Interaction of Self-Assembled Architectures formed by Amino Acids and their Derivatives with Lipid Membrane- Understanding the Molecular Basis of Specific Neurological Disorders by Spectroscopy and Microscopy

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Abstract

Mother “Nature” makes use of small molecules as the building block to structure the life-shaping important biomolecules in order for the origin and sustainment of life. For example, single amino acids are used to synthesize proteins and peptides that play basic roles in the functionalities of human physiology. The structuring of double-stranded DNA helical formation and ordering of the cellular membrane made up of phospholipids are the prime instances showing the significant architectural design for the sustainment of life. The pyramidal soup scenario, on the other hand, has proven the efficacy of molecular self-assembly in the blueprinting of “life”. However, despite the enormous pivotal role of such self-assembly of important biomolecules, like, proteins, amino acids, etc., in recent times it is reported that once such process of self-assembly becomes uncontrolled, it leads to the formation of the basis of several amyloidogenic or neurological disorders. For example, the molecular self-assembly formed by the amino acid L-Phenylalanine (L-Phe) is reported to be responsible for the Phenylketonuria (PKU) disorder. On the other hand, it is found that not only the single amino acids but also their derivatives are also equally potent to form self-assembled fibrillar structures. In this context, the artificial sweetener Aspartame (ASP), which is extensively used throughout the globe as a substituent for natural sugars, has been proved to form self-assembled fibrillar aggregates which are responsible for neurobehavioral alteration. Hence, to understand the molecular level pathogenesis of such disorders, we have put an effort to unveil the molecular level effect of such self-assemblies formed by amino acids and related molecules by understanding their effect on the mimicked model membrane system.

Phospholipid vesicles membrane has been used as a reductionist model of the real cell membrane in our work. We have studied the effect of the self-assemblies formed by either L-Phe or its derivatives on the model membrane. We have found that the membrane undergoes severe disruption once the self-assembled architectures interact with them. Such alteration of the vesicle membrane properties has been investigated using the steady-state fluorescence emission study. In addition to this, to understand the origin of electrostatic forces, zeta potential measurements have also been carried out. On the other hand, since in the biomolecular reactions, solvent molecules play a major role, the alteration of the solvation dynamics of the lipid vesicle membrane has been probed using the time-dependent Stoke’s shift technique in presence of various biomolecules

capable of forming self-assembled structures. The visual morphological alteration of such vesicle structures probed by the self-assembled architectures formed by the amino acids and their derivatives have been further captured using fluorescence lifetime imaging microscopy (FLIM), where the obtained lifetime decay analysis helped to interpret the altered rigidity of the lipid vesicle membrane. Such altered rigidity of the lipid vesicle membrane probed by the self-assembled architectures has been recommended as one of the probable reasons for the explanation of the molecular level pathogenesis of the neurological disorders originating out of the abnormal metabolic activity of such biomolecules capable of forming self-assembled architectures. Further, to understand the alteration of the membrane heterogeneity probed by the self-assembled structures, single molecular fluorescence study was performed using the fluorescence correlation spectroscopy (FCS) experiment. On the other hand, some important biomolecules, like trimethylamine N-Oxide (TMAO) have been reported to cause cardiovascular diseases (CVDs) via an unknown mechanism. We have found that TMAO forms cluster with solvent molecules that undergoes interaction with the lipid membrane, thereby making the membrane much more rigid. Hence, the reduced flexibility of the lipid membrane or the increased rigidity of the membrane probed by TMAO might be one of the prime factors for the molecular level set-up of CVDs. On the other hand, to check whether such self-assembled fibrillar structures are toxic or not, we have performed MTT-assay based cytotoxicity study on real cells, where the altered cell morphology was traced using the fluorescence microscope. Further, their effect on the red blood cells (RBCs) has also been monitored using various microscopic studies.

Taken together, we have performed a set of biophysical experimentations to understand the effect of self-assembled architectures formed by amino acids and their derivatives as well as osmolytes and investigated thoroughly the fate of the vesicle membrane due to the interaction. Therefore, we believe that discerning the dynamics and establishing the alteration of bilayer rigidity of the phospholipid vesicle in presence of the said biomolecules will not only account for the molecular level pathogenesis of such disorders but also aid to design therapeutic inhibitors so that such effect of the biomolecules on the membrane can be antagonized.

Keywords: Self-assembly, Fibrillar Morphology, Vesicle Rigidity/Fluidity, Neurological Disorders, Cells, Fluorescence, FLIM, FCS