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Preparation of Giant Unilamellar Vesicles and Solid Supported Bilayer from Large Unilamellar Vesicles: Model Biological Membranes

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Abstract

Giant Unilamellar Vesicles (GUV) and supported planar membranes are excellent model biological systems for studying the structure and functions of membranes. We have prepared GUV from Large Unilamellar Vesicles (LUV) using electroformation and Supported planar Lipid Bilayer (SLB) by vesicle fusion method. LUV was prepared using an extrusion method and was characterized using Dynamic Light Scattering (DLS) and zeta potential measurements. The techniques for obtaining GUV as well as SLB from LUV have been demonstrated. We have directly observed the formation of GUV under phase contrast microscopy. This study will provide some insights into the physico-chemical properties of both nano and micron size vesicles. We believe that this method could be extremely useful for reconstituting various bio-molecules in GUV. We have presented one example where an antimicrobial peptide NK-2 was reconstituted in GUV prepared from LUV. SLB formation was monitored and characterized using Atomic Force Microscopy (AFM).

Keywords: AFM, Dynamic Light Scattering, Model Membranes, Optical Microscopy, Solid Supported Bilayer, Vesicles

1. Introduction

Lipid bilayers are the basic building blocks of all biological membranes [1]. Phospholipids are their major constituents. Therefore, phospholipids bilayer serves as an excellent model system of bio-membranes. The complexity of biomembrane has led to the development of a wide variety of simpler model systems. As model systems, unilamellar vesicles and solid SLB are widely used for studying structure and function of membranes at physiological condition. SLB is popularly described in terms of physiological matrix and are useful to study the surface chemistry of the cell and the membrane-protein interaction [2]. A variety of experimental techniques were employed to study the fundamental as well as applied aspects of membrane structure, dynamics in the presence of various biomolecules such as proteins, cholesterol, receptors etc. [3, 4]. AFM is one of the well-established techniques for imaging SLBs at nanometer resolution. A unique feature of AFM is its ability to monitor dynamic processes, such as the interaction of membranes with proteins and other bio-active molecules [5]. Besides SLB, unilamellar vesicles are also widely used bio-mimetic systems. Vesicles are basically microscopic sac that encloses a volume with molecularly thin biomembrane. These are formed by self assembly of amphiphilic molecules, such as phospholipids, diblock co polymer etc. [6, 7]. Phosphatidylcholines (PCs) are the most abundant phospholipids in all eukaryotic cells, whereas, phosphatidylglycerols (PGs) and phosphatidylethanolamines (PEs) are found in bacterial cell [1] as well as eukaryotic cell. The phospholipids, when suitably mixed with water or similar solvent, form bilayers, commonly known as Multilamellar Vesicles (MLV). However, MLV do not serve as a good model system of biological membranes, as cellular membrane is a single bilayer of lipid molecules. Therefore, one needs to prepare model membrane containing single bilayer, known as unilamellar vesicles.

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4. Conclusion

Preparation and characterization of lipid membranes are the key steps for understanding the properties and functions of cellular membrane. In the present study, we have discussed the formation and characterization of phospholipid bilayer, an excellent model system of biological membranes, in the form of LUV, GUV and SLB. We have successfully obtained GUV from LUV using electroformation. We have also measured size distribution and zeta potential of LUV using dynamic light scattering. The several advantages of the present approach to prepare GUV over the conventional electroformation were also discussed. GUV provides the possibility to directly visualize certain interactions in a small but optically resolvable volume encapsulated by the vesicle membrane. We have presented an example, where an antimicrobial peptide NK-2 was reconstituted in GUV prepared from LUV. Formation of SLB was described using AFM study. The present study provided us an important insight into the physico-chemical properties of membranes in terms of their size and geometry. Therefore, GUV and SLB could be excellent and simple model systems whose size, geometry, and composition could be tailored with great precision.

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