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Molecular mechanism of haemolysis induced by triphenyltin chloride

Květoslava Burda[1]*, Janusz Lekki[1], Jakub Cieślak[2], Jerzy Kruk[3], Małgorzata Lekka[1], Stanisław Dubiel[2], Jan Stanek[4] and Zbigniew Stachura[1]

[1]Institute of Nuclear Physics, ul. Radzikowskiego 152, 31-342 Kraków, Poland
[2]Faculty of Physics and Nuclear Techniques, The University of Mining and Metallurgy (AGH), al. Mickiewicza 30, 30-059 Kraków, Poland
[3]Institute of Molecular Biology, Jagiellonian University, al. Mickiewicza 3, 31-120 Kraków, Poland
[4]Institute of Physics, Jagiellonian University, ul. Reymonta 4, 31-059 Kraków, Poland

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Organometals are known to cause lysis of cells, but the molecular mechanism of their action is not recognized. In this work, we have examined the interaction of triphenyltin with erythrocyte membranes. We determined the order of haemolytic activity of the investigated organometal species as being: triphenyllead > tripropyltin = triphenyltin > triethyllead > trimethyltin. Such an order suggests that the haemolytic activity increases with the increasing hydrophobicity of the organic ligands. Compounds containing lead are more toxic than the respective complexes of tin. Triphenyltin chloride (Ph_3SnCl) is very effective in lysis of erythrocytes. Using ^{119}Sn Mössbauer spectroscopy we showed that triphenyltin interacts with the protein components of pig erythrocyte membranes in a highly specific way, but we did not detect any interaction of triphenyltin with pig haemoglobin. The Mössbauer spectrum was fitted with a single doublet characterized by hyperfine parameters that differ considerably from those reported for other organotin compounds in membranes of red blood cells. Applying the point charge model of the electric field gradient for the analysis of the environment of tin bonds from the quadrupole splitting, we could indicate N_{het} from histidine and/or S_{thiol} from cysteine as the only possible ligands of Ph_3Sn(IV). We expect that protein components of erythrocyte membranes having similar cysteine and histidine arrangement, such as in cat or rat haemoglobins, which provide high-affinity binding sites for organotins, can bind triphenyltin with high affinity. We give some arguments that ankyrin and β-spectrin are the most probable targets of Ph_3Sn(IV) action and indicate its potential binding sites within the proteins. The highly specific interaction of triphenyltin with the membrane cytoskeleton components, postulated by us, should already influence the rigidity of red blood cells at the stage preceding the lysis of erythrocytes. To support this hypothesis, we carried out scanning force microscopy measurements of red blood cells elasticity. We have observed a lower stiffness for erythrocytes treated with concentrations of Ph_3SnCl that caused less than 20% of haemolysis.

KEYWORDS: erythrocyte; haemolysis; triphenyltin; Mössbauer spectroscopy; scanning force microscopy

INTRODUCTION

Among the organometallic compounds, organotins and organoleads have been found as the most common toxicants for living organisms. They leak into the environment mainly as a result of the widespread use of various chemicals, especially biocides.[1] They are accumulated in plants and animals, leading to their poisoning.[2] The organotin and organolead compounds are easily soluble in the lipid fraction of cell membranes. The degree and location of cell disruption by the organometals depends on the chemical structure of the toxicant, *viz*. the length of the alkyl chain, its hydrophobicity and the metal cation. Usually, organoleads and organotins lead to the lysis of cells, but the molecular basis of this process is not yet resolved. It is known that these

*Correspondence to: K. Burda, Fakultät für Biologie, Lehrstuhl Zellphysiologie, Universität Bielefeld, 33615 Bielefeld, Germany.
E-mail: kveta.burda@biologie.uni-bielefeld.de
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compounds affect various biochemical processes. For example, organoleads cause the liberation of arachidonic acid from biological membranes,[3] organotins mediate chloride–hydroxide exchange across membranes[4,5] and inhibit the adenosine triphosphate synthase (ATPase) complex in mitochondria[6] and chloroplasts.[7]

In this work, we studied the process of haemolysis induced by triphenyltin chloride (Ph_3SnCl) and tried to explain its high haemolytic activity at the molecular level. We also compared the haemolytic activity of triphenyltin with other organometalic species. The chemical surroundings of triphenyltin within erythrocyte membranes were examined using ^{119}Sn Mössbauer spectroscopy. The hyperfine parameters of the Mössbauer spectra indicate a highly specific binding site for tin within the protein fraction of membranes. A comparison of the elasticity of red blood cells treated with various concentrations of organotin compounds with the elasticity of the untreated cells confirmed our hypothesis, that some proteins forming the membrane cytoskeleton of erythrocytes provide binding sites for triphenyltin. Measurements performed by scanning force microscopy showed that only triphenyltin, at very low concentrations, influenced the rigidity of the erythrocyte.

We believe that the analysis presented here may be applicable to the investigations of the lysis process for a wider class of cells, because many structural and functional properties of erythrocyte membranes are similar to many other types of cell.[8]

MATERIALS AND METHODS

Erythrocytes were prepared from fresh hog blood (containing 3.8% citric acid as an anti-coagulant). The blood was centrifuged (1000*g*, 4 °C) for 15 min and plasma was removed. Precipitated cells were suspended in 5 mM phosphate buffer pH 7.4 with 0.15 M NaCl. This suspension was centrifuged (1000*g*, 4 °C) and the supernatant was removed. This procedure was repeated three times. Finally, the washed erythrocytes were suspended in the phosphate buffer with NaCl at a cell concentration of 2×10^9 red blood cells per millilitre. The suspension of washed erythrocytes was divided into several parts of 3 ml volume each. These suspensions were then incubated at 37 °C in the presence of various concentrations of the organometallic species for 30 and 60 min: trimethyltin chloride (Me_3SnCl), tri-*n*-propyltin chloride (Pr_3SnCl) and Ph_3SnCl for 30 min; triethyllead chloride (Et_3PbCl) and triphenyllead chloride (Ph_3PbCl). All the chemicals were from Alf GmbH. The compounds were added from ethanol stock solutions (except for trimethyltin) in such amounts that the final concentrations in the samples were as follows: 66, 132, 270 and 430 μM of Me_3SnCl; 33, 66, 132, 263 and 424 μM of Pr_3SnCl; 17, 33, 66, 132, 260 and 506 μM of Ph_3SnCl; 17, 33, 66, 131, 260 and 390 μM of Et_3PbCl; 8.3, 17, 33, 66, 130 and 320 μM of Ph_3PbCl. We have also tested for possible haemolysis caused by ethanol. In the case of concentrations of organometals up to 130 μM, the concentration of ethanol in samples did not exceed 1% and we did not observe haemolysis in a blank test for ethanol. At the highest applied concentration of organotin (506 μM) the ethanol concentration reached 4% in a sample. At this concentration the ethanol caused less than 20% of haemolysis in a control sample incubated at 37 °C for 30 min. Afterwards, the suspensions of red blood cells were centrifuged at 1000*g* for 15 min. The sediments were washed several times with 5 mM phosphate buffer with 0.15 M NaCl until no more haemoglobin was detected in the supernatant.

The degree of haemolysis was estimated from measurements of haemoglobin absorbance at 546 nm in the respective supernatants using an SLM Aminco DW2000 spectrophotometer.

For Mössbauer experiments we used erythrocytes (8×10^9 red blood cells per millilitre) treated with Ph_3SnCl (2.86 mM) or Me_3SnCl (3.33 mM) containing ^{119}Sn in natural abundance. The total initial volume of each sample was 10 ml. After haemolysis we centrifuged the samples and washed the sediments several times (see above), collecting the supernatants and sediments separately. For the experiments we lyophilized the fractions. The transmission ^{119}Sn Mössbauer spectra were recorded at 80 K in a continuous flow cryostat (CF506, Oxford Instruments). The temperature stability was within 0.1 K. The source of 23.9 keV gamma radiation was $Ca^{119m}SnO_3$ (1 mCi) kept at room temperature. The very weak effect of the resonant absorption in the case of samples containing erythrocyte membranes, *ca* 0.1%, required 10 days, resulting in final statistics of 73.4×10^6 counts per channel.

The elasticity of the intact red blood cells and cells treated with various toxicants, which contained about 9×10^9 red blood cells per millilitre, was measured using a home-built scanning force microscope (SFM) working in contact mode.[9] Commercial silicon nitride cantilevers were used as a probe, with a spring constant of 0.01 N m^{-1} (Atos GmbH, Germany). The tip radii were determined with a TGT01 silicon standard (NMDT, Russia) to be about 20–30 nm. In these measurements, a drop of precipitated erythrocytes, prepared in accordance with the procedure described above, was put on a glass covered with 0.015% poly-L-lysine (Sigma–Aldrich). The measurements were performed at room temperature in 5 mM phosphate buffer pH 7.4 containing 0.15 M NaCl. A separate force calibration was always performed for each series of curves. As a calibration reference, a glass plate was used. Its stiffness was assumed to be infinite in the range of applied loads (up to about 5 nN).

RESULTS

Haemolytic activity

The haemolytic activities of the organometallic compounds at various concentrations and two different incubation times are shown in Fig. 1. It can be seen that, for all cases, the

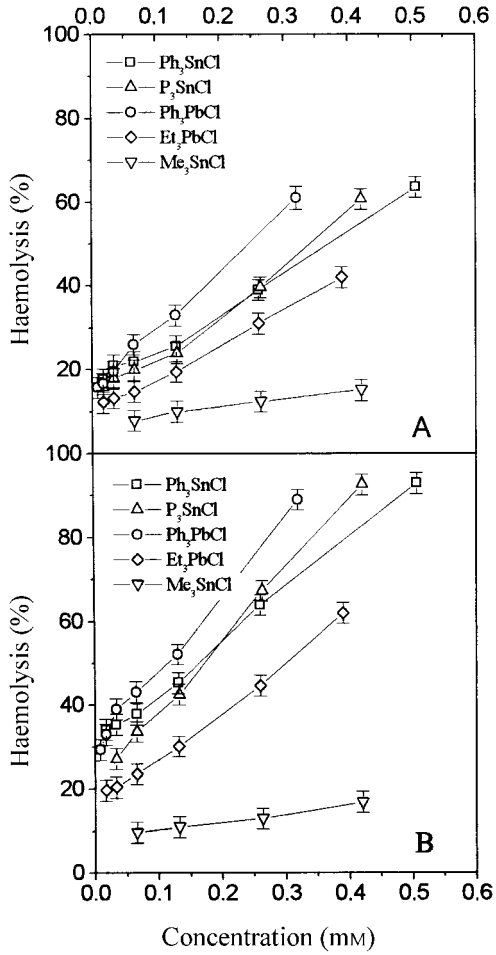

Figure 1. The haemolytic activity of organotins and organoleads as a function of their applied concentrations for 30 min (A) and 60 min (B) of incubation at 37 °C.

degree of haemolysis increases with the time of incubation. The highest haemolytic effect was observed for Ph_3PbCl. Pr_3SnCl and Ph_3SnCl exhibited similar haemolytic activities up to a concentration of 0.25 mM. At higher concentrations, the Pr_3SnCl was more efficient in haemolysis than Ph_3SnCl, especially for longer times of incubation. The lowest haemolytic activity was observed for Me_3SnCl. Et_3PbCl exhibited much lower effectiveness in haemolysis than Ph_3PbCl, Ph_3SnCl and Pr_3SnCl. This was even more pronounced for samples incubated for 60 min. However, Et_3PbCl had significantly higher haemolytic activity than Me_3SnCl for both incubation times.

To summarize, haemolytic activity increased with the size of the hydrophobic moiety of the organotin and organolead compounds. For the same organic ligation of metal, compounds containing lead cations were more effective than the corresponding tin compounds in the process of lysis, which is evident for triphenyltin and triphenyllead.

Mössbauer studies

We measured Mössbauer spectra of red blood cells treated with Ph_3SnCl at a concentration exhibiting 80% haemolytic activity, and Me_3SnCl at a concentration exhibiting 20% haemolytic activity. All spectra of organotin chlorides, as well as of the lyophilized fractions of the supernatants containing haemoglobin and sediments containing erythrocyte membranes, were fitted by single doublets. Increasing the number of fitted lines did not improve the quality of fits. The corresponding isomer shift δ quadruple splitting ΔE, and the line-width Γ of the least-squares fits are given in Table 1. In Fig. 2 we show the spectra of Ph_3SnCl and triphenyltin bound to erythrocyte membranes. The solid lines in Fig. 2 represent the theoretical curves.

It can be seen from Table 1 that most of the Me_3SnCl remained in the supernatant. On the contrary, Ph_3Sn(IV) was detected only in the sediment. Comparison of the hyperfine parameters of the solid compounds with the organotins within the lyophilized fractions of partially haemolysed red blood cells showed that trimethyltin did not interact specifically with erythrocytes, whereas triphenyltin reacted in a highly specific way with erythrocyte membranes.

The Mössbauer hyperfine parameters, namely isomer shift and quadrupole splitting, which are related to the electron density and the electric field gradient (EFG) at the nucleus respectively, give an insight into the structure, reactivity and mechanism of transformation of organotin compounds.[10,11] Using the concept of partial quadrupole splitting (p.q.s.) produced by a specified ligand,[12] some qualitative informa-

Table 1. ^{119}Sn Mössabuer parameters of organotin(IV) in lyophilized pig erythrocyte systems. Measurements were performed at 80 K. In all cases a single doublet has been fitted to the experimental data[a]

Organotin system	δ (mm s^{-1})	ΔE (mm s^{-1})	Γ (mm s^{-1})
Me_3SnCl (solid)	1.46 ± 0.01	3.48 ± 0.01	1.56 ± 0.02
Me_3SnCl in the supernatant of erythrocytes haemolysed 20%	1.28 ± 0.01	3.40 ± 0.01	1.24 ± 0.01
Me_3SnCl in the sediment of erythrocytes haemolysed 20%		Not observed	
Ph_3SnCl (solid)	1.34 ± 0.01	2.58 ± 0.01	1.22 ± 0.01
Ph_3SnCl in the supernatant of erythrocytes haemolysed 80%		Not observed	
Ph_3SnCl in the sediment of erythrocytes haemolysed 80%	1.25 ± 0.01	1.74 ± 0.01	1.56 ± 0.01

[a] δ: isomer shift with respect to $CaSnO_3$ at room temperature; ΔE: quadrupole splitting; Γ: full width at half-height of the resonance peak.

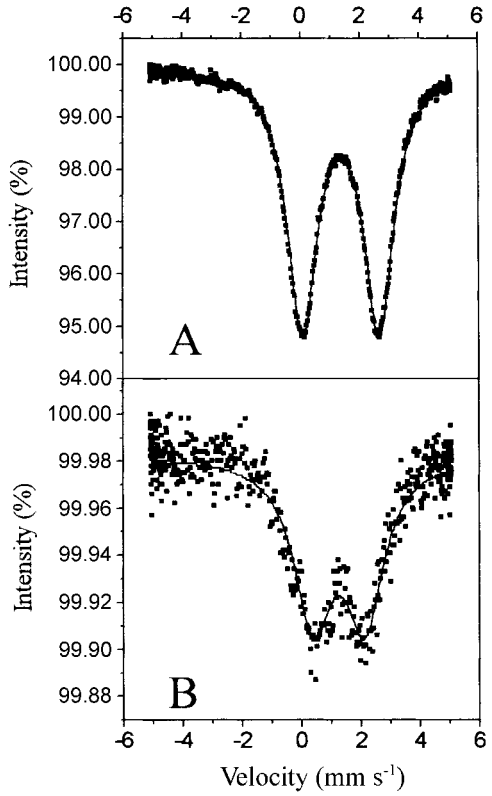

Figure 2. Mössbauer spectra of Ph_3SnCl (A) and triphenyltin bound to erythrocyte membranes (B). The solid lines represent fitted curves.

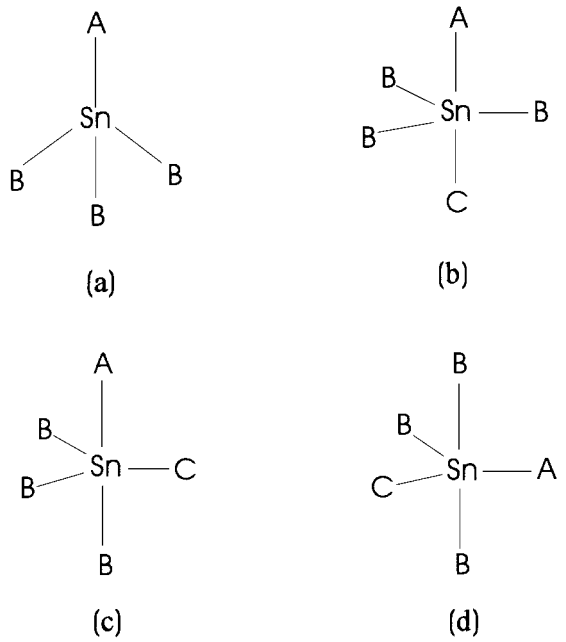

Figure 3. The possible structures of four-coordinate $SnAB_3$ (a) and isomers of five-coordinate $SnACB_3$; a trigonal bipyramidal arrangement of ligands with A and C as trans ligands (b), with A as trans ligand and C as cis ligand (c), and with A and C as cis ligands (d).

tion about the nearest neighbourhood of tin(IV) can be obtained. The applied point charge model of the EFG, restricted to the contribution from the nearest neighbours, leads to the conclusion that a certain ligand always gives the same contribution to the quadrupole splitting, or, more precisely, to the total EFG tensor, independently of the other ligands. For estimation of the quadrupole splitting, which is the measurable quantity, the complete EFG tensor was constructed for the expected four- and five-coordinate complexes of the organotins investigated. The possible isomers of $SnACB_3$ and $SnAB_3$ complexes are shown in Fig. 3, where B represents an organic ligand, and A and C are two other ligands (for example halides). We used the p.q.s. values for various ligands bound to tin(IV) at different types of environment.[13,14] The calculated values of the quadrupole splitting are given in Table 2. A comparison of these values with those obtained experimentally shows that the solid Ph_3SnCl has a four-coordinate structure and solid Me_3SnCl has a five-coordinate structure. Me_3SnCl is an example of a compound having an associated structure in the solid state,[12] whereas Ph_3SnCl has no associated structures. The experimental ΔE values for the solid chloride of methyltin are slightly higher than the theoretical values. This is probably due to small changes in angles and lengths of the Sn—Cl bonds. The isomer shift and the quadrupole splitting estimated for trimethyltin in lyophilized supernatant are slightly smaller than the hyperfine parameters for the solid compound; this is perhaps due to structural changes of bonds, which lead to some decrease in the effective number of 5s electrons within the nucleus.

The hyperfine parameters of Ph_3Sn(IV) in erythrocyte membranes were measured for the first time. As can be seen from Table 2, there are a few candidates for organotin complexes for which the theoretical values of quadrupole splitting would match the experimental ΔE. These are: tetrahedral coordination of tin with an axial ligand of imidazole nitrogen N_{het} (from histidine) or of hydroxyl; trigonal bipyramidal structure with two axial ligands of S_{thiol} (from cysteine) and N_{het}/N_{am} (amino group); five-coordinate complexes with N_{het} and hydroxyl to form axial and equatorial ligands of mixed or homogeneous character. In fact, we can exclude hydroxyl ligation of tin, since it is known that for such compounds the isomer shift does not exceed 1 mm s^{-1}.[15,16] Comparing this value with the value of the experimental isomer shift of 1.25 mm s^{-1}, it can be seen that only structures containing heterocyclic nitrogen or/and sulfur are possible ligands of tin in the systems investigated.

SFM investigations

Using scanning force microscopy, we measured the elasticity

Table 2. Calculated quadrupole splittings for four-coordinate $SnAB_3$ and five-coordinate $SnACB_3$ complexes[a]

Complex	Ligands	Calculated ΔE (mm s^{-1})
	Tetrahedral structures (Fig. 3a)	
Ph_3SnA	A = Cl	2.52
	$A = N_{het}$	1.60
	$A = S_{thiol}$	1.54
	A = OH	1.72
Me_3SnA	A = Cl	2.74
	A = OH	1.94
	Trigonal bipyramidal	
	Both ligands A and C are axial (Fig. 3b)	
Me_3SnAC	A = C = Cl	3.39
	A = Cl, C = OH	3.13
Ph_3SnAC	A = C = Cl	2.94
	A = Cl, C = OH	2.68
	A = C = OH	2.42
	$A = C = N_{am}$	2.98
	$A = C = N_{het}$	2.74
	$A = C = S_{thiol}$	0.56
	$A = S_{thiol}$, $C = N_{het}$	1.65
	Ligand A is axial and ligand C is equatorial (Fig. 3c)	
Ph_3SnAC	A = Cl, $C = N_{het}$	1.92
	$A = N_{het}$, C = Cl	2.05
	$A = C = N_{het}$	1.92
	$A = C = N_{am}$	2.06
	$A = C = S_{thiol}$	0.78
	$A = N_{het}$, $C = S_{thiol}$	0.95
	$A = S_{thiol}$, $C = N_{het}$	2.23
	A = C = OH	1.73
	A = OH, $C = N_{het}$	1.93
	$A = N_{het}$, C = OH	1.73
	$A = N_{am}$, C = OH	1.74
	A = OH, $C = N_{am}$	2.08
	$A = S_{thiol}$, C = OH	2.01
	A = OH, $C = S_{thiol}$	0.83
	Both ligands A and C are equatorial (Fig. 3d)	
Ph_3SnAC	$A = C = H_{het}$	3.42
	$A = C = S_{thiol}$	1.53
	A = C = OH	3.14
	A = C = Cl	3.61

[a] het: heterocyclic nitrogen from histidine; thiol: thiolate from cysteine; am: nitrogen from amino group.

of erythrocytes under natural conditions. We calculated the Young's modulus, characterizing the cell stiffness[9,17] in the frame of the Sneddon model for interaction, describing the case of the elastic half-space pushed by a hard axisymmetric indenter.[18] SFM tips, used in the measurements, were four-sided-shaped pyramids. Their shape could be approximated by a paraboloid or cone.

For micromolar concentrations of toxicants we did not observe any significant differences between the elasticity of

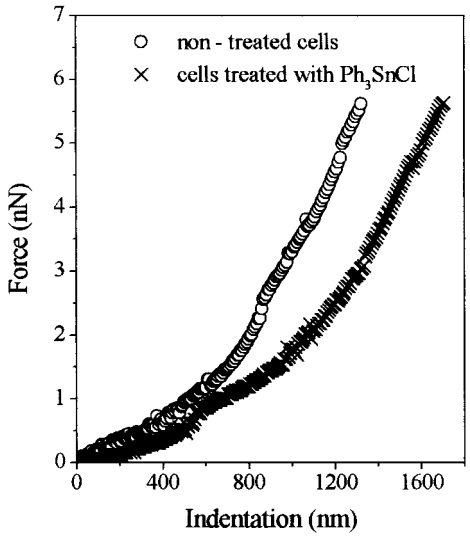

Figure 4. Typical force versus indentation curves taken for intact erythrocytes and erythrocytes incubated with 33 μM Ph_3SnCl at 37 °C for 30 min.

intact erythrocytes and erythrocytes treated with organometals. However, we found an apparent change of Young's modulus for red blood cells treated with 33 μM Ph_3SnCl (30 min incubation at 37 °C), which caused less than 20% of haemolysis (Fig. 1A). Figure 4 shows the force curves of intact erythrocytes and those influenced by Ph_3SnCl but not haemolysed. The data were collected in two independent measurements of 16 cells (ten force curves were collected for each cell). The Young's modulus of 10.1 ± 2.1 kPa for intact red blood cells falls to 4.3 ± 1.0 kPa for cells treated with Ph_3SnCl. This means that the treated erythrocytes are less stiff than the intact cells.

DISCUSSION

We can arrange the organoleads and organotins according to their haemolytic activity: triphenyllead > tripropyltin = triphenyltin > triethyllead > trimethyltin. In the homologous series, the toxicity increases with the hydrophobicity of the organic ligands. Compounds containing lead are always more effective in haemolysis than the corresponding compounds with tin. These results are in agreement with earlier reports.[19]

The distribution of Me_3SnCl and Ph_3SnCl in erythrocyte systems can be explained by the hydrophilic nature of trimethyltin (which is responsible for its relatively poor haemolytic activity) and the hydrophobic character of triphenyltin and tripropyltin (which shows a highly haemolytic action). This, in turn, suggests that the high-affinity binding site of triphenyltin is located in a very hydrophobic region of the erythrocyte membrane systems. Despite the fact that Ph_3SnCl caused 80% of haemolysis, no tin was

detected in the supernatant. This observation allowed us to conclude that pig haemoglobin has no binding sites for organotins. So far, only cat and rat haemoglobins have been found to bind organotins.[14,20,21] The S_{thiol} cysteine and N_{het} histidine were suggested to be highly specific ligands of organic compounds of tin.[14,20,21]

In our studies, the high-affinity binding site of triphenyltin, characterized by the same hyperfine parameters as those found for cat and rat haemoglobins or rat mitochondrial ATPase,[6,22] is provided by components of the erythrocyte membrane. As far as we know, such a highly specific interaction of organotins with membrane components has not previously been observed.[23] The hydrophobic triphenyltin easily penetrates the membrane and can access its inner part. Therefore, it may interact with the integral proteins as well as with the cytoskeleton proteins. The broader linewidth of our spectrum, compared with the natural one, supports the idea that the ligation of triphenyltin with erythrocyte membrane has a non-homogeneous character. This means that there are more binding sites within the protein components of erythrocyte membranes, giving perhaps more than one binding site for Ph_3Sn(IV). The high-affinity binding sites of triphenyltin can have a trigonal bipyramidal structure with two axial ligands of S_{thiol} (from cysteine) and H_{het} (from histidine), as in the case of rat and cat haemoglobins, and/or a five- or four-coordinate structure only with the heterocyclic nitrogen of histidine, as was observed in the case of ATPase in rat mitochondrial membrane. We suggest that several cytoskeletal proteins and integral membrane proteins are potential targets of triphenyltin action. For example, it is well known that ankyrin 1, present in red blood cells, is formed by 24 tandemly organized repeats, each of them containing 33 amino acids.[24] We have performed an analysis of the amino acid sequence and the tertiary structure of erythroid and non-erythroid ankyrins based on the data in the Protein Data Bank. We found that the following amino acid sequence conserved within the ankyrin repeats is one of the possible binding sites of triphenyltin:

...GSTPLHIA**C**KKN

*HVRVMELLLKTGASIDAVTESGLTPLHVASFMG***H**LPIVK...

The tertiary structure of the amino acids given provides a similar arrangement of histidine and cysteine residues to the cat (rat) haemoglobin. The underlined, bold letters indicate cysteine (C) and histidine (H) residues that are probably ligated to tin. β-Spectrin, which is a very important cytoskeleton protein, is also a highly probable candidate that can provide the binding site for the triphenyltin. It is composed of about 18 repeats, each of them containing 106 amino acids organized in a triple helical structure.[25] A possible binding site, analogous to the haemoglobin one, can be located within the structure made of:

LW**C**QMKTAGYPNVNIHN FTTS

*WRDGMAFNALIHK***H**RP

This amino acid sequence is conserved in β-spectrins.

Ankyrin has binding sites for cytoskeleton proteins. It provides a high-affinity linkage of the spectrin–actin networks to the inner surface of the plasma membrane. One ankyrin molecule is bound to one β-subunit of the spectrin heterotetramer. Erythrocyte ankyrin has two binding sites for protein band 3. It induces oligomerization of protein band 3.[26] Erythrocyte ankyrin also binds (Na,K)-ATPase.[24,27] The disruption of the binding between Na,K-ATPase and ankyrin may cause inhibition of ATP synthesis. The interaction of Ph_3Sn(IV) with ankyrin, spectrin and/or band 3 protein can explain in a consistent way the lower stiffness of red blood cells treated with the toxicant.

We observed a decrease of the Young's modulus of modified erythrocytes at a 33 μM concentration of Ph_3SnCl. We did not detect any change in the elasticity of cells treated with such low concentrations of Me_3SnCl or even Pr_3SnCl, the latter in our studies exhibiting a similar haemolytic activity to triphenyltin. This suggests that triphenyltin interacts with components of erythrocyte membranes in a different way than tripropyltin. The concentration of Ph_3SnCl applied was almost stoichiometric (10^7 Ph_3Sn per cell, before the sample was washed out several times) in relation to the protein band 3 (about 10^6 copies per cell), ankyrin (about 10^5 copies per cell) or β-spectrin (about 10^5 copies per cell), assuming more than one binding site per molecule in the two latter cases.

The cytoskeleton proteins are very important for maintaining the integrity of the erythrocyte membrane and their modification can lead to changes in the erythrocyte stiffness. There are two possible mechanisms that could modify the rigidity of the cells: an increase of the tension in the inner lipid monolayer or/and the reorganization of the spectrin–actin network and its linkage to the membrane. Under normal conditions, the entire bilayer is underlaid with a cytoskeleton.[28] In the intact cell, the skeleton is expanded by means of its interactions with the membrane bilayer,[29] but under non-physiological conditions, the area of the isolated red blood cell membrane skeleton decreases.[30–32] Hence, the lateral tension and the elastic energy of the skeleton are decreased after a partial detachment of the lipid bilayer. In our case, the interaction of triphenyltin with ankyrin or β-spectrin could be responsible for the observed changes of erythrocyte rigidity. Our results are in accordance with the knowledge that the abnormal or deficient membrane skeletal proteins responsible for the haemolytic anaemias in humans and mice lead to major defects in erythrocyte shape and mechanical stability.[33]

Summarizing, we have shown, using ^{119}Sn Mössbauer spectroscopy, that triphenyltin interacts in a highly specific

way with protein components of pig erythrocyte membranes, but not with pig haemoglobin. We suggest that some components of the cytoskeleton provide a high-affinity binding site for the toxicant. This idea is supported by measurements of the elasticity of red blood cells, which showed lower stiffness only in the case of treatment with low concentrations of Ph_3SnCl causing less than 20% of haemolysis.

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REFERENCES

1. Attar KM. *Appl. Organomet. Chem.* 1996; **10**: 317.
2. Thayer JS. *J. Organomet. Chem.* 1974; **76**: 265.
3. Krug HF. *Appl. Organomet. Chem.* 1992; **6**: 297.
4. Motais R, Cousin JL and Sola F. *Biochim. Biophys. Acta* 1977; **467**: 357.
5. Selwyn MJ, Dawson AP, Stockdale M and Gains N. *Eur. J. Biochem.* 1970; **4**: 120.
6. Farrow BG and Dawson AP. *Eur. J. Biochem.* 1978; **86**: 85.
7. Gould JM. *FEBS Lett.* 1978; **94**: 90.
8. Bennet V. *Physiol. Rev.* 1990; **70**: 1029.
9. Lekka M, Laidler P, Gil D, Lekki J, Stachura Z and Hrynkiewicz AZ. *Eur. Biophys. J.* 1999; **28**: 312.
10. Parish RV. In *Mössbauer Spectroscopy Applied to Inorganic Chemistry*, vol. 1, Long GJ (ed.). Plenum: New York, 1984; 527–575.
11. Burda K, Hrynkiewicz A, Kołoczek A, Stanek J and Strzałka K. *Hyperfia. Interact.* 1994; **91**: 891.
12. Parish RV and Platt RH. *Inorg. Chim. Acta* 1970; **4**: 65.
13. Bancroft GM, Das VGK, Sham TK and Clark GM. *J. Chem. Soc. Dalton Trans.* 1976: 643.
14. Barbieri R and Musmeci MT. *J. Inorg. Biochem.* 1988; **32**: 89.
15. Herber RH, Stöcker HA and Reiche WT. *J. Chem. Phys.* 1965; **42**: 2447.
16. Zuckerman JJ. Applications of ^{119m}Sn Mössbauer spectroscopy to the study of organotin compounds. In *Advances in Organometallic Chemistry*, Stone FGA, West R (eds). Academic Press: New York, London, 1970; 22–134.
17. Weisenhorn A, Khorsandi M, Kasas S, Gotzos V and Butt HJ. *Nanotechnology* 1993; **4**: 106.
18. Sneddon IN. *Int. J. Eng. Sci.* 1965; **3**: 47.
19. Kleszczyńska H, Hładyszowski J, Pruchnik H and Przestalski S. *Z. Naturforsch. Teil C* 1997; **52**: 65.
20. Elliot BM, Aldridge WN and Bridges JW. *Biochem. J.* 1979; **177**: 461.
21. Siebenlist KR and Taketa F. *Biochem. J.* 1986; **233**: 471.
22. Dawson AP, Farrow BG and Selwyn MJ. *Biochem. J.* 1982; **202**: 163.
23. Musmeci MT, Madonia G, Giudice MTL, Silvestri A, Ruisi G and Barbieri R. *Appl. Organomet. Chem.* 1992; **6**: 127.
24. Lux SE, John KM and Bennet V. *Nature* 1990; **344**: 36.
25. Speicher DW and Marchesi VT. *Lett. Nature* 1984; **311**: 177.
26. Hall TG and Bennett V. *J. Biol. Chem.* 1987; **262**: 10537.
27. Devarajan P, Scaramuzzino DA and Morrow JS. *Proc. Natl. Acad. Sci. U.S.A.* 1994; **91**: 2965.
28. Liu SC, Derick LH, Duquette MA and Palek J. *Eur. J. Cell Biol.* 1989; **49**: 358.
29. Steck TL. Red cell shape. In *Cell Shape: Determinants, Regulation and Regulatory Role*, Stein W, Bronner F (eds). Academic Press: New York, 1989; 205–246.
30. Lange Y, Hadesman RA and Steck TL. *J. Cell Biol.* 1982; **92**: 714.
31. Svoboda K, Schmidt CF, Branton D and Block SM. *Biophys. J.* 1992; **63**: 784.
32. Hägerstrand H, Danieluk M, Bobrowska-Hägerstrand M, Iglic A, Wróbel A, Isomaa B and Nikinmaa M. *Biochim. Biophys. Acta* 2000; **1466**: 125.
33. Bennet V. *Annu. Rev. Biochem.* 1985; **54**: 273.

