Molecular dynamics simulations to see the effect of temperature and pressure change on the dynamics of protein

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Abstract

In this work we study the effect of temperature and pressure on the dynamics of calmodulin protein using molecular dynamics simulations. The calmodulin protein is used for this study as a first step which can be extended further to other proteins. Calmodulin is a calcium binding protein and thus plays a very important role in the transport of calcium ions inside the cell. The temperature is earlier reported to play an important role in the dynamics of this protein which is applicable to the other proteins as well. In this study we therefore study the combined effect of temperature and pressure on the conformation change of this protein. The pressure alone is not observed to play a significant role in the dynamics of the protein whereas pressure along with temperature is observed to change the conformation of the protein to a large extent. The results were confirmed with the molecular dynamics simulations performed with the help of NAMD software. These results are consistent with the experimental results of the other workers performed with fourier transform infrared spectroscopy. Temperature is observed to pave pathways to expand/unfold the whole protein structure making it more susceptible to the effects of pressure.

Keywords: *Protein, Conformation, Calmodulin, Molecular Dynamics*

1. Introduction

Calmodulin, or we can say calcium-modulated protein, is a calcium-binding protein found in the cytoplasm of all eukaryotic cells. It interacts with many other proteins in the cell and acts as a regulator molecule in a large variety of cellular functions, these function includes like regulation of cell cycle, fertilization, muscle contraction, and intracellular signalling [1,2,3]. It is a family of protein along with troponin C, another essential protein.

a. Structure of protein

Calmodulin, a protein that contains 148 amino acids is highly protected shown in Figure 1. This well knows protein is widely known with two symmetrical globular domains each containing the *N*- and *C*-domain separated by a flexible linking region having four Ca^{2+} binding sites [1]. The positions of the Ca^{2+} ions can be specified in amino acids: 21–32, 57–68, 94–105 and 130–141; in each region calcium binds precisely 12 amino acids. These regions are located between two alpha-helices, the first two regions (21–

32 and 57–68) are on one side of the linker region the other two (94–105 and 130–141) are on the other side [2]. Calmodulin binds a huge variety of target proteins, making it important for flexibility. Its flexibility can be shown when it is bound to a target protein, Nuclear magnetic resonance studies tell that the linker region of Calmodulin protein is flexible, even when it is not bound to a target protein. The EF-motifs hands of calmodulin bind up to four calcium ions. This supply an electronegative environment for ion coordination. After calcium binding, the hydrophobic methyl groups exposed to the protein via conformational change. Using both X-ray spectroscopy and nuclear magnetic resonance studies, the scientist can find out the conformational changes occurred in the alpha-helices of the EF motif that changes the binding affinity for target proteins.

b. Conformation change in protein

Conformation of protein is very important in understanding the biomolecular interactions. In the simplest case, two biomolecules can interact having no change in their conformation. Molecular interactions which show conformational changes in the interacting molecules are functional. Conformational changes in the protein molecule can be produced by pH variation [4], temperature [5], pressure [6], etc. Thus, the affinity of the protein for the two phases is changed by restructuring the molecule.

Conformational changes in proteins are also possible by their intrinsic flexibility. These changes may occur with only small amount of energy. At the molecular structural level, the conformational changes in single polypeptides are produced due to the change in main chain torsional angles along with the side-chain orientations. The overall effect of such changes may be localized with reorientations of a few residues and small torsional changes in the regional main chain. On the other hand, torsional changes localised at very few critically placed residues may lead to large changes in tertiary structure.

2. Methodology

Computer simulations are carried out for understanding the structure and molecular interactions of the molecules. The two main branches of simulations are molecular dynamics and Monte Carlo. In this work we use classical molecular dynamics simulations. Computer simulation gives interference between the microscopic world to the macroscopic world to understand the bulk properties. Here we can guess the interactions to find the accurate predictions of properties. Basically, we perform simulations on a computer because they are hard to perform on the laboratory as we work at extreme pressure and temperature conditions.

a. Molecular Dynamic (MD) and its Algorithm

Molecular dynamics basically gives the idea of what is happening between the atoms, what kind of interactions are going on, what amount of forces they are facing or exerting. So here we assume a given energy potential function, and this function helps us to find the force experienced by the atom from another atom at given position and instant of time. From this calculated force and newton's law of motion, we can find out how this force is affecting the motion of the atoms. So basically we divide the time in different time steps not more than a few femtoseconds i.e 10^{-15} second. At each time step, we compute the pressure, energy, force. And after that, we move the atom a little bit and update the position and velocities of an atom using newton's law of motion.

The molecular dynamics simulation method is based on Newton's second law that is **F=ma**, where **F** is the force exerted on the particle, **m** is its mass and **a** is its acceleration. If we know the force acted on each atom we can find out the acceleration of each atom in the system. On integration of this equation if motion we can find out the position, velocities, and accelerations of the particles on a specific time. If the positions and velocities of each atom are determined, we can predict the state of the system at any instant of time. Simulations of solvated proteins are calculated up to the nanosecond time scale. In this work we perform all our MD simulations for 40 ns.

b. Softwares used

There are many molecular dynamics softwares but their core functionality are quite similar. Some widely used programs for molecular dynamics of biomolecules such as CHARMM [7] and its direct family is AMBER and GROMOS. These softwares have a great range of capabilities. These programs help users in a large extent where users can not directly find their exact solutions.

i. NANOSCALE MOLECULAR DYNAMICS (NAMD)

NAMD [8] is computer software for performing molecular dynamics simulation and it is written using the Charm++ parallel programming model. Because of its parallel efficiency, it can be used to simulate large biological systems having millions of atoms. It is a parallel molecular dynamics code enabling interactive simulation by linking to the VMD [9]. NAMD has an interface to quantum chemistry packages such as ORCA and MOPAC, as well as a scripted interface to many other quantum packages. Together with Visual Molecular Dynamics (VMD), NAMD's interface provides access to hybrid QM/MM simulations in an integrated, comprehensive, customizable, and easy-to-use suite.

ii. VISUAL MOLECULAR DYNAMICS (VMD)

Visual molecular dynamics (VMD) is a molecular modeling and visualization computer program. VMD is developed as mainly a tool to view and analyze the results of molecular dynamics simulations. It also includes tools for working with volumetric data, sequence data, and arbitrary graphics objects. VMD software is designed for modeling, visualization, and analysis of biological systems, such as proteins, nucleic acids, etc. It can be used to view more general molecules since VMD can read standard Protein Data Bank (PDB) files and display the contained structure in 3D. VMD provides a wide variety of methods for rendering and coloring a molecule. VMD can be used to animate and analyze the trajectory of a molecular dynamics (MD) simulation. VMD can act as a graphical front end for an external MD program by displaying and animating a molecule undergoing simulation.

3. Results and Discussion

The MD results of the simulations performed under different temperature and pressure conditions are discussed here. The temperature earlier was reported to play an important role in the dynamics of this protein which is applicable to the other proteins as well [4]. In this work we therefore work on performing our MD simulation at a higher pressure of 4 atm and temperature 300 K. The protein is first solvated in a water box of size 10 Å as seen in Figure 2.

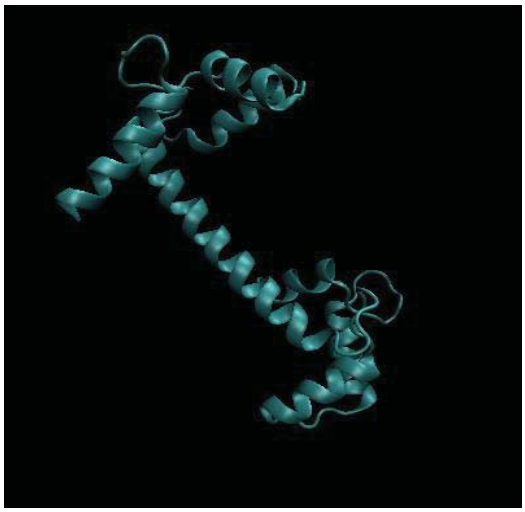


FIG 1. Schematic diagram of Calmodulin (CaM) protein

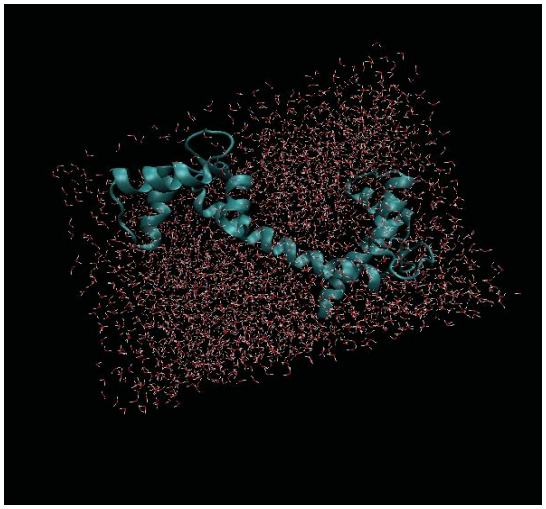


FIG 2. Schematic diagram of Calmodulin (CaM) protein

The protein is observed to behave normally at this temperature and pressure conditions not showing any significant variation in the energy. The plots are not shown here due to the brevity of space. This means that a higher value of pressure alone does not play any significant role on the dynamics of the protein. To further observe this in detail we perform MD simulation of 40 ns at a higher temperature and higher pressure value as 375 K and 4 atm pressure. This is done to see a combined effect of higher temperature and pressure values on the dynamics of protein. The root mean square deviation (RMSD) for the same is as shown in Figure 3.

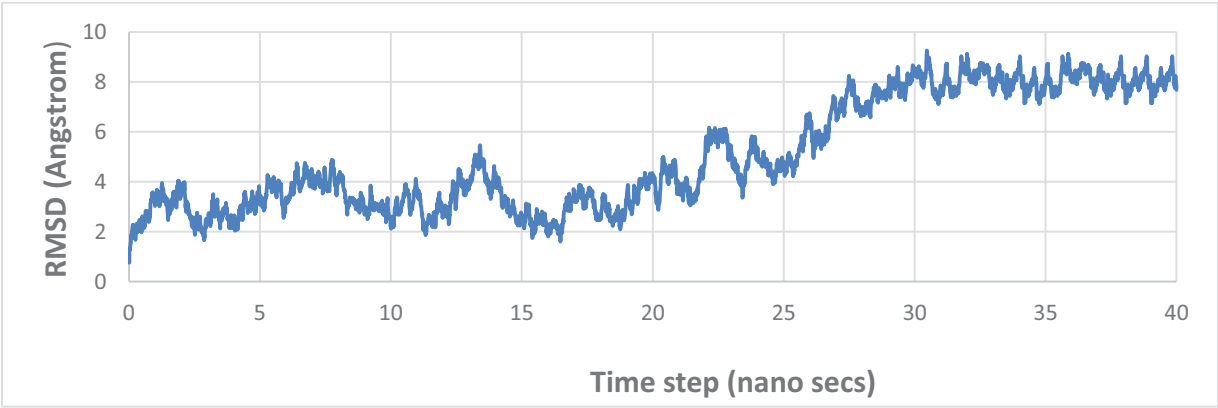
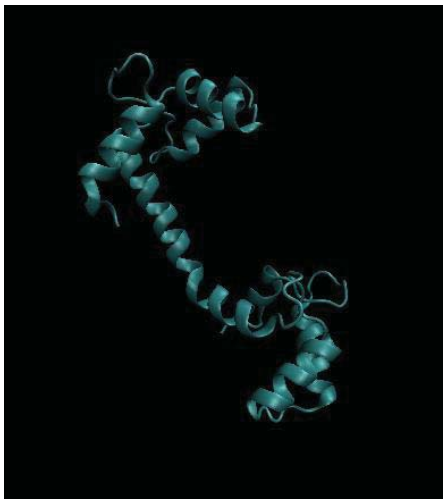
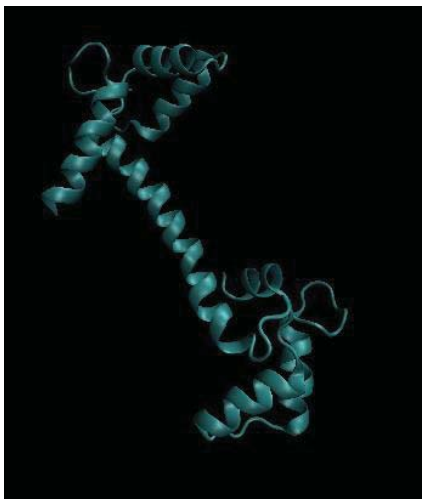


FIG 3. Room Mean Square Deviation (RMSD) of the CaM protein as function of Time Step (ns)

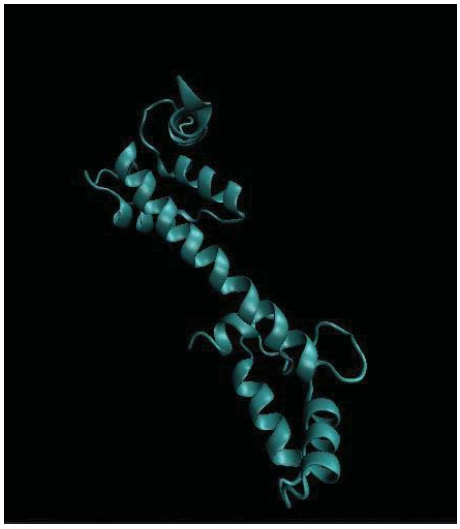
A significant variation is observed towards the end of the simulation which confirms dynamic behavior of the protein. The RMSD is observed to stabilize towards the end of the simulation. The snapshots for the same are as shown in Figure 4. The two lobes of the protein are observed to come closer during the course of the simulation. The linker is also observed to band towards the end of the simulation. This confirms a conformation change in the protein which is a collective effect of temperature and pressure variation. This is in agreement with the experimental results of the other workers performed with Fourier transform infrared spectroscopy [6].



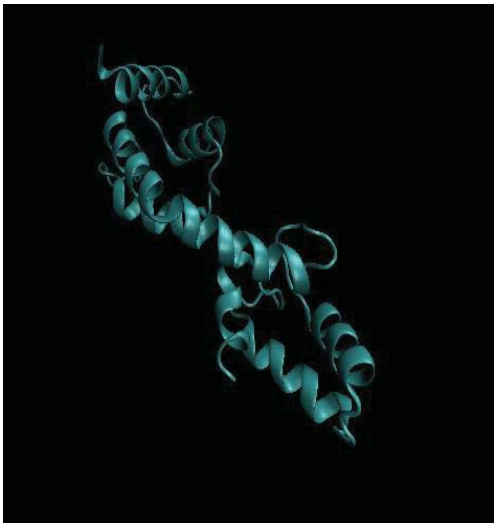
(a) 3.2 ns



(b) 10 ns



(a) 30 ns



(d) 37 ns

FIG 4. Shapshots showing a conformation change in the CaM protein at (a) 3.2 ns (b) 10 ns (c) 30 ns (d) 37 ns

4. Conclusion

The combined effect of temperature and pressure on the dynamics of calmodulin protein is studies using molecular dynamics simulations. Pressure alone is not observed to play an important role in the dynamics of this protein whereas the combined effect of temperature and pressure brings a conformation change in the protein. The linker part is observed to bend whereas the two lobes come close to each other during the simulation. The results were confirmed with the molecular dynamics simulations performed with the help of NAMD software. The results are observed to be consistent with the experimental results of the other workers performed with fourier transform infrared spectroscopy. Temperature is observed to pave pathways to expand/unfold the whole protein structure making it more susceptible to the effects of pressure.

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