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Arsenite interactions with phospholipid bilayers as molecular models for the human erythrocyte membrane

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Abstract

There are scanty reports concerning the effects of arsenic compounds on the structure and functions of cell membranes. With the aim to better understand the molecular mechanisms of the interaction of arsenite with cell membranes we have utilized bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively. The capacity of arsenite to perturb the bilayer structures was determined by X-ray diffraction and fluorescence spectroscopy, whilst the modification of their thermotropic behaviour was followed by differential scanning calorimetry (DSC). The experiments carried out by X-ray diffraction and calorimetry clearly indicated that $NaAsO_2$ interacted with DMPE and modified its thermotropic behaviour. No such information has been so far reported in the literature.

Keywords: Arsenic; Arsenite; Phospholipid bilayer; Erythrocyte membrane

1. Introduction

The drinking water contamination by arsenic is a major health problem. Most cases of human toxicity from arsenic have been associated with exposure to inorganic arsenic [1]. Despite the well documented information, there are insufficient reports concerning the effects of arsenic compounds on the structure and functions of cell membranes, particularly those of human erythrocytes [2–4]. In the course of in vitro systems search for the toxicity screening of chemicals of biological relevance, different cellular models have been applied to examine their adverse effects. The cell membrane is a diffusion barrier which protects the cell interior. Therefore, its structure and functions are susceptible to alterations as a consequence of interactions with chemical species. With the aim to better understand the molecular mechanisms of the interaction of sodium arsenite with cell membranes we have utilized molecular models of the erythrocyte membranes. Erythrocytes were chosen because although less specialized than many other cell membranes they carry on enough functions in common with them such as active and passive transport, and the production of ionic and electric gradients, to be considered representative of the plasma membrane in general. The molecular models consisted of bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively [5,6], and large unilamellar vesicles (LUV) of DMPC. The capacity of $NaAsO_2$ to perturb the multibilayer structures of DMPC and DMPE was evaluated by X-ray diffraction, the modifications of their thermotropic behavior were followed by differential scanning calorimetry (DSC), and DMPC LUV were studied

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; LUV, large unilamellar vesicles; DPH, 1,6-diphenyl-1,3,5-hexatriene; *r*, fluorescence anisotropy; GP, general polarization; DSC, differential scanning calorimetry.

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by fluorescence spectroscopy. These systems and techniques have been used in our laboratories to determine the interaction with and the membrane-perturbing effects of other inorganic compounds [7–13].

2. Materials and methods

2.1. X-ray diffraction studies of phospholipid multilayers

The capacity of $NaAsO_2$ to perturb the structures of DMPC and DMPE multilayers was evaluated by X-ray diffraction. Synthetic DMPC (lot 80 H-8371, MW 677.9), DMPE (lot 084 K-1676, MW 635.9) from Sigma, and $NaAsO_2$ (p.a., lot 2324537) from Merck were used without further purification. About 2 mg of each phospholipid was introduced into 1.5 mm diameter special glass capillaries, which were then filled with 200 ml of (a) distilled water and (b) aqueous solutions of $NaAsO_2$ in a range of concentrations (1 μM to 10 mM). The specimens were X-ray diffracted after 1 h incubation at 37 °C and 60 °C with DMPC and DMPE, respectively, in flat plate cameras. Specimen-to-film distances were 8 and 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered CuKα radiation from a Bruker Kristalloflex 760 (Karlsruhe, Germany) X-ray generator was used. The relative reflection intensities were obtained in a MBraun PSD-50 M linear position-sensitive detector system (Garching, Germany); no correction factors were applied. The experiments were performed at 18±1 °C, which is below the main phase transition temperature of both DMPC and DMPE. Each experiment was repeated three times and in case of doubts additional experiments were carried out.

2.2. Fluorescence measurements of DMPC large unilamellar vesicles (LUV)

The influence of $NaAsO_2$ on the physical properties of DMPC LUV was examined by fluorescence spectroscopy using DPH (1,6-diphenyl-1,3,5-hexatriene) and laurdan (Molecular Probe, Eugene, OR, USA) fluorescent probes. DPH is widely used as a probe for the hydrophobic regions of the phospholipid bilayers because of its favorable spectral properties. Its steady-state fluorescence anisotropy measurements were used to investigate the structural properties of DMPC LUV, as it provides a measure of the rotational diffusion of the fluorophore, restricted within a certain region such as a cone, due to the lipid acyl chain packing order. Laurdan, an amphiphilic probe, has a high sensitivity of excitation and emission spectra to the physical state of membranes. With the fluorescent moiety within a shallow position in the bilayer, laurdan fluorescence spectral shifts provide information of its molecular dynamic properties at the level of the phospholipid polar headgroups. The quantification of the laurdan fluorescence shifts was effected using the general polarization GP concept [14], which is related to the lipid polar headgroup organization in lipid bilayers.

DMPC LUV suspended in water were prepared by extrusion of frozen and thawed multilamellar liposome suspensions (final lipid concentration 0.4 mM) through two stacked polycarbonate filters of 400 nm pore size (Nucleopore, Corning Costar Corp., MA, USA) under nitrogen pressure at 37 °C, which is above the lipid phase transition temperature. DPH and laurdan were incorporated into DMPC LUV by addition of 2 μl/ml aliquots of 0.5 mM solutions of the probe in dimethylformamide and ethanol respectively in order to obtain final analytical concentrations of 2.5 μM, incubating them at 37 °C for 45 min. Fluorescence spectra and anisotropy measurements were performed on a Spex Fluorolog (Spex Industries Inc., Edison, N.J., USA) and in a phase shift and modulation Gregg-200 steady-state and time-resolved spectrofluorometer (ISS Inc., Champaign, IL, USA) respectively, both interfaced to computers. Software from ISS was used for data collection and analysis. Measurements of LUV suspensions were made at 18 °C and 37 °C using 10 mm path-length square quartz cuvettes. Sample temperature was controlled by an external bath circulator (Cole-Parmer, Chicago, IL, USA) and monitored before and after each measurement using an Omega digital thermometer (Omega Engineering Inc., Stanford, CT, USA). Anisotropy measurements were made in the *L* configuration using Glan Thompson prism polarizers (I.S.S.) in both exciting and emitting beams. The emission was measured using a WG-420 Schott high-pass filter (Schott WG-420, Mainz, Germany) with negligible fluorescence. DPH fluorescence anisotropy (r) was calculated according to the definition: $r=(I_{||}-I_{\perp})/(I_{||}+2I_{\perp})$ where $I_{||}$ and $I_{\perp}$ are the corresponding vertical and horizontal emission fluorescence intensities with respect to the vertically polarized excitation light [15]. Laurdan fluorescence spectral shifts were quantitatively evaluated using the GP concept (see above) which is defined by the expression $GP=(I_b-I_r)/(I_b+I_r)$, where I_b and I_r are the emission intensities at the blue and red edges of the emission spectrum, respectively. These intensities have been measured at the emission wavelengths of 440 and 490 nm, which correspond to the emission maxima of laurdan in the gel and liquid-crystalline phases, respectively [16]. $NaAsO_2$ was incorporated in LUV by addition of adequate (0.1 M) aliquots of $NaAsO_2$ solution in order to obtain the different concentrations used in this work. The samples thus prepared were then incubated at 18 °C for ca. 15 min. Blank subtraction was performed in all measurements using labeled samples without probes. The data presented in Table 1 represent mean values and standard error of ten measurements in two independent samples. Unpaired Student's *t*-test was used for statistical calculations.

Table 1
Effect of $NaAsO_2$ on the anisotropy (r) of DPH and the general polarization (GP) of Laurdan embedded in DMPC LUV at 18 °C and 37 °C

$NaAsO_2$ (mM)	r (DPH) 18 °C	GP (Laurdan) 18 °C	r (DPH) 37 °C	GP (Laurdan) 37 °C
0	0.326±0.001	0.542±0.002	0.085±0.002	−0.088± 0.003
0.1	0.326±0.001	0.546±0.001	0.086±0.002	−0.080±0.003
1	0.326±0.001	0.544±0.001	0.085±0.002	−0.085±0.003
5	0.325±0.001	0.544±0.001	0.085±0.002	−0.080±0.003
10	0.324±0.002	0.554±0.001	0.085±0.002	−0.074±0.003

2.3. Differential scanning calorimetry (DSC) studies of phospholipid model systems

The effect of $NaAsO_2$ on the thermal properties of phospholipid model structures was studied using multilamellar liposomes prepared from DMPC (Lot 049H5156), and DMPE (Lot 13H83681) from Sigma. The appropriate amounts of lipid dissolved in chloroform were evaporated under a stream of N_2. Further evaporation was carried out by keeping the samples under vacuum for 20 min. Dry lipid films were suspended in buffer (1 mM EDTA/ 10 mM Hepes/50 mM KCl, pH 7.7) or deionized water in case of DMPE and DMPC, respectively and aqueous solutions of $NaAsO_2$ were added in the concentration range of 1 mM up to 20 mM. The multilamellar liposomes (MLV) were prepared by vortexing the samples at the temperature above gel-to-liquid crystalline phase transition of the pure lipid (about 25 °C for DMPC and 51 °C for DMPE). The DSC measurements were performed using a differential scanning calorimeter (CSC 6100 Nano II, Calorimetry Sciences Corp., Provo, UT, USA). To avoid bubble formation during heating modes the samples were degassed prior to loading by putting the vacuum of 0.3–0.5 atm on the solution for a period of 10 min. Then the sample cell was filled with about 400 μl of MLV suspension and an equal volume of buffer itself was used as a reference. The cells were sealed and equilibrated for about 20 min below the starting temperature of the run. The scan range was 10–40 °C for DMPC and 25–80 °C for DMPE. Heating/cooling scan rates were 1 °C per minute. The heating scans were carried out first. The reference scan was subtracted from the sample scan and each data set was analyzed for the thermodynamic parameters with a CpCalc software package supplied by CSC (Provo, UT, USA). The accuracy for the main phase transition temperature and enthalpy was ±0.1 °C and ±0.8 kJ/mol, respectively.

3. Results

3.1. X-ray diffraction studies of phospholipid multilayers

Fig. 1A exhibits the results obtained by incubating DMPC with water and $NaAsO_2$. As expected, water altered the structure of DMPC; its bilayer width increased from about 55 Å in its dry crystalline form [17] to 64 Å when immersed in water, and its low-angle reflections were reduced to only the first two. On the other hand, only one strong reflection of 4.2 Å showed up in the wide-angle region which corresponds to the average distance between fully extended acyl chains organized with rotational disorder in hexagonal packing. These results were indicative of the fluid state reached by DMPC bilayers. Fig. 1A discloses that after exposure to $NaAsO_2$ (up to 10 mM) the low- and wide-angle lipid reflection intensities (indicated as (a) and (b) in the figure, respectively) practically did not show any variation. From these results it can be concluded that $NaAsO_2$ did not produce any structural perturbation of DMPC bilayers. Fig. 1B shows the results of the X-ray diffraction analysis of DMPE bilayers incubated with water and $NaAsO_2$. As it can be noticed, 5 mM and higher concentrations of $NaAsO_2$ caused a gradual weakening of all DMPE reflection

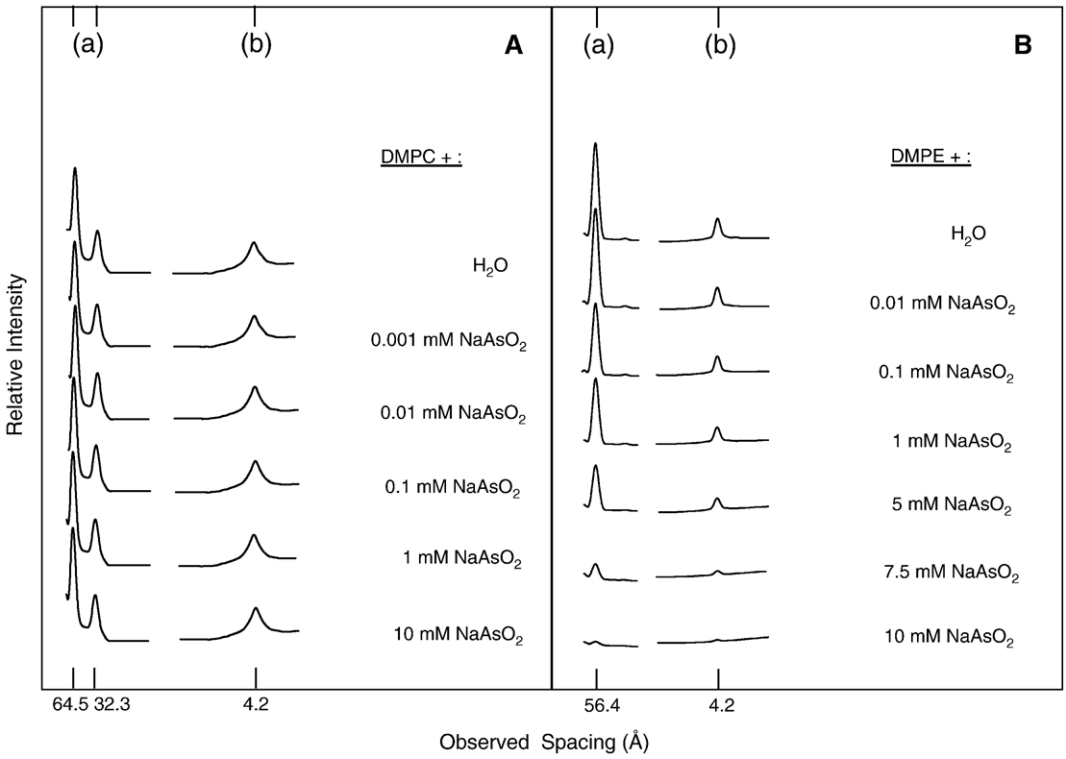

Fig. 1. Microdensitograms from X-ray diffraction patterns of dimyristoylphosphatidylcholine (DMPC) (left panel) and dimyristoylphosphatidylethanolamine (DMPE) (right panel) in water and aqueous solutions of $NaAsO_2$; (a) and (b) correspond to low-and wide-angle reflections.

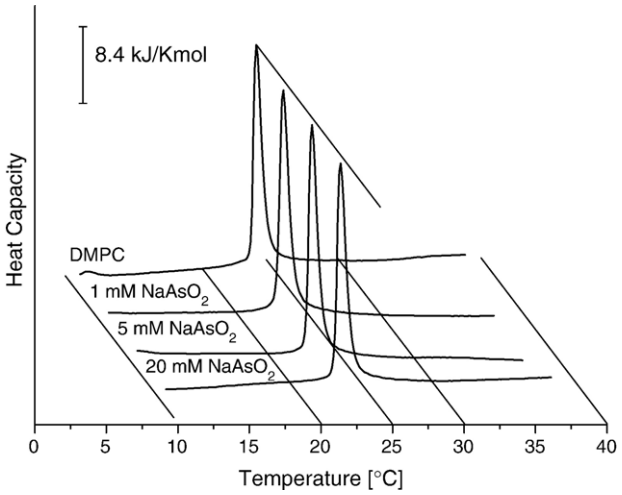

Fig. 2. Representative DSC heating curves obtained for multilamellar DMPC liposomes containing different concentrations of $NaAsO_2$. Scans were obtained at a heating rate of 1 °C min^{-1}.

intensities, which almost disappeared when $NaAsO_2$ (10 mM) was applied. In fact, 5, 7.5 and 10 mM arsenite caused a 50%± 0.8, 81%±0.9, and 93%±1.0 reduction of the reflection intensities, respectively.

3.2. Fluorescence measurements of DMPC large unilamellar vesicles (LUV)

The interactions of $NaAsO_2$ with DMPC LUV were determined at the hydrophilic/hydrophobic interface and at the acyl chain hydrophobic core regions of the phospholipid bilayer by evaluation of laurdan general polarization (GP) and DPH steady-state fluorescence anisotropy (r), respectively. Table 1 shows that increasing concentrations of $NaAsO_2$ (0 to 10 mM) did significantly change neither laurdan GP nor the anisotropy values in DMPC LUV at 18 °C and 37 °C.

3.3. Differential scanning calorimetry (DSC) studies of phospholipid model systems

In the thermal range of 10–40 °C, the pure DMPC liposomes exhibit a strong and sharp main-transition at 24.3 °C, with an enthalpy change (ΔH) of 18.8 kJ mol^{-1}, arising from the conversion of the rippled gel phase ($P_{\beta'}$) to the lamellar liquid-crystal L_α phase. The transition is reversible and the shape of the peak is roughly symmetrical, with only a slight skewing toward lower temperatures. Thermodynamical parameters found for DMPC are concurrent with the literature data [18]. In Fig. 2, DSC thermal response of pure DMPC and DMPC containing 1 mM to 20 mM $NaAsO_2$ is shown. Clearly, at all the concentrations studied no drastic changes in the thermotropic behaviour of this zwitterionic phospholipid were observed. A set of calorimetric data relative to the heating and cooling processes of DMPC liposomes in $H_2O/NaAsO_2$ mixtures with increasing amounts of $NaAsO_2$ is given in Table 2.

The representative high-sensitivity DSC heating thermograms obtained for pure DMPE multibilayer vesicles and binary

Table 2
Thermodynamic parameters of the main phase transition of pure fully hydrated DMPC multilamellar liposomes and DMPC/$NaAsO_2$ mixtures determined from heating and cooling scans collected at a heating (cooling) rate of 1 °C min^{-1}

Compound		Heating			Cooling		
	Concentration [mM]	ΔH [kJ/mol]	ΔS [kJ/kmol]	T_m [deg]	ΔH [kJ/mol]	ΔS [kJ/kmol]	T_m [deg]
DPMC		19.3	0.067	24.3	18.4	0.063	23.5
+$NaAsO_2$	1	21.8	0.071	24.2	20.5	0.071	23.3
	5	21.0	0.071	24.2	19.7	0.067	23.4
	20	20.6	0.071	24.2	19.7	0.067	23.4

mixtures of DMPE and $NaAsO_2$ at various arsenic concentrations are shown in Fig. 3. In the absence of any additives fully hydrated DMPE bilayers underwent one thermotropic phase transition in the temperature range 25 °C to 80 °C; the highly cooperative main transition ($L_\beta \rightarrow L_\alpha$ phase transition) occurred at 50.2 °C with ΔH of 26.0 kJ mol^{-1}, which corresponds to the gel-to-liquid-crystal transition. Here, the transition temperature corresponds to the transition peak at the maximal peak height and the transition enthalpy corresponds to the integrated area under the peak divided by the lipid concentration. The results for the thermodynamic data of the pure DMPE are in agreement with previous reports [19]. Considering the DSC profiles depicted in Fig. 3, it is clearly seen that incorporation of $NaAsO_2$ markedly alters the thermotropic behaviour of DMPE bilayers. Generally, arsenic induces the broadening of the phospholipid main transition, which may reflect a reduction of the cooperativity between the lipid acyl chains. This, together with the lowering of the transition temperature, is an indication that the presence of arsenic perturbs DMPE multibilayers, both in the gel L_β and in the liquid-crystalline L_α states. With the highest $NaAsO_2$ content (20 mM), the shape of the main transition peak is also modified by an additional shoulder. Apart from this, in the lower temperature range additional peak appears which could suggest the concomitant presence of

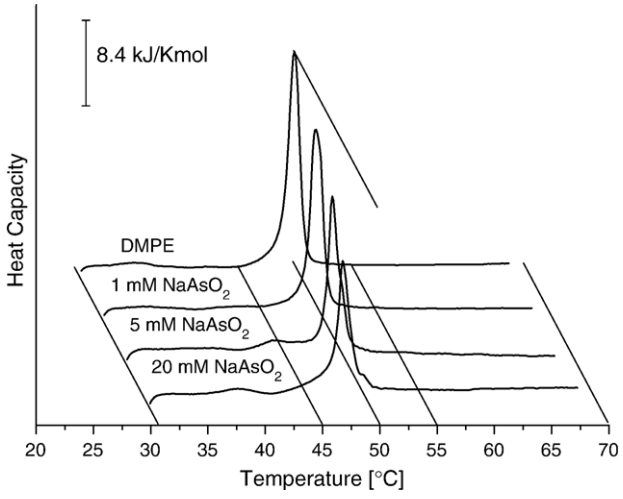

Fig. 3. Representative DSC heating curves obtained for multilamellar DMPE liposomes containing different concentrations of $NaAsO_2$. Scans were obtained at a heating rate of 1 °C min^{-1}.

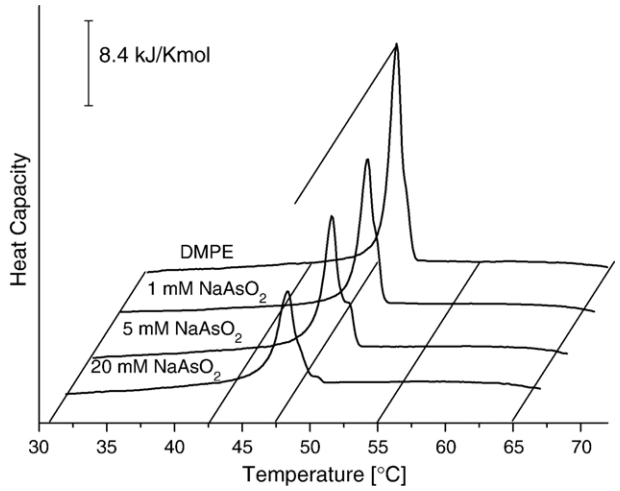

Fig. 4. Representative DSC cooling curves obtained for multilamellar DMPE liposomes containing different concentrations of $NaAsO_2$. Scans were obtained at a cooling rate of 1 °C min^{-1}.

more than one phase. Moreover, the thermograms obtained after addition of $NaAsO_2$ higher concentrations (≥ 5 mM) are skewed towards lower temperatures, indicating that arsenic distributes preferentially into liquid-crystalline regions of the membrane.

The thermotropic behaviour of pure DMPE liposomes as well as the DMPE liposomes containing $NaAsO_2$ observed during the successive cooling scans (after 10 min of equilibration at 70 °C) in the temperature range from 70 °C to 30 °C (Fig. 4) shows the peak characteristic of the $L_\alpha \rightarrow L_\beta$ transition. However, the transition pathways in heating and cooling display significant hysteresis arising from both the finite response time of calorimeter as well as from the different lateral mobility of acyl chains in the gel and in the liquid-crystal phase. The lower excess heat capacity values recorded in cooling direction may indicate that molecular rearrangements have substantially longer response time. The pattern observed for cooling of the DMPE/$NaAsO_2$ systems is essentially similar to the corresponding heating one with a progressive broadening of main transition peaks as well as their shift to the lower temperatures. However, the high temperature shoulder at about 48.5 °C is clearly observed for ≥ 5 mM arsenic concentration. The effectiveness in thermotropic phase transition perturbations exerted by various concentrations of $NaAsO_2$ was further analyzed in terms of thermodynamic parameters. Fig. 5 presents values of transition temperature, enthalpy and molar heat capacity, determined for DMPE/$NaAsO_2$ systems on the basis of heating and cooling scans. The main transition temperature appears to be noticeably affected in the presence of $NaAsO_2$ molecules and as a general feature a lowering of its value is observed. Dependencies obtained by plotting T_m, determined on the basis of heating scans as a function of $NaAsO_2$ content are shown in Fig. 5a and have an almost linear character. Interestingly, the range of changes observed for phase transition as a consequence of $NaAsO_2$ presence remains the same both for heating and cooling scans. The decrease of gel–liquid crystalline phase transition temperature generally observed in the DMPE/$NaAsO_2$ mixtures may indicate a distortion of the favorable packing of the lipid molecules in a pure DMPE multibilayer. Changes in the maximum heat capacity corresponding to the gel–liquid crystalline transition of DMPE liposomes containing varying proportions of $NaAsO_2$ are illustrated in Fig. 5b for heating and cooling scans. It is

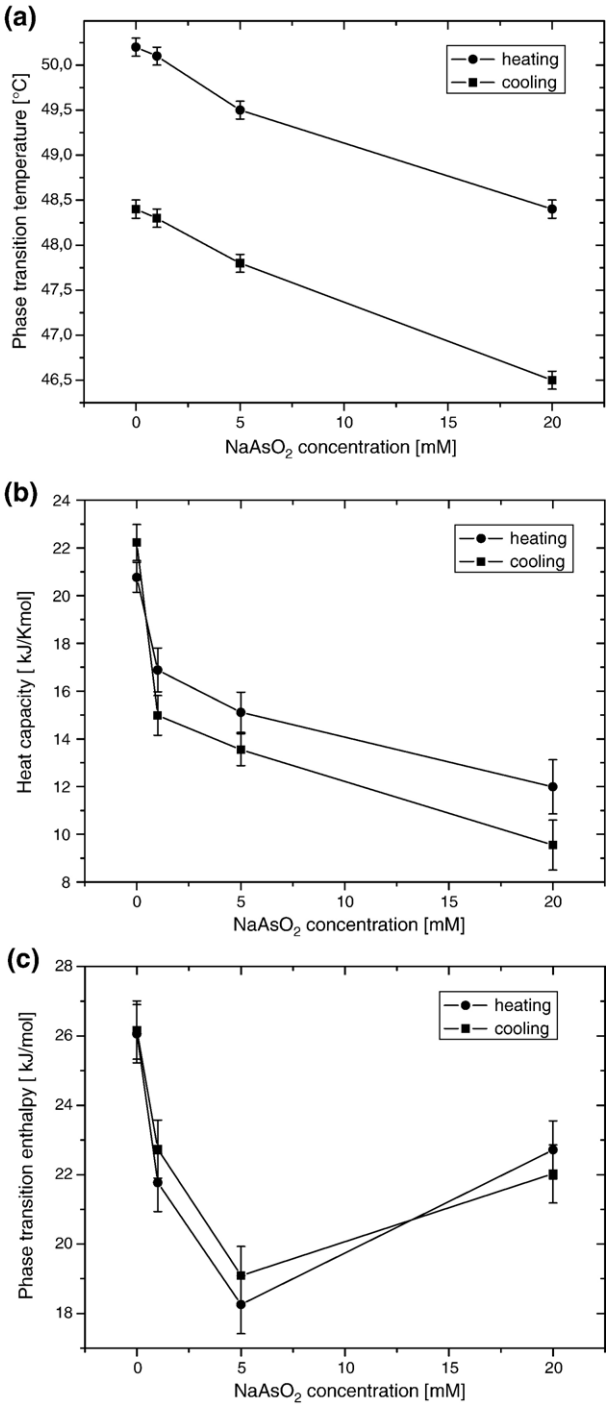

Fig. 5. The effect of different concentrations of $NaAsO_2$ on the temperature (a), height (b) and enthalpy (c) of DMPE main transition peak obtained during the DSC heating and cooling measurements.

remarkable that the presence of $NaAsO_2$ in the lipid multibilayer causes the significant reduction in height of DMPE main transition peak, which is easily observed even at a concentration as low as 1 mM. A decrease of maximum heat capacity is accompanied by an increase of half-height width ($\Delta T_{1/2}$) of the peak, which reflects the decrease in the cooperativity of the system. As a general feature $\Delta T_{1/2}$ increases with the $NaAsO_2$ content especially in cooling scans (Fig. 4). Enthalpies calculated from the endotherms for the main transition of phospholipid decrease in the presence of $NaAsO_2$ in liposomes at concentrations ≤ 5 mM to 18.4 kJ mol^{-1} and increase to a value corresponding to that obtained for a sample with 1 mM concentration of arsenic (Fig. 5c). The calorimetric profiles of the samples containing higher proportions of arsenic are not easily quantified, as it is difficult to resolve the main transition peak from the shoulder. Taking together the heats corresponding to both components one obtains the value of enthalpy of the order of 17.6–21.4 kJ mol^{-1} K^{-1}, which is slightly lower than in the case of the pure phospholipid. Enthalpies differing about 2.1 kJ mol^{-1} were measured for corresponding systems in heating and in the cooling cycles.

4. Discussion

Erythrocytes are the first target site of arsenic compounds when they attack the body after systemic absorption, and the cell membrane is the frontier facing the attack [2]. In order to understand the nature of these interactions X-ray diffraction, fluorescence and calorimetric studies were carried out on DMPC and DMPE bilayers, which represent phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively [5,6]. These phospholipids differ only in their terminal amino groups, these being $^{+}N(CH_3)_3$ in DMPC and $^{+}NH_3$ in DMPE. Moreover, both molecular conformations are very similar in their dry crystalline phases [17] with the acyl chains mostly parallel and extended, and the polar groups lying perpendicularly to them. In fact, the experimental studies on the interaction of $NaAsO_2$ with DMPC bilayers showed that the lipid was not structurally perturbed by $NaAsO_2$. However, the observations by X-ray diffraction and calorimetry that $NaAsO_2$ interacts with DMPE and perturbs its bilayer structure are rather amazing.

DSC results of DMPC measurements presented in this work show no significant changes of the $L_\beta - L_\alpha$ phase transition, even at concentrations of $NaAsO_2$ as high as 20 mM. Neither significant transition broadening, nor shift of the phase transition temperature, or any traces of lipid separation phenomenon, were noticed from DSC thermograms of DMPC in the presence of the arsenic compound. These observations indicate the absence of strong molecular interactions promoted by $NaAsO_2$ molecules at the level of phospholipid acyl chains in DMPC bilayer. The evidence confirms the fluorescence anisotropy and the X-ray diffraction data that no significant structural perturbations of DMPC bilayers were induced by arsenite.

Although $NaAsO_2$ has no influence on the structural and dynamic properties of DMPC, however it is able to modify the DMPE model membranes. Since both lipids possess the same fully saturated acyl chains of 14 carbon atoms, the reason of a dissimilar $NaAsO_2$ effect is probably related to the structural differences in their headgroup regions. It seems that $NaAsO_2$ exhibits greater affinity towards the surface of phosphatidylethanolamine than towards phosphatidylcholine. This behaviour can be attributed to the increased polarity of DMPE liposome surface, consequent to the unshielded positive charge on the nitrogen atom. The phases below the main transition temperatures are the ripple phase and the flat gel phase for lecithins such as DMPC and phosphatidyletanolamines, respectively. This difference is correlated with a structural difference in the gel phases [20]. The conformationally ordered chains of DMPE are aligned along the bilayer normal, while for the saturated DMPC, the hydrocarbons are tilted with the respect to the bilayer normal. The presence of considerable amounts of water between the bilayers makes the interbilayer interactions rather small. When the hydrocarbon chains 'freeze' at the T_m, the water is expelled bringing neighboring bilayers closer together and strengthening the interactions between them. As previous studies revealed in the case of PEs, the adjacent lipid molecules in the bilayer interact by hydrogen bonding between the amino (hydrogen-donating) and phosphate (hydrogen-accepting) groups resulting in the fact that the gel-to-liquid-crystal phase transition occurs at higher temperature than the corresponding PCs. Presumably, these intermolecular H-bonding between the lipid head groups that involves the release of hydrogen-bonded water from the bilayer surface, rather than interaction with water molecules, stabilizes PE bilayer as suggested by Kodama [21]. Moreover, the strong interaction between the PE headgroups (both intra and interbilayer) inhibit the lipid hydratation for all temperatures $T < T_m$.

Arsenite, as an amphiphilic solute, is able to distinctively modulate the properties of PEs. Such modulation could be ascribed to the so-called Hofmeister mechanism reviewed in [22] and discussed in terms of indirect effects of the solute induced through changes in the solvent properties. Accordingly, since arsenite (with its three hydrogen bonding positions) tends to disrupt the hydrogen-bonded water structure, it can be classified as a chaotrope. Bearing in mind that the L_α phase has the largest surface area among the three phases formed by the hydrated phospholipids, arsenite as a water structure breaker should be able to induce an enlargement of interfacial area of the lipid. This has good corroboration in the thermotropic behaviour of DMPE/$NaAsO_2$ mixtures. A decrease of the T_m value in the concentration-dependent manner that was observed in the presence of arsenic molecules in DSC thermograms of DMPE (Fig. 5a) may be due to a loosening of the hydrogen bonds network at the interface. This trend is in agreement with experimental data from spectrophotometry [23] and calorimetry [24]. A concentration-dependent downshift of T_m was previously observed for DMPE multibilayers in the presence of different chaotropics in the range of 0.1–1 M. Indeed, anions that were arranged in the series: $I^- < SCN^- < ClO_4^-$ for the Hofmeister effect, (i.e. dramatic disruption of the structure of water layers at the interface of the bilayers and the marked

lowering of the strength of the hydrogen bonds at the lipid interface), were also shown to induce the T_m decrease in the same order [25].

Independently of the arsenite effect on water structure, it seems reasonable to examine the significance of direct interaction between arsenite and phospholipid molecules. Taking into account the large polarizability, a specific adsorption of the arsenite anion at the interface should be considered. It is worth noticing that adsorption of chaotropic anions to PE has been reported on the basis of electrophoretic mobility measurements [26] but ^{13}C and ^{31}P-NMR data provided no evidence for binding of the chaotropic agents to the membrane surface of soy PE [27]. Arsenite anion may form hydrogen bond directly with the PO_4^- group of PE molecules in competition with hydrating water molecules as well as amino groups. Reduction of the effective PE–PE head group interaction should leave phosphorous group free and hence its mobility should increase as well as the interfacial area of lipid. Thus, the direct insertion of arsenite into the head group region can also explain the experimental observation of T_m. Such reasoning is in accordance with molecular dynamic simulations data [28] showing that the larger anions are more hydrophobic and they prefer the bilayer interior, explained by a less-structured hydratation shell. Large anions can penetrate even more deeply than Na^+ ions to within 12.5 Å of the bilayer center and unlike Cl^- and Na^+ they are energetically stable in a hydrophobic environment. Arsenite is present as $As(OH)_3$ at the almost neutral pH in which experiments were carried out [29]. Due to the non-bonded electron pair localized on the As atom, it is likely to present a polar pyramidal conformation. Therefore, it is hardly plausible that it can penetrate so deeply into the hydrophobic core of the phospholipid bilayer as to be able to affect the lateral interaction between the apolar chains, which is supported by the values of transition enthalpy. Most likely, the arsenite molecules interact with DMPE positively charged terminal amino groups disrupting their packing arrangement as discussed above, but not with those of DMPC which instead of hydrogen are provided with bulky methyl groups. This offers the explanation for the lack of effects on DMPC main phase transition observed in our DSC measurements, fluorescence studies as well as X-ray diffraction data. Nevertheless, in previous reports it was shown that on increasing concentrations (>0.5 M) chaotropic anions can suppress the pretransition of 16-carbon disaturated lecithin (DPPC) as well as stabilize its fluid state, the latter effect being stronger in DMPE. It is well established that among monovalent ions, the chaotropics are the most effective in influencing the properties of neutral lipid bilayers. However, their effects are much more evident at high concentration. The present results pointed to the fact that the chemical nature of the neutral lipid polar head group plays a crucial role in determining the type of effects that chaotropics are able to produce. In conclusion, these results indicate that $NaAsO_2$ interacts with a class of lipid present in the human erythrocyte membrane. No such information has been so far reported in the literature. On the contrary, arsenite interactions with membrane lipids have been disregarded [2].

Acknowledgements

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