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

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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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[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