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Thermal behavior of novel non-sonicated arsonolipid-containing liposomes

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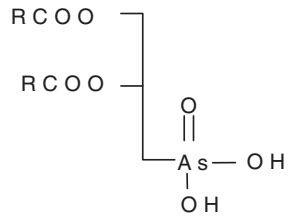
Abstract

The thermal properties of novel arsonolipid-containing liposomes in PBS pH 7.4 and in water in absence and presence of Ca^{2+} ions are reported. Liposomes composed of arsonolipids with different acyl chains (C_{12}, C_{16} and C_{18}) were prepared by the one step method. Microcalorimetry results showed that (i) the thermotropic transitions of arsonoliposomes (in PBS, pH 7.4, and in water) increase as a function of arsonolipid fatty acyl chain length, (ii) arsonoliposomes of long fatty acyl chain arsonolipids (C_{16} and C_{18}) showed higher enthalpy and transition temperature in the buffer compared to those observed in water (for arsonoliposomes of C_{12}-fatty acyl chain arsonolipid, the order was reversed which might be attributed to their different structure), and (iii) the presence of 2 mM $CaCl_2$ has more pronounced effects on the thermal properties of arsonoliposomes in distilled water than in buffer, which suggests that the ionic strength of the dispersion medium plays an important role in determining the thermal properties of arsonoliposomes.

Keywords: Arsonolipids; Arsonoliposomes; Liposome; Phase transition; Microcalorimetry

In a recent publication, more than 100 arsenolipids and their analogues and derivatives, which are naturally occurring, were reported and their potential applications in biological processes were thoroughly discussed [1]. Arsonolipids are analogues of phosphonolipids in which P has been replaced by As in the lipid head group (Fig. 1) [2,3]. The possibility of combining the demonstrated antileukemic activity of arsenic containing compounds [4] with the ability of liposomes to deliver cytotoxic drugs in an activity-enhancing and toxicity-reducing manner was the rationale for developing arsonolipid-containing liposomes. Up to date, several types of arsonoliposomes have been prepared and their size, surface charge, morphology, physical stability and membrane integrity characteristics have been evaluated [5–8]. In vitro studies with some of the arsonoliposomes have shown different toxicity levels against cancer and normal cells [9,10] as well as substantial anti-parasitic activity [11].

Herein, we report on the thermal properties of non-sonicated arsonolipid containing vesicles, in distilled water and in phosphate-buffered saline (PBS) at pH 7.4, in the absence and in the presence of Ca^{2+} ions, as monitored by microcalorimetry. We carried out the experiments in PBS pH 7.4, in order to mimic the



Lauryl: R= $C_{11}H_{23}$ (C_{12})

Palmitic: R= $C_{15}H_{31}$ (C_{16})

Stearic: R= $C_{17}H_{35}$ (C_{18})

Fig. 1. Chemical structure of arsonolipids.

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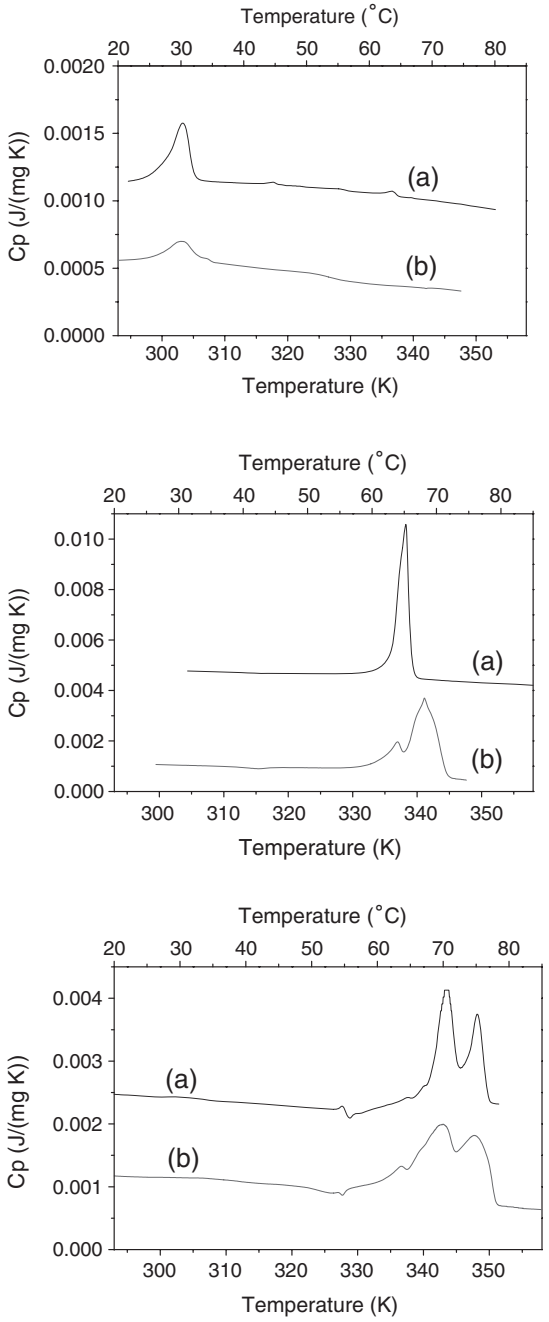

Fig. 2. Normalized thermograms of C_{12} (top panel), C_{16} (middle panel), and C_{18} (bottom panel) arsonoliposomes in PBS pH 7.4. The thermograms (a) and (b) represent heating of the liposomes with a scan rate of 30 °C/h in the absence and in the presence of 2 mM $CaCl_2$, respectively. Graphs were vertically shifted for comparison.

in vivo conditions, and in distilled water. We used 2 mM $CaCl_2$ concentration, because this is the calcium concentration in plasma.

Arsenic-containing analogues of phosphonolipids, the *rac*-2, 3-diacyloxypropylarsonic acids (given the trivial name arsonolipids [C_{12}, C_{16}, C_{18}]), were synthesized as described previously [2,3]. All reagents used were of analytical grade (Sigma-Aldrich). Using the C_{12}, C_{16}, and C_{18} arsonolipids we prepared plain arsonoliposome vesicles with the "one step method" [5]. For this, appropriate amounts of lipids (in powder form) were mixed with distilled water or with PBS, pH 7.40. Subsequently, the suspensions were stirred for 6 h at 70–90 °C, depending on the transition temperature of the arsonolipid as described previously [5].

Calorimetric studies were carried out in a VP-DSC calorimeter (MicroCal Inc., Northampton, MA). Very small heat exchanges of the liposomes suspended in water and in PBS pH 7.4, in the presence and in the absence of 2 mM $CaCl_2$, were measured in the temperature range 15–90 °C. As reference, pure water or PBS buffer were used (with or without 2 mM $CaCl_2$ co-solute, as required). All samples were degassed under vacuum for 15 min prior to loading the cells. During the calorimetric scan the liposome suspension and the reference were kept under 1.5 bar pressure to avoid boiling of the samples. The temperature increased with a heat rate between 10–60 °C/h and, after reaching the maximum temperature, the samples were down-scanned to 20 °C. In all cases, the lipid concentration was 5 mg/ml. Experiments were also performed at different lipid concentrations between 0.1 and 10 mg/ml. The normalized excess heat capacity functions were obtained after base line subtraction and manual data processing. The calorimetric data were collected at scan rates between 10 and 60 °C/h with similar peak characteristics for the transition temperature and the enthalpic exchange which suggests that the transition is not kinetically controlled in the time scale of the calorimetric experiments. Subsequent up- and down-scans of the liposome suspensions resulted in reversible endothermic and exothermic transition peaks, respectively, with good recovery of the enthalpy. A hysteresis effect in the transition temperature was observed upon the up- and down-scanning.

Typical thermograms of non-sonicated arsonoliposomes are illustrated in Fig. 2, with C_{12} (top panel), C_{16} (middle panel), and C_{18} (bottom panel) acyl chains in PBS pH 7.4 (150 mM

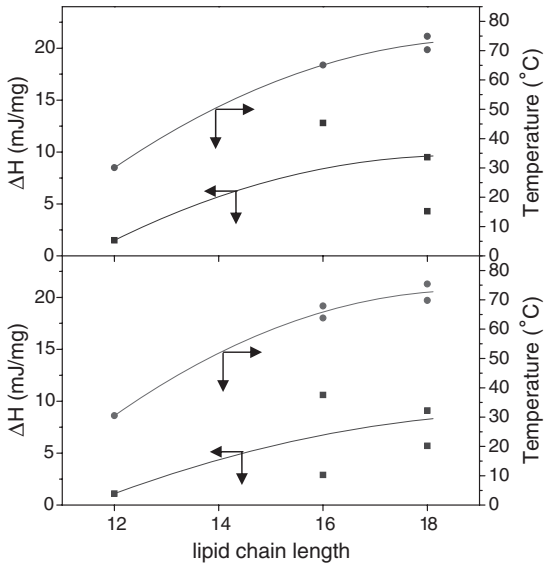

Fig. 3. The effect of the chain length of the lipids on the transition temperature and the enthalpy change of the liposomes in PBS pH 7.4 in the absence (top panel) and in the presence (low panel) of 2 mM of $CaCl_2$. The left and right *y*-axis represent the ΔH values (■) and transition temperatures (●), respectively. The lines are presented as a guide for the eye.

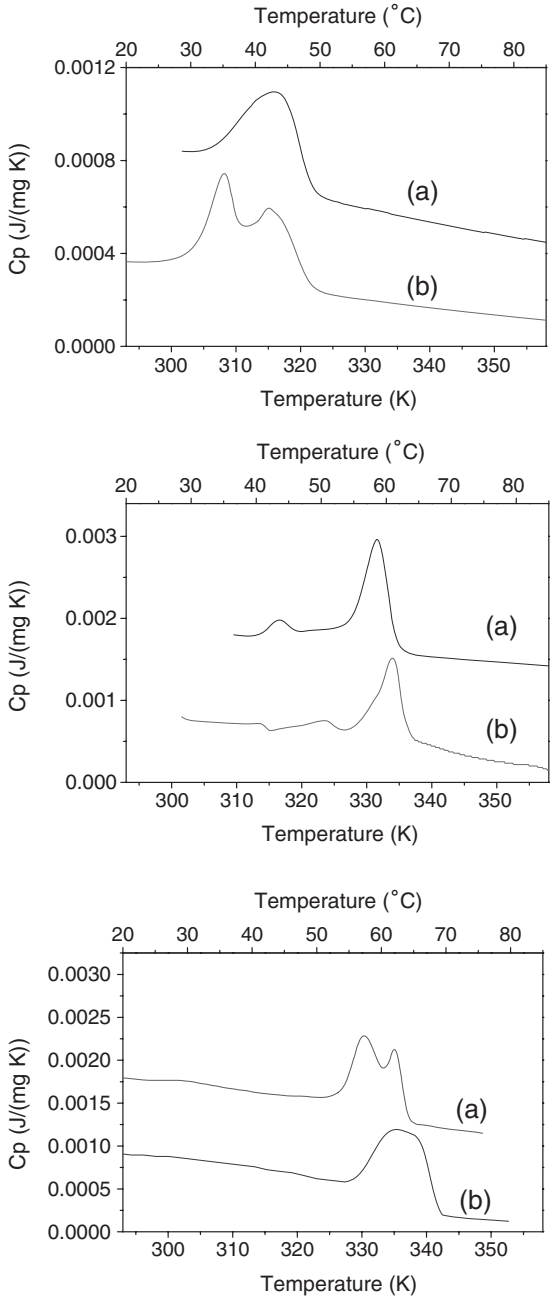

Fig. 4. Normalized thermograms of C_{12} (top panel), C_{16} (middle panel), and C_{18} (bottom panel) arsonoliposomes in water. The thermograms (a) and (b) represent heating of the liposomes with a scan rate of 30 °C/h in the absence and in the presence of 2 mM $CaCl_2$, respectively. Graphs were vertically shifted for comparison.

NaCl). The graphs presented in the manuscript were recorded at 30 °C/h both upon heating and cooling. The thermograms (a) and (b) represent heating of the liposomes in the absence and in the presence of 2 mM $CaCl_2$, respectively. The transition temperatures and the enthalpy changes of the liposomes in PBS in the absence of Ca^{2+} increased by increasing the fatty acyl chain of the arsonolipids (Table 1 and Fig. 3, top panel) with thermal transitions of 30 °C for C_{12}, 65 °C for C_{16}, and 70 and 75 °C for C_{18}. Previous studies [2,3] have shown that the melting points of dry arsonolipids increase as the fatty acyl chain length increases. The thermotropic transition characteristics of the aqueous dispersions of the arsonolipids showed that the phase transition and the enthalpy change depend on the length of the two fatty acyl chains. In the thermogram of arsonoliposomes of the C_{18} acyl chain arsonolipid, two transition peaks were observed (Fig. 2, bottom panel). Such a phenomenon could be explained by a hindrance of the hydration of the lipid head groups that are held assembled by strong intermolecular hydrogen bonds.

The interaction of externally added Ca^{2+} with arsonolipid containing liposomes and its effect on their thermotropic behavior were also investigated. The thermograms and the thermal properties (T_m, ΔÇ) of arsonoliposomes with different acyl chain lengths in the presence of 2 mM Ca^{2+} in PBS (pH 7.4, 150 mM NaCl), respectively are shown in Fig. 2 (curves b) and Fig. 3 (bottom panel). In the presence of Ca^{2+}, a broadening of the peak was observed for C_{12} and C_{18} arsonoliposomes. However, the transition temperature was essentially unchanged. In the case of C_{16} arsonoliposomes, the presence of Ca^{2+} altered the phase transition temperature of the liposomes and showed another transition peak which might indicate that the ion perturbs only the outer monolayer with no effect on the inner lipid bilayer [12], or the formation of clusters, one containing the lipid alone and the other the lipid-Ca^{2+} complex [13].

The results presented here show that the addition of Ca^{2+} ions did not result in significant changes on the transition temperature or the enthalpy released upon the transition of the C_{12} and C_{18} arsonoliposomes in PBS, at least not for the concentrations investigated. The behavior of the C_{16} arsonoliposomes cannot be explained with the current data. The minor effect of Ca^{2+} on the thermal behavior of the arsonoliposomes could be attributed to ionic interactions

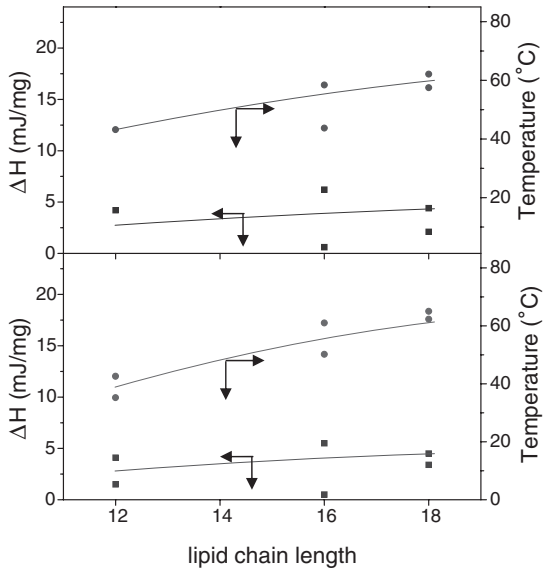

Fig. 5. The effect of the chain length of the lipids on the transition temperature and the enthalpy change of the liposomes in distilled water in the absence (top panel) and in the presence (low panel) of 2 mM of $CaCl_2$. The left and right *y*-axis represent Δ*H* values (■) and transition temperatures (●), respectively. The lines are presented as a guide for the eye.

Table 1
Transition temperatures and the enthalpy differences of non-sonicated arsonoliposomes in the absence and in the presence of 2 mM $CaCl_2$ in PBS pH 7.4 and in distilled water

Lipid	PBS pH 7.4 (150 mM NaCl)				Distilled water			
	Presence of $CaCl_2$ (2 mM)		Absence of $CaCl_2$		Presence of $CaCl_2$ (2 mM)		Absence of $CaCl_2$	
	T_m (°C)	ΔH_{cal} (mJ/mg)	T_m (°C)	ΔH_{cal} (mJ/mg)	T_m (°C)	ΔH_{cal} (mJ/mg)	T_m (°C)	ΔH_{cal} (mJ/mg)
C_{12}	30.5±0.7	1.1±0.4	30.1±0.6	1.5±0.3	35.2±0.5	4.3±0.4	43.2±0.9	4.2±0.3
					42.6±0.8	1.9±0.5		
C_{16}	63.8±0.4	2.9±0.3 [a]	65.1±0.6	12.8±0.6	50.2±0.3	0.5±0.5	43.7±0.3	0.6±0.3
	67.9±0.2	10.6±0.5 [a]			61.0±0.4	5.5±0.3	58.4±0.6	6.2±0.5
C_{18}	69.8±1.5	9.1±0.4	70.3±0.4	9.2±0.8	62.3±0.6	4.5±0.4 [a]	56.8±0.5	4.4±0.2
	75.0±0.6	5.7±0.7	74.9±0.8	4.1±0.5	65.0±0.4	3.4±0.2 [a]	62.1±0.6	2.1±0.1

[a] Values were calculated from deconvolution of the peaks using 2 components and assuming Gaussian pea.

between Ca^{2+} and the phosphate anions of the medium (i.e., PBS). In our experiments, the concentration of $CaCl_2$ was relatively low (i.e., 2 mM), and therefore, part of the Ca^{2+} ions may be associated with phosphate anions. The percentage of these complexes in the liposome suspension depends on the stability constants of the calcium–phosphate complexes. The concentration of all ionic species in the liposome solution was calculated from the pH value, the equilibrium constants for all chemical species involved in the system and the expressions for the mass and charge balance. Proton dissociation and ion pair association constants were also used in the calculations. This system of equations was solved for the solution species by successive approximations for the ionic strength [14]. For this reason a computer program software was developed [15,16]. The ionic activity coefficients of the ionic species were calculated using the modified Debye–Hückel equation [17,18]. Hence, solution speciation calculations showed that the concentration of the free Ca^{2+} ions in the arsonoliposomes suspension is ca. 1 mM. Our experimental data suggest that such a concentration is not sufficient to induce significant changes to the thermotropic behavior of non-sonicated arsonolipid vesicles.

In order to investigate the effect of the ionic strength of the medium on the thermal behavior of the arsonoliposomes we performed the same experiments in distilled water in the presence and absence of $CaCl_2$. Fig. 4 presents typical thermograms from this series of experiments. The transition temperatures and the enthalpy differences in the absence and in the presence of $CaCl_2$ in water are shown in Table 1 and in Fig. 5 (top panel). An increase in the phase transition temperature was observed as a function of the fatty acyl chain, which is in line with the data presented before for the arsonoliposomes prepared in PBS (Fig. 5, top panel). However, the presence of 2 mM of $CaCl_2$ altered the transition temperatures of the arsonoliposomes tested when the medium was distilled water (Table 1, Fig. 5 bottom panel). This might be attributed to the different level of binding of calcium to the vesicle surface in the different media. Indeed, surface charge studies showed a decrease of the ζ-potential, in absolute values, of C_{18}-arsonolipid containing non-sonicated liposomes prepared in distilled water when 1.8 mM $CaCl_2$ was present [8]. These results signify the high affinity of these vesicles for calcium ions. Hence, the changes observed in the thermal properties of the liposomes in distilled water could be explained in terms of binding of the Ca^{2+} ions to the surface of the vesicles.

Higher values of enthalpies and transition temperatures were obtained when the arsonoliposomes with long fatty acyl chains (i.e., C_{16}, C_{18}) were suspended in PBS as compared to the values observed when the same type of liposomes were suspended in distilled water, with and without $CaCl_2$. The picture was reversed for liposomes consisting of the short fatty acyl chain (C_{12}). The transition temperature and the enthalpy released were higher in distilled water as compared to PBS. It has been shown previously that liposomes consisting of C_{12} arsonolipids form long tubes when dispersed in water (instead of vesicles) which break down to cubes by sonication [5]. These vesicles might be more vulnerable to possible structural and/or thermal changes in the presence of electrolytes.

In light of these findings, the differences observed in the phase transition temperatures of arsonoliposomes in water and in PBS could be mainly attributed to the ionic strength, which is directly related with the thickness of the electric double layer, at least for the liposomes consisting of long fatty acyl chains, and possibly due to the morphology of the liposomes consisting of short fatty acyl chains.

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