Molecular Simulation of Water and Ion Motion in Lysozyme Crystals

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The dynamics of water and chloride counter ions (Cl^-) in single unit cells of orthorhombic and tetragonal hen egg white lysozyme lattices are investigated by 5 ns molecular dynamics simulations. The electrostatic interaction inside the crystal channel significantly influences the diffusion of chloride ions. The corresponding diffusion constants of Cl^- calculated by averaging over all ions during the 5 ns time scale for the orthorhombic and tetragonal lysozyme unit cells are $(1.604 \pm 0.05)\times10^{-5}$ and $(0.95 \pm 0.05)\times10^{-5}$ cm^2s^{-1}, respectively. According to our results, water molecules close to the protein surface jump between hydration sites with a broad range of site residence time, and show a higher mobility than water molecules further away from the protein surface. The results are of interest to study ion and water transport through well-defined biological nanopores and may elucidate more features of water-protein and ion-protein interactions in protein crystals.

Introduction

The behaviour of solvent and small ions near proteins plays a major role in the stabilization of the protein structure (folding, unfolding) as well as in the internal dynamics and function of proteins [1-5]. When proteins make up the lining of small pores, water and ions affect the passage of substances through such pores [6]. Protein crystals contain pores that range in width from approximately 0.3 nm up to 10 nm and occupy 25-75 percent of the crystal volume [7]. Their porosity is comparable to that of inorganic porous catalysts and sorbents such as zeolites and silica-gel [7,8]. Cross-linked protein crystals have recently been proposed for chemical and pharmaceutical applications as extremely stable catalysts [9] and as selective (chiral) separation media [10]. Properties of intracrystalline water molecules and ions, and their transport through the crystal, are essential to these applications.

Recent studies on diffusion of water molecules in protein crystals still leave open questions concerning the mobility of water near proteins that constitute the pore walls [11,12]. It is also important to know whether the properties of proteins in crystal and in solution are the same [13]. Molecular dynamics (MD) simulations have recently been used to study the properties of protein crystals [13-16]. Short MD trajectories were used to examine the interatomic distance fluctuations and displacement correlations of a single orthorhombic lysozyme unit cell [17,18]. Moreover, the analysis of rigid-body motions within a unit cell reproduces the main features of the experimental scattering studies [12,15,19]. MD studies of the dynamic properties of ions and water molecules inside protein crystals are scarce. A recent study by Walser et al. [16] evaluates different electrostatic forces by studying the protein atom fluctuations and chloride ion properties in a ubiquitin unit cell. This study, however, does not investigate long-time diffusion properties. Although MD simulations with explicit ions, solvent molecules, and proteins should in principle allow for a very realistic representation of transport phenomena in complex biological system such as protein crystals and ion channels, such atomistic simulations are still impractical for calculating meaningful diffusion properties of large molecules.

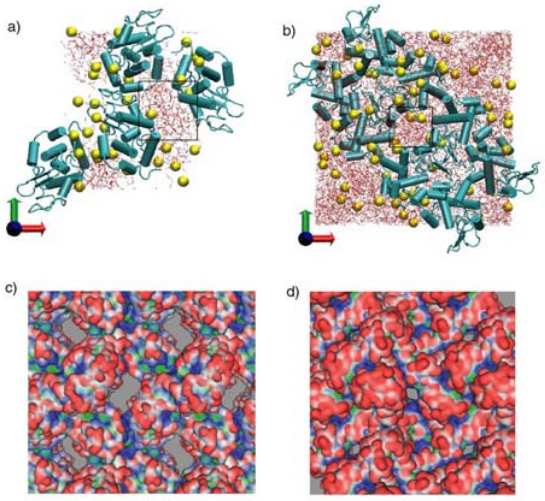

Figure 1. All-atom representation of a single unit cell of orthorhombic (a) and tetragonal (b) lysozyme lattices. The view is along the z-axis perpendicular to the (100) plane. Pore regions are labelled by squares. Lysozyme molecules are pictured as cartoons; yellow balls represent chloride ions, and water molecules are represented by red lines. (c) and (d) show the surface representation for orthorhombic (LYZO) and tetragonal (LYZT) lattices. Hydrophilic and hydrophobic regions are shown in blue and red, respectively.

The practical potential of protein crystals as a biochemical porous medium was our first motivation for the present work. Here, we present a 5 ns MD simulation of water and chloride ion dynamics and their detailed interactions with lysozyme molecules within orthorhombic and tetragonal unit cells. Water-lysozyme and chloride-lysozyme interactions on the molecular level were studied both computationally and experimentally [19,20], yet a number of fundamental questions remain unanswered, particularly in a lattice environment. Do water molecules move by translation and/or rotation? How do chloride ions move inside a unit cell? What is the detailed nature of the diffusive motion of water molecules in a lysozyme crystal? What are the length scales and the time scales (dynamics) of all those events?

Methodology

We used MD simulations to examine water and ion motions over a period of 5 ns in a single unit cell of the orthorhombic and tetragonal lattices, using periodic boundary conditions. For the sake of convenience, the orthorhombic and tetragonal lysozyme lattices are referred to as LYZO and LYZT, respectively. It was formerly shown that the dynamic properties of water and small ions in a single-unit-cell simulation are close to those in a multi-unit-cell simulation [21]. Our simulations show that the motion of water molecules and chloride ions in a periodic unit cell and over 5 ns is diffusive. Lysozyme consists of 129 amino acids with 1001 non-hydrogen atoms. Hydrogen atoms attached to aliphatic carbon atoms are incorporated with the latter, but the remaining 342 hydrogen atoms are treated explicitly, leading to 1343 (pseudo-) atoms in total. The simulations were done at pH 7. The amino acids Glu and Asp were taken to be deprotonated while Lys, Arg and His residues were protonated. This leads to +8 electron charges per protein molecule. Chloride ions were then added for electroneutrality. The crystal structures of lysozyme, entry 1AKI [22] for orthorhombic (LYZO) and 6 LYZ [23] for tetragonal (LYZT), were taken from the Brookhaven Protein Database and used as a starting point. In the case of the orthorhombic crystal, four protein molecules related by the crystallographic symmetry $P2_12_12_1$ were placed in the orthorhombic unit cell with a=5.9062 nm, b=6.8451 nm, and c=3.0517 nm. For the tetragonal lattice, eight protein molecules were placed in a unit cell of size a=7.91 nm, b=7.91 nm, and c=3.79 nm, applying a $P4_32_12$ symmetry. The total size of the LYZO system was 5372 protein atoms, 13576 water molecules and 32 chloride ions, leading to

46132 atoms. The LYZT system consisted of 10744 protein atoms, 11005 water molecules and 64 chloride ions, leading to 43823 atoms. Figure 1 shows instantaneous configurations of the atomic model of the fully hydrated LYZO and LYZT, and their crystal structures. Pore regions in single unit cells are framed in Figures 1a and 1b. Repeating the unit cell along crystallographic axes generates the pore network (Figures 1c and 1d). The system was equilibrated for τ=100 ps using harmonic position restraints (1000 kJ $mol^{-1}nm^{-2}$). The simple point charge (SPC) model was used to model water [24]. Simulations used the GROMOS96 force field [24]. The temperature was controlled by the weak-coupling algorithm, separately for protein and solvent plus ions with a time constant of 0.1 ps and a temperature of 300 K. A cutoff of 1.4 nm was used for Van der Waals interactions. To remove the artefact associated with truncation of electrostatic forces, electrostatic interactions were calculated using the Particle-Mesh Ewald (PME) method with a grid spacing of 0.12 nm and fourth order interpolation. The integration time step was 2 fs. Simulations were done with the GROMACS package [25,26] (http://www.gromacs.org). Figures 1a, 1b, 4a and 4b were made using VMD v1.8.1 [27], while Figures 1c and 1d were made using MOE [28]. The diffusion constants, *D*, of the ions and water molecules were calculated by means of the Einstein relation:

$$D = \lim_{t \to \infty} \frac{\left\langle \left| \vec{r}(t+\tau) - \vec{r}(\tau) \right|^2 \right\rangle}{6t} \qquad (1)$$

Results and Discussions

Figure 2 shows the root mean square fluctuation of C-α for each residue, calculated from the distance fluctuation matrix of a 5ns trajectory of crystalline lysozyme. The root mean square fluctuations (RMSF) from the X-ray structure were averaged over the four and eight proteins in LYZO and LYZT, respectively. Although the inclusion of counter ions in an MD simulation box might lead to some deviations, in the lattice simulations, however, addition of counter ions only has a very limited effect (fluctuations on the order of 0.01 nm). The fluctuation results in Figure 2 are completely consistent with previous works on fluctuation and correlation in crystalline lysozyme [18,19]. The RMSF patterns are similar for both systems. The largest fluctuations are for the α-helix loops. The β-strand residues show a low mobility with values of the order of 0.01 nm.

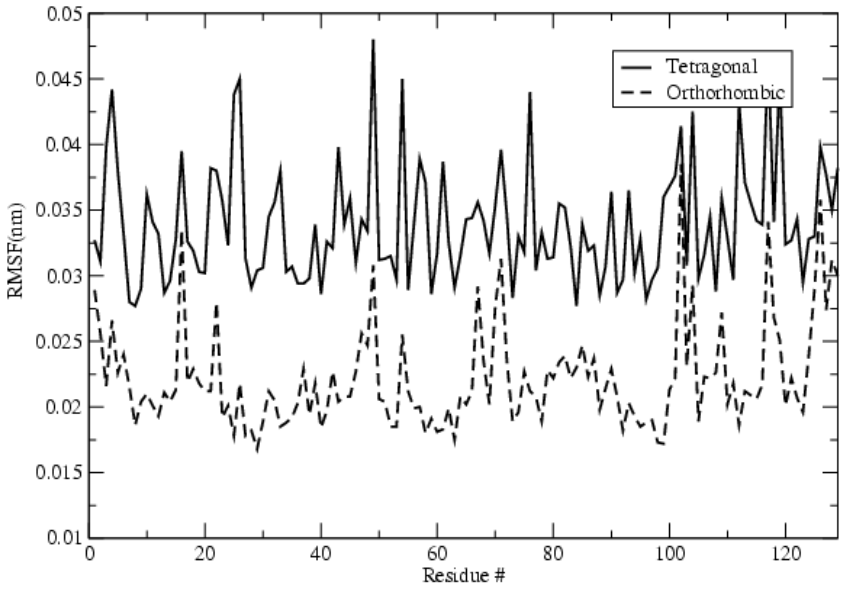

Figure 2. The root mean square fluctuation of the C-α atoms of each residue with respect to its average position. The curves are on the same scale. The full line is shifted upward by 0.01 nm.

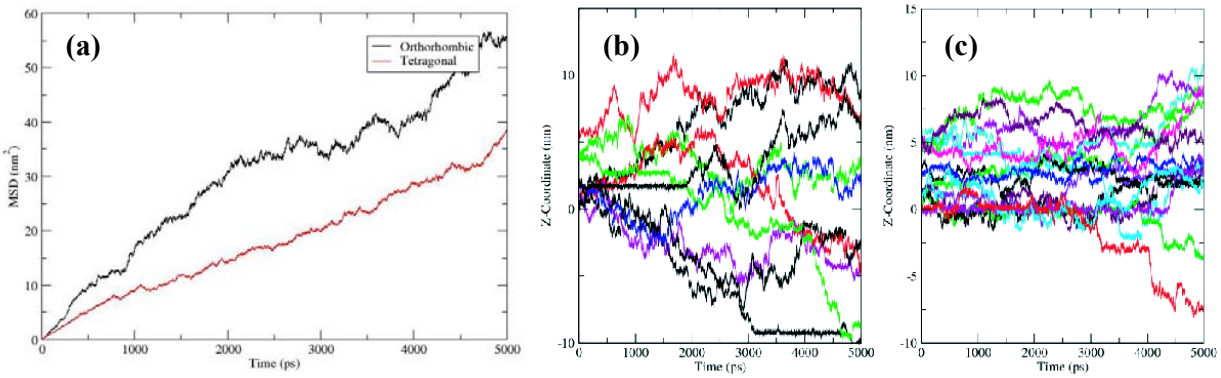

Figure 3. (a) The average mean square displacement (MSD) of chloride ions as a function of time. (b) and (c) show the displacement of chloride ions along the z-axis through the LYZO and LYZT unit cells, respectively. Different colours represent different chloride ions.

We studied the motion of the chloride counter ions in the two lattices. The motions of the counter ions around their initial positions were sampled each 20 ps during the 5 ns simulations. These average positions differ somewhat from the initial placement, and the corresponding distances remain relatively high. Although the ions sample only a small fraction of the accessible configurational space within 5 ns, their locations are sufficiently randomised during this time. Consequently, simulated properties do not depend on the (arbitrary) initial placement of the ions [15]. Figure 3a shows the mean square displacement of the ions as a function of time. It can be seen that each jump corresponds to an ion hopping to another location and undergoing only limited motions between the two jumps. Figures 3b and 3c show the time-dependent displacement of a few representative chloride ions along the z-axis of the unit cell (see Figure 1), during the 5 ns dynamics. Some chloride ions travel all the way within the unit cell, some remain in the pore region of the unit cell, and some go through the unit cell and return after some time. The corresponding diffusion constants calculated from equation (1) and averaged over all ions during the 5 ns time scale for the LYZO and LYZT systems are $(1.604 \pm 0.05)\times10^{-5}$ and $(0.95 \pm 0.05)\times10^{-5}$ cm^2s^{-1}, respectively. Our analysis shows that diffusion of chloride ions is controlled by electrostatic ion-protein site interactions. Diffusion of chloride ions in orthorhombic lysozyme (LYZO) is faster than in tetragonal lysozyme (LYZT). However, we also showed that a positively charged ion diffuses faster in LYZT despite LYZT's narrow pores [29]. This can be explained by the nature of the crystal contacts in tetragonal compared to orthorhombic lysozyme. In the case of LYZO, more negatively charged residues are involved in ion-protein interactions inside the pore space. When less positive centers are available to negative ions, the adsorption site density is lower, and, therefore the diffusivity is higher.

In order to study water motion inside a unit cell of the lysozyme lattices, we took into account that the proteins affect the dynamics of all water molecules. The analysis of water in protein crystals is very useful, as proteins and other essential biological molecules are in contact via an aqueous medium, and the water content in protein crystals is comparable to that in living cells [30]. A very recent incoherent quasielastic neutron scattering (QENS) experimental study suggested that the water molecules inside a pore region of a triclinic lysozyme crystal could be divided into two populations [31]. The first mainly corresponds to the first hydration layer, in which water molecules reorient themselves 5-10 times slower than in bulk solvent, and diffuse by jumps from hydration site

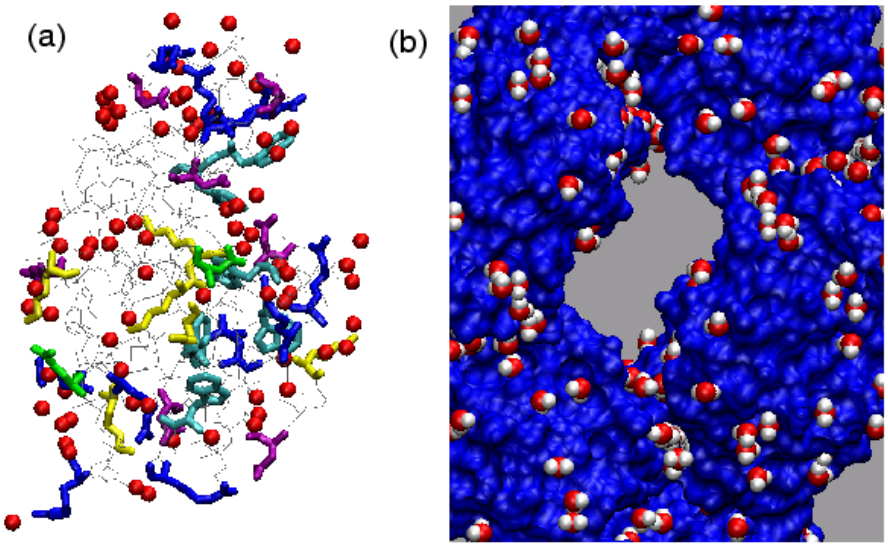

Figure 4. (a) Lysozyme hydration sites (red balls) calculated from the solvent density map. Lys: yellow; Arg: blue; Asp: purple; Glu: green; Trp: light blue. (b) A molecular view of the LYZO surface and the position of waters on the hydration sites.

to hydration site. The second group corresponds to water molecules further away from the protein surface. This second layer is actually confined between hydrated proteins. It was indicated that a “solvent stream” along the protein surface guides the substrate diffusion in the first layer. The latter leads to a two-dimensional surface diffusion rather than a three-dimensional diffusion [20,31]. MD trajectories may provide much information regarding the static and dynamic pictures of water molecules in those hydration layers, and the findings can be compared to the QENS results. In the following, we characterize the dynamic aspects of water-protein interactions during water transport through a single unit cell of orthorhombic lysozyme lattice, in terms of the residence time and the diffusion coefficient of hydration water molecules at the protein surface. Protein hydrations sites have been defined as local maxima in the water number density map, which satisfy certain conditions [2,32]. They should be no further than 3 Å away from any protein atom, and no closer than at least 1 Å. The hydration sites obtained from this method were compared to the positions of the crystallographic waters in structures obtained from the protein data bank, Figure 4. Based on this, we identified a total of 245 and 185 hydration sites around orthorhombic and tetragonal lysozyme molecules in a unit cell, respectively. Note that the number of hydration sites of protein in a crystal may be smaller than that of free proteins in solution, as many of the sites in a crystal are buried. In general, the first hydration shell follows the shape of the protein, but there are regions where the hydration sites are clustered. Figure 4 shows that both charged (LYS, ASP, GLU and ARG) and polar (TRP) residues are found in the region of high-density sites.

The residence time of water molecules on each site was calculated from 5 ns trajectories. Our analysis for calculating the mean residence time around hydration sites on protein surfaces in orthorhombic and tetragonal lysozyme crystal consists of the calculation of the relaxation time of water molecules in the hydration layer around a protein atom [2]. Using this procedure, we have calculated the residence time of water molecules in the first hydration layer of lysozyme molecules in LYZO and LYZT systems. Population analysis of the hydration sites shows that they are never all occupied simultaneously. In fact, the number of sites occupied at the same time never seems to exceed 75% of the total number and there are no cases of double occupancy. Figure 5 shows that residence times vary

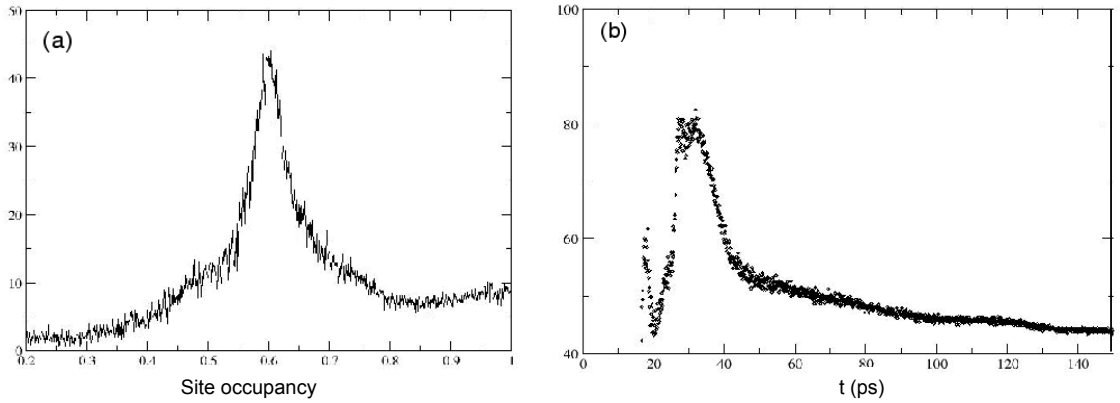

Figure 5. Distribution of site occupancy (a) and residence time (b) for LYZO.

between 5 and 500 ps, and that the average site occupancy varies from 25% (R84) to 100% (R96). For both LYZO and LYZT systems, a particular residence time distribution is evident. The broad peak on the right hand side of the residence time distributions indicates that the protein may prolong the life of the bound water molecules by up to one order of magnitude, but such sites are few. Visits of water molecules to some of these strong sites are likely to be the source of residence times on the order of hundreds of picoseconds within a 5 ns simulation.

The first water layer around the protein shows considerable orientational disorder. Such a phenomena can be observed by looking at the water molecules' dipole orientations inside the pore. On the hydration sites near the protein surface, it appears that each water molecule reorients itself much more slowly than in the bulk, as predicted in [20,31]. Within a longer time period, however, the water molecules jump from one hydration site to a neighbouring hydration site, while they reorient themselves. The long-range diffusion coefficient of these water molecules is small compared to bulk solvent. Figure 6 schematizes a model for the behaviour of water molecules close to and far from the protein surface in a pore, based on the residence time calculations and dynamics of the first water layer. Nearly no water molecules are immobile. The water molecules in the first hydration shell do not only reorient themselves, but also jump between hydration sites with a broad range of residence times, from 5 ps to 500 ps. There are few water molecules with residence times higher than 500 ps that bound to the protein surface strongly. The long-range two-dimensional surface diffusion coefficient calculated for these water molecules is about 10 times less than that for bulk water (at 25^{o}C ~2.4 $\times 10^{-5}$ cm^{2}s^{-1} [33]), and 2 times more than what is predicted for a triclinic lysozyme lattice [31]. Water molecules further away from the protein surface undergo a long-range translation. They are confined in between the hydrated proteins of the pore wall. The average mean square displacement of these water molecules shows a long-range diffusion coefficient ~50 times less than bulk water, in agreement with [31]. Therefore, they move 5 times more slowly than water molecules in the first layer. A hydrogen-bonding network interacting with protein surface atoms allows for such a dynamic behaviour. For the water molecules close to the protein surface, the diffusion is kept on the surface. They show a higher mobility because of the reduction in dimensionality at the water-protein interface.

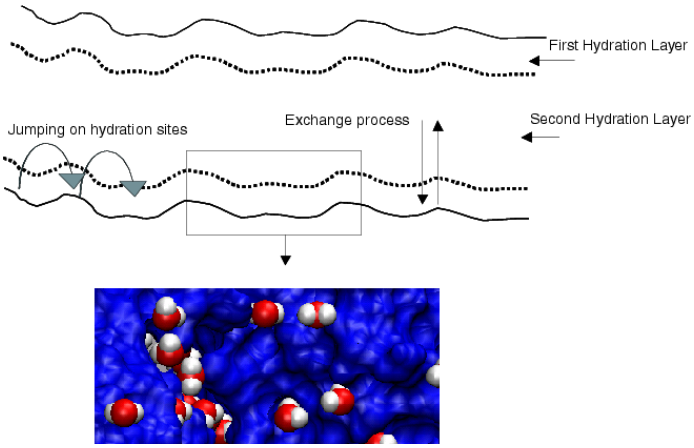

Figure 6. Model of water molecular dynamics in a pore in lysozyme crystals.

Conclusions

Molecular dynamics simulations of the single unit cells of orthorhombic and tetragonal lysozyme were performed to analyze the dynamics of water molecules and chloride ions. Based on the residence time and dynamics of water, a model for the behaviour of water molecules close to (first layer) and far from (second layer) the protein surface was presented. The water molecules in the first layer jump between hydration sites with a broad range of residence time on sites, from 5 ps to 500 ps. A two-dimensional surface motion is evident for these water molecules. Water molecules in the second layer undergo a 3D motion with a long-range diffusion coefficient 5 times less than water molecules in the first layer. Similar to the procedure applied for water, we studied the dynamics of chloride ions around the charged residues on the protein surface. No clear crystal ion sites were detected in LYZO and LYZT at the examined ionic concentration (~0.45M). The electrostatic interaction inside the crystal channel significantly influences the diffusion of chloride ions. The corresponding diffusion constants of Cl^- calculated by averaging over all ions during the 5ns time scale for the orthorhombic and tetragonal lysozyme unit cells were $(1.604 \pm 0.05)\times10^{-5}$ and $(0.95 \pm 0.05)\times10^{-5}$ cm^2s^{-1}, respectively.

Keywords: Molecular dynamics. Lysozyme crystal. Diffusion. Biological nanopores.

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