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Comparative effect of cationic gemini surfactant and its monomeric counterpart on the conformational stability of phospholipase A_2

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ABSTRACT

Herein, we have investigated the effect of cationic gemini surfactant (hexanediyl-α,ω-bis-(N-(2-hydroxyethyl)–N-methylhexadecylammonium bromide), (16-6-16MEA) and its monomeric counterpart (N-(2-hyroxyethyl)–N, N-dimethylhexadecylammonium bromide), (16MEA) on the conformational stability of phospholipase A_2 (PLA_2). The interaction of gemini surfactant and its monomeric counterpart with PLA_2 was characterized by utilizing steady-state fluorescence, time-resolved fluorescence, CD spectroscopy, and computational methods. The steady-state fluorescence results suggested the involvement of static quenching mechanism which was further supported by time resolved fluorescence measurements. The Stern-Volmer equation was utilized to calculate the value of Stern-Volmer quenching constant K_{SV}. The stoichiometric binding ratio of PLA_2 with 16MEA and 16-6-16MEA was observed to be 1:1. The far-UV CD spectra for PLA_2 revealed similar alteration in secondary structure, in presence of 16MEA and 16-6-16 MEA, which was further supported by computational methods.

1. Introduction

Phospholipase A_2 (PLA_2) is a name given to water soluble enzyme and a diverse superfamily of lipolytic enzymes that cleave the sn-2 acyl ester bond of glycerophospholipids specifically to produce free fatty acids and lysophospholipids [1,2]. According to their location, mammalian PLA_2 has been divided into high molecular mass intracellular PLA_2 and low molecular mass secreted PLA_2 ($sPLA_2$). $sPLA_2$ is deeply involved in rheumatoid arthritis, atherosclerosis, respiratory distress syndrome and septic shock [3–5]. PLA_2 catalyses the rate-limiting step in the formation of inflammatory eicosanoids by hydrolysing phospholipids and releasing their precursor arachidonic acid. In humans, cytosolic ($cPLA_2$) and secretory ($sPLA_2$) forms of PLA_2 have been identified [6]. $cPLA_2$ enzyme has the preference for arachidonic acid at sn-2-position of phospholipids. $sPLA_2$ can also hydrolyze phospholipids, which are important components of surfactant and cell membranes. This not only decreases surfactant concentrations [7,8] but also yields various pathogenic products such as lysophospholipids, which can interfere with surfactant function [9,10]. These findings imply that the investigations related to molecular mechanism of this enzyme's action and regulation of $sPLA_2$ activity are critical for controlling inflammatory diseases and have implications on chemical biology and pharmacology. The effects of environmental conditions such as the presence of surfactants/detergents, reducing agents, salt ionic strength and ligands to observe protein ligand interactions have been studied of late [11]. Surfactants are used on a large scale throughout the world in biological, medical and industrial fields. As a result of progress in industrial technology; demands for high-performance cationic surfactants are continuously increasing since decreasing the quantity of surfactant used can contribute to reduce the load on the natural purification system. Accordingly, novel cationic surfactants have been successfully designed and developed. Although cationic surfactants comprise only a small portion of the surfactant market, their importance in practical applications continues to grow. Gemini or dimeric cationic surfactants because of their tunable molecular geometry and superior performance in applications are generating interest in surfactant chemistry [12]. The choice of 16MEA and its gemini counterpart, 16-6-16MEA was based on their cationic nature. Dam

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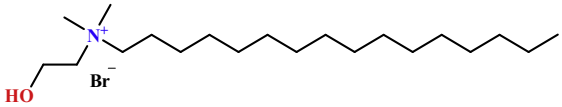
et al. have shown that cationic geminis are better solubilizers than conventional monomeric surfactants due to very low critical micelle concentration (cmc) values [13]. Moore et al. studied the role of polar head group on the protein-surfactant complex formation [14]. Protein surfactant interactions are important in a wide spectrum of applications in drug delivery, cosmetics and food industry [13,15]. Protein functions are dependent on its three dimensional structure [16]. A lot of research articles have been published in recent years on protein surfactant interaction as the property of surfactants to act as denaturants of water soluble proteins was identified [17]. The properties of proteins like surface hydrophobicity and conformational stability can be modified on interacting with surfactants in solutions [5–8]. The interactions generally involved are electrostatic or hydrophobic interactions or in some cases specific binding between surfactant and protein takes place.

The process of crystallization of proteins is influenced by specific nature of protein-surfactant interactions. The binding of surfactants to the proteins induces the conformational changes in the protein which in turn affects the polarity and stability of protein [18,19]. The recent investigations [20] have revealed that cationic gemini surfactants interact more efficiently with proteins than their corresponding monomeric counterparts. A wide range of properties is exhibited by surfactant mixtures and are known to show better performance than their individual components [21] which makes them important for biological and pharmaceutical fields. However, little attention has been paid towards their interaction with PLA_2. In this paper, we report the interaction of 16-6-16MEA and 16MEA with PLA_2.

2. Materials and methods

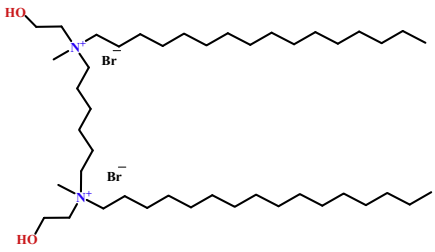
2.1. Materials

PLA_2 was purchased from Sigma Aldrich and was used as received. 10 mM phosphate buffer of pH 7.4 was used to prepare PLA_2 solutions. Doubly distilled water was used throughout the experiments. The surfactants used in the present study (16MEA and 16-6-16MEA) were synthesized in our lab using the synthetic protocol of Sharma et al. and Borse et al. [22,23]. Scheme 1.



N-(2-hydroxyethyl)-*N*,*N*-dimethylhexadecan-1-ammonium bromide

(A)



Hexanediyl-α,ω-bis-(N-(2-hydroxyethyl)-N-methylhexadecylammonium bromide)

(B)

Scheme 1. Structure of 16 MEA (A) and (B) 16 -6-16 MEA.

2.2. Steady state fluorescence measurements

Steady-state fluorescence measurements were performed at 298 K on a Cary Eclipse spectrofluorimeter (Varian, USA) equipped with a 150 W xenon lamp using 1 cm path length quartz cuvettes. Excitation and emission slits with a nominal band pass of 5 nm were used for all measurements. The excitation wavelength was set at 280 nm while as the emission wavelength was set as 290–420 nm. The concentration of PLA_2 was kept 5 μM. The background intensities of samples without PLA_2 were subtracted from each sample spectrum. The temperature was maintained by a constant temperature cell holder which is connected to water circulator (Varian).

2.3. Time resolved fluorescence measurements

Time-resolved fluorescence measurements were performed at room temperature, using a single-photon counting spectrometer equipped with pulsed nanosecond LED excitation heads at 280 nm (Horiba, Jobin Yvon, IBH Ltd, Glasgow, UK). The fluorescence lifetime data (5 μM PLA_2) were measured to 10,000 counts in the peak, unless otherwise indicated. The instrumental response function was recorded sequentially using a scattering solution and a time calibration of 114 ps/channel. Data was analyzed using a sum of exponentials, employing a nonlinear least square convolution analysis of the form [24]:

$$f(t) = \sum_{i=1}^{n} a_i \exp\left(\frac{-t}{\tau_i}\right) \quad (1)$$

where τ_i are the decay times, a_i is the relative contribution of the components at $t = 0$ and n is the number of decay times. The goodness of fit was judged in terms of both a chi-squared (χ^2) value and weighted residuals. Making use of the impulse response function (IBH DAS6 software), time-resolved fluorescence decays were analyzed [24,25].

2.4. Circular dichroism measurements

The CD measurements were carried out on a JASCO J-1500 spectropolarimeter (Tokyo, Japan). The instrument was calibrated with D-10-camphorsulfonic acid. All measurements were carried out at 298 K with a thermostatically controlled cell holder attached to a Neslab RTE-110 water bath with an accuracy of ±0.1 °C. The spectra were recorded in a quartz optical cell with a path length of 0.1 cm. The scan speed was fixed at 100 nm min^{-1} while as the response time was fixed at 1 s. All spectra were corrected for background by subtraction of appropriate blanks and were smoothed without any change in the overall shape of the spectrum. The concentration of PLA_2 for CD measurement was kept 17.8 μM.

2.5. Molecular docking and molecular dynamic simulation

To predict the structure of the PLA_2-16MEA/16-6-16MEA complex, the location of the binding sites and the interactions involved between protein and surfactants, molecular docking was performed. The crystal structure of PLA_2 (PDBID:1G4I) was obtained from protein data bank. The structure of 16MEA and 16-6-16MEA was constructed by using Chem Draw Ultra 8.0 and energy minimization of surfactants structure was done using MM2 force field. AutoDock 4.2 software was utilized to carryout molecular docking. Lamarckian genetic algorithm (LGA) was applied to calculate the possible conformation of 16MEA and 16-6-16MEA that binds to PLA_2. A maximum of 10 conformers was considered for the PLA_2.