pH-Induced Structural Changes in mRNA Lipid Nanoparticles

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Abstract - The SARS-CoV2 virus has had an enormous adverse global impact. With great efforts from scientists around the world, mRNA vaccines have been developed to effectively reduce morbidity and death due to the virus. mRNA vaccines use a nanoparticle lipid-based formulation (LBF) to deliver the mRNA to the cell interior. This LBF is a mixture of an ionizable lipid, a PEGylated lipid, a phospholipid and cholesterol. Importantly, the chemical behavior of the ionizable lipid changes with the pH conditions. The mRNA formulation process is initially carried out at acidic pH and the formulation is then raised to physiological pH. The change in charge of the ionizable lipid due to pH conditions affects the molecular arrangement of the LNPs. Therefore, understanding pH-induced structural changes of lipid nanoparticles (LNPs) in mRNA formulations will greatly assist the development of new mRNA delivery systems. Molecular dynamics (MD) is a powerful tool for exploring complex systems at the atomic level. In this study, we carried out long MD simulations of pre-assembled LNPs using the coarse-grain MARTINI force field. Our simulations provide insight into molecular packing within the LNP at acidic and physiological pH and how water molecules aggregate inside the LNP. The simulations further show that LNPs at acidic pH have ordered molecular arrangements and LNPs at physiological pH have less ordered molecular arrangements. The simulation results agree with SAXS measurements reported in the literature. The results of our study will help us understand LNP systems at different pHs and explore efficient mRNA delivery systems.

Keywords: lipid nanoparticles, mRNA, pH

I. INTRODUCTION

Starting in November 2019 the spread of the SARS-CoV2 virus has had an enormous effect on global events. To date, millions of people worldwide have died from the respiratory disease caused by this virus. However, with great efforts from scientists around the world, vaccination has become the most suitable approach fight this viral disease.

Among the various vaccine types, mRNA vaccines have been the most versatile platform so far. Formulation is an important aspect of the mRNA platform to ensure that the mRNA is delivered to the cell without degradation. The frontline COVID-19 vaccines developed by Moderna, and Pfizer use a lipid-based formulation (LBF) to surround the mRNA and deliver it to the target cells efficiently. This LBF is a mixture of ionizable or cationic lipid (pKa < 7), PEGylated lipid, phospholipid and cholesterol. Together, these lipid materials self-assemble to form a nano sized particle with spherical morphology known as lipid nanoparticles (LNPs).

The ionizable lipids within the LNPs play a critical role; they are positively charged at acidic pH and are neutral at physiological conditions (pH 7.4). The mRNA formulation process is carried out initially at pH 4 and then raised to pH 7.4. During this procedure chemical nature of the ionizable lipid influences the molecular structure of the LNPs and these structural arrangements are still not fully understood.

To explore new avenues of the LNP-based drug delivery systems, understanding the structure of mRNA-encapsulated LNP and its microenvironment is essential. However, the atomic details of such colloidal systems are difficult to obtain from experimental methods such as small-angle X-ray scattering (SAXS) or NMR spectroscopy due to the heterogeneous composition of the LNP formulations. In contrast, molecular dynamics (MD) has become an important emerging technique for exploring complex colloidal systems at the atomic level [1-2]. MD simulations model the movement of atoms and molecules by solving the Newton's equations of motion to model the dynamic evolution of a molecular system over time. To date, few experimental studies have investigated how the molecular structure of LNP systems change as the pH is varied [3-4]. Similarly, few MD studies have investigated this [5]. Thus, we are still lacking detailed atomic information on the molecular arrangement of each lipid component within LNP and the extent that water molecules are trapped inside the LNPs. Thus, the aims of the current study are, to investigate the structural changes of LNPs at acidic and physiological pHs and to study the how water is dispersed inside the LNPs. For this purpose, we modeled pre-assembled LNPs using MD simulations with the MARTINI force field for 1.5 microseconds (μs). The resulting structural features were then compared with existing experimental data in the literature.

II. MATERIALS AND METHODS

A. Topologies

Topologies for DSPC and cholesterol were obtained from the MARTINI website. The parameterization of the ionizable lipid; DLin-MC3-DMA (both protonated and neutral form), RNA (with 13 bases), and the DMG-PEG2000 was carried out following the processes given on the MARTINI website.

B. Construction of computational model systems

LNPs were constructed using 50: 38.5: 10: 1.5 molar ratios of ionizable lipid, cholesterol, DPSC and DMG-PEG2000 along with the molar ratio of ionizable lipid to nucleotide (i.e., N to phosphate) as 5. All systems were modeled as containing 20% formulation and 80% water (w/w). Changes in pH were modelled by changing the fractions of ionized lipid (DLin-MC3-DMA). At pH 4, 100% of the lipid was ionized. At pH 7.4 and 10% of the lipid was ionized. All RNA and lipid molecules except DMG-PEG2000 were placed in a random orientation in a sphere with 25 nm radius. The water content inside the LNP was varied as 1% (less water) and 10% (more water) as some literature models [6] have proposed that LNPs contain water. The PEGylated lipid (DMG-PEG200) was placed randomly on the surface of the sphere.

C. *MD simulations*

All simulations used GROMACS version 2018.4 with the isothermal-isobaric ensemble (NPT). Systems were simulated at 310 K with a reference pressure of 1 bar. Before the production run, systems were energy minimized using the steepest descent method to remove steric clashes and then the systems were subjected to an equilibration run (4 ns). The production was run for 1.5 µs with a time step of 10 femtoseconds.

D. *MD analysis*

All structures were visually inspected using VMD software and the structural details were compared with the available experiment data.

III. RESULTS AND DISCUSSION

To investigate the pH-induced structural changes of the LNPs and to study the distribution of water inside the LNPs, we carried out MD simulations for pre-assembled LNP structures. In these pre-assembled NLPs as starting configurations, lipids, RNA and water were randomly distributed in spherical morphology and particularly PEGylated lipid was placed on the surface of the morphology. The water content inside LNPs was changed to 1% and 10%. Acidic and physiological pH conditions were modeled considering 100% and 10% ionization of DLin-MC3-DMA.

We observed that randomly placed lipid molecules were re-arranged according to the hydrophobicity and lipophilicity nature of the molecular components and assembled structure stayed in the spherical morphology for 1.5 µs without any deformation. Visual inspection further revealed that DMG-PEG2000 molecules dispersed on the surface fully interacted with water irrespective to the pH condition. For LNPs simulated at acidic pH showed ordered bilayer-like arrangement composed of cholesterol, DSPC and positively charged DLin-MC3-DMA. However, the molecular arrangement of LNPs simulated at physiological pH is amorphous. Interestingly, this behavior matches with SAXS data in the literature [3-4]. The ordered and amorphous molecular arrangements inside the LNPs (with 10% water inside the LNP) at pH 4 and 7.4 are shown in Fig. 1.

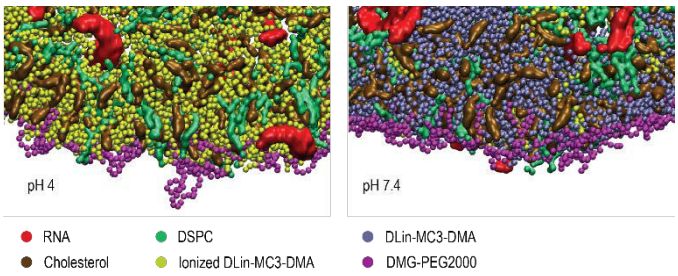

Fig.1 – Molecular arrangements inside the LNPs (at the edge) at pH 4 and pH 7.4.

LNPs simulated with 10% water inside the LNP at physiological pH formed separate water aggregates inside the LNP, while the same systems simulated at acidic pH resulted in interconnected water clusters. This behavior is the same for the systems simulated at two pH conditions with 1% water inside LNPs.

IV. CONCLUSION

Our simulations show that LNPs at acidic pH are ordered structures while LNPs at physiological pH are amorphous, which matches with the experimental observations. This result highlights that MD can be used as a tool to predict the structural features of complex colloidal systems successfully. Furthermore, our simulations reveal that the chemistry of the ionizable lipid influences the distribution of water within the LNP. Currently, we are working to find whether the chemistry of ionizable affects the distribution of RNAs within the LNP.

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