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Effect of pyrrolidinium based ionic liquid on the channel form of gramicidin in lipid vesicles

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ABSTRACT

The present work is focused on the interaction between membrane bound gramicidin and 1-butyl-1-methyl-2-oxopyrrolidinium bromide (BMOP) ionic liquid. Ionic liquids (ILs) are solvents that are often liquid at room temperature and composed of organic cation and appropriate anion. The gramicidin peptide forms prototypical ion channels for cations, which have been extensively used to study the organization, dynamics, and function of membrane spanning channels. The interaction was studied by circular dichroism, steady state, time-resolved fluorescence spectroscopy in combination with dynamic surface tension and field emission scanning electron microscopic methods (FESEM). The results obtained from circular dichroism shows that the BMOP interacts with the channel form of gramicidin in lipid vesicle without any considerable effect on its conformation. The Red-edge excitation shift (REES) also supported the above findings. In addition, the fluorescence studies suggested that BMOP makes ground state complex with ion channel, which was further supported by time resolved measurements. Furthermore, dynamic surface tension analysis shows the faster adsorption of BMOP with membrane bound gramicidin at the air–water interface. Additionally, FESEM results indicated that BMOP forms a film around the membrane bound gramicidin at higher concentration. These results are potentially useful to analyze the effect of ionic liquids on the behaviour of membrane proteins.

1. Introduction

Gramicidin is a linear peptide occurring as a natural product from bacillus brevis. It is known to form ion channels in synthetic as well as natural membranes [1]. The specificity of gramicidin lies in forming prototypical ion channels which are selective for monovalent cations. Study of these prototypical ion channels is employed to obtain a plethora of information related to the structure and function of complex membrane spanning channels [2–4]. Gramicidin channels are referred as an excellent prototype for transmembrane channels due to its small size, easy availability, and the relative ease for chemical modification. These feature's states gramicidin as unique among small membrane-active peptides and thus provide a platform to be utilized for revealing the factors responsible for folding of membrane proteins [5–7]. Gramicidin channels are ion-selective owing to its unique sequence of alternating L- and D-chirality, that renders it environment sensitive [8]. The combinations of dihedral angle generated in the conformation space by various gramicidin conformations are allowed according to the Ramachandran plot [9].

The detailed molecular mechanism of gramicidin adopting various conformations in membranes is yet unknown. Gramicidin conformation in membranes, therefore, appears to be dependent on its "solvent history" [10]. The channel conformation of gramicidin in membranes has been characterized in molecular detail by solid-state NMR [11]. It was reported that the channel form of gramicidin is the most preferred and thermodynamically stable conformation in membranes and membrane-mimetic environments [12,13]. This form is characterized as single stranded $\beta^{6.3}$ helical dimer which conducts cation, and is formed by the head-to-head (amino terminal to-amino terminal) single stranded $\beta^{6.3}$ helical dimer [14]. In this conformation, the carboxy terminus remains exposed to the membrane–water interface, while the amino terminal is buried into the hydrophobic core. Tryptophan residues in this conformation remain clustered at the membrane–water interface around the entrance to the channel [14–16]. These residues are of prime importance in ion channel activity

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and conformation of gramicidin [17]. However, the conformation of gramicidin in interdigitated lipid bilayer is not the typical $\beta^{6.3}$ helix as in the normal bilayer [18].

Ionic liquids (ILs), organic salts with a melting temperature below 100 °C, are a class of materials that have considerable potential to advance liquid formulation for protein based pharmaceuticals [19]. ILs have been developed as a successful alternatives for organic solvents in traditional chemical processes, and more recently they have been explored as replacements for aqueous media in enzyme-based processes [20]. ILs possesses mesmerizing features such as high flexibility, tunability and less volatility, in addition to chemical variety that widens the range of compounds. Due to this features, their dispersion to environment is checked to reduce the waste and pollution. Thus ILs gets an edge over other solvents and although being expensive compensates for the initial cost. [21].

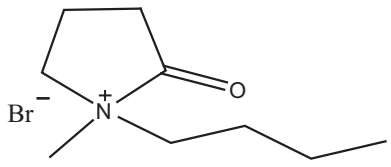
In depth investigation, computational as well as experimental studies of the interaction between ILs having different alkyl chain length and varied head groups with phospholipid membranes have been reported and the results showed disruption of the phospholipid bilayer at higher concentration of ILs with long hydrocarbon side chain [22–24]. Some recent findings also reported that ILs has the ability to fuse and aggregate lipid vesicles depending upon the length of the alkyl chain at higher concentrations [25,26]. These studies involving interaction between ILs and membranes are very informative for determining their various aspects like, mode of action of such chemicals and their effect on the biological membranes. Insertion of peptides or proteins into the frame widens the scope further for better understanding and assessing of the role of ILs in case of biological molecules. So far, very little attention was paid in regard for their use as reaction media for membrane protein. Herein, the study serves the purpose well to evaluate the effect of interaction of IL with membrane bound peptide.

In order to investigate the interaction of pyrrolidinium based IL, 1-butyl-1-methyl-2-oxopyrrolidinium bromide (BMOP) on a gramicidin ion channel in lipid vesicles, we applied a combination of spectroscopic approaches, which includes circular dichroism (CD) spectroscopy, fluorescence quenching, red edge excitation shift (REES), fluorescence lifetime decay along with dynamic surface tension and field emission scanning electron microscopy (FESEM). BMOP behaves as a room temperature ionic liquid (RTIL), and it occurs in liquid form at room temperature [27]. To the best of our knowledge, this work represents the first report of interaction of IL with the channel form of gramicidin ion channel in lipid membranes.

2. Materials and methods

2.1. Materials

Gramicidin (from Bacillus brevis) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC; diC14:0 PC) was purchased from Sigma Aldrich. IL, 1-butyl-1-methyl-2-oxopyrrolidinium bromide (BMOP) used were synthesized in our lab previously [28]. Scheme 1 shows the schematic structure of BMOP. The gramicidin typically consisted of 80–85% gramicidin A, 6–7% B, 5–14% C and <1% gramicidin D [29–31].



Scheme 1. Structure of 1-butyl-1-methyl-2-oxopyrrolidinium bromide (BMOP).

All chemicals used were of the highest purity available. Solvents used were of spectroscopic grade. Millipore water was used throughout the experiments.

2.2. Preparation of lipid vesicles

All experiments were done using unilamellar vesicles prepared as stated earlier [12]. The molar ratio of gramicidin to lipid were kept 1:50 for all experiments. The sample was subjected to a high vacuum for overnight followed by hydrating the film with pH 7.2 buffer. Afterward, the samples were vortexed and subjected for sonication. The samples were then centrifuged and were incubated for a minimum of 8 h at 65 °C to induce the channel conformation [10,15]. Background samples were prepared in the same way except that gramicidin was omitted. All experiments were carried out at 303.15 K i.e. above the phase transition temperature of DMPC [32].

2.3. Addition of BMOP

The BMOP of varying concentrations ranging from 0.125 mM to 0.495 mM for spectroscopic and 0.263 mM to 200 mM for other techniques were utilized. The samples were left in the dark for a period of four hours before each experiment.

2.4. Steady state fluorescence measurements

Steady-state fluorescence measurements were performed 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 background intensities of samples without gramicidin were subtracted from each sample spectrum. The spectral shifts obtained with different sets of samples were identical in most cases, or were within ±1 nm of the ones reported. The gramicidin/lipid ratio was kept low to avoid any instrumental errors.

2.5. Fluorescence quenching measurements

Fluorescence quenching experiments of gramicidin in lipid vesicles were carried out by measurement of fluorescence intensity in separate samples containing increasing amounts of BMOP taken from a freshly prepared 50 mM stock solution. Samples were kept in the dark for at least 4 h before measuring fluorescence. Corrections for inner filter effect were made using the equation [33]

$$F = F_{obs}\text{antilog}[(A_{ex} + A_{em})/2], \quad (1)$$

where F is the corrected fluorescence intensity and F_{obs} is the background subtracted observed fluorescence intensity. The values A_{ex} and A_{em} are the measured absorbances at the excitation and emission wavelengths. The data was analyzed by fitting of the Stern–Volmer equation [33]

$$\frac{F_0}{F} = 1 + K_{sv}[Q] = 1 + K_q\tau_0[Q] \quad (2)$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively. K_q is the quenching rate constant of the fluorophore, K_{sv} is the Stern–Volmer quenching constant, [Q] is the molar concentration of BMOP (quencher) and τ_0 is the lifetime of the fluorophore without quencher. The K_{sv} value obtained from the slope of the Stern–Volmer plot of gramicidin-BMOP system.