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Mercury Inhibits the L170C Mutant of Aquaporin Z by Making Waters Clog the Water Channel

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Abstract

We conduct *in silico* experiments of the L170C mutant of the E. coli aquaporin Z (AQPZ) with and without mercury bonded to Residue Cys 170. We find that bonding mercury to Cys 170 does not induce consequential structural changes to the protein. We further find that mercury does not stick in the middle of the water channel to simply occlude water permeation, but resides on the wall of the water pore. However, we observe that the water permeation coefficient of L170C-Hg⁺ (with one mercury ion bonded to Cys 170) is approximately half of that of the mercury-free L170C. We examine the interactions between the mercury ion and the waters in its vicinity and find that five to six waters are strongly attracted by the mercury ion, occluding the space of the water channel. Therefore we conclude that mercury, at low concentration, inhibits AQPZ-L170C mutant by making water molecules clog the water channel.

Aquaporins (AQPs) are integral membrane channel proteins that mediate the bidirectional flux of water and selected small molecules across the cell membrane. [1] Mercurial compounds were found to inhibit water transport[2] and were used for various *in vitro* analyses of aquaporins. [3–10] It is known that mercury inhibits most AQPs by interacting with the accessible cysteine residues. [7] The current literature provides two mechanisms of inhibition of AQPs by mercury: The first is simple occlusion of the water pore by the mercury atoms/ions found in the vicinity of the cysteine residues lining the water channel wall; The second is conformational change (collapse of the water pore) at the selectivity filter (SF) (namely, the aromatic/arginine (ar/R) constriction) region induced by mercury bonded to a cysteine residue nearby. The simple occlusion mechanism was elucidated from the X-ray crystallographic studies[10] of aquaporin Z from E. Coli (AQPZ)[11–14] and its mutants. The second mechanism was proposed in an *in silico* study on the basis of molecular dynamics (MD) simulations of the bovine aquaporin AQP1.[15] In this paper, we present an *in silico* study of the L170C mutant of AQPZ and demonstrate a new mechanism of mercury inhibition: The mercury ion Hg⁺ covalently bonded to the cysteine residue (Cys 170) causes water molecules to clog the water channel. This new mechanism explains L170C's high sensitivity to mercury observed in the *in vitro* functional experiments of Ref. [10].

MD simulations complement *in vitro* experimental studies by providing valuable dynamics information and connecting the dots from the static information in the X-ray structure to the functional experiments such as water transport assays. In the current literature, there are

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many *in silico* studies of AQPs, [15–58] but few on the mercury inhibition of water transport. [15] In order to reveal the mechanism of mercury inhibition of L170C, we present *in silico* studies of the L170C mutant of AQPZ in absence of and in presence of mercurials in the systems. We utilize the widely used MD engine NAMD[59] to perform *in silico* experiments. We use the well tested CHARMM27 force fields[60] to model the intra- and inter- molecular interactions of our all-atom model systems of the cell membrane containing water channel proteins. The first system of our study, illustrated in Fig. 1, consists of an AQPZ-L170C (PDB code: 2O9F) tetramer embedded in a $109 \text{ \AA} \times 109 \text{ \AA}$ patch of POPE lipid bi-layer solvated with TIP3 waters and ionized with NaCl to neutrality and physiological ion concentration. Note that Residue His 174 is set to the protonated state and carries a positive charge. The second system of our study is derived from the first system by mutating Residue Cys 170 into Residue Cys-Hg⁺ (the mercury ion Hg⁺ takes the place of the γ hydrogen of cysteine) and deprotonating Residue His 174 from the positively charged form into the neutral form. Correspondingly, no additional ions are needed to neutralize the second system. We know that the protonation state of a histidine residue is determined by its environment at a neutral pH level of the solution. Therefore, based on the fact that His 174 is in the proximity of Cys 170, we choose His 174 to be deprotonated when Hg⁺ is covalently bonded to the S atom of Cys 170. The parameters for the Cys-Hg⁺ residue are taken from Ref. [15] that contains an MD study of mercury inhibition of bovine AQP1. Note that the fine features of water-Hg²⁺ interactions have been studied in Refs. [61–63]. Our study shows that these fine features are not necessary to explain the mechanism of mercury inhibiting L170C.

After equilibration, we conducted 30 ns equilibrium MD simulations for each of the two systems from which we examine the structural stability of the protein. The first question we need to answer: Are there consequential structural changes caused by bonding mercury to Cys 170 whose side chain is on the water channel wall? More specifically, does the water pore collapse anywhere along the channel of L170C-Hg⁺ in resemblance to what was observed in Ref. [15]?

In Fig. 2, we show how the structures of L170C and L170C-Hg⁺ deviate from their crystallographic structures (PDB codes: 2O9F and 2O9G, respectively) when embedded in the lipid bilayer. Fig. 2A and Fig. 2B show the root mean square deviations (RMSDs) of the alpha carbons as a function of time. In both cases, the RMSDs stabilize around a value less than $2 \text{ \AA}$, which means that there are no significant overall changes in the solution structures of the protein mutants from their crystallographic form. However, we still need to answer the question about local structural changes because local changes to the side chains of the residues lining up the water channel can be strongly consequential. Fig. 2C and Fig. 2D demonstrate the water pore radii along the water channel at different times in comparison to the crystallographic structure. We find that nowhere along the water channel does the water pore collapse in either the L170C or the L170C-Hg⁺ system. We also find that the mercury ion bonded to Cys 170 does not stick far enough out of the channel wall to occlude the pore space, instead, it remains nearly on the channel wall. The pore radius of L170C-Hg⁺ water channel is greater than that of the crystallographic structure (PDB code: 2O9G) that has multiple mercury atoms located in the middle of the pore. At this point, we can conclude that mercury does not inhibit L170C by structural changes.

Now the next question: Does bonding mercury to Cys 170 actually inhibit water transport through the water channel? This question can be answered directly by the measurements of osmotic permeability from the *in silico* experiments of the L170C and the L170C-Hg⁺ systems. Using the theoretical formulation of Ref. [64], we computed the mean square displacements (MSD) of the water molecules in the water pore. The results are shown in Fig. 3(A). From the slope of the MSD vs time curves, we can compute the osmotic permeability:

[64] The slope is equal to $2D_n$ (D_n being the collective diffusion constant of the waters in the pore) and the osmotic permeability $p_f = v_W D_n$ where v_W is the average volume occupied by one water molecule. For L170C, we have $p_f = 4.4 \pm 0.3 \times 10^{-14} \text{ cm}^3/\text{s}$, which is identical to what was obtained in Ref. [33] for the wild-type AQPZ in POPE lipid bi-layer. For L170C-Hg⁺, we have $p_f = 2.0 \pm 0.2 \times 10^{-14} \text{ cm}^3/\text{s}$. Clearly, the osmotic permeability of L170C-Hg⁺ is only half of that of L170C. This corresponds to the half maximal inhibitory concentration, IC₅₀, along the dose-response curve for L170C and explains the high sensitivity of L170C to HgCl₂ observed in the water transport assays of Ref. [10]. Even though bonding single mercury to Cys 170 does not cause much steric hindrance in the water pore, it does significantly reduce the osmotic permeability of the channel.

In order to understand how mercury bonding to Cys 170 reduces water permeability without inducing steric hindrance in the water pore, we have computed the potential of mean force (PMF) on the basis of water density along the water channel. [16] The results are shown in Fig. 3(B). The presence of the mercury ion bonded to Cys 170 causes a significant lowering of the PMF around $z = -2\text{\AA}$ where Cys 170 resides, forming a new free-energy well on the PMF profile of L170C-Hg⁺. Accordingly, water density in the region becomes higher due to the strong electrostatic attraction between the mercury ion and the waters in its vicinity as shown in Fig. 4. This altered PMF profile divides the stochastic process of a water molecule permeating the water pore into two processes. When a water molecule enters the water pore from the vestibule on the extracellular side of the membrane, it overcomes the free-energy barrier in the ar/R region (around $z = -7\text{\AA}$). It then dwells in the free-energy well in the vicinity of the Cys-Hg⁺ 170 residue. From there, it overcomes a second free-energy barrier near the NPA motifs before traversing onto the cytoplasm side of the membrane. Thus it is the dwelling waters that clog up the water channel and hinder the permeation flow of water. It is also noted that the Na⁺ and Cl⁻ ions do not enter into the water channels, which is consistent with the known fact that aquaporin water channels do not allow permeation of ions.

Based on the *in silico* experiments of the AQPZ-L170C mutant with or without a mercury ion bonded its Cys 170 residue, we find that mercury does not inhibit L170C by inducing significant structural changes in the protein or by directly occluding the water channel but, instead, it does so by making water molecules clog the water channel. Our model system of L170C-Hg⁺ does not involve more than one mercury atoms/ions in a given water pore. It is relevant to *in vitro* experiments at low concentrations of mercury. At low mercury concentrations, the probability for multiple mercury atoms/ions to exist in any given water pore should be small. The most probable case is that one mercury ion is covalently bonded to the Cys 170 residue. Finally, in this *in silico* study, we use a planar POPE bi-layer to model the cell membrane, which is not exactly identical to the membrane of the proteoliposomes used in the *in vitro* experiments of Ref. [10]. It is true that the liposome curvature is neglected in our planar lipid bi-layer. However, considering the fact that L170C structure does not deviate significantly in presence or in absence of mercury, no significant effects are expected from the membrane curvature. Also, the phospholipid headgroups of the proteoliposomes may be charged but the POPE headgroups are neutral (though highly polar). This difference is not expected to cause any problems because both sides of the membrane is fully solvated with sufficient amount of water having appropriate concentration of Na⁺ and Cl⁻ ions.

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Fig. 1.

The all-atom model system consisting of an L170C homotetramer embedded in a patch of POPE bi-layer solvated in a $109 \text{ \AA} \times 109 \text{ \AA} \times 117 \text{ \AA}$ box of waters neutralized and ionized with NaCl to a concentration of 0.15M. (A) shows only the L170C tetramer (blue) and the patch of lipid bi-layer (cyan). (B) shows the top view and (C), the side view of the system. In (B) and (C), waters are the red dots. Sodium and chlorine ions are shown as yellow and blue spheres respectively. (D) shows one protomer of the L170C mutant of AQPZ along with waters in its pore. (E) shows one protomer of the L170C-Hg mutant of AQPZ along with waters in its pore. In both (D) and (E), Residues His 174, Arg 189, Cys 170, and Cys-Hg 170 are shown in VDW representation. Also in VDW are the waters. The graphics were rendered with VMD. [65] The MD simulations were implemented as the Langevin dynamics under constant pressure of 1 bar with periodic boundary conditions. The particle mesh Ewald was employed for long range electrostatic forces. The cutoff distance was set to $12 \text{ \AA}$ with a switching distance of $10 \text{ \AA}$. The time step was chosen as 2 fs and the damping constant 5/ps.

Fig. 2. RMSDs of L170C (A) and L170C-Hg⁺ (B) from the crystallographic structure of L170C (PDB code: 2O9F) as a function of time. The initial structure of L170C-Hg⁺ is derived from that of L170C after 6 ns of equilibration by mutating Cys 170 and deprotonating His 174. The pore radii along the channel axis (z-axis) at t=0, 6, 12, 18, 24 ns for L170C (C) and L170C-Hg⁺ (D). The t=0 snapshots are the crystal structures of L170C (PDB code: 2O9F) and of L107C-Hg (PDB code: 2O9G). (E) illustrates a representative water pore of a protomer. Rendered with VMD[65] and HOLE2 plug-in. [66]

Fig. 3.

(A) Mean square displacements (in $\text{\AA}^2$) of waters in the water channel as a function of time (in ps). The mean values and the error bars are computed from two sets of simulation paths. The first set contains 100 paths of 100 ps each that are the consecutive 100 ps-segments of the simulation sampling from 16 to 26 ns. The second set consists of the same from the simulation from 26 to 36 ns. The simulation from 0 to 16 ns was to equilibrate the system after embedding the protein to the lipid bi-layer and melting the lipid tails. (B) Potential of mean force computed from the water density profile along the water channel. The mean values and the error bars are computed from two sets of data that were obtained from simulations of 16 to 26 ns and from 26 to 36 ns for each system. The PMF was computed as $\Delta G(z) = -k_B T \ln[n(z)/n_0]$ where z is the coordinate along the water channel pointing along the direction from periplasm to cytoplasm, $n(z)$ is number density of waters in a water channel, and $n_0 = n(z = 20\text{\AA})$ is the reference density chosen to be at $z = 20\text{\AA}$.

Fig. 4. Waters near Residue 170 (Cys or Cys-Hg⁺) and their electrostatic energy with Residue 170. Shown in the top are representative snapshots of a protomer with waters its pore. Cys 170 and Cys-Hg⁺ 170 are shown in the VDW and the waters in the CPK representation. Shown in bottom left is the number of waters with in 5Å of Residue 170. Shown in bottom right is the electrostatic energy of those waters with Residue 170. Rendered with VMD. [65]