Effects of the binding of calcium ions on the structure and dynamics of the Φ X174 virus investigated using molecular dynamics

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Abstract It is known that the presence of calcium ions (Ca^{2+}) is necessary for the enterobacterial virus Φ X174 to inject its DNA into the host cell, and that some mutations in the major capsid proteins lead to better survivability at higher temperatures. Our goal in the current study is to determine the physical changes in both the wild-type and mutant virus due to the binding of Ca^{2+} . Thus, we performed molecular dynamics simulations of the Φ X174 major capsid protein complex with and without Ca^{2+} bound. Our results show that binding of Ca^{2+} leads to energetic and dynamical changes in the virus proteins. In particular, the results suggest that binding of Ca^{2+} is energetically favorable and that the mutation leads to increased fluctuations of the protein complex (especially with the calcium ions bound to the complex), which may increase the rate of genome packaging and ejection for Φ X174.

Keywords Φ X174 virus · Φ X174 major capsid protein · F242 mutation · Calcium ion binding · Viral dynamics · Viral structure & stability · Molecular dynamics simulation

1 Introduction

As simple as viruses are relative to multicellular life, they are complex biomolecular systems from both a structural and dynamical perspective. Experimentally, it is difficult

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or impossible to attain sufficient temporal or spatial resolution to elucidate the atomic-level dynamics of viral capsids [1]. Over the last 35 years, molecular dynamics (MD) simulations have evolved to provide insight into the dynamics of biological molecules [2], and multiple MD methods have been developed to study viral structures in silico [3]. Only relatively recently has an all-atom simulation for a virus become feasible, and then only on the diminutive satellite tobacco mosaic virus [4]. Earlier, Cagin et al. [5] devised the technique of rotational symmetric boundary conditions to better model an icosahedral virus while needing to simulate only one portion of the virus (see [6] for an example). Coarse-graining has also been employed by Arkhipov et al. to approximate various viral systems, including the Φ X174 procapsid [1].

Φ X174 is a small bacteriophage composed of several different proteins arranged as 12 pentameric units. Four of these proteins known as F, G, H and J form the mature capsid that is approximately 27 nm in diameter [7]. Each protein has its own function; F is the major capsid protein, G is the major spike protein, J is the DNA binding protein, and H is the DNA pilot protein (an H protein accompanies the DNA when ejected) [8, 9]. Φ X174 infects the common bacterium *E. coli* by attaching to the cell wall and injecting its DNA into the bacterium. Ca^{2+} appears to be necessary for this injection process, and Φ X174 has been shown to eject its DNA without attaching to any bacteria in the presence of high calcium chloride concentrations [10]. This is likely related to Ca^{2+} 's induction of the conformational change demonstrated by Incardona and collaborators [11]. Ca^{2+} is also known to play an important role in the assembly of most viruses, and thus is likely important for Φ X174 assembly as well [12, 17]. Due to the high viral populations in typical experiments, Φ X174 mutates frequently, allowing it to quickly adapt to different environments. One common mutation in the major capsid protein is F242, in which F protein residue 242 mutates from leucine to phenylalanine [13]. The F242 mutation is a common mutation seen during experimental adaptation of Φ X174 to high temperature [14]. Experimentally, it is known that this mutation confers increased fitness at higher temperatures at the cost of decreased fitness at normal temperatures [13].

In this study, we use MD simulations to examine the physical characteristics of capsid proteins of Φ X174, and observe effects of the binding of Ca^{2+} to the wild-type and the F242 mutant virus. Our results show that binding of Ca^{2+} lowers the potential energy of both wild-type and mutant protein complexes, and that binding of Ca^{2+} results in increased fluctuations of the mutant proteins compared to the wild-type.

2 Methods

Figure 1 shows our model system consisting of six major capsid F proteins from Φ X174 as determined by McKenna et al. (protein data bank entry 2BPA; accessed online via VIPERdb) [15, 16]. Figure 2 shows the locations of the amino acid mutation F242 and the calcium ions that were added manually using the coordinates given by Ilag et al. [17]. No experimental protein structures are available for the F242 mutant Φ X174 and thus we computationally constructed the protein structures using the “mutate residue” extension from the molecular analysis program Visual Molecular Dynamics (VMD) [17, 18].

Four systems were simulated for the current study: the wild-type F protein complex with and without Ca^{2+} bound, and the F242-mutated F protein complex with and without Ca^{2+} bound. The complexes were approximately 17.5 nm at the widest point at the start of the simulations (calculated graphically with VMD using the “bond” option). All four

Fig. 1 Φ X174 virus capsid used in the current study. The *six colored proteins* indicate the F protein complex simulated here

systems were prepared for MD simulation by first solvating the protein complex in water with periodic boundary conditions imposed for more accurate physiological conditions (see Fig. 3) using the “solvate” command in VMD. Each box of water initially allowed for 0.7 nm of space between each box face and the edge of the complex, with box dimensions approximately $18.2 \times 17.5 \times 9.0$ nm (calculated graphically with VMD). Once

Fig. 2 Simulation protein system showing the location of the mutations and calcium ion. Each ribbon color represents a different major capsid F protein. The six outer *teal spheres* indicate F242 mutation sites and the four inner *green spheres* indicate calcium ion locations. The virus capsid interior is towards the reader

Fig. 3 Full simulation system that includes the F protein complex solvated in a box of water. This system contains a total of 3.67×10^5 atoms

solvated, each full simulation system contained approximately 3.67×10^5 atoms, of which approximately 4×10^4 comprised the protein complex.

Our MD simulations were performed on a Linux Rocks computer cluster using the molecular software package NAMD with the default CHARMM force field [19, 20]. Each simulation consisted of first minimizing the energy of the system for 5×10^3 steps, then equilibrating the water by harmonically restraining the heavy atoms (S, N, O, C) in the protein and performing 5×10^5 steps of MD simulation, and finally equilibrating the system by performing 1×10^6 steps of MD without restraints. Simulations were under NPT conditions with 1 atm constant pressure via a Langevin piston and physiological 310 K constant temperature via Langevin dynamics [21, 22]. The time between each integration step was 2 fs, and the coordinates and energies for the systems were output every 1 ps (500 MD steps).

To determine the total potential energy of each system, we analyzed the log file that contains calculated values for quantities such as pressures and energies for each recorded time step using the “data_avg” procedure in the “namdstats.tcl” script in VMD. The total potential energy here includes the bond, electrostatic, and van der Waals interactions.

The trajectory files containing atomic coordinates were also analyzed for the root mean square fluctuations (RMSFs) for individual atoms using VMD’s “measure RMSF” command. In general, an RMSF is calculated as follows:

$$RMSF_{\vec{x}} = \sqrt{\frac{1}{T} \sum_{i=1}^T \left(|\vec{x}_i| - \langle |\vec{x}| \rangle \right)^2}, \quad (1)$$

where T is the total number of output frames, and x_i is the distance from the origin for frame i (here, the distance of the atom from its original position). We then averaged the fluctuations of the atoms over each residue using MATLAB to find the RMSF per residue using:

$$RMSF_{residue} = \frac{1}{N} \sum_{j=1}^N y_j, \quad (2)$$

where N is the number of atoms in a given residue, and y_j is the RMSF of atom number j [23]. The potential energies were calculated using the final 1 ns of equilibration and the RMSFs were calculated over the final 0.5 ns, i.e., after the potential energies had reached equilibrium (see Fig. 4).

Fig. 4 Potential energy for each simulation system (mutant M, mutant with calcium MC, wild-type W, wild-type with calcium WC) as a function of simulation time. These results show that once the system equilibrates, binding of Ca^{2+} to either mutant or wild-type results in a lower (more favorable) potential energy. The average potential energies after the system equilibrates (i.e., 0.6–1 ns) are shown in parentheses in the legend

3 Results and discussion

In this section, we detail our MD simulation results on the effects of Ca^{2+} binding on the potential energy and dynamics of the Φ X174 virus capsid. We find that binding of Ca^{2+} decreases the potential energy in both wild-type and mutant, and that binding of Ca^{2+} slightly decreases the flexibility of the wild-type proteins but significantly increases the flexibility of the mutant proteins.

Figure 4 shows that binding of Ca^{2+} significantly decreases the potential energy of the system for both the wild-type and the mutant by roughly the same amount. From an energetic perspective it makes some intuitive sense that binding of Ca^{2+} results in a lower energy state since we know that calcium ions spontaneously bind to Φ X174 in experiments, enabling the virus to eject its DNA [10]. In addition, three of the binding sites have a negative charge of $-1e$, and the fourth (central) site is neutral but highly polar [17]. Thus, binding of the $+2e$ charged calcium ions is electrostatically favorable.

Figure 5 shows the effects of the binding of Ca^{2+} and the F242 mutation on the dynamics of Φ X174 by displaying the residue-averaged RMSF from (2). Our results show that the mutation and binding of Ca^{2+} change the fluctuations relative to the wild-type in different ways. The mutant has somewhat larger fluctuations than the wild-type (Fig. 5b), and binding of Ca^{2+} to the mutant significantly increases its fluctuations (Fig. 5c). In contrast, binding of Ca^{2+} to the wild-type somewhat decreases its fluctuations (Fig. 5a), and the mutant

Fig. 5 Differences in the root mean square fluctuations (RMSF) between two systems (same notation as Fig. 4). The results shown are averages over 10 residues. Deviations from zero indicate a difference in the RMSF between the two systems. The *left column* shows the effect of Ca^{2+} binding and the *right column* shows the effect of the F242 mutation. The plot titles show the systems used in calculating the differences, and also the average differences in parentheses from the corresponding raw data. The *red vertical bars* at the top of each plot indicate the locations of the residues that interact with calcium ions and the *black vertical bars* at the bottom of each plot indicate the divisions between the F proteins

with Ca^{2+} bound has significantly larger fluctuations than the wild-type with Ca^{2+} bound (Fig. 5d).

These fluctuation differences do not appear to directly correlate with the Ca^{2+} binding site locations, but there does appear to be a somewhat loose correlation amongst the individual proteins. Figure 5d most clearly illustrates this point since the average RMSF differences change from one protein to the next, with the third F protein exhibiting the most marked differences.

In a recent study, we found that mutations that are located at the F protein interfaces in a G4-like phage have greater conformational entropy than the wild-type complex [24]. This qualitatively agrees with the increases in fluctuations seen for the mutant in the current study. Increases in both the fluctuations and conformational entropy suggest increased flexibility of the structure. Increased flexibility may partly explain the greater fitness of the F242 mutant at higher temperatures relative to the wild-type (this mutant exhibits lower fitness than the wild-type at normal physiological temperatures). Increased flexibility may be biologically important since it is thought that conformational changes are required for ejection of the DNA from the capsid [11]. Increased fluctuations, particularly those at protein interfaces, allow the proteins to explore more conformational space and thus could increase the rate of genome packaging or ejection.

Our results show that the F242 mutation increases the fluctuations of the complex while maintaining the potential energy of the system. It may be interesting to study other amino acid mutations to ascertain the generality of this result as it may act as a constraint to limit the types of mutations that could occur. Also, future studies could be performed to determine how the local dynamics of the proteins impact the global dynamics of the full capsid.

4 Conclusions

We used molecular dynamics simulations of the major capsid F protein complexes to study the energetic and dynamic differences between wild-type and F242 mutant variants of the Φ X174 virus and found physical clues for roles of the binding of Ca^{2+} . We found that binding of Ca^{2+} decreases the potential energy for both wild-type and mutant. Our results also show that binding of Ca^{2+} decreases the potential energy of the wild-type and mutants roughly equally.

In addition, we found that binding of Ca^{2+} can either slightly decrease or significantly increase the flexibility of the viral capsid proteins. The mutant was slightly more flexible than the wild-type, the wild-type was slightly more flexible than the wild-type with Ca^{2+} bound, the mutant was significantly less flexible than the mutant with Ca^{2+} bound, and the mutant with Ca^{2+} bound was significantly more flexible than the wild-type with Ca^{2+} bound. There were also somewhat abrupt, sustained changes in the fluctuations which roughly varied from protein to protein, resulting in some proteins with similar dynamics and some proteins with highly distinct dynamics. These increases in fluctuations, especially at protein-protein interfaces, could be important for increasing the rate of DNA packaging and ejection.

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