ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Molecular Dynamic Simulation of Single Stranded DNA and Effect of Ionic Strength.

Sahidul Islam*.

Department of Chemistry, Trivenidevi Balotia College, Raniganj, Paschim Burdwan-713347, West Bengal, India.

ABSTRACT

The structural change of nucleic acids in biological system are common phenomena. The conformational fluctuation dynamics of nucleic acids in biological media with ionic strength is profoundly impactful in understanding the bio-evolution. Here the conformational trajectories of single stranded DNA in 50 ns time span and assembly of water molecules and cations around it has been investigated through the molecular dynamic simulation method in water medium. The higher ionic strength of the medium renders the decrease of persistence length offering more structural flexibilities and stabilizing collapsed globular form. The water molecule around the single stranded DNA form tight hydration layer followed by the diffused one. The solvated metal ions form two layers, first one is tightly packed thin and sharp and second one is comparatively thick and diffused. The first layer screening the negative charge of single stranded DNA diminish intramolecular electronic repulsions thus offer more backbone conformational flexibilities. The negative charge screening effect for single stranded DNA, higher valent metal ions are much more effective compared to the lower valent metal ions.

Keywords: single stranded DNA, simulation, persistence length, ionic strength, hydration layer, screening effect

https://doi.org/10.33887/rjpbcs/2021.12.1.2

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Nucleic acids are natural bio-poly electrolytes [1]. The structural transition of a nucleic acid is associated in performing the normal biological functions such as genetic information carries and transmission, DNA- protein recognition, genetic expression, regulation and replication and genetic recombination [2]. Hence the study of its dynamics and fluctuations is of paramount importance. More than 50 years ago although the double stranded DNA structure has been elucidated by Watson and Crick and large number of researchers across the glove have had the endeavor, still the conclusive mechanistic course of function specific polymeric structural fluctuations and responsible parameters for controlling mechanical properties are not yet well established [3]

It is surprising that researchers have given much more effort of DNA polymeric structural change elucidation compared to that of RNA. In fact, DNA study has been used to understand the flexibilities of RNA due to lack of information [4]. Among many factors, the normal base paired regions in DNA offer structural rigidity while non base paired regions offer flexibility. The relation between local rigidity and structural flexibility is very natural [5]. The broader knowledge in conformation-function relationships for nucleic acids equip in the effort to understand structural modifications for replications, evolutions and gene therapy. One of the most recent endeavor of various scientific groups is the designing of drugs or inhibitors where nucleic acid-based molecules are considered as potential drug candidates [6].

Among many scientific methods, fluorescence labelling and probing is one of the most popular for understanding conformational dynamics, nucleic acid-protein interactions and quantitative estimations [7]. Förster resonance energy transfer between the labelled probes offer ample information on nucleic acid fluctuation dynamics [8]. For mechanical property study of electrolytic polymer, the popular worm like chain (WLC) model is very common [9]. The use of the WLC model is extended to investigate the DNA flexibility [10]. According to the WLC model a poly electrolyte chain is a function of contour length (L) and persistence length (l_p). The contour length is the simple tether length and persistence length is the minimum length below which a chain behaves like a stiff rod [11]. The persistence length is function of intrinsic stiffness (l_0) and repulsion parameter within the chain (l_{el}). Fitting the nucleic acids in WLC model and using the elegant combination of small angle X-Ray scattering (SAXS) and Single molecular förster resonance energy transfer (smFRET) huge information on the mechanical properties hence flexibility and folding propensity can be found. It has been established in the in vitro study; the persistence length of nucleic acid is a function of ionic strength of the medium [12]. The ionic strength of the biological fluid is very high due to the presence of many ions [13]. Hence the comprehensible knowledge from the in vitro findings could be extended to understand the in vivo phenomena. For various DNA molecules and along with several base modifications, the persistence length obtained from atomic force microscopy (AFM), single molecular FRET combined with SAXS and single molecular spectroscopy with optical tweezer are found to be almost same [14]. Fluorescence correlation spectroscopy, a non-invasive single molecular technique is also widely used for studying the flexibilities and conformational dynamics of nucleic acids [15]. The double stranded helical DNA conformational flexibility can directly be investigated by pulsed electron-electron double resonance (PELDOR) [16]. The conformational fluctuations of nucleic acids can also be studied using the theoretical techniques like Molecular dynamic (MD) simulations [17]. A new web tool, NAFlex allow to study the nucleic acid flexibilities. The pure nucleic acid or bound with other molecules can be found in protein and nucleic acid data banks. These structures can be directly or removing the unnecessary part incorporated for simulation. The varieties of methods such as colorless WLC model, base pair resolution mesoscopic model and atomistic molecular dynamics simulations with wide varieties of protocols and forcefields are available in the tool [18].

Despite having close similarities between DNA and RNA in constituting components, the X-ray crystallography study provided ample of evidences that the conformation of sugars (ribose and deoxyribose) play the critical role in differentiating backbone conformational flexibilities [19]. Several theoretical and experimental findings categorically revealed that DNA is always much more flexible than RNA [12, 20]. The RNA helix intrinsically resists to bend or twist-deformations. From the variety of physical measurements, the persistent length of double stranded RNA in presence of magnesium chloride was found to be 700-800Å approximately 1.5-2.0-fold larger than the value corresponding to double stranded DNA. The main helix flexibilities although a measure of the quantifiable measurement of the forces of interactions, the effect of the non-helix parts (such as loops, branches) cannot be ignored [10]. It is now emerging that like protein, nucleic acid functions are also dictated by tertiary folds [21].

The basic but crucial questions that captured the interest is, Why DNA plays the vital role in carrying genetic material but not RNA in that extent? Why DNA adopts stable double stranded helical structure While RNA in general is single stranded? In chemical context the only subtle differences between the skeleton of DNA and RNA are the methyl group in DNA thymine base and hydroxyl group in RNA ribose sugar. The chemically inert methyl group is supposed to be very less effective to make any dramatical difference between single stranded DNA (ss-DNA) and single stranded RNA (ss-RNA) although in totality we cannot rule out its effect, as throughout the long and entire evolutionary journey of bio-species, nature has selected it for the sake of perfect function in bio-machinery system. The hydroxyl group in RNA probably plays the key role in making ample differences between these differently entitled nucleic acids. But why and how one hydroxyl group pivotally play the crucial role in differentiating DNA and RNA in terms of chemical as well as biological activity hugely, has still been remained as an unresolved mystery in nucleic acid phylogeny of bio-genesis.

MD simulation is a growingly popular theoretical technique. The virtual detail insight of dynamics, conformational interplay and structural transitions can be studied using this tool. Although there are lot of shortcomings and limitations, there are many hope and scope in future. It can be predicted that the full fledge evolution of MD simulation will facile the study of biomolecules and ligand interactions, binding kinetics and the structural polymorphism in physiologically important environments. The evidence of MD simulated elastic model predict the regularity region in DNA, thus it can help in understanding the nucleosome positioning and chromatin plasticity. It could become an ultimate tool in deciphering the physiological code that contribute in gene regulations. The behavior of biomolecules and interactions land scape in nonaqueous media and in gas phase can easily be unraveled in atomistic level [22].

MATERIALS AND METHODS

The A-DNA oligomer having the generic sequence 5'-GGGCATGCCC-3' is directly available in Nucleic Acid Bank (NAB) in double stranded form (NDB ID: NA2228 and PDB ID: 4IZQ). Using Viewer-Lite software we have generated single stranded chain from the double strand and removed all water molecules. Thus, we have generated the PDB input file. Molecular dynamic simulations have been carried out using computational software GROMACS [23]. For the simulations, all atom CHARMM force field as typical nucleic acid potential parameters have been selected [24]. Water model used is SPC as it is one of the many accurate models. This ss-DNA oligomer has been solvated immersing in a cubic box of edge length 5.9 nm followed by addition of counter ions to neutralize the system. Total three simulations (50 ns each) have been carried out in presence and absence of NaCl and $MgCl_2$. The detail has been enlisted in the **Table 1.**

Table 1. The water molecules and ions in the box when ss-DNA molecule was simulated

Simulation	Box(nm^3)	No. of Water Molecules	Salt conc.	No. of +ve ions	No. of –ve ions
1	5.91^3	4509	0 (M)	13	4
2	5.89^3	4467	0.3 (M) NaCl	34	25
3	5.84^3	4445	0.3 (M) $MgCl_2$	30	51

After the solvation and ion addition, energy minimization of the system has been carried out to eliminate any bad forces and bad contacts that may be present in the system. With the system in place, a 2 ns long NVT equilibration has been performed at constant temperature 300K. Further a 2 ns NPT equilibration has been carried out introducing Parrinello-Rahman thermostat. The system attends the constant pressure 1 bar. For the equilibrations LINCS constraint algorithm has been used. [25]. At the end of NPT run, the cell attains a constant edge length of 5.98 nm. The data have been collected from 50 ns long NVT simulation using 2fs time step updating the coordinates each 250 fs. Minimum image convention has been employed to calculate the Lennord Jones interactions with spherical cut off distance of 1 nm. To calculate long range electrostatic interactions, particle mesh Ewald (PME) method has been used [26].

ISSN: 0975-8585

RESULTS AND DISCUSSION

In the entire course of 50 ns simulation, various configurations of ss-DNA molecule have been observed from the simulated trajectories and are displayed at different time intervals in **Figure 1.** From an ordered initial configuration with sequentially stacked base pairs, the ss-DNA undergoes a number of configurations--something that goes on to show the configurational flexibility of it in aqueous solutions. The simulated configurations show a disruption of sequential base stacking and a fluctuating collapsed coil like form. These results agree well with what experimental studies have proposed.

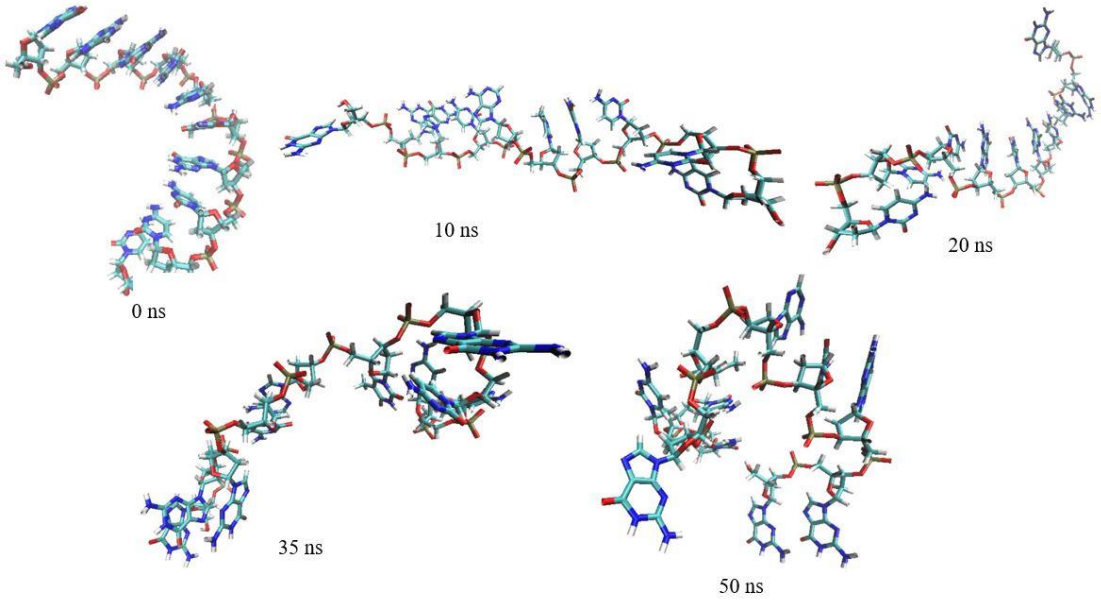

Figure 1: Snapshots of the simulated ss-DNA molecule (from top left to bottom right) at 0, 10, 20, 35 and 50 ns

To further understand the structural deviation from the initial configuration, root mean square deviations (RMSD) has been calculated. RMSD provide information about the conformational flexibility by giving a visual idea about the local motions exhibited by the molecule in the given conformational space. From the RMSD study of whole ss-DNA molecule in various salt concentrations, 0.61, 0.66 and 0.76 nm average RMSD values were observed in presence of 0 (M) salt, 0.3 (M) NaCl and 0.3 (M) $MgCl_2$ respectively.

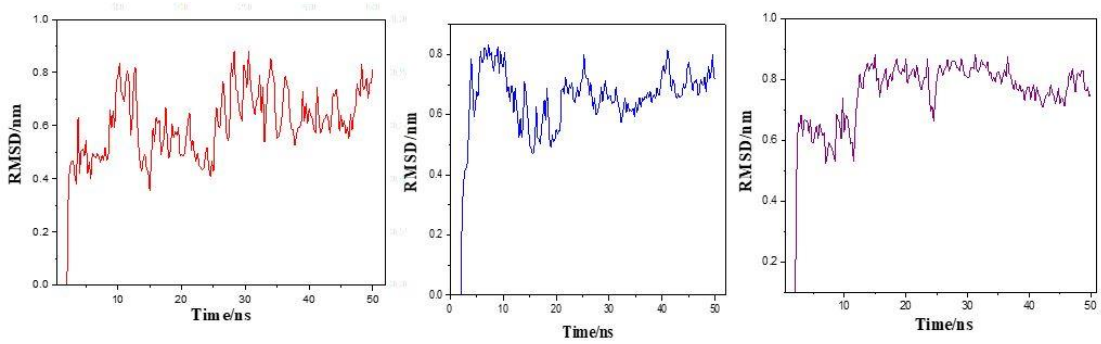

Figure 2: Time evolution plots of RMSD of the simulated ss-DNA molecule in presence of 0 (M) salt, 0.5 (M) NaCl and 0.5 (M) $MgCl_2$ in water (from left to right)

From the observed average RMSD values it is quite clear that in presence of metal ions, deviation of structure from equilibrium happen and it happen more in solution of Mg^{2+} than Na^{+} having same concentrations. This is supported by smFRET experiment [12]. This is attributed to the interaction between cationic species and the electronegative sites, which leads to screening of the negative charges responsible for backbone collapse.

ISSN: 0975-8585

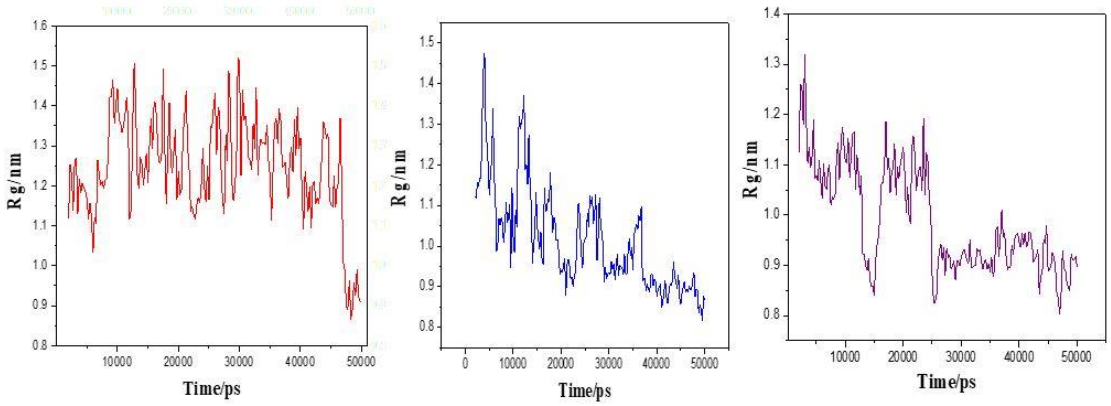

Figure 3: Time evolution plots of Radius of gyration of the simulated ss-DNA molecule in presence of 0 (M) salt, 0.5 (M) NaCl and 0.5 (M) $MgCl_2$ in water (from Left to Right)

The expansion and collapse of a polymer such as ss-DNA can also be well characterized by introducing another physical quantity radius of gyration. Higher the radius of gyration lower is the compactness of the structure. Here the calculated values of average Rg's are 1.24, 1.01 and 0.99 nm. From RMSD (**Figure 2.)** and time evolution Rg plots (**Figure 3.)** it could be seen the movement of the molecule parts got restricted in presence of salts compared to the one in absence of salt. The backbone collapsed structure get locked and persisted in salt solution and again it is seen the higher efficiency of Mg^{2+} compared to Na^{+}.

It can be speculated; the collapsed structure gets stabilized by π-staking as well as hydrogen bonding. In single stranded DNA π-staking mainly renders the stability. In presence of salt, structural body parts come in very favorable distance and get stacked. For double stranded DNA molecule hydrogen bonding is the main stabilizing factor but in case of single stranded DNA π-staking play the major role to collapsed structure stabilization. The equilibrium structure is in extended form and has the less possibility of having intramolecular hydrogen bonds but as the simulation run, intra molecular hydrogen bond forming favorable distance could be attended due to three-dimensional structural collapse. As the structure get more relaxed in presence of salt, there is a more possibility of stronger hydrogen bond formation although that may not necessarily be able to lock the conformation permanently.

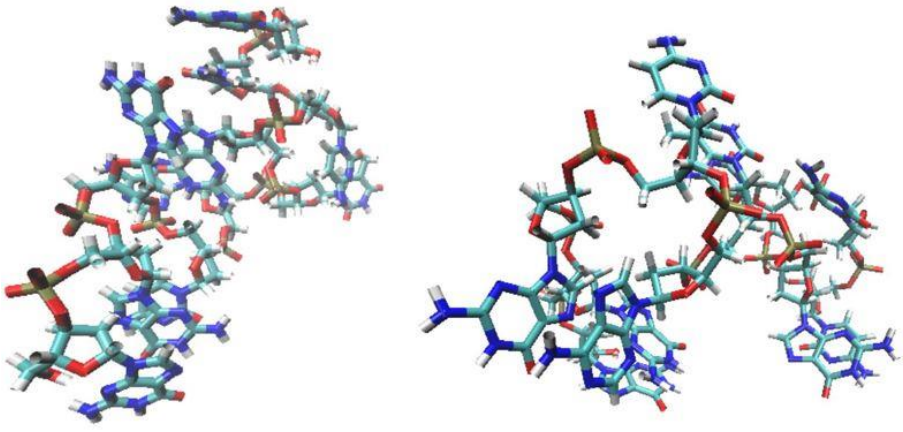

Figure 4: Snapshots of the simulated ss-DNA molecule at 50 ns in presence of 0.3(M) NaCl (Left panel) and 0.3(M) $MgCl_2$ (Right panel)

DNA-water assembly in solution

The negatively charged polyelectrolytic ss-DNA molecule leads to the aggregation of water molecules around its phosphate backbone resulting its hydration. The Radial distribution functions of oxygen atom of water

ISSN: 0975-8585

with respect to oxygen and phosphorus atoms of phosphate moiety of DNA have been computed (**Figure 5.)** The emergence of sharp peaks at 0.26 and 0.38 nm with g(r) value 1.94 and 2.44 for oxygen atoms of water molecules around the oxygen and phosphorus atom of phosphate moiety of ss-DNA clearly indicate the formation of hydration layer. The much higher number density of oxygen atom of water around oxygen atom compared to phosphorous atom of phosphate moiety, indicate ss-DNA molecule gets more hydrated through oxygen atom compared to phosphorus atom. The very reason of tight water packing around oxygen atoms of phosphate moiety in ss-DNA is hydrogen bonding with the hydrogen atom of water molecules.

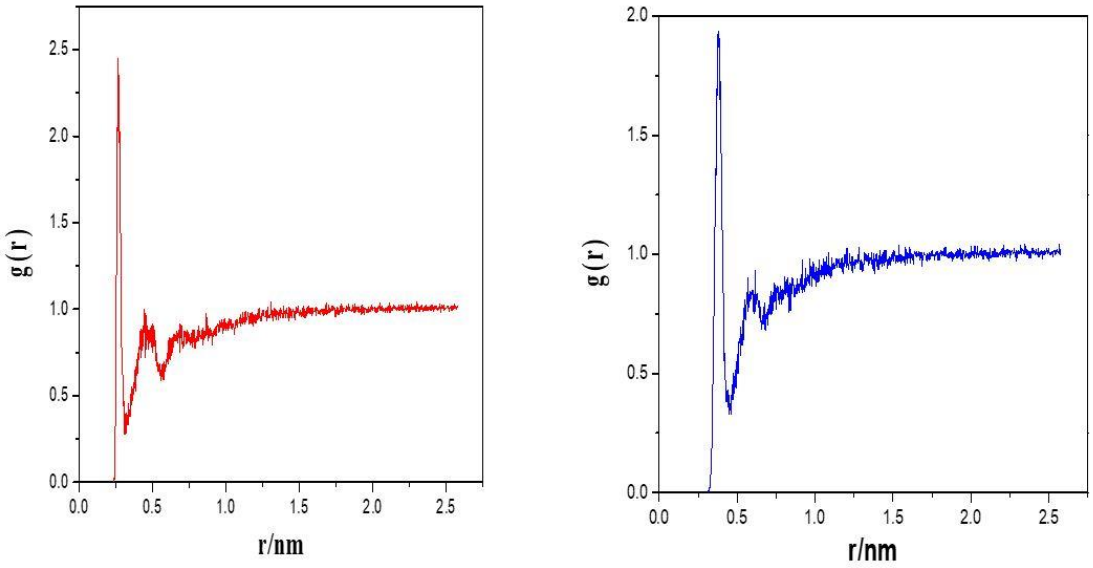

Figure 5: Radial Distribution function of oxygen atom of water with respect to the oxygen atom (Left panel) and phosphorus atom (Right panel) of phosphate moiety of ss-DNA

DNA-cation assembly in solution

To investigate the metal ion ss-DNA interactions, the radial distribution function of metal ions with respect to the oxygen and phosphorus atom of phosphate moiety of ss-DNA has been computed (**Figure 6.** and **Figure 7.).**

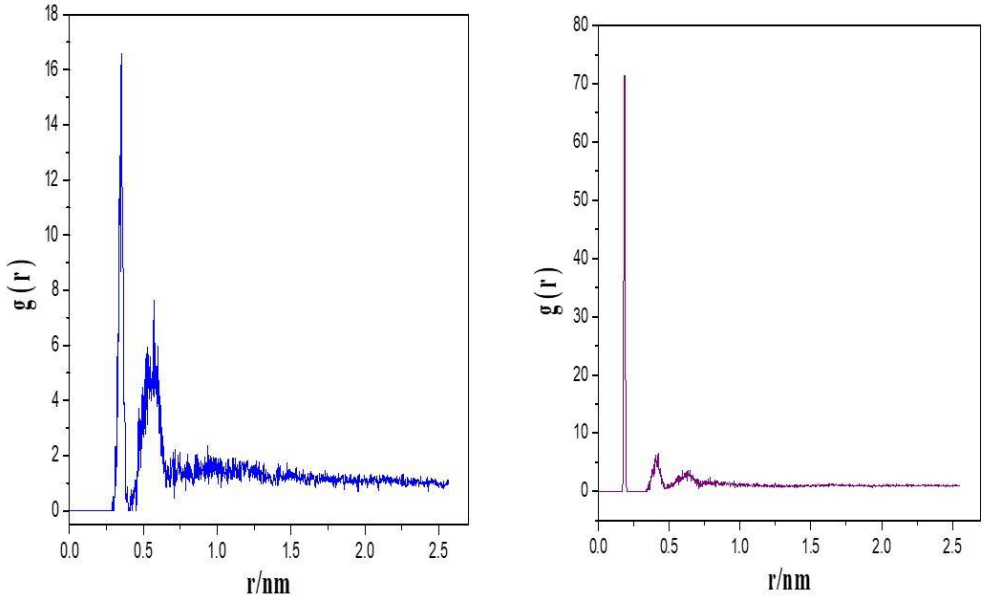

Figure 6: Radial distribution function of Na^+ (Left panel) and Mg^{2+} (Right panel) with respect to oxygen atom of phosphate moiety of ss-DNA

ISSN: 0975-8585

The emergence of two peaks (for each metal ions) at 0.21, 0.46 and 0.19, 0.41 with radial distribution intensity 12.35, 9.49 and 71.72, 6.5 indicate the formation of two layers of the metal ions around the ss-DNA molecule. The very high peak intensity in Mg^{2+} compared to Na^{+} solution of same concentration suggests the negative charge screening power of Mg^{2+} is much higher (5.80 times) than Na^{+}. This result is very reasonable, consistent and in close agreement with the value obtained from combined SAXS-smFRET experiments [12]. It could be assumed with skepticism the first layer screen the negatively charge phosphate backbone of ss-DNA.

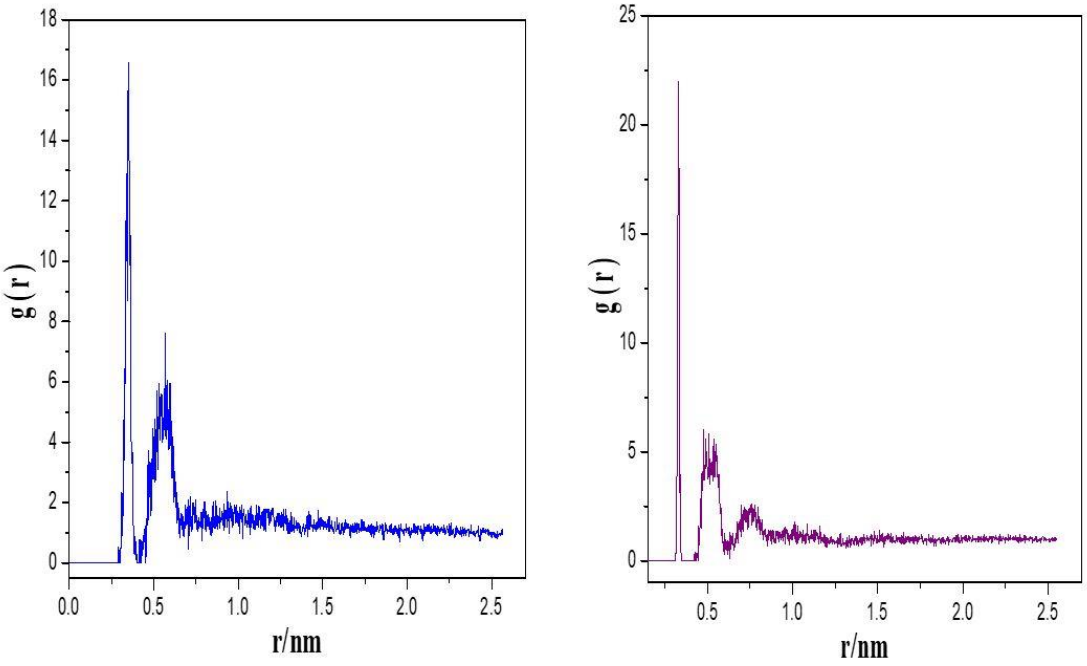

Figure 7: Radial distribution function of Na^{+} (Left panel) and Mg^{2+} (Right panel) with respect to phosphorus atom of phosphate moiety of ss-DNA

CONCLUSIONS

From the all atom simulation of ss-DNA in water and in presence of various metal ions here the structural changes have been investigated. The findings of here are also supported by the fact of decreasing persistence length of ss-DNA with ionic strength reported in literature [12]. In the course of simulation, it has been found here, the three-dimensional structural collapse of the ss-DNA from an ordered equilibrium state. The collapse of the ss-DNA structure is much more prominent in solution of Mg^{2+} compared to that in Na^{+} from which it can be concluded the negative charge Shielding efficiency of Mg^{2+} is much higher compared to Na^{+} and it has been shown quantitatively in radial distribution plots. In the solution, water molecules in the vicinity of ss-DNA molecule get tightly packed forming a hydration layer. The metal ions in solution form two layers. Comparing with the experimental findings, it could be believed, tightly packed first layer of the metal ions screen the negative charge of ss-DNA, giving a clear picture of the assembly of ions and water molecules in physiologically important environments.

ACKNOWLEDGEMENTS

The author thanks Prof. G. Naresh Patwari, Department of Chemistry, IIT Bombay for his valuable advice while conducting this research. He thanks the colleagues of Department of Chemistry Trivenidevi Bhalotia College for their suggestions and assistance. Finally, he is thankful to Department of Chemistry, IIT Bombay for providing necessary infrastructure.

REFERENCES

[1] Meisburger SP, Sutton JL, Chen H, Pabit SA, Kirmizialtin S, Elber R, Pollack L. Biopolymers 2013; 99: 1032-1045.

[2] Bao L, Zhang X, Jin L, Tan ZJ. Chin Phys B 2016; 25: 187-198.
[3] Peters JP, Maher LJ. Q Rev Biophys 2010; 43: 23–63.
[4] Schlatterer JC, Kwok LW, Lamb JS, Park HY, Andresen K, Brenowitz M, Pollack L. J Mol Biol 2008; 379: 859–870.
[5] Opron K, Xia K, Burton Z, Wei GW. J Comput Chem 2016; 37: 1283–1295.
[6] Duffy K, Arangundy-Franklin S, Holliger P. BMC Biol 2020; 18: 112-126.
[7] Michel BY, Ziuba D, Benhida R, Demchenko AP, Burger A. Front Chem 2020; 8: 1-123.
[8] Wranne MS, Füchtbauer AF, Dumat B, Bood M, El-Sagheer AH, Brown T, Wilhelmsson LM. J Am Chem Soc 2017; 139: 9271–9280.
[9] Bouchiat C, Wang MD, Allemand JF, Strick T, Block SM, Croquette V. Biophys J 1999; 76: 409–413.
[10] Kim JY, Jeon JH, Sung W. J Chem Phys 2008;128: 101-108.
[11] Mitchell JS, Glowacki J, Grandchamp AE, Manning RS, Maddocks JH. J Chem Theory Comput 2017; 13:1539–1555.
[12] Chen H, Meisburger SP, Pabit SA, Sutton JL, Webb WW, Pollack L. Proc Natl Acad Sci 2011; 109: 799–804.
[13] Liu B, Poolman B, Boersma AJ. ACS Chem Biol 2017; 12: 2510–2514.
[14] Kahn JD. Biophys J 2014; 107: 282–284.
[15] Li H, Ren X, Ying L, Balasubramanian S, Klenerman D. Proc Natl Acad Sci 2004; 101: 14425–14430.
[16] Marko A, Denysenko V, Margraf D, Cekan P, Schiemann O, Sigurdsson ST, Prisner TF. J Am Chem Soc 2011; 133: 13375–13379.
[17] Šponer J, Banáš P, Jurečka P, Zgarbová M, Kührová P, Havrila M, Otyepka M. J Phys Chem Lett 2014; 5: 1771–1782.
[18] Hospital A, Faustino I, Collepardo-Guevara R, González C, Gelpí JL, Orozco M. Nucleic Acids Res 2013; 41(W1): W47–W55.
[19] Murray LJW, Arendall WB, Richardson DC, Richardson JS. Proc Natl Acad Sci 2003; 100: 13904–13909.
[20] Noy A, Pérez A, Lankas F, Javier LF, Orozco M. J Mol Biol 2004; 343: 627-638.
[21] Marcia M, Humphris-Narayanan E, Keating KS, Somarowthu S, Rajashankar K, Pyle AM. Acta Crystallogr D 2013; 69: 2174–2185.
[22] Pérez A, Luque FJ, Orozco M. Acc Chem Res 2011; 45: 196–205.
[23] Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, Lindahl E. SoftwareX 2015; 1-2: 19–25.
[24] Baker CM, Anisimov VM, MacKerell AD. J Phys Chem B 2011; 115: 580–596.
[25] Hess B, Bekker H, Berendsen HJC, Fraaije JGEM. J Comput Chem 1997; 18: 1463–1472.
[26] Petersen HG. J Chem Phys 1995; 103: 3668–3679.

-=