[5] 最近の研究から「Molecular dynamics study of the role of ions in maintaining virus capsid stability」

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Abstract

Molecular Dynamics simulation results of modelling an entire capsid of the PCV2 virus in explicit water are presented. Particular attention has been given to the distribution of ions inside the capsid. Because the internal surface of the capsid is positively charged, the correct distribution of chloride ions is important. It is demonstrated that this distribution is ultimately connected to the stability of the capsid.

keywords：capsid, Molecular Dynamics, stability, PCV2, all-atom simulation

1. Introduction

Even though the structures of many viruses are largely known, the atomistic details of their molecular structures became available from experimental measurements only recently. The main methods include X-ray crystallography and cryo-electron microscopy [1−5]. These methods, however, necessarily measure the structure at low temperature and/or in crystalline state, that is very far from the physiological conditions present in living cells. Also, almost always some parts of the virus remain unresolved due to their high flexibility or asymmetric packing in the crystal.

An alternative is to model these systems using all-atom Molecular Dynamics (MD) [6−15]. Modelling provides the structure and (for limited intervals of time) the dynamics of the virus. The latter is being progressively increased with the development of specialised high-performance computers for MD [16-17]. Here, we report the structure of all-atom MD modelled capsid of virus porcine circovirus 2 (PCV2) without the genome inside. We investigate the structural stability of the capsid in explicitly represented water solution of 0.15M NaCl imitating biological environment.

2. Molecular systems and simulation details

The capsid of PCV2 consists of 60 copies of a protein, positioned according to icosahedral symmetry. The 3D structure of this protein was measured using X-Ray crystallography [1] (PDB ID 3R0R). The 3D structure of the first 32 residues of the N-terminus was not possible to deduce due to their high mobility or asymmetric packing. To recreate the missing parts of the structure, we used the known sequence of the amino acid residues and the Modeller software [19]. It uses homology modelling for predicting the structure. For each system, the protein subunit was copied 60 times and placed according to the transformation matrices using VIPERdb [20].

The total charge of the capsid equals 360 e (without tails) or 180 e (with tails) and is non-uniformly distributed between its inner and outer surfaces [18]. This can be seen on dependences of the sum charge of the capsid atoms within some distance from its centre of mass (COM) on this distance, presented in our paper [18]. The distinct well in one of the graphs corresponds to the tails that extend toward the capsid centre. The middle of

the capsid wall is located at 8.5 nm from its COM, but the maxima in the charge plots are situated at ~7.8 nm, which indicates that the positive charge is concentrated at the inner surface of the wall. Its magnitude was assumed equal to the value at the maximum, which is +606 for the capsid without tails and +407 for the other one. The outer surface is charged negatively with a smaller magnitude (-246 in the capsid without the tails and -227 in the other one).

Therefore, for the empty capsid, the distribution of the ions, which neutralise the charge, are expected to be important for the stability of the capsid, as they substitute the DNA in this role. It has been shown for the satellite tobacco mosaic virus that, indeed, the ions tend to be placed at the sites normally occupied by the genome [9]. To test this hypothesis, we modelled several systems that include different number of chloride ions in the capsid.

Because the capsid appeared to be highly polar, we neutralized the charge of each surface separately. Namely, 606 (for the capsid without the tails) or 407 (for the other one) Cl− ions were placed into the capsid cavity, and 246 (for the capsid without the tails) or 227 (for the other one) Na+ ions were placed in the bulk solution. Finally, 1720 Na+ and Cl− were uniformly distributed across the system to mimic 0.9% NaCl physiological saline. The systems with the stated amounts of ions will be called ‘neutralizing’ further in the text.

Also, for both capsids we performed simulations with reduced numbers of ions, where just half of the needed number of Cl− ions inside was placed (namely, 300 in the capsid without the tails and 200 in the other one). To the outer solution 60 Cl− (for the capsid without the tails) or 20 Na+ (for the other one) were added. 1720 Na+ and Cl− were distributed, as well. The described above systems will be called “low”. The numbers of added ions are summarized in Table 1 for the systems without the tails and in Table 2 for the system with the tails.

Table 1

Numbers of ions added to the capsids without the tails

System	Added ions	
	Inside	Outside
“neutralizing” systems	606 Cl^-	246 Na^+
“low” systems	300 Cl^-	60 Cl^-

Table 2

Numbers of ions added to the capsids with the tails

System	Added ions	
	Inside	Outside
“neutralizing” systems	407 Cl^-	227 Na^+
“low” systems	200 Cl^-	20 Na^+

GROMACS package has been used for performing the simulations [21]. Two forcefields were used for confidence: GROMOS53a6 together with SPC water and AMBER03 with TIP3P water. Here, the results obtained with the latter will be presented only for short. The results of the former are generally similar.

The energy minimisation was carried out initially, followed by simulations with the capsid atoms restrained to their positions. Such simulations were performed at 200 K and then at 300 K, each for 1 ns. Then, the position restrains were removed and the systems were simulated for 10 ns at 300 K. Velocity rescaling thermostat was used. The cut-off distances for direct sum of PME electrostatics and for van der Waals interactions were set to 1 nm.

3. Results

As we hypothesized, the systems with the neutralising number of chloride ions inside the capsid demonstrated stable structure after the production run of 10 ns. In contrast, the non-neutralising number of ions led to the capsid’s deformation with the tendency towards the

collapse of the structure.

3.1. The capsids with the neutralizing number of ions

The graphs of root mean square deviation (RMSD) (a) and radius of gyration (b) are shown in Fig. 1 for the neutralising system [18].

The RMSD of the capsid without the tails with respect to the crystallographic structure continues to grow (Fig, 1a), while the gyration radius (Fig 1b) changes insignificantly, which allows us to conclude that the capsid's structure is stable. The increase in RMSD can be explained by the "breathing" motion of the capsid caused by the thermal motion of water in the system that leads to insignificant dynamics of the capsid without the destruction of its structure. Similar behaviour is observed for the system with the tails: the value of RMSD (Fig. 1a) is larger than for the system without the tails because the former has 60 N-terminal domains located inside the capsid and affecting the dynamics caused by the thermal motion of water molecules.

The gyration radius of the capsid with the tails (Fig. 1b) increases to 8,35 nm at the beginning of simulation and remains constant for the rest of the simulation. The increase of diameter is caused by the N-termini rearrangement, after which the radius stabilises because of the strong interaction of the tails' charged groups with other tails, as well as with the interior of the capsid.

Compared to AMBER03 forcefield, the GROMOS53a6 one produces somewhat less stable structures, where gyration radius keeps growing, albeit very slow.

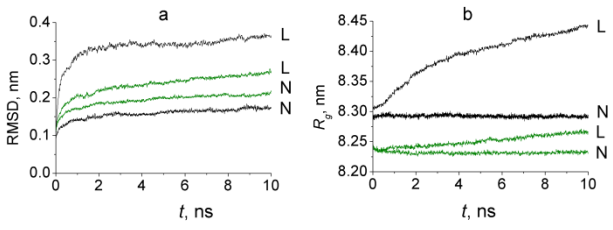

Figure 1. The dependence of RMSD (a) and gyration radius (b) on time for the systems with the tails (green curve) and without them (black curve) with respect to the initial structure. N – neutralising systems, L – low ions systems. The analysis did not include the N-termini, thus, the same number of residues was involved.

3.2. The capsids with the low number of ions

The comparison of RMSD between the simulation with the neutralising number of ions and that containing twice as few number of ions confirms the collapse of the structure in both GROMOS53a6 and AMBER03 forcefields (Fig. 1) [18]. Similarly to [8], we observed asymmetric destruction of the capsid expressed as a strong deformation along one axis.

We have analysed the distribution of Cl- and Na+ ions during the first nanosecond of the simulation for the systems with the neutralising number of ions and with the low number of ions [18]. Sodium ions tend to concentrate on the outer surface of the capsid for both low and neutralising number of ions. The chloride ions tend to concentrate at the inner surface in both cases. In the non-neutralising scenario, the shortage of ions leads to voids in their distribution in the areas around the pores. For the neutralising case, the ions are distributed uniformly covering the inner surface without gaps [18]. In the Fig. 2, the location of the ions is presented after 1ns of the simulation.

We put forward a hypothesis that connects the stability of the capsid with the presence of both sodium and chloride ion layers.

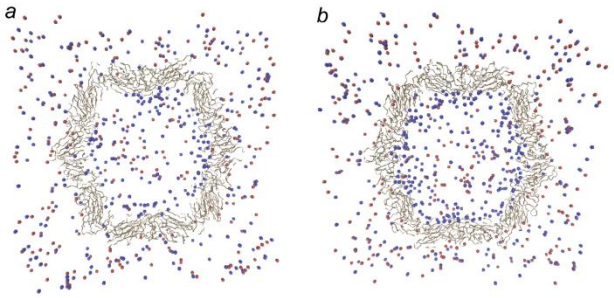

Figure 2. Distribution of ions after 1ns of the simulation for low (a) and neutralising (c) number of ions. Red is for sodium, blue is for chloride ions. The capsid is shown cross cut in the middle.

The shortage of the chloride ions leaves the positive charge of some regions of the inner wall uncompensated, which leads to weakening of the interaction between the protein subunits. In the neutralising number of ions case, the uniform layers of negative and positive charge on the inside and outside of the capsid are attracted to each other, thus stabilising the structure of the whole capsid [18].

4. Conclusions

Summarising, we showed that the correct number of ions placed inside the virus capsid is critically important for its stability in MD simulation. The number of the chloride ions should be such that they neutralise the positive charge of the capsid inner surface.

Between the two forcefields tried, AMBER03 seems to be more suitable for such simulations, possibly because of faster settling of the stable structure of the capsid.

Finally, we showed that the overall difference between the crystallographic structure and the structure in biological environment is not significant that proves the resemblance of the measured structure to the one of live virus.

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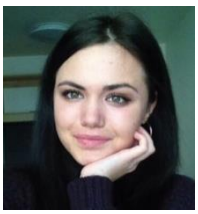

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