



: Listeriolysin O - pore or pre-pore? Study on Giant Unilamellar Vesicles using Confocal Fluorescent Microscopy – imaging and FCS

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Abstract

Among the category of cholesterol dependent cytolysins (CDC), Listeriolysin O (LLO) is the most significant pore-forming toxin due to its ability in evading the immune response and causing listeriosis. The mechanism of pore formation of the pore-forming toxin starts with binding of the protein to the cholesterol, following with oligomerisation which in turn forms pores. In our experiments, we were able to distinguish between the pore and pre-pore using confocal fluorescence microscopy to analyse the leakage of dye and its effect on the lipid diffusivities using fluorescence correlation spectroscopy on giant unilammellar vesicles (GUV). The presence of pre-pore and pore forms of the CDCs have been otherwise proved using Atomic Force Microscopy, Transmission Electron Microscopy and Cryo-Electron Microscopy. Two population of GUVs: leaked and un-leaked were observed when LLO was added to the dye filled vesicles. Interestingly, LLO was found bound to the Ld region of leaked as well as un-leaked vesicles. The presence of pore enhanced the diffusivity of the lipids in leaked vesicles whereas the pre-pore decreased them significantly in the un-leaked GUVs. Preliminary results from the Forster Resonance Energy transfer (FRET) analysis, showed that FRET between LLO and lipids could be observed in the leaked vesicle and not in the un-leaked one which is a measure of distance between them.