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Advances in Applied Science Research, 2012, 3 (3):1545-1550

ISSN: 0976-8610
CODEN (USA): AASRFC

Variable pH based molecular dynamics simulation of type II antifreeze protein

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ABSTRACT

Anti-freeze proteins(AFPs) in case of cold adapted organisms helps to avoid or reduce damage to the organism caused by freezing stress. Without the AFPs, water molecules will add to an ice lattice that results ice crystal growth and there by causes heavy damage to the tissue. Due to this property of these proteins are commercially used for cryopreservation purpose. Here in this work effect on physiological pH on the structure and function of the type II antifreeze protein has been established. The crystallography structure of the protein was obtained from Protein Data Bank. Molecular dynamics simulation of the protein was performed at 1 nano second (1000 pico seconds) in a series of physiological pH environment ranges from 2-11.The constant physiological condition was chosen for the simulation as protein in water at -5 degree Celsius and the salt concentration 0.15M.The results indicates the stability of the protein within a suitable pH range between 4 and 6.

Key words: anti-freeze proteins, Gromacs, physiological pH, protein data bank.

INTRODUCTION

The antifreeze proteins (AFPs) are also called as ice structuring proteins (ISPs) are a class of proteins produced in many vertebrates, plants, fungi and bacteria that permit their survival in subzero environments [1]. AFPs bind to small ice crystals to inhibit growth and recrystallization of ice that would otherwise be fatal for the organism [2].In comparison to widely used antifreeze substances like ethylene glycol, AFPs do not have lower freezing point in proportion to concentration. So this allows them to act as antifreeze at very less concentrations (about 1/300) of those of other dissolved solutes, hence minimizes their effect on osmotic pressure [3]. The binding capabilities of AFPs has been studied and attributed to their binding ability at specific ice crystal surfaces [4]. Many types of antifreeze glycoprotein or AFGPs are also widely found in fishes of Antarctic regions having molecular mass range around 2.6-3.3 kD [5].There are basically 4 classes of antifreeze proteins *viz.* Type I,II,III and type IV. The Type II AFPs are mostly studied and these are cysteine-rich globular proteins containing five disulfide bonds [6]. The inhibition of freezing incase of the AFPs are thought to by an adsorption–inhibition mechanism as they adsorb to non basal planes of ice, inhibiting thermodynamically favored ice growth [7]. There are many applications for antifreeze proteins are available for using these proteins in increasing freeze tolerance of crop plants and extending the harvest season in cooler climates ,improving farm fish production in cooler climates, lengthening shelf life of frozen foods ,improving cryosurgery, enhancing preservation of tissues for transplant or transfusion in medicine, therapy for hypothermia etc [8-9].As the function of these proteins resides on stability of the 3D structure hence, molecular dynamics simulation based analysis is essential to study the structural stability in different physiological environment [10-11].pH dependent molecular dynamics simulation methods are used to predict the substrate specifity and selection of enzymes thereby provides a deep insight to study about structural and functional aspect [12]. The ice binding capability in case of antifreeze protein has been studied in different environmental conditions like gas phase, solvated by water, adsorbed on the ice crystal plane in the gas phase and in aqueous solution by molecular dynamics simulation method [13].Also the temperature dependent unfolding pathway has been resolved

for type III antifreeze protein by GROMACS (Groningen Machine for Advanced Chemical Simulation) software package [14-15].

The prime objective of the study is to evaluate the effect of physiological pH on antifreeze protein. The antifreeze protein type II is considered here. The molecular dynamics simulation was performed in water in series of different pH and the structural basis was evaluated by calculating energy, root mean square deviation, radius of gyration, residue wise root mean square fluctuation, distribution of hydrogen bonds etc. The study is performed to establish the relationship between the physiological pH with the structural and functional aspect of the antifreeze protein.

MATERIALS AND METHODS

The considered antifreeze protein structure was retrieved from Protein Data Bank (PDB) (www.rcsb.org/pdb), which is the data base of protein structures. The selected protein PDB ID: 2AFP was considered [16 -17].The protonation process was done with the protein to prepare the series of protonated proteins by using H++ server which was the input for molecular dynamics simulation [18]. The server allows quickly obtaining and estimating of pKs as well as other related characteristics of bio-molecules such as isoelectric points, titration curves, and energies of protonation microstates. It also automates the process of preparing the input files for typical molecular dynamics simulations. Protons are added to the input structure according to the calculated ionization states of the chemical groups at the user specified pH. The output structure is in the PQR (PDB + charges + radii) format. In addition to this the corresponding coordinate and topology files are generated in the format supported by the molecular modeling package AMBER. The molecular dynamics simulation was performed by GROMACS 4.5.4 package by using AMBER99SB force field [19].The simulation for each protonated protein was set in temperature 268K, keeping salt concentration at 0.15M and at 1 nano second.In total 10 simulation were performed for each protonated form of protein having pH range from 2-11. The computing facility for the molecular dynamics simulation utilised is High performance cluster for Biological applications which is based on intel Xeon dual Quad core as processor, Gluster HpC 1.3 X86-64 bit edition ,total 16 nodes each having 4GB of memory [20]. After each simulation various property like total energy, rmsd, radius of gyration, hydrogen bond profiles are computed and analysed.

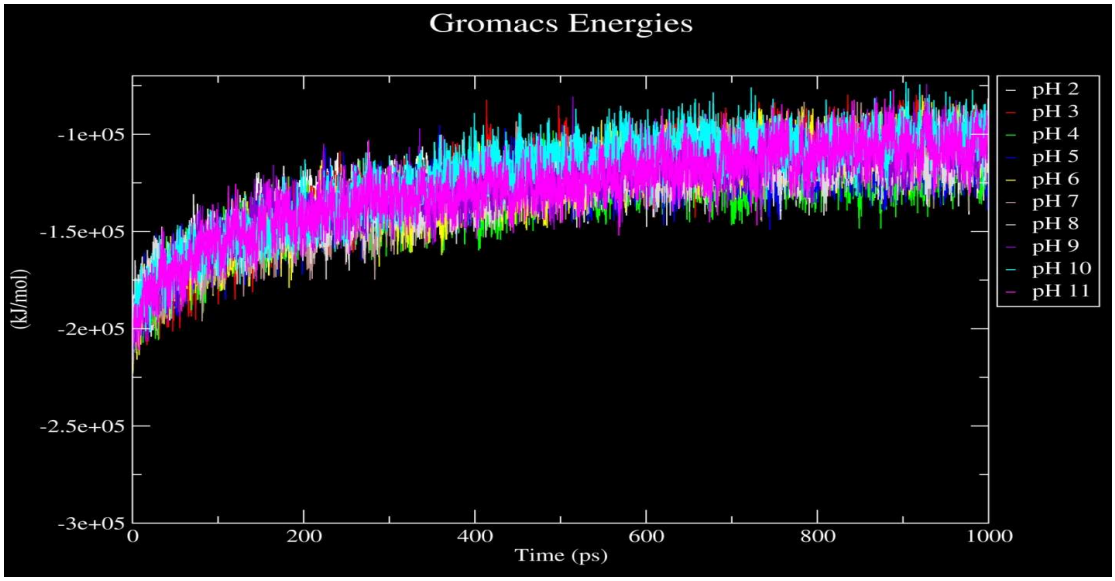

Figure 1: Total energy profile at different pH

Table 1: Showing the various parameters analysed during molecular dynamics simulation

Serial No.	Parameters	Mode of analysis	Simulation time	Tools used
1.	Total energy	Energy profile at different pH	1000 Pico second	GROMACS 4.5.4
2.	Root mean square deviation	C-alpha back bone deviation during simulation		
3.	Root mean square fluctuation	Residue wise fluctuation at different pH during simulation		
4.	Radius of gyration	Compactness of proteins during simulation		
5.	Hydrogen bonding profile	Hydrogen bonding occurrence/distribution at different pH during simulation		

RESULTS AND DISCUSSION

Like other proteins in case of antifreeze proteins, the ionisation equilibrium also influences the structural and conformational flexibility [21]. The process can be computed by molecular dynamics simulation in suitable physiological environments. The conformational sampling of the proteins leading to discover the stability state of the protein which is associated with its function. Also the effect of pH on different antifreeze protein has been studied extensively which indicates these proteins acts in a broad range of pH [22].

TOTAL ENERGY

The major objective of energy value calculation study is to check the stability of the protein during simulation at a variable pH and constant salt concentration and temperature in terms of total energy. The total energy profile of the protein in different pH is given in the figure 1.The result indicates the protein shows stability in almost all physiological pH (both in acidic and basic) in terms of the total kinetic and potential energy.

RMSD (Root Mean Square Deviation) ANALYSIS

RMSD refers to root mean square deviation from back bone of the structure to the initial starting structure.RMSD is a measure for conformational stability of the proteins. The plots of RMSD were obtained from molecular dynamics simulation and given in figure 2.Theoverall RMSD was found to be suitable and minimum deviation was obtained for acidic pH (at pH 2) in comparison to maximum deviation in alkaline pH (at pH 10).

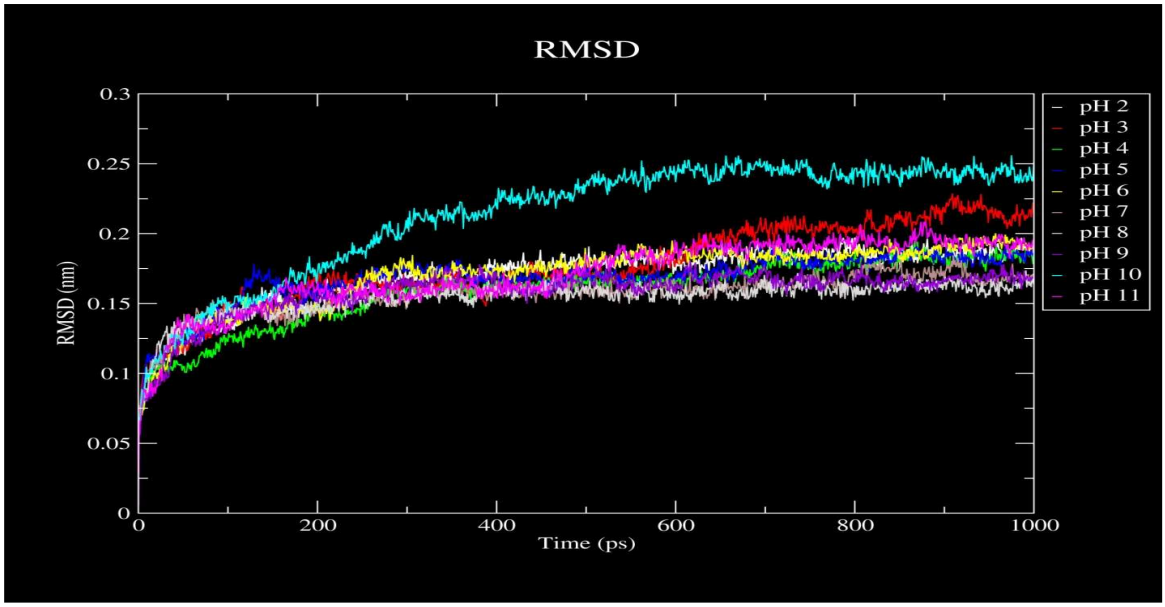

Figure 2: Backbone RMSD at different pH

RADIUS OF GYRATION CALCULATION

Radius of gyration (Rg) is the mass weighted root mean square distance of a collection of atoms from their common center of mass. Here the analysis provides the overall dimension or compactness of the protein. The plot of radius of gyration in simulation time in different protonted condition of the protein is given in figure 3.The plot shows the deviation remains within the range of 1.52-1.6 nanometer. The protein at lower pH shows greater variation than at higher pH.The maximum fluctuation in all cases during 800-1000 pico seconds may be due to complete loss of secondary structures [23].

RMSF (ROOT MEAN SQUARE FLUCTUATION) ANALYSIS

The root mean square fluctuation (RMSF) is a measure of the deviation between the position of particle and some reference position. Here residue wise RMSF was calculated for the protonated proteins during simulation as given in figure 4.The result indicates the residues 60-80 and 100-110 shows grater fluctuation at all pH.The fluctuation due to the protonation that influences the solvent accessibility in specific residues [24].

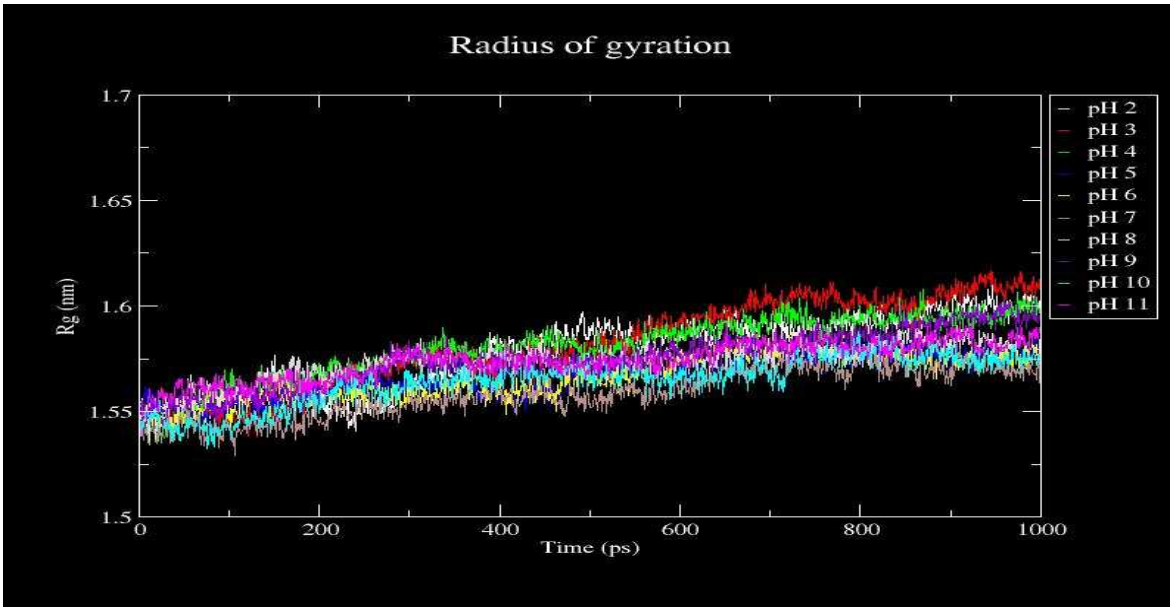

Figure 3: Radius of gyration at different pH

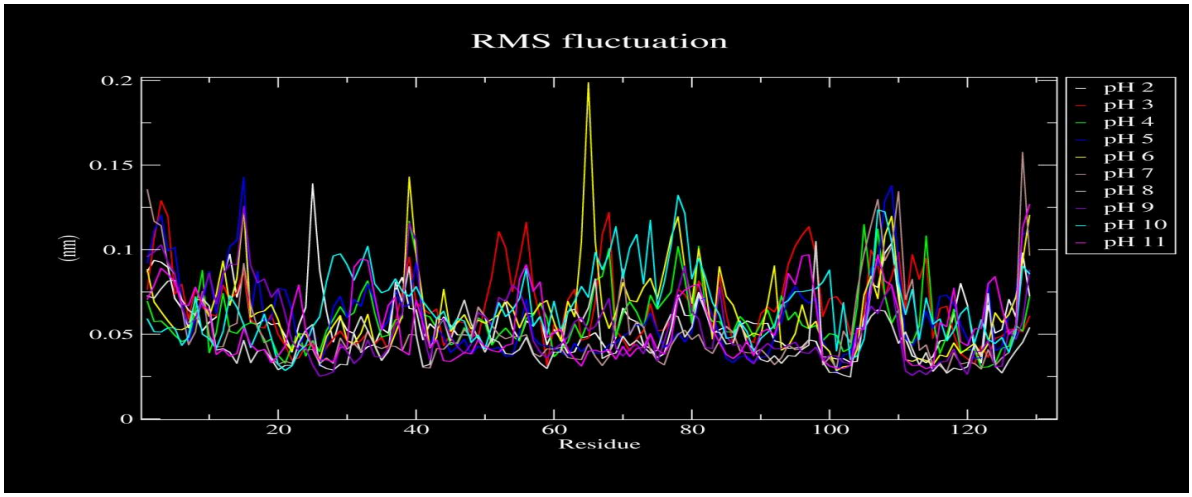

Figure 4: Root mean square fluctuation at different pH

HYDROGEN BONDING PROFILE ANALYSIS

The hydrogen bonding profile was computed from the molecular dynamics simulation as given in Figure 5.The number of hydrogen bond increases at pH 7 and 8 gradually decreases in all acidic and alkaline pH.

Many experimental methods are available to study the structural stability of biomolecules like proteins [25]. Molecular dynamics simulation at specific environment is one of the suitable methods to compute molecular property leads to prediction about protein function [26]. Since the stability of a functional protein can be predicted by the above studied parameters, hence the pH based molecular dynamics simulation suggests the best molecular mechanism of stability of these proteins.

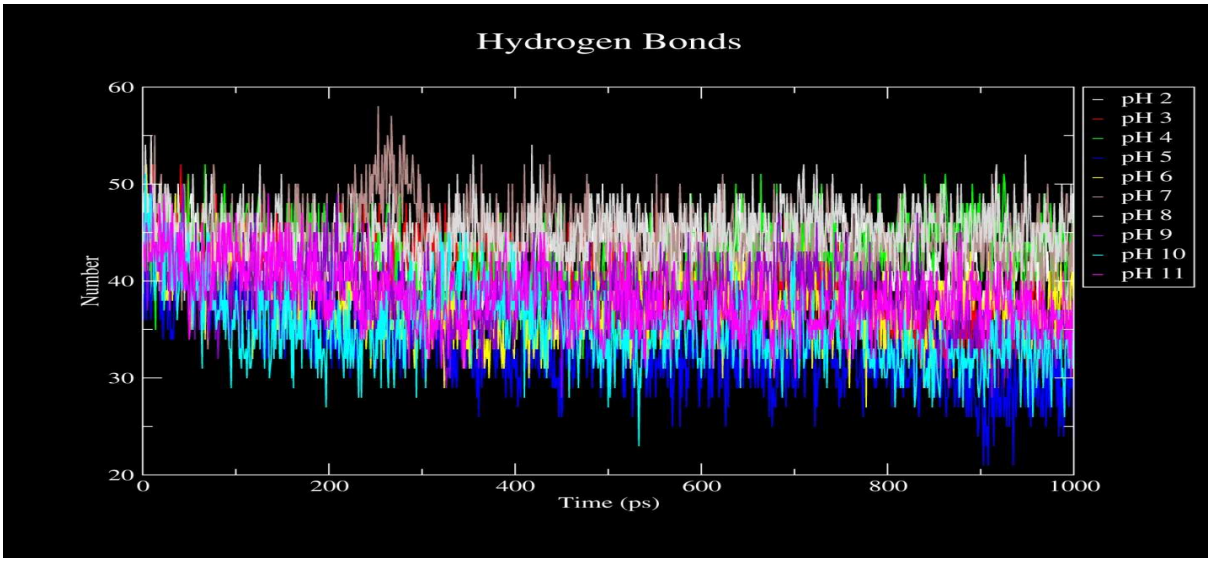

Figure 5: Hydrogen profile at different pH

CONCLUSION

The pH induced effect on structure of a type II antifreeze protein has been analysed by using molecular dynamics simulation method. The pH is considered to be a major factor in solvent medium in case of proteins for its proper function in *in-vitro/in-vivo* conditions. Observing the great importance of these antifreeze proteins particularly in cryo preservation purpose the study of structure and function of the proteins in different pH is an important issue. The overall result supports that the pH level 4-6 is suitable to maintain the structural stability of the protein. Further analysis like protein folding pathway and stability study by creating mutagenesis in residues would be highly essential to design novel AFPs with improved function and the same could be potentially used in the biomedical field.

Acknowledgment
We are thankful to Chief executive of MITS Engineering College, Rayagada, Odisha for his encouragement and providing us MIRC LAB for computing facility.

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