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# PEPTIDES CONFORMATIONAL CHANGES OF THE ERYTHROCYTE MEMBRANE INDUCED BY ORGANOMETALLIC TIN COMPOUNDS

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**The paper presents the results of a study on the effect of selected organic chlorides of tin on peptide conformations of erythrocyte ghosts from pig blood. The following compounds were used: dibutyltin dichloride (DBT), tributyltin chloride (TBT), diphenyltin dichloride (DPhT) and triphenyltin chloride (TPhT). Peptide conformation changes were determined on the basis of measurements done with the ATR FTIR technique. This method made it possible to measure the percent share of a peptide with specified conformation in the whole amount of the peptides in the membranes studied. The investigation showed that all the tin organic compounds studied cause a several-percent decrease in the quantities of both the peptides with the α-helix and turn conformation, and about a 20% increase in ghost peptides with β-sheet conformation. . It seems that the changes observed can cause disturbances in the function of proteins and, consequently, the activity of the membrane; and this may be one of the aspects of the toxic properties of organotins.**

## INTRODUCTION

The toxic properties of organic tin compounds are well known and documented in numerous papers e.g. Hoch et al., (2001); Craig et al., (2003); Antizar-Ladislao et al., (2008); Pelerito et al., (2006). However, as indicated by Buck-Koehntop et al. in a review Buck-Koehntop, (2006), the number of papers on organic tin compounds (OTC) interaction with biological macromolecules is relatively modest. Of those only a few concentrate on the possible molecular mechanisms of OTC toxicity. It was been shown, in particular, that OTC compounds reduce the stability of lipid membranes Rozycka-Roszak and Pruchnik, (2001), Chicano et al., (2001), induce hemolysis Kleszczyńska et al., (1997), and alter $Na^+/K^+$-ATPase activity Przestalski et al., (2000) activity. One possible mechanism of OTC-protein interaction is the formation of bonds between Sn (IV) atoms and thiol groups Stridh et al., (2001). The present work contains the results of an investigation into the effects of the four selected OTC on erythrocyte proteins. Comparing the data on toxicological activity presented in the above citations, the compounds chosen for the study belong to the most toxic organotin chlorides. The aim of the work was determine the changes caused in ghost proteins by the compounds, and an attempt to describing the mechanisms of the interaction on the basis of the results obtained from ATR-FTIR spectroscopy. The incentive for undertaking the study was the supposition that one of the reasons for OTC toxicity was disturbance in the active membrane functions via an effect on membrane protein conformation.

## MATERIALS

The tin compounds used - dibutyltin dichloride $(C_4H_9)_2SnCl_2$-DBT, tributyltin chloride $(C_4H_9)_3SnCl$ – TBT, diphenyltin dichloride $(C_6H_5)_2SnCl_2$ – DPhT, and triphenyltin chloride $(C_6H_5)_3SnCl$ – TPhT - were obtained from Alfa Aesar Johnson Matthey GmbH (Germany) and ABCR (Germany). The tin compounds and all other chemicals used in the present work were of analytical grade and the water used for solutions was doubly distilled.

## METHODS

The experiments were done with pig erythrocytes. Fresh heparinised pig blood was centrifuged for 3 min at 1000×g, the plasma removed and the cells washed twice with isotonic phosphate buffer solution (131 mM NaCl, 1.79 mM KCl, 0.86 mM $MgCl_2$, 11.80 mM $Na_2HPO_4{\cdot}2H_2O$, 1.80 mM $Na_2HPO_4{\cdot}H_2O$) of pH 7.4. Cell ghosts were obtained using the Dodge et al. method Dodge et al., (1963). The washed erythrocyte cells at 60% hematocrit were diluted to get 4% hematocrit and 20 mOsm suspension, then incubated for 1 hour. After incubation the hemolisat was centrifuged to removed

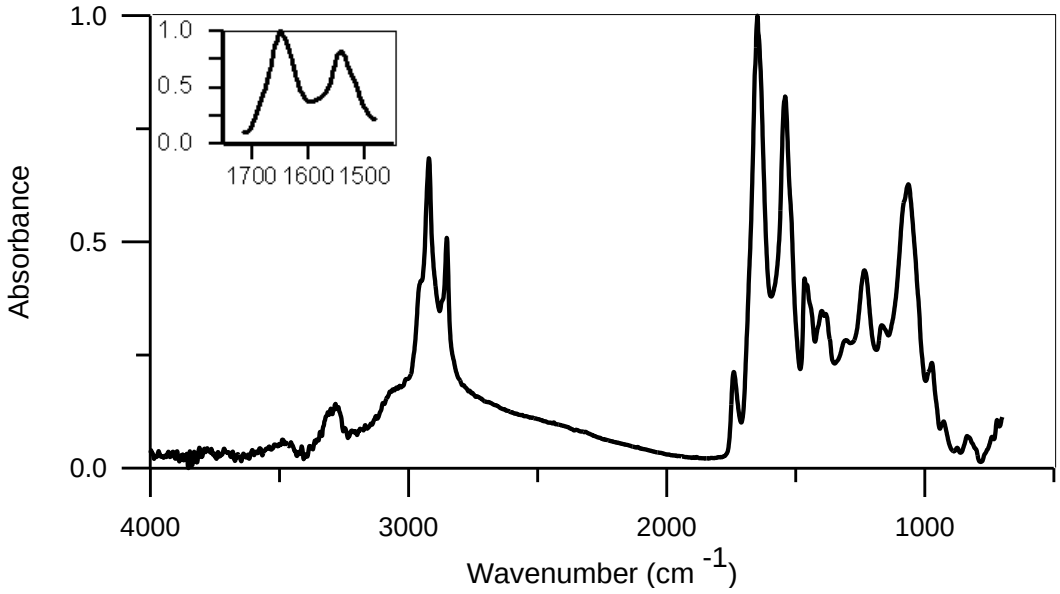


Fig. 1. A representative spectrum of unmodified ghosts after preliminary treatment. The insert shows the amide I and II band range (1750 $cm^{-1}$ – 1450 $cm^{-1}$).

haemoglobin and the cell membranes were washed 3 – 4 times and centrifuged to obtain optically white colour. After last centrifugation, the erythrocyte ghost were diluted in 310 mOsm PBS buffer again. All preparations and incubation went on at 4 $^0$C. Erythrocyte ghosts were suspended in a 310 mOsm phosphate buffer of pH 7.4 at protein concentration ca. 1 mg/ml. Erythrocyte ghosts, obtained as described above, were divided into two parts.

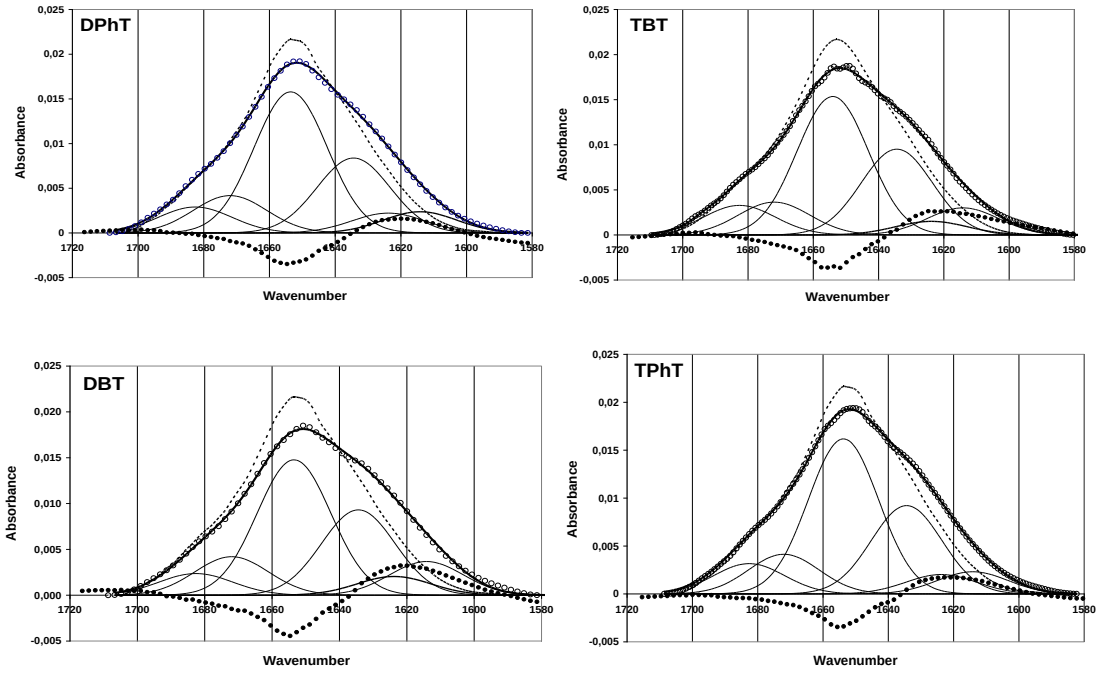


Fig. 2. Spectra of the averaged amide I bands of membranes modified by the compounds studied with their deconvolutions, the unmodified membranes spectra and difference spectra. Circle – averaged measurement points, solid thick line – summ of the deconvolutions, solid thin line – deconvolutions bands, dashed line – unmodified membranes spectra, dotted line – difference spectra.

One was the control sample and the other was treated with the compounds studied to get 0.5 mM concentration and incubated at room temperature for 24 h. After incubation the cell suspension was centrifuged for 0.5 h at 8000 g and then IR spectra were taken of such condensed suspension. The IR spectrometer used was a Thermo Nicolet product with ZnSe crystal applied. Each single spectrum was obtained from 128 records at 2 $cm^{-1}$ resolution in the range 700-4000 $cm^{-1}$. Preliminary elaboration of a spectrum was done using the EZ OMNIC v6.0 program, also of the Thermo Nicolet firm. After filtering the noise out from the spectrum of the object studied, the spectrum of the buffer solution was subtracted in order to remove the OH band of water. A representative spectrum of unmodified ghosts is shown in Fig. 1.

Next, from the spectra were taken bands of interest of the amide I (range 1550 – 1720 $cm^{-1}$) and amide II (range 1500 – 1550 $cm^{-1}$), and the base line was adjusted and bands were normalised to equal area. The measurements were repeated five to seven times for all the compounds to improve accuracy and the spectra presented here are the result of averaging. The respective parts of the amide I band are connected with protein conformation. The range of wavenumbers 1610 $cm^{-1}$ – 1640 $cm^{-1}$ corresponds to β-sheet structure, ~1645 $cm^{-1}$ to random conformation. The range 1650 $cm^{-1}$ – 1660 $cm^{-1}$ corresponds to α conformation, the components of wavenumber ~1668 $cm^{-1}$ and ~1685 $cm^{-1}$ correspond to turns conformation, and values ~1675 $cm^{-1}$ and ~1694 $cm^{-1}$ to β-sheet conformation Alvarez et al., (1987); Surewicz and Mantsch, (1988); Jackson and Mantsch, (1993); Pelton and McLean, (2000). In the amid II band two components can be distinguished: ca. 1520 $cm^{-1}$ and 1540 $cm^{-1}$, connected with anti-parallel and parallel β-sheet conformation, respectively. The mean spectra, for control and modified membranes, were subjected to deconvolution with Gauss curves into their component parts using the PeakFit program and their percent share in the entire band was determined.

## RESULTS

Finding extrema of second and fourth derivatives of spectra, the component parts of the control spectrum was determined. In this way, the unmodified (control) membranes amide I band, was divided into six component parts of wavenumbers 1615, 1624, 1634, 1653, 1672 and 1683 $cm^{-1}$. The wavenumbers 1610-1640 correspond to the β-sheet conformation, where the lower frequencies 1600-1620 corresponding to an antiparallel β-sheet, and higher wavenumbers 1630-1640 to parallel β-sheet. The wavenumber interval 1650-1660 corresponds to the α conformation, wavenumber 1672 and 1683 $cm^{-1}$ are generated by proteins in the β-sheet and turns conformations. Deconvolution of the modified membranes spectra was done preserving the same wavenumbers as for the unmodified membranes spectra. Averaged spectra with the deconvolutions and the difference spectra were used to discuss the results.

Tabele 1 Percentage share of individual components in the 1st amide band.

| Wn | structre | contrrol | C+DPhT | c+TPhT | c+DBT | c+TBT |
|---|---|---|---|---|---|---|
| 1683 | turns/β | 7,6 | 8,1 | 8,5 | 6,8 | 9 |
| 1672 | turns/β | 13,4 | 11,7 | 11,1 | 11 | 10 |
| 1653 | α | 49,4 | 44 | 43,6 | 40,8 | 42,3 |
| 1634 | β par | 23,4 | 23,4 | 24,8 | 25,8 | 26,2 |
| 1624 | β | 2,8 | 6,2 | 5,5 | 5,7 | 4,1 |
| 1615 | β apar | 3,2 | 6,6 | 6,2 | 10 | 8,3 |
| | β summ | 36,1 | 42,05 | 42,05 | 47 | 43,6 |

Fig. 2 presents a comparison of the mean amide I bands of membranes modified by the compounds studied with those of unmodified membranes and their difference. The percent share of individual components in amide I bands for control and modified ghosts are presented in Table I and Fig. 3.

For the ghosts of erythrocytes modified with the organometallic compounds there was observed a decrease in intensity of frequency 1658 $cm^{-1}$ corresponding to the α conformation and 1672 $cm^{-1}$ corresponding to turns and β-sheet, with a simultaneous increase in intensity in the range 1608-1640 $cm^{-1}$ that corresponded to β-sheet conformation. These changes correspond to a decrease in the amount of protein in the α- helix conformation from about 13% in the case of membranes treated with TPhT to about 17% for those treated with DBT and an increase in protein content of β-sheet conformation by about 18% for DPhT, TPhT

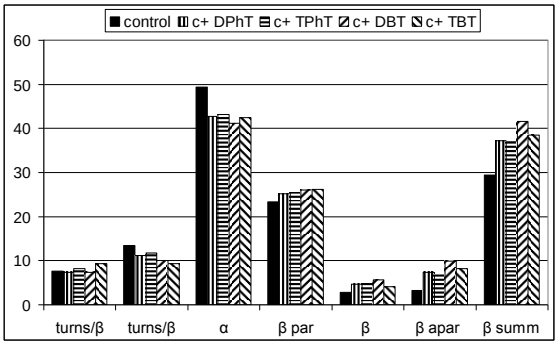


Fig. 3 Percent share of protein of specific secondary conformation in control and OTC modified erythrcyte membranes.

and TBT; and about 29% in the case of DBT. Since modifying OCT membranes causes increased protein in the β-sheet conformation, the few percent decrease in

wavenumber 1672 $cm^{-1}$ seen from the difference curves and in Table I should correspond to a decrease in the amount of the turn conformation. Comparing the II amide band of modified membranes with the band for control membranes (Fig. 4), one can notice a relative increase in the share of antiparallel β-sheet conformation in which the amount of protein of the β-sheet conformation increases, the decreasing intensity of wavenumber 1672 $cm^{-1}$ must be due to a decreasing amount of protein in the turns conformation. OTC compounds form hydrogen bonds with carbonyl groups, which leads for instance to dehydration of membrane

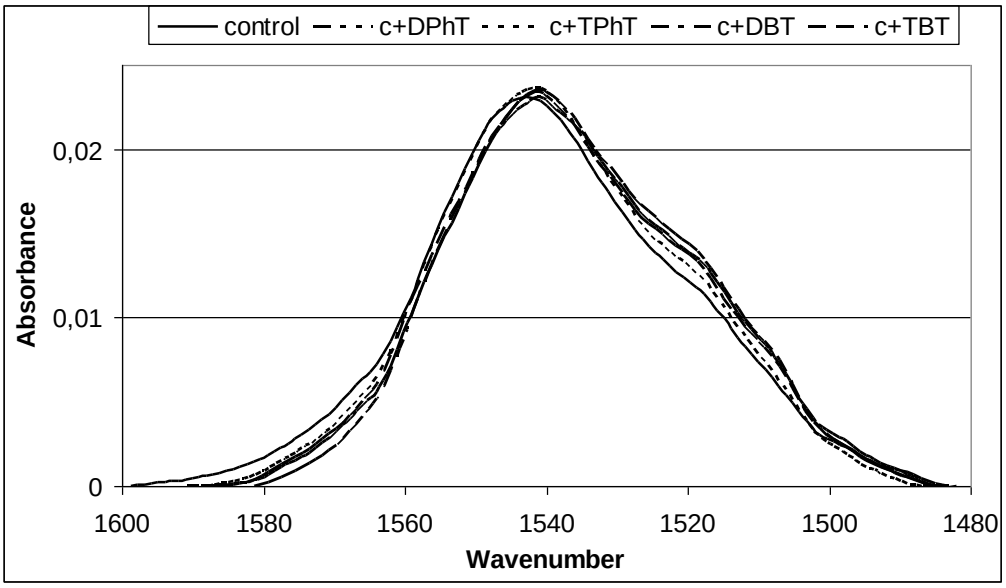


Fig. 4. Spectra of the averaged amide II bands of membranes modified by the compounds studied and the unmodified membranes spectra.

in the low frequency band of ca. 1520 $cm^{-1}$. This is in accord with the changes observed for the amide I band, where the increase in the amount of protein in antiparallel β-sheet conformation of 1614 $cm^{-1}$ wave number is larger in the 1634 $cm^{-1}$ parallel β-sheet conformation.

## DISCUSSION

The amide I band is composed chiefly of stretching vibrations of C=O groups of aminoacids. Their vibration frequencies depend on the secondary conformation of protein, which allows one to reach conclusions about conformational changes that a protein undergoes on the basis of changes observed in the I amid band Okubo and Noguchi (2007); Palaniappan and Vijayasundaram (2008); Garip et al., (2010). The changes in intensity of the frequencies of the amid band components seen in the results presented indicate that there is a decreasing number of protein molecules in the α-helis conformation and an increasing number of molecules in the β-sheet conformation. We observed also changes in the intensity of the component of wavenumber 1672 $cm^{-1}$, which is connected with β-sheet and turns conformations. Since, as it follows from the analysis of the entire amide I band lipids Żyłka et al., (2009). It seems that the conformational changes observed may arise due to the breaking of hydrogen bonds between the carboxyl and amine groups and substituting them by bonds with OTC. In the case of interaction with the proteins of the α or the turns conformation, the breaking of a carboxyl and amine hydrogen bond must result in a change of conformation that tends to become a linear protein, like the β-sheet. The OTC studied induce a greater increase in intensity of the frequency which corresponds to the antiparallel β-sheet (1615 $cm^{-1}$) rather than to the β-sheet parallel (1634 $cm^{-1}$) conformation.

The functions of proteins in cell processes are closely connected and controlled by the protein structure and change in protein conformation means a break in the process the protein participates in or a change in the process itself Kim et al., (2003), Watson et al., (2005), Dessailly et al., (2009), Baclayon et al., (2010). This necessarily results in a change in the cell functioning and consequently of the entire organism. The results obtained indicate that the toxic properties of organometallic compounds can significantly affect the membrane peptides conformation and function, which may be one of the causes of their toxicity.

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