Polish Journal of Environmental Studies Vol. 9, No. 5 (2000), 419-422

Interaction of Organo-tin Derivatives with Gramicidin Ion-conducting Channel

H. Radecka

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Division of Food Science, Tuwima 10, 10-747 Olsztyn, Poland

Received: April 20, 2000
Accepted: May 15, 2000

Abstract

The objective of this study was to determine the interaction of organo tin (IV) compounds with a gramicidin ion-conducting channel incorporated into planar lipid bilayers. The relative change of trans membrane current was used as a parameter for estimating the above interaction. This measured parameter was in good relationship with physicochemical - partition coefficients (logP) and topological - total surface area (TSA) properties of the compounds under study. The presented model might be applied for estimation of toxicity of organo metallic derivatives.

Keywords: triphenyltin(IV) chloride, trimetyltin(IV) chloride, planar lipid bilayers, gramicidin ion-conducting channel

Introduction

During the last two decades, intensive investigation on the toxicity of organo derivatives of tin and lead in relation to many living organisms has been carried out. Particularly, special attention was put on the triorganoderivatives of tin, which are the most toxic. In the literature, there have been many attempts to establish a general relationship between the structure of organo lead and tin compounds and their toxicity [1-7]. Ambrosini and co-workers established that interaction of trialkytin compounds with lipid membranes (widely used as a model of cell membrane) are differentiated both on the chemical structure of compounds and on the composition of lipids forming the membrane [8].

In our previous investigations on the molecular base of toxic properties of alkyltin derivatives, we were dealing with their influence on the structure of planar lipid bilayers, which form apart from the protein, the main constituent of the membrane cell [9-12]. We established that the compounds under study interact mainly with the hydrophilic part of lipid bilayer. Their penetration inside the hydrophobic part of the membrane is rather negligible. Similar relationships were observed by Przestalski and co-workers [13-15].

Here, the results of the investigation of the influence of alkyl - and phenyl - derivatives on the permeability of the gramicidin ion-conducting channel incorporated into bilayer lipid membrane (BLM) has been presented.

The ion-conducting channel is formed with two molecules of gramicidin due to the formation of hydrogen bounds between HN- and formyl groups [16-17]. The channel can traverse the hydrocarbon core of lipid bilayer membrane.

Also very important from channel function properties point of view, there are tryptofan residues located on the surface of the interface lipids/aqueous [18]. Nitrogen atoms from the indol rings of tryptofan residues are believed to be the probable place of interaction with tin atoms of organo derivatives.

That way, the gramicidin ion-conducting channel was selected for the investigation of the mechanism of the toxic influence of organotin compounds. The possibility of application of the above model as a toxicity test of compounds under study will be discussed.

Materials and Methods

Reagents

Azolecitin from soy beans, gramicidin, n-decane, cholesterol were purchased from Sigma-Aldrich (Poznan, Poland). Trimetyltin (IV) chloride and triphenyltin (IV) chloride were purchased from Alfa Products (Germany).

Formation of Planar Lipid Bilayers (BLM) Incorporated with Gramicidin Ion-Conducting Channel

Planar lipid bilayers (BLM) were formed using the Tien method [19, 20] from 2% of lipid solution (azolecitin + cholesterol, in ratio 4:1) in n-decane on a 1.7 mm diameter hole in Teflon partition which divided the measuring cell into two equal compartments.

Membrane formation solution also contained 10 ppm of gramicidin in relation to the total amount of lipids. The optimum concentration of gramicidin was found experimentally. 0.1 M KC1 solution was used as an electrolyte. Membrane formation was monitored optically and by measuring the membrane's electrical capacity. The typical run of capacity changes during membrane formation was illustrated in Fig. 1.

The S-µl stock solution of the examined compound in methanol was pipetted into the compartment containing 0.1 M KC1 to obtain the appropriate concentration ranges. After each dose of tin compound solution, 5 µl 0.1 M KC1 solution was added to another compartment of the measuring cell in order to avoid differences in hydrostatic pressure. The measurements of the electrical parameters of BLM were carried out by four-electrode potentiostat-galvanostat [21].

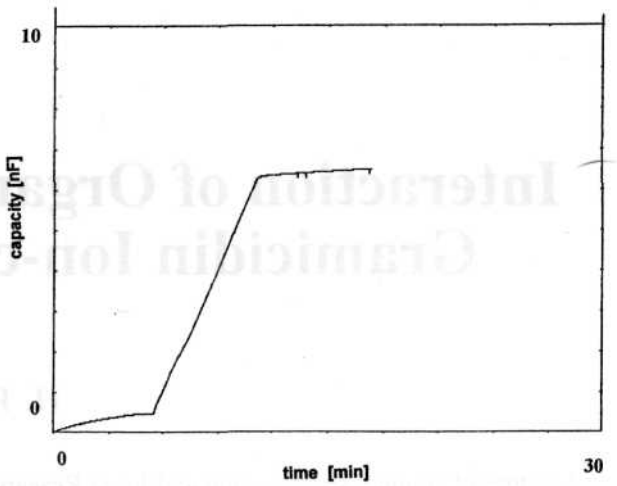

Fig. 1. Changes of capacity during BLM formation.

Results and Discussion

The typical run of changing the current flowing through BLM modified with gramicidin was illustrated in Fig. 2. (A,B). The characteristic minimum of the current was observed for triphenyltin (IV) chloride (A) and for trimetyltin (IV) chloride (B). Next, the current increased along with the increase of compound concentration. However, the triphenyltin (IV) chloride presented higher intensity of current increase. In the case of this compound, the destruction of BLM was usually observed at the concentration ca. $1O^{-5}$ M (Table 1). On the other hand, trimetyltin (IV) chloride caused no BLM destruction. This allowed for the measurements of capacity of BLM, after treatment by this compound. The capacity was almost twice larger in comparison to the initial value, when no tin compounds were in the solution.

Thus, it could be stated that trimetyltin (IV) chloride caused the decrease of thickness of BLM [22, 23].

In order to compare the influence of compounds under study on the function of gramicidin channel, the relative values of trans-membrane current decrease were used:

$$\frac{I_{min}}{I_0} \cdot 100\%$$

I_{min} - minimum value of current
I_o - initial value of current (c=0)

In the case of triphenyltin (IV) chloride, a very high decrease of intensity of current (up to 10% of the initial value) was observed, whereas a much lower decrease was caused by trimetyltin (IV) chloride (only up to 65% of the initial value) (Tablel).

The probable mode of interaction of compounds under study with the gramicidin ion-conducting channel was introduced in Fig. 3. Triphenyltin (IV) chloride characterized with higher hydrophobicity (logP = 2.65 [24]) is able to penetrate deeper of gramicidin channel entrance

Table 1. Changes of current flowing through the gramicidin ion-conducting channel incorporated into BLM under the influence of organotin (IV) derivatives (n=5)

Compounds	I_{min}/I_0 [%]	T[min]	c_m [M]	c_d [M]	C_0 [nF]	C_f [nF]
Trimetyltin chloride	67.2 ± 2.8	14 ± 1	1.6 (± 0.3) × 10^{-7} M	*	3.5 ± 0.2	6.0 ± 0.2
Triphenyltin chloride	10.8 ± 9.2	16 ± 1	6.3 (± 0.7) × 10^{-7} M	5.0 (± 0.2) × 10^{-5} M	3.8 ± 0.2	**

Imin - the minimum value of current flowing through the gramicidin channel
I_0 - the initial current value (C=0)
c_m - the concentration of compound when the minimum of current value occurred
c_d - the concentration of compounds caused the BLM destruction
T - time when minimum of current were reached
C_0 - the initial capacity of BLM
C_f - the final capacity of BLM (after treatment with organotin derivatives)
* - the destruction of BLM was no occurred
** - BLM was destroyed

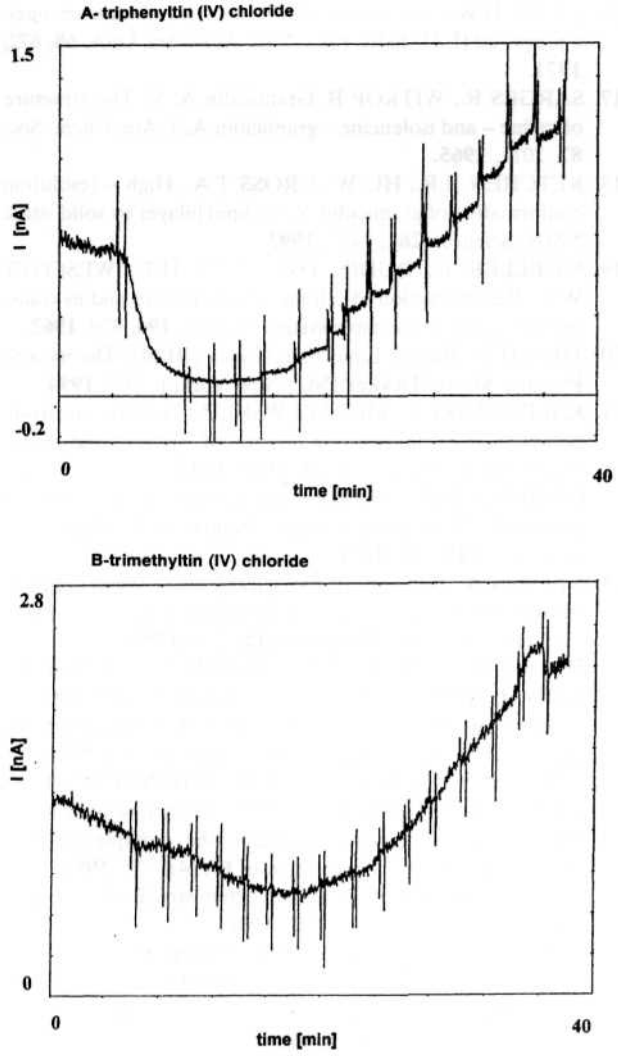

Fig. 2. The run of changes of current flowing through the BLM modified by gramicidin under influence of: A - tirphenyltin (IV) chloride, B - trimetyltin (IV) chloride.

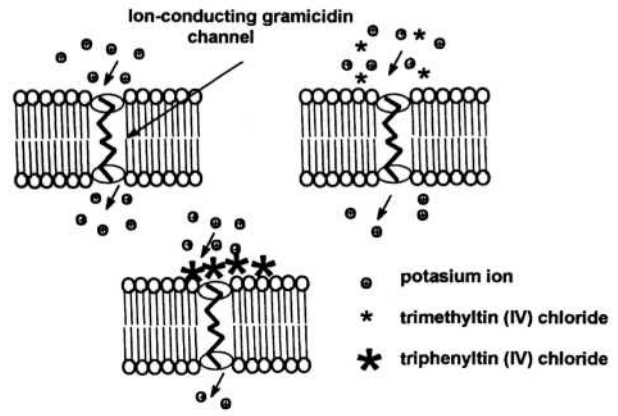

Fig. 3. The schema of function of the gramicidin ion-conducting channel in the presence of organotin (IV) compounds.

in comparison to less hydrophobic trimethyltin (IV) chloride (logP = -2.3 [24]). Also, these molecules differ from a topological properties point of view. The calculated total surface areas (TSA) are 154.5 A^2 and 329.6 A^2 for trimethyl- and triphenyltin (IV) derivatives, respectively [25]. That way, the compound with larger TSA and higher lipohilicity is able to block channel more efficiently.

The most probable place of interaction of tin derivatives with gramicidin channel are indol rings from tyrosine residues located at the interface lipids/aqueous [26]. Probably, the base of this interaction is the formation of coordination bound between tin and nitrogen atoms. Similar bound formation was observed between organo tin derivatives and residue of histidine [27].

Conclusions

The planar lipid bilayers modified with gramicidin channel could be a useful model tool for investigation of interaction of organo metal derivatives with membrane cell. The differentiated run of current changes in relation to chemical structure of organotin derivatives suggested that this method might be applied as a test for estimation of the toxicity of compounds under study.

The main advantage of BLMs is their high sensitivity. Also, this artificial model of membrane cell gives the possibility of the incorporation lipophilic molecules increasing the selectivity of the observed phenomenon. However, the inherent mechanical stability is the mine disadvantage of BLMs. The disposition of lipid layers on solid support is one of the solutions of mentioned problems and this research direction is in progress in our laboratory.

Acknowledgements

This research was supported by KBN grant No. 6 PO4 GO 4313.

References:

1. BLAKEY D.H., BAYLEY J.M., DOUGLAS G.R., Induc tion of chromosomal aberrations in Chinese hamster ovary cells by triethyllead acetate. Mutation Research, **298, 1, 1992.**
2. ENG G., TIERNEY E.J., OLSON G.J., BRINCKMANS F.E., BELLAMA J.M., Total surface areas of Group IVA organometallic compounds: predictors of toxicity to algae and bacteria. App. Organomet. Chem., 5, 33, 1991,
3. HAMASAKI T, MATSUMO H., SATO T., NAGASE H., KITO H., YOSHIKA Y., Estimation of the hemolytic effects of various organotin compounds by structure-activity rela tionships, App. Organometal. Chem., 9, 95, 1995.
4. NAGASE H., HAMASAKI T., SATO T., KITO H., YOSHIKA Y., OSE Y., Structure-activity relationships for organotin compounds on the red kilifish Oryzias Latipes. App. Organometal. Chem., 5, 91, 1991.
5. RADECKI J., Chemical structure of lead and tin com pounds and their antymitotic activity and accessibility for maize roots, Acta Acad.Agricult. Technol. Olst., 55, 4, 1993 (in Polish)

6. RADECKI J., BANASZKIEWICZ T., KLASA A., The ef fect of different tin compounds on the mitotic activity of maize root tip cells, Acta Physiologiae Plantarum, **4,** 359, **1989.**
7. RADECKI J., An investigation of the interaction of trialkyl-lead compounds with humic acids, Environmental Technol ogy Letters, **9,** 621, **1988.**
8. AMBROSINI A., BERTOLI E, ZOLESE G., Effect of organotin compounds on membrane lipids: fluorescence spec troscopy studies, Applied Organomet. Chem. **10,** 53, **1996**
9. RADECKA H., ZIELINSKA D, SARAPUK J., RADECKI J., Attempt of estimation of organotin compounds toxicity. Journal de Medicina Preventiva, **5,** (2), 60, **1997.**
10. RADECKA H, SARAPUK J., ZIELINSKA D, RADECKI J., Effects of selected metallo-organic compounds on black lipid membranes. Polish Journal of Food and Nutrition Sciences, **6/47,** (3), 97, **1997.**
11. RADECKA H., ZIELINSKA D., RADECKI J., Interaction of organic derivatives of tin(IV) and lead (IV) with model lipid membranes, The Science of the Total Environment, **234,** 147, **1999.**
12. ZIELINSKA D., RADECKA H., RADECKI J. Contribu tion of membrane surface charge in the interaction of lead and tin derivatives with model lipid membrane, Chemosphere, **40,** 327, **2000.**
13. LANGER M, GABRIELSKA J., PRZESTALSKI S, The effect of the dipalmitoylphosphatidylcholine lipid bilayer state on the adsorption of phenyltins, Appl. Organometal. Chem., **14,** 152, **2000.**
14. SARAPUK J., KLESZCZYNSKA H., PRZESTALSKI S., Stability of model membranes in the presence of organotin compounds, Appl. Organometal. Chem. , **14,** 40, **2000.**
15. LANGER M., GABRIELSKA J., PRZESTALSKI S., Ad sorption of phenyltin compounds onto phosphatidylcholine/cholesterol bilayers, Appl. Organometal. Chem., **14,** 25, **2000.**
16. URRY D.W., The gramicidin A transmembrane channel: a proposed (L,D) helix. Proc. Natl. Acad. Sci. USA. **68,** 672, **1971.**
17. SARGES R, WITKOP B. Gramicidin A. V. The structure of valine - and isoleucine - gramicidin A, J. Am. Chem. Soc. 87,2011,1965.
18. KETCHEN R.R., HU W, CROSS T.A, High - resolution conformation of gramicidin A in a lipid bilayer by solid -state **NMR,** Science,. **261,** 1457, **1993.**
19. MUELLER P., RUDIN D.O., TIEN H.T., WESCOTT W.C., Reconstruction of cell membrane in vitro and its transormation into an excitable system, Nature, **194,** 979, **1962.**
20. TIEN H.Ti. Bilayer Lipid membranes (BLM): Theory and Practise. Marcel Dekker INC., New York, p. 267, **1994.**
21. KALINOWSKI S., FIGASZEWSKI Z., A four- electrode potentiostat-galvanostat for studies of bilayer lipid mem branes. Meas. Sci. Technol. 6, 1050, **1995.**
22. GOODALL M.C., Thickness dependence in the action of gramicidin A on lipid bilayers, Archives of Biochem. and Biophys. , **147,** 129, **1971.**
23. NELSON A., Influence of biologically active compounds on the macromolecular gramicidin channel function in phos pholipid monolayers, Langmuir, **12,** 2058,1996.
24. HAMASAKI T., MASUMOTO H., SATO T, NAGASE H., KITO H., YOSHIOKA Y., Estimation of the hemolytic ef fects of various organotin compounds by structure-activity relationships, Appl. Organometal. Chem., **9,** 95, **1995.**
25. LAUGHLIN R.B. Jr., FRENCH W., JOHANNESEN R.B, GUARD H.E., BRINCKMAN F.E, Predicting toxicity us ing computed molecular topologies: the example of triorganotin compounds, Chemosphere, **13,** (4), 575, **1984.**
26. The Handbook of Environmental Chemistry. Ed. by O. Hutzinger, Vol. 3, Part E, p. 32-33, **1990.**
27. ARSENEV A.S. , LOMIZE A.L., BARSUKOV J.L., BYSTROV V.F., Gramicidin A trans-membrane ion-channel. Three- dimensional structure reconstruction based on NMR spectroscopy and energy refinement, Biol. Membr. 3 ,1077, **1986.**