Influence of Organic and Inorganic Ions on Organolead-Induced Hemolysis of Erythrocytes

Halina Kleszczyńska* and Janusz Sarapuk

Department of Physics and Biophysics, Agricultural University, Norwida 25, 50–375 Wrocław, Poland. Fax: +48-71-205172. E-mail: halina@azi.ar.wroc.pl

* Author for correspondence and reprint requests

Z. Naturforsch. **56c,** 853–856 (2001); received February 26/March 27, 2001

Organoleads, Erythrocytes, Hemolysis Inhibition

The influence of some inorganic (K^+, Mg^{2+}, and Al^{3+}) and organic $C_nH_{2n+1}SO_3^-$, n = 12 and 14) ions was studied on the hemolysis of erythrocytes (RBC) caused by organolead compounds (tripropyllead – TPL, tributyllead – TBL and triphenyllead – TPhL chlorides). It was found that sulfonate anions increased the hemolytic effect induced by triorganoleads, while inorganic cations protected RBC against the triorganoleads action, especially when the latter were used at small concentrations. This protection was weaker when the concentration of organoleads increased and depended on the kind of ion. The protective efficacy sequence was like that: $Mg^{2+} > Al^{3+} > K^+$. The less hemolytic of the triorganoleads studied was TPL. TBL was slightly more effective than TPhL. The efficacy of the sulfonate ions to increase the triorganolead chloride – induced hemolysis was practically the same for TPL and TBL. A weaker efficacy of $C_{12}H_{25}SO_3^+$ was observed when TPhL was used as RBC membrane modifier.

Introduction

There are two main reasons of environment pollution with organolead compounds. They may be introduced into the environment as pesticides or originate from antiknock additives to gasoline (Thayer 1974; Craig 1982; Kumari *et al.*, 1993). Although their concentration seems to decrease in the last years, they still constitute a menace to a living organism because of their high toxicity. To exert this toxicity the place of first contact is the cell wall and/or membrane of a biological object. As a consequence of this contact, various toxic effects may occur (Craig 1982; Zimmermann *et al.*, 1987; 1988; Endres and Faulstich 1989; Hager *et al.*, 1989; Krug 1993; Trela *et al.,* 1997). One method to determine toxicity of organoleads is to study their hemolytic activity (Kleszczyńska *et al.*, 1997; 2000; Sarapuk *et al.*, 2000). Hemolysis of erythrocytes (RBC) is thought to be the result of the interaction of exogenous compounds with the lipid phase of the RBC membrane and triorganolead compounds studied can be regarded as such compounds.

Three of the organolead compounds studied in this work were previously shown to exhibit high plant and hemolytic toxicity (Sarapuk *et al.,* 2000) and the aim of this work was to find whether this toxicity could be modified by various organic (sulfonate) and inorganic ions (K^+, Mg^{2+}, and Al^{3+}), especially if such ions may be used to partially eliminate the toxic efficacy of triorganoleads. It was already shown that some cations, especially divalent, may exert a protective effect against hemolysis of the erythrocytes induced by various factors (Bashford *et al.*, 1986; 1988; 1989; Portlock *et al.*, 1990; Song *et al.*, 1993; Park *et al.*, 1994; Rudenko and Patelaros 1995; Stasiuk and Kozubek, 1996). Also, some cations prevented leakage from living cells or liposomes treated with various substances (Horiguchi *et al.*, 1986; Bashford *et al.*, 1988; 1989; Alder *et al.*, 1991).

Methods

The following organolead compounds were used in the investigation: tri-n-propyllead chloride $(C_3H_7)_3PbCl$ – TPL; tributyllead chloride $(C_4H_9)_3PbCl$ – TBL, and triphenyllead chloride $(C_6H_5)_3PbCl$ – TPhL. All were purchased from Alfa, Johnson Matthey, Karlsruhe, Germany.

Fresh heparinized pig blood was used. For washing erythrocytes and experimentation a solu-

0939–5075/2001/0900–0853 $ 06.00

tion of 0.9% NaCl with 1 mM EDTA, buffered in 10 mM Tris [(hydroxymethyl)aminoethane]-HCl (Serva, Heidelberg/Germany/USA), pH 7.4. Erythrocytes collected from the plasma were washed four times in the isotonic phosphate solution of pH 7.4 (131 mM NaCl, 1.79 mM KCl, 0.86 mM $MgCl_2$, 11.79 mM $Na_2HPO_4{\cdot}2H_2O$, 1.80 mM $Na_2H_2PO_4{\cdot}H_2O$), and then incubated in the same solution but containing proper amounts of the triorganoleads studied.

Organoleads were also added to the phosphate solution containing salts of the ions used, one at a time. These were potassium, magnesium and aluminium chlorides, and sodium sulfonates ($C_nH_{2n+1}SO_3Na$, n = 12 and 14, Aldrich, Poznań, Poland). Concentration of the salts in the solution was 50 μM for the ions. At this concentration hemolysis of erythrocytes did not exceed 5% during the experiment. Modification was conducted at 37 °C for 4 h, the samples of 10 ml volume contained erythrocytes at 2% concentration, and the suspension was stirred continuously. The percent of hemolyzed cells was measured after 4 h of incubation. The samples taken were centrifuged and the hemoglobin content measured in the supernatant using a spectrophotometer (Specol 11, Carl Zeiss, Jena) at 540 nm (Boyer *et al.*, 1993; Hamasaki *et al.*, 1995). The hemoglobin concentration in the supernatant expressed in percentage was taken as percent of hemolyzed cells, calculated relative to a sample containing totally hemolyzed erythrocytes. All compounds studied were dissolved in ethanol in such amounts that the concentration of ethanol in the samples did not exceed 1%.

Results and Discussion

The results obtained are shown in Table I for TPL, TBL and TPhL, respectively. Note that the applied concentrations of TPL (75 μM and 100 μM) were higher than those of TBL and TPhL (50 μM and 75μM), which evidences weaker efficacy of the former compound to induce RBC hemolysis. The erythrocyte concentration in the solution was 2%, while the concentration of ions used was 50 μM in each case. Such concentration did not caused RBC hemolysis greater than 5%. The presence of triorganoleads in 50 μM concentration (TBL and TPhL) or 75 μM (TPL) caused 60–70% hemolysis of RBC. The weakest hemolysis inducer was the less hydrophilic of the triorganotins, i. e., TPL. Its concentration had to be 100 μM to bring about 100% hemolysis while TBL and TPhL gave the same effect when used at 75 μM concentration. Sulfonate ions when present in the solution synergistically increased triorganoleads efficacy to induce RBC hemolysis. This increase was essentially the same for both sodium sulfonates used ($C_{12}H_{25}SO_3Na$ and $C_{14}H_{29}SO_3Na$). Once again TPhL was found to affect RBC less in comparison to TBL when both were used together with the $C_{12}H_{25}SO_3Na$ compound (see Table I). Quite an opposite effect was observed when inorganic ions (K^+, Mg^{2+}, and Al^{3+}) were present in the incubation solution. To a different degree, they minimalized the lytic toxicity of the organoleads. The best protection of erythrocytes was obtained with magnesium ions. Potassium ions were found to be slightly more effective than aluminium when

Table I. Influence of inorganic and organic ions on hemolysis of erythrocytes induced by tripropyllead chloride (TPT), tributyllead chloride (TBT) and triphenyllead chloride (TPhT).

		Percent hemolysis					
Compounds	Concentration [μM]	Control	K^+	Mg^{2+}	Al^{3+}	S_{12}	S_{14}
$(C_3H_7)_3PbCl$	75	70	58	44	65	100	100
	100	100	88	67	91	100	100
$(C_4H_9)_3PbCl$	50	76	64	54	65	100	100
	75	100	82	77	88	100	100
$(C_6H_5)_3PbCl$	50	62	69	37	54	85	100
	75	100	92	72	79	100	100

Concentration of ions was 50 μM, S_{12} – $C_{12}H_{25}SO_3^-$, S_{14} – $C_{14}H_{29}SO_3^-$.

hemolysing compounds were trialkylorganoleads, and the efficacy was reversed when triphenyllead was used.

The results obtained indicate that the ions studied modify the lipid phase in different ways enhancening or weakening the hemolytic action of the organoleads. It seems that the long hydrocarbon chain of sulfonates while incorporating into the lipid phase of the erythrocyte membrane disturbs its organization at the hydrophobic interior and thus may ease interaction of the organoleads with the membrane. Such an approach is justified in view of the observed differences between $C_{12}H_{25}SO_3^-$ and $C_{14}H_{29}SO_3^-$ ions (see Table I). Similar hydrocarbon chain dependence is commonly observed for interaction of various biologically active compounds with biological and model membranes (Kleszczyńska *et al.*, 2000; Sarapuk 2000). Additionally, they can partially screen a positive charge of phospholipid head groups and thus to ease interaction of the triorganoleads with the lipid part of erythrocyte membrane.

On the other hand, inorganic cations should localize at the polar part of lipid phase of RBC and modify the potential barrier at the lipid bilayer surface. The modification may be the result of the electrostatic interaction between cations and negative charges of polar lipid headgroups (e. g., phosphate groups of phospholipids) making this part of membrane to become less negative which do not facilitate the organoleads to interact with membrane so efficiently as in the absence of cations. This assumption seems to be valid since the observed differences in the protective efficacy depended on the valency of the cations used. Similar valency-dependent protective properties of some cations against hemolysis of RBC induced by organotin compounds were shown earlier (Kleszczyńska *et al.*, 1997). The best potential-barrier-modifying property of magnesium ions may be due to cross-linking of phospholipid molecules by those ions, an effect well known for calcium ions (Kuczera and Żyłka, 1979). The resulting stabilization of the lipid bilayer should protect RBC membranes against hemolysis by triorganoleads. Also, the membrane becomes partially inaccessible to them after adsorption of cations. The last assumption is important in the case of the aluminium cation and, to a lesser degree, the potassium action which has the highest rate of permeation across the RBC membrane (Makino *et al.*, 1983) due to its smallest charge.

The cations bound to the membrane partially neutralise its negative charges, as has been shown by measuring electrophoretic mobility of red cells in the presence of various cations (Bashford *et al.*, 1988). The result is a diminished electric interaction driving organoleads into the polar part of phospholipid phase of RBC membranes, which in turn makes them less efficient to hemolyse the membrane. Such a view has been confirmed by our fluorimetric investigations with the use of PE-fluorescein as a probe sensitive to membrane surface charge. The compounds studied were trialkyltins and the study showed that the cations caused the negative net charge of the erythrocyte membrane to decrease, with resulting weakening of the electrostatic interaction with those organometals (data not published) and thereby making it more difficult for them to incorporate into the RBC membrane. The sulphonate ions do not influence the potential barrier in such a degree as inorganic ions do, as they are, due to their long hydrocarbon chains, "sucked" deeply into the lipid bilayer.

Alder G. M., Arnold W. M., Bashford C. L., Drake A. F., Pasternak C. A. and Zimmermann U. (1991), Divalent cation-sensitive pores formed by natural and synthetic mellitin and by Triton X-100. Biochim. Biophys. Acta **1061**, 111–120.

Bashford C. L., Alder G. M., Menestrina G., Micklem K. J., Murphy J. J. and Pasternak Ch. A. (1986), Membrane damage by hemolytic viruses, toxins, complement, and other cytotoxic agents: a common mechanism blocked by divalent cations. J. Biol. Chem. **261**, 9300–9308.

Bashford C. L., Alder G. M., Graham J. M., Menestrina G. and Pasternak Ch. A. (1988), Ion modulation of membrane permeability: effect of cations on intact cells and on cells and phospholipid bilayers treated with pore-forming agents. J. Membrane Biol. **103**, 79–94.

Bashford C. L., Rodrigues L. and Pasternak Ch.A. (1989), Protection of cells against membrane damage by haemolytic agents: divalent cations and protons act at the extracellular side of the plasma membrane. Biochim. Biophys. Acta **983**, 56–64.

Boyer M. J., Horn I., Firestone R. A., Steele-Norwood D. and Tannock I. F. (1993), pH dependent cytotoxicity of N-dodecylimidazole: a compound that acquires detergent properties under acidic conditions. Br. J. Cancer **67**, 81–87.

Craig P. J. (1982), Environmental aspects of organometallic chemistry. In: Comprehensive Organometallic Chemistry. The Synthesis, Reactions and Structures of Organometallic Compounds (Wilkinson G., Gordon F., Stone A., Abel E. W., ed.). Pergamon Press, Oxford, Vol. **2**, pp. 979–1020.

Endres K. and Faulstich H. (1989), Triethyllead inhibits mitochondrial ATPase of the endosperm and sucrose uptake in the cotyledons of *Ricinus communis*. J. Plant Physiol. **133**, 531–536.

Hager A., Moser I. and Berthold W. (1989), Uncoupling of photophosphorylation by triethyllead (Et_3Pb^+) generated from tetraethyllead (Et_4Pb) in illuminated chloroplasts. J. Plant Physiol. **134**, 5–8.

Horiguchi Y., Uemura T., Kozaki S. and Sakaguchi G. (1986), Effects of Ca^{2+} and other cations on the action of *Clostridium perfringens* enterotoxin. Biochim. Biophys. Acta **889**, 65–71.

Kleszczyńska H., Bielecki K., Sarapuk J., Dziamska A. and Przestalski S. (2000), Influence of triphenyllead chloride on biological and model membranes. Z. Naturforsch. **55c**, 764–769.

Kleszczyńska H., Hadyszowski J., Pruchnik H. and Przestalski S. (1997), Erythrocyte hemolysis by organic tin and lead compounds Z. Naturforsch. **52c**, 65–69.

Kleszczyńska H., Sarapuk J. and Różycka-Roszak B. (2000), Modification of mechanical properties of model membranes by some bifunctional surfactants. Cell. Mol. Biol. Lett. **5**, 67–73.

Krug H. F. (1992), The toxic effects of organometals on the Lands cycle in HL-60 cells. Appl. Organomet. Chem. **6**, 297–304.

Kuczera J. and Żyłka R. (1979), Calcium ion binding to lecithin vesicles. Stud. Biophys. **75**, 25–33.

Kumari A., Tandon J. P. and Singh R. V. (1993), Antimicrobial effects of newly synthesized organotin (IV) and organolead (IV) derivatives. Appl. Organomet. Chem. **7**, 655–660.

Makino K., Shiga K., Arakawa M. and Kondo T. (1983), Effect of various ion on glycerol-induced hemolysis. Yakuzaigaku **43**, 1–5 (in English).

Park J. W., Jahng T. A., Rho H. W., Park B. H., Kim N. H. and Kim H. R. (1994), Inhibitory mechanism of Ca^{2+} on the hemolysis caused by *Vibrio vulnificus* cytolisin. Biochim. Biophys. Acta **1194**, 166–170.

Portlock S. H., Clague M. J. and Cherry R. J. (1990), Leakage of internal markers from erythrocytes and lipid vesicles induced by melittin, gramicidin S and alamethicin: a comparative study. Biochim. Biophys. Acta **1030**, 1–10.

Rudenko S. V. and Patelaros S. V. (1995), Cation-sensitive pore formation in rehydrated erythrocytes. Biochim. Biophys. Acta **1235**, 1–9.

Sarapuk J. (2000), Bifunctional surfactants – their potential application as pesticides. Int. Agrophysics **14**, 319–325.

Sarapuk J., Kleszczyńska H. and Przestalski S. (2000), Stability of model membranes in the presence of organotin compounds. Appl. Organomet. Chem. **14**, 40–47.

Song L. Y., Ahkong Q. F., Baldwin J. M., O'Reilly R. And Lucy J. A. (1993), Divalent cations, phospholipid asymmetry and osmotic swelling in electrically-induced lysis, cell fusion and giant cell formation with human erythrocytes. Biochim. Biophys. Acta **1148**, 30–38.

Stasiuk M. and Kozubek A. (1996): Modulation of hemolytic properties of resorcinolic lipids by divalent cations. Cell. Mol. Biol. Lett. **1**, 189–198.

Thayer J. S. (1974), Organometallic compounds and living organisms. J. Organomet. Chem. **76**, 265–295.

Trela Z., Radecki J. and Przestalski S. (1997), Effect of triphenyllead on the resting potential and electrical conductance of Nitellopsis obtusa membrane. Polish J. Environ. Studies **6**, 37–40.

Zimmermann H. P., Plagens U. and Traub P. (1987), Influence of triethyl lead on neu-rofilaments *in vivo* and *in vitro*. NeuroToxicol. **8**, 569–578.

Zimmermann H. P., Faulstich H., Hansch G. M., Doenges K. H. and Stournaras C. (1988), The interaction of triethyl lead with tubulin and microtubules. Mutation Res. **201**, 293–302.