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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