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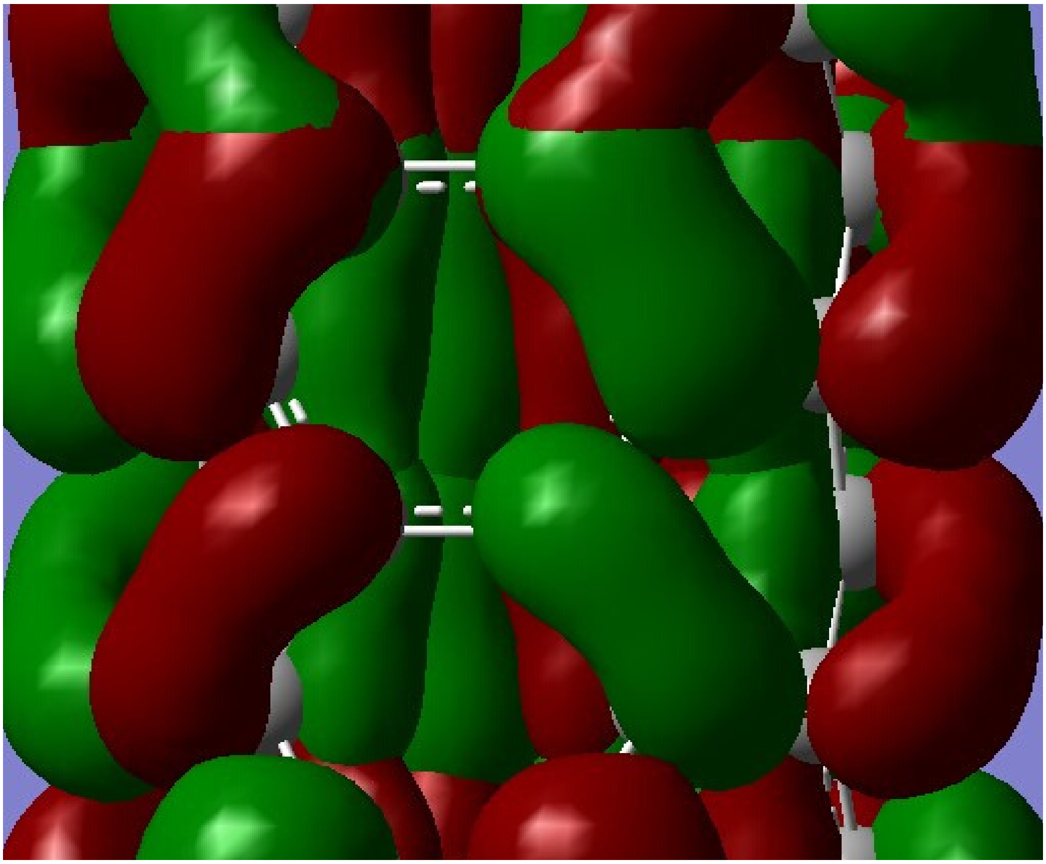

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Transport properties of cysteine dimer in water

Research Article

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Abstract: Disulphide bond in cysteine residues plays vital role in structural stability and fuctional variation of protein molecules. Study of cysteine dimer linking with disulphide bond reveals the nature of stability of tertiary and quarternary structure in polypeptide chain. In order to study the transport properties of cysteine dimer, the molecular dynamics (MD) simulations have been performed at different temperature. The self diffusion coefficients of both cysteine dimer and TIP3P water model have been estimated at four different temperature from the slope of mean square displacement (MSD) versus time plot using Einstein's relation and their binary diffusion coefficients from Darken's relation.

Keywords: Molecular dynamics • Diffusion coefficient • Disulphide bond • Dimer

1. Introduction

Cysteine is a non-essential amino acid. It contains a thiol group -SH in its side chain. It is a white crystalline solid having physical properties: molar mass 121.15 gram per mole, melting point 513 K and solubility in water is 16 gram per 100 mL at 288 K [1, 2]. Cysteine plays vital role in the absorption of nutrients in the inner wall of small intestine. Its defensive mechanism against some diseases is essential for human body. Cysteine residue in a protein molecule strengthens the immune system and defends against the dementia, parkinson and multiple sclerosis [3, 4].

Two cysteine molecules bond together via two different methods: peptide bond (CO-NH) and disulphide bond ($R-S-S-R'$) [1], where R & R' are the side chains of two cysteine residues as shown in Fig. 1. Peptide bond is ordinary bond to form a polypeptide chain, whereas the disulphide bond is formed between two side chains derived from two thiol groups. Disulphide bond in two cysteine residues are very important components to form tertiary structure of proteins, besides to some weak interactions like hydrogen bonding, hydrophobic interactions salt bridges and weakly polar interaction. Although methionine, another amino acid, contains sulphur atom in its side chain, it does not form disulphide bond. Hence, the study the characteristics of disulphide bond is important. [1, 5].

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Due to the oxidation of the sulfhydryl group of cysteine residues in a protein molecule, disulfide bond is formed. In such process, the thiol part -SH of cysteine molecules, contributes in disulfide bond formation, is deprotonated and covalent bond is formed between them [5–7]. Two cysteine molecules also covalently linked through peptide bond. Peptide bond is formed after the elimination of a water molecule during the interaction of carboxyl (C-terminus) and amine (N-terminus) regions of two amino acids. This bond has partial double bond character, i.e., stronger than single bond and weaker than double bond. It prevents the rotation of residues in protein molecules [1, 2]. This work is basically focus on disulphide bonding in cysteine dimer.

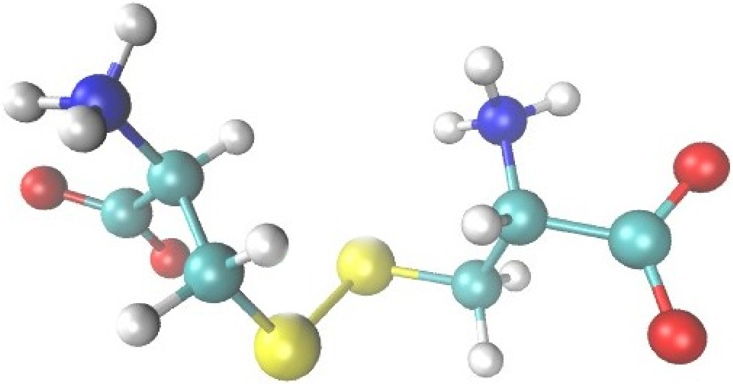

Figure 1. Cysteine dimer.

Diffusion is an important physical phenomenon to understand the rate of transport of mass from the region of higher concentration to lower concentration [8–11]. Many bio-molecules like insulin are stabilized due to the formation of disulphide bond. The detailed understanding of effect of disulphide bond in any molecule and its effect on diffusion phenomenon in aqueous environment plays an important role in different areas including drugs designing. With our best knowledge, the diffusion coefficient of cysteine dimer, covalently bonding with disulphide bond, in water using classical molecular dynamics technique has not been studied yet, which motivate us to study the diffusion phenomenon of cysteine dimer.

2. Methodology

Diffusion

Diffusion is a transport property of matter due to which particles flows from the region of higher concentration to lower concentration due to the concentration inhomogeneity [12]. The knowledge about diffusion phenomenon of biomolecules plays an important role to understand many phenomena in living organisms, like transport of biomolecules at different part of body through body fluids. The diffusion in a homogeneous system in the absence of chemical concentration gradient is called self diffusion and is measured in terms of self diffusion coefficient [13] which is estimated from slope of mean square displacement(MSD) versus time graph using Einstein's equation as [14, 15];

$$D = \lim_{t\to\infty} \frac{\langle [r(t) - r(0)]^2 \rangle}{6t}. \tag{1}$$

In equation (1), $r(t) - r(0)$ is the displacement of particle from reference point during the course of time t, $[r(t) - r(0)]^2$ is the square of displacement and $\langle ... \rangle$ represents the ensemble average and hence $\langle [r(t) - r(0)]^2 \rangle$ gives MSD of particle.

Further, the binary diffusion, the diffusion of particles in the mixture of two different species, is measured in terms of binary diffusion coefficient using Darken's relation as [16];

$$D_{12} = N_2 D_1 + N_1 D_2 \tag{2}$$

In equation (2), D_{12} is the binary diffusion coefficient, D_1 and D_2 are the self-diffusion coefficients of substances 1 and 2 respectively, and N_1 and N_2 are the corresponding mole fractions.

Computational Details

In this work, we performed classical molecular dynamics (MD) simulations for the system of 1 cysteine dimer and 1984 water molecules in cubic simulation box of size about 3.90 nm at four different temperature; 288 K, 298 K, 303 K and 308 K using GROMACS 5.1.2 software package [17]. The cysteine dimer was extracted from the insulin molecule (pdb entry: 3i40) taking seventh residue of chain A and also seventh residue of chain B. The input files for MD simulation were generated from CHARMM-GUI online software program [18]. The dimer was solvated in a periodic boundary condition (pbc) box with TIP3P water sample. All the bonded and non-bonded parameters are used, assigned by CHARMM36 force field [19].

MD simulation was begun from energy minimization run using Steepest-descent algorithm [17]. This run removes steric clashes in which the undesirable coordinates of atoms of side chain or backbone that may occupy wrong position in the same coordinate space and bring the system in the condition of minimum potential energy state. Since the transport properties like diffusion depends on the thermodynamic parameters like temperature, pressure etc. of the system [20], the system should be stabilized for thermodynamic parameters. Then, the equilibration run was carried out to stabilize the temperature, pressure, density etc. The equilibrated bio-molecular system saves the computation efforts by removing external unwanted forces [21, 22]. To bring the system in thermodynamics equilibrium, equilibration run of 100 ns with time step of 1 fs was carried out in NPT ensemble for the system at each temperature. During the equilibration run; LINCS algorithms, Berendsen barostat with coupling time of 0.8 ps and Velocity rescaling thermostat with coupling time of 0.01 ps were used to constraint all the bonds, maintain constant pressure and maintain constant temperature respectively [17]. Also, 1 nm cut-off distance was taken for Lennard-Jones and Coulomb interactions; and PME (Particle-mesh Ewald) method was used to handle long range coulomb interaction. Furthermore, Maxwell-Boltzmann distribution and was used to assign initial velocities of each particle; and the new positions and velocities of the particles was calculated using leapfrog algorithm [17] after each time step respectively.

After equilibration of the system, production run was performed in NVT ensemble for 100 ns with time step of 1 fs for each temperature. During the equilibration run, velocity-rescaling thermostat with coupling time of 0.01 ps was used and the initial velocities of each particle was taken from the final step of equilibration run.

3. Results and Discussion

Diffusion coefficient

In this section, we present the self diffusion coefficients of water and cysteine dimer as well as their binary diffusion coefficient at different temperature. The self diffusion coefficient of both solute as well as solvent are estimated from their MSD versus time graph. Although, all the production run were carried out for 100 ns, we have plotted the MSD graph for 3 ns and 5 ns for cysteine dimer and water respectively due to the region that statistics is better in beginning region of the graph. Figs. 2 and 3 show the msd versus time graph for cysteine dimer and water respectively at four different temperatures: 288 K, 298K, 303 K and 308 K. From the Figures, it is clearly seen that the slope of the graph increases with temperature for both cysteine dimer as well as water. This indicates that self diffusion increases with temperature.

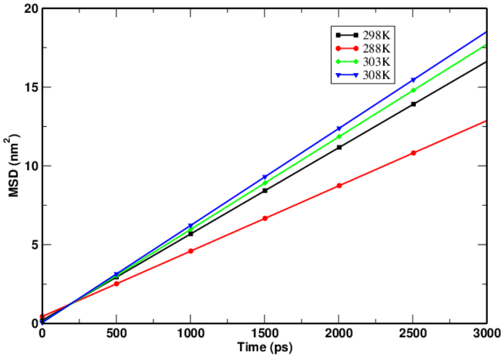

Figure 2. MSD versus time plot of cysteine dimer at four different temperature.

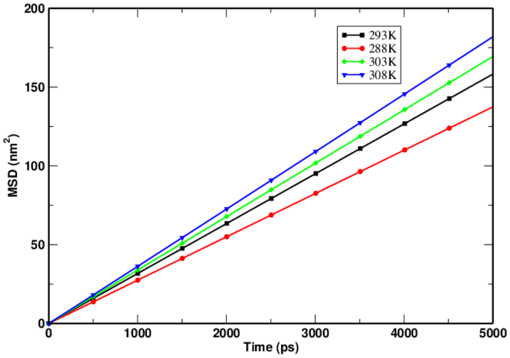

Figure 3. MSD versus time plot of water at four different temperature.

Also, the estimated values of the self diffusion coefficients of cysteine dimer and water estimated from the slope of the MSD versus time graph using Einstein relation; and their binary diffusion coefficients using Darken's relation are presented in Table 1.

From the Table 1, it is seen that both self and binary diffusion coefficients increases with increase in temperature. This is due to the increase in random velocity of the particles and decrease in density of the system with increase in temperature. As a result, the available space for diffusion increases with temperature. The simulated value of self diffusion coefficient at 298 K is in closely agreement with previously reported simulated value

[23]. Also, from the Table, we observe that the estimated values of self diffusion coefficient of water at different temperature are greater than the previously reported experimental values. The reason for this observation is that we used TIP3P water model during the simulations which overestimate the value of diffusion coefficient. Furthermore, we observed that the binary diffusion coefficient of the system equals to corresponding value of self diffusion coefficient of cysteine dimer, which is due to infinite dilute concentration of cysteine dimer in the solution.

Table 1. Estimated values of self and binary diffusion coefficients at different temperature.

SN	Temp.	Diffusion coefficients $(D_{PBC})(10^{-9}m^2\ s^{-1})$				
		Self				Binary
		For cysteine dimer	For Water			
	(K)	MSD	MSD	Reference[23]	Experiment [24]	Calculated
1.	288	0.69	4.58		1.77	0.69
2.	298	0.91	5.27	5.4	2.30	0.91
3.	303	0.98	5.65		2.60	0.98
4.	308	1.02	6.06		2.90	1.02

Temperature Dependency of Diffusion

The Table 1 shows the temperature dependent behavior of diffusion coefficient. In order to check whether the behavior is Arrihenius or not, we have plotted graphs between $ln(D)$ versus $(1/T)$. Fig. 4 shows the temperature dependent behavior of self diffusion of water which follows the Arrhenius equation [25]:

$$\ln D = \ln D_0 - \frac{E_a}{N_A k_B T} \tag{3}$$

In equation (3), D is the diffusion coefficient, D_0 represents pre-exponential factor, E_a is the activation energy for diffusion, N_A is Avogardo's number whose value is 6.022×10^{23} mol^{-1}, k_B is the Boltzmann's constant whose value is 1.38×10^{-23} JK^{-1} and T is the absolute temperature. The intercept when extrapolated to the $1/T \rightarrow 0$ in the Arrhenius plot gives the pre-exponential factor.

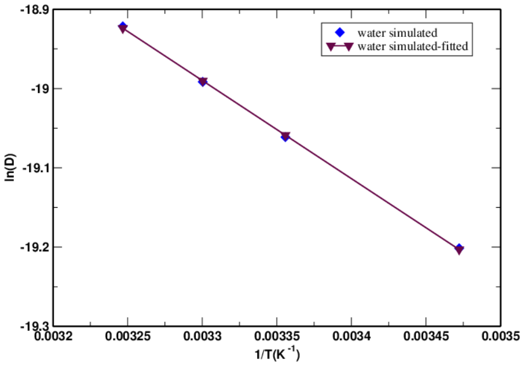

Figure 4. Arrhenius diagram for simulated values of self diffusion coefficient of water.

4. Conclusions

In this work, molecular dynamics simulation has been carried out to study the diffusion properties of cysteine dimer with disulphide bond in infinite dilute solution of water. This study is performed solvating single cysteine dimer molecule in 1984 water molecules at temperature 288 K, 298 K, 303 K, 308 K using GROMACS 5.1.2 software package. CHARMM36 force field parameters and TIP3P water model are used throughout the simulations. Einstein′s equation is used to determine the self diffusion coefficient of cysteine dimer and the water. The estimated values of self diffusion coefficients are found to be higher than the previously reported experimental values. This is due to the fact that TIP3P water model overestimate the diffusion coefficient. Also, the diffusion of binary mixture of cysteine dimer and water was calculated from Darken′s relation. The temperature dependence of diffusion coefficient has been tested from Arrihenous plot.

In near future, we are intended to study the transport properties of poly-cysteine with peptide bond in aqueous medium.

5. Acknowledgements

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