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Thermochimica Acta 458 (2007) 132–137
thermochimica acta
www.elsevier.com/locate/tca

Interaction of arsenic compounds with model phospholipid membranes

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Available online 12 January 2007

Abstract

This study is part of a project aimed at examining the influence of arsenic on biological membranes. By the use of differential scanning calorimetry (DSC) we have followed the thermotropic behavior of multilamellar vesicles prepared from dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) upon incorporation of sodium arsenite (AsI), disodium arsenate (AsII), cacodylic acid (AsIII) and disodium methyl arsenate (AsIV).

The effectiveness of perturbations exerted by various arsenic compounds on thermotropic phase transition was further analysed in terms of thermodynamic parameters: transition temperature, enthalpy and molar heat capacity, determined for lipid/As systems on the basis of heating and cooling scans. It is found that while it only has a slight influence on the thermotropic properties of DMPC, arsenic is able to significantly modify DMPE model membranes.

Keywords: Sodium arsenite; Sodium arsenate; Cacodylic acid; Phospholipid bilayer; Phase transition; Differential scanning calorimetry

1. Introduction

Arsenic (As) is a metalloid widely distributed in the earth's crust and present at an average concentration of 2 mg/kg. It occurs as a trace element in all rocks, soil, water and air. While the pure form of arsenic does not usually occur in the natural environment, in combination with oxygen, chlorine and/or sulphur it is commonly found and referred to as inorganic arsenic (iAs). Organic arsenic compounds (combined with carbon and hydrogen) are mainly present in marine organisms although some traces of these compounds have also been found in terrestrial species.

About one third of the atmospheric flux of arsenic is of natural origin. Volcanic activity is the most important natural source of arsenic, followed by low-temperature volatilization. Mining, smelting of non-ferrous metals and burning of fossil fuels are the major industrial processes that contribute to the anthropogenic arsenic contamination of air, water and soil. Historically, the use of arsenic-containing pesticides has left large tracts of agricultural land contaminated. It has been estimated that 70% of the world arsenic production is used in timber treatment as copper chrome arsenate, 22% in agricultural chemicals and the remainder in glass, pharmaceuticals and non-ferrous alloys. Inorganic As of geological origin is found in groundwater used as drinking water in several parts of the world, for example, Bangladesh, Argentina, Chile, India, Mexico, Taiwan and Thailand. Arsenic enters water sources through the dissolution of minerals and ores, from industrial effluents and from atmospheric deposition. The two factors generally thought to release the arsenic are pyrite oxidation and oxyhydroxide reduction. Although under normal ecological conditions the level of arsenic bioavailability is not a threat to human health, there is a trend towards a significant increase in human exposure to As. Arsenic poisoning in drinking water remains a global problem.

Arsenic can exist in four valency states: −3, 0, +3, +5 [1]. Under reducing conditions, arsenite (As^{3+}) is the dominant form; arsenate (As^{5+}) is a generally stable form in oxygenated environments. Arsenic can be inhaled, absorbed through the skin or by ingestion. Once absorbed, As rapidly combines with the globin portion of haemoglobin and is transported to the liver, kidney, spleen, lung and gastrointestinal tract, with a lesser accumulation in muscle and nervous tissue. Arsenic exerts its toxic effect through several mechanisms as reviewed in Ref. [2], the most significant of which is a reversible combination with sulphydryl

Abbreviations: As, arsenic; iAs, inorganic arsenic; AsI, sodium arsenite; AsII, disodium arsenate; AsIII, cacodylic acid; AsIV, disodium methyl arsenate; DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; MLV, multilamellar vesicles; DSC, differential scanning calorimetry; T_m, temperature of main phase transition

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doi:10.1016/j.tca.2007.01.004

groups. It binds to the pyruvate dehydrogenase complex, blocks the Krebs cycle and interrupts oxidative phosphorylation, resulting in a marked depletion of cellular ATP and eventually the death of the metabolizing cell. Through sulphydryl group binding, As also interferes with the transformation of thiamine and inhibits numerous cellular enzymes involved in glucose uptake, gluconeogenesis, fatty acid oxidation and the production of glutathione. However, binding of arsenite at nonessential sites in proteins may be a detoxication mechanism [3]. A second major form of toxicity is termed arsenolysis. As a phosphate analog, arsenate is known to affect oxidative phosphorylation by competition with phosphate. As a consequence, arsenate diminishes the formation of ATP and uncouples many reactions, which results in a loss of energy by the cell. Arsenate anions can also replace phosphate in the sodium pump and the anion exchange transport system of the human red blood cell [4].

The metabolism of As has an important role in its toxic effects. Many mammalian species methylate inorganic As. Although there is variation between different species in the rate and the extent of methylation, iAs is metabolized by a sequential process involving a two-electron reduction of pentavalent As to trivalent As, followed by oxidative methylation to monomethylarsonic acid and dimethylarsinic acid (reviewed in Ref. [5]), which are excreted, along with residual inorganic arsenic, in the urine. However, if the dose of As is very large, the elimination half-life is prolonged.

Aquatic and terrestrial biota show a wide range of sensitivity to different arsenic species (see [6], for a review on arsenic toxicity in terrestrial plants). In general, inorganic arsenicals are more toxic than organoarsenicals, and arsenite is more toxic than arsenate. Soluble inorganic arsenic is acutely toxic and the ingestion of large doses leads to gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions and eventually death. In survivors, bone marrow depression, haemolysis, hematomegaly, melanosis and encephalopathy may be observed. Long-term exposure to arsenic in drinking water is causally related to an increased risk of cancer in the skin, lungs, bladder and kidney [7–10] as well as other skin changes such as hyperkeratosis and pigmentation changes [5,11].

The mechanism or mode of action by which inorganic arsenic causes toxicity, including cancer, is not well elucidated. Arsenic exposure may cause DNA hypomethylation due to continuous methyl depletion, facilitating an aberrant gene expression that results in carcinogenesis. Further, though arsenic is nonmutagenic, it interacts synergistically with genotoxic agents in the production of mutations and also induces chromosome abnormalities and cell proliferation [12]. Noncancer effects are thought to be related to the inhibitory effects on cellular respiration at the mitochondrial level. Oxidative stress might also have an important role both in cancer and noncancer effects.

In order to elucidate the molecular mechanisms of the interaction of arsenic compounds with cell membranes we have utilized well-established models consisting of multibilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of the phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively.

2. Experimental

2.1. Preparation of phospholipid vesicles

1,2-Dimyristoyl-*sn*-glycero-3 phosphocholine (DMPC) and 1,2-dimyristoyl-*sn*-glycero-3 phosphoethanolamine (DMPE) (purity > 99%) from Sigma were used without further purification. Sodium arsenite ($NaAsO_2$), disodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) were purchased from Merck, cacodylic acid ($C_2H_7AsO_2$) and disodium methyl arsenate ($CH_3AsNa_2O_3{\cdot}6H_2O$) were from Sigma and Chemical Service, respectively. Appropriate amounts of lipid dissolved in chloroform were prepared in a glass test tube. The organic solvents were gently evaporated to dryness under a stream of nitrogen until a thin film on the wall of the test tube was formed and the sample was further desiccated under vacuum for 2–3 h. The lipid film was suspended in 1 ml of buffer (1 mM EDTA/10 mM Hepes/50 mM KCl (pH 7.7)) in case of pure DMPC and DMPE samples whilst aqueous solution of arsenic was added for lipid/As systems. Multilamellar vesicles (MLV) were formed by vortexing the mixture at about 30 °C (DMPC) and 55 °C (DMPE), i.e., above the main phase transition temperature of the pure phospholipid. Vortexing was continued until a homogeneous and uniform suspension was obtained (usually 10 min). The final DMPC and DMPE concentration was 1 mM and arsenic content in the samples was 5 mol%. The resulting suspension was allowed to equilibrate at room temperature for at least 15 min. The liposomes obtained were heterogeneous in size. With respect to phase transition measurements, this heterogeneity was of no relevance, as has been shown earlier [13].

2.2. Differential scanning calorimetry measurements

Differential scanning calorimetry (DSC) experiments were performed using a CSC Model 6100 Nano II differential scanning calorimeter (Calorimetry Sciences Corporation, Provo, UT, USA) with cylindrical cells. The calorimeter was equipped with the original data acquisition and analysis software. In order to avoid bubble formation during heating mode the samples were degassed prior to being loaded by pulling a vacuum of 30–50 kPa on the solution for a period of 10–15 min. Then the sample cell was filled with about 400 μl of MLV suspension and an equal volume of buffer was used as a reference. The cells were sealed and thermally equilibrated for about 20 min below the starting temperature of the run. All measurements were made on samples under 3 bar pressure. After the measurements the samples were checked by thin layer chromatography and no degradation of the lipids was detected. The data were collected in the range of 10–40 °C (DMPC/As) and 30–70 °C (DMPE/As) at a scan rate of 1 °C min^{-1} both for heating and cooling. Scans of buffer as sample and reference were also performed to collect the apparatus baseline. Each sample was prepared and recorded at least three times to check for reproducibility. Each data set was analysed for thermodynamic parameters with a CpCalc software package supplied by CSC.

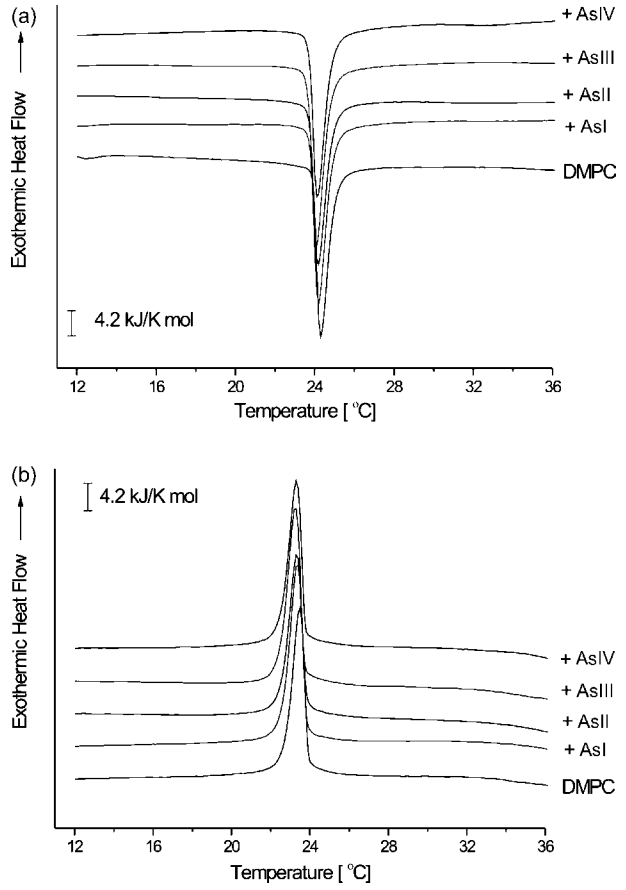

Fig. 1. Representative DSC heating (a) and cooling (b) thermograms obtained for multilamellar DMPC liposomes and DMPC liposomes containing sodium arsenite (AsI), disodium arsenate (AsII), cacodylic acid (AsIII) and disodium methyl arsenate (AsIV) at a concentration of 5 mM. Scans were obtained at a heating (cooling) rate of 1 °C min^{-1}.

3. Results

The representative high-sensitivity DSC heating and cooling thermograms obtained for pure DMPC multibilayer vesicles and binary mixtures of DMPC and different arsenic compounds at 5 mM concentration are shown in Fig. 1. In the thermal range of 10–40 °C, fully hydrated DMPC bilayers in the absence of any additives, undergo a strong and sharp main transition at 24.3 °C, with an enthalpy change (ΔH) of 18.8 kJ mol^{-1}, which corresponds to the conversion of the rippled gel phase ($P_{\beta'}$) to the lamellar liquid-crystal L_α phase. Here, the transition temperatures correspond to the transition peak at the maximal peak heat and the transition enthalpies correspond to the integrated area under the peak divided by the lipid concentration. The results for the thermodynamic data of the pure DMPC are in agreement with previous reports (for reviews see [14,15]).

As can be clearly seen, the presence of the four arsenic compounds does not perturb the thermotropic behavior of vesicles formed from DMPC. Neither the phase transition temperature and enthalpy nor the thermogram shape shows any marked changes.

Illustrated in Fig. 2 are representative high-sensitive DSC heating and cooling thermograms obtained for DMPE and As-containing DMPE liposomes. The pure DMPE liposomes measured in the temperature range from 30 to 70 °C undergo one thermotropic phase transition. We found a highly cooperative main transition arising from the gel-to-liquid-crystal transition ($L_\beta \rightarrow L_\alpha$) and the values of $T_m = 50.2$ °C and $\Delta H = 26.0$ kJ mol^{-1}, which is consistent with data in the literature ([14,15]). It is clear that the presence of arsenic markedly alters the calorimetric behavior of DMPE bilayers. AsI, AsII and AsIV produce a significant increase in the width of the observed heating and cooling thermograms, AsIV being the most effective in this respect. Concurrently, the height of the phase transition peak is reduced. Thus, the decrease in cooperativity of the transition is not accompanied by a lowering of the enthalpy value. Moreover, in the case of AsIV an additional shoulder is observed in a higher temperature region both in heating and cooling scans. Different behavior of DMPE liposomes is exhibited in the presence of AsIII. While the phase transition peak is strongly sharpened in the heating scan, during cooling its half-height width increases even more than in case of other examined As. Furthermore, the shape of the peak is modified showing two hardly resolved maxima.

In pure DMPE liposomes, T_m showed a hysteresis ($\Delta T = T_m - T_{m'}$) of about 1.8 °C between heating and cooling cycles, arising from the finite response time of the calorimeter as well as from the different lateral mobility of acyl chains in the gel and in the liquid crystal phase. A small increase in hysteresis (up to 2.1 °C) was observed upon incorporation of AsII. In contrast, samples containing AsIII had this ΔT value reduce to 1 °C. The presence of AsIV in the DMPE bilayer had almost no effect in this respect.

Moreover, some difference between the thermograms relevant to the first and subsequent heating and cooling scans could be noticed, if all cycles were consecutive (Fig. 2 insets). For pure DMPE, T_m appeared at a slightly higher temperature in the first scan than in the subsequent ones and the observed $\Delta T_m = (T_{mfirst} - T_{msubs})$ value is about 0.3 °C. While it remains unchanged upon addition of AsIV, in the case of the liposomes containing AsIII and AsII, ΔT_m is abolished.

The influence of various As compounds in thermotropic phase perturbations was further analysed in terms of thermodynamic parameters. The enthalpy and entropy determined for DMPE/As liposomes on the basis of heating and cooling scans were 20.9 kJ mol^{-1}, 0.084 kJ K^{-1} mol^{-1}, and 19.7 kJ mol^{-1}, 0.080 kJ K^{-1} mol^{-1}, respectively. Within an estimated error range they had the same value for all examined DMPE/As binary systems, regardless of the kind of As supplementation. Fig. 3 illustrates the effect on transition temperature exerted by various As compounds. As a general feature, in the presence of all examined As compounds except for AsIII, a decrease in T_m is observed, roughly of the same degree in heating and cooling processes. AsIV is shown to cause the highest phase transition shift ranging 1.4 °C both for heating and cooling. Again, AsIII shows a different effect among arsenic compounds. Its presence in the DMPE bilayer induces a slight upward shift of T_m up to

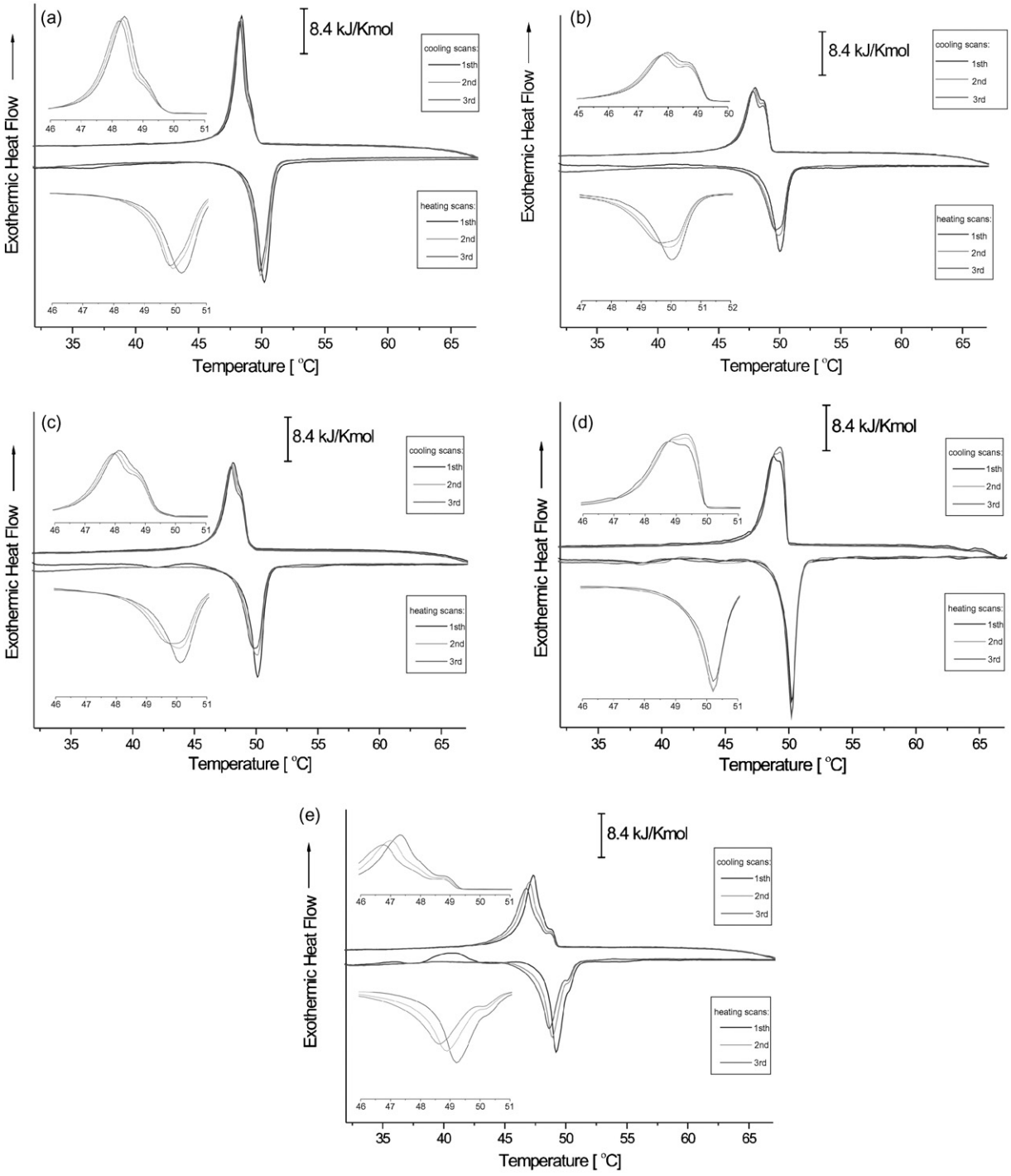

Fig. 2. Representative DSC successive heating and cooling thermograms obtained for: (a) multilamellar DMPE liposomes and DMPE liposomes containing: (b) sodium arsenite (AsI), (c) disodium arsenate (AsII), (d) cacodylic acid (AsIII) and (e) disodium methyl arsenate (AsIV) at a concentration of 5 mM. Insets show enlargements of the phase transition region. Scans were obtained at a heating (cooling) rate of 1 °C min^{-1}. Subsequent scans were collected after 10 min equilibration at 30 °C (heating) or 70 °C (cooling).

50.4 °C during heating and an even higher one (T_m = 48.8 °C) as determined for cooling scans.

4. Discussion

The molecular models we used in our DSC studies on the interaction of As with biological membranes consist of bilayers of DMPC and DMPE, which are representative of the phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively. Erythrocytes could be in turn considered as representatives of the plasma membrane. Moreover, they seem to be the most relevant system from the point of view of arsenic toxicity, since they can be regarded as the target of arsenic when it attacks a body after systemic absorp-

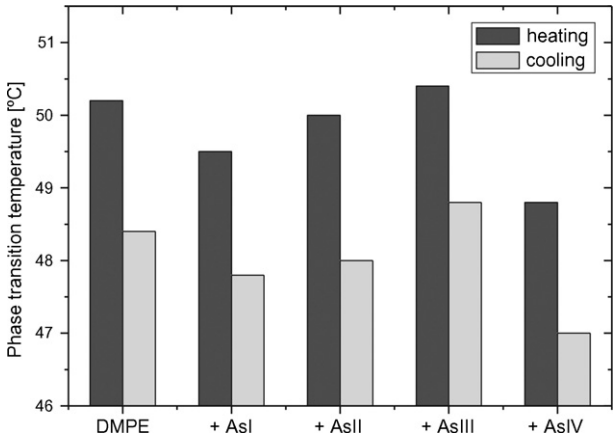

Fig. 3. The effect of sodium arsenite (AsI), disodium arsenate (AsII), cacodylic acid (AsIII) and disodium methyl arsenate (AsIV) at a concentration of 5 mM on the temperature of DMPE main transition peak obtained during the DSC heating and cooling measurements.

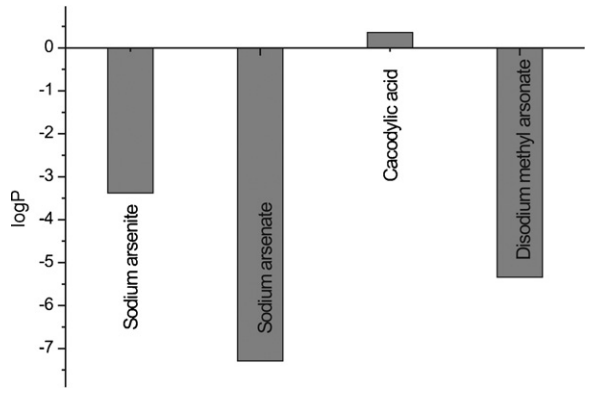

Fig. 4. The values of $\log P$ in octanol/water determined for various arsenic compounds. The value of $\log P$ (P stands for partition coefficients) is used as a measure of molecular hydrophobicity. Data taken from http://chem.sis.nlm.nih.gov/chemidplus/.

tion [16]. The cell membrane constitutes the frontier facing this attack [17].

Among calorimetric studies, human erythrocyte ghosts and extracted lipids were investigated by Landbrooke et al. [18] who found that after the removal of cholesterol a broad endothermic transition could be detected. Reinvestigations of the thermotropic properties of human red cell ghosts using more sensitive instruments [19,20] allowed the detection of four endothermic transitions in the region of 45–80 °C, which turned out to be irreversible mainly due to the denaturation processes. To the best of our knowledge there are no DSC studies on the effect of any chemicals of biological relevance on the thermotropic behavior of red blood cells.

The significance of phase transitions in the biomembranes is not to be overestimated. In the region of phase transition, domains of fluid and ordered lipids coexist. Due to different packing of the lipids in the two phases on the boundaries of these domains, structural faults are formed, which cause an increase in membrane permeability, which facilitates the action of hydrolytic enzymes. Moreover, in the behavior of many enzymes, the nonlinear dependence of enzymatic activity on temperature has been attributed to the phase transition of the membrane lipids. Ordering of the lipids will cause an aggregation of proteins and a change in their microenvironment resulting in an increase in activation energy. Taking into account that in human erythrocyte membranes there are more than 20 kinds of protein, of which some are critical enzymes [17], the above considerations are important in assessing the effect of arsenic on the human body. It is worth noticing that as with other biological membranes although the amount of lipids undergoing melting in erythrocytes is very small, those lipids strongly influence the microenvironment of the enzymes. This is revealed by the change in the activation energy of the enzymes. That is why we decided as a first approach to focus our studies on As-lipid interactions.

In this work, by studying the effect of As on phospholipid bilayers, we have shown that arsenic compounds are able to modify to various extents DMPE phase transition. Thus, they belong to a group of membrane modifiers [23] together with many metal ions, drugs and other chemical compounds (including prenylquinones [21] and carotenoids [22] investigated by DSC in our laboratory), which can trigger local phase transition or phase separation at constant temperature. Even though the phase changes may occur in 'microdomains', these factors alter membrane fluidity and modify the microenvironment of membrane proteins, thereby having a profound influence on the properties of biomembranes.

The most apparent difference in the effectiveness of phase transition perturbation displayed by As compounds upon incorporation into lipid bilayers is related to the class of phospholipids that build the bilayer. DMPC liposomes, which turned out to be insensitive to As presence are formed from a lipid possessing the same fully saturated acyl chain of 14 carbon atoms as in DMPE. These phospholipids differ only in their terminal amino groups, which are $^{+}N(CH_3)_3$ and $^{+}NH_3$ in DMPC and DMPE, respectively. Thus, it seems that it is the greater affinity displayed by As towards the surface of ethanolamine – than choline – containing liposomes that determine the kind of interactions leading to the changes in thermotropic behavior clearly seen in case of DMPE and not observed for the DMPC bilayer.

The localisation of the modifier within the lipid layer is determined by the presence of polar and apolar groups in the compound and by the geometric arrangements of these groups within the molecule. Comparing the structure of the examined As compounds has not given a clear answer as to their preferences and arrangement into the phospholipids bilayer. Thus, in the search for the physicochemical properties of arsenic compounds that could be of crucial importance from the point of view of the various effects they displayed on the thermotropic behavior of the DMPE bilayer we found partition coefficients (P) (http://chem.sis.nlm.nih.gov/chemidplus/) as illustrated in Fig. 4. The value of $\log P$ is used as a measure of molecular hydrophobicity, a parameter that affects drug absorption, bioavailability, hydrophobic drug–receptor interactions, metabolism of molecules as well as their toxicity. It has also become a key parameter in studies of the environmental fate of chemicals. Furthermore, octanol being relatively non-polar

(dielectric constant of 10.3) is often used as a model solvent for understanding partitioning from water into lipid membranes [24]. The most characteristic feature arising from Fig. 4 is the highly negative values of log *P* for As I, AsII and AsIV, which means the extremely low hydrophobicity of these compounds in contrast to log *P* determined for AsIII as 0.360. Such a distinction in partition coefficient setting apart cacodylic acid from other arsenic compounds corroborates well with the effect the latter exhibits on DMPE phase transition. The significant increase in the height of the main phase transition observed for the DMPE/AsIII system indicates that structural changes took place resulting in an increase in heat capacity and the cooperativity of transition, similar to the effect of a small concentration of Fe^{2+} and Fe^{3+} ions [25]. However, it is still the increased polarity of the DMPE liposome surface, consequent to the unshielded positive charge on the nitrogen atom which seems to be decisive in delineating the scope of perturbations displayed by various arsenic compounds on the phase transition of phospholipids.

Admittedly, the membrane model used is simplified and does not account for the role of proteins. However, we believe that a more detailed analysis of the calorimetric data supported by X-ray diffraction, fluorescence spectroscopy studies as well as scanning electron microscopy of human erythrocytes will allow a comprehensive analysis of the interaction of arsenic with biological membranes. The obtained results will assist an insight into the arsenic–lipid matrix interactions, providing a basis for the toxicological studies on living cells.

Furthermore, considering the data provided by Pickering et al. [26], which show that As^{5+} enters the roots of plants growing hydroponically as a phosphate analog and is promptly reduced to As^{3+}, our data may provide some background knowledge, valid for bioaccumulation studies that offer a way of removing arsenate from contaminated soils. However, to optimize this process it is important to thoroughly understand the biological mechanism involved.

Acknowledgements

This work was financed from the budget of the Polish Ministry of Science for the years 2005–2008 under project No. 2 P04A 063 28 and supported by FONDECYT (1060990) and UDEC-PUCV (205.024.031-1S).

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