Poster

BIOPHYSICAL AND FUNCTIONAL CHARACTERIZATION OF AN ION CHANNEL PEPTIDE CONFINED IN A SOL-GEL MATRIX

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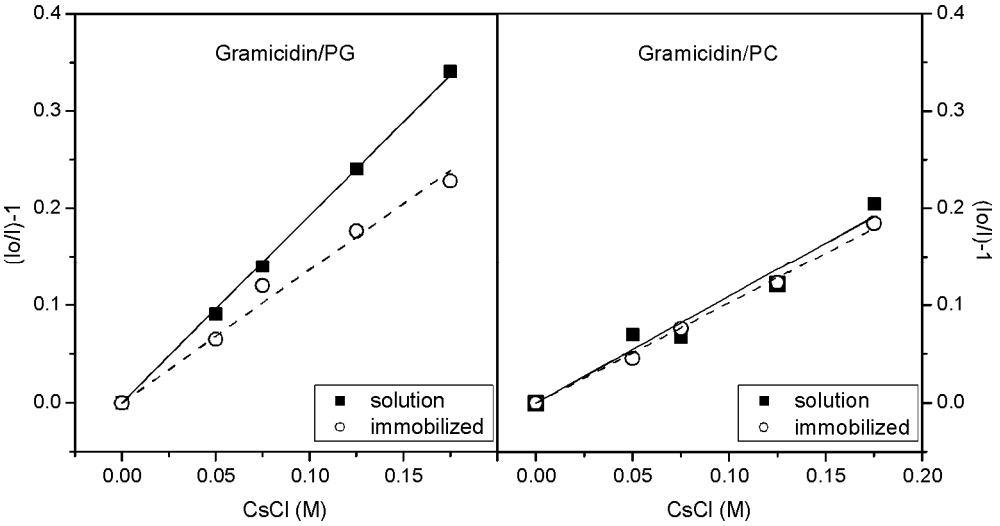
Ion channels are membrane proteins involved in the maintenance of the appropriate ion balance across the biological membrane, connecting the inside of the cell to its outside in a selective fashion. The ability to immobilize these proteins in inorganic matrices represent a significant step forward in developing a new generation of biologically active materials with potential applications in areas such as high throughput drug screening and for new generation of sensors. [1]

The major problems limiting the immobilization of the lipid membrane-ion channel systems are their lower stability, as well as the necessity to develop a methodology able to retain the physical properties of the lipid bilayer, since it is the media where membrane proteins perform their activity. Recently, we have demonstrated that use of alcohol-free sol-gel routes combined with negatively charged lipids could minimize effects exerted by host matrix on liposome structure, meanwhile for pure zwitterionic liposomes, even the use of these routes affects the lipid phase transition [2] Here we use the same experimental protocol to immobilize the transmembrane ion channel peptide gramicidin in a sol-gel matrix. Gramicidin was reconstituted in anionic and zwitterionic liposomes and the effects of sol-gel immobilization on the biophysical properties of gramicidin were determined from changes in the photophysical properties of its tryptophan residues. In addition, the physical state of the lipid membrane was analysed by measuring the spectral shift of the fluorescent probe laurdan. Finally, the ion channel activity of the immobilized gramicidin was monitored through a fluorescence quenching assay using the fluorescent dye pyrene-1,3,6,8-tetrasulphonic acid (PTSA) (see Figure). Results show that, the ionic channel properties of the immobilized gramicidin are preserved in both zwitterionic and anionic liposomes, even though the zwitterionic polar heads interact with porous surface of the host matrix.

References:

[1] Dunn, B., Zink, J.I., Accounts of Chemical Research, **40** ,9, (2007) 747.
[2] Esquembre, R., Ferrer, M.L., Gutiérrez, M.C., Mallavia, R., Mateo, C.R., Journal of Physical Chemistry B, **111** ,14, (2007) 3665.

Figure:



Representative data for Stern-Volmer analysis of cesium quenching of PTSA fluorescence. PTSA was encapsulated in sol-gel immobilized (void circle) and free (square) proteoliposomes of Gramicidin and PG or PC. Io is the fluorescence intensity in absence of quencher, and I is the fluorescence intensity in the presence of quencher. Concentration of PTSA inside the liposomes was 4mM and the ratio of gramicidin to lipid was 1:60(mol/mol). The excitation wavelength was fixed at 355 nm and emission was monitored at 385 nm.