ISSN 0974 - 9373

Vol. 24 No. 1 (2020) **Journal of International Academy of Physical Sciences** pp. 105- 114

Molecular Dynamical Investigation of a YodA Protein Signifies Zinc Ion-Residues Interactions

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(Received February 02, 2020)

Abstract: YodA protein is well known lipocalin-like protein which shows interaction with Zinc (Zn^{2+}) ions, so called metal binding protein. The synchronization of Zn^{2+} ions on protein surface was investigated by molecular dynamics (MD) simulation. A systematic MD study of crystal structure (1OEK.pdb) of YodA protein was taken into account. Moreover, simulations of two more systems, *i.e.* entry of free (i) 4 Zn^{2+} ions (ii) and 2 Zn^{2+} ions in the bulk of Apo form of YodA protein were considered to emphasize the binding event and encounter of ions on residues of protein surface. Stabilities of each system were probed and further investigations revealed, Zn^{2+} ions show robust interactions with glutamate and histidine residues. This revelation was corroborated by distance calculation during simulation. By comparing with other amino acid residues, glutamate and histidine demonstrate the better coordination with Zn^{2+} ions. This study might enhance to understand the Zinc interaction with YodA protein and investigation can propose significant hypothesis on metal resistance and bioremediation strategy.
Keywords: Protein, crystal, glutamate, strategy, lipocalin-like.

1. Introduction

Many bacterial systems are competent of resisting toxic metal levels of indispensable metals like copper, cadmium, zinc, iron by reducing the uptake, storage in metal particles and organic ligands and/or by increased

outflow[1-3]. The proper uptake of metals participates an important role in the host pathogen interaction. However, uptake with increasing concentrations of ions, such as zinc (Zn^{2+}), is reported in many organisms[4]. About decade before, determination of Zinc binding protein crystal structure was reported to confirm the Zn^{2+} binding in Escherichia coli, which is commonly text as YodA protein. It has been identified as part of the response of the bacterium to a challenge with Zinc. Experiment suggested that the expression of YodA may show general stress response. 1OEK.pdb, was reported as YodA protein in protein databank database and in this crystal structure, four Zn^{2+} ions were bound with the two amino acids residues of protein in which three were bound with 3 residues of glutamate, histidine and aspartate along with water molecule as a heteroatoms and last one was observed to interact with three histidine molecules. Various studies were involved on the metal protein coordination by using different computational approaches. Molecular dynamics (MD) simulations generate a wealth of data in which MD trajectory requires analysis in terms of the individual positions (and possibly velocities and forces) of all atoms or a selected subset of atoms for each time frame of a trajectory. MD approach was also provided better result in terms of protein-metal, protein cofactors and/or protein-ligand complex. Previously, different protein models were generated for bacterial system Salmonella enterica and investigated the zinc binding affinity with these models by molecular dynamics simulation[5]. In this study, it is shown trajectory analysis of YodA protein and addition of Zn^{2+} ions. Main focus of this work is to highlight the stability factors and propensities of ions with particular amino acid residues in protein and make comparative study with other systems.

2. Materials and Method

Classical molecular dynamics (MD) simulation was taken into consideration[6]. All MD simulations were performed using the MD simulation package Gromacs4.0.6[7, 8]. The structure of the YodA as a wild type protein (1OEK.pdb) was taken[9] and found protein databank database. For graph visualization, XMGRACE (http://plasmagate.weizmann.ac.il) was used. PyMOL[10] package was used for visualization and system inspections.

2.1 Simulation Details: Gromos96 53a6 force field[11,12] was applied for calculation of MD performance. The YodA protein was solvated with Simple point charge (SPC) water model [13,14] and centred it at least in 1nm

from the box edge, box was defined as cube. System was treated with periodic boundary conditions. The bond lengths in the protein were constrained using the LINCS algorithm[15]. Energy minimization, temperature and pressure bath ensemble were conducted for 500 pico second (ps) simulation steps. The equations of motion were integrated using a leap frog algorithm with a time step of 2 fs. Simulations to equilibrate the system were performed during 500ps using Berendsen coupling algorithms[16] to obtain a temperature of 300 K and the isotropic pressure of 1 atm. The PME algorithm[17] was used for electrostatic interactions with a cut-off of 1 nm. The Lennard-Jones interactions were truncated at 1.0 nm. Neighbour searching was performed every 5 steps. In the production MD runs the V-rescale thermostat[18] was employed instead of the non-stochastic thermostat used during equilibration. A single cut-off of 1 was used for Van der Waals interactions. Temperature coupling was done with the V-rescale algorithm. Pressure coupling was done with the Parrinello-Rahman algorithm[19].

3. Result and Discussion

The list of simulation was represented in table 1.

Table 1: List of simulations performed in this study

Serial number	Systems (1OEK.pdb)	Simulation time (nano-second)
1	4 Zn^{2+}+ YodA (crystal)	50
2	4 Zn^{2+} (in the bulk) +Apo-YodA	50
3	2 Zn^{2+} (in the bulk) + Apo-YodA	100

3.1 Interaction of Zn^{2+} ions in Crystal structure of YodA Protein: Four Zn^{2+} ions interaction was found by visualizing the YodA protein crystal structure. Glutamate, histidine and aspartate residues were shown interaction with three Zn^{2+} ions separately along with water molecules. In addition, with, three Histidine residues with different specifier were found to get interacted together with an ion (Figure1). One residue of surface protein are glutamate, histidine and aspartate, which were specifically interacted with ion even more tendency.

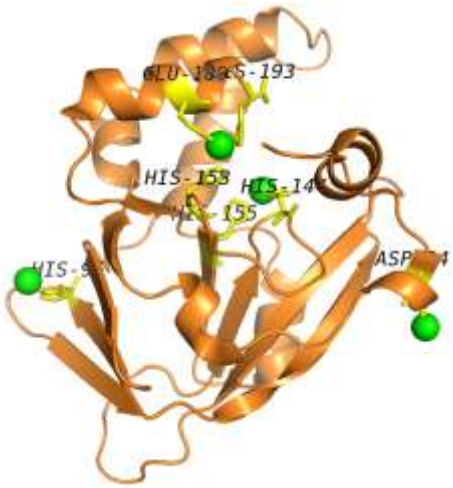

Figure 1. The presented structure is ID: 1OEK.pdb, a YodA protein crystalized in presence of Zn^{2+} ions (green balls). For the purpose of simulations, the crystal Zn^{2+} ions were taken into consideration, however, crystal ions were removed for system of 2, 3 (Table 1) and entry of free Zn^{2+} ions were added in bulk. Protein surface is depicted in orange cartoon while interacted amino acid residues are coloured with yellow stick around ions.

One of the most important fundamental properties to analyse protein stability and its stability close to the crystal structure, the standard way to measure this is by the root mean square displacement (RMSD). RMSD calculations for Holo-YodA protein was depicted in Figure 2. The RMSD values of each system increased at the beginning with respect to its native structure which was shown few mild fluctuations between ~35ns and end of simulation time. Average RMSD value was obtained as 0.39 nm on comparison with crystal structure.

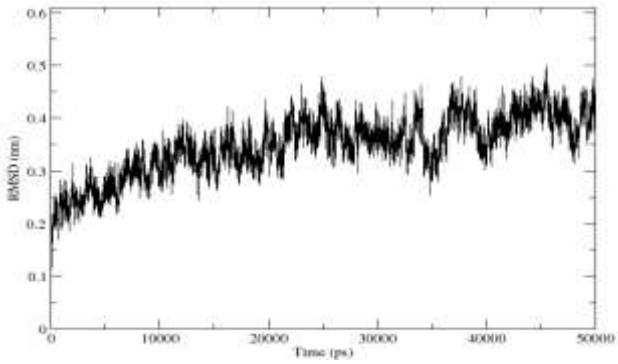

Figure 2. RMSD of Holo-YodA (1OEK.pdb) crystal during the simulation, relative to the starting crystal structure.

Convergence of the simulation towards equilibrium was inspected from the structure relaxation. Usually, such relaxation is only considered in terms of the Euclidean distance from a crystal structure (Figure 2). It increases rapidly in the first part of the simulation, but stabilizes around 0.38 nm, roughly the resolution of the x-ray structure. The difference is partly caused by limitations in the force field, but also because atoms in the simulation are moving and vibrating around an equilibrium structure. We employed a better measure, in which first created a running average structure from the simulation and compared the running average with the x-ray structure, which gives a more realistic RMSD.

3.2 Entry of Free Zn^{2+} ions into the Bulk of the Apo-YodA Protein: The mechanism, by which Zn^{2+} travel to the surface of the protein and find the binding domain, was investigated by the present experiment (system 2, 3 table-1). Moreover, specific binding residue was also revealed while examining the prominsties of ions's encounter. The four and two Zn^{2+} were added to the water box of the apo-YodA structure, which was initially in bulk, and found their positions randomly. Entry led to an average frequency of the encounter, where the Zn^{2+} approached the solvating shell and found preferable carboxylate moieties. Residues that were identified in the first and second encounters of a Zn^{2+} was mainly glulamate. In the first observation, to quantize this event, we approximated the minimum distance between the identified residue and, collectively, all Zn^{2+} (Figure 3).

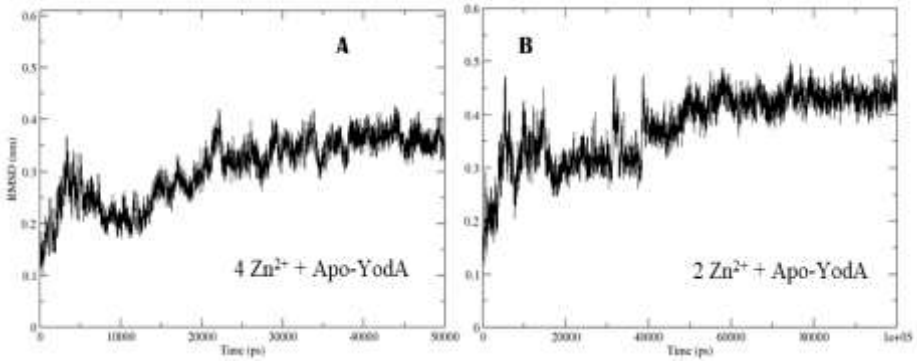

Figure 3. RMSD of systems 2 and 3 in table 1. **A.** Free entry of 4 Zn^{2+} ions into the bulk of Apo-YodA system. **B.** RMSD of system of free entry of 2 Zn^{2+} ions into the bulk of Apo-YodA system. Entry of 4 ions in the bulk shows stability in last ~15ns during simulation of 50ns, while entry of 2 ions represents plateau from ~50ns to end of simulation.

3.2.1 System of entry of 4 Zn ions:

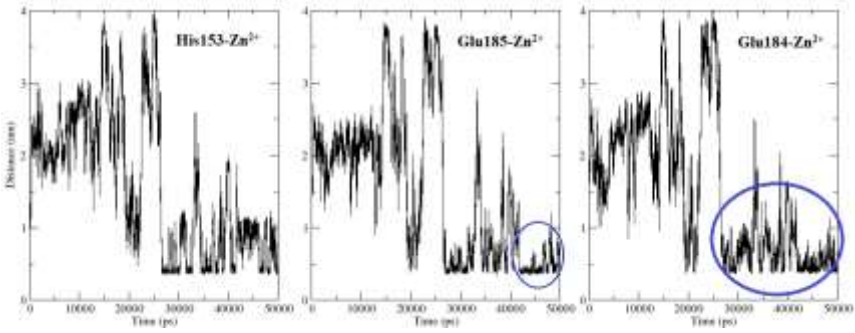

Figure 4. Distance calculations from Zn^{2+} to specific Histidine (His), Glutamate (Glu) residues depicted as figure ligand. Specifically, carboxylate moieties in protein surface shows better propensities with ions where Zn^{2+} reaches at ~27ns time and maintain till end of simulation time. Ions maintain proper distance with Glutamate, highlighted in blue circles. Although few major fluctuations of ion are shown on interaction with His.

Figure 4 confirms that the Zn^{2+} ions were in bulk initially and discovered crucial surface residues within the time period from ~27 ns to ~50 ns with exception of few fluctuations. His153 and E184-185 were identified as crucial moieties, which obtained significant propensities in the direction of Zn^{2+} ions. These residues of Apo-Yoda protein were reached with an appropriate distance of <0.30 nm (solvation shell) and remained horizontal at the same distance until the end of simulation.

3.2.2 System of entry of 2 Zn ions:

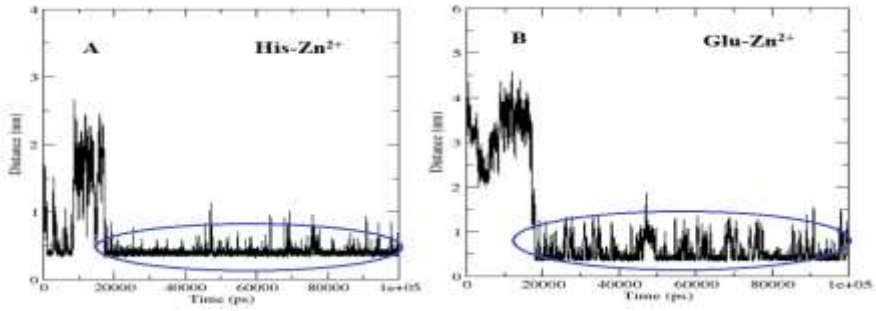

Figure 5. Calculations of distance during simulation between Zn^{2+} ions and His and Glu residue. Blue eclipses are shown minimum distances maintained by ions within 0.4nm. Ions demonstrate equal residence time to reach protein surface.

Figure 5 reports occurrence of ions within the solvation shell of protein which was evidenced by calculation of distance between ions and specific residue. Basically, the experiment of entry of 2 Zn^{2+} ions in the bulk of the box of Apo-YodA protein, recruited histidine and glutamate residues as crucial residues that maintain a distance of approximately 0.30 nm within Zn^{2}+ ions. The binding time of Zn^{2+} is the contact time at which these two moieties possess distances less than 0.4 nm and the time explains the total staying time of ions on the residues after the encounters with the surface of protein. Mostly carboxylate moieties, histidine and glutamate, were identified in protein surface by means of high residence time with Zn^{2+} ions. This study revealed the motion of the Zn^{2+} and binding robustness of ions on the histidine and glutamate. The maximum residence time between residues and ions are approximated as simulation time of 80 ns.

The conclusions drawn for these experiments of entry of Zn^{2+} in bulk were supposed to be significant for ion travel on the surface of the YodA protein. In addition, all four Zn^{2+} were started disappearing from the bulk very rapidly after ~25 ns. In case of the histidine residue, the ions achieved appropriate distances at the very beginning of the simulation like glutamate. A comparison of the binding phenomenon using the crystal structure revealed that the ions accumulated on the glutamate and histidine residues regions and also on residues of the adjacent domain to facilitate their contribution in the role of YodA activity. The response of microorganisms to metals ions, such as zinc, copper, mercury and cadmium, has been studied in some detail[20-25]. The composition of YodA protein is made up of two domains: a major domain that is structurally related to the lipocalin/calycin family of proteins[26] and a smaller helical domain. Lipocalins are a growing family of small, usually secreted proteins whose main structural feature is an up-down beta-barrel[27, 28].

Recently, calcium ions interaction with protein residues were proposed through MD simulation[29]. The MD simulations is reported to provide a structural dynamical picture of the YodA protein and experiments of entry of Zn^{2+} ions in bulk. MD study of protein molecule has solved various structural problems. The MD simulations of protein molecule has been demonstrated various interaction problems. Zn^{2+} ions binding residues in protein was highlighted and evidenced significantly by distance calculations.

4. Conclusion

These findings are appreciated for understanding the behaviour of zinc ions on lipocalin-protein, the results may be supportive for Zn^{2+}-mediated YodA activity and propensities of zinc on carboxylate moieties. Study shows a focus on structural insight about interaction improvement between zinc binding residues in YodA protein. Further, study provides an explanation for access to the Zn^{2+} preferably by the histidine and glutamate residues. Because of the role of the ion as a key regulator of protein function, results suggest that the role of YodA protein might be to decrease the concentration level of zinc ions in *E. coli* cells during cadmium stress by its ability to bind heavy metal.

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