VERSITA

CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 11, (2006) pp 56 – 61
http://www.cmbl.org.pl

DOI: 10.2478/s11658-006-0005-7

Received: 26 October 2005
Revised form accepted: 16 December 2005

Short communication

THE INFLUENCE OF TIN COMPOUNDS ON THE DYNAMIC PROPERTIES OF LIPOSOME MEMBRANES: A STUDY USING THE ESR METHOD

DARIUSZ MAN*, MARIAN PODOLAK and GRZEGORZ ENGEL
Institute of Physics, Opole University, Oleska 48, 45-052 Opole, Poland

Abstract: The influence of organic and inorganic compounds of tin on the dynamic properties of liposome membranes obtained in the process of dipalmitoylphosphatidylcholine (DPPC) sonication in distilled water was investigated. This was carried out by means of the spin ESR probe method. The probes were selected in such a way as to penetrate different areas of the membrane (a TEMPO probe, 5-DOXYL stearic acid, 16-DOXYL stearic acid). Four compounds of tin were chosen: three organic ones, $(CH_3)_4Sn$, $(C_2H_5)_4Sn$ and $(C_3H_7)_3SnCl$, and one inorganic one, $SnCl_2$. The investigated compounds were added to a liposome dispersion, which was prepared prior to that. The concentration of the admixture was changed within the values from 0 to 10%-mole in proportion to DPPC. The studies indicated that the chlorides of tin display the highest activity in their interaction with liposome membranes. Since these compounds have ionic form in a water solution, the obtained result can mean that this form of admixture has a considerable influence on its activity. Furthermore, it was found that there is a slightly stronger influence of tin compounds with a longer hydrocarbon chain on changes in the probes' spectroscopic parameters.

Key words: DPPC liposomes, ESR, Tin compounds

INTRODUCTION

With the development of civilization and industry came an increase in the amounts and types of toxins contaminating the natural environment. Among these toxins are compounds containing heavy metals, for example, tin. Found as

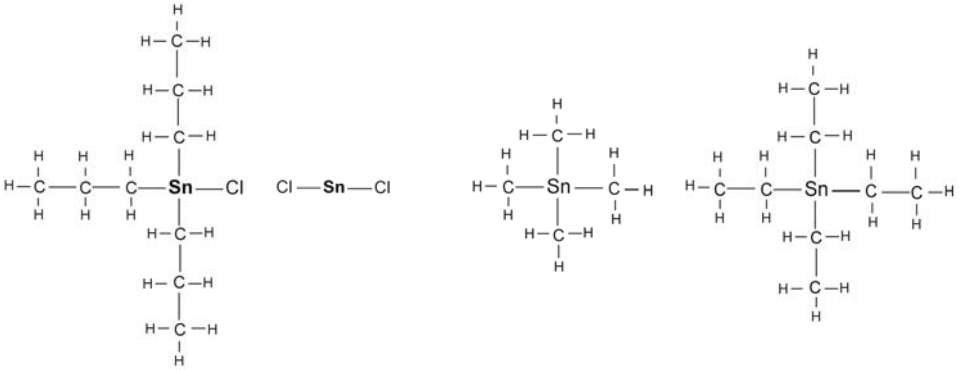
* Corresponding author, e-mail: dariusz.man@uni.opole.pl

a component in many alloys, tin is applied as an anti-corrosion agent to metal sheets, and used to construct elements of electric and electronic devices. Tin is treated as a metal of low toxicity, but it can poison organisms due to its ability to accumulate in living tissue and its commonness in nature. Symptoms of tin poisoning include neurological disorders. Alkyl compounds of tin are particularly toxic, and the more methyl groups they contain, the more harmful they are. Among their other effects, organic compounds of tin inhibit the process of oxidative phosporylation and accelerate the hemolysis of erythrocytes [1-3]. They also change the physical properties of lipid model membranes. Ionized compounds of tin (e.g. chlorides) show a particular kind of activity in their exertion of influence on lipid membranes [4-6]. The aim of this study was to investigate the influence of selected tin compounds (organic and inorganic) on the dynamic properties of liposome membranes.

MATERIALS AND METHODS

Liposomes were obtained via the process of dipalmitoylphosphatidylcholine synthetic lecithin (DPPC) sonication in distilled water. The total sonication time of a sample of 1.5 ml was 5 minutes, and was carried out in one-minute cycles (sonication and cooling). The DPPC concentration in the sample was 0.04 M, and the molar ratio of spin probe to DPPC was 0.01. Four compounds of tin were chosen for the study, and they can be divided into two categories based on the structure of the particles. The first consisted of two chloride compounds, one organic $(C_3H_7)_3SnCl$ and one inorganic $SnCl_2$, while the second included two organic, non-chloride compounds, $(CH_3)_4Sn$ and $(C_2H_5)_4Sn$. They differed in the number of hydrocarbon groups surrounding the tin atom. The compounds in the first class (chlorides) dissociate in a water solution into negative ions of chlorine and bi-positive inorganic ions of tin, or into unipositive organic ions. The structural formulae of the investigated compounds are:

Class I Class II



$(C_3H_7)_3SnCl$ $SnCl_2$ $(CH_3)_4Sn$ $(C_2H_5)_4Sn$

Three spin were probes used: 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 2-(3-Carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxy (5-DOXYL-stearic acid), and 2-Ethyl-2-(15-methoxy-15-oxopentadecyl)-4,4-dimethyl-3-oxazolidinyloxy (16-DOXYL-stearic acid). Each penetrates different regions of liposome membranes. The TEMPO probe dissolves both in the hydrophobic part of the membrane and in a water environment. On the basis of an ESR spectrum, the spectroscopic partition parameter (F) of the probe between the membrane and its surrounding was determined. The measure of parameter F is the ratio of the high-field line amplitude in the ESR spectrum of the probe dissolved in a lipid environment (H) to the sum of the high-field line amplitude in a lipid (H) and water environment (P) (Fig. 1). For the control sample free from an admixture of the investigated compounds, the partition parameter was marked with the symbol Fo. The value of this parameter (F) is very often related to the fluidity of the membrane [7].

The 5-DOXYL probe dissolves in the hydrophobic part of the membrane and is located in the vicinity of the lipid bilayer surface. On the basis of the ESR spectrum of this probe, an ordering coefficient was determined, the value of which is directly proportional to the probe order parameter (S) [8]. The measure of this coefficient is the distance between the extreme lines of the spectrum of the 5-DOXYL probe.

The 16-DOXYL probe, similarly to the 5-DOXYL one, dissolves in the hydrophobic part of the membrane, but is located in the center of the lipid bilayer. On the basis of the ESR spectrum of this probe, parameter τ was determined; its value is inversely proportional to the rotation speed of the probe [9]. In order to emphasize the changes going on in the samples under the influence of the investigated compounds, we analyzed the relative values of the determined spectroscopic parameters.

Each of the measurements was repeated 10 times. The values of the parameters presented here are the arithmetic mean of the measurements. Measurement errors in the spectroscopic parameters amounted to: 5% for the TEMPO probe, 6% for the 16-DOXYL probe, and 4% for the 5-DOXYL probe.

RESULTS AND DISCUSSION

Fig. 1 presents the dependence of the relative value of the spectroscopic parameter F/Fo of the TEMPO probe dissolved in a water suspension of DPPC liposomes, containing admixtures of compounds of the first (chlorides) (Fig. 1A) or the second (non-chlorides) class (Fig. 1B) on the concentration of the admixtures. It follows from the data that all the investigated compounds caused a rise in the fluidity of liposome membranes (an increase in the value of F/F_0), although in the case of $(CH_3)_4Sn$, this rise was minimal. The biggest changes in F/F_0 occurred within the range of low concentrations of the admixture (from 0 to 4%). At higher concentrations, the value of the parameter became stable. A local fluidity minimum was observed around a concentration of 1-1.5% for all of the compounds. In the case of $(CH_3)_4Sn$, this minimum meant a stiffening of the

membranes ($F/F_0 < 1$). The occurrence of the minimum for membranes within the range of low concentrations of the admixtures was reported on earlier [10].

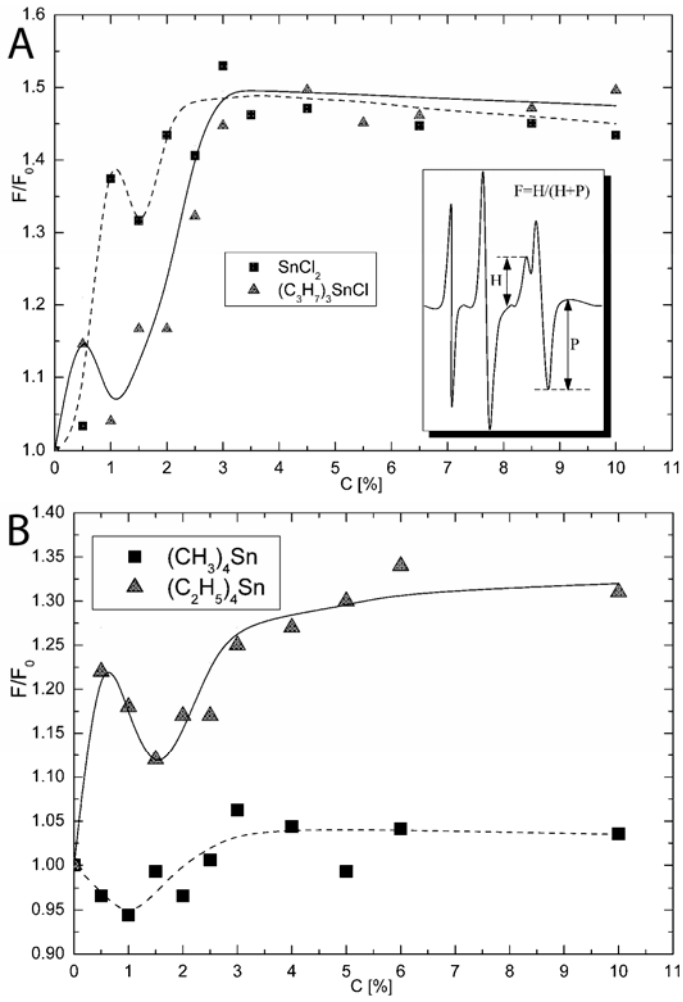


Fig. 1. The dependence of the relative value of the spectroscopic parameter F/Fo of the TEMPO probe dissolved in a water suspension of DPPC liposomes, containing admixtures of compounds of the first class (chlorides) (A) and compounds of the second class (non-chlorides) (B), on the concentration of the admixtures.

For the chlorides (organic and inorganic), F/F_0 stabilized at a value of 1.5 (the highest value). For $(C_2H_5)_4Sn$, (a compound of class II, with a greater number of CH_2 groups) the value of F/F_0 stabilized at 1.3, while for $(CH_3)_4Sn$ (with a lower number of CH_2 groups) at 1, which demonstrates a lack of influence on the part of the admixture on the value of parameter F (on the fluidity of the membranes) within the range of higher concentrations.

The TEMPO probe, which was used in the investigations in this study, yields information on the dynamics of the surface layer of liposome membranes. This layer is formed by lipid polar heads, which, in the case of DPPC, give rise to electric dipoles. For this reason, interactions with ionic admixtures locating in this region of the membranes may, to a great extent, determine the results of measurements taken by means of the TEMPO probe.

In the case of probes penetrating the hydrophobic layer of the membrane (5-DOXYL, 16-DOXYL), the changes in the spectroscopic parameters induced by admixtures of the investigated salts were insignificant in comparison with the changes observed by means of the TEMPO probe. The ordering coefficient, which was registered using the 5-DOXYL probe, changed within the range of values from 0% to 1% for compounds of Class II, at concentrations of the compounds in the membrane higher than 3%. For lower concentrations, the changes in the parameter were of chaotic nature and remained within the range of values from 0% to 2.5%. Similar results were obtained for compounds of Class I, the difference being that $(C_3H_7)_3SnCl$ induced greater changes in the parameter (about 4%) in comparison with the changes induced by admixtures of $SnCl_2$ (about 1%). Similar tendencies were observed in the changes in the spectroscopic parameter t of the 16-DOXYL probe (the value of the parameter being inversely proportional to the rotation speed of the probe) induced by admixtures of the investigated compounds. In this case, no differences in the influence of the compounds of Class I on parameter t were found. It was also for this probe that the changes in parameter t induced by admixtures of Class I and II compounds at concentrations lower than 3% were of chaotic character. It follows from the investigations that the changes in the spectroscopic parameters of the 16-DOXYL and 5-DOXYL probes influenced by admixtures of compounds of Class I and II were similar. At low concentrations of the admixtures (lower than 3%), the changes were chaotic and could not be interpreted in a unequivocal way. It is likely that the effect was caused by the measurements having been taken at 25ºC, below the temperature of the main phase transition of lecithin DPPC (40.5ºC). For concentrations higher than 3%, the 5-DOXYL and 16-DOXYL probes displayed only a slight increase in the fluidity of the investigated area of the membrane.

The biggest changes in the spectroscopic partition parameters, as observed for the TEMPO probe, which was located in the surface layer of the membrane, could be induced by the biggest changes in the fluidity or the presence of admixture ions in this layer of the membrane. It follows from the research carried out earlier [11] that the rise in the pH of the solution of the water environment of liposomes containing admixtures of tin ions, among others, caused a slight rise in parameter F of the TEMPO probe. The presence of the examined compounds in the environment of the liposomes may have a similar influence of this parameter to the changes in pH. Due to this, it can be supposed that the partition parameter of the TEMPO probe should not be unequivocally interpreted as the indicator of membrane fluidity.

REFERENCES

1. Gray, B.H., Porvaznik, M., Flemming, C. and Lee, L.H. Organotin-induced hemolysis, shape transformation and intramembranous aggregates in human erythrocytes. **Cell Biol. Toxiol.** 3 (1987) 23-38.
2. Kleszczyńska, H., Hładyszowski, J., Pruchnik, H. and Przestalski, S. Erythrocyte hemolysis by organic tin and lead compounds. **Z. Naturforsch.** 52c (1997) 65-69.
3. Hamasaki, T., Masumoto, H., Soto, T., Nagase, H., Kito, H. and Toshioka, T. Estimation of the hemolytic effects of various organotin compounds by stucture – active relationships. **Appl. Organomet. Chem.** 9 (1995) 95-104.
4. Gabrielska, J., Sarapuk, J. and Przestalski, S. Role of hydrophobic and hydrophilic interactions of organotin and organolead compounds with model lipid membranes. **Z. Naturforsch**. 52c (1997) 209-216.
5. Radecka, H., Zielińska, D. and Radecki, J. Interaction of organic derivatives of tin (IV) and lead (IV) with model lipid membranes. **Sci. Total Environ.** 234 (1999) 147-153.
6. Kleszczyńska, H., Sarapuk, J. and Przestalski, S. Destabilization of model membranes by organotin compounds. **Folia Histochem. Cytobiol.** 37 (1999) 1-31.
7. Shimshick, E.J. and McConnell, H.M. Lateral phase separation in phospholipid membranes, **Biochemistry** 12 (1973) 2351-2360.
8. Schreier, S., Polnaszek, C.F. and Smith, I.C.P. Spin labels in membranes problems in practice, **Biochim. Biophys. Acta** 515 (1978) 375-436
9. Hemminga, M.A. Interpretation of ESR and saturation transfer ESR spectra of spin labeled lipids and membranes, **Chem. Phys. Lipids** 32 (1983) 323-383.
10. Podolak, M., Man, D., Waga, S. and Przestalski, S. Bimodal Effect of Amphiphilic Biocide Concentrations on Fluidity of Lipid Membranes, **Z. Naturforsch.** 51c (1997) 853-858.
11. Podolak, M., Man, D. and Przestalski, S. Effect of solution pH on the organic tin compounds-liposome membranes interaction, Abstracts of the 7 th Conference on Cell Biology, Kraków, **Folia Histochem. Cytobiol.** 37 (1999) sup. 1, 36.