The protonation state of Glu202 in acetylcholinesterase

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June 5, 2021

Abstract

Acetylcholinesterase (AChE) is the crucial enzyme in the central nervous system. It is the target of various organophosphorus nerve agents and pesticides, and the inhibition of AChE is a therapeutic strategy for the treatment of various neurological-related diseases. The Glu202 is a key residue adjacent to the catalytic His447 and plays important role in catalysis. Although the Glu202 has long been considered as negatively charged in many studies, more and more evidences support a protonated Glu202. However, Glu202 is freely accessible by solvent, and thus it seems more reasonable for Glu202 to majorly take the deprotonated state. In the present work, we carried out a series of molecular dynamics simulations with the Glu202 adopting different protonation states. Our results show that the protonated Glu202 is important in maintaining the key hydrogen bond network that supports the catalytic triad, whereas the deprotonated Glu202 results in the collapse of the key hydrogen bond network which consequently destabilizes the catalytic His447. We also notice that different protonation states of Glu202 merely alters the binding mode of ACh. However, since the catalytic His447 is disrupted if Glu202 is deprotonated, His447 can not facilitate the nucleophilic attack performed by Ser203. Therefore, the catalytic efficiency of ACh hydrolysis should be remarkably decreased if Glu202 is deprotonated. Our findings suggest that, when designing and developing highly active AChE inhibitors or proposing mechanistic hypotheses for AChE-catalyzed reactions, the protonated state of Glu202 should be considered.

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Acetylcholinesterase (AChE) is the crucial enzyme in the central nervous system. It is the target of various organophosphorus nerve agents and pesticides, and the inhibition of AChE is a therapeutic strategy for the treatment of various neurological-related diseases. The Glu202 is a key residue adjacent to the catalytic His447 and plays important role in catalysis. Although the Glu202 has long been considered as negatively charged in many studies, more and more evidences support a protonated Glu202. However, Glu202 is freely accessible by solvent, and thus it seems more reasonable for Glu202 to majorly take the deprotonated state. In the present work, we carried out a series of molecular dynamics simulations with the Glu202 adopting different protonation states. Our results show that the protonated Glu202 is important in maintaining the key hydrogen bond network that supports the catalytic triad, whereas the deprotonated Glu202 results in the collapse of the key hydrogen bond network which consequently destabilizes the catalytic His447. We also notice that different protonation states of Glu202 merely alters the binding mode of ACh. However, since the catalytic His447 is disrupted if Glu202 is deprotonated, His447 can not facilitate the nucleophilic attack performed by Ser203. Therefore, the catalytic efficiency of ACh hydrolysis should be remarkably decreased if Glu202 is deprotonated. Our findings suggest that, when designing and developing highly active AChE inhibitors or proposing mechanistic hypotheses for AChE-catalyzed reactions, the protonated state of Glu202 should be considered.

Keywords

acetylcholinesterase, protonation state, Glu202, catalytic triad

Introduction

Acetylcholinesterase (AChE) is the enzyme responsible for the termination of impulse transmission in the central nervous system. It rapidly hydrolyzes acetylcholine (ACh), the neurotransmitter in the synaptic cleft at the neuromuscular junction and at cholinergic synapses.[1] The catalytic efficiency is very high, approaching the rate of a diffusion-controlled reaction.[2,3]AChE is the target of various natural and synthetic compounds such as organophosphorus (OP) nerve agents and pesticides.[4] By inhibiting AChE, OP compounds cause accumulation of ACh, resulting in paralysis, seizures, and other symptoms of the cholinergic syndrome, and even lead to death by respiratory arrest. Since the level of ACh is associated with various neurological disorders, the inhibition of AChE is also a therapeutic strategy for the treatment of related diseases. The AChE inhibitors have provided the principal drugs approved by the FDA for the management of Alzheimer's disease.[4,5]

The binding pocket of AChE is a long and narrow gorge, extending from the surface of the enzyme down to the catalytic site. In this site, the catalytic triad (Ser203, His447, Glu334) and the oxyanion hole (Gly121, Gly122, Ala204) are essential in the catalytic reaction. The Glu202, a residue adjacent to the catalytic His447, also plays important role in catalysis.[6-8] With substitutions at Glu202, the catalytic rate constant was decreased up to 80-fold compared with wild-type AChE.[9] It has also been suggested that Glu202 is a key residue facilitating the HI6-induced reactivation of the sarin-inhibited AChE.[10] In addition, Glu202 has been reported as a key residue of AChE with an important role in phosphorylation, [7] spontaneous reactivation[11], and aging [8]. Although Glu202 has long been considered as negatively charged in many studies, there exists evidence suggesting that Glu202 is more likely to be protonated. For example, AChE is more stable when both Glu202 and Glu450 are protonated.[12] Also, it has been suggested that Glu202 needs to be protonated for reactivation to occur.[13]However, Glu202 is on the surface of the catalytic site and is freely accessible by water molecules. It seems reasonable for Glu202 to majorly take the deprotonated state. Why Glu202 more likely to adopt the protonated state is still not fully understood.

Butyrylcholinesterase (BChE) is a plasma cholinesterase. It is structurally very similar to AChE. In BChE, the Glu197 is a residue corresponding to the Glu202 in AChE. In our previous study, we found that the protonation state of Glu197 in BChE is crucial in maintaining the catalytic triad.[14] Shown in Figure 1A

is the crystal structure of BChE. Our study demonstrated that the Glu197 needs to be protonated to join and stabilize a key hydrogen bond network, which has a highly conserved water molecule at its center. Without the support of this key hydrogen bond network, the catalytic His438 dramatically shifts away from its crystal structure position, resulting in a distorted catalytic triad that is unable to perform catalysis. If Glu197 is deprotonated, then repulsion occurs between the negatively charged water oxygen and the negatively charged carboxyl group of Glu197, pushing Glu197 away from the key hydrogen bond network. Without the stabilization effect from Glu197, the key hydrogen bond network eventually collapses, affecting the stability of catalytic H447 and consequently caused a distorted catalytic triad.

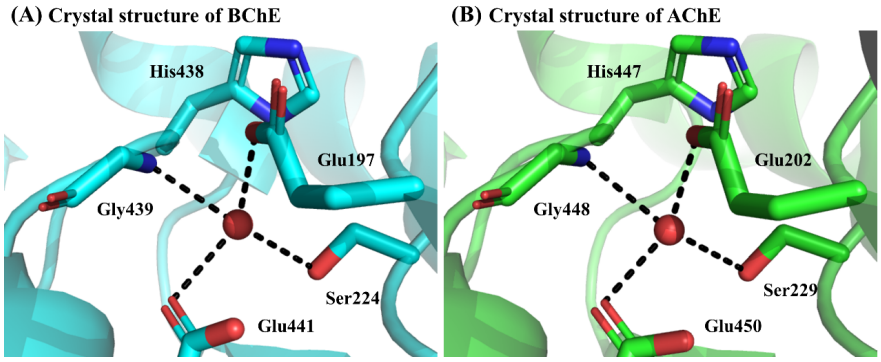

Figure 1. The key hydrogen bond network in (A) the crystal structure of BChE (PDB ID 4BDS), and in (B) the crystal structure of AChE (PDB ID 4EY4). The oxygen and nitrogen atoms are rendered with red and blue colors, respectively. The carbon atoms in BChE and AChE are rendered with cyan and green colors, respectively. The black dashed lines indicate hydrogen bonds, and the red spheres at the center are the water molecules.

In the crystal structure of AChE, as we can see from Figure 1B, such a key hydrogen bond network also exists, and it is highly similar to that in BChE. Therefore, just as that in BChE, the Glu202 in AChE is very likely to be protonated in order to join and stabilize the key hydrogen bond network. Interestingly, this hydrogen bond network has been pointed out to be critical in stabilizing the active center, but the protonation state of Glu202 was not mentioned.[15] Thus, it is of great interest to know what protonation state is adopted by Glu202 in stabilizing the key hydrogen bond network, and what consequence is if Glu202 adopts a different protonation state. In the present work, we carried out a series of molecular dynamics simulations to investigate the dynamical behaviors of the key hydrogen bond network with the Glu202 adopting different protonation states. Their effects on the stability of catalytic His447 are also examined.

Materials and Methods

Structures preparation

The initial structures for molecular dynamics (MD) simulations were constructed based on the crystal structure of AChE with the PDB ID 4EY4. All buffer ions and glycerol were deleted. Missing residues were fixed by using the Galaxyfill standalone program.[16] The protonation states for the titratable residues at pH 7.4 were assigned by using pdb2pqr[17] and had been carefully verified by visualization. The ACh substrate was optimized by performing *ab initio* quantum mechanical calculations using Gaussian09 program[18] at HF/6-31G* level, followed by calculations of electrostatic potentials on the molecular surfaces also at HF/6-31G* level. The calculated electrostatic potentials were used to determine partial atomic charges of ACh by using the standard restrained electrostatic potential (RESP) fitting procedure.[19]The missing force field parameters were taken from the general Amber force field (GAFF) implemented in Amber 18.[20] Each system was neutralized by adding counter ions of 0.15 M NaCl and then was solvated in a rectangular box of TIP3P water molecules with a minimum solute wall distance of 10 Å. The number of counterions was determined by using the SLTCAP algorithm, which was supposed to more accurately capture screening effects.[21] All

inserted sections, including the missing residues, hydrogen atoms, waters, and counter ions, were subjected to energy-minimization followed by 1 ns conventional MD simulations to remove possible bad contacts and to equilibrate the waters and counter ions. The positional constrains with the weight of 100 kcal/(mol· Å^2) were applied on the remaining atoms, *i.e.* , all atoms resolved in the crystal structure.

For the MD simulations of AChE-ACh complex systems, the substrate ACh was docked into the active site of AChE by using the Autodock vina program.[22] Note that, to initiate the hydrolysis of ACh, the ACh carbonyl carbon atom should be close enough to the catalytic Ser203, and the carbonyl oxygen atom should be close enough to the oxyanion hole. Thus, to ensure an appropriate starting structure, we had also employed NMR constraints with the weight of 10 kcal/(mol· Å^2) on the key distances between the ACh carbonyl carbon atom and the catalytic Ser203 hydroxyl oxygen atom, and on the key distances between the ACh carbonyl oxygen atom and the amide hydrogen atoms of oxyanion hole. The NMR constraints would take effect only when the distance to Ser203 was larger than 3.5 Å, or the distances to the oxyanion hole were larger than 2.5 Å.

The last snapshots of each MD simulation were taken as the initial structures for the subsequent conventional MD simulations.

MD simulations

All MD simulations were carried out with Amber ff14SB force field by using the PMEMD module of Amber 18.[20] The Particle Mesh Ewald method was used and nonbonded interactions were calculated within the cutoff value of 10 Å. Each structure was relaxed through energy-minimization with 4000 steps steepest descent followed by 1000 steps conjugate gradient. The minimized structure was then heated by varying the temperature linearly from 0 to 300K in the NVT ensemble and then subjected to the 10 ns production run under the NPT ensemble. The SHAKE algorithm was used to constrain the bond length of hydrogen atoms. A time step of 2 fs was used and the Langevin Thermostat with a collision frequency of 5 per ps was employed to control the temperature.

Results and Discussion

Protonated Glu202 stabilizes the hydrogen bond network

To investigate the effects of the protonation states of Glu202, we have performed two sets of ˜100 ns MD simulations, where the only difference is the protonation state of Glu202. Shown in Figure 2A is the RMSD traces of protonated Glu202 with respect to its crystal structure position. Clearly, the protonated Glu202 is pretty stable and stays at its initial crystal position throughout the entire simulation. However, as we can see from Figure 2B where Glu202 is deprotonated, the RMSD traces show that Glu202 is quite unstable, and moves away from its crystal structure position for most of the time during the simulation. To understand why the protonated Glu202 is much more stable than the deprotonated Glu202, we have compared the representative MD-simulated structures to the crystal structure. In Figure 2C, the carbon atoms of the representative structure and of the crystal structure are rendered with orange and green colors, respectively. We can see that the key hydrogen bond network in the simulated structure (orange carbons) is almost identical to that in the crystal structure (green carbons). There are four stable hydrogen bonds associate with the centered water molecule. This water molecule points its two hydrogen atoms toward Glu450 and Ser229, respectively. Thus, for the remaining two hydrogen bonds, the water oxygen needs to accept the hydrogen atoms from Gly448 and Glu202, respectively, evidently showing the Glu202 needs to be protonated. Clearly, if the Glu202 is deprotonated, then repulsion occurs between the water oxygen atom and the Glu202 carboxyl group, pushing the Glu202 away from its crystal structure position. As we can see from Figure 2D, where the carbon atoms of the representative MD-simulated structure are rendered with light blue color, the deprotonated Glu202 significantly deviates from its crystal structure position. The Glu450 also deviates largely from its crystal position. Importantly, the key water molecule is disappeared, suggesting the key hydrogen bond network is collapsed. This observation is consistent with what we discovered in the previous study, where the key hydrogen bond network in BChE eventually collapses without the support from Glu202.

Apparently, due to the multiple hydrogen bonds associate with the centered water molecule, the Glu202 needs to be protonated to join and stabilize the hydrogen bond network, whereas the deprotonated Glu202 can not join it, eventually resulting in the collapse of the key hydrogen bond network.

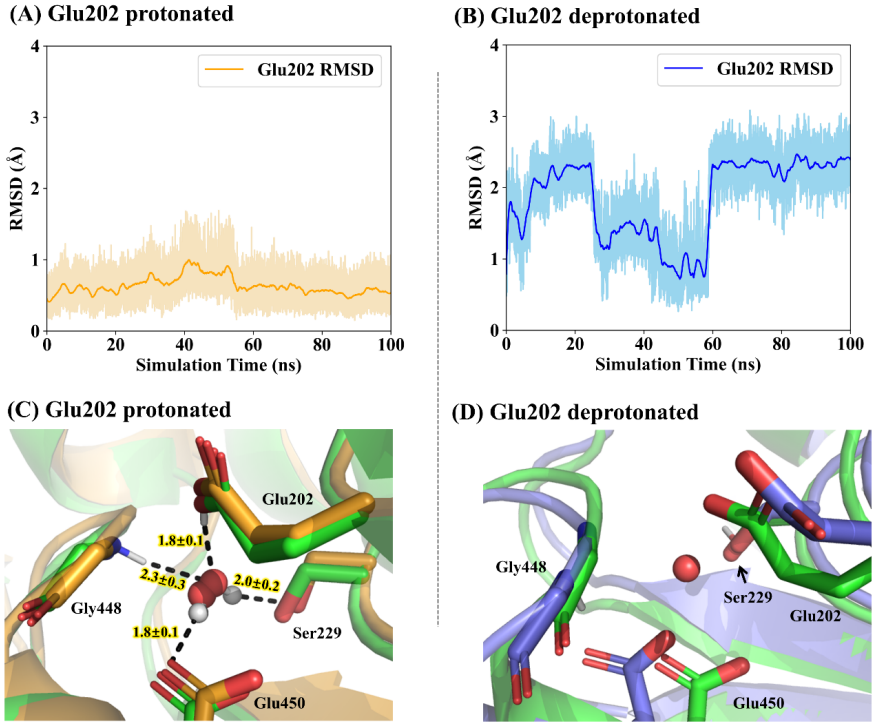

Figure 2. (A) The RMSD traces of the protonated Glu202. (B) The RMSD traces of the deprotonated Glu202. (C) The representative structure of the key hydrogen bond network with Glu202 being protonated in comparison to that in the crystal structure. (D) The representative structure of the key hydrogen bond network with Glu202 being deprotonated in comparison to that in the crystal structure. The carbon atoms in the crystal structure are colored with green, and the carbon atoms in the Glu202-protonated and Glu202-deprotonated structures are colored with orange and light blue, respectively. The oxygen, nitrogen, and hydrogen atoms are colored red, blue, and white, respectively. The key water molecules are rendered as ball and stick. The black dashed lines are the simulated hydrogen bonds associate with the key water molecule. The distances of the hydrogen bonds are labeled with yellow background and are in the angstrom unit. All nonpolar hydrogen atoms are not shown for clarity.

The catalytic His447 is more stable with Glu202 being protonated

In our previous study, we found that the catalytic His438 of BChE is eventually distorted after the collapse of the key hydrogen bond network. It is thus interesting to know whether the same effects can be observed in AChE. Shown in Figure 3A is the RMSD traces of the catalytic His447 of AChE with Glu202 being protonated. Clearly, the His447 stays firmly at its crystal structure position throughout the entire simulation. However, from Figure 3A where Glu202 is deprotonated, the RMSD traces indicate that the catalytic His447 stays at its crystal structure position for only the initial 20 ns. Then, the His447 deviates significantly from its crystal structure position. Shown in Figure 3C is the His447 in the representative MD-simulated structure compared to that in the crystal structure. Similar to what is observed for the key hydrogen bond network, the simulated His447 is also almost identical to that in the crystal structure. The distances of the two hydrogen bonds in the catalytic triad are 2.4±0.8 Å and 1.8±0.1 Å, respectively, indicating a stable catalytic triad throughout the simulation. However, this catalytic triad is observed as disrupted in a simulation with

Glu202 being deprotonated. As we mentioned above, due to the repulsion between the deprotonated Glu202 and the key water molecule, the key hydrogen bond network is eventually collapsed. From Figure 4D, we can see that the collapsed key hydrogen bond network produces an empty space for the catalytic His447. This empty space decreases the stability of His447 and promotes it to deviate largely from its crystal structure position, causing the disruption of the catalytic triad.

Therefore, the key hydrogen bond network plays an important role in stabilizing the catalytic triad through supporting the His447. Without the key hydrogen bond network, the His447 could deviate largely to occupy the space that originally belongs to the hydrogen bond network, and this large deviation of His447 apparently results in the distortion of the catalytic triad.

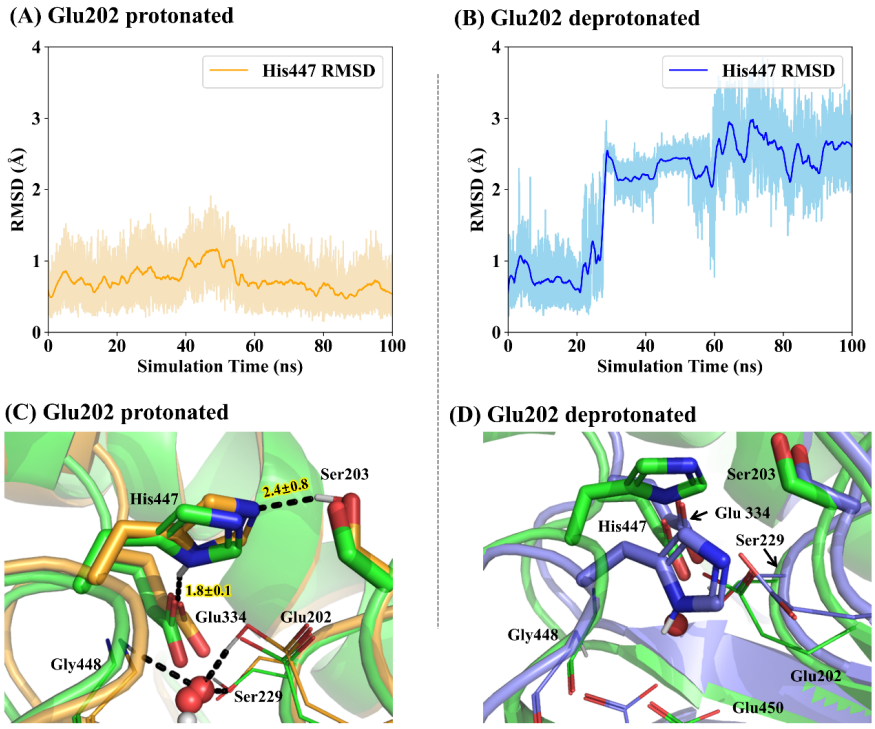

Figure 3. (A) The RMSD traces of the catalytic His447 in simulation with Glu202 being protonated. (B) The RMSD traces of the catalytic His447 in simulation with Glu202 being deprotonated. (C) The representative structure of the catalytic His447 with Glu202 being protonated in comparison to that in the crystal structure. (D) The representative structure of the catalytic His447 with Glu202 being deprotonated in comparison to that in the crystal structure. Atoms are colored and rendered in the same style as in Figure 2.

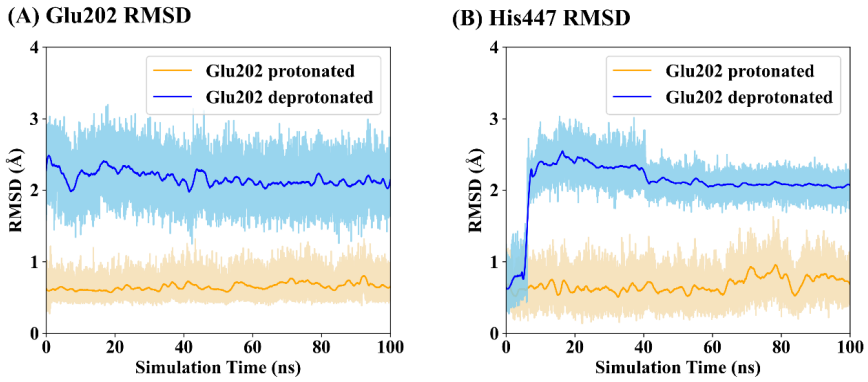

Figure 4. (A) The RMSD traces of Glu202 in simulations with Glu202 taking different protonation states. (B) The RMSD traces of the catalytic His447 in simulations with Glu202 taking different protonation states. The traces with Glu202 being protonated and deprotonated are colored with orange and blue, respectively. The substrate is ACh.

The effects of Glu202 protonation states on AChE-ACh complex

Now that it is clear the protonation states of Glu202 play important role in stabilizing the key hydrogen bond network and consequently stabilizing the catalytic triad in *apo* AChE. Whether the same effects could be observed when AChE binds a substrate remains uncertain. To answer this question, we have performed ˜100 ns MD simulations with ACh binding at the active site. As we can see from Figure 4A, the RMSD trace of the protonated Glu202 vibrates around small values, indicating a stable Glu202 that stays firmly at its crystal structure position, whereas the RMSD trace of the deprotonated Glu202 vibrates around large values, showing a Glu202 that moves away from its crystal structure position throughout the entire simulation. Similar behaviors are observed for the catalytic His447. In Figure 4B, we can see that His447 also stays firmly at its crystal position when Glu202 is protonated, and dramatically moves away when Glu202 is deprotonated.

Shown in Figure 5 are the comparisons between the representative MD-simulated structures and the crystal structure. In Figure 5A, the key hydrogen bond network is almost identical to that in the crystal structure. The lengths of four hydrogen bonds are 1.8±0.1 Å, 1.9±0.2 Å, 1.8±0.1 Å, and 2.4±0.3 Å, respectively, implying a very stable hydrogen bond network throughout the entire simulation where Glu202 is protonated. As expected, Figure 5B shows that the hydrogen bond network is collapsed when Glu202 is deprotonated, illustrating that the deprotonated Glu202 causes the collapse of the key hydrogen bond network no matter there is ACh binding at the active site or not. As for the catalytic His447, the representative MD-simulated structure, which is given in Figure 5C, clearly shows that the His447 is very close to that in the crystal structure. The distances of the two hydrogen bonds in the catalytic triad are 1.9±0.2 Å, and 1.7±0.1 Å, respectively, indicating a very stable catalytic triad with Glu202 being protonated. However, if Glu202 is deprotonated, as shown in Figure 5D, the catalytic His447 deviates significantly away from its crystal structure position, and the hydrogen bond between His447 and Ser203, which is critical in maintaining the catalytic triad, is lost due to the shift of His447. Shown in Figure 5E and F are the binding modes of ACh with Glu202 being protonated and deprotonated, respectively. In both structures, the averaged distances between the carbonyl oxygen of ACh and the oxyanion hole are no more than 3.2 Å, and their standard deviations are very small, indicating that ACh binds tightly with the oxyanion hole regardless of the protonation state of Glu202. Also, the distances between the carbonyl carbon of ACh and the hydroxyl oxygen of Ser203 are 3.4±0.4 Å with Glu202 being protonated and 3.2±0.2 Å with Glu202 being deprotonated, suggesting the Ser203 is at an appropriate position for the nucleophilic attack no matter what the protonation state of Glu202 is. Apparently, the protonation states of Glu202 barely alter the binding mode of ACh. Actually, if Glu202 is deprotonated, ACh binds slightly tighter with the oxyanion hole and also slightly closer to

the nucleophilic hydroxyl group of Ser203. However, as clearly shown in Figure 5F, the catalytic His447 significantly moves away from Ser203, and thus lost its role in assisting the nucleophilic attack on ACh performed by Ser203. As a result, the catalytic hydrolysis of ACh hardly starts and the catalytic efficiency should be remarkably decreased if Glu202 is deprotonated.

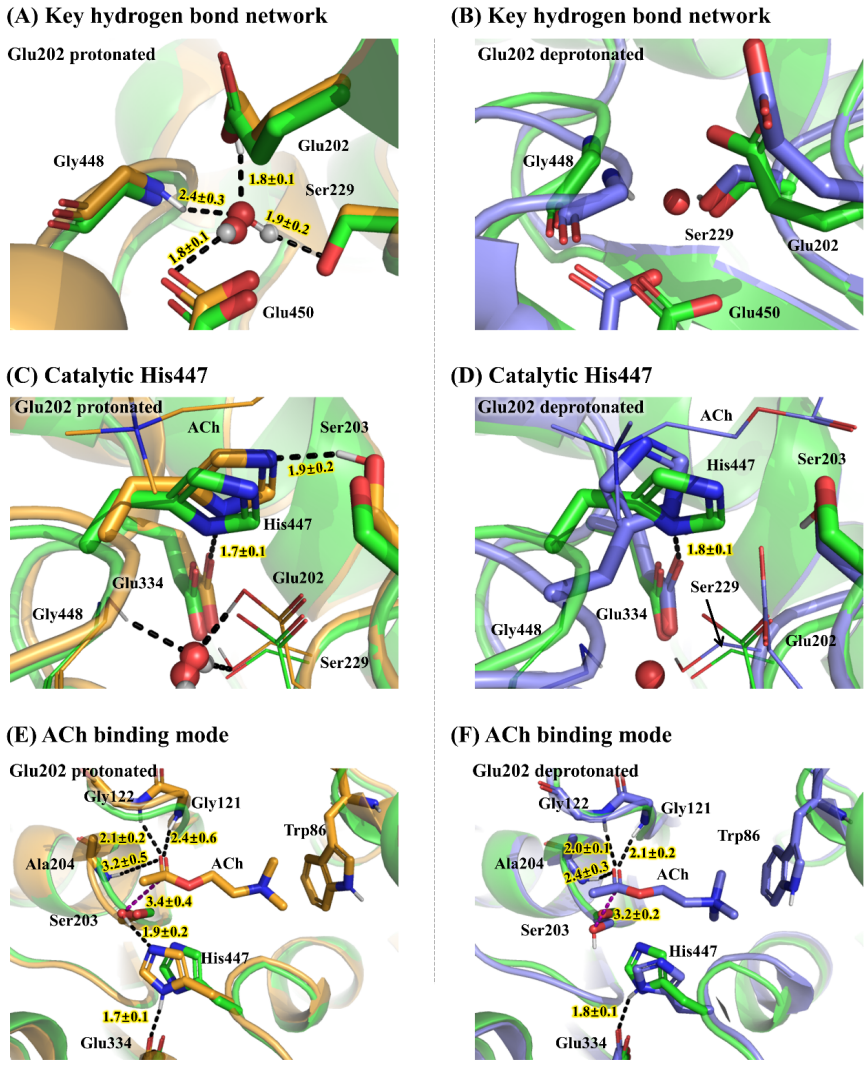

Figure 5. The comparisons between the representative MD-simulated structures and the crystal structure. (A) The key hydrogen bond network with Glu202 being protonated. (B) The key hydrogen bond network with Glu202 being deprotonated. (C) The catalytic His447 with Glu202 being protonated. (D) The catalytic His447 with Glu202 being deprotonated. (E) The binding mode of ACh with Glu202 being protonated. (F). The binding mode of ACh with Glu202 being deprotonated. The purple dashed lines indicate distances between the hydroxyl oxygen of catalytic Ser203 and the carbonyl carbon of ACh. Atoms are colored and rendered in the same style as in Figure 2.

Conclusion

In this study, we demonstrate that the protonated Glu202 is important in maintaining the key hydrogen bond network and hence supporting the catalytic triad. The key hydrogen bond network has a water molecule sitting at the center. Due to the multiple hydrogen bonds associate with the centered water

molecule, the Glu202 needs to be protonated to join and stabilize this hydrogen bond network, whereas the deprotonated Glu202 results in the collapse of the key hydrogen bond network which consequently destabilizes the catalytic His447. Although different protonation states of Glu202 merely alters the binding mode of ACh in the enzyme, the catalytic efficiency of ACh hydrolysis should be remarkably decreased if Glu202 is deprotonated, since the disrupted His447 can not facilitate the nucleophilic attack performed by Ser203.

Our findings suggest that it is necessary to take into account the protonation states of Glu202 for AChE related studies. In particular, when designing and developing highly active AChE inhibitors, or proposing mechanistic hypotheses for AChE-catalyzed reactions, the protonated state of Glu202 should be considered.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported in part by the Open Project Program of Key Laboratory of Drug Prevention and Control Technology of Zhejiang Province (No. 2020005), and by the National Natural Science Foundation of China Grants (21102050). The computational work in this paper is supported by the public computing service platform provided by the Network and Computing Center of HUST.

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