Molecular dynamics simulations and density functional theory studies of NALMA and NAGMA dipeptides

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(Classical molecular dynamics (MD) simulations using fixed charged force field (AMBER ff03) and density functional theory method using the M05-2X/6-31G** level of theory have been used to investigate the plasticity of the hydrogen bond formed between dipeptides of N-Acetyl-Leucine-MethylAmide (NALMA), N-Acetyl-Glycine-MethylAmide (NAGMA), and vicinity of water molecules at temperature of 300 K. We have noticed that 2–3 water molecules contribute to change in the conformations of dipeptides NAGMA and NALMA. The self-assembly of 11 water molecules leads to the formation of water bridge at vicinity of the dipeptides and it constrain the conformations of dipeptides. We have found that the energy balance between breaking of the C=O...H–N H bonds and the formation of the C=O...H–O (wat) H bonds may be one of the determining factors to control the dynamics of the folding process of protein molecules.

Keywords: AMBER ff03; M05-2X/6-31G**; NALMA; NAGMA; water bridge and conformers

1. Introduction

The protein folding is one of the most important active areas of research in biophysics (Ball, 2008; Chandler, 2005; Chaplin, 2006; Mittal, Jayaram, Shenoy, & Singh Bawa, 2010). The importance of water in biological systems has been studied over a century using wide range of physical techniques. The presence of water molecules along with biomolecules is responsible for the most of the characteristic structures and function of biomolecules, due to its unique hydrogen bond capability (Franks & Mathias, 1982). The association of water with biomolecules is classified into three categories: Internal or structural water; hydration; and free water. The internal or structural water is relatively immobile within the solute, and its relaxation dynamics are often restricted to slow rotation (Yokomizo, Higo, & Nakasako, 2005). The hydration water consists of structured water shells which depend upon the protein–surface interaction; its diffusion dynamics are slow, and it can exchange with outer shells (Helle, 2004; Hunt, Kattner, Shanks, & Wynne, 2007; Raschke, 2006). Free water is beyond the third hydration shell which, in principle, is not strongly influenced by the protein surface other than due to the excluded volume effect of the protein molecules (Russo, Hura, & Head-Gordon, 2004). From this perspective, the internal and hydration waters are more important for protein stability and functions.

Despite, large number of experimental and theoretical studies (Cheng & Rossky, 1998; Perticaroli et al., 2011; Ramalho & Da Cunha, 2011; Sterpone, Stirnemann, Hynes, & Laage, 2010; Zhang, Yang, Kao, Wang, & Zhong, 2009), some fundamental issues, such as characteristic mobility of interfacial water and spatial extent of the induced perturbation, are strongly debatable (Cheng & Rossky, 1998; Perticaroli et al., 2011; Sterpone et al., 2010; Zhang et al., 2009) and mechanism of protein–water coupling dynamics was studied over the nanosecond range. The hydrophobic and hydrophilic nature of the amino acid comprised of protein structure and the hydrophobic peptides are contributing the folding process in water environment. Born, Weingartner, Brudermann, and Havenith (2009) have suggested that the activation of intermolecular network motions of water will be a key step in the activation of protein function. In the present study, we have chosen A. N-Acetyl-Leucine-Methylamide (NALMA) which comprises a hydrophobic amino acid side chains, \((CH_3)_2-\text{CH–CH}_3\) attached to the \(C_x\) atom of a polar-blocked polypeptide backbone with \(CH_3\) endcaps, \((CH_3–CO–NH–C_xH–CO–NH–CH_3)\) and B. N-Acetyl-Glycine-Methylamide (NAGMA) which comprises the polar-blocked backbone and a hydrogen atom is attached to the \(C_x\) atom. These two peptides were chosen because their chemical composition is similar; but
NALMA is more hydrophobic than NAGMA, because of the hydrophobic leucine side chain (Russo et al., 2011). The structures of the two peptides are shown in Figure 1.

Many spectroscopic techniques such as neutron scattering (Matardier-Jugroot & Head-Gordon, 2007; Russo, Murarka, Copley, & Head Gordon, 2005; Russo, Teixeira, & Ollivier, 2009), terahertz (THz) absorption spectroscopy (Born et al., 2009), dielectric spectroscopy (Murarka & Head-Gordon, 2008), nuclear magnetic resonance (NMR) relaxation (Qvist, Persson, Mattea, & Halle, 2009), optical Kerr effect (Muzur, Heisler, & Meech, 2010), Extended frequency range depolarization light scattering (EDLS) (Perticaroli et al., 2011) have been used to study the above molecules. The molecular dynamics (MD) simulations (Johnson, Malardier-Jugroot, Murarka, & Head-Gordon, 2009) were also performed close to room temperature. Masman, Lovas, Murphy, Enriz, and Rodriguez (2007) have investigated the conformational preference of NALMA using \textit{ab initio} and DFT methods. Russo et al. (2009) have found that the rotation, diffusion motions, and dynamical transitions have occurred at around 250 K for long side chain peptide (NALMA), while for peptide with short side chain (NAGMA) there were no dynamical transitions but only rotational motion was observed by elastic and quasi elastic neutron scattering (QENS) experiment. Born et al. (2009) have found that the minimum number of hydration water required for activating water–peptide (NALMA and NAGMA) network motion probed by THz spectroscopy. Russo et al. (2011) have investigated that the changes in the hydrogen bond network of hydration water molecules which depend on the sites of biomolecules (NAGMA and NALMA) at low frequency mode by inelastic scattering experiment and MD simulations.

Thus, despite, good amount of experimental and simulation results, the protein folding process in water environment is not yet clear. Since the motion of water molecules is intimately connected to the process of hydrogen bond forming and breaking, a deep knowledge of the H-bond kinetics in the complex system is essential to elucidate overall dynamical characteristics. So, we have decided to probe further on these two model dipeptides, by calculating radial distribution functions (RDFs), conformational analysis, rupture time of hydrogen bonds, hydration water shell analysis, and interaction energy of dipeptides using MD simulation and density functional theory (DFT) methods. We have addressed three specific questions: one concern with the ongoing conflict of minimum number of water molecules required to activate the conformational changes of dipeptides. Second concern is to investigate the plasticity of intermolecular and intramolecular hydrogen bonds of NALMA and NAGMA. The third concern is to predict the rupture time of intramolecular hydrogen bond dipeptides in water and vacuum medium.

2. Computational methods

2.1. MD simulation

The AMBER package was used to run MD simulations with ff03 force field (Case et al., 2004; Hornak, Abel, Okur, Stockb nude, & Roitberg, 2006). As TIP3P water model is found to be well balanced (Guimaraes, Barreiro, Augusto, De Olliviera, & De Alencastro, 2004; Jorgensen, Chandrasekhar, Madura, Impey, & Klein, 1983) with Amber force field, it is used here for water modeling. This force field has proved to be useful in studying the propensity of peptides (Abiram & Kolandaivel, 2010; Praveena & Kolandaivel, 2008). The equations of motion were integrated using leapfrog (Morris et al., 1984) with a time step 2 fs. The langvin dynamics was used to control the temperature at 300 K with collision frequency of 1.0 \textit{ps}^{-1}. The Barendson pressure coupling method

Figure 1. The chemical composition of (A) NAGMA and (B) NALMA dipeptide.
Table 1. Simulations setup prepared to perform MD for NALMA and NAGMA dipeptide.

| Solute | Force field | No. of waters | Volume of the simulation (Å³) | Solvent | Simulation length (ns) | Temp (K) | Dimension of the PBC box (Å³) |
|--------|-------------|---------------|-------------------------------|---------|------------------------|----------|-------------------------------|
| NALMA  | Leaprcff03  | 598           | 29,434.06                    | Water   | 10                     | 300      | 32.9 × 35.3 × 29.6            |
| NAGMA  |             | 698           | 33,972.60                    |         | 10                     |          | 30.6 × 26.8 × 26.6           |

(Barendsen, Postme, Vangunsteren, Dinola, & Haak, 1984) was applied to describe the barostat with a constant pressure of 1 atm. The Particle-Mesh Ewald (Darden, York, & Pederson, 1993) method was employed to treat the long-range electrostatic interactions. The hydrogen atoms were constrained to the equilibrium bond length using SHAKE algorithm (Ryckaert, Ciccotti, & Barendsen, 1977). The nonbonded cutoff distance of 1 nm is subjected to simulations over the 10 ns. We performed MD runs (trajectories) for NALMA and NAGMA embedded in the TIP3P water model at 300 K temperature and the detail of the simulation procedure is given in Table 1. The dipeptides attained equilibrium and minimization, respectively, at 20 and 100 ps of MD simulation.

2.2. DFT method

The five most stable conformers of NALMA and NAGMA dipeptides were identified by MD simulations. The performance of M05-2X (Mathew, Corninboeuf, Schreiner, Fokin, & Schleyer, 2007; Wodrich, Corninboeuf, & Schleyer, 2006; Zhao, Schultz, & Truhlar, 2006; Zhao & Truhlar, 2006) function has been documented in the literature by simultaneous optimization of exchange and correlation function including kinetic energy density. Hence, the five most stable conformers of NALMA, NAGMA, and their complexes (NALMA ... (H2O)n, NAGMA ... (H2O)n); n=0, 2, 3...11 were optimized using M05-2X method employing 6-31G (d,p) basis set. Each optimized geometries of dipeptides and their complexes were subjected to frequency calculations at the M05-2X/6-31G (d,p) level of theory to confirm that their minimum energy is being true. The interaction energies were corrected for basis set superposition error using the counterpoise method (Boys & Bernardi, 1970; Wang, 2008; Wodrich, Corninboeuf, & Schleyer, 2006) and it is calculated using the ensuing equation

\[ E = E_{dib} - (E_A + E_B) \]  

where \( E_{dib} \) is the total energy of the dipeptide–water complex and \( E_A, E_B \) are the energies of the monomers, respectively. The interactions between the model dipeptide and water molecules have further explored through the electrostatic potential, \( V(r) \) which is defined as energy of interaction of a positive point charge located at \( P \) with the nuclei and electrons of a molecule (Wodrich et al., 2006).

\[ V(r) = \sum_A Z_A R_{AP} - \sum_{\mu} \sum_{\nu} P_{\mu\nu} \int \frac{q_\mu(r)q_\nu(r)}{r_p} \, dr \]  

The first summation is over the nuclei A. Z is atomic number and \( R_{AP} \) is distance between the nuclei and the point charge. The second summation is over basis functions, \( \phi_\mu, \phi_\nu \) is the density matrix, and the integrals reflect the columnic interactions between the electron and point charge, where \( r_p \) is the distance separating the electron and point charge. All the above quantum chemical calculations were carried out using the Gaussian 09 program (Frisch et al., 2009) package. The graphical program Argus lab was used to plot electrostatic potential map.

3. Results and discussion

3.1. Strength of hydrogen bonds

The RDFs of the intermolecular hydrogen bonds between O (dipeptide)...H (water), C (dipeptide)...H (water), N (dipeptide)...H (water) of the NALMA, and NAGMA are shown in Figure 2 and their structural data are given in Table 2. The hydrogen atom of water is abbreviated as H (wat). The hydrogen bond length between nitrogen atom of NALMA, NAGMA, and hydrogen of water molecules beyond 3.5 Å found to be negligible. The RDFs of O–H formed that a peak has been observed at 1.85 Å and lower point at 2.45 Å indicated the hydrogen bond of NAGMA. A peak at 2.05 Å and a lower point at 2.35 Å have been noticed for NALMA. The positions of the maxima and minima \( r^{O-H}, \ r^{C-H}, \ r^{N-H} \) and of the amplitudes \( g^{O-H}, \ g^{C-H}, \ g^{N-H} \) and \( g^{O-H}, \ g^{C-H}, \ g^{N-H} \) for the NAGMA, NALMA with hydrogen atom of the water molecules are reported in Table 2.

The ratio \( g^{O-H}/g^{N-H} = 4.73 \), \( g^{C-H}/g^{O-H} = 1.19 \), \( g^{N-H}/g^{C-H} = 1.03 \), and \( g^{O-H}/g^{N-H} = 1.24 \), \( g^{C-H}/g^{N-H} = 0.96 \), \( g^{O-H}/g^{N-H} = 1.02 \) for NAGMA and NALMA, respectively. The weak hydrogen bond interaction has been noted between the oxygen atoms of the dipeptides and the surrounding water molecules (O (dipeptide)...H (wat)) and nitrogen atom of dipetide with H atom of water molecules (N (dipeptide)...H (wat)). The order of the H-bond strength for both dipetides are observed as O–H > C–H > N–H. This investigation indicates that the hydrogen bond strength is higher for NAGMA compared with the NALMA dipeptide. The
O–H interaction energy of the hydrogen bond is 5.61 and 5.91 kcal/mol, respectively, for NALMA and NAGMA. From the DFT method, we have calculated the energies as 10.30 and 14.65 kcal/mol between C = O1 (dipeptide) and H (wat) for NALMA and NAGMA complexes, respectively. The energy values are 9.41 and 12.60 kcal/mol between C = O2 (dipeptide) and H (wat) for NALMA and NAGMA complexes, respectively. In order to investigate the O–H interaction energy of NALMA which is higher than NAGMA complexes and their energy values were confirmed by MD and DFT methods.

Another measurement of the intramolecular plasticity of hydrogen bond strength between the C=O1…H–N2, C=O2…H–N1 of the dipeptides (NAGMA and NALMA) were investigated in water and vacuum environment, which are shown in Figures 3 and 4. The ratio of $\frac{g_{O1-H}^{max}}{g_{O1-H}^{min}}$ is equal to 2.67 for NAGMA and 3.37 for NALMA dipeptide. The ratio of $\frac{g_{O2-H}^{max}}{g_{O2-H}^{min}}$ is observed to be 1.33 for NAGMA and 6.88 for NALMA, which are shown in Table 3. The hydrogen bond strength of the backbone–backbone interaction (C=O…H–N) is found to be in the reversible process of the intermolecular hydrogen bond strength O (dipeptide)...H (wat). The hydrogen bond strengths of NALMA and NAGMA are calculated as 0.67 and 0.5 kcal/mol, respectively, in water medium. Measurement of the above values for model compounds by Klontz (1993) indicated that the N–H…O=C hydrogen bond, even in CCl4 environment, has a value of 4.2 kcal/mol but this value is decreased to 0.5 kcal/mol in water medium. Similarly, Williams, Searle, Mackay, Gerhard, and Mapstone (1993) estimated the same as 0.5–1.5 kcal/mol and Fersht et al. (1985) have calculated as 1.5 kcal/mol. This decrease in energy from the value of the isolated system is being attributed to the various hydrophobic effect of water.

The intramolecular hydrogen bond strength of dipeptide is shown in Figure 3. The energy value of C=O1…H–N2 is 3.41 and 5.06 kcal/mol for NAGMA and NALMA, respectively, in vacuum. The intramolecular hydrogen bond strength was found to be higher in vacuum when compared with water environment. The hydrogen bond strength between O (dipeptide)...H (wat) is greater among the other interactions (C (dipeptide)...H (wat), N (dipeptide)...H (wat)) and its energy is 5.61 and 5.91 kcal/mol for NALMA and NAGMA. This energy helps to break the intramolecular hydrogen bond between C=O…H–N and forms the hydrogen bond with surrounding water molecules of NAGMA and NALMA. This is one of the reasons for folding process of protein. These results are in agreement with the previous experimental (Franks

Table 2. The positions of the maxima and minima $r_{max}$, $g_{max}$, $r_{min}$, $g_{min}$ (Å) and of the amplitudes $g_{O-H}^{max}$, $g_{C-H}^{max}$, $g_{N-H}^{max}$ and $g_{O-H}^{min}$, $g_{C-H}^{min}$, $g_{N-H}^{min}$ for the NAGMA and NALMA dipeptides of the first peak of the RDFs.

|          | $r_{max}$ | $r_{min}$ | $g_{max}$ | $g_{min}$ | $\frac{g_{max}}{g_{min}}$ |
|----------|-----------|-----------|-----------|-----------|---------------------------|
| NAGMA    | O–H       | 1.85      | 2.45      | 2.45      | 0.51                      | 4.73                     |
|          | C–H       | 4.05      | 2.04      | 5.55      | 1.72                      | 1.11                     |
|          | N–H       | 5.15      | 1.91      | 6.15      | 1.88                      | 1.03                     |
| NALMA    | O–H       | 2.05      | 0.83      | 2.35      | 0.67                      | 1.24                     |
|          | C–H       | 4.35      | 1.68      | 5.75      | 1.73                      | 0.96                     |
|          | N–H       | 7.75      | 1.99      | 9.65      | 1.95                      | 1.02                     |

Figure 2. Oxygen (dipeptide)...Hydrogen (water), Carbon (dipeptide)...Hydrogen (water), and Nitrogen (dipeptide)...Hydrogen (water) RDFs of (A) NALMA and (B) NAGMA dipeptide.
3.2. Hydration shell analysis

The interaction of water molecules with dipeptides made changes in the conformation of dipeptides at room temperature. The water molecules form the first hydration shell at 3.5 Å and the second hydration shell is within 5.0 Å. The two and three water molecules have played a role for the conformational changes in the NALMA and NAGMA, respectively. It is observed from Figure 5(A), the five water molecules interact with NALMA in the first coordination at 1.50 ns and number of water molecule decreases as the time increases. Perticaroli et al. (2011) have investigated the NALMA using the dynamics of water by EDLS experiment as the function of tem-

Table 3. The positions of the maxima and minima $r_{\text{max}}^{\text{O1-H}}$, $r_{\text{max}}^{\text{O2-H}}$ and $r_{\text{min}}^{\text{O1-H}}$, $r_{\text{min}}^{\text{O2-H}}$ and of the amplitudes $g_{\text{max}}^{\text{O1-H}}$, $g_{\text{max}}^{\text{O2-H}}$, $g_{\text{min}}^{\text{O1-H}}$ and $g_{\text{min}}^{\text{O2-H}}$ for the NAGMA, NALMA dipeptides of the first peak of the RDFs.

|       | $r_{\text{max}}$ | $g_{\text{max}}$ | $r_{\text{min}}$ | $g_{\text{min}}$ | $\frac{g_{\text{max}}}{g_{\text{min}}}$ |
|-------|-----------------|-----------------|-----------------|-----------------|---------------------|
| NAGMA | O1–H            | 2.55            | 0.23            | 2.85            | 0.12                | 2.67                |
|       | O2–H            | 2.45            | 0.23            | 2.85            | 0.17                | 1.33                |
| NALMA | O1–H            | 2.55            | 0.43            | 3.35            | 0.17                | 3.37                |
|       | O2–H            | 2.55            | 2.55            | 0.41            | 0.06                | 6.88                |
temperature ranges from 5 to 65 °C and it has been reported that 50–65 water molecules perturbed around the second hydration layer of the dipeptide. This result agrees with our simulation results. We have found that average of 65–75 water molecules are falling into the second solvation shell. Those water molecules do not directly interact with NALMA residue, but they also contribute to some extent on the conformations of NALMA. This strong perturbation suggested the existence of collective effect of hydrophobic molecules on the surrounding water molecules.

We observed that six water molecules interact with the NAGMA at 2.06 and 3.03 ns and five water molecules are seldom interacting with NAGMA at 0.23, 2.13, 3.03, 3.80, 4.16, 4.80, 5.95, 5.32, 5.53, 6.93, 7.13, 8.25, 8.42, 8.61, 9.51, 9.63 ns, etc. which are shown in Figure 5(B). An average of 50–65 water molecules have been found to be in the second solvation shell. Russo et al. (2009) have investigated the above peptides and reported that the number of water molecules around the NALMA is higher than that of NAGMA and this result agrees with the hydration shell analysis.

3.3. Conformational analysis

The different conformational changes of NAGMA and NALMA dipeptides have been analyzed by the conformational analysis plot in water and vacuum environment over the 10 ns MD simulation. The description of conformation of model dipeptides by two torsion angles, \( \psi(N-C_\alpha-C-N) \) and \( \phi(C-N-C_\alpha-C) \) proposed by Ramachandran and Sasikharan (1968) and they have been used to investigate the secondary structures of protein (alpha helical region, beta sheet region, and turn region). The \( \varphi \) and \( \psi \) values for the different conformations of NALMA and NAGMA are shown in Figures 6 and 7.

The conformations of NAGMA take place in the region around \( \varphi = \psi = \pm 180^\circ \), which is considered as the beta sheet. The torsion angle values of the beta sheet are \( 135^\circ < \varphi < 180^\circ \), \( -180^\circ < \psi < -135^\circ \); \( 135^\circ < \varphi < 180^\circ \), \( -135^\circ < \psi < -180^\circ \); \( 135^\circ < \varphi < -180^\circ \), \( 135^\circ < \psi < 180^\circ \); \( 135^\circ < \varphi < 180^\circ \), \( -135^\circ < \psi < 180^\circ \). The beta sheet of NAGMA is less populated in vacuum environment around 3.0, 4.0, 4.2, 5.0, and 6.5 ns which is displayed in Figure 6(A). The turn region (called left-handed alpha helical region) around \( -25^\circ < \varphi < -180^\circ \), \( -90^\circ < \psi < 90^\circ \), and \( 25^\circ < \varphi < 180^\circ \), \( 90^\circ < \psi < 90^\circ \) have been observed in the conformational analysis plot at vacuum medium. The conformational changes of the same dipeptide established in water environment are shown in Figure 6(B). It has indicated that the turn region are less populated in water than vacuum environment around the torsion angles \( -130^\circ < \varphi < -180^\circ \), \( 130^\circ < \psi < 180^\circ \), \( -130^\circ < \varphi < -180^\circ \), \( -130^\circ < \psi < -180^\circ \), \( 130^\circ < \varphi < 180^\circ \), \( 130^\circ < \psi < 180^\circ \) and \( 130^\circ < \varphi < 180^\circ \), \( -130^\circ < \psi < 180^\circ \). The disallowed regions are observed at 2.4, 4.7, 5.1, and 8.4 ns and this is treated as beta sheet region. The population of the beta sheet is in water compared with vacuum environment. This result agrees with the Klewegt (1999) studies. The conformational changes of the NAGMA displayed in Figure 6 shows the conversion of alpha helix into beta sheet and vice versa. So the C=O and N–H bonds of NAGMA backbone interact with water molecules in a different way.

The conformational changes of NALMA have been analyzed using conformational analysis plot shown in Figure 7. It has been observed that the alpha helical region in Figure 7(A) and its corresponding torsion angles are \( -30^\circ < \varphi < -180^\circ \), \( -60^\circ < \psi < -45^\circ \). The beta sheet is exhibited in the region \(-140^\circ < \varphi < -45^\circ \), \(-45^\circ < \psi < 80^\circ \). It has been observed that the
alpha helical conformations occurred from the beginning to 3.2 ns and often beta sheets were found to be exhibited above 3.2 ns. The alpha helical region of the NALMA dipeptide in vacuum medium is shown in Figure 7(A) and the corresponding torsion angles are \( \psi_{14} \approx 30 \), \( \psi_{14} \approx 60 \) and this is converted into the beta sheet in water environment at 300 K temperature. From Figure 7, it is observed that NALMA exhibits the alpha helix in vacuum. The beta sheets are appeared in the water environment and shown in Figure 7(B). This result agrees well with experiment result of QENS and NMR experiment (Qvist & Halle, 2008).

It has been found that number of conformers is less for NALMA than NAGMA dipeptide. So the probability of interaction of water molecules with side chains of NALMA is less (Figure 7(B)), since the interaction of water molecules with NAGMA is less (Johnson et al., 2009).

3.4. Rupture time of hydrogen bond

The dynamics of hydrogen bond breaking and reforming in vacuum and in different environments is an important area for theoretical and experimental investigations (Fersht et al., 1985; Sheu, Yang, Selzle, & Schlag, 2002). Sheh-yi sheu et al. (2002) have reported that the hydrogen bond energy is 5 kcal/mol for protein in the absence of water medium and 1.6 kcal/mol in the presence of water environment. The dynamics of protein folding and enzymatic reactions are intrinsically related to the stringy of the hydrogen bond. The breaking and reformation of hydrogen bonds is one of the key steps in all protein dynamics. The primary and immediate origin of these structural factors in protein are contributing to formation and breaking of hydrogen bonds. It is of interest to look for a possible direct connection between

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Figure 6. The backbone angles (\( \varphi \) and \( \psi \)) of NAGMA dipeptide changes over the 10 ns MD simulations in (A) vacuum and (B) water environment.

Figure 7. The backbone angles (\( \varphi \) and \( \psi \)) of NALMA dipeptide changes over the 10 ns MD simulations in (A) vacuum and (B) water environment.
solvent and primary hydrogen bond by directly observing the process on a picoseconds time scale using MD.

The rupture time of hydrogen bond is defined as the time required for dissociating the hydrogen bond between the proton acceptor atom and hydrogen atom. The hydrogen bond length between the C=O and N–H atoms of dipeptide (NAGMA and NALMA) were measured over the time and represented in Figure 8. The hydrogen bond length is found to be approximately between 2.8 and 3.0 Å (Karle, 1999) and if the value increased beyond 3.0 Å, the rupture process gets started. From Figure 8, blue color indicates the NALMA and block represents for NAGMA. The sharp peaks of NALMA and NAGMA represent the making and breaking of hydrogen bonds (C=O…H–N) shown in Figure 8 (A). This happened at approximately 13 ps time scale for NALMA and around 11 ps for NAGMA in vacuum environment. The energy of hydrogen bond is 3.41 kcal/mol for NAGMA and 5.06 kcal/mol for NALMA which is closer to gas phase \textit{ab initio} computation results (Kim

![Diagram A](image1.png)

![Diagram B](image2.png)

Figure 8. The rupture time of hydrogen bond processes for NALMA and NAGMA dipeptides of backbone–backbone interaction (C=O…H–N) in (A) vacuum and (B) water environment.
& Friesner, 1997; No, Kwon, Kim, Jhon, & Scheraga, 1995). We have employed 598 water molecules around NALMA and 698 water molecules around NAGMA for the simulation. The time series of rupture process for NAGMA and NALMA in water environment at 300 K are shown in Figure 8(B), which represents the presence of hydrogen bond between C=O—H⋯N of dipeptide (NALMA and NAGMA) up to 30 ps, where the hydrogen bond length is found to be around 2.5 Å. After the above time, the hydrogen bond is stabilized at around 3.0 Å and completely dissociate at 4.5 Å. The average rupture time of the hydrogen bond is approximately 8.0 ps for NAGMA and 5.0 ps for NALMA, which has been calculated based on the hydrogen bond formation and breaking. The lifetime of hydrogen bond is larger for NAGMA than NALMA in water environment up to 30 ps is shown in Figure 8(B). The energy of the dipeptide reduced for both NALMA and NAGMA as 0.67 kcal/mol and 0.5 kcal/mol in water environment which is in excellent agreement with commonly accepted value 0.5–1.5 kcal/mol (Wodrich et al., 2006).

4. DFT study
The maximum probable conformers of NALMA and NAGMA along with five successive minimum energy conformers were identified by MD method (Figure 9). We determined five lowest energy conformers and have maximum probability of occurrence throughout the simulation. These conformers are represented as NALMA1, NALMA2, NALMA3, NALMA4, NALMA5 and NAGMA1, NAGMA2, NAGMA3, NAGMA4, and NAGMA5, respectively, are shown in Figures S1 and S7. The structural analysis of NALMA has revealed 43 different conformations at B3LYP/6-31G* level of theory in the water environment using Poisson–Boltzmann self-consistent reaction field (PB-SCRF) method, where five global minimum conformers.

Figure 9. Total energy (kcal/mol) of (A) NALMA and (B) NAGMA dipeptides throughout the simulation of 3 ns time scale.
mers have been found among 43 conformers. The relative energies were calculated for NALMA structure at M05-2X/6-31G** level of theory and AMBER ff03 force field in water environment using the global minimum structure determined at PB-SCRF/B3LYP/6-31G* level of theory (Masman et al., 2007) as reference point. Table 4 reported the calculated relative energy with respect to the most minimum energy conformers (Masman et al., 2007). The minimum energy conformers calculated by AMBER ff03 force field is close to the conformers calculated by Masman et al. (2007) than M05-2X/6-31G** level of theory. We found the following conformational energies −456.497, −456.496, −456.506, −456.495, −456.498 for NAGMA and −613.75, −613.76, −613.73, −613.74, −613.73 for NALMA, respectively. The five most stable conformers of NALMA and NAGMA dipeptide predicted from DFT and MD simulations at water environment. These conformers energy is shown in Figure 10 and it confirmed that the MD simulation is capable of predicting the energetically favorable conformers than the DFT method. The NAGMA (Figure 10(A)) conformers energy is higher than NALMA (Figure 10(B)) conformers. To gain deep understanding on its atomic behavior, DFT studies have been carried out starting from two and have been extended upto 11 water molecules. The conformers interact with 2, 3, 4, 5…11 water molecules are represented as W2, W3, W4, W5…W11, respectively. All the calculations were performed at M05-2X/6-31G** level of theory. All the optimized structures and complexes are given as the supporting information in Figures S1–S12, wherein all the bond length have been mention as Å.

### 4.1. Hydrogen bond interaction

We have performed hydrogen bond survival analysis based on the following points. We have identified all the hydrogen bonds formed between the active site of the acceptor dipeptides and donor of the water molecules in the system using the following geometric criteria to decide whether the hydrogen bond is formed at a distance < 3.5 Å between acceptor and donor of the atom and the angle between the donor and acceptor atoms of the water molecules is 150°–180°. The hydrogen bond formed between the active sites of backbone and water molecules are C6–O1…H (wat), C8–O2…H (wat), N1–H16…O (wat), and N2–H14…O (wat); and sometimes water molecules tend to form the hydrogen bond in the side chain of NAGMA. These structures are then made to interact with 2–11 water molecules. We have found that the hydrogen bond distance varies from 1.8 to 2.5 Å for NALMA complexes and 1.8 to 2.0 Å for NAGMA complex. The hydrogen bond length of C=O…H (wat) and N–H…O (wat) of NALMA is higher than NAGMA dipeptides.

From the MD simulations method, it has been revealed that the minimum number of conformers has found to exist in water environment. This result agrees with the experimental result of NMR and X-ray studies (Hura, Sorenson, Glaeser, & Gordon, 1999; Qvist & Halle, 2008). The protein folding process is mainly due to the contribution of change of backbone (\(\phi, \psi\)) angles and side chain angles. By increasing the number of water molecules, the conformational changes were observed. In NALMA, it is due to the variations of \(\phi, \psi\), and side chain angles (C5–C4–C1, C4–C1–C2, and C4–C1–C3), but in case of NAGMA it is due to variations of \(\phi\) and \(\psi\) only. The angle C5–C4–C1 is varied from 108° to 113°, C4–C1–C2 is varied from 113° to 118°, and C4–C1–C3 is varied from 110° to 113°. The side chain angles were found to varying around 3°–5° when number of water molecules increased and it partially contributed to protein folding but backbone dihedral angles \(\phi, \psi\) is the good approximation to describe protein folding. The dihedral angles \(\phi\) and \(\psi\) vary from −64.63° to 122.31° and from −152.81° to 90.36°, respectively; for NAGMA and for NALMA, it varies from −144.62 to −85.05° and from −3.77° to 64.09°, respectively. From this, it has been found that minimum number of conformers has found to exist in the water environment. These results were confirmed by MD simulation.

The water molecules form the water bridge around NALMA and NAGMA, which constrains the conformational change of the dipeptides. This study will help to
probe dynamics experimentally on a femtosecond time scale (Perticaroli et al., 2011). The effort has been made to understand the time scale and detailed dynamics involved in the process of making and breaking of hydrogen bonds in bulk and aqueous solution (Williams et al., 1993). The hydrogen bond length between the

Figure 10. The optimized of five most stable conformers for (A) NAGMA and (B) NALMA at MD (AMBER ff03) and DFT (M05-2X) methods.
dipeptides and water molecules are displayed in Figure 11, which confirms the change of hydrogen bond length during the simulations.

In the case of NAGMA dipeptides, it has been noticed that an average of two to three water molecules have an influence on the structure of the complexes than four, five, etc. While increasing the water molecules from 2 to 11, the water molecules try to align itself in the form of a water bridge around the structure (Figure 12). Since the side chain group of leucine does not have electronegative atoms, they do not show hydrogen bonding at short distances and also moves the water molecules away from them. Though dynamics study say that an average of two and three water molecules has profound interaction with the structures, in the NALMA, NAGMA dipeptides cases, the increase in water molecules, upto 11, has also produced some changes in the conformations of the dipeptides. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) map for NAGMA and NALMA dipeptides are shown in Figures 13 and 14, respectively.

Electrostatic potential map (Boyd, 1997) is very useful three–dimensional (3D) diagram of model dipeptide. They are used to visualize the size and shape of the model dipeptides. The electrostatic potential energy map for NALMA and NAGMA dipeptides are displayed in Figure 15. The potential energy values are projected on the electron isodensity surface ($\rho = 0.002$ a.u) showing 3D shape of the model dipeptides. The electrostatic potential is computed from the ZNDO wave function. The carboxylic
The oxygen of NALMA and NAGMA is colored as red corresponding to its strong negative potential charge ($\rho = -0.1$ a.u.) and strong attraction to the probe. The amine group of model dipeptides is white, corresponding to its strong positive potential charge ($\rho = 0.1$ a.u.) and strong repulsion by the probe. This color region is intimated that should be able to provide hydrogen bond and to interact electrostatically through the C=O and N–H groups of model dipeptides. The hydrogen bonds are interacted electrostatically through the amine (N–H), carboxylic (C=O), and neighbor water molecules. Figure 15 observed that the blue color represents the slightly positive charge ($\rho = 0.01$ a.u.) and weak repulsion (green, violet, and pink represents the intermediate values of the potential). The larger electron density in that red color region confirmed the presence of electrostatic interaction.

From Figure 15, it has been observed that the lack of electronegative atom present in the NALMA dipeptides (Figure 15(A)) is compared to NAGMA (Figure 15(B)).

### 4.2 Interaction energy

The interaction energy of water molecules with the NALMA and NAGMA was calculated at M05-2X/6-31G** level of the theory. We have carried out the minimum energy conformations of the dipeptides (NALMA and NAGMA) and their interaction with water molecules are calculated and tabulated in Table 5. The interaction energies were found to be increasing as the number of water molecules increased around the dipeptides (Figure 12). The overall DFT study revealed that these struc-
tures are hydrophobic and so cannot accommodate more water molecules with dipeptides.

5. Conclusion

The MD simulations have been performed to analyze the structural behavior of NALMA and NAGMA dipeptides. It has predicted that the beta sheet conformation is found to have maximum probability of occurrence in water environment for NAGMA and NALMA dipeptides, respectively, through simulation of 10 ns time scale. The above peptides interact with water molecules and observed that average of two water molecules interact with NALMA and three water molecules with NAGMA. The intramolecular hydrogen bond strength between the backbone–backbone interaction of dipeptides (C=O…H–N) has been reduced to 0.5, 0.67 kcal/mol in water environment compared to that of vacuum. The rupture time of the hydrogen bond is found to higher value of 11 and 13 ps in vacuum than water environment, respectively, for NAGMA and NALMA. From this, we observed that the dipeptides have interacted with water molecules through hydrogen bonding and altered the geometry. The presence of hydrogen bond interaction due to water molecules helpful to break the intramolecular C=O…H–N hydrogen bond of dipeptides. The energy balance of breakage of the C=O…H–O (wat), N–H…O–H (wat) hydrogen bonds may be one of the determining factors to control the dynamics of the folding process. The conformational changes of dipeptide (φ, ψ) values lead the folded state of protein (alpha sheet) into the unfolded state (beta sheet) after the influence of water molecules through hydrogen bond. The characteristic feature of the C=O…H–N hydrogen bond and its strength smaller than C=O…H–O (wat), N–H…O–H (wat) hydrogen bond shown in this study may shed new

Table 5. Interaction energy (in kcal/mol) for the five most stable conformers of NAGMA, NALMA, and their hydrated complexes calculated at M05-2X/6-31G** level of theory.

| Conformer I     | Conformer II | Conformer III | Conformer IV | Conformer V |
|-----------------|--------------|---------------|--------------|-------------|
| NAGMA + W2      | −14.369      | −29.053       | −13.177      | −13.177     | −27.359     |
| NAGMA + W3      | −39.093      | −38.905       | −32.316      | −39.093     | −35.266     |
| NAGMA + W4      | −44.929      | −51.016       | −52.710      | −49.447     | −45.493     |
| NAGMA + W5      | −55.409      | −54.596       | −52.961      | −61.935     | −61.935     |
| NAGMA + W6      | −76.744      | −66.766       | −66.264      | −65.637     | −71.159     |
| NAGMA + W11     | −131.714     | −66.264       | −65.637      | −71.159     | −71.159     |
| NALMA + W2      | −11.671      | −12.361       | −12.550      | −18.072     | −15.122     |
| NALMA + W3      | −22.213      | −27.108       | −16.754      | −27.108     | −25.916     |
| NALMA + W4      | −43.423      | −46.623       | −33.8227     | −44.7414    | −44.804     |
| NALMA + W5      | −62.813      | −62.750       | −51.455      | −50.828     | −57.417     |
| NALMA + W6      | −67.605      | −67.668       | −67.3317     | −74.736     | −63.252     |
| NALMA + W11     | −124.435     | −67.668       | −74.736      | −63.252     | −63.252     |
light into the detailed understanding of the folding converted to unfolding dynamics of protein.

The influence of water molecules on the structure and stability of hydrated NALMA, NAGMA complexes have been analyzed by the DFT method. The intermolecular and intramolecular hydrogen bonds of dipeptides are depicted in the electrostatic potential map. The incremental process of water helps to understand the minimum number of water molecules needed to change conformational behavior of protein.

**Supplementary material**
The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2012.698380.

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