Insights from studying the mutation-induced allostery in the M2 proton channel by molecular dynamics

Jing-Fang Wang1,2,3,4 and Kuo-Chen Chou1,3

1Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, 800 Dongchuan, Shanghai 200240, China, 2Shanghai Center for Bioinformation Technology, 100 Qinzhou Road, Shanghai 200235, China and 3Gordon Life Science Institute, 13784 Torrey Del Mar Drive, San Diego, CA 92130, USA

*To whom correspondence should be addressed.
E-mail: jfwang8113@sjtu.edu.cn

Received April 22, 2010; revised May 25, 2010; accepted May 26, 2010

Edited by Marius Clore

As an essential component of the viral envelope, the M2 proton channel plays a central role in the virus replication and has been a key target for drug design against the influenza A viruses. The adamantane-based drugs, such as amantadine and rimantadine, were developed for blocking the channel so as to suppress the replication of viruses. However, patients, especially those infected by the H1N1 influenza A viruses, are increasingly suffering from drug-resistance problem. According to the findings revealed recently by high-resolution NMR studies, the drug-resistance problem is due to the structural allostery caused by some mutations, such as L26F, V27A and S31N, in the four-helix bundle of the channel. In this study, we are to address this problem from a dynamic point of view by conducting molecular dynamics (MD) simulations on both the open and the closed states of the wild-type (WT) and S31N mutant M2 channels in the presence of rimantadine. It was observed from the MD simulated structures that the mutant channel could still keep open even if binding with rimantadine, but the WT channel could not. This was because the mutation would destabilize the helix bundle and trigger it from a compact packing state to a loose one. It is anticipated that the findings may provide useful insights for in-depth understanding the action mechanism of the M2 channel and developing more-effective drugs against influenza A viruses.

Keywords: influenza A virus/M2 proton channel/drug resistance/molecular dynamics/four-helix bundle

Introduction

The surface membrane proteins of influenza A virus are constituted by three important components: hemagglutinin, neuraminidase and the M2 proton channel. The M2 proton channel is a homotetrameric protein with 97 residues per subunit, each of which comprises an intracellular C-terminal domain formed by 54 residues, a transmembrane domain formed by 19 residues and an extracellular N-terminal domain formed by 24 residues (Pielak and Chou, 2010). Functioning as a proton channel, the M2 protein is regulated by the surrounding pH values in endosomes (Chizhmakov et al., 1996). Owing to its ability in controlling the entrance of protons into the virion, the M2 channel is thought to play a crucial role in the viral life cycle and has been an attractive drug target against influenza A virus. For instance, amantadine and rimantadine were the first effective drugs for influenza treatment, and both are adamantane-based drugs designed to inhibit the channel. However, more and more patients, especially those infected by the recent H1N1 influenza virus, are suffering from drug resistance to adamantane-based drugs (Bright et al., 2006).

Recently, using the high-resolution NMR spectroscopy (Schnell and Chou, 2008), the long-sought 3D structure of the M2 protein channel has been successfully determined that may eventually lead to the solution of the drug-resistance problem and help design more-effective medications (Borman, 2008).

According to the NMR structure (Schnell and Chou, 2008), the M2 proton channel has a minimalist architecture with four equivalent rimantadine binding sites accessible from the lipid bilayer at the interface between two subunits. The mutation S31N has been detected in most of the resistant viruses and hence was thought to be the arch-criminal for resisting the adamantane-based drugs. However, the mechanism underlying the resistance of influenza viruses to adamantane-based drugs is not quite straightforward. This is because the resistance-conferring mutations, such as L26F, V27A, A30T, G34E and L38F, are spread over three helical turns of the transmembrane helix, covering a region much larger than the dimensions of the aforementioned drug. To reveal such a puzzle, a novel allosteric mechanism was proposed for the drug inhibition and drug resistance of influenza A M2 channel (Schnell and Chou, 2008), and it was further confirmed by a series of follow-up studies from different angles (Huang et al., 2008; Du et al., 2009; Pielak et al., 2009b). Meanwhile, the mechanism has also been used to guide the design of new drugs (Wei et al., 2009; Du et al., 2010; Zarubaev et al., 2010).

The present study was initiated in an attempt to investigate into the allosteric mechanism from the dynamic point of view. There exist low-frequency motions in biomacromolecules as inferred by a series of theoretical studies (Chou and Chen, 1977; Chou, 1983, 1984a,b, 1985; Chou et al., 1989) and observed by experimental observations (Painter et al., 1981, 1982; Martel, 1992; Urabe et al., 1983, 1998). Many marvelous biological functions in proteins and DNA and their profound dynamic mechanisms, such as cooperative effects (Chou, 1989), allosteric transition (Chou, 1987), intercalation of drugs into DNA (Chou and Mao, 1988) and assembly of microtubules (Chou et al., 1994), can be revealed by investigating their internal motions as elaborated in a comprehensive review (Chou, 1988). Such inferences have been later observed by NMR (Chou et al., 2001) and applied in medical treatments (Gordon, 2007, 2008; Madkan et al., 2009). Meanwhile, investigation into the internal...
motion in biomacromolecules and its biological functions is deemed as a ‘genuinely new frontier in biological physics’, as recently announced by the Vermont Company at its website at http://homepages.sover.net/~bell/newFrontierpics.htm. In view of this, to better understand the novel allosteric inhibition mechanism of the M2 channel, we should consider not only the static structural information of the channel but also its dynamical information obtained by simulating its internal motions and dynamic process.

To realize this, the molecular dynamics (MD) simulation (Brunger et al., 1985; Karplus and McCammon, 2002; Concet et al., 2009; Gonzalez-Diaz et al., 2009; Perez-Montoto et al., 2009) was utilized to investigate the dynamical behaviors for the M2 proton channel and its mutated structures in hopes to provide useful insights for understanding the allosteric mechanism at a deeper level.

Materials and methods

The structure for the wild type (WT) of the M2 channel was generated from the atomic coordinates of 2rlf.pdb (Schnell and Chou, 2008), whereas for its S31N mutant from 2kjh.pdb (Pielak et al., 2009a). Both were downloaded from the RCSB Protein Data Bank (Berman et al., 2000).

Because no ligand was detected in the original S31N mutant structure (2kjh.pdb) to generate a comparable counterpart to the 2rlf.pdb complex, using AutoDock 3.0 (Morris et al., 1998), we docked four rimantadine to the corresponding sites of 2kjh.pdb, where the drug molecule was detected in 2rlf.pdb as a ligand.

To investigate the action mechanism of the proton channel, let us consider the two different types (Schnell and Chou, 2008): one is with non-protonated His-37 tetrads and the other is with fully protonated His-37 tetrads. The latter can be easily converted from the former by using the ‘pdb2gmx’ module in the GROMACS 3.3.3 (van der Spoel et al., 2005).

Thus, we have the following four different types of channels: (i) the WT channel with non-protonated His-37 tetrads, (ii) the WT$^+$ channel with fully protonated His-37 tetrads, (iii) the S31N mutant channel with non-protonated His-37 tetrads and (iv) the S31N$^+$ mutant channel with fully protonated His-37 tetrads.

Subsequently, each of the above four complexes was inserted into 108 DPPC bilayer membranes, followed by being solvated with the explicit SPC water model embedded in a 6.5 × 6.5 × 7.5-nm$^3$ simulation box with 16 chloride ions to neutralize the system concerned.

Before starting MD simulations, each of the four systems formed via the above procedures was subjected to a steepest descent energy minimization until a tolerance of 100 kJ/mol. After energy minimization, each was subject to steepest descent energy minimization until a tolerance of 0.001 kJ/mol. Subsequently, each of the above equilibrated systems for both WT and mutant channels. Meanwhile, the electrostatic interactions were calculated by the PME algorithm with the interpolation order of 4 and 0.12 nm. The van der Waals interactions were treated by using a cutoff of 12 Å. The MD simulations in our study were performed with a time step of 2 fs (10$^{-15}$ s), and the coordinates were saved every 1 ps (10$^{-12}$ s).

In this study, the MD simulations were performed by the free software GROMACS 3.3.3 with GROMACS force field parameters, periodic boundary conditions and NPT ensemble (van der Spoel et al., 2005). The topology file, force field parameters and atomic charges for rimantadine were generated by the online tool PRODRG (Schuttelkopf and van Aalten, 2004; van Aalten et al., 1996). Similar methods and procedures were also successfully used to investigate the protein folding, protein-ligand interaction, protein–protein interaction and protein conformational transitions (see, e.g. Wang et al., 2007a, 2008, 2009a, b; Wang and Chou, 2009).

Results and discussion

It was reported that the M2 channel involved two different configurations with different protonations at His-37 (Schnell and Chou, 2008; Pielak and Chou, 2010). In order to investigate the resistances to the adamantine-based drugs, we calculated the MD simulated pore radius along the axis of the four-helix bundle channel in the presence of rimantadine for (i) the WT channel with non-protonated His-37 tetrads (see the blue curve in Fig. 1), (ii) the WT$^+$ channel with fully protonated His-37 tetrads (see the pink curve), (iii) the S31N mutant channel with non-protonated His-37 tetrads (see the red curve) and (iv) the S31N$^+$ mutant channel with fully protonated His-37 tetrads (see the green curve). The following can be seen from Fig. 1.

When the His-37 tetrads are not protonated, the channel pore radius around the position $-68$ or $-53$ along the WT channel is smaller than the radius of a water molecule (cf.

---

**Fig. 1.** The MD simulated pore radius along the axis of the four-helix bundle (from N- to C-terminus) calculated by the HOLE package (Smart et al., 1993) for the WT and S31N mutant M2 channel with non-protonated and fully protonated His-37 tetrads in the presence of rimantadine, where the blue, pink, red and green curved lines represent for the WT channel, WT channel with fully protonated His-37 tetrads (WT$^+$), S31N channel and S31N channel with fully protonated His-37 tetrads (S31N$^+$), respectively. For facilitating comparison, the van der Waals radius for a water molecule was shown in the dashed lines. See the text for further explanation.
the blue curve of Fig. 1), and hence no water molecule can go through the WT channel, as observed by experiments.

For the S31N channel when its His-37 tetrad is not protonated, the channel pore radius around the position $-67$ or $-51$ along the WT channel is still smaller than the radius of a water molecule (cf. the red curve of Fig. 1), and hence no water molecule can go through the channel without any block. That is why the virus with such mutant M2 channel can be resistant to the adamantane-based drugs, fully consistent with the observation in Pielak et al. (2009a).

The aforementioned phenomena were caused by the structural allostery due to the S31N mutation, weakening the interactions between two adjacent helices of the four-helix bundle channel. This is because Asn has a larger side chain with more amino groups to receive protons than Ser, particularly in high pH conditions. This would loosen the assembly of the four-helix bundle. To quantitatively examine the assembly of the four-helix bundle with a dynamic approach, we calculated the average distances between the mass centers of two adjacent helices from 15 000 simulated structures obtained by the MD simulations. The results are listed in Table I, from which we can see that the distances between the mass centers of two adjacent helices in the mutant channel are all greater than those in the WT channel, clearly indicating that the four-helix bundle (Chou et al., 1988) in the mutated channel is more loosely packed (Chou et al., 1992).

The MD simulated results of Fig. 1 and Table I can be further elucidated as follows. Shown in Fig. 2 is a cartoon drawing for the four-helix bundle in the WT channel before binding with the rimantadine drug (upper left), that in the WT channel after binding with the drug (upper right), that in the S31N mutant channel before binding the drug (lower left) and that in the S31N channel after binding with the drug (lower right). As we can see from the figure, in the WT channel, its four constituent helices are tightly packed in such a manner that, after binding with the rimantadine drug, the His-37 and Asp-44 tetrads (Schnell and Chou, 2008) can act as a gate by the hydrogen-bonding interactions between the adjacent two helices (Huang et al., 2008a) to prevent the water molecules from going through the channel pore. In the S31N mutant channel, however, its four helices were packed in a relatively much looser manner so that, even after binding with rimantadine drug, the channel gate formed by the His-37 and Asp-44 tetrads would become more tolerable for water molecules to go through.

<table>
<thead>
<tr>
<th>Table I. List of the average distances between the mass centers of two adjacent helices over 15 000 MD simulated four-helix bundle structures for the WT and S31N mutant channels, respectively</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
</tr>
<tr>
<td>WT</td>
</tr>
<tr>
<td>S31N</td>
</tr>
</tbody>
</table>

Conclusion

Owing to its vitally important role in the replication cycle of influenza viruses, the M2 proton channel has been an attractive target for designing drugs against flu. For example, the adamantaine-based drugs were developed aimed at inhibiting the channel. However, more and more patients, especially those infected by the new H1N1 influenza virus recently, are suffering from the drug-resistance problem, which is caused by the mutations (L26F, V27A and S31N) in the four-helix
bundle of the channel. It was observed by the MD simulations in this study that the mutant channel could still open even if with the presence of the adamantane-based drugs such as rimantadine. This is remarkably different from the WT channel that can be inhibited by rimantadine. The results of our dynamic study indicate that the aforementioned mutations would induce the four-helix bundle in the original WT M2 channel from a tightly packing state to become a loose packing one, so as to allow the water molecules pass through the channel even if it has been bound with rimantadine, fully consistent with the allosteric mechanism as revealed by the NMR studies (Schnell and Chou, 2008; Pielak et al., 2009a). The findings reported here may also stimulate new strategies to deal with the drug-resistance problem, designing more potent drugs against influenza viruses.

Acknowledgements

The authors wish to thank the two anonymous reviewers, whose constructive comments are very helpful for strengthening the presentation of this study.

Funding

This work was supported by the grants from Shanghai Natural Science Foundation under the Contract No. 10ZR1421500 and “Chen Guang” project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation.

References