



Effect of glycine betaine on the hydrophobic interactions in the presence of denaturant: A molecular dynamics study



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ABSTRACT

Hydrophobic interactions are very important in a variety of systems such as proteins. Osmoprotectant glycine betaine (GB) stabilizes proteins against denaturation and counteracts harsh conditions such as presence of high concentration of urea. Therefore, it is interesting to specifically investigate the effect of osmolytes on hydrophobic interactions to explore whether the latter plays any role in the counteraction of denaturing property of urea. In this work, molecular dynamics simulations have been performed on neopentane (NEP), a non-polar molecule, in aqueous GB, urea, and GB-urea mixture to understand the effect of GB on hydrophobic interactions in the presence of urea. The results were analyzed by calculating radial distribution function, potential of mean force, coordination number, and interaction energies. The results indicate that the presence of GB decreases hydrophobic interactions between urea and NEP. We also find that GB and urea molecules strongly interact with each other through H bonding and electrostatic interactions. However, GB and urea have negligible effect on hydrophobic interactions between the NEP molecules. This study provides insights into the role of hydrophobic interactions in osmolyte induced counteraction of harsh conditions.

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1. Introduction

The hydrophobic interactions play a key role in various phenomena such as formation of oil droplets in water, membrane and micelle formation, protein stabilization, protein-ligand interactions, and drug delivery. Some of the applications of the hydrophobic interactions include cleaning of the laundry, formation of micro-emulsions to make new materials, and removal of grease with detergents [1–3]. Hydrophobic interactions among non-polar moieties of proteins are the main driving force for protein-folding and protein-stabilization [4–8]. The protecting osmolytes such as glycine betaine (GB) are known to stabilize proteins and counteract the harsh conditions such as high temperature, high pressure, and presence of high concentration of urea [9–15]. There have been many studies investigating the mechanism of protein stabilization and counteraction of harsh conditions by osmolytes [11,16–19]. However, the exact mechanism by which osmolytes stabilize proteins and counteract the harsh conditions is still a matter of debate [9, 20–29]. The counteraction of denaturing property of urea by osmolytes has been one of the most studied problem by many research groups where different interactions among co-solutes, solvent, and proteins have been investigated to explore the mechanism of osmolyte action [9–11,17–19,24,28,30–35]. In this study, we specifically explore the

effect of GB (protecting osmolyte) on hydrophobic interactions in the presence of urea, and investigate whether counteraction of denaturing property of urea by GB is governed by change in hydrophobic interactions between different molecules.

To specifically explore the hydrophobic interactions, neopentane (NEP) (Fig. 1), a non-polar molecule, has been used to mimic the hydrophobic moieties of proteins, though we do not intend to make any direct comparisons with proteins. Urea, a denaturing agent, is known to denature proteins when present at approximately 8 M concentration [4,9,22, 36,37]. Therefore, our studies have been carried out in approximately 8 M urea concentration. We used GB (Fig. 1) as a protecting osmolyte as it is one of most effective natural osmolyte for protein stabilization and is known to counteract the denaturing property of urea [11–13,38,39].

In this study, effect of GB on hydrophobic interactions has been investigated in the presence and absence of urea by using NEP as a hydrophobic molecule. The results provide insights into the role of hydrophobic interactions in the counteraction of denaturing property of urea by osmolytes.

2. Methods

2.1. Computational details

Molecular dynamics (MD) simulation studies were performed for neopentane (NEP) in glycine betaine (GB)-water, urea-water, and GB-urea-water mixtures to understand the effect of osmolyte on the hydrophobic interactions in the presence and absence of denaturing agent urea. The details of the simulated systems are provided in Table 1. NEP

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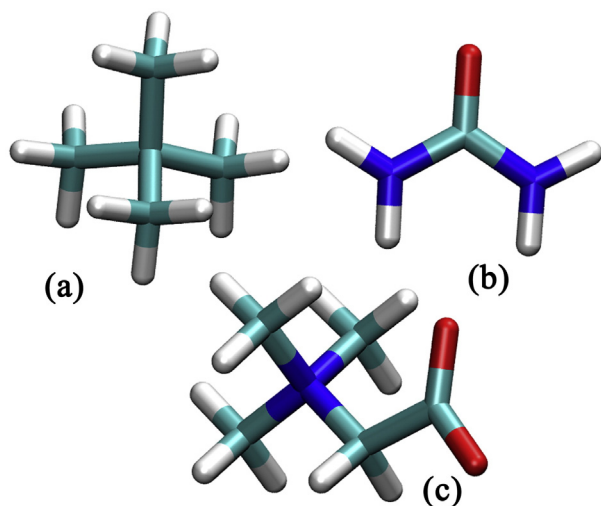


Fig. 1. Licorice model of (a) neopentane (NEP), (b) urea, and (c) glycine betaine (GB). Red, blue, cyan and gray colors represent oxygen, carbon, nitrogen and hydrogen atoms, respectively.

at 0.5 M concentration has been simulated in GB and GB-urea mixture where the concentrations of GB varies from 0.5 M to 3 M. All the simulations were performed using GROMACS 4.5.4 molecular simulation package [40–42] with OPLS all atom force field [43]. The extended single point charge (SPC/E) [44] water model was used. We used Duffy et al. model for urea [45,46] following the other studies [27,47–50]. The charge parameters for GB were taken from literature [51]. For all the simulations, canonical ensemble (NVT) was used at $T = 298.15$ K. The temperature of the systems was maintained using v-rescale thermostat [52] with a relaxation time of 0.1 ps. A cut-off of 1.2 nm was used for van der Waals (vdw) and electrostatic interactions. The long range electrostatic interactions were modeled using Particle-Mesh Ewald (PME) method [53]. Here, the grid spacing of 0.24 nm and interpolation order of six was used. The equations of motion were integrated using leap-frog algorithm with a time step of 2 fs. The covalent bonds with hydrogen atoms were constrained using LINCS algorithm [54]. Periodic boundary conditions with minimum image convention were used to remove the edge effects.

All the systems were steepest-descent energy minimized for 20,000 steps followed by 10,000 steps of conjugate gradient energy minimizations. Again all the systems were steepest-descent energy minimized with a maximum of 10,000 steps. Before the final runs, the simulations were performed using NPT ensemble at $T = 298.15$ K and $p = 1$ bar for 2 ns and then finally the systems were simulated for 100 ns in NVT ensemble for production runs. The coordinates were saved every 2 ps generating 50,000 configurations. Last 20 ns of the simulation runs were used for the analysis purpose. The equilibration of the systems was checked by ensuring that the observables such as interaction energies,

Table 1

Overview of the systems simulated. NEP, GB, M, V, N, and d represent neopentane, glycine betaine, molarity, volume, number of molecules, and simulation density of systems, respectively.

System	N_{NEP}	N_{GB}	N_{Urea}	N_{Water}	$V(\text{nm}^3)$	$d(\text{gcm}^{-3})$	$M_{GB}(\text{M})$	$M_{Urea}(\text{M})$
N	50			5294	167.5	0.981		
N0.5GB	50	50		5030	169.2	0.992	0.5	
N1GB	50	100		4809	163.3	1.022	1.0	
N2GB	50	200		4221	168.0	1.022	2.0	
N3GB	50	300		3694	168.2	1.041	3.0	
UN	50		800	3449	168.8	1.124		7.9
UN0.5GB	50	50	800	3215	168.7	1.136	0.5	7.9
UN1GB	50	100	800	2926	168.2	1.143	1.0	7.9
UN2GB	50	200	800	2395	169.1	1.161	2.0	7.9
UN3GB	50	300	800	1849	169.0	1.181	3.0	7.9

radial distribution functions, and available surface area of NEP molecules fluctuated around their equilibrium values.

2.2. Analysis

2.2.1. Radial distribution function

The local structuring and the interactions between two molecules can be investigated by using radial distribution function ($g(r)$). The $g(r)$ between two atoms X and Y can be calculated by using the following equation[55]:

$$g_{XY}(r) = \frac{\langle \rho_Y(r) \rangle}{\langle \rho_Y \rangle_{bulk}} = \frac{1}{\langle \rho_Y \rangle_{bulk}} \frac{1}{N_X} \sum_{i \in 1}^{N_X} \sum_{j \in Y} \frac{\delta(r_{ij} - r)}{4\pi r^2} \quad (1)$$

where r represents the distance between the atoms X and Y, and $\langle \rho_Y(r) \rangle$ and $\langle \rho_Y \rangle_{bulk}$ represent the number density of type Y atoms around type X atoms at a distance r and the bulk density of type Y atoms, respectively. The bulk density of type Y atoms was calculated by averaging out all the spheres around type X atoms from zero to half of the box length. As r approaches half of the box length, $\langle \rho_Y(r) \rangle$ approaches the bulk density, therefore, the $g(r)$ approaches 1.

2.2.2. Hydrogen bond definition

A geometric criteria was used to define a hydrogen bond between donor and acceptor atoms as suggested by Luzar et al. [56]. A hydrogen bond was considered if the donor-hydrogen-acceptor angle was less than or equal to 30° and the donor-acceptor distance was less than or equal to 0.35 nm [57,58]. In this study, we have calculated the hydrogen bond angle and distance distribution between water molecules to investigate the effect of osmolytes on water hydrogen bonding network.

2.2.3. Coordination number

The coordination number of an atom Y around an atom X at a distance r represents the total number of Y atoms present in a shell of radius r where X atom is positioned in the center of the shell. The coordination number of water, GB, and urea around the NEP molecule were calculated as follows:

$$C.N. = 4\pi \rho_Y \int_0^{r_c} r^2 g_{XY}(r) dr \quad (2)$$

where $g_{XY}(r)$ represents the radial distribution function between molecules X and Y atoms, and ρ_Y is bulk number density of Y atoms. The cut-off distance which has been taken as the first minima of corresponding RDFs is represented r_c .

3. Results and discussion

3.1. NEP-GB interactions in the absence and presence of urea

The radial distribution functions (RDFs) between central carbon atom of NEP (CP) and methyl carbon atoms of GB (CG) were plotted to investigate the hydrophobic interactions between NEP and GB in the presence and absence of urea (Fig. 2a-c). The small first and second peaks of CP-CG RDFs suggest weak hydrophobic interactions between NEP and GB which further decrease in the presence of urea (Fig. 2c). In the Fig. 2a, the CP-CG RDFs decrease with increase in the concentration of GB indicating decrease in the hydrophobic interactions between NEP and GB. This decrease can be attributed to higher GB-GB interactions in comparison to weak NEP-GB interactions. The increase in the GB concentration leads to more intermolecular GB interactions i.e. more involvement of GB molecules among themselves leading to decrease in the hydrophobic interactions between NEP and GB. This is expected as GB has cationic and anionic sites which can lead to favorable electrostatic interactions. In the presence of urea, however, CP-CG

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