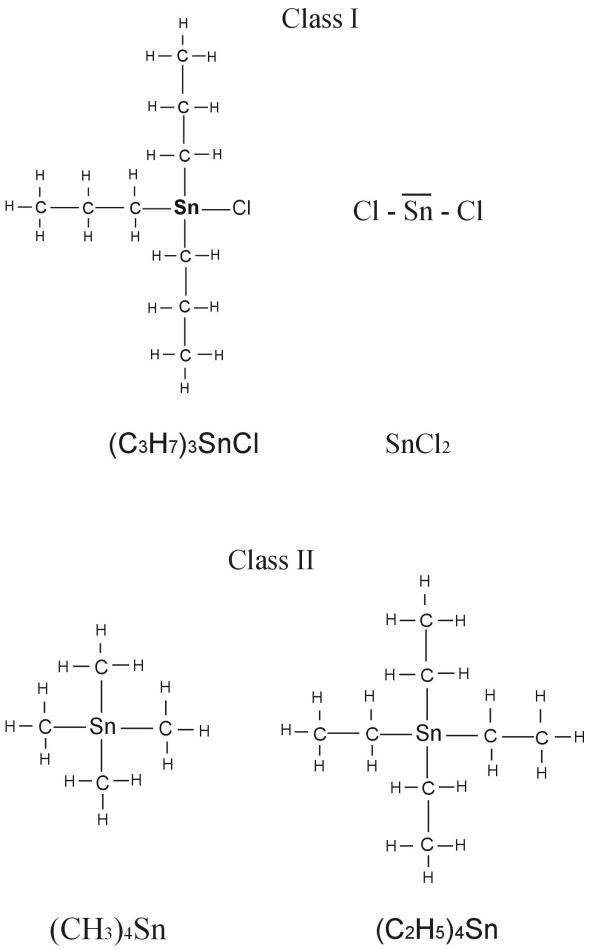

Fig. 1. Structures of compounds of class I (chlorides) and class II (devoid of chlorine).

In the study, two spin probes, differing as far as their places of location in the liposome membranes are concerned, were used: TEMPO and 16-DOXYL-stearic acid. The first (TEMPO) dissolves both in the hydrophobic part of the membrane and in water environment, whereas the other (16-DOXYL-stearic acid) locates itself deep in the hydrophobic layer of the membrane.

On the basis of the ESR spectrum obtained by means of TEMPO, the spectroscopic parameter of partition (F) of this probe in the membrane and its environs was determined. F is defined as the ratio of the high-field line amplitude of the ESR spectrum of a spin probe placed in lipid medium (H) and the amplitude of the high-field line of a probe placed in aqueous medium (P). The value of F is connected, among others, with the fluidity

of the membrane (Shimshick and McConnell, 1973). F for the control sample, devoid of an admixture of the investigated compounds, was marked Fo.

On the basis of the spectrum obtained from a 16-DOXYL-stearic acid probe, the spectroscopic parameter τ was determined. The value of the parameter depends, among others, on the degree of fluidity of the membrane and is the higher, the stiffer (the more ordered) the environment in which the probe remains is (Hemminga, 1983) (in the case of isotropic environment, τ is the rotation correlation time of the probe). For the control sample without an admixture of the investigated compounds parameter τ was marked τ_o.

Each of the measurements was repeated 10 times. The values of the spectroscopic parameters, which are presented in this work, are the arithmetic means of the measurements. The errors of measurement of the spectroscopic parameters amounted to 5% for TEMPO and 6% for 16-DOXYL-stearic acid, respectively.

In order to underline the changes occurring in liposomes under the influence of admixtures of the investigated compounds, there are relative values of the spectroscopic parameters F/Fo and τ/τ_o discussed in the paper.

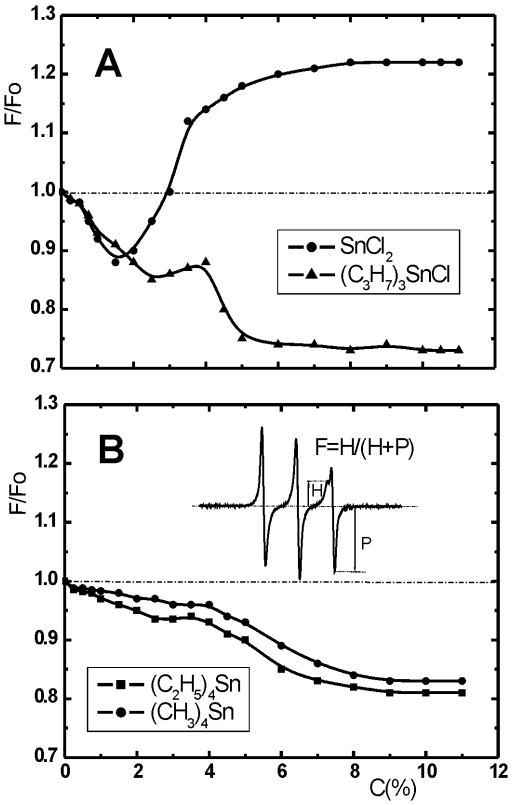

Fig. 2. Dependence of the relative value of spectroscopic parameter F/Fo of TEMPO dissolved in a water suspension of EYL liposomes containing admixtures of (A) compounds of class I (chlorides) and (B) compounds of class II (devoid of chlorine) on the content C of the admixtures.

Results and Discussion

Figs. 2A and 2B present the dependence of the relative value of the spectroscopic parameter F (F/Fo) of TEMPO probe dissolved in water suspension of EYL liposomes containing admixtures of the compounds belonging to class I (Fig. 2A) and those of class II (Fig. 2B) on the content of the admixtures. It follows from the figures that for the admixtures of all the investigated compounds, with the exception of the inorganic chloride $SnCl_2$, decreased the value of the parameter F/Fo, which can testify the lowering of the fluidity of the liposome membranes (Shimshick and McConnell, 1973). The chlorine-free compounds (class II) stiffened the liposome membrane only very slightly – parameter F/Fo was lower than 1 (Fig. 2B). The minimal value of this coefficient amounted to 0.83 for the $(CH_3)_4Sn$ and 0.81 for the $(C_2H_5)_4Sn$ admixture, respectively. Hence, it can be concluded that the compound with the longer hydrocarbon chain was a little more active in the interaction with liposome membranes. The course of the curves describing changes in the parameter F/Fo in dependence on the content of the admixture, for both compounds devoid of chlorine, was very similar and displayed tendencies towards stabilizing over 7% of the admixture. The course of the curves in Fig. 2A is of much different character. The inorganic tin chloride $SnCl_2$, at low contents of the admixture, stiffened the membranes, yet beyond a content of 1.5% (for which the minimum of the value of parameter F/Fo, amounting to *ca.* 0.88, was observed) it caused a rise in its value. For a content of 3% of the admixture, coefficient F/Fo reached the value of 1, whereas beyond this content the coefficient began to rise (the fluidity of the liposome membrane increases) up to 1.22, which was reached at the admixture contents of 8–11%. The organic chloride $(C_3H_7)_3SnCl$, within the whole range of investigated content, caused the membrane to stiffen, but the dynamics of this

process was changeable. Initially, parameter F/Fo decreased (similarly as in the case of the compounds of class II) to reach 0.85 at a content of the admixture amounting to about 2.5%, then it rose slightly only to rapidly drop at a content of about 4% to the value of 0.75 at 5% admixture content. Beyond this content, parameter F/Fo kept decreasing slightly to the value of 0.73 which was reached at 11% content level. The rapid change in the parameter F/Fo at 4% of $(C_3H_7)_3SnCl$ admixture content was accompanied by the appearance of an additional line in the ESR spectrum of TEMPO (Fig. 3). The amplitude of this line was rising along with the increase in the admixture content. The appearance of an additional line in the spectrum can be explained by the formation of domains of the compound in the lipid environment of the membrane. Another cause of the observed effect could be the formation of micelles by $(C_3H_7)_3SnCl$ particles in the water environment surrounding the liposomes. Nevertheless, investigations of water dispersions of the compound (within the range of 0.0001 to 10% in the mol proportion to water), carried out by means of the TEMPO probe, did not show the presence of micelles in those dispersions.

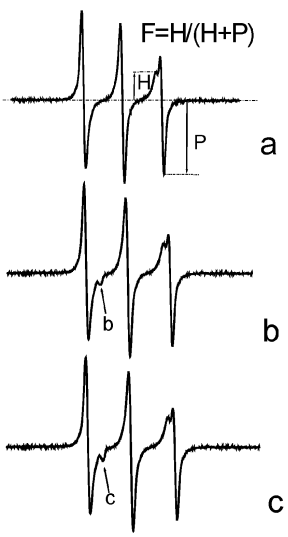

Fig. 3. Spectroscopic spectra of the TEMPO probe dissolved in a water suspension of EYL liposomes admixed with $(C_3H_7)_3SnCl$. (a) Liposomes dispersion spectrum without the admixture; (b) liposomes dispersion spectrum with a 4% admixture of the investigated compound; (c) liposomes dispersion spectrum with 8% admixture of the investigated compound.

The influence of the compounds studied on the spectroscopic parameters of the 16-DOXYL-stearic acid probe are shown in Figs. 4A and 4B. Compounds of class II (devoid of chlorine) increased the value of parameter τ/τ_o of this probe to only a slight degree within the whole range of the investigated contents, which might prove the weak stiffening of the internal area of liposome membranes (the rise in the parameter τ is connected with inhibition of the rate of oscillation movement of the probe by the environment surrounding it) (Hemminga, 1983). The compound of longer hydrocarbon chains showed a slightly stronger influence on the rise of parameter τ. The maximum value of parameter τ/τ_o amounted to 1.18 for $(C_2H_5)_4Sn$ and 1.15 for $(CH_3)_4Sn$ (Fig. 4B). In the case of tin chlorides the changes in parameter τ followed a two-way course: for low

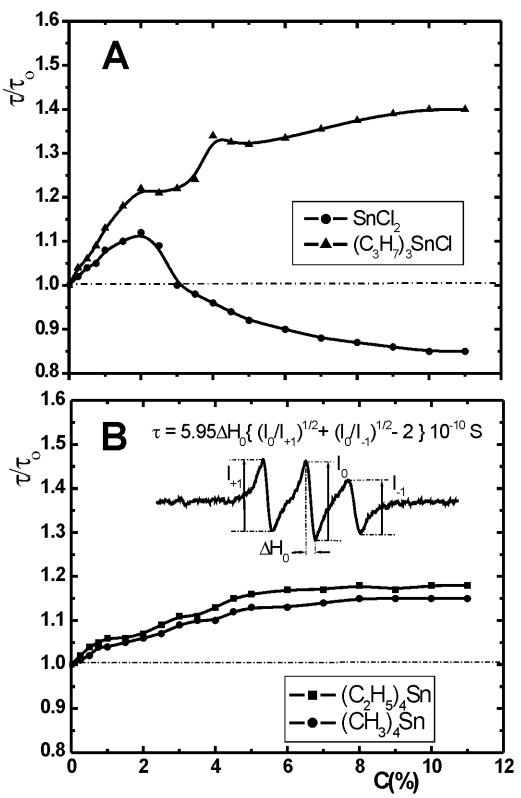

Fig. 4. Dependence of the relative value of spectroscopic parameter τ (τ/τ_o) of a 16-DOXYL-stearic acid probe dissolved in a water suspension of LJ liposomes containing admixtures of (A) class I (chlorine) compounds and (B) class II (devoid of chlorine) on the content C of the admixtures.

contents of the admixture the observed rise of τ/τ_o amounted to 1.12 for a 2% $SnCl_2$ admixture and to 1.22 for a 2% admixture in the case of $(C_3H_7)_3SnCl$. Above these contents there followed a gradual decrease in the value of parameter τ/τ_o. For $SnCl_2$ the parameter reached the value of 1 at 3% admixture content and then decreased insignificantly to the value of 0.85 at 11% of its content. The organic chloride $(C_3H_7)_3SnCl$, above 2% of its content, caused an insignificant decrease in the values of parameter τ and a growth again above 3% content, forming the first local maximum ($\tau = 1.22$, Fig. 4A). At the compound content of 4% there was observed a second local maximum, the value of parameter $\tau/\tau_o = 1.34$, and after its slight decrease within the range of contents between 4% and 5% there again followed its monotonic rise to the value 1.4 at 11% admixture content.

The organic chloride turned out more active in the interaction with liposomes within the whole range of content of the applied admixtures. It displayed a considerable influence on the changes in the spectroscopic parameter of both TEMPO and 16-DOXYL-stearic acid probes introduced into water dispersions of EYL liposomes.

On the basis of the conducted research the following conclusions can be drawn: The tin compounds containing chlorine showed greater activity in the interaction with membranes of EYL liposomes (they changed the values of the spectroscopic parameters of spin probes to a greater extent) in comparison with the compounds devoid of chlorine. This property can be connected with dissociation of chlorine particles in the water environment and introduction of an electric charge into the membranes, the charge interacting very strongly with polar (hydrophilic) heads of lipid particles. This character of interactions was studied by the authors of the present work in a computer model of the surface layer of liposome membranes and displayed a considerable influence of the value of electric charge of the admixture and its concentration on the binding energy of the dipole system simulating a hydrophilic layer of liposome membranes. The results of the investigations (Man *et al.*, 2001) were confirmed experimentally (Shimshick and McConnell, 1973). Of the two tin chlorides (organic and inorganic), examined in the work, the organic compound $(C_3H_7)_3SnCl$ proved far more active in the interaction with membranes of EYL liposomes. As one could conclude, the compound, due to the hydrophobic properties and relatively big size of the particles, penetrates into the hydrophobic interior of the membranes in an easy manner and causes considerable disturbance in their structure and fluidity. An exceptionally high activity of the compound (in comparison with compounds devoid of chlorine) in the interaction with membranes was also observed in a study by Podolak *et al.* (2006) which was carried out by means of the electric method on filters impregnated with butylene ester of lauric acid ($C_{16}H_{32}O_2$).

The occurrence of extremes in the course of the curves illustrating changes in the spectroscopic parameters for the TEMPO and 16-DOXYL-stearic acid probes, in the case of admixtures containing chlorine (Figs. 2A and 4B), testifies to a local (because of the content of the admixture) stiffening of membranes for precisely determined contents of the investigated compound. The contents remain within the range of values between 1.5 and 2.5%. The obtained results are both qualitatively and quantitatively in accordance with those obtained by the authors in their earlier research, both theoretically (computer simulation) and experimentally (where ammonia salts constituted the admixtures introduced into lecithin) (Podolak and Man, 2002; Podolak *et al.*, 1997).

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