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Biophysical Chemistry 132 (2008) 1 – 8

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Monomethylarsonate (MMA^V) exerts stronger effects than arsenate on the structure and thermotropic properties of phospholipids bilayers

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Received 18 July 2007; received in revised form 25 September 2007; accepted 26 September 2007
Available online 3 October 2007

Abstract

Methylation of inorganic arsenic has been regarded as a detoxification mechanism because its metabolites monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) are supposed to be less toxic than inorganic arsenite and arsenate. In recent years, however, this interpretation has been questioned. Additionally, there are insufficient reports concerning the effects of arsenic compounds on cell membrane structure and functions. With the aim to better understand the molecular mechanisms of the interaction of MMA^V and arsenate with cell membranes, we have utilized molecular models consisting in bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of many cell membranes including that of the human erythrocyte. The capacity of MMA^V and arsenate to perturb the bilayer structures of DMPC and DMPE was evaluated by X-ray diffraction; the modifications of their thermotropic behavior were followed by differential scanning calorimetry (DSC), while DMPC large unilamellar vesicles (LUV) were studied by fluorescence spectroscopy. It was found that MMA^V and arsenate did not structurally perturb DMPC bilayers; however, DMPE bilayers did suffer structural perturbations by MMA^V. DSC measurements also revealed that DMPE's thermotropic properties were significantly affected by arsenicals, where MMA^V was more effective than arsenate, whilst only slight modifications were observed in the case of DMPC-MMA^V system.

Keywords: Monomethylarsonate; Arsenate; Phospholipid bilayer; Phase transition

1. Introduction

Arsenic (As) is a metalloid with oxidation states of −3, 0, +3 and +5. It is present in the environment in a wide variety of different chemical forms. Although it occurs naturally, it is also introduced through use as herbicides and as a byproduct of a number of industrial processes. Drinking water contamination by arsenic is a major health problem. In well oxygenated waters, the most common species present is the pentavalent arsenic, while under reducing conditions such as in ground waters the trivalent species are the predominant forms. Most cases of human toxicity from arsenic have been associated with exposure to inorganic arsenic [1]. Each of its different chemical forms posses different physical and chemical properties, toxicities, mobilities, etc., and it is only when these are known that their molecular mechanisms of toxicity can be understood. Chronic exposure to arsenic causes a wide range of toxic effects on the skin including keratoses, hyperpigmentation, and cancer

Abbreviations: MMA^V, disodium monomethylarsonate; DMA^V, monosodium dimethylarsonate; DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; Laurdan, 6-dodecanoyl-2-dimethylaminonaphthalene; MLV, multilamellar vesicles; LUV, large unilamellar vesicles; *r*, fluorescence anisotropy; GP, fluorescence generalized polarization; DSC, differential scanning calorimetry; T_m, temperature of main transition.

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doi:10.1016/j.bpc.2007.09.012

[1,2]. More recently, studies have raised the possibility that ingestion of arsenic in drinking water is also a cause of several internal cancers including bladder, kidney, liver and lung cancers [3–6]. Epidemiological studies have also demonstrated that arsenic exerts other adverse effects such as diseases of the peripheral vascular, cardiovascular and cerebrovascular systems, hypertension, diabetes mellitus, goiter, hepatomegaly, respiratory system dysfunctions, injury to the peripheral and central nervous systems, malformation, growth retardation and death [2,7,8]. A basic tenet is that the acute toxicity of trivalent arsenic is greater than pentavalent arsenic [7]. Despite its well-known toxicity, arsenic derivatives are used in traditional Chinese medicine to devitalize the pulp of diseased teeth and in regimes for psoriasis, rheumatic diseases, and syphilis [9]. Moreover, arsenic was rediscovered recently by modern medicine and is now in use worldwide in the form of arsenic trioxide (As_2O_3) in patients with relapsed or refractory acute promyelocytic leukemia (APL) [10].

Historically, methylation of inorganic arsenic has been regarded as a detoxification mechanism because its metabolites monomethylarsonic acid (MMA^v) and dimethylarsinic acid (DMA^v) are supposed to be less toxic than inorganic arsenite and arsenate [11]. In recent years, however, this interpretation has been questioned [12–14]. Additionally, an study on arsenic-exposed humans concluded that individuals with the coexistence of high homocysteinemia level and high urinary MMA may exacerbate atherosclerosis formation caused by arsenic in the carotid artery in humans [12]. Thus, there is increasing debate on whether the metabolic methylation of arsenic should be considered a detoxification process.

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 [15–17]. The most important function of any biological membrane is to serve as a general diffusion barrier. Therefore, its structure and functions are susceptible to alterations as a consequence of interactions with chemical species. In the course of in vitro system search for the toxicity screening of chemicals of biological relevance, different cellular models have been applied to examine their adverse effects. With the aim to better understand the molecular mechanisms of the interaction of MMA^v and arsenate with cell membranes, we have utilized molecular models. They consisted of multibilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of many cell membranes including that of the human erythrocyte [18,19], multilamellar vesicles (MLV) of DMPC and DMPE, and large unilamellar vesicles (LUV) of DMPC. The capacity of MMA^v and arsenate to perturb the bilayer structures of DMPC and DMPE was evaluated by X-ray diffraction, the modifications of their thermotropic behavior were followed by differential scanning calorimetry (DSC), while DMPC LUV were studied by fluorescence spectroscopy.

2. Materials and methods

2.1. X-ray diffraction studies of DMPC and DMPE multilayers

The capacity of MMA^v and arsenate to perturb the structures of DMPC and DMPE multibilayers was evaluated by X-ray diffraction. Synthetic DMPC (lot 80H-8371, MW 677.9), DMPE (lot 084K-1676, MW 635.9) from Sigma (MO, USA), disodium monomethylarsonate (MMA^v) (Lot 320-136A, MW 291.9) from Chem Service (West Chester, PA, USA) and disodium arsenate (Lot 427382) from Merck (Darmstadt, Germany) 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 μl of (a) distilled water and (b) aqueous solutions of MMA^v and arsenate in a range of concentrations (1 μM to 10 mM). The specimens were X-ray diffracted after 30 min. incubation at 37 °C and 60 °C with DMPC and DMPE, respectively. 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 an MBraun PSD-50 M linear position-sensitive detector system (Garching, Germany); no correction factors were applied. The experiments were performed at 18 °C ± 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 MMA^v and arsenate 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 because it provides a measure of the rotational diffusion of the fluorophore. In the case of hindered rotations as in membranes, the anisotropy is related to the lipid acyl chain packing order as the rotational diffusion is restricted within a certain region such as a cone. Laurdan, an amphiphilic probe, has a high sensitivity of excitation and emission spectra for the physical state of membranes. With the fluorescent moiety within a shallow position in the bilayer, laurdan fluorescence spectral shifts provide information about the polarity and/or molecular dynamic of its fluorophore environments at the glycerol backbone level of the phospholipid polar head groups. The quantification of the laurdan fluorescence shifts were effected using the generalized polarization GP concept [20].

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 0.45 μm 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 steady-state and time-resolved spectrofluorometer (ISS Inc., Champaign, IL, USA) 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: $r=(I_{\parallel}-I_{\perp})/(I_{\parallel}+2I_{\perp})$ where $I_{\parallel}$ and $I_{\perp}$ are the corresponding vertical and horizontal emission fluorescence intensities with respect to the vertically polarized excitation light [21]. 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 nm and 490 nm, which correspond to the emission maxima of laurdan in the gel and liquid-crystalline phases, respectively [22]. MMA^v and arsenate were incorporated in LUV by addition of adequate (0.1 M) aliquots of their solutions in order to obtain the different concentrations used in this work. The samples thus prepared were then incubated at 37 °C for ca. 15 min. Blank subtraction was performed in all measurements using labeled samples without probes. The data presented in Tables 1 and 2 represent mean values and standard error of ten measurements in two independent samples. Unpaired Student's t-test was used for statistical calculations.

2.3. Differential scanning calorimetry (DSC) studies of DMPC and DMPE multilamellar liposomes (MLV)

The effect of MMA^v and arsenate 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 1 h. Dry lipid films were suspended with 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 MMA^v were added to obtain final 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 applying 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 was used as a reference. The cells were sealed and equilibrated for about 20 min below starting temperature of the run. The scan range was 10 °C–40 °C for DMPC and 25 °C–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 CpCalc software package supplied by CSC (Provo, UT, USA). The heat capacity of each transition is given by $C_p(T)=dH(T)/dT$, while the entropy of transition is defined as: $\Delta S(T)=\Delta H(T_m)/T_m$. The accuracy for the main phase transition temperature and enthalpy was ± 0.1 °C and ± 0.8 kJ/mol, respectively.

Table 1
Effect of monomethylarsonate (MMA^v) ($Na_2CH_3As_2O_3$) on the anisotropy (r) of DPH and on the generalized polarization (GP) of laurdan embedded in DMPC LUV at 18°C and 37°C

MMA^v (mM)	r (DPH) 18 °C	GP (Laurdan) 18 °C	r (DPH) 37 °C	GP (Laurdan) 37 °C
0	0.327±0.001	0.548±0.003	0.084±0.002	−0.057±0.002
0.1	0.328±0.001	0.540±0.001	0.086±0.002	−0.056±0.002
1	0.323±0.001	0.540±0.002	0.090±0.002	−0.058±0.001
10	0.329±0.001	0.541±0.002	0.088±0.002	−0.061±0.002
20	0.331±0.001	0.541±0.002	0.087±0.002	−0.062±0.002

Table 2
Effect of arsenate (Na_2HAsO_4) on the anisotropy (r) of DPH and on the generalized polarization (GP) of laurdan embedded in DMPC LUV at 18 °C and 37 °C

Na_2HAsO_4 (mM)	r (DPH) 18 °C	GP (Laurdan) 18 °C	r (DPH) 37 °C	GP (Laurdan) 37 °C
0	0.332±0.001	0.549±0.001	0.083±0.002	−0.087±0.003
0.1	0.317±0.001	0.550±0.002	0.083±0.002	−0.086±0.003
1	0.304±0.001	0.552±0.002	0.083±0.002	−0.086±0.003
5	0.305±0.001	0.552±0.002	0.083±0.002	−0.084±0.002
10	0.310±0.002	0.553±0.002	0.084±0.001	−0.080±0.003
20	0.316±0.002	0.552±0.002	0.084±0.002	−0.080±0.003

3. Results

3.1. X-ray diffraction studies of DMPC and DMPE multibilayers

Fig. 1A presents the results obtained by incubating DMPC with water and MMA^v. As expected, water altered the structure of DMPC; its bilayer repeat (bilayer width plus the width of water layer between bilayers) increased from about 56.4 Å in its dry crystalline form [23] to 64.5 Å when immersed in water, and its low-angle reflections (indicated as (a)) 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 (indicated as (b)) which corresponds to the average distance between fully

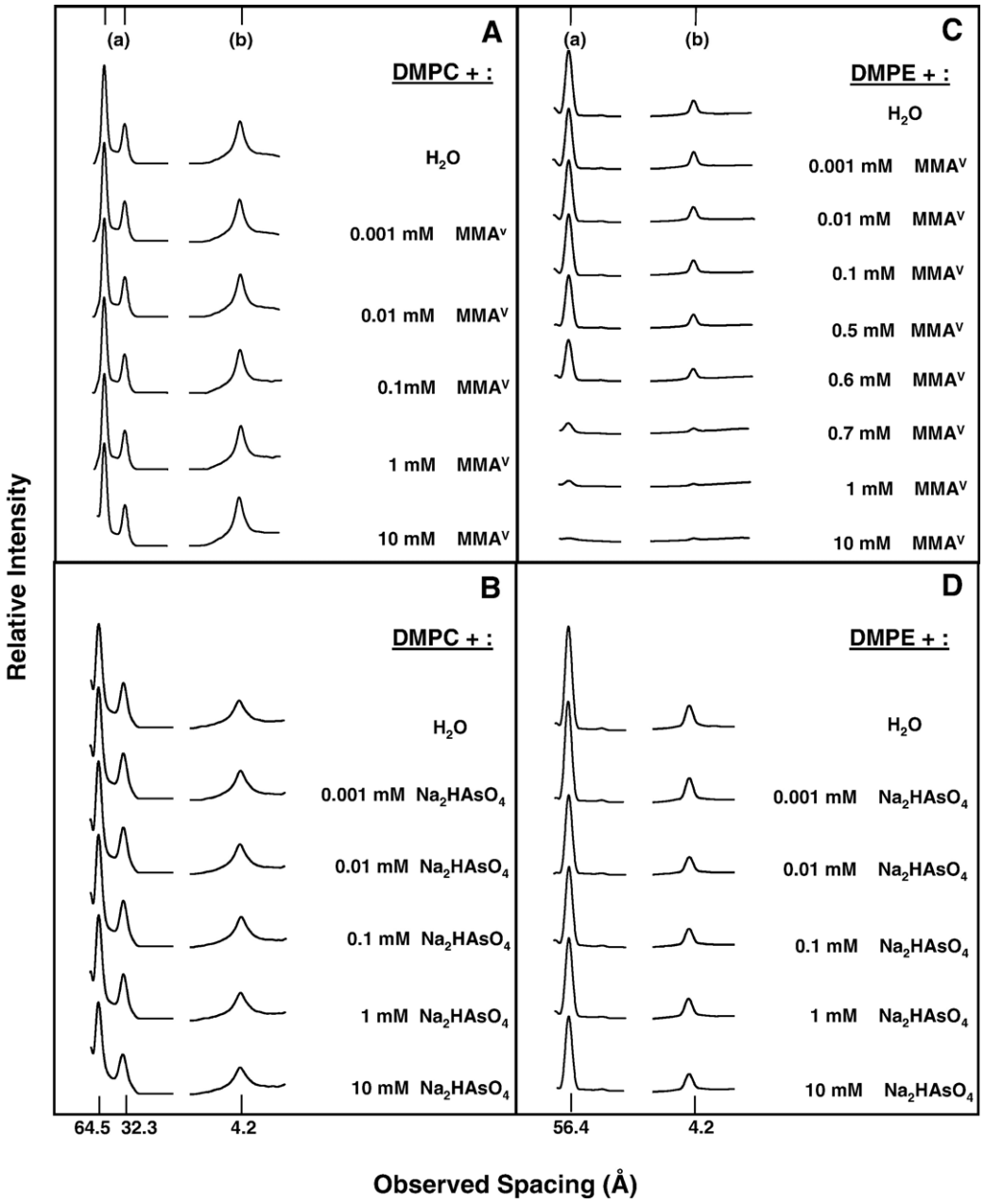

Fig. 1. Microdensitograms from X-ray diffraction patterns of dimyristoylphosphatidylcholine (DMPC) (left panel) and dimyristoylphosphatidylethanolamine (DMPE) (right panel) in water and aqueous solutions of disodium monomethylarsonate (MMA^V) (A and C) and disodium arsenate (Na_2HAsO_4) (B and D).

extended acyl chains organized with rotational disorder in hexagonal packing. These results were indicative of the more fluid state reached by DMPC bilayers, which changed from the crystalline phase to the rippled $P_{\beta'}$ gel state. Fig. 1A discloses that after exposure to MMA^V (up to 10 mM), the low-and wide-angle lipid reflection intensities practically did not show any variation. From these results, it can be concluded that MMA^V did not produce any structural perturbation of DMPC bilayers. Fig. 1B shows the results of the X-ray diffraction analysis of DMPC bilayers incubated with water and Na_2HAsO_4. As it can be observed, arseniate also did not induce any structural perturbation to DMPC bilayers. When these experiments were repeated with DMPE, 0.6 mM and higher concentrations of MMA^V caused a gradual weakening of all DMPE reflection intensities, which disappeared when 10 mM MMA^V was applied (Fig. 1C). Finally, Fig. 1D discloses that arsenate up to 10 mM did not cause any structural change to DMPE.

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

The interactions of MMA^V and Na_2HAsO_4 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 generalized polarization (GP) and DPH steady-state fluorescence anisotropy (r), respectively.

Table 3
Thermodynamic parameters of the main phase transition of pure, fully hydrated DMPC multilamellar liposomes, DMPC/Na_2HAsO_4 and DMPC/MMA^v mixtures determined from heating and cooling scans collected at a heating (cooling) rate of 1 °C min^{-1}

Compound	Concentration [mM]	Heating			Cooling		
		ΔH [kJ/mol]	ΔS [kJ/K mol]	T_m [°C]	ΔH [kJ/mol]	ΔS [kJ/K mol]	T_m [°C]
DPMC		19.3	0.067	24.3	18.4	0.063	23.5
+Na_2HAsO_4	1	20.9	0.071	24.2	20.0	0.067	23.4
	5	19.70	0.067	24.2	18.8	0.063	23.3
	20	20.0	0.067	24.2	19.7	0.067	23.4
+MMA^v	1	20.9	0.071	24.1	20.0	0.067	23.3
	5	19.7	0.067	24.1	19.7	0.067	23.3
	20	19.3	0.063	24.0	18.0	0.063	23.2

The accuracy for the main phase transition temperature and enthalpy was ± 0.1 °C and ± 0.8 kJ/mol, respectively.

The results, presented in Tables 1 and 2, indicate that in the assayed range of concentrations (0 to 20 mM), the effects of both MMA^v and arsenate on DMPC bilayers were almost negligible, results that agree with those obtained by X-ray diffraction.

3.3. Differential scanning calorimetry (DSC) studies of DMPC and DMPE multilamellar liposomes (MLV)

From the thermograms of DMPC dispersions in excess of water with increasing concentrations of monomethylarsonate and arsenate (data not shown) it appears that in the thermal range of 10 °C–40 °C, one transition can be observed in each heating scan, which is designated as transformation of the rippled gel phase ($P_{\beta'}$) to the lamellar liquid-crystal (L_α) phase. The values of the transition temperature and an enthalpy change in the absence of arsenic compounds were determined to be 24.3 °C and 18.8 J mol^{-1}, respectively. Here, the transition temperature corresponds to the transition peak at the maximal peak, and the transition enthalpy corresponds to the integrated area under the peak divided by the lipid concentration. These are in good agreement with published data [24,25]. The most obvious feature observed in the thermograms is that the presence of arsenate and MMA^v has very little effect on the thermal properties of DMPC MLV. As can be seen from the data shown in Table 3, there is no significant effect on the main phase transition temperature in either the heating or cooling scans upon incorporation of arsenate, and only a slight (not exceeding 0.3 °C) shift to the lower values during heating in case of MMA^v. Calculations of the transition enthalpy show

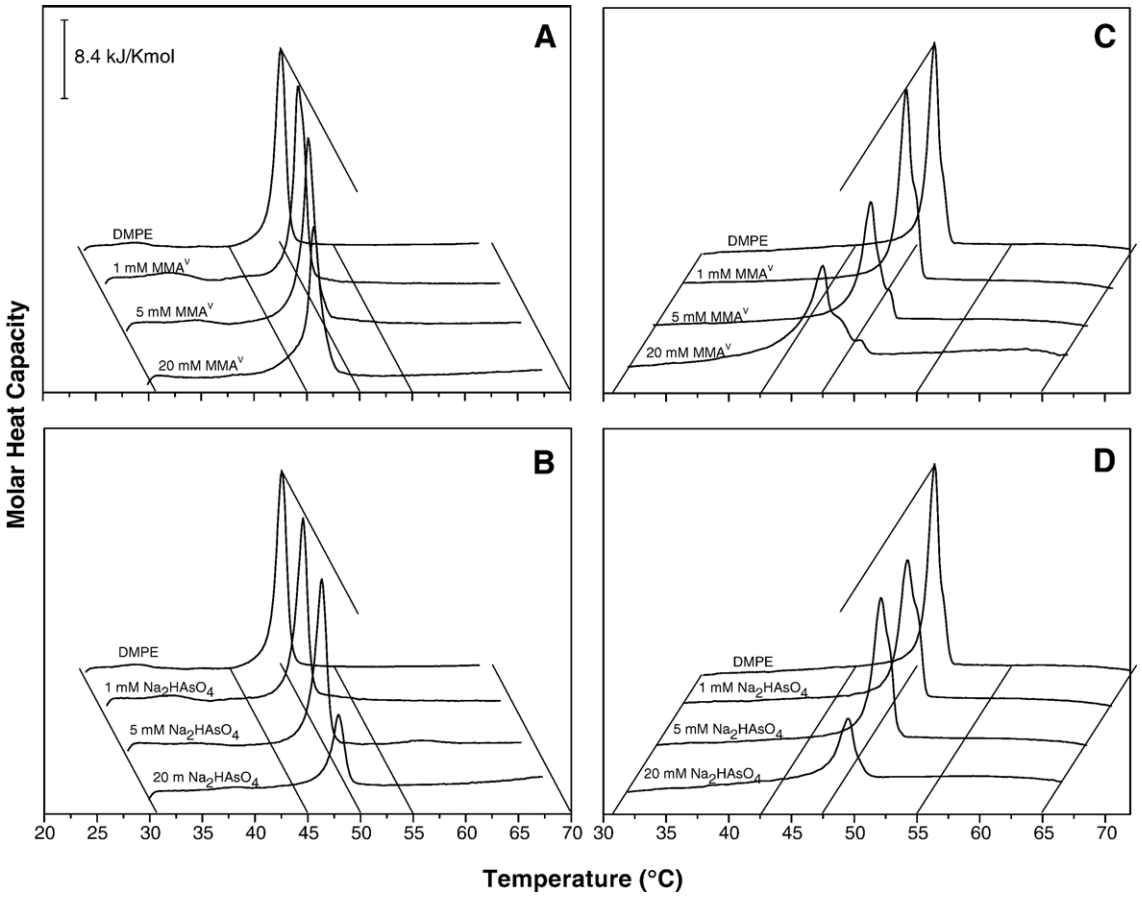

Fig. 2. Representative DSC heating and cooling curves obtained for multilamellar DMPE liposomes containing different concentrations of disodium monomethylarsonate (MMA^v) and disodium arsenate (Na_2HAsO_4). A: heating curves of MMA^v; B: heating curves of Na_2HAsO_4; C: cooling curves of MMA^v; D: cooling curves of Na_2HAsO_4. Scans were obtained at heating and cooling rates of 1 °C min^{-1}.

Table 4
Thermodynamic parameters of the main phase transition of pure, fully hydrated DMPE multilamellar liposomes, DMPE/Na_2HAsO_4 and DMPE/MMA^v mixtures determined from heating and cooling scans collected at a heating (cooling) rate of 1 °C min^{-1}

Compound	Concentration [mM]	Heating			Cooling		
		ΔH [kJ/mol]	ΔS [kJ/K mol]	T_m [°C]	ΔH [kJ/mol]	ΔS [kJ/K mol]	T_m [°C]
DMPE		26.4	0.084	50.2	26.0	0.080	48.4
+Na_2HAsO_4	1	21.8	0.075	50.2	20.5	0.075	48.2
	5	20.9	0.075	50.0	19.7	0.075	48.0
	20	20.5	0.034	49.6	19.7	0.029	47.5
+MMA^v	1	21.8	0.088	49.8	20.5	0.088	48.1
	5	20.9	0.084	48.8	19.7	0.080	47.0
	20	20.5	0.075	47.3	19.7	0.075	45.4

The accuracy for the main phase transition temperature and enthalpy was ± 0.1 °C and ± 0.8 kJ/mol, respectively.

inconsiderable variations, with MMA^v being more effective in that respect.

Aqueous dispersion of DMPE in the lamellar phase can undergo a gel-to-liquid-crystalline 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 [24,25]. Representative DSC heating traces of DMPE preparations doped with arsenate and MMA^v are depicted in Fig. 2 (A and B). The incorporation of increasing concentrations of arsenic compounds results in a progressive shift of the observed transition to lower temperatures and the lowering of the corresponding peaks. As shown in Table 4, MMA^v exhibits the most profound effect in terms of temperature shift ($\Delta T = 2.9$ °C and 3.0 °C in heating and cooling scans, respectively). On the other hand, the most significant reduction in peak height is revealed for the sample containing arsenate at 20 mM concentration. In the cases of the two arsenic compounds, a broadening of the peak transition is observed and the enthalpy values decrease as the concentrations increased from 1 to 20 mM. Interestingly, as shown in Fig. 2 (C and D), the pattern displayed in cooling scans differs markedly from that in the heating ones. For DMPE-arsenate mixture, an additional shoulder is noticeable in a higher temperature region of the thermograms and an even more complicated feature (two distinctive shoulders) is displayed in the case of DMPE-MMA^v samples.

4. Discussion

The experimental studies on the interaction of MMA^v and Na_2HAsO_4 with DMPC bilayers by X-ray diffraction and fluorescence spectroscopy showed that the lipid was not structurally perturbed by any of them. On the other hand, DMPE bilayers suffered structural perturbations by MMA^v. A somewhat similar result was observed with arsenite ions, which interacted with DMPE but not with DMPC [26]. The explanation might lie in the fact that in both DMPE and DMPC the adjacent lipid molecules in the bilayer are held by electrostatic interactions and hydrogen bonds between neighboring amino and phosphate groups. Given DMPE's smaller $^+NH_3$ group, and therefore higher effectively charged than the bulkier $^+N(CH_3)_3$ of DMPC, it preferentially interacts with MMA^v ions with the consequent disruption of the whole DMPE bilayer structure. On the other hand, given that MMA^v has a methyl group that confers it an amphiphilic character, it might then locate in the lipid precisely between the polar head groups and the initial part of the acyl hydrophobic chains with the ensuing perturbation of both DMPE regions.

Among the effects that may explain the changes in the thermotropic behavior of liposomes upon incorporation of additives, there are two which seem to be of importance for arsenic: (i) specific and direct interaction between phospholipids and polar centers on foreign substances resulting from the presence of the polar head group in the phospholipids, and (ii) the ability to modify the interfacial energy of the lipid–water interface and induce the change in water structure due to the presence of chaotropic solutes. It seems that in DMPE lipid systems treated with arsenicals, the observed behavior emerges from the simultaneous presence of both effects discussed above. Moreover, the differences found between the effects caused by Na_2HAsO_4 and MMA^v cannot be only due to the differences of their chaotropic effect on the water structure. Although arsenate destabilizes the characteristic three-dimensional structure of water more than MMA^v, the T_m decrease is probably partially compensated by the setting up of direct interactions with the polar centers of lipid molecules.

Considering the calorimetric features, apart from the main transition peak, additional shoulder (or two peaks in the case of MMA^v), are apparent in the cooling curves of DMPE/arsenicals systems. Such thermotropic behavior can be explained in terms of the existence of spatial inhomogeneities or phase separation. We can assume that it may result from the lower partition coefficient of arsenic compound at lower temperatures, which requires some of the arsenic molecules to be transferred back into the aqueous phase. Transient formation of arsenic domains of high concentration within the bilayer during the cooling probably leads to spatial inhomogeneities.

The question of a slight penetration of arsenicals into the bilayer interior can also be examined. Judging from the value of partition coefficient between octanol and water (log P), which is used as a measure of molecular hydrophobicity, both arsenic compounds are hydrophilic in nature. Hence, it is more likely that their molecules get intercalated between the phospholipid polar groups. Indeed, it was found that the transition enthalpy decreased with increasing arsenic concentration (Table 4), indicating that both arsenate and MMA^v are unable to increase the acyl chain order. A good confirmation of this fact is also offered by the data from our fluorescence anisotropy measurements. This is consistent with a more superficial interaction of As with both phospholipids. Thus, we can conclude that arsenicals are located closer to the interfacial region than to the hydrophobic core of the bilayer. One possible mode of insertion is the location of the arsenate polar hydroxyl group near the phospholipid carbonyl groups with which it could form hydrogen bonds. On the other hand, it is well known that the

substitution of a hydroxyl group for a methyl group in arsenate can have dramatic effects on molecular properties of the compound. Its dipole moment, as well as the ionization energy, increases while the molecular volume available for the delocalization of the electron density decreases. Since there is generally a direct correlation of the dipole moment and log *P*, it can be supposed that the log *P* increases with addition of a methyl group to Na_2HAsO_4, and this is the case [27]. However, with the log P value of −5.5, although significantly higher than that found for arsenate (−7.3), MMA^V must still be regarded as a hydrophilic compound. It is worth mentioning that for choline log P was found to be −5.16. Consequently, following the idea of Bonora et al. [28] we may assume that the MMA molecules can form in the vicinity of the bilayer surface a 'less hydrophilic' surrounding shell that interacts with the lipid head groups, involving in some way, apart from direct interactions, the apolar region of the lipid chains closest to the lipid interface.

The DSC results also indicate that the effect of arsenate and its methyl derivative MMA^V is pronounced in DMPE but not in DMPC, proving the strong effect of these arsenical compounds on the morphological organization of the membrane. A similar observation was previously made for sodium arsenite [26]. The key point seems to lie in that respect in a different degree of hydration characteristic for the surfaces of the membranes composed of these two classes of lipids. Hydrogen bonding between the lipid head groups in DMPE results in a more tightly packed and less hydrated structure that could resist the penetration of arsenicals beyond the superficial membrane interface. The decreased main transition temperature value of the arsenic-doped DMPE dispersion suggests that the presence of the modifier decreases the head group-head group interaction of the neighboring DMPE molecules. This effect is due to hydrogen bonding and/or electrostatic interactions between the polar groups of arsenic and DMPE molecules, which reduce the effective interaction between lipids in the head group region. Interactions between arsenicals and lipid molecules involve As=O groups and hydroxyl groups in the case of MMA^V and arsenate, respectively. Being classified as weakly donating the CH_3 group has also being shown to affect the stability of As–OH (As–ONa) bonds by its inductive effect [29]. The transfer of a negative charge from the methyl group increases the electron density on the central arsenic atom and reduces the ionic interaction of AsOH (AsONa).

In the case of the more polar ethanolamine group, the main role seems to be played by the formation of strong polar interactions involving the As=O groups, leading to an effective dehydration. In contrast, in the presence of the less polar choline liposome surface, a hydration–dehydration equilibrium between H_2O and arsenicals takes place, modulated by the different hydrophobicities of arsenate and MMA^V. In an attempt to explain the ability of arsenate to perturb the thermotropic behavior of DMPE not evidenced in DMPC bilayer, one could also consider self-association of Na_2HAsO_4 with intermolecular bonding of its polar hydroxyl groups that might result in a more hydrophobic behavior of As. Interestingly, such differences are clearly seen at high concentrations when self-association is more probable to occur. The fact that it could be enhanced in DMPE bilayers by intermolecular hydrogen bonding between the lipids together with hydrophobic mismatch may account for the observed effects.

It has been previously shown that in forming toxic and carcinogenic arsenic species, reduction from the pentavalent state to the trivalent state is of major importance. However, as suggested by Kitchin [30] for inorganic arsenic, oxidative methylation followed by reduction to trivalency may be an activation rather than a detoxification pathway, which would be particularly true for arsenate. In the light of these findings, the results of our studies, even when performed on model membranes that constitute a simplified system, are worth taking into account in the toxicological considerations because they open up the possibility that methyl derivatives of arsenate could exert more toxic effects through their interactions with the lipids of membrane bilayers.

Acknowledgements

This work was supported by FONDECYT 1060990, UDEC-PUCV 205.024.031-1S and Ordered Research Project PBZ-KBN-110/P04/19.

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