Investigating chitosan-encapsulated cholera spray-dried vaccine: a molecular modelling study

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ABSTRACT

Cholera is a disease that is still prominent in developing countries, as these areas have poor sanitation and difficulty with accessing clean water. This infectious disease is caused by the Vibrio cholerae bacterium that produces enterotoxins, causing diarrhea and fluid loss. Vaccines are vital as they can prevent unnecessary and deadly outbreaks in countries or areas where the disease is not endemic; therefore, easy-to-transport and administer vaccines are being developed in order to solve these issues. Spray drying is a method being investigated for vaccine development as an alternative to consistent cold chain and injectional delivery. Chitosan, a nontoxic biopolymer with intriguing properties, is of interest in being utilized as a vehicle or encapsulating system for spray-dried vaccines, as multiple studies have shown improved efficacy and appropriate immunogenicity responses. Molecular modelling can be implemented before actual wet-lab experiments to obtain significant insights and correlations that can be applied for the optimization of the proposed vaccine system. Through molecular modelling, a vaccine system will be built and simulated. After simulation, data showed that the vaccine is not destroyed under high acidic environment (pH 1.5 to 2.0). Additionally, the chitosan cage is stable.

INTRODUCTION

Traditional vaccines require consistent cold chain and injectional delivery, which can make administration and distribution hard and expensive. Spray drying is a method being investigated as an alternative because it is less susceptible to degeneration caused by temperature, easier to administer, and easier to distribute. Oral delivery is a mode of delivery being considered for spray-dried vaccines as it does not require inhalers and or any sophisticated mechanisms. Chitosan, a nontoxic biopolymer with intriguing properties, is of interest in being utilized as a vehicle or encapsulating system for spray-dried vaccines. Multiple studies have shown improved efficacy and appropriate immunogenicity responses in chitosan-encapsulated vaccines as it can substantially improve stability, membrane permeability, mucoadhesivity and the controlled behavior during release. Cholera, an intestinal disease that causes severe diarrhea and dehydration, is still prominent in developing countries such as the Philippines. In 2011, cholera was in the top 10 leading causes of deaths in the Philippines. Molecular modelling, the process of simulating and mimicking the behavior of individual molecules with the help of computer software, provides useful insights and correlations that can be applied for the optimization of the proposed vaccine system.

METHODOLOGY

01 Materials

The researchers will be using the softwares, UCSF Chimera 1.14, Free Maestro Schrodinger 12.6, gVim Editor, Groningen Machine for Chemical Simulations 5.1 (GROMACS) and charmm-gui. The chitosan fragment is gathered from PubChem while the protein structure of the cholera vaccine (1f4x) to be encapsulated is extracted from RCSB Protein Data Bank (PDB).

02 Preparation and Simulation

Using UCSF Chimera, a model of an encapsulating cage made out of chitosan units will be built in UCSF Chimera 1.14. The model of the encapsulating cage will be properly bonded using Free Maestro Schrodinger 12.6. Chitosan fragments will be bonded using the 1-4 connection of carbon atoms and will be crosslinked using a phosphorus atom. Hyaluronic acid will also be used to link chitosan as a connector. The vaccine file is prepared by uploading it to the solution builder of charmm-gui. Then, the file is assessed using gVim Editor where all unnecessary residues, atoms, and molecules are removed. After that, the preparation, minimization, equilibration, and simulation of the vaccine system will be done on GROMACS.

03 Data Analysis

Data from GROMACS will be used for statistical analysis as well as to analyze the system stability of the chitosan cage with the vaccine. This includes analysis on RMSD, RMSF, ROG (Radius of Gyration), RDF, interactions between the molecules and the structural stability of the chitosan cage. The parameters of the experiment will be cross-referenced with studies on spray-dried vaccines. Xmgrace, a tool that can be used to view the graphs of the generated NVT and NPT ensembles in GROMACS, can enable this study to determine the constant volume and pressure of the simulation. PyMOL viewer and visual molecular dynamics (VMD) can be insightful in showing the structure and interaction of the generated system in a cartoon-like manner.

RESULTS

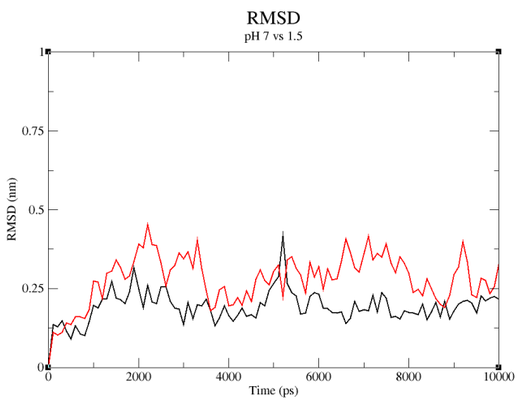

Figure 1. RMSD Graph

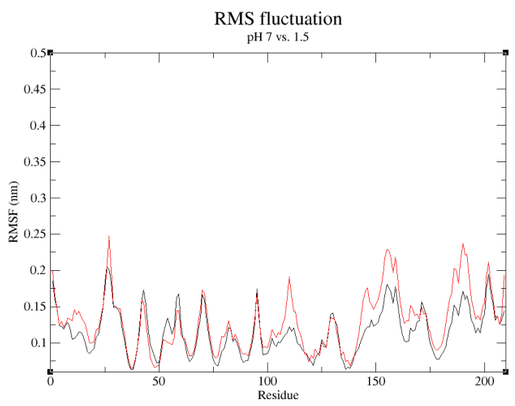

Figure 2. RMSF Graph

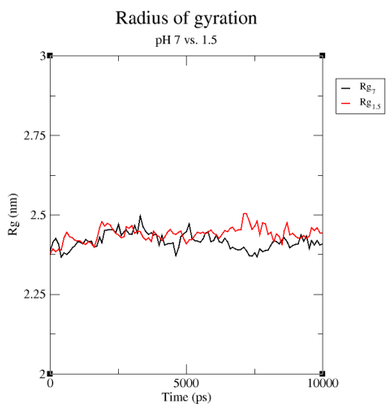

Figure 3. ROG Graph

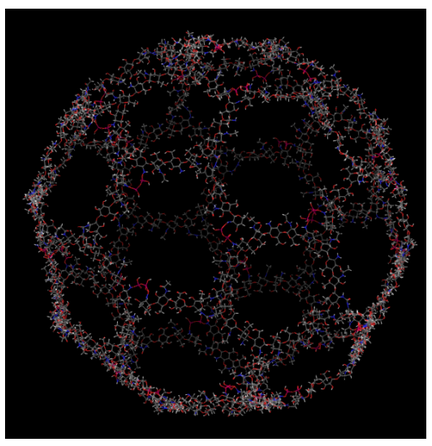

Figure 4. Chitosan Cage

Due to technical and troubleshooting problems, the result or data analysis produced only compares the root mean square deviation (RMSD) (Figure 1.), root mean square fluctuation per residue (RMSF) (Figure 2.), and radius of gyration (ROG) (Figure 3.) of the vaccine protein at pH 1.5 and pH 7. Analyzing the graphs above, it can be seen that the protein file is fairly stable under acidic conditions, as the fluctuation seen in the plots are not far apart. However, it is important to note that there are still some instability coming from the protein file at pH 1.5 for it is experiencing denaturation. All in all, this comes to show that the vaccine is not destroyed under high acidic environment, and only shows minimal denaturation.

Additionally, the chitosan cage (Figure 4.), which was initially created to protect the protein file from acidic environments for it to deliver the desire immunization along the GI tract., was made stable by ensuring proper bonds, bond length, and structure. Upon seeing the data analysis in the first two setups, the cage is still important in this study as it can be the agent to minimize or protect the vaccine against denaturation.

CONCLUSION

Currently, there are two more setups to be performed to fully determine if the chitosan cage created is able to reduce or remove the fluctuations seen in the plots. In addition, the cage is necessary in encapsulating the protein to protect it in the process of spray-drying. Based on the review article of Kanojia, et al. (2017), the parameters that fit the current experiment is carbon dioxide-assisted nebulization with bubble drying. This process places the aqueous vaccine under "supercritical conditions" of high pressure and temperature. Then, the mixture turns into spray of droplets that is to be dried under 25 to 65 degrees Celsius. The first two setups discussed in the results are under 300 K; thus, the vaccine has a chance of being compatible with this process for it can survive fluctuating pH levels and fairly high temperatures. Nonetheless, the parameters need more details and research to be reflected on the experiments or setups. Hence, there is more to be done in terms of simulation and experimentation because the current data is not sufficient enough to completely determine if the vaccine in this study is confidently valid for spray drying and it has not yet been integrated with the constructed chitosan cage.