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Molecular Dynamics Simulation Study of the Structural Changes of Calcitonin in an Explicit Solvent

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Abstract

Calcitonin is a 32 amino acids polypeptide hormone found in the osteoclast to lower the calcium and phosphorus level in different animals. This study employed molecular dynamics simulation technique to investigate the structural changes of calcitonin in an explicit solvent using the GROMACS 2016.5 software. The modeled structure of calcitonin was used during MD simulation using the Amber ff99-SB force field, TIP3P water model under isothermal-isobaric condition with periodic boundary condition imposed on x, y, z direction. The analyses were done using GROMACS software, grace and visual molecular dynamics (VMD) packages. The Root Mean Square Deviation (RMSD) Root Mean Square fluctuation (RMSF), hydrogen bonds and Radius of Gyration and end to end distance were computed from the reference initial structures. The RMSD values ranges from 0.5 nm to 1.5 nm during the simulation. The 6^{th} residue (Thr) and 20^{th} (His) residue has the lowest fluctuation at 0.4 nm. The RMSF values of the residues show that the polar residues tend to have a higher fluctuation than the hydrophobic residues with cysteine (1^{st} residue having the highest RMSF value of 0.8 nm and threonine 6^{th}residue with the lowest RMSF value of 0.4 nm. Twenty-six intramolecular hydrogen bonds were found which indicates the stability of the protein during the simulation. Radius of gyration was computed to determine its compactness with water. The end to end distance of the protein decreases from 45.77 nm in the starting structure to 15.28 nm in the final MD structure and this indicates that the protein was undergoing folding during the simulation. From the RMSD, RMSF values, calcitonin tends to be relatively stable at a conformational state, the hydrogen bonds also supports its stability. The radius of gyration reveals it compactness with water and the end to end distance also supports its folding process.

Keyword: Calcitonin**,** Root Mean Square Deviation**,** visual molecular dynamics (VMD)**,** Root Mean Square fluctuation (RMSF)**.**

Introduction

Computational chemistry is the scientific method of applying computers to gain chemical information. It is the link between theoretical and experimental chemistry. Theoretical chemistry is mainly concerned with the development of mathematical models which allow one to derive chemical properties from calculations and to interpret experimental observations. The mathematical models developed in theoretical chemistry are usually validated by comparism with experiment.

Theoretical chemistry existed before the arrival of electronic computers. Computational chemistry, however, relies heavily on powerful microelectronics to cope with huge computational tasks. It focuses on the application of theoretical methods which require calculation treatments which are by far too large to be done without fast computers (Hoffmann *et al.*, 2003).

Of course, the strict separation of theoretical, computational, and experimental chemistry is of an academic nature. In practice, theoreticians often not only develop a new method but also need to design more efficient algorithms to make the method applicable. Before computational results can be interpreted, computational chemists need to undertake benchmark studies to determine the limitations of a method. Without the knowledge about the accuracy of the applied mathematical model, any computational study is without scientific significance (Leach, 2001).

Aim and Objectives

The aim of the study is to employ Molecular Dynamics Simulation technique to investigate the structural stability of Calcitonin in explicit solvent within the 250 nanosecond time of the experiment.

The objectives of the study include:

- To measure the structural stability of calcitonin by determining the Root Mean Square Deviation (RMSD)
- To determine the compactness of Calcitonin in water
- To determine the intramolecular H-bond present
- To check for possible secondary structure of Calcitonin

Methodology

Starting Structure

Calcitonin structure was built using CHIMERA software. UCSF Chimera (or simply Chimera) is an extensible programme for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, trajectories, and conformational ensembles (Petterson *et al.*, 2004). The molecule was

built using the thirty-two amino acid letter sequence CGNLSTCMLGTYTQDFNKFHT FPQAIGCGAP.

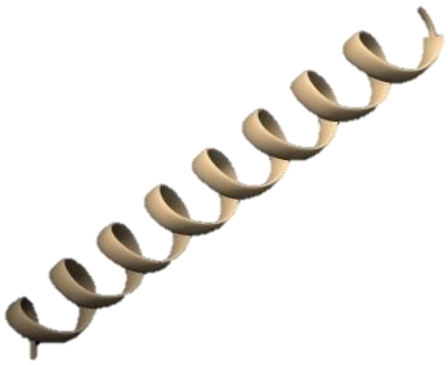

Figure 1.Cartoon Structure of Calcitonin Drawn with Chimera

Protocol of the Molecular Dynamic Simulation

All the simulations were carried out using the Groningen machine for chemical simulations (GROMACS 2016) (Majid *et al.*, 2019). The AMBER99SB protein (Hassan *et al.*, 2017) nucleic AMBER94 force field and TIP3P water model (Jorgensen *et al.*, 1988) were used. The molecule in the pdb format was converted to gro file using the "pdb2gmx" command line and the configurations were edited before solvation. The protein structure was solvated with an explicit solvent in a cubic box of water to imitate the natural environment of Calcitonin in the body. After solvation, the Calcitonin system has a net charge of -1.00e- so a Sodium ion was added to counter the ions (which was assumed to be Chlorine) to make the system neutral.

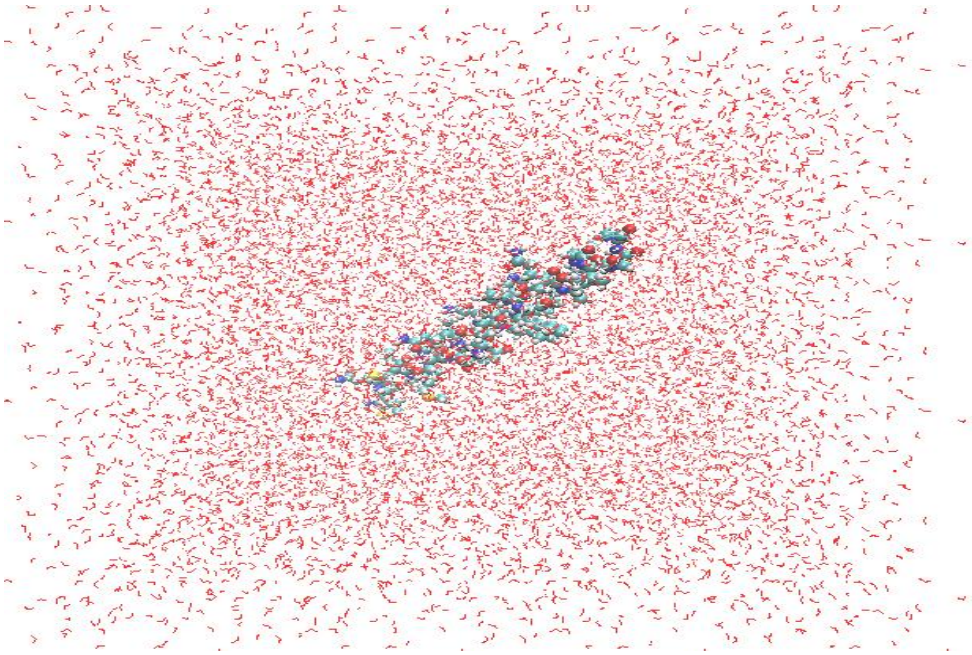

Figure 2.Solvated Calcitonin

Energy Minimisation

Two rounds of minimization were carried out on the system. The first energy minimization was done using the steepest descents method which was weakly coupled to an external bath using the Berendsen's thermostat. The temperature was maintained at 300 K. The energy minimized structure was subjected to a position constraint during the simulation for 20ps.

The input files stating the value of the parameters for the first and second energy minimization are given in appendix I and II respectively.

Equilibration

Two rounds of equilibration were done on the system. The first equilibration was done after the first energy minimization and the second after the second minimization. All bonds were constrained in the first equilibration with Lincs and molecular dynamics integrator while in the second equilibration Nose-Hoover temperature coupling and Parrinello-Raman pressure coupling with isotropic type was used. A verlet cut-off scheme was used to maintain the system at 300 K. The input files stating the value of the parameters for the first and second equilibration steps are given in appendix III and IV respectively.

Production

The production was carried out using the Isobaric-Isothermal ensemble i.e Constant Temperature and pressure (NPT) of 300 K and 1 atm for 250ns with time-step of 2fs. The temperature and the pressure were controlled by coupling the system with the Nose-Hoover thermostat (Hoover, 1985) and the Parrinello Rahman barostat using isotropic conditions respectively. The input files stating the value of the parameters for the production is given in appendix V.

Analysis

The analysis was done using the resulting trajectory and data files to obtain information on the structural properties of the molecules. Some important quantities were calculated. The quantities include Root Mean Square difference, Root Mean Square fluctuations using the Xmgrace software, Ramachandran plot with the aid of VMD and Radius of gyration (Rg) which is defined as the distribution of atoms of a protein around its axis (Stadler *et al*., 2012). Hydrogen Bond, temperature and pressure were also determined.

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Results and Discussions

Verification of Isobaric-Isothermal Ensemble

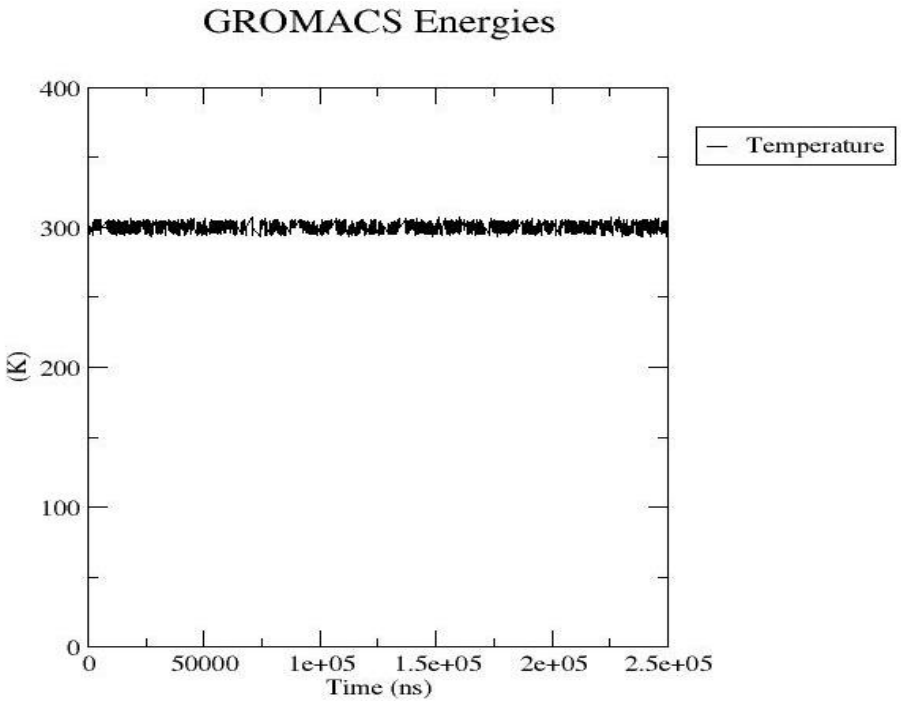

Figure 3.Temperature of the Protein

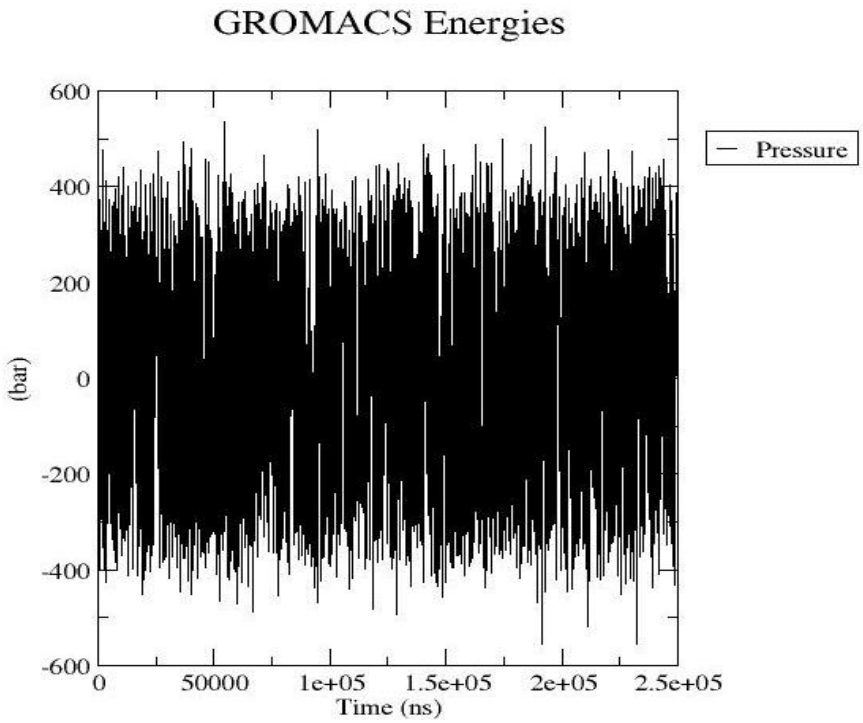

Figure 4.Pressure of the Protein

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The stability of the systems were analyzed by monitoring the temperature and pressure, because the simulation was performed under standard condition using NPT ensemble. The temperature and pressure of the calcitonin remained stable throughout the simulation. The temperature remained at 300K which confirms the effectiveness of Hoover thermostat as in figure 3 above and the pressure remained at 1 atm which confirms the effectiveness of Parrinello-Rahman barostat as in figure 4 above.

Root Mean Square Deviation (RMSD)

The Root Square Deviation (RMSD) is used to measure the average change in the displacement of a selection of atoms for a particular frame with respect to a reference frame (Coutsias *et al*., 2004). It is calculated for all frames in the trajectory. RMSD is defined by the equation below

RMSD: $\sqrt{\frac{1}{N}\sum_{i=1} N\ \delta_i^2}$

Where δ_i is the distance between atom i and either a reference structure or the mean position of the N equivalent atoms (Coutsias *et al*, 2004). This is often calculated for the backbone heavy atoms C, N, O and $C_\alpha$or sometimes just the C_α atoms. RMSD was computed based on C_α atoms and taking the frames of 4001 of the simulation as the reference. The RMSD were also computed at the backbone and all-atom levels.

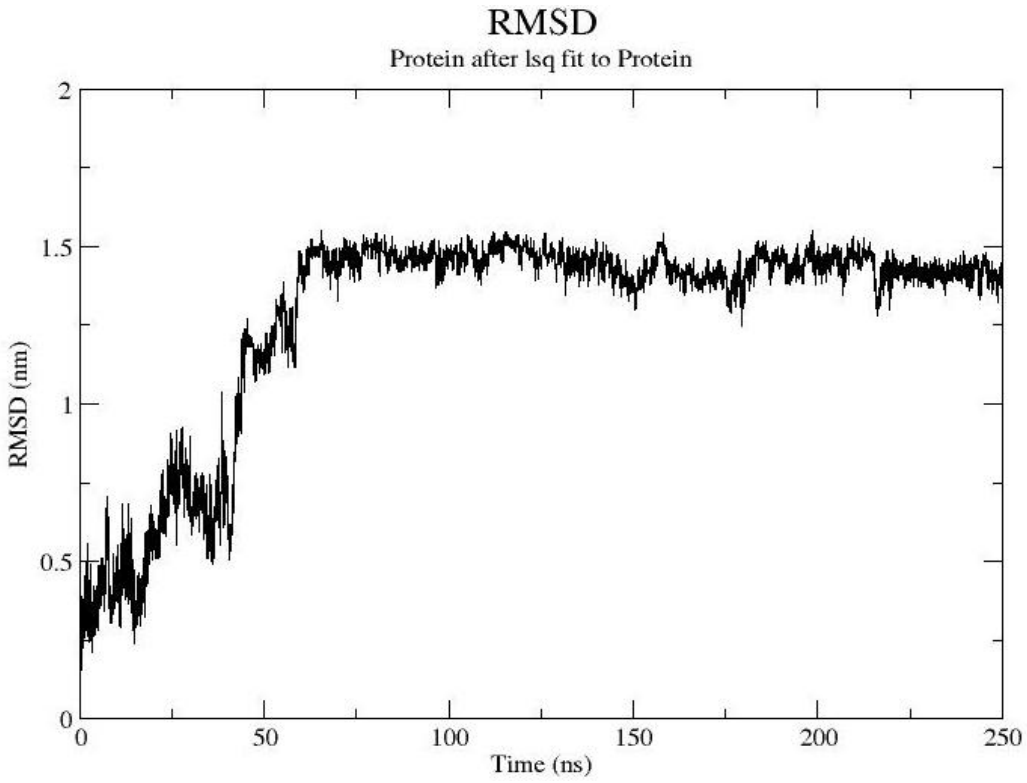

Figure 5.Graphical representation of the protein RMSD of calcitonin

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In the above graph it can be deduced that the molecule visited four conformational states at 25ns, 50ns, 75ns and maintained stability at 100ns. Calcitonin shows a gradual increase in fluctuation and stabilizing at 1.5nm.

Hydrogen Bonds

Hydrogen bond is an interaction involving a hydrogen atom located between a pair of other atoms having affinity for electrons. Hydrogen bonds can occur between molecules intermolecularly or within same molecule intramolecularly (Thomas, 2002). Before formation of Hydrogen bond there must be a donor and acceptor. The distance between the donor and the acceptor should be less than that of the cut-off distance with 3.0Å by default.

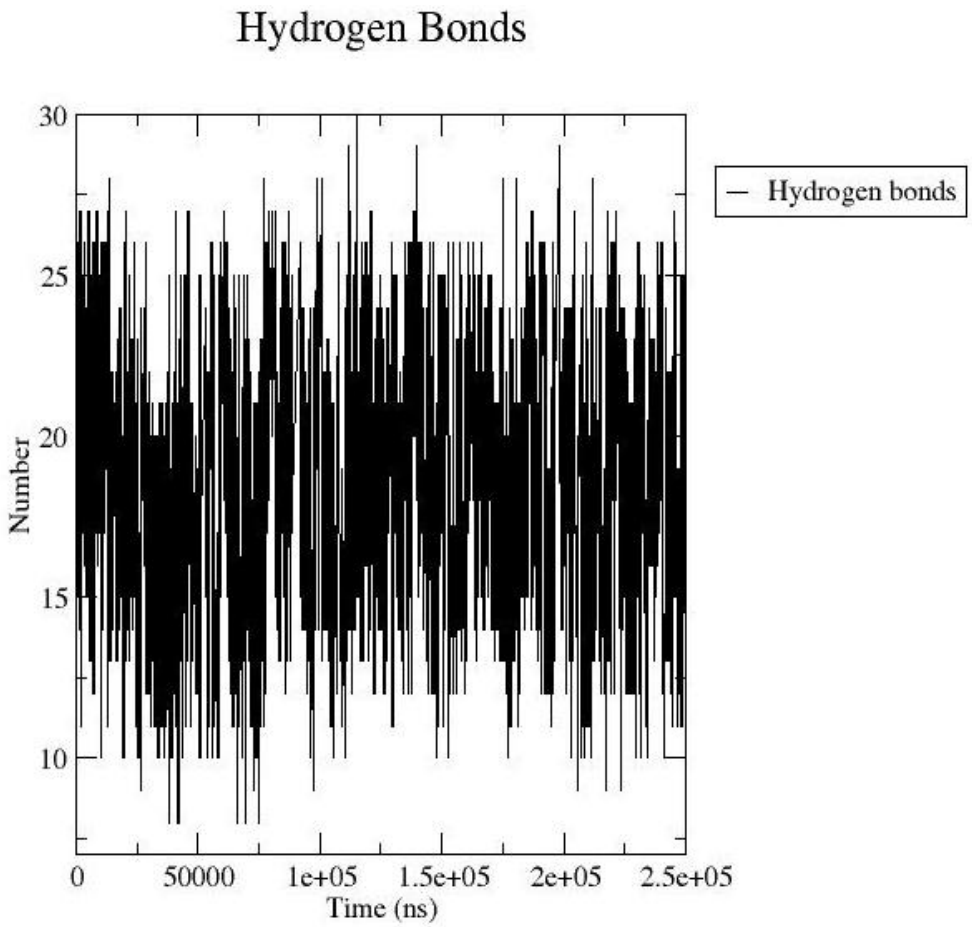

Figure 6.Graphical representation of the hydrogen bond present in calcitonin

From figure 6, there are 26 hydrogen bonds which are formed as a result of hydrogen bonds between donor and acceptor atom of protein as shown in the table below. Hydrogen bonds with occupancy less than 30% are regarded. It also supports the stability of the molecule.

Table 1.Showing the Donor, Acceptor and Occupancy of the H-Bond Formed

Donor	Acceptor	Occupancy
THR6-Main-N	GLY2-Main-O	9.09%
LEU9-Main-N	SER5-Main-O	9.09%
MET8-Main-N	LEU4-Main-O	9.09%
PHE16-Main-N	TYR12-Main-O	18.18%
ALA31-Main-N	ILE27-Main-O	9.09%
THR25-Side-OG1	THR21-Main-O	36.36%
GLN14-Main-N	GLY10-Main-O	9.09%
THR13-Main-N	LEU9-Main-O	18.18%
GLY10-Main-N	THR6-Main-O	27.27%
THR11-Side-OG1	CYS7-Main-O	27.27%
TYR12-Main-N	LEU9-Main-O	9.09%
THR6-Side-OG1	ASN3-Side-OD1	9.09%
PHE22-Main-N	LYS18-Main-O	27.27%
ASN17-Side-ND2	THR25-Side-OG1	9.09%
GLN14-Side-NE2	THR11-Main-O	18.18%
THR13-Side-OG1	LEU9-Main-O	36.36%
THR6-Side-OG1	GLY28-Main-O	36.36%
ASN17-Side-ND2	THR13-Side-OG1	27.27%
ALA26-Main-N	PHE22-Main-O	9.09%
Donor	Acceptor	Occupancy
ASN17-Main-N	THR13-Main-O	9.09%
ASN3-Side-ND2	SER5-Side-OG	9.09%
GLN24-Side-NE2	HIS20-Main-O	9.09%
TYR12-Main-N	MET8-Main-O	9.09%
THR6-Main-N	ASN3-Side-OD1	18.18%
THR11-Main-N	CYS7-Main-O	9.09%
THR6-Side-OG1	VAL29-Main-O	9.09%

Root Mean Square Flunctuation

RMSF is a measure of the displacement of a particular atom or group of atom that is related to the reference structure averaged over the number of atoms (Tarantino *et al*., 2014). Trajectories variability can be investigated by plotting the root mean square fluctuation against number of atoms or residues as plotted in Fig.7a and 7b.

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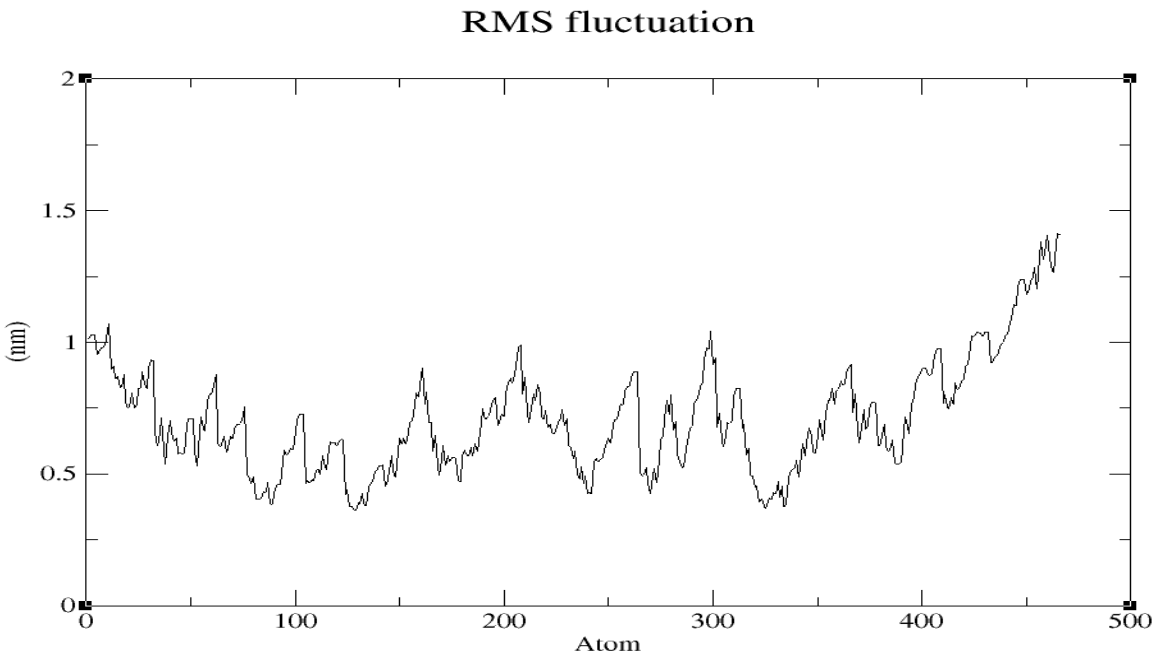

Figure 7a.RMS fluctuation plot of calcitonin in (nm) against the number of atoms

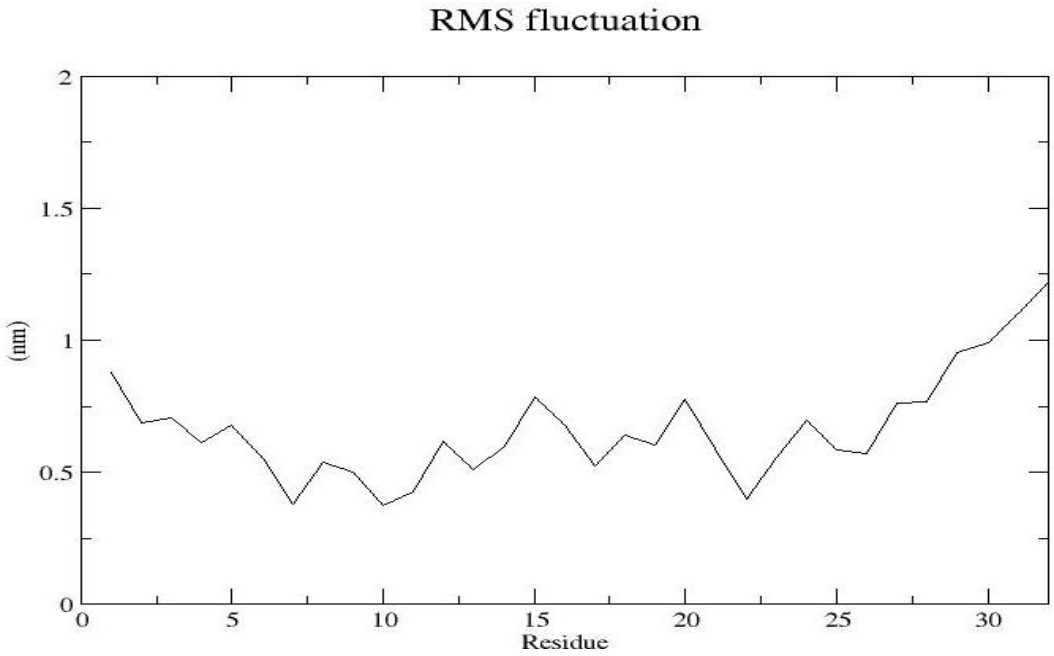

Figure 7b.RMS fluctuation plot of calcitonin in (nm) against the number of residue

From the above graph, the center of mass of each protein revolves round a fixed position ranging from 0.3nm to 1.45nm. The hydrophobic residue tends to have a higher peak than the polar residue. Proline at the protein side chain which is hydrophobic was buried in the water solvent which also supports the stability of the molecule.

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End to End Distance

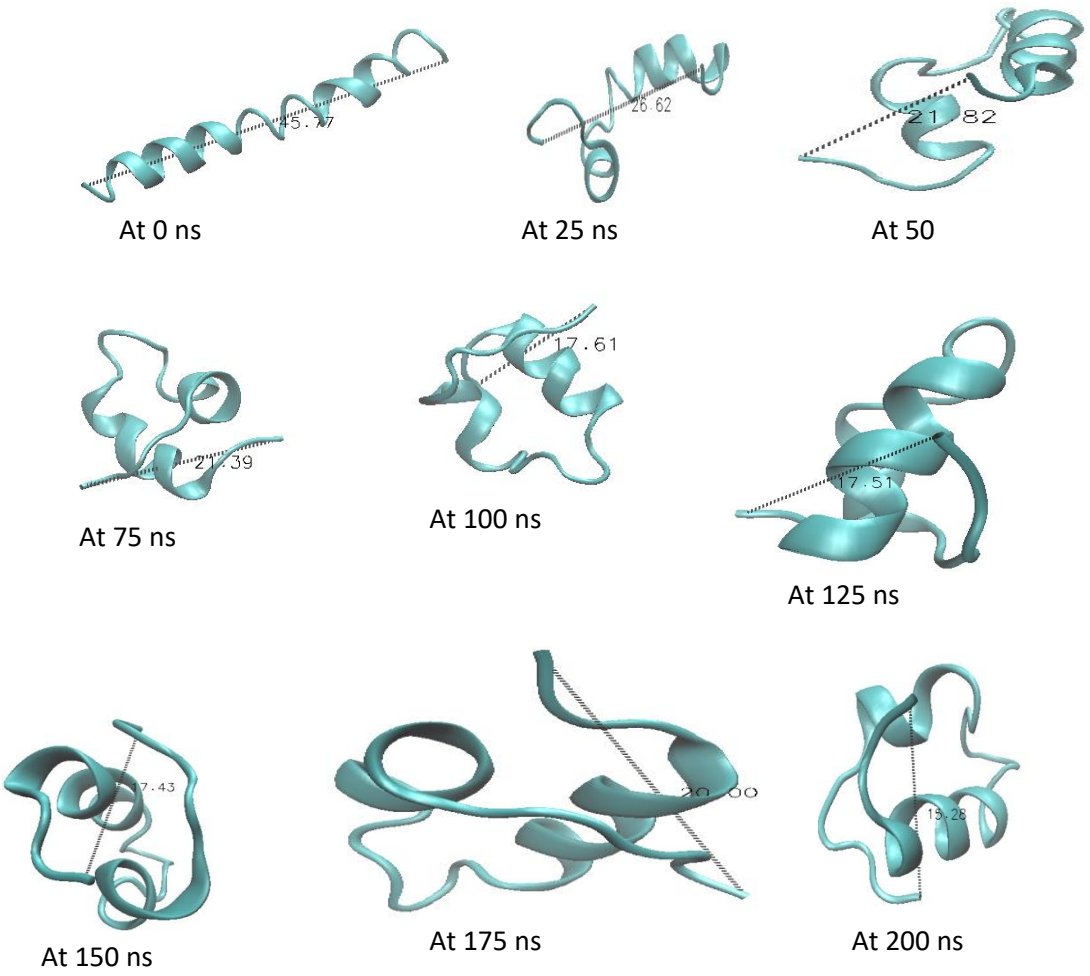

Figure 8.End to End Distance of Calcitonin at Different Timescale

From the diagram above it can be deduced that the protein with a bond distance of 45.77nm at 0 ns decreases to 26.77nm at 25 ns which is to show that the protein was undergoing a folding process. Protein folding in molecular dynamics also supports its stability. Protein folding is originated from the breakdown of the polypeptide chain, which is driven by the "desire" of hydrophobic amino acids to escape the polar solvent water. Protein folding is however a very fast process within the range of seconds or even millisecond.

Radius of Gyration (ROG)

Radius of gyration (ROG) is defined as the distribution of atoms of a protein around its axis. It gives the length that represents the distance between the point when it is rotating and the point where the transfer of energy has the maximum effect. The calculation of ROG is the most significant indicators that are widely used in predicting the structural activity of a macromolecule and its compactness.

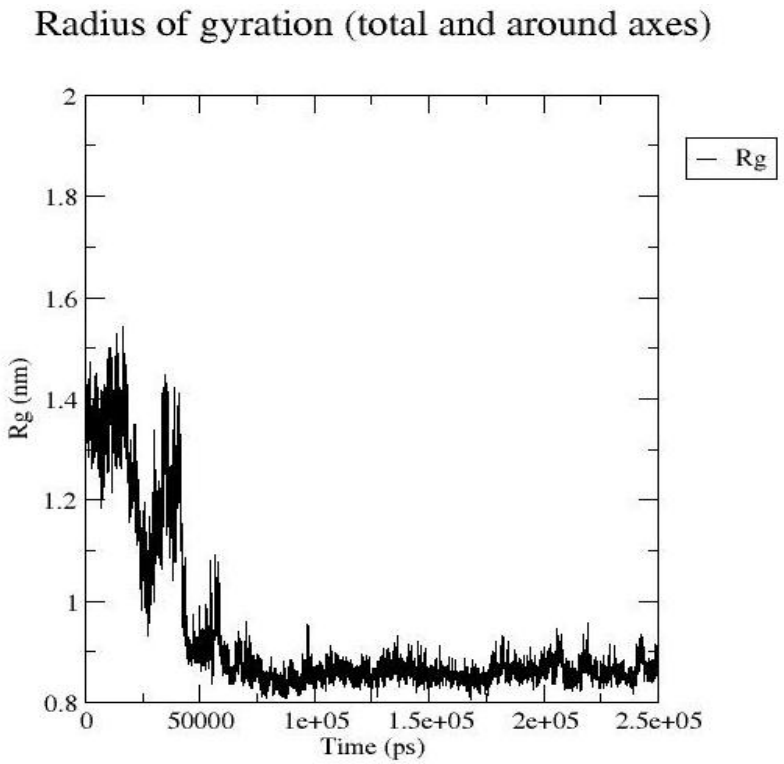

Figure 9.Radius of gyration of calcitonin

From the plot above, the ROG ranges from 0.8nm to 1.5nm. At 50ns the molecule adopts the lowest energy i.e. more compactness can be observed and folding process begins there.

Conclusion

Molecular dynamics was carried out in all atoms explicit solvent simulation studies of Calcitonin. The dynamics of the Calcitonin affects the conformational changes of molecules and the global dynamics was analyzed and discussed. From the structural analysis of the Calcitonin using RMSD and RMSF values, Calcitonin tends to be more stable at a conformational state. The presence of H-bonds supports the conclusion on structural stability of calcitonin. The end to end distance also reveals that the structure was undergoing a folding process.

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