

Computational Approach Study of a Versatile Model Peptide: Lessons from N-Methylacetamide

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ABSTRACT

In this mini review, we report a brief overview of results of molecular dynamics simulations of N-methylacetamide and its aqueous solution at different thermodynamic conditions. The structure and dynamical properties of neat NMA are analysed at different temperatures and pressures. The results are highlighted in terms of various dynamical properties associated with translational and rotational motion of neat NMA. The results of solvation structure and dynamics of different alkali metal and halide ions in liquid NMA are also mentioned. Then we proceed for discussing the behaviour of NMA and its aqueous solutions with varying salt concentration. How the structure and dynamics of aqueous NMA solution are changing in presence of different cosolvents are also highlighted. Finally, the recent study of the influence of dimethyl sulfoxide, ethanol and trifluoroethanol on hydrogen bonding behaviour of aqueous NMA system is discussed.

Keywords: Molecular dynamics simulation, N-methylacetamide, Hydrogen bond dynamics.

The peptide group which is an essential repeated unit of proteins plays an essential role in the biological environment. N-methylacetamide (NMA) is a model peptide to study the peptide linkage which forms the backbone of proteins and polypeptides [1]. It has been frequently used in several simulation studies to gain microscopic understanding of the environment around peptide linkages. NMA has also been used in optimization of Chemistry at Harvard Macromolecular Mechanics (CHARMM) and optimized potentials for liquid simulations (OPLS) force fields [2,3]. NMA was chosen as the focus of our research owing to its properties like hydrogen bond forming ability (suggested from X-ray and electron diffraction data) as well as intrinsic properties like high dipole moment and dielectric constant [4-6]. An initial molecular dynamics simulation study was conducted to study the changes in hydrogen bonding structure of liquid NMA with variation of temperature and pressure [7]. Our results revealed that while the number of hydrogen bonds may increase with application of pressure at a given temperature, the stability of the same decreased at higher temperature and pressure. Higher thermal motion of molecules at higher temperatures caused the self-diffusion coefficient of NMA to increase but the dependence of translational motion on pressure was found to be greater than that of rotational motion, which is eventually attributed to excluded volume effects rather than hydrogen bond pressure

dependence. It was noted that coordination number of NMA decreased due to thermal disorder induced by high temperatures but tended to increase at higher pressures due to compression effects. This study was further added in dimension by Sarma and Paul [8] who investigated pressure-induced alteration of hydrophobic as well as hydrogen bonding in aqueous solutions of N-methylacetamide. Comparison of $O_{NMA} \dots H_{NMA}$ radial distribution structure (RDF) obtained in this study with that of neat NMA results found in our work showed that peak positions at 1.9 Å remained unchanged but the structure of the RDF was weakened due to aqueous solvation of NMA. The aqueous environment around NMA also decreased the average number of NMA-NMA hydrogen bond (0.08) as compared to neat NMA (1.0) on applying pressure.

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Also, they noticed that on applying pressure, peak heights for NMA-NMA site-site correlations were reduced. They contrasted these results to our work, suggesting that the

overall change in surrounding water molecules may alter how pressure affects NMA-NMA association.

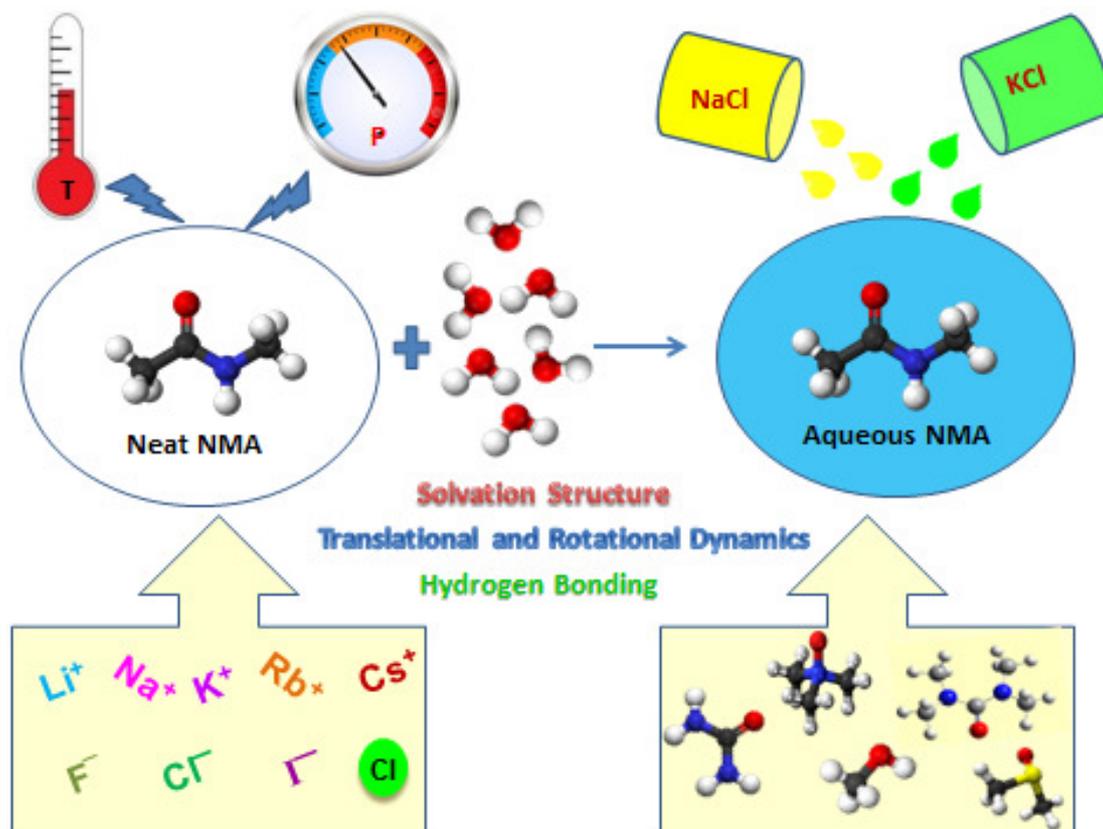


Figure 1. Studies on NMA: A Graphical Overview

In biologically active systems, peptide linkages are often immersed in aqueous system with presence of several ions. Keeping this concept in mind, we designed a study where we could zoom on to subtle interplays of hydrogen bonding along with ion coordination and dynamics behaviour in aqueous environment of peptide bonds [9]. Theoretical and experimental investigations on interactions of NMA with water were devoted to influence of aqueous environment changes on amide-I vibrations of N-methylacetamide [19-24]. In our study, *Intra*- and *inter*-molecular hydrogen bonds were calculated between NMA-water with the help of certain geometric criteria [25-31] where it is assumed that a hydrogen bond between two species exists, if the following distance and angular criteria are satisfied, *i.e.*, $R^{(OX)} < R_c^{(OX)}$, $R^{(OH)} < R_c^{(OH)}$, and $\theta < \theta_c$. For example, $R^{(OX)}$ and $R^{(OH)}$ denote the oxygen (water)-nitrogen (NMA) and oxygen (water)-hydrogen(NMA) distances, and the corresponding quantities with subscript “c” denote the cutoff values. The angle θ ($=\theta^{(NOH)}$) is the nitrogen-oxygen-hydrogen angle, and the θ_c is the upper limit that is allowed for hydrogen bond to

exist between oxygen of water and the hydrogen of the NMA molecule. The cutoff values for O-N and O-H distances are determined from the positions of the first minimum of the corresponding radial distribution functions. We have maintained the cutoff angle to be 45° instead of giving 30° to allow for flexibility induced by thermal effects. It was found that with dilution, though stability of both kinds of hydrogen bonds decrease, average number of *inter*-molecular NMA-water hydrogen bonds are found to increase in contrast to decrease of NMA-NMA *intra*-molecular hydrogen bonds. Even when water *intra*-molecular hydrogen bonding becomes prevalent at higher dilution range, even then stability of NMA-water hydrogen bonds continues to dominate. Hydrogen bond energies for $>C=O_{NMA} \cdots H_{WAT}$ and $N-H_{NMA} \cdots O_{WAT}$ hydrogen bonds have been calculated by Sarma and Paul [8] to be nearly similar and they have also commented that this is consonant with the results of our study. Recently, Yadav and Chandra [32] observed slower vibrational spectral diffusion of water molecules in solvation shells of N-methylacetamide and also proposed that average lifetime and residence time for $O_{NMA} \cdots H_{WAT}$ hydrogen bonds were higher than that for $H_{NMA} \cdots O_{WAT}$ hydrogen bonds.

In the same study Pattanayak and Chowdhuri [9], considered biologically important ions (Na^+ , K^+ , Cl^-) and a neutral solute modelled by Cl^- were considered for the study and it was revealed that while the positive and negative ions were strongly solvated by pure NMA, as water is gradually introduced into the system, they had higher inclination to keep water in their neighbourhood. On the other hand, the neutral solute showed more affinity towards NMA due to its ability to be solvated by NMA's methyl groups. Strong solvation shells of the ionic solutes made the translational dynamics slower for them as compared to faster motion of the neutral solute. Around the same time, Algaey and Vegt compared interactions of aqueous salt solutions with N-isopropylacrylamide (NiPAM) to that with NMA. While NMA formed hydrogen bonds with all Hofmeister anions, NiPAM did not show such hydrogen bonding interactions [33]. Encouraged by these results, several simulations were conducted by Pattanayak and Chowdhuri to explore behaviour of five alkali metal and halide ions in liquid NMA at two different temperatures [34]. Smaller sized ions had stronger solvation shells while increasing ion size led to monotonic increase in coordination number which was further increased for lower temperature. At higher temperatures, less structuring of solvation shells were noted and the effect was more apparent for anionic moieties where interactions with NMA were disrupted. Anomalous ion size dependence of diffusion coefficients were observed which were attributed to high dielectric friction in NMA and also for neutral solutes, higher Stokes friction was postulated to be a factor in the diffusion being less in NMA than for water or methanol. Beck et al. studied small clusters formed of an ion, N-methylacetamide and incrementing number of water molecules of the form $\text{Cl}^-(\text{NMA})_1(\text{H}_2\text{O})_{0-2}\text{Ar}_2$, through Infrared Predissociation (IRPD) spectra combined with Born-Oppenheimer Molecular Dynamics (BOMD) IR spectra. It was demonstrated how Cl^- forms a strong ionic hydrogen bond to the H-N(NMA), due to which the N-H stretch is weakened and shifted to lower frequency [35].

The next step to enhance our understanding of behaviour of solutes in presence of NMA was to observe structure and dynamics of salt solutions in liquid as well as aqueous solution of NMA. Initially, different concentrations of NaCl were taken to observe variation of hydrogen bonding structure in liquid NMA [36]. From the amide-amide radial distribution functions, hydrogen bond energy and number variation trends, it was clear that with increasing salt concentration, hydrogen bonding in NMA was perturbed and destabilised. Contact-ion pair influences were evident when ion-NMA site-site correlations were investigated and residence times were found to be higher for sodium ion near NMA, relative to the anion. Friction due to ion atmosphere was regarded as the main reason behind slowing of translational as well as rotational dynamics of NMA as well as the ions.

Subsequently, effects of NaCl and KCl were explored in aqueous solutions of NMA which more realistically mimic the biological and cellular environments [37]. Previously, Jungwirth and co-workers [38] had investigated solvation shell of ions in aqueous NMA solutions containing 1M concentration of various salt solutions. We found that with increasing salt concentration, NaCl enhances ion interactions with NMA and water at the cost of NMAs interactions with itself and water while the opposite effect was seen in case of KCl , which had stronger contact-pair formation probability. Slower translational and rotational dynamics as well as structural relaxation times of NMA-water hydrogen bonds were more noticeable in concentrated NaCl in comparison to KCl solution.

Having concluded such studies on behaviour of liquid as well as aqueous NMA solutions in presence of ion and salt concentrations, we shifted our focus to various cosolvents and cosolutes which may surround the vicinity of aqueous environment of peptide linkages. We started our journey in this area [39] by considering system of NMA in methanol-water solution. Methanol was chosen owing to its wide range of biological activity including structural effects on proteins; its extensive hydrogen bonding network in water as well as experimental evidences of effects on NMA [40,41]. It was found that addition of even high concentration of methanol could not alter the preference of NMA to hydrogen bond with water than with methanol, as was evident from strong interaction energies and stable NMA-water hydrogen bonds. While water oxygen showed preference to stay in vicinity of methyl group of NMA, methanol strongly interacted with the non-polar sites of NMA with its methyl groups instead.

We then conducted a comparative investigation of effects of important cosolutes like urea, trimethylamine N-oxide (TMAO) and tetramethylurea (TMU) on behaviour of aqueous NMA solution [42]. These co-solutes form part of a special class of molecules known as osmolytes which protect proteins against environmental stresses and also affect their folding/conformational equilibria [43-50]. Earlier, several experimental studies had been conducted on the structural dynamics of water in presence of such osmolytes [51-53]. Gao et al set precedence by exploring solution structure of specific protein backbones in presence of these osmolytes [54]. In our study, we found that NMA-NMA hydrogen bonding tendency were enhanced by TMAO and TMU relative to urea and number of water molecules near methyl surface of NMA were significantly reduced in presence of TMU. An interesting observation came through, that NMA partners with TMU to give rise to water self-segregation but NMA-water hydrogen bonds were more stable in TMU solution. Urea affected NMA's tendency to donate hydrogen to water oxygen but did not have any appreciable effects on hydrogen bonding structure and dynamics of aqueous NMA solution. Since the molecule of TMAO could be described by three force field models i.e Kast, Garcia and Netz, we

[55] carried out a separate study to compare them in their relative interactions with aqueous NMA solution. Compared to the widely used Kast model, Garcia and Netz model increased hydrogen bonding with NMA amide hydrogen with oxygen of TMAO. Particularly, Netz model decreased $H_{\text{NMA}} \dots O_{\text{WAT}}$ hydrogen bonding interactions owing to affinity of TMAO oxygen towards amide site of NMA. Three-hydrogen bonded water complexes of TMAO were more favoured by Garcia and Netz models in comparison with Kast model. Paul and Paul [56,57] studied the effects of trehalose on aqueous solvation of NMA and also investigated its role in counteracting effects of urea on hydrogen bonding in N-methylacetamide solution. It was observed that water-NMA hydrogen bonds were gradually replaced by NMA-trehalose hydrogen bonds and NMA-urea hydrogen bonds in their binary solutions but in ternary solutions, trehalose molecules were preferred to be in vicinity of NMA surfaces than urea and a decrease in interaction energy between NMA-urea molecules was seen in ternary urea-trehalose-NMA system.

Recently, influence of DMSO on hydrogen bonding behaviour of aqueous NMA system was studied by Chand and Chowdhuri [58] owing to diverse range of effects that DMSO has on proteins. In aqueous environment, NMA forms the strongest hydrogen bonds to DMSO than to water or itself with orientation of DMSO molecule towards amide hydrogen site of NMA. At high DMSO concentrations, lower lifetimes of $H_{\text{NMA}} \dots O_{\text{WAT}}$ hydrogen bonds were noted, where the stability of $H_{\text{NMA}} \dots O_{\text{DMSO}}$ hydrogen bond was found to increase. Methyl-methyl interaction of DMSO and NMA molecules reduces but presence of high DMSO concentrations dehydrates nearby water molecules from the methyl site of NMA. In a recent study, Chand and Chowdhuri [59] have also examined the behaviour of ethanol and trifluoroethanol on hydrogen bonding properties of aqueous N-methylacetamide. It is observed that ethanol favours aqueous solvation of N-methylacetamide but trifluoroethanol maintains NMA-NMA hydrogen bonding while also donating hydrogen bond to carbonyl oxygen of NMA.

This review highlights some recent computational investigations on the structure and hydrogen bond dynamics of NMA and its aqueous solution. Presently, our endeavours are directed at more such explorations of influence of mixtures of such co-solutes on aqueous environment of N-methylacetamide in an effort to bring us one step closer to the microscopic investigations of the peptide bond and the hydrogen bonding networks and dynamics therein, which will bring clarity in comprehension of protein-solvent interactions in future.

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