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Glycine Amino Acid Transport inside the Nanopores of Lysozyme Protein Crystal

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Transport of glycine amino acid molecules inside the fully hydrated nanopores of a lysozyme protein crystal was investigated using molecular dynamics (MD) simulations. Mean square displacement (MSD) analysis of glycine molecules suggested that there are different regimes during glycine transport inside the lysozyme nanopores. Glycine molecules undergo a diffusive behavior along the main pore of the protein crystal with the self-diffusion coefficient of about $1.98 \times 10^{-13}\,m^2\,s^{-1}$, four orders of magnitude less than that in pure water. This observation is in good agreement with available experimental data. Moreover, analyses based on density and radial distribution functions (RDFs) showed that most interactions of glycine molecules with lysozyme take place on LYS96, ARG14, and ASP87 residues. These interactions are the result of hydrogen-bonding interactions between both amino and carboxylic groups of glycine molecules and active sites of protein residues. These interactions change the effective pore size of the lysozyme crystal.

Highly porous crosslinked protein crystals (CLPCs) have recently been proposed as a novel class of nanoporous molecular sieves.[1] They contain pores that range from approximately 0.3–10 nm and occupy 25–75% of the crystal volume. Robust CLPCs have been successfully applied as extremely stable biocatalysts[2] and as selective separation media. Understanding the nature of transport of solvents and solutes in CLPC is somehow related to many biotechnological processes.[2] Properties of intracrystalline water molecules, ions, and solutes and their transport properties are required for many applications of CLPC. Several studies have been focused on the experimental determination of the solute and water transport in protein crystals.[3] Current understanding of fluid behavior in protein crystals is largely incomplete. Recent studies on diffusion in protein crystals still leave open questions on the mobility of solutes and protein–solute interactions near proteins that constitute the pore walls.[3] In addition to advanced experimental techniques, versatile computational tools are generally needed to correlate reactivity of the protein and transport of solute with nanoporosity of the enzyme crystals at the atomistic level.[4] Molecular simulations have been playing an increasingly important role in providing atomistic/molecular pictures that would otherwise be experimentally intractable or impossible. Among these, molecular dynamics simulations have recently been used to study the properties of protein crystals.[4] Malek et al.[4] applied the first extensive molecular dynamics simulations to consolidate the main features of water and solute diffusion in CLPCs and after that these studies were continued by others.

Here, orthorhombic lysozyme was used as a simple and known model of the crosslinked protein crystals to study the diffusion of glycine molecules through the nanopores of the protein as well as its interactions with protein surface. Lysozyme consists of 129 amino acids with 1001 non-hydrogen atoms. The crystal structure of lysozyme, Entry 1 AKI, is taken from Brookhaven Protein Data Bank (PDB)[5] and was used as a starting point. In this crystal, four protein molecules related by the crystallographic symmetry $P2_12_12_1$ were placed in the unit cell with crystallographic indices of $a = 5.9062$ nm, $b = 6.8451$ nm, and $c = 3.0517$ nm.

Glycine (Gly) is a natural amino acid with chemical formula NH_2CH_2COOH. Figure 1a shows its chemical structure. Since this amino acid contains both amino and carboxyl groups, the net charge of the molecule is pH dependent. At low pH, the amino group is protonated and the molecule carries a positive charge. At high pH, the carboxylic group is dissociated and the molecule has a negative charge. At pH equal to the isoelectric point, the amino acid has a zwitterionic form, where the molecule contains a protonated amino cation and a carboxylate anion and the net charge is zero. Therefore, in this study, transport of glycine molecule was considered in its neutral form.

The simulated system consisted of one unit cell of lysozyme which is composed of 4 protein molecules, 1528 water molecules, 32 Cl^- ions which act to neutralize the system, and 4 glycine molecules to prepare 0.1 M solution. Figure 1b shows one unit cell of lysozyme protein crystal with four glycine molecules.

Molecular dynamics simulation was applied to investigate the interactions of glycine molecules with the lysozyme protein

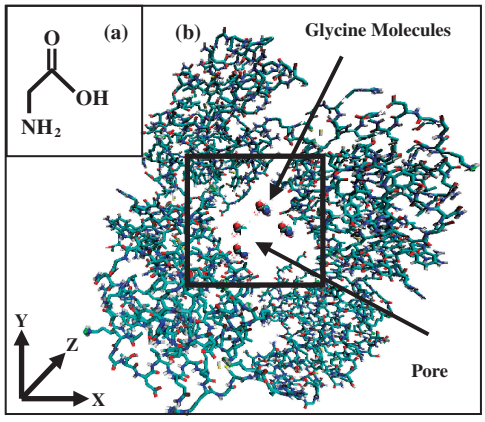

Figure 1. Chemical structure of the glycine amino acid (a), one unit cell of lysozyme protein crystal with four glycine molecules (b).

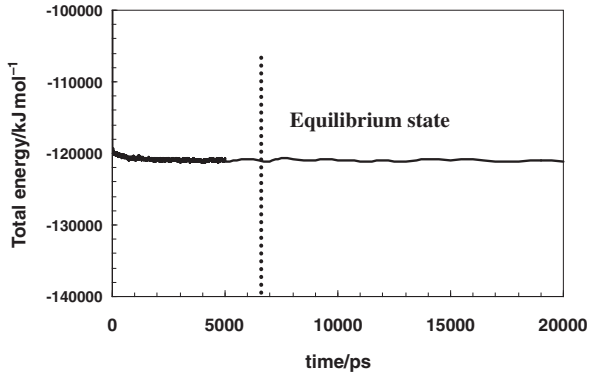

Figure 2. Calculated total energy of the simulated system.

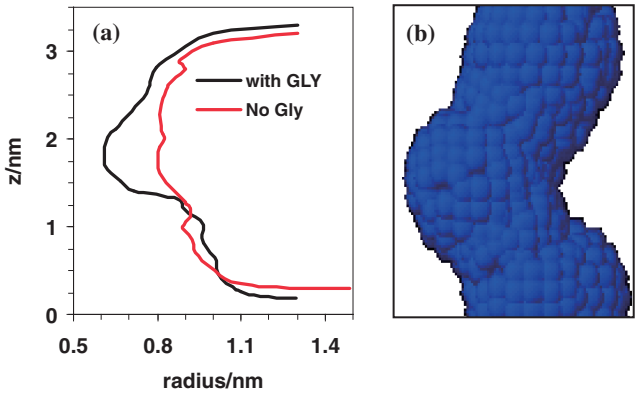

Figure 3. Pore size distribution along the z axis (a), visualized pore size by HOLE algorithm (b).

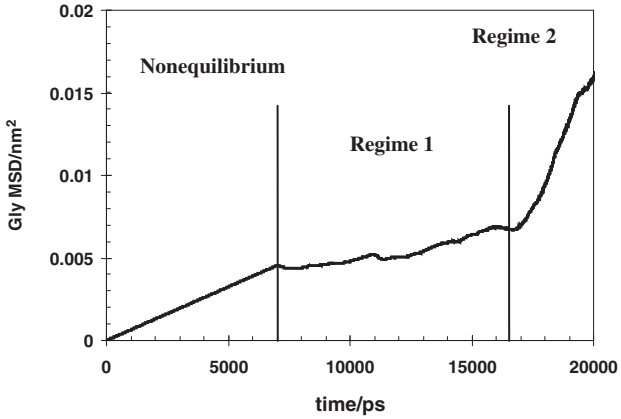

Figure 4. Mean square displacement of glycine molecule along the main pore of lysozyme crystal.

crystal. The simple point charge (SPC) model was used to represent water molecules. GROMOS 96 force field was also used for protein and glycine molecules.[6] A cut off of 1 nm was used for van der Waals interactions and the periodic boundary condition was applied in all three directions. The simulation box size is calculated based on the unit cell of lysozyme crystallography indices as: $a = 5.9062$ nm, $b = 6.8451$ nm, and $c = 15.2585$ nm. At the beginning of the simulations, the system was minimized for energy for $t = 200$ ps using harmonic position restraints (1000 kJ mol^{-1} nm^{-2}). Then MD simulation was performed in a canonical ensemble (*NVT*) at 300 K for 20 ns. The temperature was controlled by the Berendsen weak-coupling algorithm. Simulation was carried out using GROMACS software[7] and visualization was done using VMD v.1.8.6 commercial package.[8]

To compare the diffusion behavior of glycine molecules in the protein crystal to that in pure water, another simulation was set up. A water box of dimensions $a = 5.9062$ nm, $b = 6.8451$ nm, and $c = 15.2585$ nm with 4 glycine molecules (0.1 M solution) was constructed, and simulation was performed by using similar interaction parameters as the previous simulations.

During simulation, the equilibration of the system was monitored by calculating the total energy of the system. Calculated results showed that the system reaches the equilibrium state after 7 ns (Figure 2).

In the next stage, the pore-size distribution was calculated by using HOLE algorithm.[9] Results show that there is a main pore along the z-axis with the averaged pore size 0.82 nm. Figure 3 shows the pore-size distribution of the protein crystal in two statuses: without glycine molecules[10] and with glycine molecules. According Figure 3, it can be concluded that protein pore size changes to the narrowest state due to glycine transport.

At the next stage, the diffusion behavior of glycine molecules along the main pore was investigated. As Figure 4 illustrates, MSD of glycine molecules versus time has different regimes. The system reaches the equilibrium state after 7 ns which is in good agreement with the energy curve (Figure 2). The most effective regime is related to the time interval of $7\text{ ns} < t < 16.5$ ns. The MSD vs. time in this regime is linear demonstrating a diffusive behavior of glycine molecules in the protein crystal (MSD is proportional to t with a slope nearing unity). So Einstein equation can be used to calculate the self-diffusion coefficient (eq 1):[4]

$$D = \lim_{\Delta t \to \infty} \frac{\langle |\Delta \bar{r}|^2 \rangle_{t_0}}{2d\Delta t} \quad (1)$$

where $\langle |\Delta \bar{r}|^2 \rangle_{t_0}$ is the MSD of solvent and solute molecules during the time interval Δt, averaged over the ensemble of molecules in d dimensional space. The calculated diffusion coefficient of glycine molecules in the lysozyme protein crystal is equal to 1.98×10^{-13} m^2 s^{-1} which is in good agreement with experimental data.[11] The last regime is due to the box dimension limitation. Calculated results show that glycine molecules diffuse along the main pore of lysozyme protein crystal four orders of magnitude slower than their transport inside the pure water. This is because of the special interactions between these molecules and the special residues of lysozyme crystal. Our conclusions in this stage are based on the limited applied time frame.

To investigate the interactions of glycine molecules with the lysozyme residues, the density profile and radial distribution function of glycine molecules were calculated. To obtain the solute density profile, the averaged number of glycine molecules in a cylindrical segment within a slice of ≈0.2 nm thickness was divided to the volume of the segment. Figure 5 shows the density profile of glycine molecules along the different axes. As Figure 5 indicates glycine molecules spend most of their times on the protein surface near LYS96, ARG14, and ASP87 residues

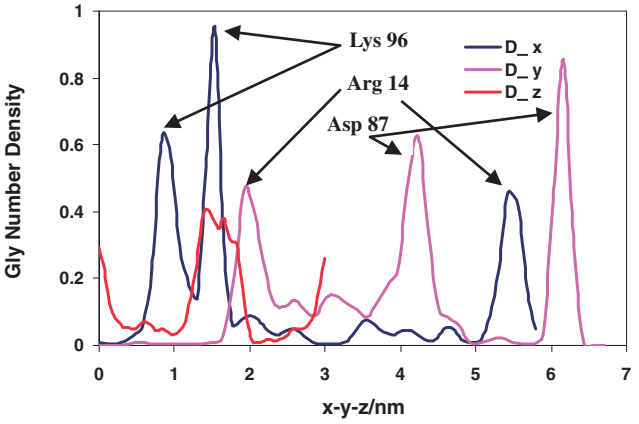

Figure 5. Density profile of glycine molecules.

of lysozyme protein crystal which are located at the internal surface of the main pore. Radial distribution function analyses of amino and carboxylic groups of glycine molecules with mentioned residues showed that there are considerably high interactions between amino and carboxylic group side chains of the glycine molecules and LYS96, ARG14, and ASP87 amino acids of the lysozyme protein crystal (Figure 6).

In conclusion, simulation results indicate that pore morphology does not change during the simulation time, but the pore size decreases during glycine transport. This behavior is likely a result of glycine molecule interactions with the residues located at the internal surface of the pore wall. Moreover, glycine molecules in protein channels posses a slower dynamic motion than that in bulk water. This shows that the pore structure has significant effect on the diffusion process of solutes in the protein crystal.

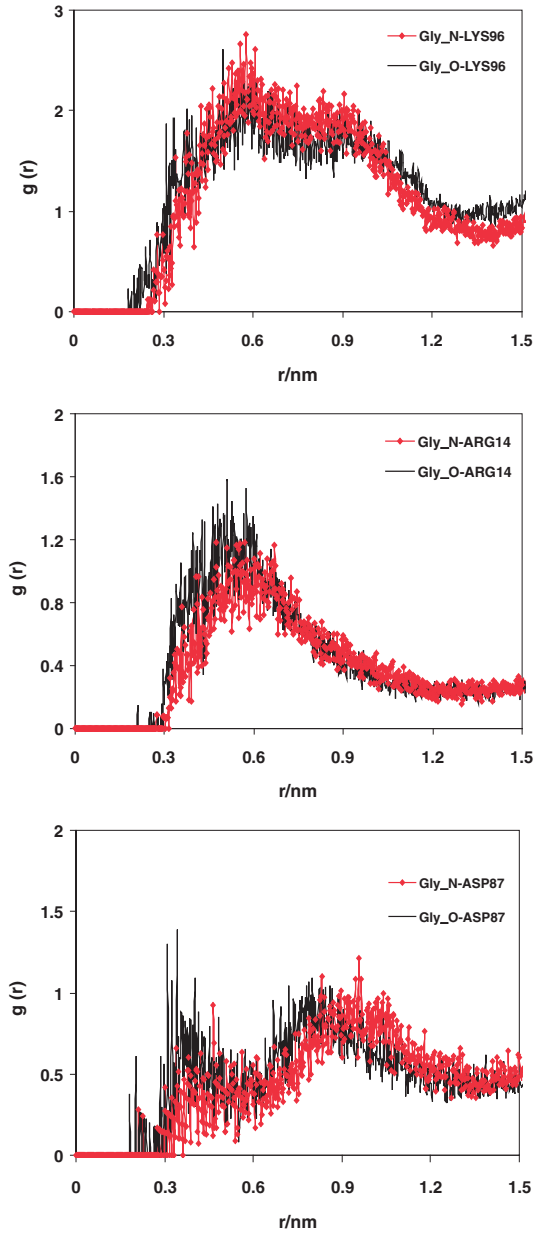

Figure 6. Radial distribution function of amino and carboxylic groups of glycine with LYS96, ARG14, and ASP87.

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