Review Article

Biomolecules in Binary Solvents: Computer Simulation Study of Lysozyme Protein in Ethanol-Water Mixed Solvent Environment

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Abstract

Proteins are building blocks of biological systems and play an important role from the health and medical perspective in the drug reactions and efficiency. Proteins function well in their natural water solvent environments and are influenced by modified solvent environments such as alcohol. Effect of protein-solvent interaction on the protein structure is widely studied with experimental and computational techniques. However, molecular level understanding of proteins interaction with many solvents is still not fully understood. The present work aims to obtain a detailed understanding of solvent effect on lysozyme protein, using water, ethanol, and different concentrations of water-ethanol mixtures as solvents. We use detailed atomistic molecular dynamics simulations to study using GROMACS code. Compared to neat water environment, the lysozome structure shows remarkable changes in water-ethanol mixed solvent, with increasing ethanol concentration. Significant changes were observed in the protein secondary structure involving alpha helices. We found that increasing ethanol concentration results in a systematic increase in total energy, enthalpy, root mean square deviation (RMSD), and radius of gyration of lysozyme protein. A polynomial interpolation approach is presented to determine these quantities for any intermediate alcohol percentage, and compared with the values obtained from a full MD simulation. Results from MD simulation were in good agreement with those obtained from the interpolation approach. The polynomial approach eliminates the need for computationally intensive full MD analysis for the concentrations within the range (0-12%) studied.

INTRODUCTION

Protein molecules are important in the formation of most biological building blocks inside the cell. The structure of protein plays an important role in biological functions [1]. For example, proteins like collagen support the cell structure due to its coiled helical shape which is long, strong and stringy [2]. Hemoglobin, a globular protein with a folded and compact shape can maneuver through the blood vessel providing a function to supply oxygen to the body cells [3]. Proteins function well in their natural water solvent environments and other solvent environments such as alcohol alter the behavior of protein structures and influence the therapeutic drug interactions from a medical perspective, starting from their fundamental molecular structures. Previous experimental studies have shown that the alcohol exerts significant influence on the protein structure.

Excess concentration of alcohol may denature the protein structure by disrupting the secondary structure of the protein. Such changes in the structure of the protein may also reduce the efficiency of drug reactions that target such proteins. For this very reason, alcohol intake is not advised along with medication. In order to gain further insights into the effect of alcohol on protein structure and dynamics, a deeper understanding of this phenomenon is needed. In this work, we try to obtain a molecular level picture of this problem by carrying out detailed molecular dynamics simulation studies.

Several experimental, theoretical and computational studies focused on understanding the structure-function relationship of proteins. Heat, acids and bases, reducing agents, alcohol etc. influence protein structure thereby its function. Ping *et al.* observed that the tertiary structure of lysozyme was destroyed

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as the temperature increased to 80 °C that also affected protein's function [4]. Solvents also influence the protein structure to a great extent. For example, a protein adapts dissimilar structure in a hydrophobic solvent compared to that in water. Despite numerous experimental and simulation studies, protein behavior in mixed solvents such as ethanol-water mixtures has not been fully understood from a molecular viewpoint. In this work, we conduct extensive molecular dynamics simulation studies of a lysozyme protein in ethanol-water mixture at various concentrations of ethanol in water to understand the solvent influence on protein structure and dynamics. This provides insight into the fundamental understanding of the protein structure in an alcohol environment. The understanding of the variations in the protein molecular structure in an alcohol concentration environment can provide further insights into their molecular interactions and efficiency under therapeutic drugs. For this purpose we use GROMACS molecular dynamics simulation analysis code. We chose Lysozyme as it closely resembles protein structure found in humans. Secondly it is moderate in size for molecular dynamics simulation analysis compared to most other proteins. A cartoon representation of lysozyme protein (protein data bank file 1AKI.pdb) is shown in (Figure 1).

Water plays an important role in maintaining cell membrane and enzyme activities acting as lubricant for protein movements in cells. More importantly, proteins need water to function and are their natural environment. Hence, we first simulate protein in water to understand the behavior of lysozyme in a water solvent environment. This is followed by the simulation of lysozyme in an ethanol-water mixed environment at different ethanol-water ratios. The hydroxyl group of ethanol (C_2H_5OH) can bond with hydrogen from other ethanol to make it less volatile and more viscous than lower polar organic compound with almost same molecular weight for example, propane. It is miscible with water and some other organic compounds. The focus of this

work is to study and understand the effect of different solvent environments (ethanol-water mix) on protein. We anticipate an understanding of the effect of ethanol on lysozyme provides insights into similar other protein-solvent interactions as well. Several experimental studies have been carried out before to study the effect of ethanol on protein and the effect of water on protein with tangible results [5-8]. However, to our knowledge, the effect of ethanol and ethanol-water mixtures has not been studied computationally in detail. In this work, we explore the effect of ethanol on lysozyme by carrying out detailed atomistic molecular dynamics simulation studies.

In a series of simulation studies, Bagchi and coworkers examined transport properties of binary mixtures [9-11]. They have shown that the specific solute solvent interaction play important role in determining the properties of such solvents. Wensink *etal* [12] studied binary mixtures of alcohol and water using molecular dynamics simulation. They computed the shear viscosity using non-equilibrium molecular dynamics simulation. The diffusion constant was studied along the rotational correlation time, and was found that mobility correlates with viscosity data i.e. the viscosity is maximal at intermediate alcohol concentration [13]. It was found that at maximal viscosity, mobility was minimal. They combined viscosity and diffusion calculations to compute the effective hydrodynamic radius of the particles in the mixture using Stoke- Einstein relation [14]. The analysis indicated that there is no collective diffusion of molecular clusters in the mixture and the pure liquid. The present work examines lysozyme protein structure and dynamics in various alcohol-water mixtures by using series of molecular dynamics simulations at different alcohol-water solvent ratios. A brief background of the molecular dynamics analysis is presented next for completeness. The remainder of this article is organized as follows. Section 2 provides the details of the molecular dynamics modeling method employed in this study. Section 3 contains simulation results on protein structure in water, ethanol and water-ethanol mixed environments. Section 4 presents a detailed discussion and analysis of the results. We close the article with few concluding remarks in the summary section.

MOLECULAR DYNAMICS SIMULATIONS

GROMACS is the MD analysis code employed in the present work. It is most commonly used open source software for molecular dynamics studies of materials and biological systems. Importantly, it is one of the fastest MD codes among the open source codes for MD simulation that are currently available.

The force field and the initial configuration can be used to estimate or calculate the motion and position of the particles/atoms in a molecular system. The sum of the intermolecular interaction and intra-molecular interaction is equal to the total potential energy of the system. In the following, we describe these two interactions in detail.

The bond stretching and bond bending energy equation is based on Hooke's law [15].

$$E_b = \sum k_b (r - r_0)^2 \qquad (1)$$

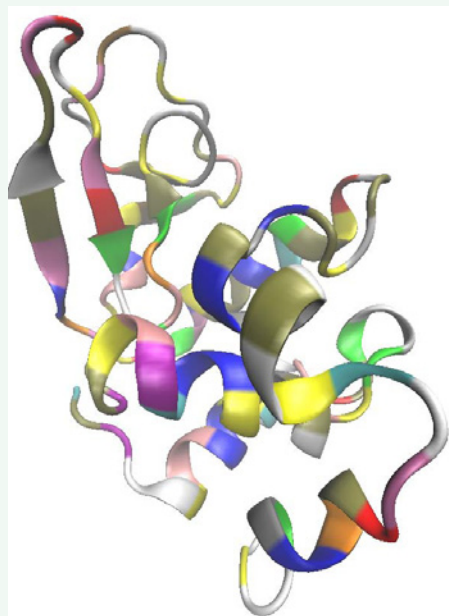Where E_b is bond energy, K_b is bond-interaction constant, and

Figure 1 A cartoon depiction of lysozyme protein structure is shown. Distinct colors represent different residues present in the protein.

r_0 is the equilibrium position length between two bonded atoms.

$$E_\theta = \sum k_\theta (\theta - \theta_0)^2 \qquad (2)$$

where E_θ is bond-angle energy, K_θ is the corresponding force constant, θ_0 is the equilibrium angle.

The interaction of the nonbonded molecules were modeled using the Lennard Jones potential and the Coulumbic potential [16] as described in the following equation

$$v_{nonbonded(i,j)} = v_{lernad(i,j)} + v_{coulomb(i,j)} \qquad (3)$$

where v is the potential for non bonded interaction between atoms of different molecules i and j with the total potential is given as the sum of the coulomb potential and the Lennard Jones potential

$$v_{coulomb(i,j)} = \frac{1}{4\pi\varepsilon_0} \frac{q_i q_j}{r_{ij}} \qquad (4)$$

$$v_{lernard(i,j)} = 4\varepsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right) \qquad (5)$$

ε_0 is the permittivity of free space, $q_{(i,j)}$ are charges, ε is the energy parameter with reference to the depth of the potential well, σ_{ij} is the finite distance for which inter-particle potential is zero. In the present study, we use the OPLS force field parameters that are widely accepted for the biomolecular simulation studies.

Molecular dynamics simulation of lysozyme protein in water

We begin with simulating lysozyme protein in neat water environment, which has been extensively studied both by experimental and computational techniques. For this purpose, we analyze various thermodynamic quantities in addition to structural parameters such as radius of gyration and RMSD (Root Mean Square Deviation) for the molecular system. This initial study helps us verify the consistency of our simulation method and also serves as reference to compare and contrast the protein structure and its dynamics under other solvent environments such as the ethanol-water mixture focused in the present work.

The protein structure obtained from PDB was solvated with TIP3P [17] water and the whole system was minimized using steepest descent method. All computations were performed using in-house computation cluster located on the NC A & T campus. Both NVT and NPT equilibration simulations were carried out for 200ps with a 1fs time step. We used temperature and pressure values of 300K and 1 bar, respectively. The final configuration from NPT equilibration was taken as the starting structure for the production MD.

The simulation system contained one lysozyme protein molecule of 129 residues with 1960 atoms and 12,365 water molecules (37,095 atoms). The system contained a total of 39,055 atoms. We used the Berendsen thermostat [18] to control the temperature. For the MD production simulation, the system was run for 50 million time steps corresponding a total duration of 50ns.

The structure of the protein was examined with commonly used structural properties such as radius of gyration (R_g) root mean square deviation (RMSD) etc. which are described below.

Radius of gyration helps understand the compactness of the protein structure and is given by [15],

$$R_g = \left(\frac{\sum_i \|r_i\|^2 m_i}{\sum_i m_i}\right)^{\frac{1}{2}} \qquad (6)$$

m is the mass of the atom r_i and *i* are the positions specific to a particular atom with the reference point being the center of mass [19]. The R_g value for the lysozyme protein obtained from present simulation study is shown as a function of time in (Figure 2(A)). As shown, the R_g value fluctuates around an average value of 1.42nm throughout the simulation, suggesting that the protein maintains relative compact size during the entire dynamics simulation time.

The RMSD (root-mean square deviation) is the measure of the average distance between the atoms of the back bone of superimposed proteins. The RMSD can be calculated with the equation [20],

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2} \qquad (7)$$

RMSD could be used for quantitative comparison between the structure of the native state of protein and its partially folded state. For the present lysozyme-water system, RMSD value for the entire simulation time (50ns) is shown in (Figure 2(B)). The average RMSD value is less than 1nm, indicating a relatively folded structure for the protein in this natural water environment, consistent with the existing simulation studies [21]. Combined with the result of R_g for the protein in the present study indicates a compact structure for lysozyme in water during the entire simulation, in good agreement with earlier simulation analysis reported in the literature [22].

The next sections discuss the lysozyme protein in different solvent conditions that include ethanol, and ethanol-water mixture and comparison with the data from pure water environment.

Molecular dynamic simulation of protein in ethanol

This section focuses on the analysis of lysozyme in ethanol solvent environment. Ethanol is miscible with water. It is also known to have profound effect on proteins and biomolecules. This in turn could have significant impact on the interaction with and efficiency of therapeutic drugs. The behavior of lysozyme in a 100% ethanol solvent condition is studied here. For this purpose, lysozyme protein was solvated with 100% ethanol. The lysozyme-ethanol molecular system employed in the present study contained one lysozyme protein (1,960 atoms) and 2,289 ethanol molecules (20,601 atoms), resulting in a total of 22, 561 atoms in the simulation system. This system was simulated for 50ns with a 1fs timestep size.

At the end of the simulation we analyzed and compared the thermodynamic quantities following the same approach as in the case of water environment. The initial and final structures

of lysozyme in ethanol are shown in (Figure 3(A) and 3(B)), respectively. As shown, the protein structure showed significant changes at the end of the dynamics simulation in a full ethanol environment. The final structure appears expanded / swollen compared to the initial structure. For comparison, in (Figure 3(C)), we also show the final structure obtained from lysozyme in water simulations.

From (Figure 3), lysozyme in ethanol appears to be swollen compared to that of water. A closer examination reveals significant difference in the protein alpha-helix content in ethanol compared to water. The alpha-helix structures were broken into relatively shorter helices in ethanol compared to water. In other words, longer helix components were no longer stabilized when solvent environment was changed to ethanol. One of the factors that stabilize protein secondary structure is the hydrogen bonding between protein and solvent. As the solvent changed from water to ethanol, the hydrogen bond network between protein and water, responsible for stabilizing alpha-helix structure was broken in case of ethanol. This led to the instability of protein secondary structure in ethanol solvent. To further confirm our findings, we calculated and compared thermodynamic quantities such as total energy and enthalpy and structural quantities such as radius of gyration (R_g) and RMSD. The results of these quantities are compared with the results from water environment.

The total energy of the lysozyme protein system in water was -443,901 (KJ/Mol) and that of ethanol is -59,164(KJ/Mol). This significant energy difference clearly indicates that the lysozyme protein in water system is more stable compared to the same protein in the ethanol system. This is in accordance with the significant change in the final protein structure in ethanol and water as shown in (Figures 3(B) and 3(C)). To further verify our results we analyzed the compactness of the protein structure by plotting the radius of gyration as a function of time as shown in the (Figure 5).

We find the radius of gyration of protein in water is approximately 1.4 nm as shown in (Figure 4(B)) and that of ethanol in (Figure 4(A)) is approximately 3.12nm. The significant difference between the R_g values indicates a significant change in the compactness of the protein. Hence, it can be inferred that the protein molecule is swollen in ethanol compared to that in water. The structural stability of protein molecule in water and ethanol was compared by analyzing the root mean square deviation (RMSD) as shown in (Figure 5).

From (Figure 5), the RMSD for protein in water and ethanol were found to be approximately 0.162 nm and 4.15nm respectively. This is a significant change in RMSD value of the lysozyme in ethanol compared to that of water. In their folded native structure, proteins typically have RMSD values of about 0.1 to 0. 2 nm [23-24]. RMSD value as high as 4.15nm shows a significant deviation of the lysozyme protein from its native structure in an ethanol solvent environment.

The simulation results showed an increase in total energy, enthalpy, radius of gyration and root mean square deviation
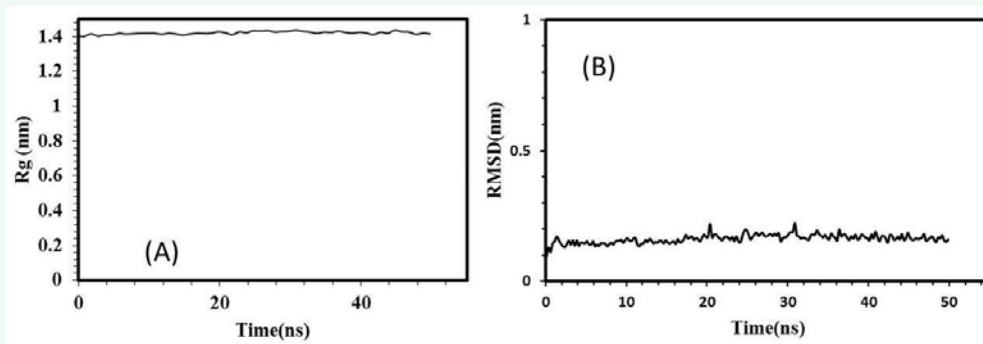

Figure 2 Structural properties of lysozyme protein in water as observed in molecular dynamics simulations (A) Radius of gyration and (B) RMSD of lysozyme protein in water.
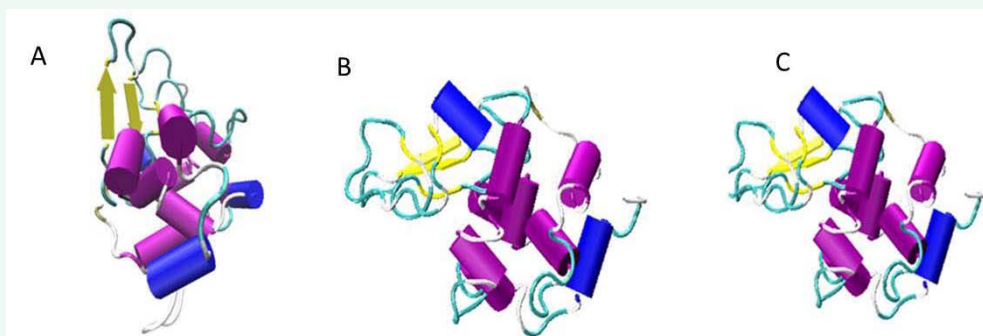

Figure 3 Comparing the protein structures: (A) initial structure (B) final structure in ethanol and (C) final structure in water.
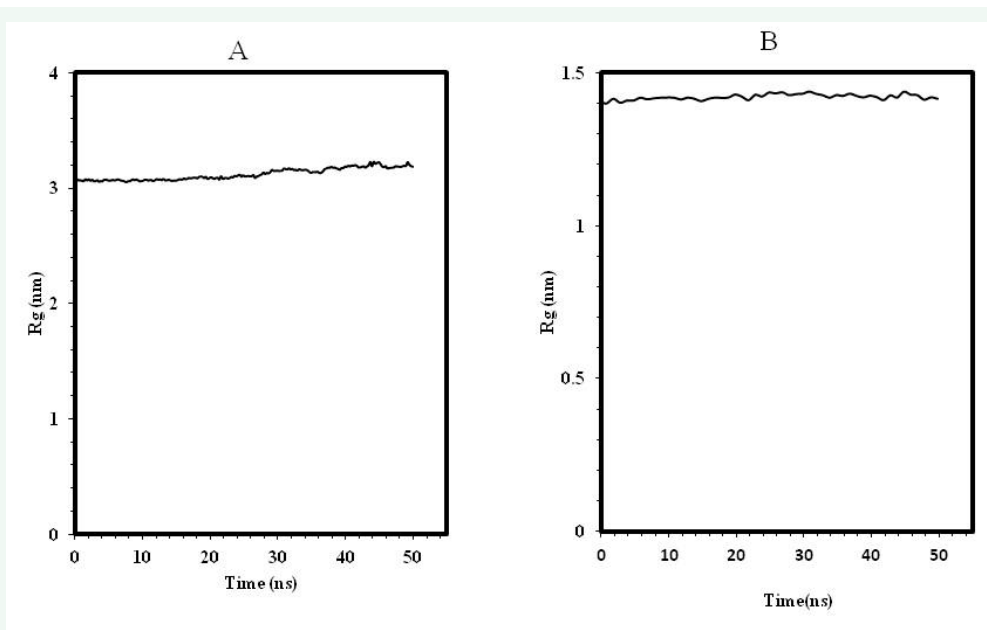
Figure 4 Effect of solvent on protein as reflected in the structural property radius of gyration (R_g) of the protein: (A) R_g of protein in ethanol and (B) R_g of protein in water.
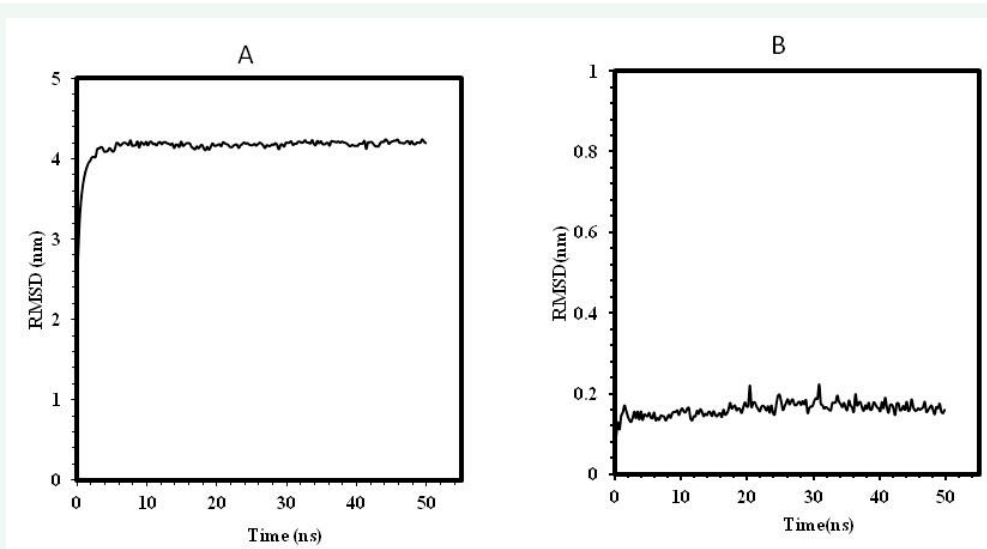

Figure 5 RMSD values for lysozyme protein: (A) in ethanol and (B) in water.

for lysozyme in ethanol compared to water. The significant increase in the calculated quantities affirms the changes in the stability of the system and compactness of the protein structure. Simulations thus reveal marked changes in protein structure and energy when solvated in ethanol compared to that in water. In order to quantify such changes in a systematic fashion, we proceed to study the effect of ethanol and water mixtures on lysozyme protein. For this purpose we use similar simulation setup as discussed in the previous sections to create and study lysozyme protein at different ethanol concentrations in water. In the present study, low ethanol concentrations in the range of 0–12% in the ethanol–water mixtures are considered. Lysozyme protein behavior in the ethanol-water mixture environment at various percentages is compared to that of pure water and 100% ethanol environments, and is presented next.

Molecular dynamics simulation of lysozyme protein in ethanol-watermixtures

In the previous sections, we have described the effect of pure water and pure ethanol on lysozyme protein. Here we conduct MD simulation of protein in ethanol-water mixtures over a range of ethanol concentration in a systematic fashion. For this purpose we set up six different ethanol concentrations of approximately 2%, 4%, 6%, 8%, 10% and 12%. This concentration range of ~2 to ~12% was selected based on the prior experimental

investigations in the literature [25]. We aim to qualitatively analyze the lysozyme protein behavior as a function of ethanol concentration and to understand the changes with the addition of low concentration of ethanol to its natural water environment. We begin the simulation set up as before but solvating the protein with both water and ethanol in specified compositions.

MD analysis was conducted as before by solvating the lysozyme protein in both ethanol and water molecules in appropriate ratios so as to obtain different concentrations of ethanol. The details are shown in (Table 1). We chose approximate percentage based on the mass of ethanol molecules and water molecules for the required percentages of water and ethanol mixture. We equilibrated the lysozyme in each ethanol-water mixture system and conducted full simulation for 50ns with a time step of 1 fs, at a pressure of 1 bar, and a temperature

of 300K. At the end of the simulation process, we calculated and compared the thermodynamic and structural quantities similar to the quantities calculated as before. The initial and final structures after the MD analysis of the lysozyme protein at different ethanol-water mixture concentrations are shown in (Figure 6). In this Figure only the protein is shown while water and ethanol are not shown for the clarity purpose. As shown in (Figure 6), noticeable changes in the initial and final structure of protein at different water -ethanol concentrations can be found.

In order to understand the role of solvent, in particular that of ethanol, we have tracked the ethanol molecules motion over the course of simulation. Corresponding snapshots showing intial and final configurations for different concentration of ethanol-water mixture are shown in (Figure 7). This Figureshows that

Table 1: Details of lysozyme ethanol water system simulated in the present study. Binary mixtures with approximately 2%, 4%, 6%, 8%, 10% and 12% ethanol concentration in water are listed in the Table.

| Ethanol concentration	Number of water molecules	Molecular weight of water (g/mol)	Number of ethanol molecules	Molecular weight of ethanol (g/mol)	Total number of atoms in simulation
2.01%	6977	125586	56	2576	23395
3.93%	6928	124705	111	5113	23743
6.13%	6612	119016	169	7774	23317
8.212%	6455	116190	226	10396	23359
10.262%	6302	113436	282	12972	23404
12.333%	6140	110520	338	15548	23422
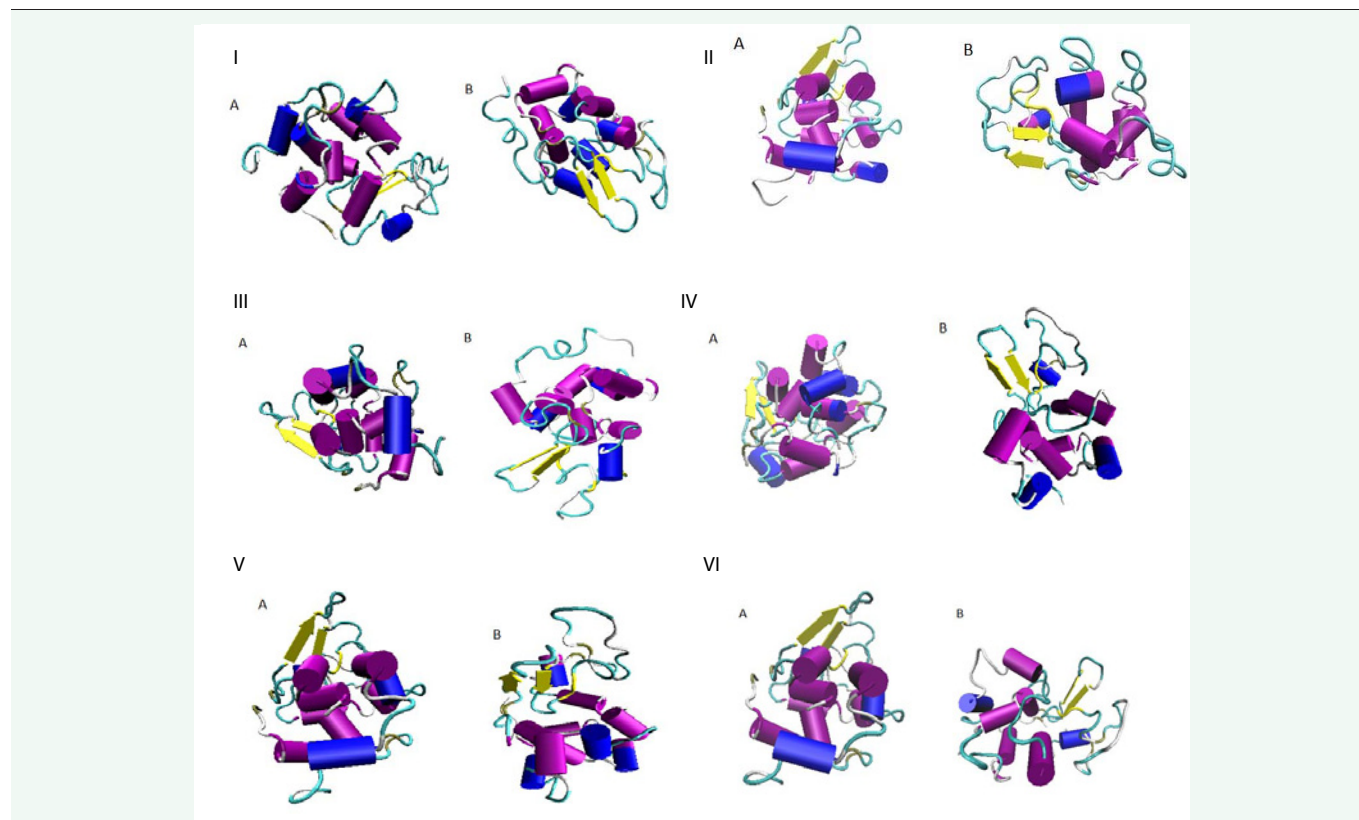
Figure 6 (A) initial and (B) final structures of protein in ethanol-water mixture at different concentrations of ethanol approximated at (I) 2% (II) 4%(III) 6%(IV) 8%(V) 10% and (VI) 12%.

the diffusion of ethanol molecules into the lysozyme causing the protein molecule to swell as the protein gets increasingly destabilized with increase in ethanol concentration. Both lysozyme and ethanol molecules are shown in this figure. Together from (Figures 6 and 7), we notice that the protein secondary structure gets altered as ethanol progressively replaces water molecules with increasing ethanol concentration. This leads to the decrease in alpha helical content of the protein in the same direction. Together, these observations reveal that the protein increasingly gets deviated from its native folded structure as the ethanol concentration increases. To further verify our observation, we calculated and compared the thermodynamic quantities starting with the total energy as presented in (Figure 8). An increase in the total energy of the system with increase in the concentration of ethanol is noticed. This also indicates decrease in the stability of the system as the concentration of ethanol increases.

We observed a significant difference in the key structural and thermodynamic quantities of protein in pure water and that in ethanol. This is shown in (Figure 8). In the range of ethanol-water mixture concentrations studied, we observed a trend of increase in the energy and enthalpy with increase in ethanol concentration. This increase in thermodynamic quantities further supports the observed swelling in protein structure. The dynamic variation in the key parameters over the MD analysis time duration is plotted in (Figure 8). The dynamic changes also showed similar behavior that of the time averaged values for the key parameters studied.

Figure 8 shows the average thermodynamic quantities as a function of mole fraction. From this figure we notice a significant increase in all the calculated quantities during the progressive passage from pure water to pure ethanol with higher absolute values at 100% ethanol. These changes further confirm the observed decrease in protein stability with increase in ethanol concentration. The summarized time average values of the thermodynamic quantities of protein in different concentration of ethanol are presented in (Table 2).

POLYNOMIAL INTERPOLATION METHOD

The present computational analysis took an average of eighty four hours of computing time for each concentration, with thirty six processors, using GROMACS software on the multi-processor computing system at North Carolina A & T State University. To obtain the thermodynamic and structural quantities for any new percentages, a complete simulation will be required. Such a complete simulation would require a significant computing time and resources. However, based on our present analysis, a relatively smooth variation of the time averaged values of the key parameters is clearly noticed. This could allow one to potentially interpolate the required values from the present generated data for a different ethanol concentration within the range of ethanol concentrations studied. Based on these observations, we propose and present an interpolation methodology for the quantitative key parameters studied as an alternative way by which the need for additional computer simulation and/or experiments can be avoided. The interpolation approach uses our simulation data and interpolating to obtain the unknown values for another ethanol concentration percentage that is within the range of present study (0 to 12%). The effectiveness of this interpolation methodology was tested as follows.

1. Select an intermediate percentage that was not used in the simulations. For this purpose, we selected ~7% ethanol concentration.
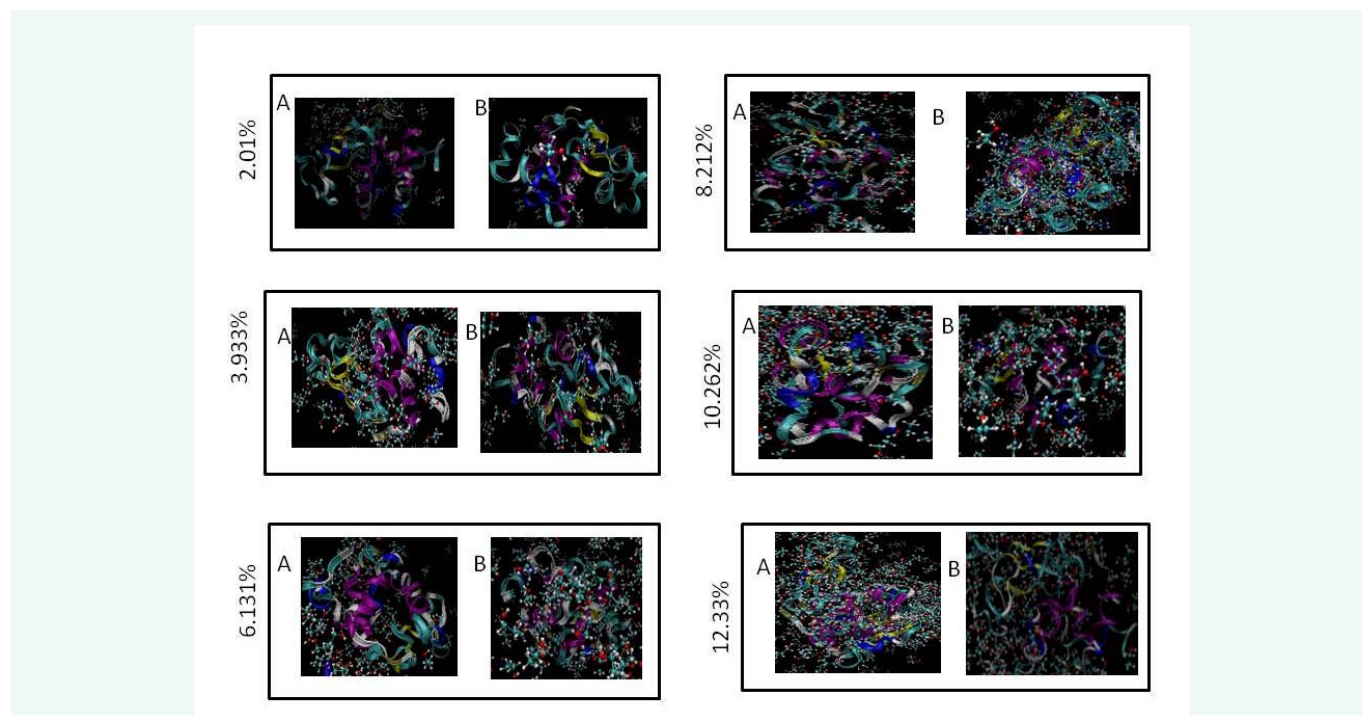

Figure 7 (A) Initial structure and (B) final structure of protein at various concentration of ethanol. Diffusion of ethanol molecules into the protein increases with increasing ethanol concentration as shown in the figure.
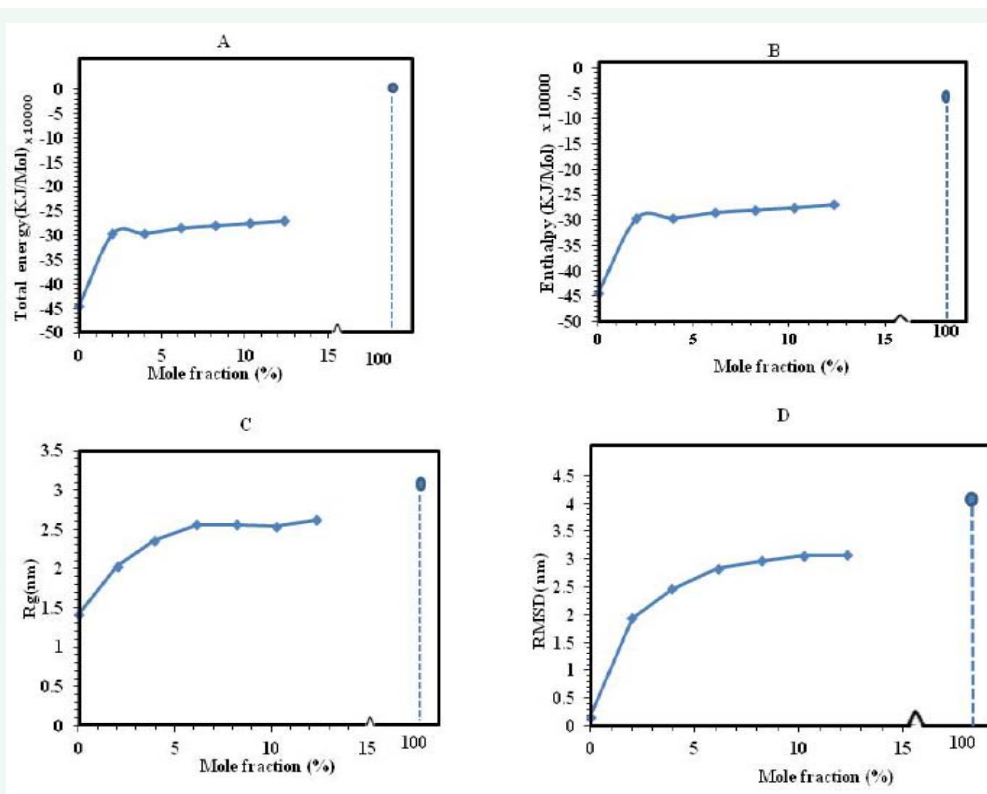
Figure 8 Time average of (A) total energy (B) enthalpy (C) radius of gyration and (D) RMSD of lysozyme protein in ethanol-water mixture is shown as a function of ethanol mole fraction.

Table 2: Time average values for the parameters calculated for various concentrations of ethanol in ethanol-water binary mixtures.

Ethanol Concentration	Radius of gyration (nm)	Total Energy (kJ/Mol)	Enthalphy (kJ/Mol)	RMSD (nm)
0%	1.42	-443865.8	-443842.0	0.17
2.01%	2.03	-296529.6	-296515.7	1.94
3.933%	2.36	-295755.5	-295741.4	2.47
6.131%	2.42	-284809.8	-284796.0	2.86
8.212%	2.56	-279755.0	-279741.2	3.00
10.262%	2.54	-275197.3	-275183.4	3.08
12.33%	2.62	-270054.9	-269361.5	3.10
100%	3.12	-54189.38	-59108.76	4.17

2. Using an appropriate interpolation method and exiting values in (Table 2), calculate the interpolated values of the key parameters at this intermediate ethanol – water concentration range (7% ethanol case).

3. Subsequently, a complete MD analysis was performed at this concentration level and compared to the obtained interpolated values.

Using polynomial method to interpolate the intermediate ~7% ethanol

In order to quantitatively determine the behavior of simulated quantities as a function of ethanol concentration, we have used a polynomial fitting as explained below. The following second order polynomial equation was used to fit the simulated quantities,

$$c_2 x^2 + c_1 x + c_0 = f(x) \tag{8}$$

where c_0, c_1 and c_2 are constants. The resulting fits for simulation results are shown in (Figure 9). The values of these constants obtained by fitting to the simulation results for all thermodynamic and structural quantities are presented in Table 3.

We generated our data using equation 8.

Using the coefficients c_0, c_1 and c_2 one can determine the above mentioned thermodynamic and structural quantities at any ethanol concentration (from 0-12%), without carrying out the full scale additional actual simulations. In order to verify this approach, we selected an intermediate ethanol concentration that has not been used in fitting procedure. By using the polynomial equation (Eq 8) we first theoretically determine
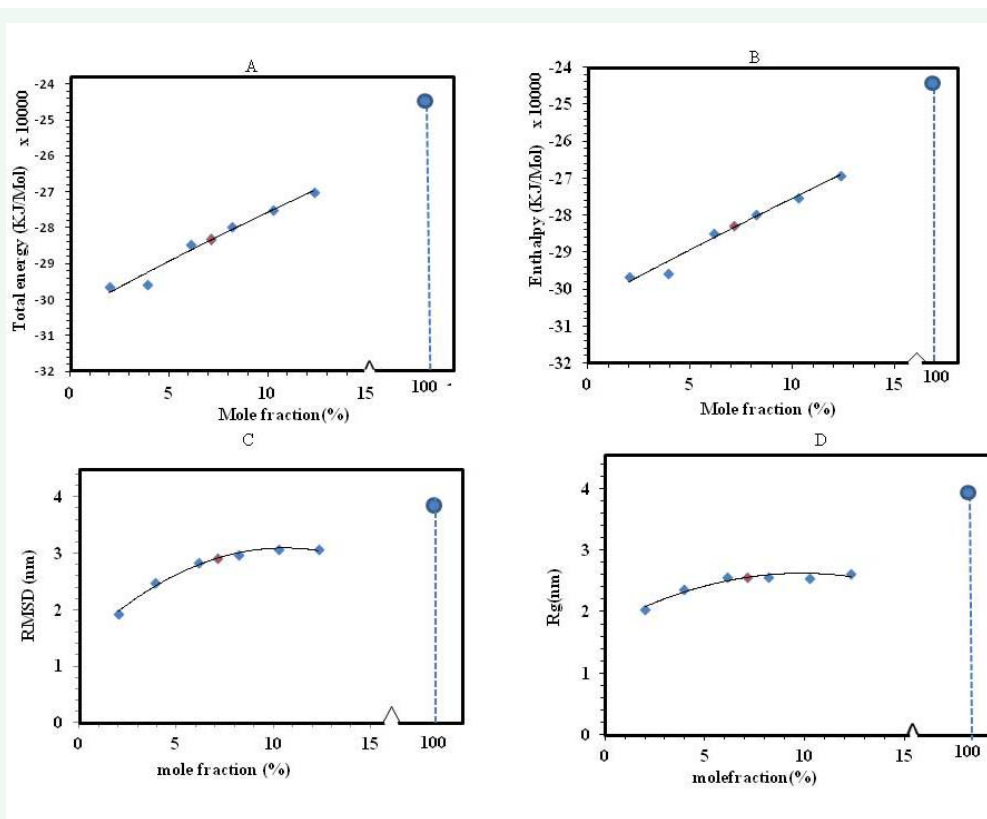
Figure 9 Values obtained from interpolation method for (A) total energy (B) enthalpy(C) radius of gyration and (D) RMSD for approximately 7% of ethanol. (in each figure red symbol corresponds to ~ 7% ethanol case) are shown along with the values obtained from simulations.

Table 3: Table of constants for polynomial in equation 5.1 for approximately 7% ethanol.

Property	C_0	C_1	C_2
Total energy	-596.247	10980.613	-329718.709
Enthalpy	-596.073	10978.794	-329700.3
Radius of gyration	2.477	0.066	0.009
RMSD	3.669	-0.564	0.058

the thermodynamics and structural quantities. In the next step, we conducted a complete simulation study as before for the selected ethanol concentration. The simulation results were then compared with that obtained from polynomial fitting procedure. We have selected ~7% ethanol as our test case, which was not used in simulations.

Figure 9 shows the fitted interpolation of simulated quantities as a function of ethanol concentration. For the analyzed quantities: total energy, enthalpy, radius of gyration and RMSD. For the intermediate percentage of ethanol (~7%) chosen is also plotted in each figure, indicated by the red symbol (Figure 9). Table4 shows the interpolated values obtained for the intermediate concentration of 7% ethanol using polynomial method.

Validating the results from interpolation method

A full MD analysis run at approximately 7% ethanol in ethanol – water mixture was employed to compute the key

parameters of energy, enthalpy, R_g and RMSD and compared with the corresponding interpolated value at this intermediate percentage. Actual percentage of 7.153 percent by mass of ethanol was employed. The system was set up similar to the other percentages we studied before. The details of the lysozyme-ethanol-water mixture simulation system are presented in (Table 4). The comparison between calculated thermodynamic quantities and key parameters from the present simulation is presented in (Table 4). To further validate the interpolation result for 7.153%, we computed the percentage error between the interpolated values and the simulated values for the 7.153% ethanol concentration as shown in (Table 4). The values from the interpolated result and the actual simulation are in good agreement. The error margin found to be within the acceptable range. Such good agreement validates the interpolation method for any other percentage within our initial percentage range which is from 0 to 12 percent ethanol. This is a remarkable result, since it can potentially avoids the need for computational or

Table 4: Simulated results vs Interpolated results for the 7.153% concentration of ethanol in water.

Property	Calculated	Simulated	Percentage error
Radius of gyration(nm)	2.51	2.53	0.79
RMSD(nm)	2.86	2.84	0.63
Total energy(KJ/Mol)	-283714.93	-282421.43	0.46
Enthalpy (KJ/Mol)	-282313.54	-276895.71	1.91

experimental procedures for other concentration values within the concentration range studied in this work.

The simulations of protein at different concentration of ethanol in the range of ~2% - 12% ethanol in ethanol-water mixture reveal that the most of thermodynamics and structural quantities show an increase with increasing ethanol concentration, thereby revealing the destabilization of folded native protein structure with increasing ethanol concentration. The analysis of structural quantities such as RMSD and radius of gyration revealed the protein structural deviation from folded state. Together, these results demonstrate a uniform trend in increase with increasing ethanol concentration. Hence, we proceed to quantify this behavior as a function of ethanol concentration. For this purpose, we obtained optimal polynomial fit for each thermodynamic and structural quantity as a function of ethanol concentration. By using such polynomial expression along with the determined coefficients, we could obtain the results for any arbitrary concentration that is within the range of 0-12% ethanol without a need for additional full scale simulation analysis.

CONCLUSION

In the present paper, we have presented a detailed study of structure and dynamics of lysozyme protein in water, ethanol and water-ethanol binary mixtures by conducting extensive computational molecular dynamics simulation studies. In each case, we performed detailed molecular dynamic simulation and analysis on the following thermodynamic and structural quantities of lysozyme: total energy, enthalpy, radius of gyration and RMSD. MD analysis studies were carried out using GROMACS MD simulation code. We carried out the simulation process for protein in water environment and performed thermodynamic and structural analysis by calculating the total energy, enthalpy, radius of gyration, and root mean square deviation. The results from the analysis showed that protein was relatively stable in water environment, without showing significant deviations from its native folded structure. We proceed to simulate and analyze protein in pure ethanol under the same thermodynamic conditions. At the end of the analysis, we observed a significant change in protein structure between the water environment and the ethanol environment. We find the protein molecule relatively swollen in ethanol solvent compared to that in water environment. To further understand the effect of solvent on protein structure in more systematic fashion, we performed full simulations on different percentages of ethanol-water mixture (2%, 4%, 6%, 8%, 10%, and 12%) and carried out similar thermodynamic analysis as before. We observed changes in protein molecule with increase in ethanol concentration as the molecule seems to increase in size based on the visual structure of protein obtained from VMD and thermodynamic analysis. We observed a trend in the variation of the derived thermodynamic quantities analyzed for the various ethanol percentages studied. Based on this variation, we proceed to check the possibility of utilizing interpolation method for intermediate percentage within our range of percentages. We chose an intermediate percentage of seven percent for this purpose. With polynomial interpolation method, we were able to calculate the values of the thermodynamic quantities for seven percent using existing data

from our previous simulation. In order to verify these results, we constructed a system of lysozyme protein in ~7% ethanol and performed a full scale molecular dynamic simulation. The results from the full simulation were compared with the interpolated results. We found both were in good agreement within the level of acceptable error.

Based on our simulation findings we conclude that the ethanol has a significant effect on lysozyme protein structure. The deviation of protein structure from its native environment suggests that the protein molecule is likely to function better in water environment compared to ethanol environment. We envisage, such molecular level insights into protein-solvent interactions can be used as guidelines in studying similar other protein-solvent interactions as well.

One of the main contributions of this work is that for subsequent percentages within a known range of percentage values, interpolation method can be successfully used to obtain the results. As demonstrated in the present work, it eliminates need for the large computing time and resources involved in such full scale simulations.

In the present work we have simulated and analyzed the dynamics of protein in ethanol-water solvent. With the knowledge acquired from this work, future studies can explore the effect of other components on proteins and other biological molecules employing the MD analysis methodology. Along these lines, one can study the effect of anesthetic molecules on proteins as well. Despite the routine usage of anesthetic molecules in medical and surgical procedures, this procedure is not devoid of side-effects. Understanding the molecular level interaction between anesthetic molecules and proteins may provide further insights on the side effects. Similar to the study here, nature and concentration of anesthetic molecules may affect the protein structure thereby its function and provide practical extension of the present work.

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